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FOCUS PAPER

Highly conserved protein kinases involved in the regulation of carbon and amino acid metabolism

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Abstract

It has been clear for over a decade and a half that ancient signalling pathways controlling fundamental cellular processes are highly conserved throughout the eukaryotes. Two plant protein kinases, sucrose non-fermenting 1 (SNF1)-related protein kinase (SnRK1) and general control non-derepressible 2 (GCN2)-related protein kinase are reviewed here. These protein kinases show an extraordinary level of conservation with their fungal and animal homologues given the span of time since they diverged from them. However, close examination of the signalling pathways in which they operate also reveals intriguing differences in activation and function.

Key words: Amino acid metabolism, carbon metabolism, plant protein kinases, regulation, signalling.

The SNF1 family of protein kinases: similarities in structure and function conserved throughout the eukaryotes

The *SNF1* (sucrose non-fermenting-1) gene of budding yeast (*Saccharomyces cerevisiae*) encodes a protein kinase (Celenza and Carlson, 1986) that is activated in response to low cellular glucose levels. It was first identified genetically in a screen of mutants that fail to express the invertase gene, *SUC2*, in response to glucose deprivation, although its functions have since been found to be far more wide-ranging. *Snf1* mutants essentially require glucose to survive and will starve on a medium containing other sugars, including sucrose, galactose, and maltose or non-fermentable carbon sources such as glycerol or ethanol. The animal homologue of SNF1 is AMP-activated protein

kinase (AMPK), while the plant homologue is SnRK1 (SNF1-related protein kinase-1).

The striking similarities between these protein kinases, will be considered first. Plants, animals and fungi are estimated to have diverged approximately 1.5 billion years ago. Yet SNF1, AMPK and SnRK1 are instantly recognizable as members of the same family. All three protein kinases are, in fact, heterotrimeric complexes (Fig. 1A). In animals these have a logical name, AMPK α , β and γ (Woods *et al.*, 1996). The 63 kDa α subunit contains the protein kinase catalytic domain in the N-terminal half and a regulatory domain in the C-terminal half that interacts with the 36–38 kDa γ subunit. The third member of the complex is the 38–40 kDa β subunit. The catalytic subunit in yeast is 72 kDa and is encoded by the *SNF1* gene itself. The regulatory subunit homologous to AMP γ is a 36 kDa protein called SNF4 (Celenza *et al.*, 1989).

The interaction between SNF1 and SNF4 appears to be regulated by glucose (Fig. 1A) and it has been proposed that SNF4 activates SNF1 by counteracting autoinhibition by the SNF1 regulatory domain (Jiang and Carlson, 1996). The third interacting protein in the SNF1 complex is one of 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 (association with SNF1 complex) (Yang *et al.*, 1994; Jiang and Carlson, 1997) and the KIS domain (kinase interacting sequence) (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).

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The plant homologue of SNF1 and AMPK α is a 58 kDa protein called SnRK1 (SNF1-related protein kinase-1). An *SnRK1* gene was cloned for the first time in 1991 (Alderson *et al.*, 1991) and homologues have since been cloned and characterized from many

plant species (reviewed by Halford and Hardie, 1998; Halford *et al.*, 2000). A SNF4/AMP γ homologue called *AtSNF4* has been cloned from arabidopsis by partial complementation of a *snf4* mutant (Kleinow *et al.*, 2000).

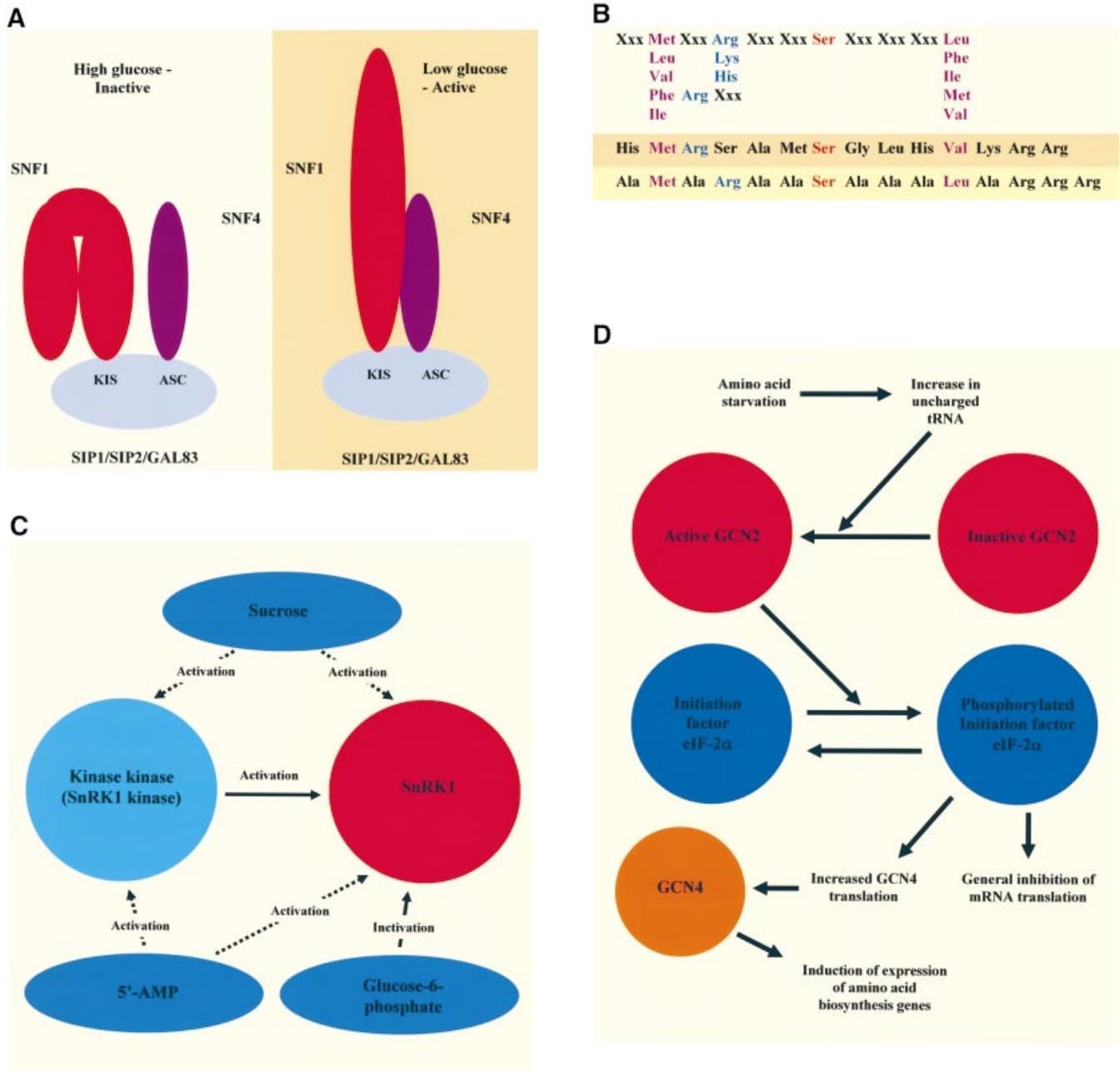


Fig. 1. (A) Cartoon showing yeast SNF1 complexes in conditions of high and low glucose. Glucose causes the catalytic subunit SNF1 and regulatory subunit SNF4 to dissociate. The regulatory domain of the catalytic subunit folds over the catalytic domain, rendering the kinase inactive. Under conditions of glucose limitation the regulatory domain of the catalytic subunit binds to SNF4 and the kinase becomes active. Homologues of all three subunits are present in animals and plants as well as fungi. (B) Phosphorylation sites for SNF1, AMPK and SnRK1. Requisite residues (coloured) are the target serine residue, hydrophobic residues at +4 and -5 with respect to the serine and a basic residue at -3 or (less preferably) at -4. The top panel shows the consensus sequence and alternatives. The middle and bottom panels show the sequences of the SAMS and AMARA peptides, respectively, that are used as standard substrates in measurements of SNF1, AMPK and SnRK1 activity. (C) Cartoon showing the known mechanisms (unbroken arrows) and inferred mechanisms (dotted arrows) for activation of SnRK1. (D) Cartoon showing the interactions of protein kinase GCN2, translation initiation factor eIF2 α and transcription factor GCN4 in general amino acid control in yeast.

Genes related to the SIP1/SIP2/GAL83/AMPK β family have been cloned from arabidopsis (*AKIN β 1* and *AKIN β 2*) and potato (*StubGAL83*) (Bouly *et al.*, 1999; Lakatos *et al.*, 1999). *AKIN β 1* and *AKIN β 2* interact with SnRK1 in the two-hybrid system, but also with the yeast SNF1 and SNF4 proteins (Bouly *et al.*, 1999). Potato *StubGAL83* was isolated by screening a yeast two-hybrid cDNA library with a potato SnRK1 cDNA (Lakatos *et al.*, 1999).

A maize homologue of AtSNF4 was given the name *AKIN β γ* because it was found to contain an N-terminal KIS domain fused with a C-terminal domain similar to SNF4, AMPK γ and AtSNF4 (Lumbreras *et al.*, 2001). Reanalysis of the arabidopsis *AtSNF4* gene shows that it too encodes a protein with this N-terminal KIS domain. The reason for this domain fusion is not clear.

The degree to which these protein kinases have been conserved is most evident in the catalytic subunit. SNF1, AMPK and SnRK1 show approximately 62% amino acid sequence identity in the kinase catalytic domain and 48% overall. There are also similarities in substrate specificity. All three recognize the target site shown in Fig. 1B (Halford and Hardie, 1998) although AMPK tolerates threonine in place of serine more readily than SNF1 or SnRK1. Peptides based on this sequence, such as the SAMS and AMARA peptides shown in Fig. 1B, are good substrates for all three protein kinases, enabling activity to be measured in a relatively easy assay (Davies *et al.*, 1989).

SNF1, AMPK and SnRK1 all act in part through the inactivating phosphorylation of biosynthetic enzymes. SnRK1, for example, phosphorylates and inactivates 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMG-CoA reductase) (sterol/isoprenoid synthesis), sucrose phosphate synthase (SPS) (sucrose synthesis) and nitrate reductase (NR) (nitrogen assimilation) *in vitro* (reviewed by Halford and Hardie, 1998). As the different systems have diverged, however, some substrates have come under their control whereas some have moved out of it. Animal HMG-CoA reductase, for example, is a substrate (reviewed by Hardie and Carling, 1997), just as the plant one is, whereas yeast HMG-CoA reductase is not. Yeast and animal acetyl Co-A carboxylases (fatty acid synthesis) are substrates whereas plant acetyl Co-A carboxylase is not. SnRK1 has also been shown to be involved in the activation of ADP-glucose pyrophosphorylase (AGPase), but through redox modulation rather than direct phosphorylation (Tiessen *et al.*, 2003).

All three protein kinases also exert their effects through the regulation of gene expression. Indeed, the classic *snf1* phenotype of inability to use carbon sources such as sucrose, maltose, galactose, ethanol, glycerol, and other non-fermentable carbon sources derives from an inability to switch on the requisite genes in response to glucose deprivation (reviewed by Dickinson, 1999), not through direct effects on the phosphorylation state of metabolic

enzymes. Clearly, transcription factors must be at the end of this branch of the signal transduction pathway and one, MIG1, has been identified as a substrate for SNF1 (Treitel *et al.*, 1998). AMPK has 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.

A role for SnRK1 in regulating gene expression was first shown by expressing an antisense *SnRK1* sequence in transgenic potato. This caused a dramatic reduction in sucrose synthase gene expression in tubers and loss of sucrose-inducibility of sucrose synthase gene expression in leaves (Purcell *et al.*, 1998). Subsequently, co-bombardment with an antisense SnRK1 gene was found to repress transient activity of an α -amylase (*α -Amy2*) gene promoter in cultured wheat embryos (Laurie *et al.*, 2003).

Intriguing differences in interacting proteins, activation and influence

The similarities in structure between the SNF1, AMPK and SnRK1 complexes have been described above. However, there is evidence that the situation in plants is somewhat more complicated than in fungal and animal systems. For example, two families of plant proteins, in addition to AtSNF4, show similarity with SNF4. These are the PV42 family, which includes PV42 from bean (*Phaseolus vulgaris*) and *AKIN γ* 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*. However, they do not complement the *snf4* mutation in yeast and are unique to plants.

Both PV42 and SnIP1 will align with SNF4 and AMPK γ , but they show little sequence similarity with each other apart from 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**) resembles 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).

SNF1, AMPK and SnRK1 also differ in their mechanisms of activation. Of the three, AMPK is probably the best understood in this respect. As its name suggests, AMPK is activated allosterically by 5'AMP (Carling *et al.*, 1987, 1989). It is also regulated by phosphorylation through the action of 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.

SNF1 activity responds sensitively to glucose levels, but the exact mechanisms involved in sensing glucose levels and initiating a signal through the SNF1 pathway are not understood. The *SNF1* gene is expressed all of the time at the same level, so regulation occurs post-transcriptionally. Glucose deprivation results in rapid phosphorylation and activation of SNF1 (Wilson *et al.*, 1996). SNF1 can be inactivated by protein phosphatases and reactivated by AMPKK, and there is evidence of the presence of an AMPKK homologue in yeast. AMP levels correlate closely with SNF1 activity, but AMP does not itself activate SNF1 allosterically in the way that it does AMPK.

SnRK1 is regulated transcriptionally and post-transcriptionally. Like AMPK and SNF1 it is activated through phosphorylation by an upstream protein kinase. It is not activated directly by AMP, but AMP does affect its phosphorylation state (Sugden *et al.*, 1999a). There is also evidence that SnRK1 is inhibited by glucose-6-phosphate (Toroser *et al.*, 2000). Furthermore, it can be inferred from the fact that it is required for sucrose synthase gene expression and AGPase redox modulation that SnRK1 responds to sucrose as well as glucose levels, since these are sucrose- (not glucose-) inducible processes. This is summarized in Fig. 1C.

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 antagonistic signals in some tissues.

The plant SnRK family has diverged and expanded

Another striking difference between animals, fungi and plants is that the family of SNF1-related protein kinases in plants has expanded and diverged into subfamilies. The SnRK1 gene family itself comprises three members in arabidopsis (Halford *et al.*, 2003a), five to ten in potato (Man *et al.*, 1997) and 10–20 in barley (Halford *et al.*, 1992). The gene family in barley and other cereals subdivides into SnRK1a and b. SnRK1a is more closely related to the homologue present in dicotyledonous plants and is expressed throughout the plant, whereas SnRK1b is unique to cereals and is expressed at highest levels in the seed (reviewed by Halford and Hardie, 1998).

The divergence does not stop there. Plants contain two other subfamilies, SnRK2 and SnRK3, that are clearly within the SNF1 family, but are significantly less similar to SNF1 and AMPK than SnRK1 is. SnRK2s and 3s have 42–45% amino acid sequence identity with SnRK1, SNF1 and AMPK in the catalytic domain. They are unique to plants and the gene families are relatively large and diverse compared with SnRK1; analysis of the arabidopsis genome sequencing project identified 10 members of the SnRK2 family and 29 members of the SnRK3 family (Halford

et al., 2003a). The family members that have been characterized (most have not) have different functions. For example, the SnRK2 subfamily 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 (Halford *et al.*, 2000; Liu *et al.*, 2000).

The use of peptide substrates for SnRK1 (Fig. 1B) allowed SnRK1 activity to be measured using a convenient assay. SnRK2 and SnRK3 might be expected to have similar substrate specificity to SnRK1. However, 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.*, 1999b). This suggests that SnRK2 and SnRK3 require different recognition sequences to SnRK1.

GCN2 (general control non-derepressible)

Amino acid starvation of yeast causes a general reduction in protein synthesis and initiates changes in expression of a huge number of genes in a process known as general amino acid control (Hinnebusch, 1992) (Fig. 1D). Fundamental to general amino acid control is the protein kinase, GCN2 (general control non-derepressible 2) (Wek *et al.*, 1989). GCN2 phosphorylates the α subunit of eukaryotic translation initiation factor-2 (eIF2 α) at serine-51 (Samuel, 1993). eIF2 can bind either GDP or GTP, but recycling of the GDP to GTP is essential for eIF2 to bind Met-tRNA to the 40S ribosomal subunit. Phosphorylation of eIF2 α inhibits this recycling, thereby decreasing the rate of protein synthesis.

A wonderfully elegant mechanism enables the expression of amino acid biosynthesis genes to be activated despite this general reduction in protein synthesis. The expression of a transcriptional activator, GCN4 (Hinnebusch, 1997), is up-regulated at the translational level through the reduced availability of amino acid-tRNA molecules. Short open reading frames at the 5' end of the GCN4 transcript that are translated under normal conditions are bypassed and translation starts from the initiation codon at the 5' end of the GCN4 coding sequence (Hinnebusch, 1992, 1994). 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.

The regulatory domain of GCN2 shows significant sequence similarity with histidyl-tRNA synthetases (Wek *et al.*, 1989) and is believed to interact with uncharged tRNA leading to activation of GCN2. Adjacent eIF2 α

kinase and histidyl tRNA synthetase domains are characteristic of GCN2-type protein kinases.

As with the SNF1 family, homologues of GCN2 have been identified in a wide range of eukaryotes, including *Drosophila melanogaster* (Santoyo *et al.*, 1997), *Neurospora crassa* (Sattlegger *et al.*, 1998) and arabidopsis (Zhang *et al.*, 2003). So the GCN2-type protein kinases are another ancient family that evolved before the divergence of plants, animals and fungi. There are also two other eIF2 α kinases that have similar catalytic domains to GCN2, but do not contain a histidyl-tRNA synthetase-like domain and respond to different stimuli. These are the haem-regulated inhibitor (HRI) that has been cloned from rabbit and rat (Chen *et al.*, 1991; Mellor *et al.*, 1994) and the double-stranded RNA-dependent kinase (PKR) that has been cloned from human (Meurs *et al.*, 1990).

The arabidopsis GCN2 homologue, AtGCN2, has been characterized only recently (Zhang *et al.*, 2003). As with the other members of the GCN2 family it includes adjacent protein kinase and histidyl tRNA synthetase-like domains and shows 45% sequence identity with GCN2 in the protein kinase domain. Expression of AtGCN2 in yeast *gcn2* mutants complements the mutation, enabling growth in the presence of sulfometuron methyl, an inhibitor of branched chain amino acid biosynthesis, and 3-aminotriazole, an inhibitor of histidine biosynthesis.

A key question is whether or not the identification of a GCN2 homologue in arabidopsis indicates that plants have a general amino acid control system similar to that of yeast. So far the evidence is conflicting. The target phosphorylation site at serine-51 of yeast eIF2 α has been shown to be conserved in eIF2 α from wheat. Yeast GCN2 will phosphorylate wheat eIF2 α at this site (Chang *et al.*, 1999, 2000) and a protein kinase activity present in wheat seedlings has been shown to do the same (Langland *et al.*, 1996). This activity appears to be PKR- rather than GCN2-like, which is confusing since *AtGCN2* is the only eIF2 α kinase gene in the arabidopsis genome. Perhaps wheat contains a PKR-like activity that arabidopsis lacks, but this requires further investigation.

Further evidence supporting the hypothesis that plants have a system of general amino acid control comes from experiments showing co-ordinated regulation of genes encoding enzymes of amino acid biosynthesis. For example, arabidopsis genes encoding tryptophan biosynthesis pathway enzymes have been shown to be induced by amino acid starvation caused by glyphosate and other treatments (Zhao *et al.*, 1998). Furthermore, blocking histidine biosynthesis in arabidopsis with a specific inhibitor, IRL 1803, has been shown to increase the expression of eight genes involved in the synthesis of aromatic amino acids, histidine, lysine and purines (Guyer *et al.*, 1995). However, this study also revealed a difference between the yeast and plant systems in that

starvation for aromatic or branched-chain amino acids did not initiate a general response. The question of general amino acid control in plants has also been addressed by measuring amino acid levels in wheat, potato and barley leaves taken from plants that were grown under different photosynthetic conditions (Noctor *et al.*, 2002). Linear relationships were observed between the contents of most minor amino acids, consistent with (but not proving) the existence of a system that regulates expression of key enzymes co-ordinately.

Perhaps the main reason for doubt over the degree of conservation between yeast and plant amino acid control systems is the lack of a clear candidate for the role of GCN4 in plants. There is a database entry describing a GCN4-complementing gene called *GCPI* from arabidopsis (accession number AJ130878), but, to our knowledge, a paper describing this gene has not been published.

C/N interactions: cross-talk between carbon and amino acid signalling pathways

It seems 'obvious' that signalling pathways controlling carbon and amino acid metabolism should cross-talk, since amino acids are based on carbon skeletons. Indeed, evidence that sucrose feeding causes an increase in the rate of nitrogen assimilation and amino acid synthesis in tobacco leaves has already been reported (Morcuende *et al.*, 1998). These effects resulted from an increase in nitrate reductase (NR) activation (though not activity) and activation of amino acid biosynthesis pathways.

NR, of course, assimilates the nitrogen used for amino acid biosynthesis and is a substrate for SnRK1 at least *in vitro* (note that unlike sucrose phosphate synthase and HMG-CoA reductase, inactivation of NR by SnRK1 requires the binding of a 14-3-3 protein to the phosphorylation site). Regulation of NR is complex and undoubtedly responds to nitrogen, in addition to carbon availability, as well as other signals. Indeed, SnRK1 is one of several protein kinases that phosphorylate NR (Douglas *et al.*, 1997). Nevertheless, this is one possible conduit through which the SnRK1 signalling pathway could regulate nitrogen assimilation and thereby amino acid biosynthesis.

Another potential route by which carbon availability could influence amino acid biosynthesis is through GCN2 and GCN4. In yeast, glucose limitation has been shown to induce GCN4 through the action of GCN2, but independently of the amino acid deprivation response (Yang *et al.*, 2000). However, investigations into GCN2 activation and function in plants have only just begun and there is no evidence so far that plant GCN2 is activated in response to glucose limitation.

The importance of studying SnRK1 and GCN2 in plants

SNF1 and GCN2 signalling pathways in fungal and animal systems have attracted a lot of attention in the last decade and a half. Both have wide-ranging and profound effects on metabolism. Understanding how AMPK is activated and functions has implications for human health because AMPK is involved in the regulation of insulin action and secretion, type 2 diabetes mellitus and obesity. Mutations in AMPK are associated with a severe heart defect (hypertrophy and arrhythmia).

Studies of SnRK1 and GCN2 in plants lag well behind parallel studies in fungal and animal systems. The same, of course, is true for most studies involving genes that are not unique to plants. So is it necessary to study these genes and their associated signalling pathways in plants at all? We submit that it is. The differences between the plant systems and their yeast and animal counterparts that have been described are reason enough. In addition, we cite the profound effects on plant development that result from genetic manipulation of SnRK1 activity. For example, antisense SnRK1 potato tubers do not sprout at all if kept at 5 °C (Halford *et al.*, 2003b), possibly because mobilization of stored starch to support sprouting is impaired. Furthermore, 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. It is possible that they are unable to respond to their carbon status and starve in a similar fashion to yeast *snf1* mutants starving on sucrose medium. However, it would have been impossible to predict that this would happen without doing the experiment.

The continuing elucidation of SnRK1 and plant GCN2, their modes of action and the signalling pathways that they operate in will allow the design of more sophisticated and strategic modifications of their activity. Potentially, this could be used to affect carbohydrate metabolism, secondary metabolism, protein synthesis, nitrogen use efficiency and the partitioning of resources between and within different crop organs.

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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.
- 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.
- Bouly JP, Gissot L, Lessard P, Kreis M, Thomas M. 1999. *Arabidopsis thaliana* homologues of the yeast SIP and SNF4 proteins interact with AKIN α 1, a 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.
- Chang L-Y, Yang WY, Browning K, Roth D. 1999. Specific *in vitro* phosphorylation of plant eIF2 α by eukaryotic eIF2 α kinases. *Plant Molecular Biology* **41**, 363–370.
- Chang L-Y, Yang WY, Roth D. 2000. Functional complementation by wheat eIF2 α in the yeast GCN2-mediated pathway. *Biochemical and Biophysical Research Communications* **279**, 468–474.
- Chen J-J, Throop MS, Gehrke L, Kuo I, Pal JK, Brodsky M, London IM. 1991. Cloning of the cDNA of the heme-regulated eukaryotic initiation factor-2 alpha (eIF2 α) kinase of rabbit reticulocytes: homology to yeast GCN2 protein kinase and human double-stranded RNA-dependent eIF2 α kinase. *Proceedings of the National Academy of Sciences, USA* **88**, 7729–7733.
- Davies SP, Carling D, Hardie DG. 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. *European Journal of Biochemistry* **186**, 123–128.
- Dickinson JR. 1999. Carbon metabolism. In: Dickinson JR, Schweizer M, eds. *The metabolism and molecular physiology of Saccharomyces cerevisiae*. London, Philadelphia, PA: Taylor and Francis: 23–55.
- 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 Ca²⁺ ions. *Biochemical Journal* **325**, 101–109.
- 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.
- Guyer D, Patton D, Ward E.** 1995. Evidence for cross-pathway regulation of metabolic gene expression in plants. *Proceedings of the National Academy of Sciences, USA* **92**, 4997–5000.
- 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.
- Halford NG, Hardie DG.** 1998. SNF1-related protein kinases: global regulators of carbon metabolism in plants? *Plant Molecular Biology* **37**, 735–748.
- Halford NG, Hey S, Jhurreea D, Laurie S, McKibbin RS, Paul MJ, Zhang Y.** 2003a. Metabolic signalling and carbon partitioning: role of Snf1-related (SnRK1) protein kinase. *Journal of Experimental Botany* **54**, 467–475.
- Halford NG, Hey S, Jhurreea D, Laurie S, McKibbin RS, Zhang Y, Paul M.** 2003b. Dissection and manipulation of metabolic signalling pathways. *Annals of Applied Biology* **142**, 25–31.
- Halford NG, Vicente-Carbajosa J, Sabelli PA, Shewry PR, Hannappel U, Kreis M.** 1992. Molecular analyses of a barley multigene family homologous to the yeast protein kinase gene *SNF1*. *The Plant Journal* **2**, 791–797.
- 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, Carling D.** 1997. The AMP-activated protein kinase: fuel gauge of the mammalian cell? *European Journal of Biochemistry* **246**, 259–273.
- 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.
- Hinnebusch AG.** 1992. General and pathway-specific regulatory mechanisms controlling the synthesis of amino acid biosynthetic enzymes in *Saccharomyces cerevisiae*. In: Jones EW, Pringle JR, Broach JB, eds. *Molecular and cellular biology of the yeast Saccharomyces*, Vol. 2. *Gene expression*. New York: Cold Spring Harbor Laboratory Press, 319–414.
- Hinnebusch AG.** 1994. Translational control of GCN4: an *in vivo* barometer of initiation factor activity. *Trends in Biochemical Sciences* **19**, 409–414.
- Hinnebusch AG.** 1997. Translational regulation of yeast GCN4: a window on factors that control initiator-tRNA binding to the ribosome. *Journal of Biological Chemistry* **272**, 21661–21664.
- 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.
- Langland JO, Langland L, Zeman C, Saha D, Roth DA.** 1996. Phosphorylation of plant eukaryotic initiation factor-2 by the plant encoded double-stranded RNA-dependent protein kinase, pPKR, and inhibition of protein synthesis *in vitro*. *Journal of Biological Chemistry* **271**, 4539–4544.
- Laurie S, McKibbin RS, Halford NG.** 2003. Antisense SNF1-related (SnRK1) protein kinase gene represses transient activity of an α -amylase (α -Amy2) gene promoter in cultured wheat embryos. *Journal of Experimental Botany* **54**, 739–747.
- Leclerc I, Kahn A, Doiron B.** 1998. The 5'-AMP-activated protein kinase inhibits the transcriptional stimulation by glucose in liver cells, acting through the glucose response complex. *FEBS Letters* **431**, 180–184.
- 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.
- 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.
- Mellor H, Flowers KM, Kimball SR, Jefferson LS.** 1994. Cloning and characterization of cDNA encoding rat hemin-sensitive initiation factor-2 alpha (eIF2 α) kinase: evidence for multi-tissue expression. *Journal of Biological Chemistry* **269**, 10201–10204.
- Meurs E, Chong K, Galabru J, Thomas NSB, Kerr IM, Williams BRG, Hovanessian AG.** 1990. Molecular cloning and characterization of the human double stranded RNA-activated protein kinase induced by interferon. *Cell* **62**, 379–390.
- Morcuende R, Krapp A, Hurry V, Stitt M.** 1998. Sucrose feeding leads to increased rates of nitrate assimilation, increased rates of α -oxoglutarate synthesis, and increased synthesis of a wide spectrum of amino acids in tobacco leaves. *Planta* **206**, 394–409.
- Natarajan K, Meyer MR, Jackson BM, Slade D, Roberts C, Hinnebusch AG, Marton MJ.** 2001. Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during amino acid starvation in yeast. *Molecular and Cellular Biology* **21**, 4347–4368.
- Noctor G, Novitskaya L, Lea PJ, Foyer CH.** 2002. Co-ordination of leaf minor amino acid contents in crop species: significance and interpretation. *Journal of Experimental Botany* **53**, 939–945.
- 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.
- Samuel CE.** 1993. The eIF2 α protein kinases, regulators of translation in eukaryotes from yeasts to humans. *Journal of Biological Chemistry* **268**, 7603–7608.
- Santoyo J, Alcalde J, Mendez R, Pulido D, de Haro C.** 1997. Cloning and characterization of a cDNA encoding a protein synthesis initiation factor-2 alpha (eIF2 α) kinase from

- Drosophila melanogaster*: homology to yeast GCN2 protein kinase. *Journal of Biological Chemistry* **272**, 12544–12550.
- Sattlegger E, Hinnebusch AG, Barthelmess IB.** 1998. cpc-3, the *Neurospora crassa* homologue of yeast GCN2, encodes a polypeptide with juxtaposed eIF2 α kinase and histidyl-tRNA synthetase-related domains required for general amino acid control. *Journal of Biological Chemistry* **273**, 20404–20416.
- 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.
- 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.
- 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.
- Tiessen A, Prescha K, Branscheid A, Palacios N, McKibbin R, Halford NG, Geigenberger P.** 2003. Evidence that SNF1 related kinase and hexokinase are involved in separate sugar signalling pathways modulating post-translational redox activation of ADP-glucose pyrophosphorylase in potato tubers. *The Plant Journal* **35**, 490–500.
- Toroser D, Plaut Z, Huber SC.** 2000. Regulation of a plant SNF1-related protein kinase by glucose-6-phosphate. *Plant Physiology* **123**, 403–411.
- Treitel MA, Kuchin S, Carlson M.** 1998. Snf1 protein kinase regulates phosphorylation of the mig1 repressor in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology* **18**, 6273–6280.
- Wek RC, Jackson BM, Hinnebusch AG.** 1989. Juxtaposition of domains homologous to protein kinases and histidyl transfer RNA synthetases in GCN2 protein suggests a mechanism for coupling GCN4 expression to amino acid availability. *Proceedings of the National Academy of Sciences, USA* **86**, 4579–4583.
- Wilson WA, Hawley SA, Hardie DG.** 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. *Current Biology* **6**, 1426–1434.
- Woods A, Azzout-Marniche D, Foretz M, Stein SC, Lemarchand P, Ferre P, Fougere F, 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. *Molecular and Cellular Biology* **20**, 6704–6711.
- 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.
- 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.
- Yang RJ, Wek SA, Wek RC.** 2000. Glucose limitation induces GCN4 translation by activation of gcn2 protein kinase. *Molecular and Cellular Biology* **20**, 2706–2717.
- Zhang Y, Dickinson JR, Paul MJ, Halford NG.** 2003. Molecular cloning of an arabidopsis homologue of GCN2, a protein kinase involved in co-ordinated response to amino acid starvation. *Planta* **217**, 668–675.
- 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.
- Zhao J, Williams CC, Last RL.** 1998. Induction of *Arabidopsis* tryptophan pathway enzymes and camalexin by amino acid starvation, oxidative stress, and an abiotic elicitor. *The Plant Cell* **10**, 359–370.