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Metabolic signalling and carbon partitioning: role of Snf1-related (SnRK1) protein kinase

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Abstract

A protein kinase that plays a key role in the global control of plant carbon metabolism is SnRK1 (sucrose non-fermenting-1-related protein kinase 1), so-called because of its homology and functional similarity with sucrose non-fermenting 1 (SNF1) of yeast. This article reviews studies on the characterization of SnRK1 gene families, SnRK1 regulation and function, interacting proteins, and the effects of manipulating SnRK1 activity on carbon metabolism and development.

Key words: Carbon partitioning, metabolic signalling, protein kinase, SnRK1.

Introduction

SnRK1 (**S**NF1-**R**elated Protein **K**inase-1) is a plant protein kinase with a catalytic domain similar to that of SNF1 (**S**ucrose **N**on-**f**ermenting-1) of yeast and AMPK (**AMP**-activated protein **k**inase) of animals. The SNF1 family of protein kinases are a distinct group within the protein kinase superfamily, but are closely related to the calcium-dependent protein kinase (CDPK) group, which includes the animal calmodulin-dependent protein kinases and the plant calmodulin-like domain protein kinases (Hardie, 2000).

SNF1 (Celenza and Carlson, 1986) is activated in response to low cellular glucose levels and is required for the derepression of a battery of genes that are repressed by glucose (Gancedo, 1998; Ronne, 1995; Dickinson, 1999). It also directly modulates the phosphorylation state of a number of metabolic enzymes, including acetyl-CoA carboxylase (Woods *et al*., 1994) and glycogen synthase (Hardy *et al*., 1994), and is required for the arrest of growth and the cell cycle under conditions of glucose deprivation (Thompson-Jaeger *et al*., 1991).

AMPK is activated by AMP (Carling *et al*., 1987, 1989) and by phosphorylation by an upstream protein kinase (AMP-activated protein kinase kinase (AMPKK)) (Hawley *et al*., 1996). Activation of AMPK by AMP is antagonized by high (mM) concentrations of ATP and a high AMP:ATP ratio is symptomatic of low cellular energy levels. For this reason, AMPK has been likened to a cellular fuel gauge (Hardie and Carling, 1997). When activated, it acts to conserve ATP by phosphorylating and inactivating regulatory enzymes of ATP-consuming, anabolic 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).

The SnRK gene family

The first plant SnRK1 sequence to be reported was a cDNA isolated from a rye endosperm cDNA library (Alderson *et al*., 1991). It encoded a 57.7 kDa protein of 502 amino acid residues showing 48% amino acid sequence identity with SNF1 and AMPK, rising to 62–64% amino acid sequence identity in the kinase catalytic domain.

SnRK1 genes have since been identified and characterized in many plant species (reviewed by Halford and Hardie, 1998). They are present in small to medium-sized gene families, comprising, for example, three members in arabidopsis and 10–20 in barley. The *SnRK1* gene family of cereals can be subdivided further into two groups,

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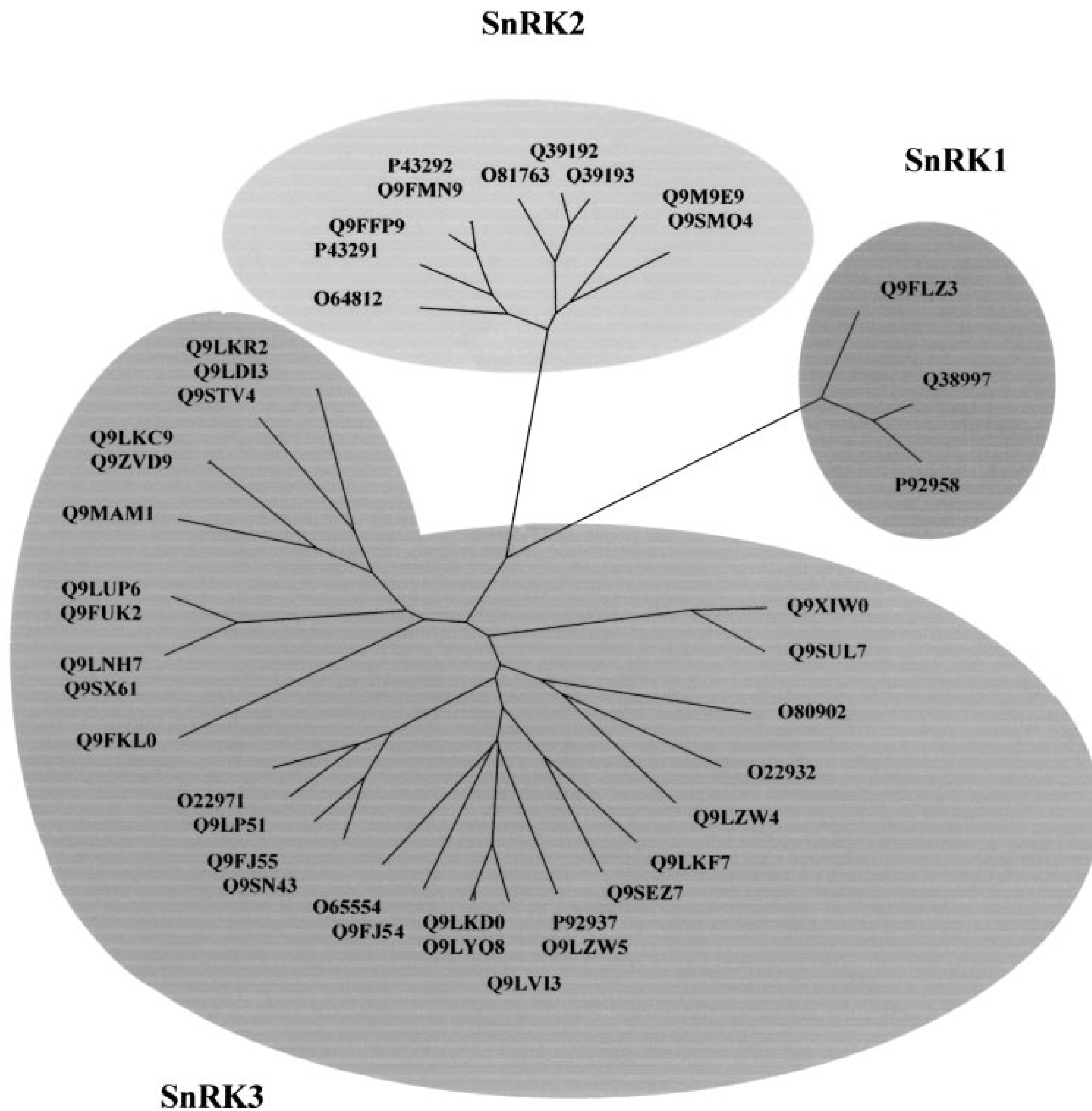

Fig. 1. Dendrogram showing the evolutionary relationship of members of the arabidopsis SNF1-related protein kinase (SnRK) family. Each protein kinase is identified by a database accession number (http://ukcrop.net/agr/). Alignments were produced using the PILEUP program (Wisconsin Package version 10, Genetics Computer Group, Madison, Wisconsin) with a gap creation penalty of 3.0 and a gap extension penalty of 1.0. Evolutionary distances were calculated using the DISTANCES program, correcting for multiple substitutions at a single site by the method of Kimura (1980), and displayed by the unweighted pair group method using arithmetic averages with the GROWTREE program. The shading superimposed over the dendrogram shows the division of the family into SnRK1, SnRK2 and SnRK3 sub-families.

SnRK1a and *SnRK1b*, on the basis of amino acid sequence similarity and expression patterns (Halford and Hardie, 1998). SnRK1a is expressed throughout the plant and is more closely related to SnRK1 from dicotyledonous plants. SnRK1b is expressed at the highest levels in the seed, although low levels of expression can be detected elsewhere, and is only present in monocotyledonous plants. The functional significance of this divergence of the gene family in cereals is not known.

Plants contain two other subfamilies of protein kinases, SnRK2 and SnRK3, containing catalytic domains with sequences that place them clearly within the SNF1 family. They have 42–45% amino acid sequence identity with SnRK1, SNF1 and AMPK in this region; they are, therefore, significantly less similar to SNF1 and AMPK than SnRK1 is. The SnRK2 and SnRK3 gene subfamilies appear to be unique to plants and are relatively large and diverse compared with SnRK1. The SnRK2 subfamily

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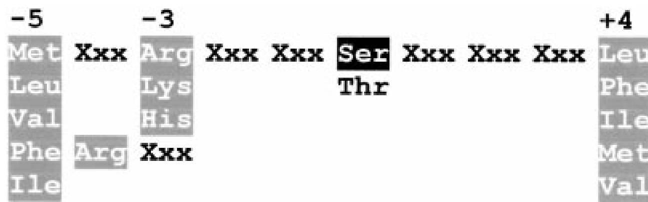

Fig. 2. Consensus sequence for phosphorylation by SnRK1. The residues required for recognition are highlighted as follows: (1) phosphorylated serine (highlighted in black); (2) hydrophobic residues at P-5 and P+4 (highlighted in grey); (3) basic residues at P-3 or P-4 (highlighted in grey).

includes PKABA1 from wheat, which is involved in mediating ABA-induced changes in gene expression (Anderberg and Walker-Simmons, 1992; Gómez-Cadenas *et al.*, 1999). The SnRK3 gene family includes SOS2, an arabidopsis protein kinase involved in conferring salt tolerance (Halfter *et al.*, 2000; Liu *et al.*, 2000). The completion of the arabidopsis genome sequencing project means that the full complement of the arabidopsis SnRK family can now be identified. These are shown as a dendrogram in Fig. 1.

SnRK1 activity

The cloning of SnRK1 genes and cDNAs allowed antisera to be raised to heterologously expressed SnRK1 and to synthetic peptides based on the predicted SnRK1 peptide sequence. Use of these antibodies led to the confirmation that a plant protein kinase related to AMPK, that was being studied independently at the biochemical level, was, indeed, SnRK1 (Ball *et al.*, 1995; Barker *et al.*, 1996). This made SnRK1 the first plant protein kinase for which biochemical and molecular biological studies came together. It had already been shown to phosphorylate the 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 (MacKintosh *et al.*, 1992) and had been purified from cauliflower (Ball *et al.*, 1994). Although it was not activated by AMP, it was found to have similar biochemical properties to those of mammalian AMPK in other respects (Ball *et al.*, 1994; MacKintosh *et al.*, 1992). It was shown to phosphorylate and inactivate a bacterially-expressed arabidopsis HMG-CoA reductase (Ball *et al.*, 1994).

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 (Fig. 2). 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.*, 1995*b*). 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 identification of peptide substrates for SnRK1 allowed SnRK1 activity to be measured using a convenient assay. Interestingly, although SnRK2 and SnRK3 might be expected to have similar substrate specificity to SnRK1, whenever SAMS or AMARA peptide kinase activity has been purified SnRK1 has accounted for most of it. A minor SAMS peptide kinase activity has been tentatively assigned to SnRK2, but has not been characterized in detail (Ball *et al.*, 1994; Barker *et al.*, 1996; Sugden *et al.*, 1999*b*). This suggests that SnRK2 and SnRK3 might require slightly different recognition sequences to SnRK1.

Regulation of SnRK1 activity

There is evidence of differential transcriptional regulation of SnRK1 gene expression. In potato, for example, the 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 evidence for post-transcriptional regulation of SnRK1 activity. SAMS peptide kinase activity in mini-tubers was found to be over 40 times higher than in mature tubers while the levels of transcript were approximately the same (Man *et al.*, 1997). This post-transcription regulation probably occurs through phosphorylation by an upstream kinase kinase. Spinach SnRK1 is regulated *in vitro* by phosphorylation on a threonine residue within the so-called T-loop that is conserved in many protein kinases (Sugden *et al.*, 1999*a*).

The exact nature of the signal that brings about changes in SnRK1 gene expression or activation state is not known. Dephosphorylation and inactivation of spinach SnRK1 has been found to be inhibited by low concentrations of 5′-AMP (Sugden *et al.*, 1999*a*). There is also evidence that SnRK1 may be inhibited by glucose-6-phosphate (Toroser *et al.*, 2000), although others have attributed the apparent inhibition of SnRK1 by glucose-6-phosphate to the presence of a contaminant (Sugden *et al.*, 1999*b*). However, these remain the only tentative links between induction or repression of SnRK1 activity and metabolic status. Further evidence that SnRK1 is involved in the control of carbon metabolism comes through the identification of its substrates and of the genes that it regulates. This has led to the hypothesis that SnRK1 is activated is response to high intracellular sucrose and/or low intracellular glucose levels (Halford and Dickinson, 2001).

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SnRK1 substrates

The first plant protein to be identified as a substrate for SnRK1 was arabidopsis HMG-CoA reductase (Dale *et al*., 1995*a*). This enzyme catalyses the NADH-dependent reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonic acid. The phosphorylation site is Ser-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. SPS is phosphorylated at Ser-158 and NR at Ser-543 (Bachmann *et al*., 1996*b*; Douglas *et al*., 1995; Su *et al*., 1996). In both cases 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*., 1996*a*; Moorhead *et al*., 1996). The sequences around both sites conform to the SnRK1 consensus recognition motif and immunological evidence confirmed that SnRK1 was one of the protein kinases responsible for these phosphorylations (Crawford *et al*., 2001; Douglas *et al*., 1997; Sugden *et al*., 1999*b*).

Clearly, if SnRK1 plays a role in controlling the activity of HMG-CoA reductase, NR and SPS, then uncoupling them from SnRK1 regulation by removing the phosphorylation site could be important in the metabolic engineering of plants with these enzymes. This might explain why the over-expression of HMG-CoA reductase in arabidopsis led to a 40-fold increase in transcript levels compared with wild-type plants, but only a 3-fold increase in HMG-CoA reductase activity (Re *et al*., 1995) and no increase in isoprenoids, although over-expression of HMG-CoA reductase in tobacco did increase enzyme activity 3–8-fold (Schaller *et al*., 1995; Chappell *et al*., 1995). Tobacco plants expressing a mutant HMG-CoA reductase gene lacking a SnRK1 target site have been produced and are being analysed (S Hey, M Beale, NG Halford, unpublished data).

HMG-CoA reductase, NR and SPS are unlikely to represent the final list of metabolic enzyme substrates for SnRK1. It is difficult to perform comprehensive database searches for the presence of the motif because different hydrophobic and basic residues are tolerated at the signature positions relative to the phosphorylated serine. However, a cursory search revealed the presence of a putative site, **Phe Arg** Gln Phe Ile **Ser** Ile Gln Cys **Val**, in an arabidopsis cyclopocyclopropane fatty acid synthase (accession No. AAL66922) and two sites, **Met** Gly **Arg** Leu Glu **Ser** Val Leu Asn **Leu** and **Ile** Phe **Lys** Gly Ile **Ser** Leu Lys Leu **Ile**, in arabidopsis trehalose 6-phosphate synthase (accession No. AAL60031). The significance of these sites would have to be tested experimentally, but there is a strong possibility that these enzymes are substrates for SnRK1.

SnRK1 regulates gene expression

One of the functions of SNF1 in yeast is the transcriptional regulation of genes encoding enzymes of carbohydrate metabolism. SnRK1 will perform this role in yeast *snf1* mutants to the extent that the yeast can utilize sucrose and non-fermentable carbon sources such as ethanol and glycerol (Alderson *et al*., 1991; Muranaka *et al*., 1994). The possibility that SnRK1 might perform a similar role in regulating gene expression in plants has been investigated by expressing an antisense potato *SnRK1* sequence in the tubers and leaves of transgenic potato (Purcell *et al*., 1998). This resulted in a reduction of up to 79% in SAMS peptide kinase activity in the tubers, confirming that SnRK1 is responsible for most if not all of the SAMS peptide kinase activity in this tissue. It also resulted in the reduction of sucrose synthase activity. Sucrose synthase gene expression was shown to have decreased dramatically in transgenic tubers and to be uninducible by sucrose in excised leaves expressing the antisense sequence. In wild-type plants, the sucrose synthase gene, *Sus4*, is expressed in tubers and is induced in excised leaves by incubation with sucrose (Fu and Park, 1995).

The role of SnRK1 in regulating the expression of other genes encoding enzymes of carbohydrate metabolism is still under investigation. For example, changes in invertase gene expression have been observed in transgenic arabidopsis plants overexpressing SnRK1 (P Lessard, M Thomas, unpublished data) and a transient expression system has been used to show that antisense SnRK1 represses α-amylase promoter activity in wheat embryos (S Laurie, RS McKibbin, NG Halford, unpublished data).

SnRK1 and development

Expression of antisense SnRK1 sequences in different plant species has had profound effects on development. For example, sprouting was found to be affected in potato tubers expressing antisense SnRK1 (RS McKibbin, NG Halford, unpublished data) and pollen development was arrested in barley plants expressing antisense SnRK1 (Zhang *et al*., 2001). The pollen grains were small, pear-shaped, contained little or no starch and were non-viable. Intriguingly, the transgene was not passed on to the T_1 generation at all, suggesting that ovule development was also affected. Pollen infertility is often associated with a lack of starch accumulation and has been suggested to be caused, in some cases, by an inability to metabolize incoming sucrose (Dorion *et al*., 1996; Sheoran and Saini, 1996; Saini, 1997). This type of male sterility in wheat and rice is associated predominantly with a decrease in acid invertase rather than sucrose synthase activity (Dorion *et al*., 1996; Sheoran and Saini, 1996) and SnRK1 has not previously been associated with the regulation of invertase activity in plants. However, antisense repression of

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extracellular invertase gene expression in tobacco anthers resulted in a similar pollen phenotype to that observed in the antisense SnRK1 transgenic barley lines (Goetz *et al.*, 2001).

According to the hypothesis that SnRK1 is activated in response to high sucrose/low glucose and causes the induction of gene expression, the pollen grains in the antisense SnRK1 plants would be unable to respond to their carbon status by expressing invertase and utilizing imported sucrose. They would starve in a similar fashion to yeast *snf1* mutants starving on sucrose medium.

Identification of SnRK1-interacting proteins

Both SNF1 and AMPK form complexes with other proteins. The SNF1 complex includes a 36 kDa protein, termed SNF4, which is a regulatory subunit required for full activity of the protein kinase (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 SNF1 complex also includes a second interacting protein from a class of proteins that comprises SIP1 (110 kDa), SIP2 (54 kDa) and GAL83 (64 kDa). These three related proteins are interchangeable in the SNF1 kinase complex and may target the complex to different substrates (Yang *et al.*, 1994). They contain two conserved domains, the ASC domain (**A**ssociation with **S**NF1 **C**omplex) (Yang *et al.*, 1994; Jiang and Carlson, 1997). and the KIS domain (**K**inase **I**nteracting **S**equence) (Jiang and Carlson, 1997). Three further interacting factors have been identified (SIP3, SIP4 and MSN3) that may couple SNF1 complexes to transcriptional regulation (Hubbard *et al.*, 1994; Lesage *et al.*, 1994, 1996). The AMPK complex includes a β-subunit (38–40 kDa), which corresponds to the yeast SIP proteins, and a γ-subunit (36–38 kDa) that is closely related to SNF4 (Woods *et al.*, 1996).

Homologues of SNF4 and the SIP1/SIP2/GAL83 family have now been identified in plants. The presence of a SNF4-like protein had been suggested by the demonstration that SnRK1 interacted with yeast SNF4 in two-hybrid experiments (Jiang and Carlson, 1997; Bouly *et al.*, 1999). Moreover, SNF4 was shown to be needed for complementation of *snf1* mutants by SnRK1 (Muranaka *et al.*, 1994). The gene, called *AtSNF4*, was finally cloned from arabidopsis by partial complementation of an *snf4* mutant (Kleinow *et al.*, 2000).

Two other families of plant proteins show 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. They do, however, share a short, hydrophobic motif, called the SnIP motif (Halford *et al.*, 2000; Slocombe *et al.*, 2002). Part of this motif (**Hyd**-Xxx-**Bas**-Xxx-Xxx-Xxx-Xxx-Xxx-Xxx-**Hyd**) bears some resemblance to the SnRK1 recognition sequence without the target serine residue, and could represent a pseudosubstrate site similar to those observed in the regulatory subunits of the cAMP-dependent kinase, PKA, of mammals (Taylor *et al.*, 1990).

Genes related to 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 and AKINγ in the two-hybrid system but also with the yeast SNF1 and SNF4 proteins (Bouly *et al.*, 1999). Potato *StubGAL83* was isolated by screening of a yeast two-hybrid cDNA library with a potato SnRK1 cDNA (Lakatos *et al.*, 1999).

Plants also contain a protein, AKINβγ, that contains an N-terminal KIS domain fused with a C-terminal domain similar to that of SNF4 and AMPK γ-subunit (Lumbreras *et al.*, 2001). This protein complements the *snf4* mutation and interacts with SnRK1 in two-hybrid assays. It appears to be unique to plants.

Two other proteins that have been found to interact with SnRK1 in two-hybrid experiments are a small heat shock protein that is a substrate, though a relatively poor one, for SnRK1 (SP Slocombe *et al.*, unpublished data) and PRL1 (Bhalerao *et al.*, 1999). In arabidopsis, mutation of *PRL1* has pleiotropic effects in sugar-, light- and stress-regulated gene expression, as well as in starch and sugar accumulation and in response to several phytohormones. Binding of PRL1 with SnRK1 was found to be enhanced in the absence of glucose and to inhibit autophosphorylation of the kinase expressed in *E. coli*. Putative SnRK1 activity was also slightly higher in a *PRL1* mutant than in the wild type (Bhalerao *et al.*, 1999), although the activity that was measured was not well characterized and was several orders of magnitude lower than activities measured in plant extracts using the SAMS or AMARA peptides as substrate.

Two-hybrid screening also identified a transcription factor that interacts with SnRK1 (S Laurie, RS McKibbin, NG Halford, unpublished data). This transcription factor is a member of the Apetela2/ethylene-responsive element binding protein family and full-length cDNAs encoding it have been cloned from barley, wheat and potato. Its function and the significance of its interaction with SnRK1 are not yet known, but its identification is a significant development given that SnRK1 has been shown to regulate gene expression.

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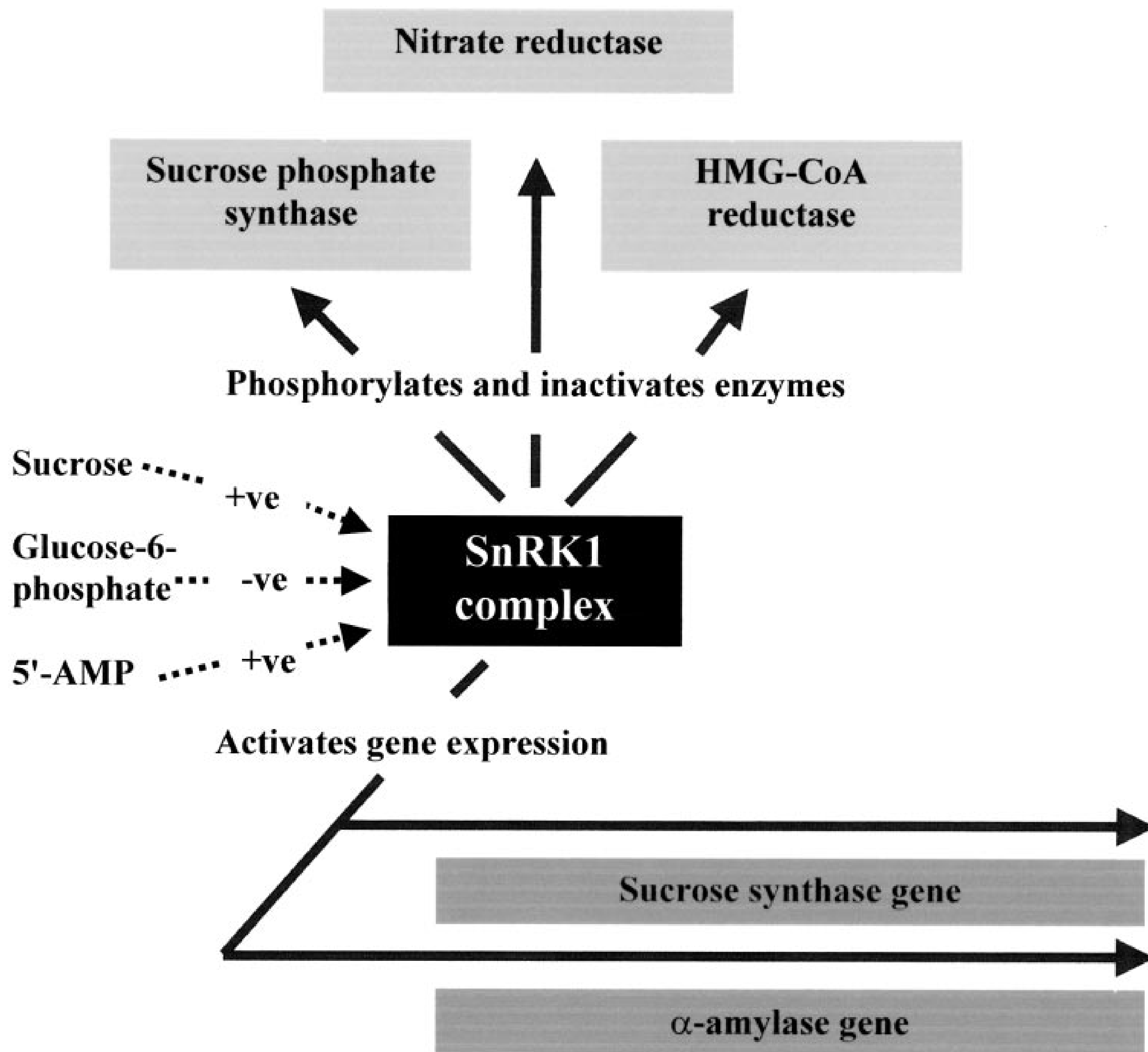

Fig. 3. SnRK1 is at the heart of the control of carbon partitioning in plants, regulating the activity of important enzymes by affecting their activation state or the activity of the genes that encode them.

Evidence that SnRK1 is a conduit for cross-talk between metabolic and cell cycle signalling

Several yeast protein kinases that are involved in cell cycle control are closely related to SNF1. They include NIM1 from fission yeast (*Schizosaccharomyces pombe*) and its budding yeast homologue NIK1 (**ni**m1-like **k**inase). NIM1 is a mitotic inducer in fission yeast that phosphorylates and thereby inactivates the cell cycle inhibitory protein kinase, WEE1 (Russell and Nurse, 1987). NIK1 is a negative regulator of SWE1 (***S**accharomyces* homologue of **we**e1) that is involved in the calcium-dependent control of mitosis (Tanaka and Nojima, 1996). Furthermore, SNF1 itself has been implicated in cell cycle control, since *snf1* mutants fail to arrest the cell cycle when they are in a state of nutrient deprivation, in other words they do not enter a normal stationary phase (Thompson-Jaeger *et al.*, 1991). Other data suggest that SNF1 is also involved in the control of the onset of meiosis (Honigberg and Lee, 1998).

Over-expression of SnRK1 in yeast resulted in a dramatic reduction in yeast cell size, suggesting that the yeast cells were completing their cell cycles too early (Dickinson *et al.*, 1999). One possible interpretation of these results is that SnRK1 was behaving like NIM1 or NIK1 in the yeast cells (over-expression of NIK1 has been shown to have a similar effect (Tanaka and Nojima, 1996)). Another interpretation is that acting as a conduit for cross-talk between metabolic and cell cycle signalling is part of the normal activity of SnRK1 and SNF1, and that this system was perturbed in the yeast cells by over-expression of the SnRK1 protein.

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Concluding remarks

The functions of SnRK1 are summarized in Fig. 3. The identification of substrates for and genes that are regulated by SnRK1 is undoubtedly far from complete. Nevertheless, SnRK1 can already be placed at the heart of the control of carbon metabolism and partitioning.

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References

Abe H, Kamiya Y, Sakurai AA. 1995. cDNA clone encoding yeast SNF4-like protein from *Phaseolus vulgaris*. *Plant Physiology* **110,** 336.

Alderson A, Sabelli PA, Dickinson JR, Cole D, Richardson M, Kreis M, Shewry PR, Halford NG. 1991. Complementation of *snf1*, a mutation affecting global regulation of carbon metabolism in yeast, by a plant protein kinase cDNA. *Proceedings of the National Academy of Sciences, USA* **88,** 8602–8605.

Anderberg RJ, Walker-Simmons MK. 1992. Isolation of a wheat cDNA clone for an abscisic acid-inducible transcript with homology to protein kinases. *Proceedings of the National Academy of Sciences, USA* **89,** 10183–10187.

Bachmann M, Huber JL, Liao PC, Gage DA, Huber SC. 1996*a*. The inhibitor protein of phosphorylated nitrate reductase from spinach (*Spinaea oleracea*) leaves is a 14-3-3 protein. *FEBS Letters* **387,** 127–131.

Bachmann M, Shiraishi N, Campbell WH, Yoo BC, Harmon AC, Huber SC. 1996*b*. Identification of Ser-543 as the major regulatory phosphorylation site in spinach leaf nitrate reductase. *The Plant Cell* **8,** 505–517.

Ball KL, Barker J, Halford NG, Hardie DG. 1995. Immunological evidence that HMG-CoA reductase kinase-A is the cauliflower homologue of the RKIN1 subfamily of plant protein kinases. *FEBS Letters* **377,** 189–192.

Ball KL, Dale S, Weekes J, Hardie DG. 1994. Biochemical characterization of two forms of 3-hydroxy-3-methylglutaryl-CoA reductase kinase from cauliflower (*Brassica oleracea*). *European Journal of Biochemistry* **219,** 743–750.

Barker JHA, Slocombe SP, Ball KL, Hardie DG, Shewry PR, Halford NG. 1996. Evidence that barley 3-hydroxy-3-methylglutaryl-Coenzyme A reductase kinase is a member of the sucrose non-fermenting-1-related protein kinase family. *Plant Physiology* **112,** 1141–1149.

Bhalerao RP, Salchert K, Bako L, Okresz L, Szabados L, Muranaka T, Machida Y, Schell J, Koncz C. 1999. Regulatory interaction of PRL1 WD protein with *Arabidopsis* SNF1-like protein kinases. *Proceedings of the National Academy of Sciences, USA* **96,** 5322–5327.

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

Carling D, Clarke PR, Zammit VA, Hardie DG. 1989. Purification and characterization of the AMP-activated protein kinase—co-purification of acetyl-CoA carboxylase and 3-hydroxy-3-methylglutaryl CoA reductase kinase activity. *European Journal of Biochemistry* **186,** 129–136.

Carling D, Zammit VA, Hardie DG. 1987. A common bicyclic protein kinase cascade inactivates the regulatory enzymes of fatty acid and cholesterol biosynthesis. *FEBS Letters* **223,** 217–222.

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

Celenza JL, Eng FJ, 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. *Molecular and Cellular Biology* **9,** 5045–5054.

Chappell J, Wolf F, Proulx J, Cuellar R, Saunders C. 1995. Is the reaction catalyzed by 3-hydroxy-3-methylglutaryl Coenzyme A reductase a rate-limiting step for isoprenoid biosynthesis in plants? *Plant Physiology* **109,** 1337–1343.

Clarke PR, Hardie DG. 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 Journal* **9,** 2439–2446.

Crawford RM, Halford NG, Hardie DG. 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 Molecular Biology* **45,** 731–741.

Dale S, Arró M, Becerra B, Morrice NG, Boronat A, Hardie DG, Ferrer A. 1995*a*. 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. *European Journal of Biochemistry* **233,** 506–513.

Dale S, Wilson WA, Edelman AM, Hardie DG. 1995*b*. 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 Letters* **361,** 191–195.

Davies SP, Carling D, Munday MR, Hardie DG. 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. *European Journal of Biochemistry* **203,** 615–623.

Davies SP, Sim ATR, Hardie DG. 1990. Location and function of three sites phosphorylated on rat acetyl-CoA carboxylase by the AMP-activated protein kinase. *European Journal of Biochemistry* **187,** 183–190.

Dickinson JR. 1999. Carbon metabolism. In: Dickinson JR, Schweizer M, eds. *The metabolism and molecular physiology of* Saccharomyces cerevisiae. London and Philadelphia PA: Taylor and Francis, 23–55.

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

Dorion S, Lalonde S, Saini HS. 1996. Induction of male sterility in wheat by meiotic-stage water deficit is preceded by a decline in invertase activity and changes in carbohydrate metabolism in anthers. *Plant Physiology* **111,** 137–145.

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

Douglas P, Pigaglio E, Ferrer A, Halford NG, 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. *Biochemical Journal* **325,** 101–109.

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

Gancedo JM. 1998. Yeast carbon catabolite repression. *Microbiology and Molecular Biology Reviews* **62,** 334–361.

Gillespie JG, Hardie DG. 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 Letters* **306,** 59–62.

Goetz M, Godt DE, Guivarc'h A, Kahmann U, Chriqui D, Roitsch T. 2001. Induction of male sterility in plants by metabolic engineering of the carbohydrate supply. *Proceedings of the National Academy of Sciences, USA* **98,** 6522–6527.

Gómez-Cadenas A, Verhey SD, Holappa LD, Shen Q, Ho T-HD, Walker-Simmons MK. 1999. An abscisic acid-induced protein kinase, PKABA1, mediates abscisic acid-suppressed gene expression in barley aleurone layers. *Proceedings of the National Academy of Sciences, USA* **96,** 1767–1772.

Halford NG, Dickinson JR. 2001. Sugar sensing and cell cycle control: evidence of cross-talk between two ancient signalling pathways. In: Francis D, ed. *The plant cell cycle and its interfaces.* Sheffield: Sheffield Academic Press, 87–107.

Halford NG, Hardie DG. 1998. SNF1-related protein kinases: global regulators of carbon metabolism in plants? *Plant Molecular Biology* **37,** 735–748.

Halford NG, Bouly J-P, Thomas M. 2000. SNF1-related protein kinases (SnRKs)—regulators at the heart of the control of carbon metabolism and partitioning. *Advances in Botanical Research Incorporating Advances in Plant Pathology* **32,** 405–434.

Halfter U, Ishitani M, Shu J-K. 2000. The arabidopsis SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein, SOS3. *Proceedings of the National Academy of Sciences, USA* **97,** 3535–3740.

Hardie DG. 2000. Plant protein serine/threonine protein kinases: classification into sub-families and overview of function. *Advances in Botanical Research Incorporating Advances in Plant Pathology* **32,** 1–44.

Hardie DG, Carling D. 1997. The AMP-activated protein kinase: fuel gauge of the mammalian cell? *European Journal of Biochemistry* **246,** 259–273.

Hardy TA, Huang D, Roach PJ. 1994. Interactions between cAMP-dependent and SNF1 protein kinases in the control of glycogen accumulation in *Saccharomyces cerevisiae*. *Journal of Biological Chemistry* **269,** 27907–27913.

Hawley SA, Davison M, Woods A, Davies SP, Beri RK, Carling D, Hardie DG. 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. *Journal of Biological Chemistry* **271,** 27879–27887.

Honigberg SM, Lee RH. 1998. SNF1 kinase connects nutritional pathways controlling meiosis in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology* **18,** 4548–4555.

Huang JZ, Huber SC. 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 and Cell Physiology* **42,** 1079–1087.

Hubbard EJ, Jiang R, Carlson M. 1994. Dosage-dependent modulation of glucose repression by MSN3 (STD1) in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology* **14,** 1972–1978.

Jiang R, Carlson M. 1996. Glucose regulates protein interactions within the yeast SNF1 protein kinase complex. *Genes and Development* **10,** 3105–3115.

Jiang R, 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. *Molecular and Cellular Biology* **17,** 2099–2106.

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

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

Lesage P, Yang X, Carlson M. 1994. Analysis of the SIP3 protein identified in a two-hybrid screen for interaction with the SNF1 protein kinase. *Nucleic Acids Research* **22,** 597–603.

Lesage P, Yang X, Carlson M. 1996. Yeast SNF1 protein kinase interacts with SIP4, a C6 zinc cluster transcriptional activator: a new role for SNF1 in the glucose response. *Molecular and Cellular Biology* **16,** 1921–1928.

Liu J, Ishitani M, Halfter U, Kim C-S, Shu J-K. 2000. The *Arabidopsis thaliana SOS2* gene encodes a protein kinase that is required for salt tolerance. *Proceedings of the National Academy of Sciences, USA* **97,** 3730–3734.

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

Mackintosh RW, Davies SP, Clarke PR, Weekes J, Gillespie JG, Gibb BJ, Hardie DG. 1992. Evidence for a protein kinase cascade in higher plants: 3-hydroxy-3-methylglutaryl-CoA reductase kinase. *European Journal of Biochemistry* **209,** 923–931.

Man AL, Purcell PC, Hannappel U, Halford NG. 1997. Potato SNF1-related protein kinase: molecular cloning, expression analysis and peptide kinase activity measurements. *Plant Molecular Biology* **34,** 31–43.

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

Muranaka T, Banno H, Machida Y. 1994. Characterization 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*. *Molecular and Cellular Biology* **14,** 2958–2965.

Purcell PC, Smith AM, Halford NG. 1998. Antisense expression of a sucrose non-fermenting-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. *The Plant Journal* **14,** 195–202.

Re EB, Jones D, Learned RM. 1995. Co-expression of native and introduced genes reveals cryptic regulation of HMG-CoA reductase expression in *Arabidopsis*. *The Plant Journal* **7,** 771–784.

Ronne H. 1995. Glucose repression in fungi. *Trends in Genetics* **11,** 12–17.

Russell P, Nurse P. 1987. The mitotic inducer nim1$^+$ functions in a regulatory network of protein kinase homologs controlling the initiation of mitosis. *Cell* **49,** 569–576.

Saini HS. 1997. Effects of water stress on male gametophyte development in plants. *Sexual Plant Reproduction* **10,** 67–73.

Schaller H, Grausem B, Benveniste P, Chye ML, Tan CT, Song CT, Chua NH. 1995. Expression of the *Hevea brasiliensis* (HBK) mull. Arg. 3-hydroxy-3-methylglutaryl-Coenzyme A reducatse-1 in tobacco results in sterol over-production. *Plant Physiology* **109,** 761–770.

Sheoran IS, Saini HS. 1996. Drought-induced male sterility in rice: changes in carbohydrate levels and enzyme activities associated

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with the inhibition of starch accumulation in pollen. *Sexual Plant Reproduction* **9,** 161–169.

Slocombe SP, Laurie S, Bertini L, Beaudoin F, Dickinson JR, Halford NG. 2002. Molecular cloning of SnIP1, a novel protein that interacts with SNF1-related protein kinase (SnRK1). *Plant Molecular Biology* **49,** 31–44.

Su W, Huber SC, Crawford NM. 1996. Identification *in vitro* of a post-translational regulatory site in the hinge 1 region of *Arabidopsis* nitrate reductase. *The Plant Cell* **8,** 519–527.

Sugden C, Crawford RM, Halford NG, Hardie DG. 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. *The Plant Journal* **19,** 433–439.

Sugden C, Donaghy P, Halford NG, Hardie DG. 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 Physiology* **120,** 257–274.

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

Taylor SS, Buechler JA, Yonemoto W. 1990. cAMP-dependent protein kinase: framework for a diverse family of regulatory enzymes. *Annual Reviews in Biochemistry* **59,** 971–1005.

Thompson-Jaeger S, Francois J, Gaughran JP, 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.

Toroser D, Plaut Z, Huber SC. 2000. Regulation of a plant SNF1-related protein kinase by glucose-6-phosphate. *Plant Physiology* **123,** 403–411.

Weekes J, Ball KL, Caudwell FB, Hardie DG. 1993. Specificity determinants for the AMP-activated protein kinase and its plant homologue analyzed using synthetic peptides. *FEBS Letters* **334,** 335–339.

Woods A, Cheung PC, Smith FC, Davison MD, Scott J, Beri RK, Carling D. 1996. Characterization of AMP-activated protein kinase beta and gamma subunits. Assembly of the heterotrimeric complex *in vitro*. *Journal of Biological Chemistry* **271,** 10282–10290.

Woods A, Munday MR, Scott J, Yang X, Carlson M, Carling D. 1994. Yeast SNF1 is functionally related to mammalian AMP-activated protein kinase and regulates acetyl-CoA carboxylase *in vivo*. *Journal of Biological Chemistry* **269,** 19509–19515.

Yang X, Jiang R, Carlson M. 1994. A family of proteins containing a conserved domain that mediates interaction with the yeast SNF1 protein kinase complex. *EMBO Journal* **13,** 5878–5886.

Zhang Y, Shewry PR, Jones H, Barcelo P, Lazzeri PA, Halford NG. 2001. Expression of antisense SnRK1 protein kinase sequence causes abnormal pollen development and male sterility in transgenic barley. *The Plant Journal* **28,** 431–442.

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