Plant Biotechnology Journal (2003) **1**, pp. 381–398 Doi: 10.1046/j.1467-7652.2003.00046.x

Review

Carbon metabolite sensing and signalling

Nigel G. Halford* and Matthew J. Paul

Crop Performance and Improvement, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

Received 27 June 2003; revised 14 August 2003; accepted 14 August 2003.
**Correspondence* (fax: +44 1582 763 010; e-mail: nigel.halford@bbsrc.ac.uk)

Abstract

The regulation of carbon metabolism in plant cells responds sensitively to the levels of carbon metabolites that are available. The sensing and signalling systems that are involved in this process form a complex web that comprises metabolites, transporters, enzymes, transcription factors and hormones. Exactly which metabolites are sensed is not yet known, but candidates include sucrose, glucose and other hexoses, glucose-6-phosphate, trehalose-6-phosphate, trehalose and adenosine monophosphate. Important components of the signalling pathways include sucrose non-fermenting-1-related protein kinase-1 (SnRK1) and hexokinase; sugar transporters are also implicated. A battery of genes and enzymes involved in carbohydrate metabolism, secondary metabolism, nitrogen assimilation and photosynthesis are under the control of these pathways and fundamental developmental processes such as germination, sprouting, pollen development and senescence are affected by them. Here we review the current knowledge of carbon metabolite sensing and signalling in plants, drawing comparisons with homologous and analogous systems in animals and fungi. We also review the evidence for cross-talk between carbon metabolite and other major signalling systems in plant cells and the prospects for manipulating this fundamentally important aspect of metabolic regulation for crop improvement.

Keywords: carbohydrates, cross-talk, hexokinase, SnRK1, sugar sensing, sugar transporters, trehalose.

Introduction

It is difficult to think of an area of plant cell biology that is not affected by carbon metabolism and its regulation. For this reason, mechanisms for the regulation of carbon metabolism are fundamental to the survival and productivity of plants. They determine the equilibrium position of enzyme-catalysed reactions in plant cells and in so doing the flux of carbon compounds through metabolic pathways. This ensures that the plant's structure and biochemistry can be established, maintained and reproduced. Understanding metabolic regulation provides the basis for rational modification of products synthesized through these pathways and of productivity itself. Evolution has adapted the basic metabolic framework found in all organisms so that plants can survive and flourish in the most marginal of environments. Knowledge of such adaptations is enabling improvements to crop stress tolerance.

When we were undergraduates in the early 1980s, metabolic regulation was still viewed as a function almost entirely of the intrinsic properties of enzymes and the levels of different metabolites in a pathway. Now we know that the activity of many enzymes responds to carbon supply and energy status, through activation and deactivation by phosphorylation, the thioredoxin system and the control of gene expression. When such control mechanisms were first discovered they were often described as blunt instruments, slow to respond and playing no part in the fine tuning of metabolic pathways. Now we know that the 'fine' and 'coarse' control of metabolism involves the co-ordinated regulation of genes and enzymes at the level of transcription, translation, post-translational modification and protein turnover. We also know that carbon metabolite signalling pathways cross-talk with other pathways, including hormone response pathways, cell cycle control mechanisms and nitrogen response systems, amongst others. Plant signalling is a complex network and true understanding will come only when we know how the different strands are integrated and how they relate to the development, survival and productivity of the whole plant.

In this review we deal with the sensing and signalling mechanisms that control plant metabolism in its response to carbon availability. These mechanisms are only being dissected now and many gaps in our knowledge still remain. In spite of this, the genetic modification of key signalling components has already been employed successfully to make strategic changes to metabolism that could improve the performance of crop plants. What is more, the rapid advances in genomics, transcriptomics, proteomics and metabolomics mean that there has never been a better opportunity to fill in the gaps.

Evidence that sugars are signalling molecules in plants – a brief history

It has been known for over a decade that sugar levels affect the expression of plant genes. In groundbreaking work as far back as 1990, Sheen used photosynthetic gene promoter/reporter gene fusions to show that seven maize photosynthetic genes were repressed by glucose or sucrose in a maize protoplast system (Sheen, 1990). Further evidence of the feedback control of photosynthetic gene expression was obtained in experiments where a yeast invertase was expressed in tobacco and Arabidopsis (von Schaewen *et al*., 1990) or tomato (Dickinson *et al*., 1991). This resulted in the accumulation of hexoses in the leaves of the transgenic plants and caused inhibition of photosynthesis.

In other early experiments, expression of the ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit (*RBCS*) gene was shown to be reduced by glucose in cell suspensions of *Chenopodium rubrum* (Krapp *et al*., 1993). Chlorophyll a/b binding protein (*CAB*) and thylakoid ATPase delta subunit gene expression was also reduced by glucose in the *Chenopodium rubrum* cell suspension, and *CAB* gene expression was shown to be reduced by sucrose in oilseed rape cell cultures (Harter *et al*., 1993; Krapp *et al*., 1993).

These experiments demonstrated that expression of photosynthetic genes was under feedback regulation by sugars, a landmark discovery that was reviewed by Sheen in 1994 (Sheen, 1994). The list of genes that respond to sugar levels continued to grow. In 1994, genes encoding isocitrate lyase and malate synthase, the two key enzymes involved in the glyoxylate cycle, were shown to be repressed by glucose (Graham *et al*., 1994) and genes encoding enzymes involved in carbohydrate metabolism swelled the numbers further. The expression of genes encoding α-amylase, the enzyme responsible for starch breakdown and thereby mobilization of the major carbohydrate storage reserve in most plants, was shown to be induced by sugar starvation in rice suspension cultures and repressed by feeding with sucrose, fructose or glucose (Yu *et al*., 1991). A similar result was obtained with isolated rice embryos (Karrer and Rodriguez, 1992), and for the expression of α-amylase promoter/reporter gene constructs in transgenic rice cell cultures (Huang *et al*., 1993). In contrast, the expression of a gene encoding β-amylase has been shown to be induced by sugars in the rosette leaves of Arabidopsis (Mita *et al*., 1995).

Analyses of the expression of genes encoding sucrose synthase, another enzyme of carbohydrate metabolism, led to an important observation. Sucrose synthase catalyses the reversible conversion of sucrose and UDP to UDP-glucose and fructose. Its activity is closely correlated with starch accumulation in potato and maize (Chourey and Nelson, 1976; Zrenner *et al*., 1995). In 1989, Salanoubat and Belliard had shown that sucrose synthase activity increased in response to sucrose but was not affected by glucose (Salanoubat and Belliard, 1989). This correlated exactly with the later findings of Sowokinos and Varns (1992), who measured sucrose synthase activity in potato cell cultures fed with sucrose, glucose or fructose. Subsequent to this, Fu and Park (1995) described two differentially expressed potato sucrose synthase genes. One of these (*SUS4*) was only expressed in tubers under normal conditions but could be induced in detached leaves in response to incubation with high concentrations of sucrose (Fu and Park, 1995). The other (*SUS3*) was expressed predominantly in stems and roots and was not induced by sucrose treatment. Neither gene responded to glucose. Clearly, sucrose could be sensed independently of glucose and presumably cause the initiation and transduction of a separate signal.

This important and perhaps not surprising finding (at least in hindsight) is often ignored. Expression of α-amylase, for example, is often reported as being repressed by sucrose as well as by glucose and other hexoses. However, this is based in the main on experiments performed with cell cultures (Huang *et al*., 1993; Yu *et al*., 1991), and sucrose has been shown to be cleaved extracellularly by a cell wall-associated invertase prior to uptake in cultured rice cells (Amino and Tazawa, 1988). Clearly in this case sucrose feeding will result in an increase in cellular hexose, not sucrose levels.

Other examples of sucrose acting independently of glucose are in the induction of expression of a patatin gene in potato (Grierson *et al*., 1994) (patatin is the major storage protein in potato tubers) and of a nitrate reductase gene in Arabidopsis (Cheng *et al*., 1992). Furthermore, fascinating recent work has shown that sucrose signalling leads to the redox modulation of ADP-glucose pyrophosphorylase (AGPase) activity (Tiessen *et al*., 2002). Sucrose and glucose may even act antagonistically to each other. Experiments on developing cotyledons of *Vicia faba* (Weber *et al*., 1996) showed that a

steep rise in sucrose synthase activity and the onset of starch biosynthesis in the cotyledons coincided with an increase in sucrose levels and a decrease in hexose levels. This suggests that the sucrose : glucose ratio could be sensed. Untangling the effects of sucrose and hexoses may be made more difficult by the fact that the levels of these metabolites within the cell may or may not be linked, depending on the presence or absence of enzymes such as invertase, sucrose synthase and sucrose phosphate synthase that interconvert them.

Carbon metabolite signal transduction pathways

The experiments that we described in the previous section showed irrefutably that carbon metabolite sensing, often referred to as sugar sensing, plays an important role in the regulation of plant metabolism. Inevitably, comparisons were made with other organisms, particularly budding yeast (*Saccharomyces cerevisiae*), in which studies on glucose sensing and signalling systems were already well advanced. Sucrose is not sensed in yeast, whereas it clearly is in plants, but nevertheless, the knowledge gained from yeast studies has proved to be an excellent starting point for the analysis of carbon metabolite sensing and signalling in plants.

A brief description of carbon metabolite sensing in yeast

The metabolism and physiology of budding yeast are dictated by the carbon source and its concentration (Dickinson, 1999). The over-riding regulatory mechanism is glucose repression, whereby the expression of genes and processes involved in aerobic metabolism or the metabolism of carbon sources other than glucose is prevented if glucose is present above a concentration of about 0.2% (w/v). This affects the synthesis of dozens of enzymes, the utilization of alternative carbon sources, gluconeogenesis, respiration and the biogenesis of mitochondria and peroxisomes, even if other carbon sources are provided. It ensures that glucose is always consumed first, regardless of what other carbon sources are available, and that the glucose is consumed entirely by fermentation to ethanol. Aerobic metabolism, in which the ethanol is consumed, commences only when glucose levels have fallen.

Glucose also affects gene regulation via a glucose induction system. This process ensures that when this sugar is available the cell possesses an optimal complement of the glucose transporters and glycolytic enzymes required for glucose utilization. *S. cerevisiae* has 17 hexose transporters encoded by *HXT* genes that are expressed differentially depending on the concentration of glucose that is available; high affinity, low capacity transporters are expressed when glucose concentration is low, whereas low affinity, high capacity transporters are expressed when glucose concentrations are high (Boles and Hollenberg, 1997; Kruckeberg, 1996; Reifenberger *et al*., 1997). Low and high affinity glucose sensors, SNF3 and RGT2, are located in the membrane. Binding of glucose to the sensors causes a signal to be generated that results in the inactivation of a protein called RGT1, a transcriptional repressor of *HXT* gene promoters. The promoters of the genes of the high affinity glucose transporters also bind another repressor, MIG1, which is active at high glucose concentrations (Ozcan and Johnston, 1996). When no glucose is present, none of the *HXT* genes are expressed because of the action of RGT1. At low concentrations neither RGT1 nor MIG1 are active, so the HXT genes are expressed, while at high concentrations only the low affinity transporters are expressed because of the action of MIG1.

Sucrose non-fermenting-1

At the heart of the glucose repression signalling pathway in budding yeast is the sucrose non-fermenting-1 (SNF1) protein kinase trimeric complex. Although not itself calcium-dependant, SNF1 is closely related to the calcium dependant protein kinase (CDPK) group, which includes the animal calmodulin-dependent protein kinases and the plant calmodulin-like domain protein kinases (Hardie, 2000; Hrabak *et al*., 2003). The catalytic subunit, SNF1 itself, was cloned and identified as a protein kinase in 1986 (Celenza and Carlson, 1986). The second subunit in the complex is a protein called SNF4, a regulatory subunit that is required for full activity (Celenza *et al*., 1989). The interaction between SNF1 and SNF4 appears to be regulated by glucose, and it has been proposed that SNF4 activates SNF1 by counteracting autoinhibition by the SNF1 regulatory domain (Jiang and Carlson, 1996). The third subunit is one from a class of proteins that comprises SIP1, SIP2 and GAL83. These three related proteins are interchangeable and may target the complex to different substrates (Yang *et al*., 1994). They contain two conserved domains, the ASC domain (Association with SNF1 Complex) (Jiang and Carlson, 1997; Yang *et al*., 1994) and the KIS domain (Kinase Interacting Sequence) (Jiang and Carlson, 1997).

The AMP-activated protein kinase of animals

In 1994, AMP-activated protein kinase (AMPK), an animal protein kinase that had been studied biochemically for decades, was purified to homogeneity and shown to be a

heterotrimeric complex of three subunits, a catalytic α subunit and accessory β and γ subunits (Davies *et al*., 1994; Mitchelhill *et al*., 1994). Amino acid and DNA sequencing of the three subunits revealed that α was closely related to SNF1, γ to SNF4, and β to the SIP1/SIP2/GAL83 subfamily (Carling *et al*., 1994; Gao *et al*., 1995, 1996; Mitchelhill *et al*., 1994; Woods *et al*., 1996). In other words AMPK is the animal homologue of SNF1.

This was something of a surprise, in that AMPK was not at that time regarded as being involved in sugar sensing *per se*. Its known function was to conserve ATP by phosphorylating and inactivating regulatory enzymes of ATP-consuming pathways such as acetyl-CoA carboxylase (fatty acid synthesis) (Davies *et al*., 1990, 1992) and HMG-CoA reductase (sterol/isoprenoid synthesis) (Clarke and Hardie, 1990; Gillespie and Hardie, 1992). However, AMPK has since been shown to inhibit gene activation by glucose in liver cells (Leclerc *et al*., 1998; Woods *et al*., 2000). Genes that respond to glucose levels in the liver include those involved in glucose and lipid metabolism, including, for example, pyruvate kinase and fatty acid synthase.

SNF1-related protein kinase-1 of plants

A plant homologue of SNF1 had already been identified before the connection between AMPK and SNF1 was made. It was first cloned from rye in 1991 and shown to complement the *snf1* mutation when expressed in yeast (Alderson *et al*., 1991). It was later given the name SnRK1 (SNF1-related protein kinase-1) and has now been identified in a wide range of plants species (Halford and Hardie, 1998; Halford *et al*., 2003a). *SnRK1* genes are present in small to medium sized gene families but are highly conserved in their structure and in the nature of the proteins that they encode (Halford and Hardie, 1998). The SnRK1 protein from Arabidopsis to barley is between 57 and 59 kDa in size and shows ≈ 48% sequence identity with SNF1 and AMPK.

Homologues of the SIP1/SIP2/GAL83/AMPKβ family have been cloned from Arabidopsis (*AKINβ1* and *AKINβ2*) and potato (*StubGAL83*) (Bouly *et al*., 1999; Lakatos *et al*., 1999). AKINβ1 and AKINβ2 interact with SnRK1 in the two-hybrid system but also with the yeast SNF1 and SNF4 proteins (Bouly *et al*., 1999).

A SNF4/AMPKγ homologue, called AtSNF4, was cloned from Arabidopsis by partial complementation of a *snf4* mutant (Kleinow *et al*., 2000). Subsequently a maize homologue was given the name AKINβγ because it was found to contain an N-terminal KIS domain fused with a C-terminal domain similar to SNF4, AMPKγ and AtSNF4 (Lumbreras *et al*., 2001). Re-analysis of the arabidopsis *AtSNF4* gene shows that it too encodes a protein with this N-terminal KIS domain (Julia Buitink, personal communication). It is not clear why this apparent domain fusion should have occurred in plants, particularly since the plant catalytic subunit encoded by SnRK1 appears to interact with yeast SNF4 in exactly the same way that SNF1 does (Jiang and Carlson, 1997; Muranaka *et al*., 1994).

The picture in plants is further complicated because two other families of plant proteins show a similarity with SNF4. These are the PV42 family, which includes PV42 from bean (*Phaseolus vulgaris*) and AKINγ from Arabidopsis (Abe *et al*., 1995; Bouly *et al*., 1999), and the SnIP1 family (Slocombe *et al*., 2002). These show 20–25% amino acid sequence identity with SNF4 and interact with SnRK1 in two-hybrid assays and *in vitro*. The PV42 and SnIP1 classes of SNF4-like proteins appear to be unique to plants. Although both will align with SNF4 and AMPKγ, they show little sequence similarity with each other (Slocombe *et al*., 2002).

Activation and substrate specificity of the SNF1/AMPK/SnRK1 family of protein kinases

AMPK was one of the first protein kinases to be studied biochemically. It was originally characterized as activities present in crude protein fractions that caused time- and ATP-dependent inactivations of HMG-CoA reductase (Beg *et al*., 1973) or acetyl-CoA carboxylase (Carlson and Kim, 1973), although at the time it was not known that these activities were functions of the same protein kinase. As its name suggests, AMPK is activated allosterically by AMP (Carling *et al*., 1987, 1989). It is also activated by phosphorylation by an upstream protein kinase, called AMP-activated protein kinase kinase (AMPKK) (Hawley *et al*., 1996). As well as acting allosterically on AMPK itself, AMP causes the allosteric activation of AMPKK. It also binds to AMPK, making it a better substrate for AMPKK and a worse substrate for protein phosphatase-2C, which reverses the action of AMPKK (Carling *et al*., 1989; Corton *et al*., 1995; Davies *et al*., 1995; Hawley *et al*., 1995). Activation of AMPK by AMP is antagonized by high concentrations of ATP.

Levels of ATP, AMP and ADP are held in equilibrium by the enzyme adenylate kinase, which catalyses the interconversion of ATP and AMP to two molecules of ADP. Continuous production of ATP when a cell is well-nourished leads to a high ATP : ADP ratio, pushing this reaction in the direction of ADP and depleting the pool of AMP. Conversely, when ATP is not being supplied in sufficient amounts by glycolysis, the TCA cycle and respiration the ATP : ADP ratio falls and AMP levels rise. A high AMP : ATP ratio is therefore symptomatic

of low cellular energy levels and AMPK has been likened to a cellular fuel gauge (Hardie and Carling, 1997).

The activation of AMPK has been demonstrated in response to a variety of stresses in mammalian cells. These include the treatment of cells with deoxyglucose or high levels of fructose (Moore *et al*., 1991; Sato *et al*., 1993), heat shock (Corton *et al*., 1994), arsenite and other inhibitors of oxidative metabolism (Corton *et al*., 1994; Witters *et al*., 1991), exercise in skeletal muscle (Winder and Hardie, 1996) and interruption of the blood supply in heart muscle (Kudo *et al*., 1995).

As well as its physiological substrates, AMPK was shown to phosphorylate the so-called SAMS peptide (His Met Arg Ser Ala Met Ser Gly Leu His Leu Val Lys Arg Arg), a synthetic peptide based on the sequence around the primary phosphorylation site for AMPK on rat acetyl-CoA carboxylase. This enabled a relatively simple assay for AMPK activity to be developed that measured the rate of phosphorylation of the SAMS peptide using radiolabelled ATP as the phosphate donor (Davies *et al*., 1989).

The finding that SNF1 and SnRK1 were homologues of AMPK enabled workers who were studying them to take advantage of the biochemical knowledge that had already been built up on AMPK. Indeed, a protein kinase activity that would phosphorylate the SAMS peptide had already been purified from plant sources and shown to have similar biochemical properties to AMPK (Ball *et al*., 1994; MacKintosh *et al*., 1992), including a very similar specificity for protein and peptide substrates (Ball *et al*., 1994; Dale *et al*., 1995b; MacKintosh *et al*., 1992). Since it phosphorylated and inactivated a bacterially expressed arabidopsis HMG-CoA reductase (HMG1) (Dale *et al*., 1995a), it was given the name HMG-CoA reductase kinase (HRK)-A, the suffix (-A) being used to distinguish it from another, minor SAMS peptide kinase activity (HRK-B) which had a much smaller native molecular mass (MacKintosh *et al*., 1992). Immunological studies confirmed that HRK-A was SnRK1 (Ball *et al*., 1995; Barker *et al*., 1996)

A recognition motif for SnRK1 was established using variant peptide substrates (Weekes *et al*., 1993). It comprises the phosphorylated serine (SnRK1 will phosphorylate threonine but phosphorylates serine much more efficiently), hydrophobic residues at positions –5 and +4 relative to the serine, and at least one basic residue which could be at –3 or –4. The AMARA peptide (Ala **Met** Ala **Arg** Ala Ala **Ser** Ala Ala Ala **Leu** Ala Arg Arg Arg), in which the minimal recognition motif is retained but other residues are alanine apart from the basic C-terminus, which is not essential, appears to be a better substrate than the SAMS peptide (Dale *et al*., 1995b). More recently, basic residues at positions –6 and +5 have been shown to enhance activity and a proline residue at position –4 has been found to favour phosphorylation by SnRK1 relative to CDPKs (Huang and Huber, 2001).

The clearest difference between SnRK1 and AMPK is that SnRK1 is not activated allosterically by AMP. However, like AMPK, SnRK1 is regulated by phosphorylation: it can be inactivated by treatment with protein phosphatases and, if phosphatase inhibitors are then added, will reactivate in a time-dependent manner in the presence of MgATP. This reactivation is lost if the kinase is purified further, presumably due to the removal of an endogenous upstream kinase (MacKintosh *et al*., 1992). Phosphorylation of SnRK1 *in vitro* is now known to occur on a threonine residue within the so-called T-loop that is conserved in many protein kinases (Sugden *et al*., 1999a).

Interestingly, a novel protein phosphatase, AtPTPKIS1, that contains a KIS domain, has been identified in Arabidopsis (Fordham-Skelton *et al*., 2002). The KIS domain was shown to interact with SnRK1 in two-hybrid experiments and *in vitro*. AtPTPKIS1 is a tyrosine phosphatase and so could not be responsible for dephosphorylating the threonine residue in the T-loop of SnRK1. Nevertheless, the significance of its interaction with SnRK1 demands further investigation.

There is also evidence of differential transcriptional regulation of *SnRK1* gene expression. In potato, for example, highest levels of expression occur in stolons as they begin to develop into tubers (Man *et al*., 1997). Expression gradually declines in maturing tubers, but is lowest in leaves. Experiments in potato also provided further evidence for the post-transcriptional regulation of SnRK1 activity. SAMS peptide kinase activity in mini-tubers was found to be over 40-fold higher than in mature tubers, while the levels of transcript were approximately the same (Man *et al*., 1997).

Like SnRK1, SNF1 is not regulated allosterically by AMP but is activated by phosphorylation. SNF1 will phosphorylate the SAMS peptide, and using the SAMS peptide phosphorylation assay SNF1 activity was shown to increase dramatically within minutes of glucose removal (Wilson *et al*., 1996; Woods *et al*., 1994). The activation was due to phosphorylation, and the effect could be mimicked *in vitro* by a distinct upstream protein kinase from yeast, or by mammalian AMPKK.

Enzyme substrates of SnRK1

As we have described above, the first plant protein to be identified as a substrate for SnRK1 was Arabidopsis HMG-CoA reductase (Dale *et al*., 1995a). This enzyme catalyses the NADH-dependent reduction of 3-hydroxy-3-methylglutaryl-coenzyme

A (HMG-CoA) to mevalonic acid. The phosphorylation site in the Arabidopsis HMG-CoA reductase HMG1 is serine-577 and the phosphorylation results in inactivation of the enzyme.

Subsequently, two other important enzymes, sucrose phosphate synthase (SPS) and nitrate reductase (NR) were shown to be substrates for SnRK1 *in vitro* (Bachmann *et al*., 1996b; Crawford *et al*., 2001; Douglas *et al*., 1995; Su *et al*., 1996; Sugden *et al*., 1999b). As with HMG-CoA reductase, phosphorylation results in inactivation of the enzyme, although the inactivation of NR also requires the binding of a 14-3-3 protein to the phosphorylation site (Bachmann *et al*., 1996a; Moorhead *et al*., 1996). The sequences at both sites conform to the SnRK1 consensus recognition motif.

HMG-CoA reductase and SPS are key enzymes in important biosynthetic pathways (isoprenoid and sucrose biosynthesis, respectively), while NR is involved in the assimilation of nitrogen into organic compounds. Inactivation of these enzymes will therefore conserve carbon, and parallels can be drawn with the roles of AMPK and SNF1. It is interesting, however, that NR and SPS have apparently been brought into the SnRK1 regulatory system as it has evolved in plants. Acetyl CoA carboxylase, a key enzyme in fatty acid biosynthesis, on the other hand, is regulated by AMPK and SNF1 in the mammalian and fungal systems, but not by SnRK1 in plants.

SnRK1 has been shown to be involved in the regulation of activity of another enzyme, ADP-glucose pyrophosphorylase (AGPase), a key enzyme in starch biosynthesis, but through a completely different mechanism. AGPase activity in growing potato tubers is subject to redox modulation in response to the availability of sucrose within the cell (Tiessen *et al*., 2002). However, redox activation of AGPase in response to sucrose (or trehalose) feeding does not occur in tuber discs prepared from potato lines expressing an antisense *SnRK1* sequence (Tiessen *et al*., 2003).

Genes regulated by SnRK1

Just like AMPK and SNF1, SnRK1 exerts its control through the regulation of gene expression as well as having direct effects on enzyme activities. However, as with the range of enzyme substrates, the genes involved are different. Expression of an antisense *SnRK1* sequence in the tubers of transgenic potato plants resulted in a dramatic reduction in sucrose synthase gene expression (Purcell *et al*., 1998). Sucrose synthase gene expression was also found to be not inducible by sucrose in excised leaves expressing an antisense *SnRK1* sequence (Purcell *et al*., 1998), in contrast to wild-type plants (Fu and Park, 1995).

This means, of course, that SnRK1 is potentially affecting starch biosynthesis at two points in the pathway, through the regulation of sucrose synthase gene expression and AGPase redox activation. In contrast to the glucose de-repression system of yeast, this area of SnRK1 function is clearly responding to sucrose and is activated by the sugar rather than repressed by it. However, there is also evidence that SnRK1 regulates genes in response to low glucose. Perhaps surprisingly, the gene in question encodes α-amylase, an enzyme that breaks starch down, but the evidence is clear. A reporter gene comprising the wheat alpha amylase *α-AMY2* gene promoter and *UIDA* (Gus) was shown to be active when bombarded into cultured wheat embryos but was tightly repressed when co-bombarded with an antisense *SnRK1* gene (Laurie *et al*., 2003). In contrast, actin and ubiquitin gene promoter activity was unaffected by the antisense *SnRK1* gene. Expression of *α-AMY2* was shown to be up-regulated by sugar starvation in the same system.

SnRK1 could only have roles in controlling the expression of genes encoding enzymes of starch synthesis and degradation in different plant organs and stages of development and in response to different sugar signals if its effects were modulated by the presence or absence of different transcription factors. Unfortunately, no plant transcription factors have yet been identified as substrates for SnRK1.

A yeast transcription factor that is almost certainly a substrate for SNF1 is the transcriptional repressor MIG1. MIG1 contains several potential SNF1 phosphorylation sites and the mutation of these results in constitutive repression of transcription (Östling and Ronne, 1998). The cellular location of MIG1 is determined by glucose: addition of glucose causes it to move to the nucleus and removal of glucose causes it to return to the cytosol (DeVit *et al*., 1997). This movement is associated with its phosphorylation state. However, MIG1 is the only yeast transcription factor to be identified as a substrate for SNF1 thus far and it has no clear homologue in plants.

Hexokinase as a sensing and signalling molecule

Another protein that is ubiquitous amongst eukaryotes and has been linked with sugar sensing and signalling is hexokinase. Hexokinase has a metabolic function, of course, in catalysing the conversion of glucose to glucose-6-phosphate (the first stage in glycolysis). It was first proposed to have a role in sensing and signalling glucose levels in yeast, in which it is found not only in the cytosol where it performs its catalytic function but also in the nucleus (Herrero *et al*., 1998; Randez-Gil *et al*., 1998). The hypothesis appeared to be

supported by the finding that mutations in *HXK2*, which encodes the major hexokinase PII isoform in *S. cereviseae*, caused partially constitutive expression of glucose-repressed genes (Entian, 1980). However, further work showed a good correlation between the overall residual hexokinase catalytic activity of different mutants and their ability to exhibit glucose repression (Ma *et al*., 1989; Rose *et al*., 1991). This suggested that hexokinase PII has a role in producing the signal molecule, but does not itself sense or initiate a signal. Despite this, hexokinase is now widely regarded as a ubiquitous glucose sensor in eukaryotes (Rolland *et al*., 2001).

In plants, hexokinase has been associated particularly with photosynthetic metabolism as a mechanism that links the carbohydrate status of leaves to photosynthetic gene expression as a means of feedback control (Jang *et al*., 1997; reviewed by Jang and Sheen, 1997). As with yeast, however, while the role of hexokinase in influencing metabolism and gene expression has been quite clear, more contentious has been whether the impact of hexokinase on metabolism and resource allocation is a consequence of the impact of its activity on metabolic flux or of a direct role in signalling (Halford *et al*., 1999). As with the experiments performed with yeast, for example, any attempt to alter hexokinase activity in transgenic or mutant plants or with inhibitors may cause changes in other important signalling molecules downstream of hexokinase, such as AMP. Similarly, feeding non-metabolisable glucose analogues to separate metabolic from signalling roles (Jang and Sheen, 1994) is problematic, as this may cause nonspecific effects such as changes in the phosphate status of cells. Phosphate itself, or ATP/AMP levels may again impact on signalling downstream of hexokinase. Thus precise interpretations of results are difficult, particularly in the complex system of the multicellular plant.

To address this, Moore *et al*. (2003) used the targeted mutagenesis of one of the Arabidopsis hexokinase genes, *HXK1*, to produce enzymes that retain signalling functions but not catalytic activities. Two distinct catalytically inactive *hxk1* mutants were obtained where glucose binding still occurred but glucose-6-phosphate was not formed. Transgenic plants were generated expressing the two catalytically inactive mutant alleles in a null mutant background. Both mutant alleles mediated glucose-dependent developmental arrest and repression of chlorophyll accumulation without altering catalytic activity or glucose-6-phosphate and fructose-6-phosphate content.

Such separation of catalytic and signalling functions of hexokinase as a result of painstaking research is as good evidence as can be obtained to demonstrate the role of hexokinase as a signalling protein. Nevertheless, it is still not known how this signalling mechanism operates and this must be the next challenge, in particular to find out how hexokinase interacts with downstream components of the signalling pathway and in what way it is affected by glucose binding.

The trehalose system

The biosynthetic pathway for the disaccharide trehalose has a regulatory function in yeast that controls hexokinase activity and signalling (Thevelein and Hohmann, 1995). The pathway involves two enzymes, trehalose phosphate synthase (TPS) and trehalose phosphate phosphatase (TPP). TPS catalyses the formation of trehalose-6-phosphate (T6P) from glucose-6-phosphate and UDP-glucose, while TPP catalyses the dephosphorylation of T-6-P to trehalose. Trehalose is cleaved by another enzyme, trehalase. Both the enzyme TPS and the metabolite T6P have an inhibitory effect on hexokinase. This regulatory system stands at the gateway to glycolysis (both T6P and TPS are necessary for glycolytic function) and ensures that cellular ATP levels are not depleted by the 'overactivity' of hexokinase (Bonini *et al*., 2000; Noubhani *et al*., 2000).

Trehalose metabolism is more widespread in nature than sucrose metabolism. Plants are the exception rather than the rule in favouring sucrose, and up to a decade or less ago were believed to have discarded trehalose for sucrose metabolism. However, it is now apparent that the capacity to synthesize and degrade trehalose is ubiquitous in plants, even though trehalose itself, with the exception of a few resurrection plants, is found in only trace abundance because of the relatively high activity of trehalase (Bianchi *et al*., 1993; Drennan *et al*., 1993; Goddijn and van Dun, 1999). Furthermore, recent evidence has shown the trehalose pathway to be an essential component of metabolic signalling in plants (Eastmond *et al*., 2002; Goddijn and Smeekens, 1998; Schluepmann *et al*., 2003).

Arabidopsis contains 11 putative *TPS* genes, of which *TPS1* is the best characterized. *TPS1* has been shown to be indispensable for carbohydrate utilization (Eastmond *et al*., 2002) and compelling evidence has been obtained through combined genetic and biochemical approaches that this is due to T6P rather than trehalose or a property of TPS (Schluepmann *et al*., 2003). This work showed that the function of T6P in plants is analogous to that in yeast in that T6P regulates carbon flux into glycolysis. Transgenic plants expressing the *E. coli OTSB* gene encoding TPP and the *TREC* gene encoding trehalose phosphate hydrolase (TPH) both have a low T6P content. Seedlings of these plants cannot grow on sugar-containing media because they accumulate large pools of metabolites while ATP levels become depleted. This work

parallels that on yeast mutants with lesions in the *TPS1* gene that cannot grow on sugar-containing media because carbon flow through glycolysis is unregulated. Phosphate is sequestered as intermediates accumulate and ATP cannot be synthesized (Hohmann *et al*., 1996; Van Aelst *et al*., 1993; van de Poll and Schamhart, 1974).

In contrast, transgenic plants with elevated T6P activity through expression of the *E. coli OTSA* gene encoding TPS actually grow better than the wild-type on sugars, something not previously accomplished by manipulation of metabolism. The levels of metabolic intermediates in these plants are low, with the exception of ATP. Moreover, expression of *OTSA* in the Arabidopsis *tps1* mutant completely restores the phenotype (Schluepmann *et al*., 2003).

Regulation of carbon flux into glycolysis by T6P may have evolved specifically to cope with sudden and large variations in carbohydrate availability, increasing the dynamic range of regulation under highly variable environmental conditions (Teusink *et al*., 1998). The abundance of *TPS* and *TPP* genes in the Arabidopsis genome may reflect the requirement for carefully regulated carbohydrate utilization in different tissues under different conditions and developmental stages. However, despite the clear similarities between the signalling functions of T6P in yeast and plants, no direct effect of T6P on hexokinase activity has ever been observed in plants, and the precise site and nature of the interaction remains to be established.

The accumulation of trehalose itself in transgenic plants has been associated with improvements in stress tolerance (Garg *et al*., 2002; Pilon-Smits *et al*., 1998), and this strategy may be important for engineering crops for marginal environments. Furthermore, trehalose feeding stimulates starch accumulation (Fritzius *et al*., 2001; Wingler *et al*., 2000), suggesting that it has signalling properties which are distinct from those of T6P. The use of transgenic plants and mutants should enable the roles of each molecule to be dissected.

T6P-dependent effects on metabolism (Schluepmann *et al*., 2003) and photosynthesis (Paul *et al*., 2001) imply interactions with hexokinase-dependent signalling and metabolism, while the effects of trehalose on starch metabolism (Fritzius *et al*., 2001) and sucrose synthase (Muller *et al*., 1998) suggest a possible interaction with SnRK1. It is therefore possible that the trehalose pathway interacts with and links both signalling systems.

Sucrose transporters

We have already described the role of plasma membrane hexose transporter (HXT) proteins and the related glucose sensors SNF3 and RGT2 in glucose transport in yeast. Mammalian cells contain a homologous family of glucose transporters (GLUT) and humans contain a combined sensor/transporter, GLUT2 (Thorens, 2001).

Plants transport sucrose, of course, not glucose. Sucrose transport is mediated by proton-coupled sucrose transporters (SUT) (Lalonde *et al*., 1999, 2003). These SUT proteins are now known to be structurally related to the HXT and GLUT transporters and related sensors of yeast and mammals. Just like their fungal and mammalian counterparts, the SUT transporters have different affinities for sucrose, SUT1 being the high affinity transporter and SUT4 the low affinity transporter, while SUT2 is a combined transporter and sensor (Barker *et al*., 2000; Weise *et al*., 2000). All three proteins have been localized in sieve elements (Reinders *et al*., 2002).

Hexose transporters

In some plant tissues, sucrose is hydrolysed to glucose and fructose by cell wall invertases, and it is glucose and fructose that are imported into cells. Glucose is taken up by monosaccharide symporters called sugar transport proteins (STPs). Despite their name, these transporters are not involved in the uptake of sucrose itself, and they do not transport fructose either; the route for fructose import still remains unclear. Arabidopsis has six cell wall invertase genes and 14 STP genes (reviewed by Sherson *et al*., 2003). The best characterized, *AtSTP1*, encodes a protein that transports galactose, xylose and mannose as well as glucose and responds sensitively to extracellular sugar availability.

It has been proposed that the expression of different invertases and STPs enables the plant to change its hexose utilization in response to different environmental conditions. It has also been suggested that STPs have a role in hexose sensing and signal initiation (Sherson *et al*., 2003), but this has not been demonstrated experimentally.

Which metabolites are sensed?

Perhaps the biggest gap in our knowledge of carbon metabolite sensing and signalling in plants is that we do not know which metabolites are sensed. We have described experiments that showed clearly that glucose and sucrose cause a response in plant cells, but is it these metabolites themselves that are sensed or something else? Other candidate molecules include other hexoses and hexose phosphates. As we have described above, trehalose and trehalose 6-phosphate have also entered the picture.

The attraction of glucose as a signalling molecule is that it is universal to all organisms as a source of energy and carbon skeletons. It would appear to make sense that organisms should be able to respond to its availability. As we have described above, budding yeast does sense glucose levels through the membrane-located sensors SNF3 and RGT2, but these only initiate signals through the glucose induction pathway. The metabolite that is sensed to initiate a signal through SNF1 and the more important glucose repression pathway remains unknown.

If glucose is itself a signalling molecule, then one possible mechanism through which it could be sensed is through its interaction with hexokinase, and we have described the evidence for hexokinase as a sensing and signalling molecule above. However, hexokinase-independent hexose sensing systems must exist as well. We know this because elevated hexose levels in the vacuole or apoplast of transgenic tobacco plants expressing a yeast invertase were clearly sensed, whereas high hexose levels in the cytosol were not (Heineke *et al*., 1994; Herbers *et al*., 1996). Hexokinase, of course, is a cytosolic enzyme in plants.

Whether or how hexokinase-dependent or independent hexose signalling pathways involve SnRK1 is not clear. In budding yeast, although the sensing mechanism is not known, SNF1 activity does respond rapidly and sensitively to glucose levels (Wilson *et al*., 1996). SnRK1 has been shown to be inhibited by glucose-6-phosphate *in vitro* (Toroser *et al*., 2000) but we have not been able to obtain unambiguous results from measurements of SnRK1 activity in feeding studies on plants or plant cell cultures with any sugars (unpublished data). Nevertheless, SnRK1 has been shown to be required for α-amylase (*α-AMY2*) gene expression in cultured wheat endosperms (Laurie *et al*., 2003). The *α-AMY2* gene is glucose-repressible and starvation-inducible, making it similar to glucose-repressed, SNF1-controlled genes in yeast.

Two quite different studies that we have referred to before have implicated SnRK1 in the transduction of signals initiated in response to sucrose. These studies were conducted on the same transgenic potato plants expressing an antisense *SnRK1* gene. The first showed that SnRK1 is required for sucrose synthase (*SUS4*) gene expression in potato tubers (Purcell *et al*., 1998); *SUS4* is a sucrose-inducible, not glucose-repressible gene. The second showed that SnRK1 is involved in sucrose-specific sensing, leading to redox modulation of ADP-glucose pyrophosphorylase (AGPase) in potato tubers (Tiessen *et al*., 2003). Interestingly, the same sensing mechanism responds to trehalose as well as sucrose. In contrast, redox activation of AGPase by glucose occurred in both wild-type and antisense SnRK1 tuber discs, so SnRK1 is apparently involved in sucrose but not glucose signalling in this system.

This more complicated mechanism for activation might explain why it has not been possible to demonstrate a clear response of SnRK1 activity to sugars supplied exogenously and is a reminder that sucrose and hexoses initiate different signals in some tissues. The fact that independent sensing of sucrose has evolved in plants should come as no surprise. Sucrose, which is found in all plants (and some cyanobacteria), is the end-product of photosynthesis, the major transported sugar and the starting point for energy metabolism and the synthesis of structural components of cells. Animals and fungi, on the other hand, do not make it at all.

We are still left with the question of which metabolites are sensed, and in the absence of a mechanism we cannot answer it. There is no such problem for those studying animal systems, of course. Starvation will result in an increase in the AMP : ATP ratio and cause the activation of AMPK. So could AMP be sensed in yeast and plant cells as well? Neither SNF1 nor SnRK1 is activated allosterically by AMP in the way that AMPK is. Nevertheless, large increases in AMP have been measured in yeast under conditions where SNF1 is activated (Wilson *et al*., 1996). AMP has also been shown to affect the phosphorylation state of SnRK1 (Sugden *et al*., 1999a), and it is possible that it acts on the protein kinase upstream of SnRK1, but this cannot be proven until that protein kinase has been identified.

Cross-talk between carbon metabolite and other signalling pathways

Evidence for cross-talk between carbon metabolite and ABA/stress signalling pathways

We have already touched on the interactions between metabolic and other signalling pathways, a phenomenon often referred to as cross-talk. A good example is the emergence over the last few years of a number of hypotheses linking ABA and sugar signalling (reviewed by Rook and Bevan, 2003). These are based on the results of screens carried out by several independent groups to identify Arabidopsis mutants that are impaired in their response to sugar (sugar response mutants). Several of the mutants identified in these screens turned out to be ABA-related. They therefore represent convincing evidence of cross-talk between ABA and metabolic signalling pathways. It has even been proposed that sugar signalling is directly mediated by ABA (Arenas-Huertero *et al*., 2000; Smeekens, 2000). An alternative hypothesis is that ABA modulates sugar signalling by priming tissues to respond to sugars (Rook *et al*., 2001). We propose

a different hypothesis, in which ABA and sugar signalling are essentially separate but converge and cross-talk through specific factors. Whichever hypothesis is correct, it is clear that the control of developmental events such as germination and seedling establishment are sensitive to both ABA and sugars.

The factors through which ABA and sugar signalling might cross-talk have not been identified, but one candidate is a protein kinase, PKABA1, which was first identified in wheat embryos and implicated in stress tolerance (Anderberg and Walker-Simmons, 1992). Incubation of embryos in ABA results in increased *PKABA1* gene expression, and both *PKABA1* transcript and endogenous ABA levels increase during water stress. The constitutive expression of *PKABA1* in barley aleurone layers resulted in suppression of α-amylase and proteinase gene expression (Gómez-Cadenas *et al*., 1999).

This is significant, because PKABA1 is a SNF1-related protein kinase belonging to a family called SnRK2. SnRK2s have 42–45% amino acid sequence identity with SnRK1, SNF1 and AMPK in the kinase catalytic domain (they are significantly less similar to SNF1 and AMPK than SnRK1 is) but have a truncated C-terminal domain. SnRK2s are relatively diverse compared with SnRK1, and the Arabidopsis family has 10 members (Halford *et al*., 2003a).

Plants contain a third SNF1-related protein kinase family, called SnRK3. These are even more diverse than SnRK2, the Arabidopsis family comprising 29 members (Halford *et al*., 2003a), most of which have not been characterized in any detail. The SnRK3 gene family includes SOS2, an Arabidopsis protein kinase involved in conferring salt tolerance (Halfter *et al*., 2000; Liu *et al*., 2000).

The SnRK2 and SnRK3 gene families appear to be unique to plants. Unlike SnRK1, they do not complement the *snf1* mutation of yeast. They have not been characterized biochemically, although a minor SAMS peptide kinase activity has been tentatively assigned to SnRK2 (Ball *et al*., 1994; Barker *et al*., 1996; Sugden *et al*., 1999a). Nevertheless, the fact that they are clearly related to SnRK1 suggests that they could link metabolic and ABA/stress signalling pathways.

Cross-talk involving hexokinase signalling

Hexokinase signalling appears to affect gene expression, cell proliferation, root and inflorescence growth, leaf expansion and senescence. Some of these effects may be mediated through cross-talk with light and hormone signalling. For example, there is good evidence for interaction and antagonism between hexokinase signalling, light and cytokinin in the regulation of leaf senescence (Hwang and Sheen, 2001; Wingler *et al*., 1998). There is also evidence that glucose signalling interacts with auxin signalling to promote or inhibit growth depending on tissue or glucose concentration (Moore *et al*., 2003). Thus, many of the growth promoting and hormonal-like properties of sugars may be explained in part through interactions between hexokinase and hormone signalling pathways.

Carbon metabolite sensing and cell cycle control

Sugar availability has been shown to control the expression of Cyclin D2 and D3 genes in the G1 phase of Arabidopsis (Riou-Khamlichi *et al*., 2000). Addition of sucrose to the growth medium to a concentration of 10 mM induced CycD2 and CycD3 gene expression within 4 h in cell suspension cultures and 6 h in intact seedlings. Glucose was as effective for CycD3 and slightly more effective for CycD2.

Prior to these experiments, considerable evidence had already been obtained to support the hypothesis that there is cross-talk between sugar sensing and cell cycle control in yeast. First, it had been known since 1991 that *snf1* mutants fail to arrest in G1 phase of the mitotic cell cycle under conditions of nutrient deprivation (Thompson-Jaeger *et al*., 1991). This was reinforced by later studies in which *snf1*, *snf4* and *mig1* mutants were shown to have a shorter G1 period than the wild-type (Aon and Cortassa, 1999). Second, SNF1 was found to be related to NIK1 and its fission yeast homologue NIM1. NIK1 is a negative regulator of SWE1, which in turn is involved in calcium-dependent control of mitosis (Tanaka and Nojima, 1996). Third, it had been shown that expression of the cyclin gene, *CLN3*, is induced by glucose (Parviz and Heideman, 1998).

While glucose is required for the onset of mitosis, it inhibits meiosis, but SNF1 is also involved here. It controls the activity of IME1, a transcription factor that is required for the initiation of meiosis (Honigberg and Lee, 1998; Purnapatre *et al*., 2002).

Intriguingly, over-expression of SnRK1 in yeast cells results in a reduction in cell volume to one-third of the normal (Dickinson *et al*., 1999). It may be that as well as performing the functions of SNF1, the over-expression of SnRK1 was functionally equivalent to over-expression of NIK1, which couples entry into mitosis with morphological development (Barral *et al*., 1999); hence the yeast cells were prematurely pushed into mitosis before normal amounts of growth had occurred.

Evidence for cross-talk between carbon metabolite and amino acid signalling pathways

Amino acids are based on carbon skeletons and it seems logical that amino acid metabolism should respond to carbon

availability. Amino acid levels in yeast are maintained by a mechanism known as general amino acid control. Two important components of this system are a protein kinase called GCN2 (general control non-derepressible-2) and a transcription factor called GCN4 (reviewed by Hinnebusch, 1992). GCN2 phosphorylates the α subunit of eukaryotic translation initiation factor-2 (eIF2α), bringing about a decrease in the rate of protein synthesis and thereby conserving amino acids.

An elegant mechanism leads to an increase in GCN4 synthesis, despite this general reduction in protein production (Hinnebusch, 1992, 1994, 1997). The slow-down in translation causes open reading frames in the 5′ region of the *GCN4* transcript to be bypassed so that translation can start from the initiation codon at the 5′ end of the GCN4 coding sequence. A total of 539 yeast genes have been shown to be induced through the action of GCN4 (Natarajan *et al*., 2001), including genes in every amino acid biosynthetic pathway except cysteine.

GCN2 is activated and GCN4 synthesized in response to the binding of uncharged tRNA to the GCN2 regulatory domain, in other words the system becomes active as amino acid levels decline. However, a mechanism that is distinct from this amino acid response but acts through GCN2 to induce the synthesis of GCN4 is initiated by glucose limitation (Yang *et al*., 2000). Induction of GCN4 during carbon starvation is believed to enhance the storage of amino acids in the vacuoles and thereby facilitate re-entry into exponential growth when glucose becomes available.

A homologue of GCN2 has now been identified in Arabidopsis and shown to complement the *gcn2* mutation in yeast, enabling growth in the presence of inhibitors of amino acid biosynthesis (Zhang *et al*., 2003). However, its ability to fulfil the role of yeast GCN2 in the glucose regulation of amino acid biosynthesis has not been tested. Furthermore, doubt still remains over the degree of conservation between yeast and plant amino acid control systems because of the lack of a clear candidate for the role of GCN4 in plants.

Another possible point of convergence for carbon metabolite and amino acid signalling systems is nitrate reductase. Nitrate reductase (NR) is involved in the assimilation of nitrogen used in amino acid biosynthesis. Its regulation is complex and undoubtedly responds to nitrogen as well as other signals. It is a substrate for several protein kinases but one of these protein kinases is SnRK1 (Douglas *et al*., 1997; Sugden *et al*., 1999a), suggesting that NR could be regulated in part in response to carbon availability.

Concluding remarks

We have summarized what we know about metabolite signalling in plants in Figure 1. The system is complex and no doubt more pieces of the jigsaw will be discovered in the coming years. Perturbations in metabolite signalling, whether accidental or deliberately induced, can have profound effects on development. This is clearly illustrated in humans, where AMPK is implicated in type 2 diabetes mellitus and obesity, and mutations in AMPK are associated with a severe heart defect (hypertrophy and arrhythmia). The genetic manipulation of SnRK1 activity in plants also affects development. For example, antisense SnRK1 potato tubers do not sprout at all if kept at 5 °C (Halford *et al.*, 2003b), possibly because the mobilization of stored starch to support sprouting is impaired. Furthermore, the expression of an antisense SnRK1 sequence causes abnormal pollen development and male sterility in barley (Zhang *et al.*, 2001). The pollen grains are small, pear-shaped, contain little or no starch and are non-viable. We hypothesize that they are unable to respond to their carbon status. In effect they starve in a similar fashion to yeast *snf1* mutants starving on sucrose medium.

These effects were obtained by manipulating SnRK1 activity in carbon sink organs, and since such organs include seeds, tubers and fruits they are of particular interest to biotechnologists. Storage carbohydrates such as starch in potato tubers and cereal grain, fructans in forage grasses and sucrose in sugar beet and sugar cane are obvious targets for manipulation, both in terms of total amount and, for the complex polysaccharides, structure and processing properties (6.1 million tonnes of starch from wheat, maize and potato is used by food and non-food industries per year in Europe). The carbon/nitrogen balance is another important parameter, manipulation of which could enable the tailoring of crop varieties to suit different end uses. For example, farmers producing barley for malting require varieties that favour the accumulation of starch over protein in the seed, while high starch lines of potato are required for the production of French fries and crisps. In contrast, farmers producing barley, wheat and maize for animal feed require varieties that favour seed protein production. Failure to increase the protein content and quality of UK barley varieties in the last quarter of a century has led to the loss of most of the animal feed market to imported American soybeans.

Minor components of foods are also possible targets for manipulation by biotechnologists. One of these is the isoprenoid group of compounds and metabolite signalling may play a part in the regulation of carbon flux into the isoprenoid biosynthetic pathway through the action of SnRK1 on

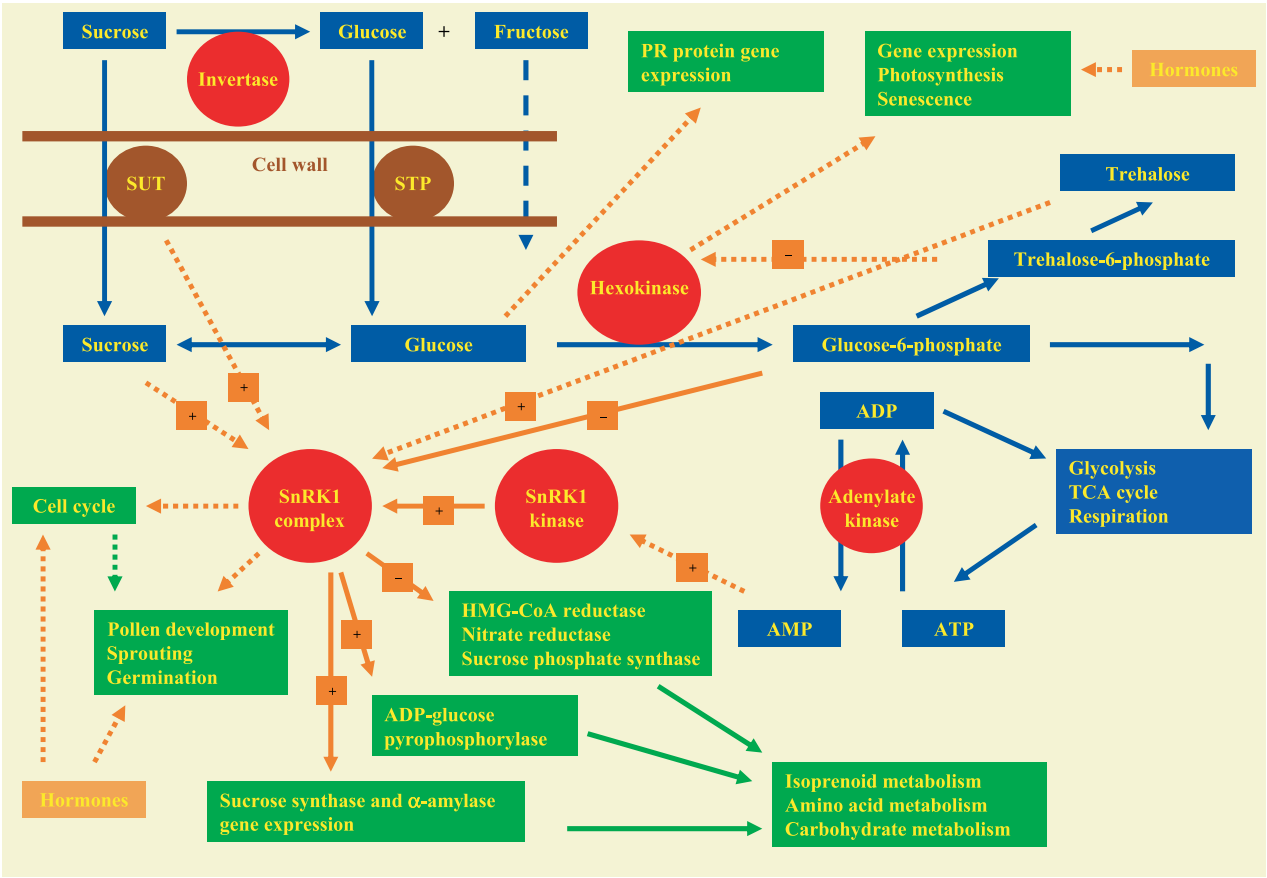

Figure 1 The complex web of metabolites, sucrose transporters (SUT), sugar (hexose) transport proteins (STP), enzymes and hormones that play a part in, impact on or are regulated by carbon metabolite signalling pathways in plants. Metabolites are shown in blue boxes, enzymes in red, transporters in brown and downstream targets green. Blue arrows show the movement of carbon through metabolism, orange arrows show signalling pathways (broken if the factors or mechanisms are not known) and green arrows show downstream effects.

HMG-CoA reductase. Plant isoprenoids are a large family of compounds, including fat-soluble vitamins (such as vitamins E and K) and sterols, both of which are believed to reduce coronary heart disease. Some phytosterols, for example, have been shown to lower serum cholesterol levels when increased in the diet. Vitamin K is also important in bone metabolism and the prevention of postmenopausal osteoporosis. Removal of the target phosphorylation site in HMG-CoA reductase could uncouple it from SnRK1 regulation (Halford *et al.*, 2003b), possibly circumventing one of the regulatory barriers to increasing isoprenoid accumulation.

Carbon source (mature leaf) processes are also important in determining crop yield and quality because they provide the carbohydrates and amino acids necessary for growth and storage. Plant breeding during the 20th century achieved substantial increases in crop yield by increasing the proportion of biomass in the harvested product (harvest index); in other words, by changing the partitioning of assimilated carbon and nitrogen within the plant in favour of the parts of the plant that were harvested. This was augmented by improvements in agronomic practice, in particular the use of nitrogenous fertilisers. However, there is little evidence that these yield increases were associated with improvements in source processes such as photosynthesis, and there is an opportunity for biotechnologists to make improvements in this area, particularly now that yield increases through 'traditional' plant breeding are predicted to be approaching their limit (Mann, 1999).

Recent research suggests that control exerted by the source on whole plant partitioning can be substantial, accounting for up to 80% of the total source-to-sink carbon flux regulation, at least in potato (Sweetlove and Hill, 2000). The critical question to understanding this control is which enzymatic steps are limiting. Much research during the 1990s established that increasing the activity of many so-called 'key' enzymes would not improve photosynthesis because of the sharing of control amongst enzymes and because the feedback regulation of photosynthesis by metabolite signalling

could neutralize any improvement (Paul *et al*., 2001). For example, many studies showed that the benefits of high atmospheric CO_2 concentrations could be short-lived because of such feedback mechanisms (Van Oosten and Besford, 1996). This emphasizes the importance of metabolite signalling in the regulation of photosynthesis and carbon metabolism in the context of the whole plant. Indeed, the manipulation of signalling processes looks to be a promising way forward in the improvement of complex physiological processes such as these, and there is already an example of this in the increase in photosynthetic capacity in transgenic plants over-expressing TPS (Paul *et al*., 2001).

Acknowledgements

Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council (BBSRC) of the UK.

References

Abe, H., Kamiya, Y. and Sakurai, A.A. (1995) cDNA clone encoding yeast SNF4-like protein from *Phaseolus vulgaris*. *Plant Phys*. **110**, 336.

Alderson, A., Sabelli, P.A., Dickinson, J.R., Cole, D., Richardson, M., Kreis, M., Shewry, P.R. and Halford, N.G. (1991) Complementation of *snf1*, a mutation affecting global regulation of carbon metabolism in yeast, by a plant protein kinase cDNA. *Proc. Natl Acad. Sci. USA*, **88**, 8602–8605.

Amino, S. and Tazawa, M. (1988) Uptake and utilization of sugars in cultured rice cells. *Plant Cell Phys*. **29**, 483–487.

Anderberg, R.J. and Walker-Simmons, M.K. (1992) Isolation of a wheat cDNA clone for an abscisic acid-inducible transcript with homology to protein kinases. *Proc. Natl Acad. Sci. USA*, **89**, 10183–10187.

Aon, M.G. and Cortassa, S. (1999) Quantitation of the effects of disruption of catabolite (de) repression genes on the cell cycle behaviour of *Saccharomyces cerevisiae*. *Curr. Microbiol*. **38**, 57–60.

Arenas-Huertero, F., Arroyo, A., Zhou, L., Sheen, J. and Leon, P. (2000) Analysis of Arabidopsis glucose insensitive mutants, gin5 and gin6, reveals a central role of the plant hormone ABA in the regulation of plant vegetative development by sugar. *Genes Dev*. **14**, 2085–2096.

Bachmann, M., Huber, J.L., Liao, P.C., Gage, D.A. and Huber, S.C. (1996a) The inhibitor protein of phosphorylated nitrate reductase from spinach (*Spinaea oleracea*) leaves is a 14-3-3 protein. *FEBS Letts*. **387**, 127–131.

Bachmann, M., Shiraishi, N., Campbell, W.H., Yoo, B.C., Harmon, A.C. and Huber, S.C. (1996b) Identification of Ser-543 as the major regulatory phosphorylation site in spinach leaf nitrate reductase. *Plant Cell*, **8**, 505–517.

Ball, K.L., Barker, J.H.A., Halford, N.G. and Hardie, D.G. (1995) Immunological evidence that HMG-CoA reductase kinase-A is the cauliflower homologue of the RKIN1 subfamily of plant protein kinases. *FEBS Letts*. **377**, 189–192.

Ball, K.L., Dale, S., Weekes, J. and Hardie, D.G. (1994) Biochemical characterisation of two forms of 3-hydroxy-3-methyl-glutaryl CoA reductase kinase from cauliflower (*Brassica oleracea*). *Eur. J. Biochem*. **219**, 743–750.

Barker, L., Kühn, C., Weise, A., Schulz, B., Gebhardt, C., Hirner, B., Hellmann, H., Schulze, W., Ward, J.M. and Frommer, W.B. (2000) SUT2, a putative sucrose sensor in sieve elements. *Plant Cell*, **12**, 1153–1164.

Barker, J.H.A., Slocombe, S.P., Ball, K.L., Hardie, D.G., Shewry, P.R. and Halford, N.G. (1996) Evidence that barley 3-hydroxy-3-methylglutaryl-Coenzyme A reductase kinase is a member of the sucrose nonfermenting-1-related protein kinase family. *Plant Physiol*. **112**, 1141–1149.

Barral, Y., Parra, M., Bidlingmaier, S. and Snyder, M. (1999) Nim1-related kinases coordinate cell cycle progression with the organization of the peripheral cytoskeleton in yeast. *Genes Dev*. **13**, 176–187.

Beg, Z.H., Allmann, D.W. and Gibson, D.M. (1973) Modulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity with cAMP and with protein fractions of rat liver cytosol. *Biochem. Biophys. Res. Commun*. **54**, 1362–1369.

Bianchi, G., Gamba, A., Limiroli, R., Pozzi, N., Elster, R., Salamini, F. and Bartels, D. (1993) The unusual sugar composition in leaves of the resurrection plant *Myrothamnus flabellifolia*. *Physiol. Plantarum*, **87**, 223–226.

Boles, E. and Hollenberg, C.P. (1997) The molecular genetics of hexose transport in yeasts. *FEMS Microbiol. Rev*. **21**, 85–111.

Bonini, B.M., Van Vaeck, C., Larsson, C., Gustafsson, L., Ma, P., Winderickx, J., Van Dijck, P. and Thevelein, J.M. (2000) Expression of *Escherichia coli otsA* in a *Saccharomyces tps1* mutant restores growth and fermentation with glucose and control of glucose influx into glycolysis. *Biochem. J*. **350**, 261–268.

Bouly, J.P., Gissot, L., Lessard, P., Kreis, M. and Thomas, M. (1999) *Arabidopsis thaliana* homologues of the yeast SIP and SNF4 proteins interact with AKINα1, a SNF1-like protein kinase. *Plant J*. **18**, 541–550.

Carling, D., Aguan, K., Woods, A., Verhoeven, A.J.M., Beri, R.K., Brennan, C.H., Sidebottom, C., Davison, M.D. and Scott, J. (1994) Mammalian AMP-activated protein kinase is homologous to yeast and plant protein kinases involved in the regulation of carbon metabolism. *J. Biol. Chem*. **269**, 11442–11448.

Carling, D., Clarke, P.R., Zammit, V.A. and Hardie, D.G. (1989) Purification and characterisation of the AMP-activated protein kinase – co-purification of acetyl-CoA carboxylase and 3-hydroxy-3-methylglutaryl CoA reductase kinase activity. *Eur. J. Biochem*. **186**, 129–136.

Carling, D., Zammit, V.A. and Hardie, D.G. (1987) A common bicyclic protein kinase cascade inactivates the regulatory enzymes of fatty acid and cholesterol biosynthesis. *FEBS Lett*. **223**, 217–222.

Carlson, C.A. and Kim, K.H. (1973) Regulation of hepatic acetyl coenzyme A carboxylase by phosphorylation and dephosphorylation. *J. Biol. Chem*. **248**, 378–380.

Celenza, J.L. and Carlson, M. (1986) A yeast gene that is essential for release from glucose repression encodes a protein kinase. *Science*, **233**, 1175–1180.

Celenza, J.L., Eng, F.J. and Carlson, M. (1989) Molecular analysis of the Snf4 gene of *Saccharomyces cerevisiae* – evidence for the physical association of the Snf4 protein with the Snf1 protein kinase. *Molec. Cell. Biol*. **9**, 5045–5054.

Cheng, C.-L., Acedo, G.N., Cristinsin, M. and Conkling, M.A. (1992) Sucrose mimics the light induction of Arabidopsis nitrate reductase gene transcription. *Proc. Natl Acad. Sci. USA*, **89**, 1861–1864.

Chourey, P.S. and Nelson, O.E. (1976) The enzymatic deficiency conditioned by the *Shrunken-1* mutations in maize. *Biochem. Genet*. **14**, 1041–1055.

Clarke, P.R. and Hardie, D.G. (1990) Regulation of HMG-CoA reductase: identification of the site phosphorylated by the AMP-activated protein kinase *in vitro* and in intact rat liver. *EMBO J*. **9**, 2439–2446.

Corton, J.M., Gillespie, J.G. and Hardie, D.G. (1994) Role of the AMP-activated protein kinase in the cellular stress response. *Curr. Biol*. **4**, 315–324.

Corton, J.M., Gillespie, J.G., Hawley, S.A. and Hardie, D.G. (1995) 5-Aminoimidazole-4-carboxamide ribonucleoside: a specific method for activating AMP-activated protein kinase in intact cells? *Eur. J. Biochem*. **229**, 558–565.

Crawford, R.M., Halford, N.G. and Hardie, D.G. (2001) Cloning of DNA encoding a catalytic subunit of SNF1-related protein kinase-1 (SnRK1-α1) and immunological analysis of multiple forms of the kinase in spinach leaf. *Plant Molec. Biol*. **45**, 731–741.

Dale, S., Arró, M., Becerra, B., Morrice, N.G., Boronat, A., Hardie, D.G. and Ferrer, A. (1995a) Bacterial expression of the catalytic domain of 3-hydroxy-3-methylglutaryl CoA reductase (isoform HMGR1) from Arabidopsis, and its inactivation by phosphorylation at serine-577 by *Brassica oleracea* 3-hydroxy-3-methylglutaryl CoA reductase kinase. *Eur. J. Biochem*. **233**, 506–513.

Dale, S., Wilson, W.A., Edelman, A.M. and Hardie, D.G. (1995b) Similar substrate recognition motifs for mammalian AMP-activated protein kinase, higher plant HMG-CoA reductase kinase-A, yeast SNF1, and mammalian calmodulin-dependent protein kinase I. *FEBS Letts*. **361**, 191–195.

Davies, S.P., Carling, D. and Hardie, D.G. (1989) Tissue distribution of the AMP-activated protein kinase, and lack of activation by cyclic AMP-dependent protein kinase, studies using a specific and sensitive peptide assay. *Eur. J. Biochem*. **186**, 123–128.

Davies, S.P., Carling, D., Munday, M.R. and Hardie, D.G. (1992) Diurnal rhythm of phosphorylation of rat-liver acetyl-CoA carboxylase by the AMP-activated protein kinase, demonstrated using freeze-clamping – effects of high-fat diets. *Eur. J. Biochem*. **203**, 615–623.

Davies, S.P., Hawley, S.A., Woods, A., Carling, D., Haystead, T.A.J. and Hardie, D.G. (1994) Purification of the AMP-activated protein kinase on ATP-g-Sepharose and analysis of its subunit structure. *Eur. J. Biochem*. **223**, 351–357.

Davies, S.P., Helps, N.R., Cohen, P.T.W. and Hardie, D.G. (1995) 5′-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP-activated protein kinase. Studies using bacterially expressed human protein phosphatase-2Cα and native bovine protein phosphatase-2A(C). *FEBS Letts*. **377**, 421–425.

Davies, S.P., Sim, A.T.R. and Hardie, D.G. (1990) Location and function of three sites phosphorylated on rat acetyl-CoA carboxylase by the AMP-activated protein kinase. *Eur. J. Biochem*. **187**, 183–190.

DeVit, M.J., Waddle, J.A. and Johnston, M. (1997) Regulated nuclear translocation of the Mig1 repressor. *Molec. Biol. Cell*, **8**, 1603–1618.

Dickinson, J.R. (1999) Carbon metabolism. In: *The Metabolism and Molecular Physiology of Saccharomyces Cerevisiae* (Dickinson, J.R. and Schweizer, M., eds), pp. 23–55. London & Philadelphia, PA: Taylor & Francis.

Dickinson, C.C., Altabella, T. and Chrispeels, M.J. (1991) Slow growth phenotype of transgenic tomato expressing apoplastic invertase. *Plant Physiol*. **95**, 420–425.

Dickinson, J.R., Cole, D. and Halford, N.G. (1999) A cell cycle role for a plant sucrose nonfermenting-1-related protein kinase (SnRK1) is indicated by expression in yeast. *Plant Growth Reg*. **28**, 169–174.

Douglas, P., Morrice, N. and MacKintosh, C. (1995) Identification of a regulatory phosphorylation site in the hinge 1 region of nitrate reductase from spinach *(Spinacea oleracea)* leaves. *FEBS Letts*. **377**, 113–117.

Douglas, P., Pigaglio, E., Ferrer, A., Halford, N.G. and MacKintosh, C. (1997) Three spinach leaf nitrate reductase/3-hydroxy-3-methylglutaryl-CoA reductase kinases that are regulated by reversible phosphorylation and/or Ca^{2+} ions. *Biochem. J*. **325**, 101–109.

Drennan, P.M., Smith, M.T., Goldsworthy, D. and van Staden, J. (1993) The occurrence of trehalose in the leaves of the desiccation tolerant angiosperm *Myrothamnus flabellifolius Welw*. *J. Plant Physiol*. **142**, 493–496.

Du Grierson, C.J.S., Zabala, M.D., Beggs, K., Smith, C., Holdsworth, M. and Bevan, M. (1994) Separate *cis* sequences and *trans* factors direct metabolic and developmental regulation of a potato tuber storage protein gene. *Plant J*. **5**, 815–826.

Eastmond, P.J., van Dijken, A.J., Spielman, M., Kerr, A., Tissier, A.F., Dickinson, H.G., Jones, J.D., Smeekens, S.C. and Graham, I.A. (2002) Trehalose-6-phosphate 1, which catalyses the first step in trehalose synthesis, is essential for *Arabidopsis* embryo development. *Plant J*. **29**, 225–235.

Entian, K.D. (1980) Genetic and biochemical evidence for hexokinase PII as a key enzyme involved in carbon catabolite repression in yeast. *Mol. Gen. Genet*. **178**, 633–637.

Fordham-Skelton, A.P., Chilley, P., Lumbreras, V., Reignoux, S., Fenton, T.R., Dahm, C.C., Pages, M. and Gatehouse, J.A. (2002) A novel higher plant protein tyrosine phosphatase interacts with SNF1-related protein kinases via a KIS (kinase interaction sequence) domain. *Plant J*. **29**, 705–715.

Fritzius, T., Aeschbacher, R., Wiemken, A. and Wingler, A. (2001) Induction of *APL3* expression by trehalose complements the starch-deficient *Arabidopsis* mutant *adg2-1* lacking ApL1, the large subunit of ADP-glucose pyrophosphorylase. *Plant Physiol*. **126**, 883–889.

Fu, H. and Park, W.D. (1995) Sink- and vascular-associated sucrose synthase functions are encoded by different gene classes in potato. *Plant Cell*, **7**, 1369–1385.

Gao, G., Fernandez, S., Stapleton, D., Auster, A.S., Widmer, J., Dyck, J.R.B., Kemp, B.E. and Witters, L.A. (1996) Non-catalytic β- and γ-subunit isoforms of the 5′-AMP-activated protein kinase. *J. Biol. Chem*. **271**, 8675–8681.

Gao, G., Widmer, J., Stapleton, D., The, T., Cox, T., Kemp, B.E. and Witters, L.A. (1995) Catalytic subunits of the porcine and rat 5′-AMP-activated protein kinase are members of the SNF1 protein kinase family. *Biochim. Biophys. Acta*, **1266**, 73–82.

Garg, A.K., Kim, J.-K., Owens, T.G., Ranwala, A.P., Choi, Y.D., Kochian, L.V. and Wu, R.J. (2002) Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. *Proc. Natl Acad. Sci. USA*, **99**, 15898–15903.

Gillespie, J.G. and Hardie, D.G. (1992) Phosphorylation and inactivation of HMG-CoA reductase at the AMP-activated protein kinase site in response to fructose treatment of isolated rat hepatocytes. *FEBS Letts*. **306**, 59–62.

Goddijn, O.J. and Smeekens, S.C.M. (1998) Sensing trehalose biosynthesis in plants. *Plant J*. **14**, 143–146.

Goddijn, O.J. and van Dun, K. (1999) Trehalose metabolism in plants. *Trends Plant Sci*. **4**, 315–319.

Gómez-Cadenas, A., Verhey, S.D., Holappa, L.D., Shen, Q., Ho, T.-H.D. and Walker-Simmons, M.K. (1999) An abscisic acid-induced protein kinase, PKABA1, mediates abscisic acid-suppressed gene expression in barley aleurone layers. *Proc. Natl Acad. Sci. USA*, **96**, 1767–1772.

Graham, I.A., Denby, K.J. and Leaver, C.J. (1994) Carbon catabolite repression regulates glyoxylate cycle gene expression in cucumber. *Plant Cell*, **6**, 761–772.

Halford, N.G. and Hardie, D.G. (1998) SNF1-related protein kinases: global regulators of carbon metabolism in plants? *Plant Mol. Biol*. **37**, 735–748.

Halford, N.G., Hey, S., Jhurreea, D., Laurie, S., McKibbin, R.S., Paul, M.J. and Zhang, Y. (2003a) Metabolic signalling and carbon partitioning: Role of Snf1-related (SnRK1) protein kinase. *J. Exp. Bot*. **54**, 467–475.

Halford, N.G., Hey, S., Jhurreea, D., Laurie, S., McKibbin, R.S., Zhang, Y. and Paul, M. (2003b) Dissection and manipulation of metabolic signalling pathways. *Ann. Appl. Biol*. **142**, 25–31.

Halford, N.G., Purcell, P.C. and Hardie, D.G. (1999) Is hexokinase really a sugar sensor in plants? *Trends Plant Sci*. **4**, 117–120.

Halfter, U., Ishitani, M. and Shu, J.-K. (2000) The arabidopsis SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein, SOS3. *Proc. Natl Acad. Sci. USA*, **97**, 3535–3740.

Hardie, D.G. (2000) Plant protein serine/threonine protein kinases: Classification into subfamilies and overview of function. *Adv. Bot. Res. Inc. Adv. Plant Path*. **32**, 1–44.

Hardie, D.G. and Carling, D. (1997) The AMP-activated protein kinase: fuel gauge of the mammalian cell? *Eur. J. Biochem*. **246**, 259–273.

Harter, K., Talke-Messerer, C., Barz, W. and Schäfer, E. (1993) Light and sucrose-dependent gene expression in photomixotrophic cell suspension cultures and protoplasts of rape. *Plant J*. **4**, 507–516.

Hawley, S.A., Davison, M., Woods, A., Davies, S.P., Beri, R.K., Carling, D. and Hardie, D.G. (1996) Characterization of the AMP-activated protein kinase kinase from rat liver, and identification of threonine-172 as the major site at which it phosphorylates and activates AMP-activated protein kinase. *J. Biol. Chem*. **271**, 27879–27887.

Hawley, S.A., Selbert, M.A., Goldstein, E.G., Edelman, A.M., Carling, D. and Hardie, D.G. (1995) 5′-AMP activates the AMP-activated protein kinase cascade, and Ca^{2+}/calmodulin the calmodulin-dependent protein kinase I cascade, via three independent mechanisms. *J. Biol. Chem*. **270**, 27186–27191.

Heineke, D., Wildenberger, K., Sonnewald, U., Willmitzer, L. and Heldt, H.W. (1994) Accumulation of hexoses in leaf vacuoles: studies with transgenic tobacco plants expressing yeast-derived invertase in the cytosol, vacuole or cytoplasm. *Planta*, **194**, 29–33.

Herbers, K., Meuwly, P., Frommer, W.B., Metraux, J.P. and Sonnewald, U. (1996) Systemic acquired resistance mediated by the ectopic expression of invertase: Possible hexose sensing in the secretory pathway. *Plant Cell*, **8**, 793–803.

Herrero, P., Martinez-Campa, C. and Moreno, F. (1998) The hexokinase 2 protein participates in regulatory DNA-protein complexes necessary for glucose repression of the *SUC2* gene in *Saccharomyces cerevisiae*. *FEBS Lett*. **434**, 71–76.

Hinnebusch, A.G. (1992) General and pathway-specific regulatory mechanisms controlling the synthesis of amino acid biosynthetic enzymes in *Saccharomyces cerevisiae*. In: *Molecular Cell Biology Yeast Saccharomyces*, Vol. 2 *Gene Expression* (Jones, E.W., Pringle, J.R. and Broach, J.B., eds), pp. 319–414. New York: Cold Spring Harbor Laboratory Press.

Hinnebusch, A.G. (1994) Translational control of GCN4 – an *in vivo* barometer of initiation factor activity. *Trends. Biochem. Sci*. **19**, 409–414.

Hinnebusch, A.G. (1997) Translational regulation of yeast GCN4 – A window on factors that control initiator-tRNA binding to the ribosome. *J. Biol. Chem*. **272**, 21661–21664.

Hohmann, S., Bell, W., Neves, M.J., Valckx, D. and Thevelein, J.M. (1996) Evidence for trehalose 6-phosphate dependent and independent mechanisms in the control of sugar influx into yeast glycolysis. *Mol. Microbiol*. **20**, 981–991.

Honigberg, S.M. and Lee, R.H. (1998) SNF1 kinase connects nutritional pathways controlling meiosis in *Saccharomyces cereviseae*. *Mol. Cell. Biol*. **18**, 4548–4555.

Hrabak, E.M., Chan, C.W.M., Gribskov, M., Harper, J.F., Choi, J.H., Halford, N., Kudla, J., Luan, S., Nimmo, H.G., Sussman, M.R., Thomas, M., Walker-Simmons, K., Zhu, J.-K. and Harmon, A.C. (2003) The Arabidopsis CDPK-SnRK superfamily of protein kinases. *Plant Physiol*. **132**, 666–680.

Huang, N., Chandler, J., Thomas, B.R., Koizumi, N. and Rodriguez, R.L. (1993) Metabolic regulation of α-amylase gene expression in transgenic cell cultures of rice (*Oryza sativa* L.). *Plant Molec. Biol*. **23**, 737–747.

Huang, J.Z. and Huber, S.C. (2001) Phosphorylation of synthetic peptides by a CDPK and plant SNF1-related protein kinase. Influence of proline and basic amino acid residues at selected positions. *Plant Cell Physiol*. **42**, 1079–1087.

Hwang, I. and Sheen, J. (2001) Two-component circuitry in Arabidopsis signal transduction. *Nature*, **413**, 383–389.

Jang, J.-C., Leon, P., Zhou, L. and Sheen, J. (1997) Hexokinase as a sugar sensor in higher plants. *Plant Cell*, **9**, 5–19.

Jang, J.C. and Sheen, J. (1994) Sugar sensing in higher plants. *Plant Cell*, **6**, 1665–1679.

Jang, J.C. and Sheen, J. (1997) Sugar sensing in higher plants. *Trends Plant Sci*. **2**, 208–214.

Jiang, R. and Carlson, M. (1996) Glucose regulates protein interactions within the yeast Snf1 protein kinase complex. *Genes Dev*. **10**, 3105–3115.

Jiang, R. and Carlson, M. (1997) The Snf1 protein kinase and its activating subunit, Snf4, interact with distinct domains of the Sip1/Sip2/Gal83 component in the kinase complex. *Molec. Cell. Biol*. **17**, 2099–2106.

Karrer, E.E. and Rodriguez, R.L. (1992) Metabolic regulation of rice α-amylase and sucrose synthase genes in plants. *Plant J*. **2**, 517–523.

Kleinow, T., Bhalerao, R., Breuer, F., Umeda, M., Salchert, K. and Koncz, C. (2000) Functional identification of an Arabidopsis SNF4 orthologue by screening for heterologous multicopy suppressors of *snf4* deficiency in yeast. *Plant J*. **23**, 115–122.

Krapp, A., Hofmann, B., Schäfer, C. and Stitt, M. (1993) Regulation of the expression of *rbcS* and other photosynthetic genes by

carbohydrates: a mechanism for the 'sink' regulation of photosynthesis? *Plant J*. **3**, 817–828.

Kruckeberg, A.L. (1996) The hexose transporter family of *Saccharomyces cerevisiae*. *Arch. Microbiol*. **166**, 283–292.

Kudo, N., Barr, A.J., Barr, R.L., Desai, S. and Lopaschuk, G.D. (1995) High rates of fatty acid oxidation during reperfusion of ischemic hearts are associated with a decrease in malonyl-CoA levels due to an increase in 5′-AMP-activated protein kinase inhibition of acetyl-CoA carboxylase. *J. Biol. Chem*. **270**, 17513–17520.

Lakatos, L., Klein, M., Hofgen, R. and Banfalvi, Z. (1999) Potato StubSNF1 interacts with StubGAL83: a plant protein kinase complex with yeast and mammalian counterparts. *Plant J*. **17**, 569–574.

Lalonde, S., Boles, E., Hellmann, H., Barker, L., Patrick, J.W., Frommer, W.B. and Ward, J.M. (1999) The dual function of plant sugar carriers in transport and in nutrient sensing. *Plant Cell*, **11**, 707–726.

Lalonde, S., Tegeder, M., Throne-Holst, M., Frommer, W.B. and Patrick, J.W. (2003) Phloem loading and unloading of amino acids and sugars. *Plant Cell Environ*. **26**, 37–56.

Laurie, S., McKibbin, R.S. and Halford, N.G. (2003) Antisense SNF1-related (SnRK1) protein kinase gene represses transient activity of an α-amylase (*α-Amy2*) gene promoter in cultured wheat embryos. *J. Exp. Bot*. **54**, 739–747.

Leclerc, I., Mar. Albà, M., Kleinow, T., Koncz, C. and Pagès, M. (1998) The 5′-AMP-activated protein kinase inhibits the transcriptional stimulation by glucose in liver cells, acting through the glucose response complex. *FEBS Lett*. **431**, 180–184.

Liu, J., Ishitani, M., Halfter, U., Kim, C.-S. and Shu, J.-K. (2000) The *Arabidopsis thaliana SOS2* gene encodes a protein kinase that is required for salt tolerance. *Proc. Natl Acad. Sci. USA*, **97**, 3730–3734.

Lumbreras, V., Mar. Albà, M., Kleinow, T., Koncz, C. and Pagès, M. (2001) Domain fusion between SNF1-related kinase subunits during plant evolution. *EMBO Rep*. **2**, 55–60.

Ma, H., Bloom, L.M., Walsh, C.T. and Botstein, D. (1989) The residual enzymatic phosphorylation activity of hexokinase II mutants is correlated with glucose repression in *Saccharomyces cerevisiae*. *Mol. Cell. Biol*. **9**, 5643–5649.

MacKintosh, R.W., Davies, S.P., Clarke, P.R., Weekes, J., Gillespie, J.G., Gibb, B.J. and Hardie, D.G. (1992) Evidence for a protein kinase cascade in higher plants: 3-hydroxy-3-methylglutaryl-CoA reductase kinase. *Eur. J. Biochem*. **209**, 923–931.

Man, A.L., Purcell, P.C., Hannappel, U. and Halford, N.G. (1997) Potato SNF1-related protein kinase: molecular cloning, expression analysis and peptide kinase activity measurements. *Plant Mol. Biol*. **34**, 31–43.

Mann, G.C. (1999) Crop Scientist seeks a new revolution. *Science*, **283**, 310–314.

Mita, S., Suzuki-Fujii, K. and Nakamura, K. (1995) Sugar-inducible expression of a gene for β-amylase in Arabidopsis. *Plant Physiol*. **107**, 895–904.

Mitchelhill, K.I., Stapleton, D., Gao, G., House, C., Michell, B., Katsis, F., Witters, L.A. and Kemp, B.E. (1994) Mammalian AMP-activated protein kinase shares structural and functional homology with the catalytic domain of yeast Snf1 protein kinase. *J. Biol. Chem*. **269**, 2361–2364.

Moore, F., Weekes, J. and Hardie, D.G. (1991) AMP triggers phosphorylation as well as direct allosteric activation of rat liver AMP-activated protein kinase. A sensitive mechanism to protect the cell against ATP depletion. *Eur. J. Biochem*. **199**, 691–697.

Moore, B., Zhou, L., Rolland, F., Hall, Q., Cheng, W.-H., Liu, Y.-X., Hwang, I., Jones, T. and Sheen, J. (2003) Role of the *Arabidopsis* glucose sensor HXK1 in nutrient, light and hormonal signalling. *Science*, **300**, 332–336.

Moorhead, G., Douglas, P., Morrice, N., Scarabel, M., Aitken, A. and MacKintosh, C. (1996) Phosphorylated nitrate reductase from spinach leaves is inhibited by 14-3-3 proteins and activated by fusicoccin. *Curr. Biol*. **6**, 1104–1113.

Muller, J., Boller, T. and Wiemken, A. (1998) Trehalose affects sucrose synthase and invertase activities in soybean (Glycine max L. Merr.) roots. *J. Plant Physiol*. **153**, 255–257.

Muranaka, T., Banno, H. and Machida, Y. (1994) Characterisation of the tobacco protein kinase NPK5, a homologue of *Saccharomyces cerevisiae* SNF1 that constitutively activates expression of the glucose-repressible *SUC2* gene for a secreted invertase of *S. cerevisiae*. *Mol. Cell. Biol*. **14**, 2958–2965.

Natarajan, K., Meyer, M.R., Jackson, B.M., Slade, D., Roberts, C., Hinnebusch, A.G. and Marton, M.J. (2001) Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during amino acid starvation in yeast. *Mol. Cell. Biol*. **21**, 4347–4368.

Noubhani, A., Bunoust, O., Rigoulet, M. and Thevelein, J.M. (2000) Reconstitution of ethanolic fermentation in permeabilised spheroplasts of wild type and trehalose 6-phosphate synthase mutants of the yeast *Saccharomyces cerevisiae*. *Eur. J. Biochem*. **267**, 4566–4576.

Östling, J. and Ronne, H. (1998) Negative control of the Mig1p repressor by Snf1p-dependent phosphorylation in the absence of glucose. *Eur. J. Biochem*. **252**, 162–168.

Ozcan, S. and Johnston, M. (1996) Two different repressors collaborate to restrict expression of yeast glucose transporter genes HXT2 and HXT4 to low levels of glucose. *Mol. Cell. Biol*. **16**, 5536–5545.

Parviz, F. and Heideman, W. (1998) Growth-independent regulation of *CLN3* mRNA levels by nutrients in *Saccharomyces cerevisiae*. *J. Bacteriol*. **180**, 225–230.

Paul, M.J., Pellny, T. and Goddijn, O. (2001) Enhancing photosynthesis with sugar signals. *Trends Plant Sci*. **6**, 197–200.

Pilon-Smits, E.A.H., Terry, N., Sears, T., Kim, H., Zayed, A., Hwang, S., van Dun, K., Voogd, E., Verwoerd, T.C., Krutwagen, R.W.H.H. and Goddijn, O.J.M. (1998) Trehalose-producing transgenic tobacco plants show improved growth performance under drought stress. *J. Plant Physiol*. **152**, 525–532.

Purcell, P.C., Smith, A.M. and Halford, N.G. (1998) Antisense expression of a sucrose nonfermenting-1-related protein kinase sequence in potato results in decreased expression of sucrose synthase in tubers and loss of sucrose-inducibility of sucrose synthase transcripts in leaves. *Plant J*. **14**, 195–202.

Purnapatre, K., Piccirillo, S., Schneider, B.L. and Honigberg, S.M. (2002) The CLN3/SWI6/CLN2 pathway and SNF1 act sequentially to regulate meiotic initiation in *Saccharomyces cerevisiae*. *Genes Cells*, **7**, 675–691.

Randez-Gil, F., Herrero, P., Sanz, P., Prieto, J.A. and Moreno, F. (1998) Hexokinase PII has a double cytosolic-nuclear localisation in *Saccharomyces cerevisiae*. *FEBS Lett*. **425**, 475–478.

Reifenberger, E., Boles, E. and Ciriacy, M. (1997) Kinetic characterization of individual hexose transporters of *Saccharomyces cerevisiae* and their relation to the triggering mechanisms of glucose repression. *Eur. J. Biochem*. **245**, 324–333.

Reinders, A., Schulze, W., Kühn, C., Barker, L., Schulz, A., Ward, J.M. and Frommer, W.B. (2002) Protein–protein interactions between

sucrose transporters of different affinities co-localized in the same enucleate sieve element. *Plant Cell*, **14**, 1567–1577.

Riou-Khamlichi, C., Menges, M., Healy, J.M.S. and Murray, J.A.H. (2000) Sugar control of the plant cell cycle: differential regulation of Arabidopsis D-type cyclin gene expression. *Mol. Cell. Biol*. **20**, 4513–4521.

Rolland, F., Winderickx, J. and Thevelein, J.M. (2001) Glucose-sensing mechanisms in eukaryotic cells. *Trends Biochem. Sci*. **26**, 310–317.

Rook, F. and Bevan, M.W. (2003) Genetic approaches to understanding sugar-response pathways. *J. Exp. Bot*. **54**, 495–501.

Rook, F., Corke, F., Card, R., Munz, G., Smith, C. and Bevan, M.W. (2001) Impaired sucrose-induction mutants reveal the modulation of sugar-induced starch biosynthetic gene expression by abscisic acid signalling. *Plant J*. **26**, 421–433.

Rose, M., Albig, W. and Entian, K.D. (1991) Glucose repression in *Saccharomyces cerevisiae* is directly associated with hexose phosphorylation by hexokinase PI and PII. *Eur. J. Biochem*. **199**, 511–518.

Salanoubat, M. and Belliard, G. (1989) The steady-state level of potato sucrose synthase mRNA is dependant on wounding, anaerobiosis and sucrose concentration. *Gene*, **84**, 181–185.

Sato, R., Goldstein, J.L. and Brown, M.S. (1993) Replacement of Serine-871 of hamster 3-hydroxy-3-methylglutaryl CoA reductase prevents phosphorylation by AMP-activated protein kinase and blocks inhibition of sterol synthesis induced by ATP depletion. *Proc. Natl Acad. Sci. USA*, **90**, 9261–9265.

von Schaewen, A., Stitt, M., Sonnewald, U. and Willmitzer, L. (1990) Expression of a yeast-derived invertase in the cell wall of tobacco and arabidopsis plants leads to accumulation of carbohydrate and inhibition of photosynthesis and strongly influences growth and phenotype of transgenic tobacco plants. *EMBO J*. **9**, 3033–3044.

Schluepmann, H., Pellny, T., van Dijken, A., Smeekens, S. and Paul, M. (2003) Trehalose 6-phosphate is indispensable for carbohydrate utilization and growth in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA*, **100**, 6849–6854.

Sheen, J. (1990) Metabolic repression of transcription in higher plants. *Plant Cell*, **2**, 1027–1038.

Sheen, J. (1994) Feedback control of gene expression. *Photosynth. Res*. **39**, 427–438.

Sherson, S.M., Alford, H.L., Forbes, S.M., Wallace, G. and Smith, S.M. (2003) Roles of cell-wall invertases and monosaccharide transporters in the growth and development of *Arabidopsis*. *J. Exp. Bot*. **54**, 525–531.

Slocombe, S.P., Laurie, S., Bertini, L., Beaudoin, F., Dickinson, J.R. and Halford, N.G. (2002) Molecular cloning of SnIP1, a novel protein that interacts with SNF1-related protein kinase (SnRK1). *Plant Mol. Biol*. **49**, 31–44.

Smeekens, S. (2000) Sugar-induced signal transduction in plants. *Ann. Rev. Plant Physiol. Plant Molec. Biol*. **51**, 49–81.

Sowokinos, J.R. and Varns, J.L. (1992) Induction of sucrose synthase in potato tissue culture: Effect of carbon source and metabolic regulators on sink strength. *J. Plant Physiol*. **139**, 672–679.

Su, W., Huber, S.C. and Crawford, N.M. (1996) Identification *in vitro* of a post-translational regulatory site in the hinge 1 region of Arabidopsis nitrate reductase. *Plant Cell*, **8**, 519–527.

Sugden, C., Crawford, R.M., Halford, N.G. and Hardie, D.G. (1999a) Regulation of spinach SNF1-related (SnRK1) kinases by protein kinases and phosphatases is associated with phosphorylation of the T loop and is regulated by 5′-AMP. *Plant J*. **19**, 433–439.

Sugden, C., Donaghy, P., Halford, N.G. and Hardie, D.G. (1999b) Two SNF1-related protein kinases from spinach leaf phosphorylate and inactivate 3-hydroxy-3-methylglutaryl-coenzyme A reductase, nitrate reductase and sucrose phosphate synthase *in vitro*. *Plant Physiol*. **120**, 257–274.

Sweetlove, L.J. and Hill, S.A. (2000) Source metabolism dominates the control of source to sink carbon flux in tuberising potato plants throughout the diurnal cycle and under a range of environmental conditions. *Plant Cell Environ*. **23**, 523–529.

Tanaka, S. and Nojima, H. (1996) Nik1, a Nim1-like protein kinase of *S. cerevisiae*, interacts with the Cdc28 complex and regulates cell cycle progression. *Genes Cells*, **1**, 905–921.

Thevelein, J. and Hohmann, S. (1995) Trehalose synthase: guard to the gate of glycolysis in yeast? *Trends Biochem. Sci*. **20**, 3–10.

Thompson-Jaeger, S., Francois, J., Gaughran, J.P. and Tatchell, K. (1991) Deletion of *SNF1* affects the nutrient response of yeast and resembles mutations which activate the adenylate cyclase pathway. *Genetics*, **12**, 697–706.

Thorens, B. (2001) GLUT2 in pancreatic and extra-pancreatic gluco-detection. *Mol. Membrane Biol*. **18**, 265–273.

Tiessen, A., Hendriks, J.H.M., Stitt, M., Branscheid, A., Gibon, Y., Farre, E.M. and Geigenberger, P. (2002) Starch synthesis in potato tubers is regulated by post-translational redox modification of ADP-glucose pyrophosphorylase: a novel regulatory mechanism linking starch synthesis to the sucrose supply. *Plant Cell*, **14**, 2191–2213.

Tiessen, A., Prescha, K., Branscheid, A., Palacios, N., McKibbin, R., Halford, N.G. and Geigenberger, P. (2003) Evidence that SNF1 related kinase and hexokinase are involved in separate sugar signalling pathways modulating posttranslational redox activation of ADP-glucose pyrophosphorylase in potato tubers. *Plant J*., **35**, 490–500.

Toroser, D., Plaut, Z. and Huber, S.C. (2000) Regulation of a plant SNF1-related protein kinase by glucose-6-phosphate. *Plant Physiol*. **123**, 403–411.

Teusink, B., Walsh, M.C., van Dam, K. and Westerhoff, H.V. (1998) The danger of metabolic pathways with turbo design. *Trends Biochem. Sci*. **23**, 162–169.

Van Aelst, L., Hohmann, S., Bulaya, B., de Koning, W., Sierkstra, L., Neves, M.J., Luyten, K., Alijo, R., Ramos, J. and Coccetti, P. (1993) Molecular cloning of a gene involved in glucose sensing in the yeast *Saccharomyces cerevisiae*. *Mol. Microbiol*. **8**, 927–943.

Van de Poll, K.W. and Schamhart, D. (1974) Characterisation of a regulatory mutant of fructose 1,6-bisphosphate in *Saccharomyces carlsbergensis*. *Mol. Gen. Genet*. **154**, 61–66.

Van Oosten, J.-J. and Besford, R.T. (1996) Acclimation of photosynthesis to elevated CO_2 through feedback regulation of gene expression: climate of opinion. *Photosynth. Res*. **48**, 353–365.

Weber, H., Buchner, P., Borisjuk, L. and Wobus, U. (1996) Sucrose metabolism during cotyledon development of *Vicia faba* L. is controlled by the concerted action of both sucrose phosphate synthase and sucrose synthase: expression patterns, metabolic regulation and implications for seed development. *Plant J*. **9**, 841–850.

Weekes, J., Ball, K.L., Caudwell, F.B. and Hardie, D.G. (1993) Specificity determinants for the AMP-activated protein kinase and its plant homologue analyzed using synthetic peptides. *FEBS Lett*. **334**, 335–339.

Weise, A., Barker, L., Kühn, C., Lalonde, S., Buschmann, H., Frommer, W.B. and Ward, J.M. (2000) A new subfamily of sucrose

transporters, SUT4, with low affinity/high capacity localized in enucleate sieve elements of plants. *Plant Cell*, **12**, 1345–1355.

Wilson, W.A., Hawley, S.A. and Hardie, D.G. (1996) The mechanism of glucose repression/derepression in yeast: SNF1 protein kinase is activated by phosphorylation under derepressing conditions, and this correlates with a high AMP: ATP ratio. *Curr. Biol*. **6**, 1426–1434.

Winder, W.W. and Hardie, D.G. (1996) Inactivation of acetyl-CoA carboxylase and activation of AMP-activated protein kinase in muscle during exercise. *Am. J. Physiol*. **270**, E299–E304.

Wingler, A., Fritzius, T., Wiemken, A., Boller, T. and Aeschbacher, R.A. (2000) Trehalose induces the ADP-glucose pyrophosphorylase gene, *ApL3*, and starch synthesis in *Arabidopsis*. *Plant Physiol*. **124**, 105–114.

Wingler, A., von Schaewen, A., Leegood, R.C., Lea, P.J. and Quick, W.P. (1998) Regulation of leaf senescence by cytokinin, sugars and light. Effects on NADH-dependent hdroxypyruvate reductase. *Plant Physiol*. **116**, 329–335.

Witters, L.A., Nordlund, A.C. and Marshall, L. (1991) Regulation of intracellular acetyl-CoA carboxylase by ATP depletors mimics the action of the 5′-AMP-activated protein kinase. *Biochem. Biophys. Res. Commun*. **181**, 1486–1492.

Woods, A., Azzout-Marniche, D., Foretz, M., Stein, S.C., Lemarchand, P., Ferre, P., Foufelle, F. and Carling, D. (2000) Characterization of the role of AMP-activated protein kinase in the regulation of glucose-activated gene expression using constitutively active and dominant negative forms of the kinase. *Mol. Cell. Biol*. **20**, 6704–6711.

Woods, A., Cheung, P.C.F., Smith, F.C., Davison, M.D., Scott, J., Beri, R.K. and Carling, D. (1996) Characterisation of AMP-activated protein kinase β and γ subunits: assembly of the heterotrimeric complex *in vitro*. *J. Biol. Chem*. **271**, 10282–10290.

Woods, A., Munday, M.R., Scott, J., Yang, X., Carlson, M. and Carling, D. (1994) Yeast SNF1 is functionally related to mammalian AMP-activated protein kinase and regulates acetyl-CoA carboxylase *in vivo*. *J. Biol. Chem*. **269**, 19509–19515.

Yang, X., Jiang, R. and Carlson, M. (1994) A family of proteins containing a conserved domain that mediates interaction with the yeast Snf1 protein kinase complex. *EMBO J*. **13**, 5878–5886.

Yang, R.J., Wek, S.A. and Wek, R.C. (2000) Glucose limitation induces GCN4 translation by activation of gcn2 protein kinase. *Mol. Cell. Biol*. **20**, 2706–2717.

Yu, S.-M., Kuo, Y.-H., Sheu, G., Sheu, Y.-J. and Liu, L.-F. (1991) Metabolic derepression of α-amylase gene expression in suspension-cultured cells of rice. *J. Biol. Chem*. **266**, 21131–21137.

Zhang, Y., Dickinson, J.R., Paul, M.J. and Halford, N.G. (2003) Molecular cloning of an arabidopsis homologue of GCN2, a protein kinase involved in co-ordinated response to amino acid starvation. *Planta*, DOI: 10.1007/s00425-003-1025–4.

Zhang, Y., Shewry, P.R., Jones, H., Barcelo, P., Lazzeri, P.A. and Halford, N.G. (2001) Expression of antisense SnRK1 protein kinase sequence causes abnormal pollen development and male sterility in transgenic barley. *Plant J*. **28**, 431–442.

Zrenner, R., Salanoubat, M., Willmitzer, L. and Sonnewald, U. (1995) Evidence of the crucial role of sucrose synthase for sink strength using transgenic potato plants (*Solanum tuberosum* L.). *Plant J*. **7**, 97–107.