Is hexokinase really a sugar sensor in plants?

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The molecular mechanisms by which plant cells sense sugar levels are not understood, but current models (adapted from models for sugar sensing in yeast) favour hexokinase as the primary sugar sensor. However, the hypothesis that yeast hexokinase has a signalling function has not been supported by more recent studies and the idea that hexokinase is involved in sugar sensing in plants has yet to be proven.

Sugar sensing clearly plays an important role in plant cell metabolism and the expression of many genes, including several involved in photosynthesis and carbohydrate metabolism, is affected by sugars. Unravelling the molecular mechanisms of plant sugar sensing is of fundamental importance to understanding plant metabolism and might have biotechnological applications, for example, in manipulating partitioning of carbon between different storage products (starch, proteins, oils) in crop seeds and tubers. At present, however, our understanding of this process is far from complete, and it is worth reviewing the much greater (although incomplete) body of work on sugar sensing in fungi.

Sugar sensing and glucose repression in fungi

In Saccharomyces cerevisiae, genes required for growth on carbon sources other than glucose are repressed by the presence of glucose in the medium, and can be derepressed when it is removed. This is the phenomenon of glucose repression (also known as catabolite repression), which requires a mechanism for sensing the availability of glucose. In spite of intensive study, the intracellular signals that mediate repression and derepression of genes in response to glucose in yeast remain unknown. Possible routes through which glucose could be sensed and a signal transduced are shown in Fig. 1. The signal for relief from catabolite repression in eubacteria is cyclic AMP, which also increases in response to glucose in S. cerevisiae and can regulate the expression of a few glucose-sensitive genes. However, a variety of evidence indicates that cyclic AMP is not a key component of the pathway controlling repression and derepression of most glucose-regulated genes in this organism. The idea that glucose-6-phosphate might be a signal appears to have originated with experiments that demonstrated that 2-deoxyglucose or glucosamine could mimic the effect of glucose in gene repression. Because these hexose sugars were not metabolized at significant rates beyond the hexose phosphate stage, this suggested that the signal for glucose repression was either the hexose itself or the hexose phosphate formed from it.

A flaw in this approach is that sugars that are rapidly phosphorylated by hexokinase, but for which the hexose phosphate is metabolized slowly or not at all, cause depletion of cellular ATP. The hexose phosphate builds up to high levels in the cell, trapping phosphate and preventing rephosphorylation of ADP to ATP. In our experience, incubation of yeast with deoxyglucose or glucosamine could mimic the effect of glucose in gene repression. Because these hexoses are repressed by the presence of glucose and can regulate the expression of many genes, including several involved in photosynthesis and carbohydrate metabolism, is affected by sugars. The idea that glucose repression and hexokinase functions could be dissociated, and this led to the proposal that hexokinase PII had a glucose-sensing function independent of its enzymic activity. However, this did not stand up to further investigation and there is, in fact, a good correlation between the overall hexokinase activity of different mutants and their ability to exhibit glucose repression. These results suggest that hexokinase PII has a role in producing the signal molecule(s) but does not support the idea that it has a sensing role per se. The glucose repression phenotype of hxk2 mutants might merely be because the slow rate of glucose phosphorylation restricts overall glucose metabolism and ATP production when the major hexokinase isoform is missing or defective.

The idea that the signal molecules were glucose and/or glucose-6-phosphate suggested that the enzyme hexokinase might be the sensor protein. The hexokinase hypothesis gained credence in the yeast-research community when mutations (hex1) found to cause partial constitutive expression of glucose-repressed genes were mapped to the gene HXK2, encoding the major hexokinase isoenzyme (Ref. 4). Initial work with hxk2 alleles suggested that the glucose repression and hexokinase functions could be dissociated, and this led to the proposal that hexokinase PII had a glucose-sensing function independent of its enzymic activity. However, this did not stand up to further investigation and there is, in fact, a good correlation between the overall hexokinase activity of different mutants and their ability to exhibit glucose repression. These results suggest that hexokinase PII has a role in producing the signal molecule(s) but does not support the idea that it has a sensing role per se. The glucose repression phenotype of hxk2 mutants might merely be because the slow rate of glucose phosphorylation restricts overall glucose metabolism and ATP production when the major hexokinase isoform is missing or defective.

Heldt’s group2 favoured the idea that changes in hexose or hexose phosphate were the key signal, they also discussed changes in ATP levels as an alternative possibility.

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Work in other fungi also suggests that hexokinase does not play a critical role in glucose repression. In Aspergillus nidulans, a mutant (ΔA1), which lacks hexokinase activity, although still expressing glucokinase, exhibits normal glucose regulation of several genes. In Candida utilis, under conditions where hexokinase activity is reduced to 10% by the addition of an inhibitor to the medium, glucose repression of α-glucosidase is not affected. Although the nature of the signal molecules remains unclear, genes involved in reversal of glucose repression in S. cerevisiae are well characterized. In particular, a functional sucrose nonfermenting-1 (SNF1) gene is essential for derepression of all glucose-repressed genes. The SNF1 gene encodes a protein-serine/threonine kinase, and the active kinase is a high-molecular-mass complex (which we shall term the SNF1 complex) containing the products of the SNF1 and SNF4 genes, and one of the products of the SIP1, SIP2 or GAL83 genes. These are the homologues of the α, β and γ subunits, respectively, of mammalian AMP-activated protein kinase (AMPK) [10]. Both SNF1 and SNF4 are essential for the reversal of glucose repression. The products of the three separate genes, SIP1, SIP2 and GAL83, represent alternate forms of the β subunit, giving rise to at least three forms of the kinase complex. Surprisingly, all three can be deleted in S. cerevisiae without apparent effect, although in the yeast, Kluyveromyces lactis, the FOG1 gene – encoding a β subunit homologue – is essential for reversal of glucose repression [11].

Of several genes shown to act downstream from the SNF1 complex in S. cerevisiae, MGR1 encodes a DNA-binding protein that represses transcription of glucose-repressed genes in the presence of glucose. The amino acid sequence of Mig1 contains several consensus sites for phosphorylation by the SNF1 complex, and mutation of these results in constitutive repression of transcription [12]. Mig1 translocates from the nucleus to the cytoplasm on glucose removability. Surfaces of cells that can function within the glucose-sensing signalling pathway in yeast [13].

Regulation of AMP-activated protein kinase
Regulation of the mammalian AMPK complex is understood better than that of its yeast counterpart. AMPK is in the downstream component of a kinase cascade, being activated by an upstream kinase, AMPK kinase (AMPKK) [14]. The system is activated in a highly sensitive manner by elevation of 5′-AMP and depletion of ATP, via a complex mechanism involving activation of both AMPK and AMPKK, promotion of phosphorylation of AMPK and inhibition of its dephosphorylation [15]. The effects of AMP are antagonized by high (mM) concentrations of ATP, so that the system responds to changes in the AMP:ATP ratio rather than merely to changes in AMP. Because adenylate kinase maintains its reaction (2ADP ↔ ATP + AMP) close to equilibrium, the AMP:ATP ratio varies approximately as the square of the ADP:ATP ratio [16], and is a very sensitive indicator of the cellular energy charge.

The AMPK cascade is activated by environmental stresses that deplete cellular ATP (Ref. 12) and also, at least in pancreatic β cells, by glucose deprivation [17]. This raises the intriguing possibility that the SNF1 complex might be activated in a similar manner in response to glucose deprivation of yeast, and that changes in AMP and ATP might be the signals responsible. The SNF1 complex was indeed found to be activated by glucose removal [18]. It appears to be due to phosphorylation by an upstream kinase, and to be associated with large increases in the cellular AMP:ATP ratio [11]. Although AMP does not allosterically activate the SNF1 complex [19], it remains possible that it could activate the system through one of the other mechanisms established for the AMPKK system, such as activation of the upstream kinase. Glucose-6-phosphate and a wide range of other sugar phosphates do not affect the activity of the SNF1 kinase complex in vitro [20].

Sucrose nonfermenting-1-related protein kinase 1s
Plant homologues of SNF1 and AMPK, SNF1-related protein kinase 1 (SnRK1), share considerable amino acid sequence identity with SNF1 (~67% in the protein kinase catalytic domain) [21]. They complement snf1 mutants, enabling them to use carbon sources such as glycerol, ethanol and sucrose, indicating that they can function within the glucose-sensing signalling pathway in yeast [22,23].

Is hexokinase a hexose sensor in plants?
The notion that hexokinase is a primary-sugar sensor and that it is capable of initiating a signal has been adopted enthusiastically by a substantial part of the plant science community [24]. The hypothesis has been tested in
transgenic Arabidopsis by antisense and over-expression of hexokinase. Plants with higher hexokinase levels were more sensitive to the cytotoxic of glucose, using photosynthetic gene expression as a marker for glucose repression, whereas plants with lower activity were less sensitive. However, these results could be explained in a similar way to those involving yeast hexokinase mutants; that is, alterations in hexokinase activity might simply affect the rate of metabolism of hexoses and therefore the production of ATP. In this latter model, hexokinase is a rate-limiting enzyme for glucose metabolism, but not a sugar sensor per se.

Another line of evidence that supports the hypothesis that hexokinase is a sugar sensor involved treating maize protoplasts with different sugars, again using photosynthetic gene expression as a marker for sugar sensing. Similar experiments were performed on a cucumber cell line using isocitrate lyase and malate synthase-gene expression as markers. These two genes are repressed by sugars in both plants and yeast. In the maize protoplasts and the cucumber callus cultures 2-deoxyglucose causes a marked degree of gene repression, whereas the non-phosphorylatable sugar, 3-O-methyl glucose does not. These results were interpreted as evidence that metabolism beyond the hexose-6-phosphate is not required to generate the signal. However, the same caveat applies to the use of ‘non-metabolizable’ sugars in plants as in yeast; even if they produce only subtle alterations in cellular ATP (and hence ADP and AMP), they could, as a secondary consequence, affect the level of almost any cellular metabolite.

It has also been reported that the introduction of glucose into cells via electroporation causes repression, whereas introduction of glucose-6-phosphate does not. This could be taken as evidence that phosphorylation of glucose is not necessary to generate the signal. However, in our view, this is not a valid comparison because glucose would still be able to enter the cells by facilitated transport once the electroporation pores had sealed, whereas glucose-6-phosphate would only enter for the brief period when the pores were open.

Another reason for caution in interpreting these results is that they were performed on protoplast cultures or cell lines. We have measured very high levels of Snf1K activity in plant mesophyll cell cultures. For example, activity in potato mini-tubers and callus cultures is ~50- and 25-fold higher, respectively, than in mature leaves, and eight- and four-fold higher, respectively, than in any tissue from whole potato plants. This elevated Snf1K activity is likely to have a significant effect on the signalling pathway. Doubts on a role for hexokinase in sugar sensing also come from evidence that high hexose levels in the presence of hexokinase do not necessarily generate a signal. Expression of yeast invertase in the cytosol of transgenic tobacco plants results in high levels of glucose and fructose but, in spite of the fact that hexokinase is a cytosolic enzyme, the elevated levels of hexoses do not appear to be sensed. However, when the invertase is targeted to the vacuole or apoplast, the high levels of glucose and fructose that are produced in these compartments are sensed and changes in gene expression are observed. These results suggest that the signal is sensed at a membrane location rather than in the cytoplasm.

Sucrose sensing in plants

One clear difference between yeast and plant cells is the importance of sucrose as a carbon source. Whereas for yeast, sucrose is merely one carbon source among many that can be turned to if insufficient glucose is available; for plants, sucrose is the major transported sugar, linking carbon source organs (mature leaves) with sink tissues (developing leaves, roots and storage organs such as seeds and tubers, Fig. 2). Of course, sucrose and hexose levels might be linked, depending on the sucrose-metabolizing enzymes present in the cell, and sucrose and glucose treatments have had similar effects in some experiments. For example, j-isomaltase gene expression can be induced by sucrose or glucose. However, it is clear that sucrose itself can act as an effector in plant cells, generating a signal independent of that generated by glucose. Sucrose synthase gene expression in potato cell cultures, for example, is induced by sucrose but not by glucose. This induction appears to require Snf1K activity, because antisense inhibition of Snf1K gene expression in potato results in a dramatic decrease in sucrose synthase gene expression in tubers and in excised leaves incubated with sucrose. It seems likely that Snf1K regulates the expression of other enzymes as well, in a role analogous to that of SNF1 in yeast, and we have shown Snf1K a central position in Fig. 2. However, this is not proven.

Further evidence that sucrose and glucose can affect sugar sensing in different ways has been obtained from experiments on developing cotyledons of Vicia faba. During the early stages of cotyledon development, a seed coat-associated invertase cleaves sucrose to maintain high levels of hexoses in the cotyledon. This activity disappears as the cotyledons begin to differentiate into storage tissue, and the cellular hexose levels fall and sucrose levels rise. This coincides with a steep rise in sucrose synthase activity and the onset of starch biosynthesis in the cotyledons.

Concluding remarks

It is now clear that there are multiple sugar-sensing pathways in plants and that sucrose and hexoses can initiate different signals in some tissues in a way that has not been shown in yeast. The term ‘sugar sensing’ is, therefore, imprecise as far as plants are concerned and terms such as hexose and sucrose sensing are more appropriate. Clearly, some of these pathways do not involve hexokinase and the hypothesis that hexokinase plays any role in hexose sensing in plants has yet to be supported by unequivocal experimental evidence.

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References

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