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SNF1/AMPK pathways in yeast

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TABLE OF CONTENTS

1. ABSTRACT

The SNF1/AMPK family of protein kinases is highly conserved in eukaryotes and is required for energy homeostasis in mammals, plants, and fungi. SNF1 protein kinase was initially identified by genetic analysis in the budding yeast *Saccharomyces cerevisiae*. SNF1 is required primarily for the adaptation of yeast cells to glucose limitation and for growth on carbon sources that are less preferred than glucose, but is also involved in responses to other environmental stresses. SNF1 regulates transcription of a large set of genes, modifies the activity of metabolic enzymes, and controls various nutrient-responsive cellular developmental processes. Like AMPK, SNF1 protein kinase is heterotrimeric. It is phosphorylated and activated by the upstream kinases Sak1, Tos3, and Elm1 and is inactivated by the Reg1-Glc7 protein phosphatase 1. Further regulation of SNF1 is achieved through autoinhibition and through control of its subcellular localization. Here we review the current understanding of SNF1 protein kinase pathways in *Saccharomyces cerevisiae* and other yeasts.

2. INTRODUCTION

SNF1 protein kinase, a founding member of the SNF1/AMPK family, was identified genetically in *Saccharomyces cerevisiae* in 1981 when the *snf1* mutation was recovered in a search for mutants unable to utilize sucrose (*snf*, *s*ucrose-*n*on*f*ermenting) (1). The mutant had defects in expression of the *SUC2* (invertase) gene (2), and *snf1* was later recognized as allelic to *ccr1* and *cat1* (3, 4). The *SNF1* gene was cloned and found to encode the catalytic subunit of a protein-serine/threonine kinase (5, 6). Subsequent studies identified the noncatalytic beta subunits (7-9) and gamma subunit (10, 11). We will refer to the heterotrimeric kinase as SNF1 and to the catalytic subunit as Snf1. In 1994, a cDNA encoding an AMPK catalytic subunit was cloned and was identified as the mammalian ortholog of Snf1 (12-14). This finding resulted in the convergence of yeast and mammalian studies.

SNF1 is required for the yeast cell to adapt to glucose limitation and to utilize alternate carbon sources that are less preferred than glucose, such as sucrose,

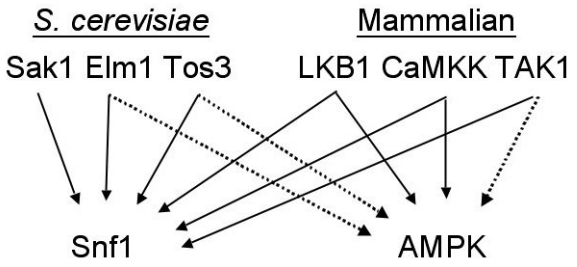

Figure 1. Conservation of SNF1/AMPK kinase cascades. Solid arrows indicate activation *in vivo* and *in vitro*. Dotted arrows indicate activation *in vitro*. Evidence suggests that TAK1 also activates AMPK *in vivo* (45, 127).

galactose, and ethanol (15, 16). SNF1 also has roles in various nutrient-responsive, cellular developmental processes, including meiosis and sporulation (1, 17), aging (18), haploid invasive growth (19), and diploid pseudohyphal growth (20). In addition to its primary role in responses to nutrient stress, SNF1 is involved in the cellular responses to other environmental stresses, including sodium ion stress, heat shock, alkaline pH, oxidative stress, and genotoxic stress (21-26). SNF1 affects cellular regulatory processes through a variety of mechanisms, including a major role in control of the genomic transcriptional program and direct effects on the activity of metabolic enzymes.

SNF1 is activated by glucose limitation (23, 27-29) and other stresses (26). SNF1 catalytic activity is regulated by three upstream kinases, Sak1, Elm1, and Tos3 (30-32), by the Reg1-Glc7 protein phosphatase 1 (33, 34), and by autoinhibition (10, 28). In addition, the subcellular localization of SNF1 is highly regulated (35-37).

The SNF1/AMPK pathway is remarkably conserved among eukaryotes. Upstream kinases were first identified in yeast (30-32), and Tos3 was shown to also activate AMPK *in vitro* (30). The catalytic domains of the three yeast kinases are similar to those of Ca^{2+}/calmodulin-dependent protein kinase kinases (CaMKKs) and the tumor suppressor kinase LKB1. This similarity led to the identification of LKB1 as an AMPK-activating kinase (30) and its verification as an authentic upstream kinase in mammalian cells (38-40). Both LKB1 and CaMKK alpha and beta, which were identified subsequently as AMPK kinases (41-43), also function in yeast cells as Snf1-activating kinases (41, 44) (Figure 1). This conserved cross-species functionality has been further exploited to identify mammalian transforming growth factor-beta activated protein kinase (TAK1) as a Snf1-activating kinase and putative AMPK kinase (45).

SNF1/AMPK pathways have also been characterized in other yeasts. In the aerobic yeast *Kluyveromyces lactis*, the homologous KlSnf1 (Fog2) pathway has roles in glucose signaling and carbon source utilization (46, 47). In the pathogenic yeasts *Candida albicans* and *Candida tropicalis*, CaSnf1 is essential for viability (48, 49). The pathway has been less well characterized with respect to function in the fission yeast *Schizosaccharomyces pombe,* but a crystal structure has been determined for a partial heterotrimeric AMPK lacking the catalytic domain (50).

3. SUBUNIT STRUCTURE

Like AMPK, the yeast orthologs are heterotrimeric. The *S. cerevisia*e genome encodes the catalytic alpha subunit Snf1, three alternate beta subunits, Sip1, Sip2 and Gal83, and the gamma subunit Snf4. There are therefore three alternate SNF1 complexes.

3.1. Snf1 catalytic subunit

The Snf1 subunit is a constitutively expressed 633 amino acid protein comprising a kinase domain near the N terminus and a C-terminal regulatory region (5, 6, 51) (Figure 2). Deletion of the C-terminal region bypasses the requirement for Snf4, suggesting that Snf4 antagonizes autoinhibition of the kinase domain by the C terminus (10, 28). The C-terminal region interacts directly with both Snf4 and the kinase domain, and deletion analysis identified residues 392 to 495 of Snf1 as sufficient for interaction with Snf4, and residues 392 to 518 for interaction with the kinase domain (28). Within this overlapping region, alteration of Leu470 to Ser abolishes interaction with Snf4 but maintains interaction with the kinase domain (28). The autoinhibitory sequence (AIS) was further defined by evidence that deletion of residues 381 to 488 bypasses the requirement for Snf4, whereas deletion of residues 381 to 414, which includes an autoinhibitory motif identified in AMPK (52), has a partial effect (53). Alterations in the kinase domain of Gly53 to Arg (10, 54) and Leu183 to Ile (53) partially alleviate the requirement for Snf4. The C-terminal region also interacts with the beta subunits, and deletion analysis identified the C-terminal residues 515 to 633 of Snf1 as sufficient for interaction with Sip2 (55).

Activation of the Snf1 catalytic subunit requires phosphorylation of Thr210 in the activation-loop segment (23, 54), and alteration of Thr210 to Ala, Glu, or Asp results in an inactive kinase (54, 56). Upstream kinases (30, 32) and Reg1-Glc7 protein phosphatase 1 (23, 33, 34) control Thr210 phosphorylation (see section 4). Neither the Snf1 C-terminal region, Snf4, nor the beta subunit is required for phosphorylation of Thr210 or its regulation by glucose signals (23, 30, 57, 58), consistent with genetic evidence regarding function of the truncated Snf1 kinase domain (10, 56).

The crystal structure of the kinase domain of Snf1 has been determined and revealed a dimer in which the activation loop Thr210 is buried (59, 60). Full-length Snf1 was also shown to self-associate in coimmunoprecipitation experiments (60). The crystal structure of a partial trimeric *S. pombe* AMPK, lacking the kinase domain, also revealed a dimer (50). The biological significance of dimerization remains uncertain.

3.2. Beta subunits

The *S. cerevisiae* genome encodes three beta subunits, Sip1, Sip2, and Gal83 (Figure 2). Sip1 and Sip2

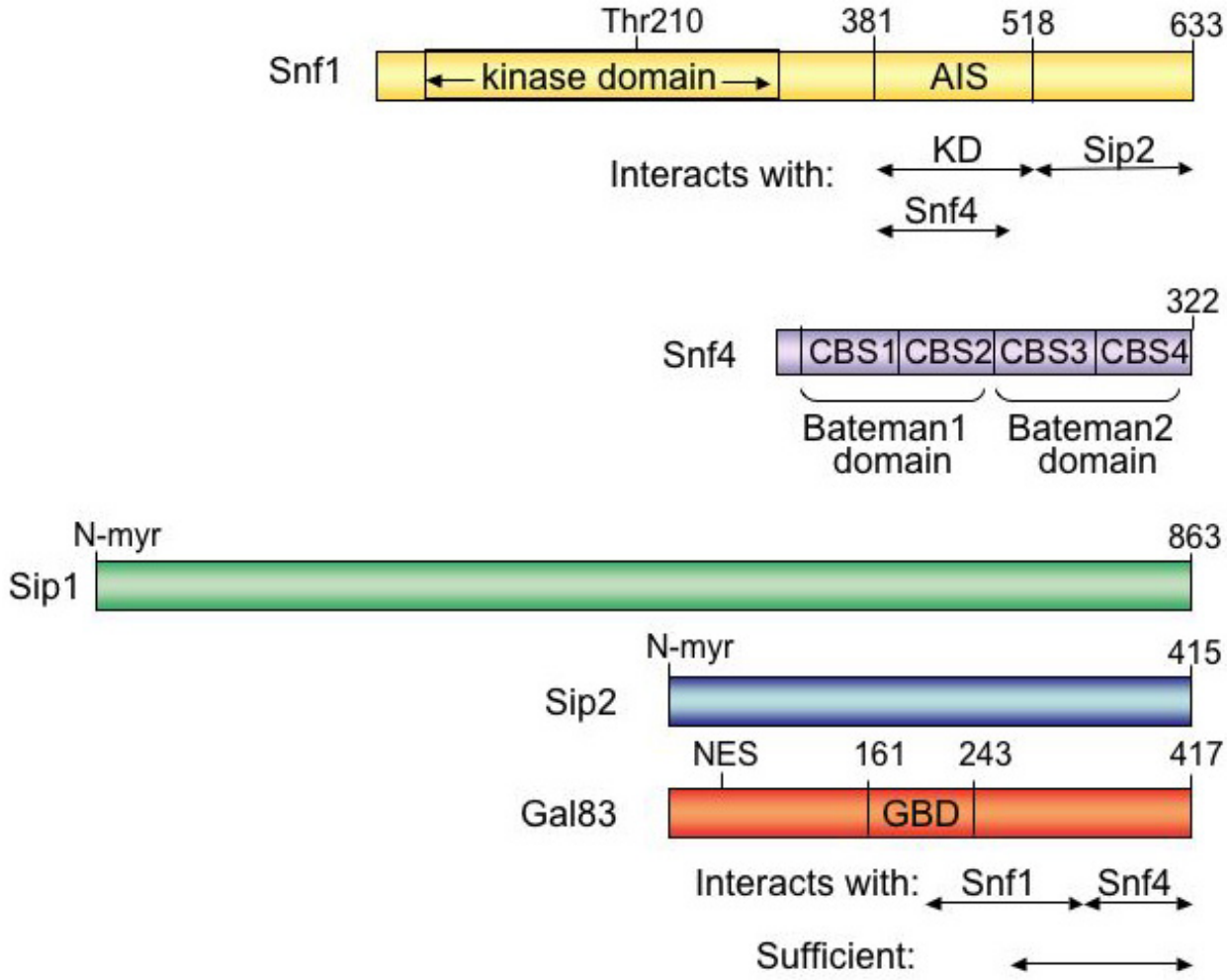

Figure 2. Structure of SNF1 subunits. Numbers, amino acid residues. Arrows, regions mapped by deletion analysis as sufficient for interaction with kinase domain (KD), Snf1, Snf4, or Sip2, as indicated. AIS, autoinhibitory sequence; CBS, cystathionine-beta-synthase repeat; N-myr, N-myristoylation consensus sequence; N ES, nuclear export signal; GBD, glycogen binding domain.

were found in a two-hybrid screen as *S*nf1-*i*nteracting *p*roteins (7, 8). Gal83 was originally named for its involvement in regulation of *GAL* genes (61) and was found to have sequence similarity with Sip2 (9). The beta subunits exhibit considerable functional overlap as each one alone is sufficient for growth under various conditions where SNF1 activity is required (62). The beta subunits contain conserved C-terminal sequences that mediate their interaction with the SNF1 complex (8). Deletion analysis identified residues 198 to 350 of Gal83 and residues 154 to 335 of Sip2 as sufficient for interaction with Snf1 (55). It is now apparent that this region, formerly designated the kinase-interacting sequence (KIS), includes part of the glycogen-binding domain (GBD, see below), as well as Snf1-interacting sequences (Figure 2). Residues 771 to 863 of Sip1 and residues 332-415 of Sip2 (designated the ASC domain, for *a*ssociation with *S*NF1 kinase *c*omplex) suffice for interaction with Snf4 (8, 55). Consistent with these results, the C-terminal 139 residues of Gal83 (distal to residue 278) are sufficient for beta subunit function (62).

The sequence of the GBD in the AMPK beta1 subunit is conserved in Gal83 (residues 161-243) and Sip2 (residues 163-245), and Gal83 binds glycogen strongly *in vitro*, whereas Sip2 binds very weakly (63). Alteration of Gal83 residues Trp184 to Ala together with Arg214 to Gln, which abolish glycogen-binding of AMPK beta1, similarly abolished glycogen binding of Gal83, as did the alteration Gly235 to Arg (63), which confers partial glucose-insensitivity to *GAL* gene expression (9, 61) and function of the Sip4 transcriptional activator (64). In cells, both mutant Gal83 proteins caused upregulation of various SNF1-dependent processes, including glycogen accumulation, expression of RNAs encoding glycogen synthase, haploid invasive growth, the transcriptional activator function of Sip4, and activation of the carbon source-responsive promoter element (63). Moreover, the GBD mutations

conferred phenotypes even in the absence of glycogen, in a mutant strain lacking glycogen synthase, indicating that they positively affect SNF1 function by a mechanism that is independent of glycogen binding.

The beta subunits have divergent N termini that confer unique subcellular localization patterns (see section 5). All three are cytoplasmic in conditions of abundant glucose. Upon glucose depletion, Sip1 relocalizes to the vacuolar membrane, Gal83 relocalizes to the nucleus, and Sip2 remains cytoplasmic (35). The beta subunits direct the localization of the Snf1 subunit (35-37).

The abundance of the beta subunits is differently regulated by glucose availability. Gal83 is the major isoform during growth on glucose, and levels of Sip2 increase during shifts to nonfermentable carbon sources. Sip1 is less abundant than either Gal83 or Sip2, and its level remains constant (35, 65). All three isoforms of SNF1 are equally active (66), but consistent with its greater abundance, the Gal83 isoform contributes the most to SNF1 activity in response to glucose limitation (37). Sip2 is N-myristoylated (67), and Sip1 contains an N-myristoylation consensus sequence that is required for its localization to the vacuolar membrane (36).

Although the beta subunits have overlapping functions, they also exhibit various differences. All the beta subunits have distinct roles in haploid invasive growth (68). Sip2 has been implicated in aging (18, 69). Gal83 mediates the interaction of Snf1 with the transcriptional activator Sip4 (62, 64), and possibly with the transcriptional apparatus (70). The different beta subunits also display stress-dependent preferences for activation by Sak1, Tos3, and Elm1 (71).

There is conservation of beta subunits among different yeast species. *K. lactis* contains only one beta subunit, KlGal83 (Fog1), which has the strongest sequence similarity to Gal83 (46). *C. albicans* has two beta subunits, one of which is N-myristoylated (72). *S. pombe* has a beta subunit that is most similar to Sip2. In *S. pombe*, the crystal structure of the partial trimer shows extensive interactions between the alpha and beta subunits, and the beta subunit contains a highly mobile polar loop at residues 244-255 that covers the pore in the gamma subunit where AMP and ATP bind; the sequence corresponding to this loop is not conserved in *S. cerevisiae* (50).

3.3. Snf4 subunit

The gene encoding the *S. cerevisiae* gamma subunit, *SNF4*, was identified by isolation of a sucrose-nonfermenting mutant (73) and is allelic to *CAT3* (74). Snf4 is a constitutively expressed protein of 322 residues that binds the Snf1 and beta subunits, independent of glucose availability (10, 11, 55). Snf4 contains two pairs of cystathionine-beta-synthase (CBS) repeats (75), called Bateman domains (Figure 2), which in various proteins have been shown to bind adenosine derivatives (76, 77). Unlike AMPK, SNF1 is not allosterically activated by AMP *in vitro* (14, 27, 29, 76), and Snf4 has a substitution at a residue that is important for the AMP-dependence of AMPK (76). The crystal structure of a dimer of the C-terminal Snf4 Bateman domain has been determined and a possible binding pocket was identified (59). This structure is similar to that of the *S. pombe* gamma subunit, which binds a single molecule of AMP or ATP in a hydrophobic cleft between CBS repeats 3 and 4 (50). The surface charge of the gamma subunit changes in response to nucleotide binding, suggesting that binding could affect interactions between subunits and thereby regulate catalytic activity (50). It remains to be determined whether *S. pombe* AMPK is regulated by nucleotides and whether Snf4 binds a ligand.

Snf4 is required for catalytic activity of the heterotrimeric kinase *in vivo* and *in vitro* (10, 11, 27). Snf4 binds to the C-terminal regulatory region of Snf1, and genetic studies showed that when this region is truncated, Snf4 is not required for the glucose-regulated function of the remaining Snf1 catalytic domain; these findings indicate that Snf4 counteracts autoinhibition by the AIS (10, 28, 53). The simple model is that Snf4 binds to the AIS when cells are limited for glucose and prevents binding of the AIS to the kinase domain. Consistent with this view, the interaction of overexpressed Snf1 and Snf4 hybrid proteins in the two-hybrid system was strongly inhibited by glucose (28); this inhibition could also reflect, at least in part, glucose inhibition of the nuclear localization of Snf1 (35) as two-hybrid interaction is assayed by *lacZ* reporter expression. Analysis of mutants lacking Snf4 indicated that it is not required for glucose-regulated phosphorylation of full-length Snf1 on its activation loop Thr210 (23, 58). At least one glucose regulatory mechanism is therefore independent of Snf4. However, recent studies showed that alteration of various residues of Snf4 relieved glucose inhibition of Thr210 phosphorylation and SNF1 activity (S.H. Iram and M.C., unpublished results). Thus, although in the absence of Snf4, phosphorylation of Snf1 Thr210 is glucose regulated, in the context of the SNF1 heterotrimer, Snf4 is required for regulated phosphorylation. These findings indicate that Snf4 has roles in regulating both autoinhibition and the functional interactions of SNF1 with upstream kinases and/or protein phosphatase in response to glucose availability. Finally, it has recently been reported that Snf4 contains a pseudosubstrate sequence that is recognized by Snf1, suggesting that Snf4 also directly inhibits Snf1 (78).

Evidence suggests that a subpopulation of Snf4 is not associated with Snf1. Snf4 is constitutively localized to both the nucleus and the cytoplasm, whereas Snf1 and the beta subunits are excluded from the nucleus during growth in high glucose (35) (see section 5). Ghaemmaghami *et al* reported a 20-fold excess of Snf4 molecules in the cell relative to other subunits (65), but others have found that Snf1 and Snf4 are present at similar levels (35) and that most, although not all, Snf4 is in complex with Snf1 (58). These observations raise the possibility that Snf4 may have additional functions in the cell independent of its functions in SNF1 protein kinase.

In this regard, it is interesting that Snf4 contains a consensus elongin C binding site and binds to the yeast

elongin C, the Elc1 protein (79). Elc1 is a homolog of Skp1, of the Skp1-Cullin-F-box ubiquitin ligase. Mutation of Cys136 in the consensus elongin C-binding sequence relieved glucose inhibition of SNF1 protein kinase; however, deletion of the *ELC1* gene did not affect SNF1 catalytic activity (S.H. Iram and M.C., unpublished results). The *elc1* mutant displayed no SNF1-related growth defect (79). These results do not support a regulatory role for Elc1 in the SNF1 pathway, but it remains possible that Elc1 affects an unidentified function of Snf4.

4. REGULATION OF SNF1 CATALYTIC ACTIVITY

SNF1 is phosphorylated and activated in response to glucose limitation (23, 27-29), in accord with its central role in adaptation to glucose depletion and utilization of alternate carbon sources, but the signal(s) regulating SNF1 has not yet been identified. As mentioned above, SNF1 is not allosterically activated by AMP (14, 27, 29, 76), but activation correlates with increased cellular AMP:ATP ratios (29). It is important to note that responses are often transient. For example, SNF1 activity is rapidly elevated when cells are transferred from medium containing glucose to sucrose, but after adaptation, minimal SNF1 activity is required for continued growth (80) and, correspondingly, the *snf1* mutant exhibits a severe growth defect on sucrose only under nonrespiratory conditions.

Snf1 is also phosphorylated and activated in response to other environmental stresses including sodium ion stress, alkaline pH, oxidative stress, but not treatment with sorbitol or heat shock (26). Some of these responses are also transient; for example, Snf1 is rapidly and strongly activated in response to alkaline stress, but activity returns to basal levels within 1 hr (26). Additionally, Snf1 Thr210 phosphorylation occurs in response to nitrogen limitation, and also to the addition of rapamycin, which implicates the target of rapamycin (TOR) kinase as a negative regulator (81).

The roles of the Snf1-activating kinases and protein phosphatase in regulating phosphorylation of Thr210 are discussed below. It is not yet clear whether the activity of these upstream kinases or the phosphatase, or both, is regulated. Alternatively, signals may be received directly by the kinase complex and affect its accessibility to upstream kinases or phosphatase. Autoinhibition is also an important control point, involving alternate binding of the AIS to the Snf1 kinase domain or to Snf4, as described in section 3.

4.1. Upstream kinases Sak1, Elm1, and Tos3

Three protein kinases with related kinase domains, Sak1, Elm1, and Tos3, activate Snf1 by phosphorylation of Thr210 (30-32). Sak1 (*S*nf1-*a*ctivating *k*inase) was formerly known as Pak1 but was renamed to avoid confusion with the p21-activated kinase family. These upstream kinases are highly similar and exhibit overlapping function as all three must be deleted to abolish SNF1 activity *in vivo* (30, 32). Phosphorylation of Snf1 Thr210 does not require the Snf1 C-terminal domain, Snf4, or the beta subunits (30, 57, 58). There is no evidence to date that activity of any of the upstream kinases is regulated by glucose signals. Evidence suggests that the three kinases make different contributions to cellular regulation under conditions of different carbon source availability; however, in all cases tested, Sak1 was the major Snf1-activating kinase (30, 37, 71, 82). While Tos3 alone is sufficient for growth on nonpreferred carbon sources, the only condition tested in which the absence of Tos3 causes a significant defect was during continuous growth on glycerol plus ethanol (82). The SNF1 beta subunit also confers some specificity for a particular upstream kinase under different growth conditions (71). Analysis of mutants indicated that Sak1, Tos3, and Elm1 are also required for activation of SNF1 in response to other environmental stress conditions (see section 6.4); each kinase suffices for activation, but again Sak1 has the major role (26).

Sak1 associates with SNF1 to form a stable complex, whereas Tos3 and Elm1 apparently associate with SNF1 more transiently (57). This stable interaction between Snf1 and Sak1 is not regulated by glucose (57), contrary to an earlier report (31). Most of the Sak1 protein in the cell appears to be associated with Snf1, but only a fraction of the total Snf1 is in a complex with Sak1 (57). The nonconserved C-terminal domains of Sak1 and Tos3 and the N terminus of Sak1 are important for SNF1 signaling (83).

Sak1 is found in the cytoplasm in glucose-grown cells, but exhibits some relocalization to the vacuolar membrane in response to glucose depletion, similar to Sip1 (37). Tos3 is cytosolic under both conditions (82). Elm1 localizes to the bud neck (84), consistent with its SNF1-independent roles in cell morphology, septin organization, and cell cycle progression (84-87).

4.2. Reg1-Glc7 protein phosphatase 1

Snf1 catalytic activity is negatively regulated by the Reg1-Glc7 protein phosphatase 1 (PP1); the Glc7 catalytic subunit is directed to SNF1 through the targeting protein Reg1 (33, 34, 56, 88). Different sequences of Reg1 interact with Snf1 and with Glc7 (34, 89). PP1 dephosphorylates the activation loop Thr210 residue during conditions of ample glucose availability (23), and in a *reg1* mutant cell, Snf1 catalytic activity is resistant to glucose inhibition (44). Reg1 is cytoplasmic and nuclear excluded in both glucose-grown and glucose-depleted cells(89). It is phosphorylated in response to glucose limitation in a Snf1-dependent manner (34).

Although there is as yet no direct biochemical evidence that the activity of Reg1-Glc7 is regulated, genetic evidence supports a regulatory role for the phosphatase. Expression of the heterologous mammalian AMPK-activating kinases LKB1, CaMKK alpha, or TAK1 in *sak1 tos3 elm1* mutant yeast cells restores glucose-regulated SNF1 activity (44, 45). Moreover, the *sak1 tos3 elm1 reg1* quadruple mutant expressing mammalian activating kinases showed glucose-insensitive SNF1 activity, and the absence of Reg1 had no effect on activity

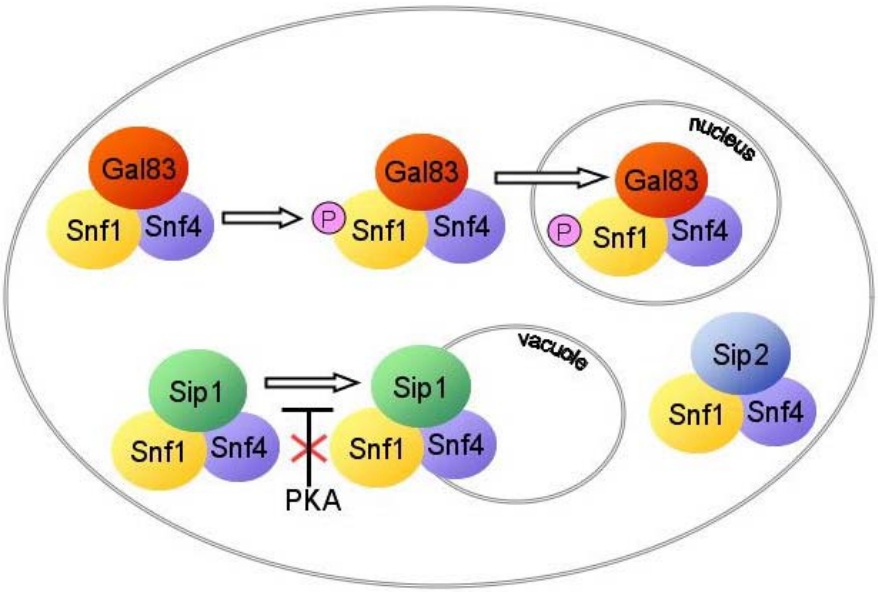

Figure 3. Relocalization of alternate forms of SNF1 in response to carbon stress. In conditions of high glucose, all three alternate SNF1 complexes are cytoplasmic. In response to carbon stress, PKA no longer inhibits the localization of Sip1 to the vacuolar membrane. Sip2 remains cytoplasmic. Relocalization of Snf1 protein kinase containing Gal83 to the nucleus in response to carbon stress requires phosphorylation (P) and activation of Snf1 and also a Snf1-independent stress signal that promotes nuclear localization of Gal83.

in glucose-limited cells (44). Although it is possible that the three yeast upstream kinases as well as LKB1 and CaMKK are all similarly glucose regulated, the simple model is that they are constitutively active and that the function of Reg1-Glc7 towards SNF1 is positively regulated by glucose signals that affect either the phosphatase or the SNF1 complex (44).

In similar experiments, in *sak1 tos3 elm1* cells expressing mammalian CaMKK alpha, SNF1 was activated by both sodium ion and alkaline stress. These results suggest that these stress signals regulate SNF1 activity by a mechanism that is independent of the upstream kinase.

5. REGULATION OF SUBCELLULAR LOCALIZATION

SNF1 is also regulated at the level of subcellular localization, which presumably affects substrate access (Figure 3). In high glucose, Snf1 and all three beta subunits are cytoplasmic (35). When glucose becomes limiting, the beta subunits take on unique subcellular localization patterns, dependent on their divergent N-terminal sequences (35, 37, 80). Sip2 remains cytoplasmic, while Sip1 relocalizes to the vacuolar membrane, and Gal83 relocalizes to the nucleus. The beta subunits also direct the localization of Snf1. In the *sip1* mutant, no vacuolar localization of Snf1 is evident, and in the *gal83* mutant, dependent on yeast strain background, Snf1 either fails to accumulate, or accumulates to a lesser extent, in the nucleus. The Snf4 subunit, which is present in excess, is both cytoplasmic and nuclear regardless of nutritional status (35). The effects of environmental stress on localization of the Snf1 and Gal83 subunits has also been examined; both become enriched in the nucleus in response to alkaline pH but not salt stress (26). Sip2 has also been reported to associate with the plasma membrane in cells that have been subjected to a magnetic bead sorting procedure (67).

The localization of Sip1 to the vacuolar membrane is inhibited by protein kinase A (PKA) activity (36). In a mutant lacking the three PKA catalytic subunits, Sip1 is constitutively at the vacuole, independent of glucose availability, and conversely, in a mutant with high PKA activity due to absence of the Bcy1 regulatory subunit, Sip1 is always cytoplasmic. Localization to the vacuolar membrane also requires the N-myristoylation consensus sequence in Sip1 (36).

Gal83 contains a leucine-rich nuclear export signal (NES) in its N terminus, which is required for its cytoplasmic distribution, and export depends on the Crm1 export receptor (80). Nuclear localization of Snf1 and Gal83 depends not only on Gal83 but also on activation of the Snf1 catalytic subunit; evidence indicates that catalytically inactive Snf1 promotes the cytoplasmic retention of Gal83 in glucose-grown cells through its interaction with the C terminus of Gal83 (80). The requirement for activation of Snf1 serves to retain inactive Snf1 in the cytoplasm, where it can be phosphorylated by the activating kinases, none of which is nuclear (37, 82, 84). Nuclear accumulation also requires Sak1, which is the major Snf1-activating kinase, but activation of Snf1 by a heterologous kinase did not rescue the localization defect (44). In contrast, in the *snf1* mutant, Gal83 exhibits

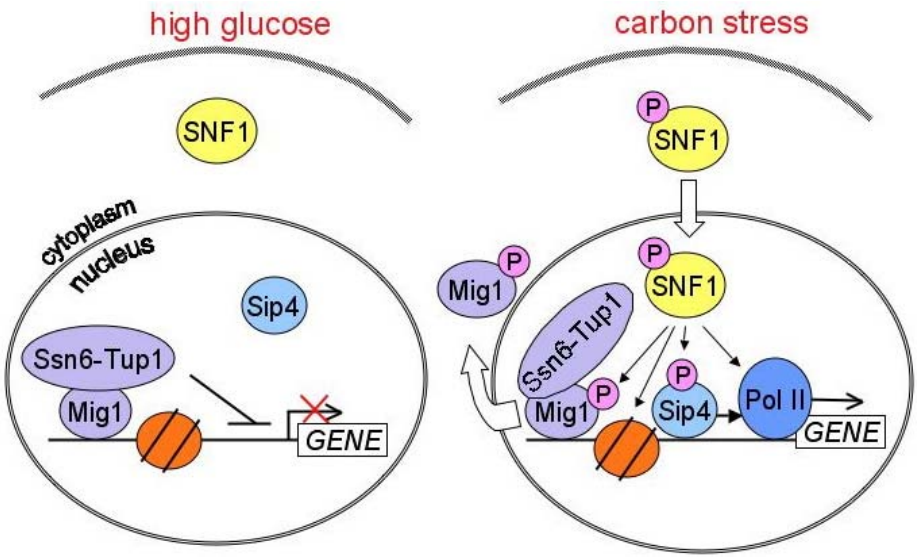

Figure 4. Mechanisms by which SNF1 regulates transcription of glucose-repressed genes. Yeast cells are depicted during growth in high glucose and after exposure to carbon stress. In high glucose, the repressor Mig1 is nuclear and represses transcription of many glucose-repressed genes (*GENE*) in conjunction with the corepressor Ssn6-Tup1. In response to carbon stress, SNF1 is phosphorylated (P) and moves into the nucleus. Sip4, a representative transcriptional activator, is phosphorylated and activates transcription of a subset of glucose-repressed genes. SNF1 phosphorylates Mig1, which alters its interaction with Ssn6-Tup1 to alleviate repression and promotes its nuclear export. SNF1 is also thought to modify chromatin (depicted by a single orange nucleosome) and affect the RNA polymerase II (Pol II) transcriptional apparatus.

glucose-regulated nuclear accumulation, and Sak1 is dispensable (37). While phosphorylation of glucose is required for cytosolic localization, evidence suggesting glucose-6-phosphate as a candidate signal regulating Gal83 localization (35) can be accounted for by its inhibitory effects on Snf1 activity (80). Studies of an N-terminal fragment of Gal83, which does not interact with Snf1, showed that addition of 2-deoxyglucose to glucose-limited cells or addition of glucose to cells lacking phosphoglucose isomerase did not cause relocalization from nucleus to cytoplasm (80). The signaling mechanism remains unclear.

6. FUNCTION OF SNF1 PROTEIN KINASE

6.1. Control of transcription in response to carbon stress

SNF1 regulates the transcription of a large set of genes, including those involved in the metabolism of alternative carbon sources, gluconeogenesis, respiration, transport, and meiosis. Genomic expression studies showed that expression of over 400 genes was SNF1-dependent under conditions of limiting glucose, including 29 of the 40 most highly glucose regulated genes (90). The largest class of SNF1-dependent genes functions in transcription and signal transduction, reflecting the central regulatory role of this kinase (90). SNF1 exerts direct effects on transcription through repressors (91, 92), activators (93-95), chromatin (67, 96), and most likely the transcriptional apparatus (70, 97) (Figure 4). Many genes are coregulated by SNF1 and the activators Adr1 and Cat8 (90). The *K. lactis* Snf1 and Gal83 homologs are similarly required for transcriptional activation of glucose-repressed genes (46).

SNF1 affects many glucose-repressed genes through control of the transcriptional repressor Mig1, which functions in conjunction with the corepressor Ssn6 (Cyc8)-Tup1 (98, 99). SNF1 phosphorylates Mig1 (91, 92, 100), which promotes its nuclear export (101). However, evidence suggests that phosphorylation of Mig1 alters its interaction with Ssn6-Tup1 to alleviate repression, but does not release DNA-bound Mig1 from the promoter (102).

SNF1 also regulates various transcriptional activators. SNF1 is required for activation by Cat8 and Sip4, which bind to the carbon source-response element found in gluconeogenic genes (93, 94, 103, 104). Sip4 is phosphorylated in response to glucose limitation, dependent on Snf1 and Gal83 (62, 64, 94). In *K. lactis*, phosphorylation of KlCat8 is dependent on KlSnf1, and mutation of a consensus SNF1 phosphorylation site in KlCat8 altered glucose regulation of its activation function, as did mutation of the conserved site in *S. cerevisiae* Cat8 (105). SNF1 promotes the binding of Adr1 to chromatin in the absence of glucose (95). SNF1 affects activation by heat shock transcription factor (HSF) in response to glucose starvation, but not heat stress (106). HSF is a direct target of SNF1 *in vitro*, and phosphorylation of HSF and binding of HSF to certain low-affinity promoters in response to glucose starvation is Snf1-dependent (107). The stress-response transcription factor Msn2 is another target of SNF1; upon glucose depletion, SNF1 phosphorylates Msn2, thereby inhibiting its nuclear accumulation (108, 109). Finally, SNF1 is required for phosphorylation and nuclear accumulation of Gln3, a GATA transcription factor, in response to glucose starvation, and SNF1 phosphorylates Gln3 *in vitro* (110).

Genetic evidence suggests that SNF1 has direct effects on the transcriptional apparatus. Recruitment of Snf1 to a promoter results in glucose-regulated transcription, and SNF1 stimulates transcription by RNA polymerase II holoenzyme that has been recruited to an artificial promoter (70). Mutations in *SNF4* were recovered as dominant suppressors that restored *INO1* transcription caused by a mutant TATA-binding protein (TBP) (111), and Snf1 was found to affect the recruitment of TBP to the *INO1* promoter (97). Snf1 also affects preinitiation complex formation *in vitro* (95).

Various lines of evidence implicate SNF1 in regulating modification of chromatin. At the *INO1* promoter, it has been reported that SNF1 phosphorylates histone H3 on Ser10, which leads to acetylation of Lys14 by the acetyltransferase Gcn5 and recruitment of TBP, thereby inducing transcription (96), and SNF1-mediated phosphorylation of Ser10 is also required for *GAL1* transcription (112). However, another study of the effect of substitution of Ala for Ser10 did not reveal a requirement for histone H3 Ser10 phosphorylation for *INO1* transcription (97). Genetic evidence connects SNF1 to Gcn5 in control of *HIS3* transcription, and Snf1 phosphorylates Gcn5 *in vitro*; however, histone H3 Ser10 phosphorylation does not contribute to Gcn5- and SNF1-mediated *HIS3* expression(113). *In vitro,* SNF1 phosphorylates a peptide containing the histone H3 Ser10 site, and this activity increases in aging cells (67). Finally, the role of SNF1 in chromatin binding by Adr1 may reflect direct effects of SNF1 on chromatin (95).

6.2. Control of metabolic enzymes and transporters in response to carbon stress

As part of its function in controlling cellular energy usage, SNF1 regulates the activity of metabolic enzymes involved in fatty acid metabolism and carbohydrate storage (14, 114, 115). SNF1 phosphorylates and inactivates acetyl-CoA carboxylase (Acc1) (27), and thereby inhibits fatty acid biosynthesis during glucose-limiting conditions. Phosphorylation of Acc1 also affects transcription of *INO1*, a key gene in phospholipid biosynthesis (116). SNF1 is required for accumulation of glycogen, an important storage carbohydrate in yeast (117), and controls the phosphorylation of glycogen synthase (21, 114, 118, 119). SNF1 also appears to affect maintenance of glycogen stores by promoting autophagy (115). SNF1 also controls expression of hexose transporters (120, 121), and in *K. lactis*, KlSnf1 is involved in the intracellular sorting of the galactose/lactose transporter Lac12 to the plasma membrane (122).

6.3. Roles of SNF1 in response to other environmental stresses

SNF1 is important for responses to many other environmental stresses besides carbon stress. The *snf1* mutant shows defects during starvation for other nutrients, including phosphate, sulfate, and nitrogen (21). Notably, diploid mutant cells fail to undergo pseudohyphal differentiation in response to nitrogen limitation (20). Phosphorylation of Snf1 Thr210 is stimulated by nitrogen limitation and negatively regulated by TOR kinase, which has known roles in nitrogen signaling (81).

The *snf1* mutant is sensitive to sodium and lithium ion stress (22, 25, 123), other toxic cations such as hygromycin B (25), alkaline pH and heat shock (124), and genotoxic stress caused by hydroxyurea, methylmethane sulfonate, and cadmium (24). The *snf1* mutant also shows reduced thermotolerance in stationary phase (21). SNF1 catalytic activity and phosphorylation of Thr210 increase as a result of exposure of cells to sodium ion stress, alkaline pH, oxidative stress, or antimycin A, which inhibits respiratory metabolism, but not sorbitol or heat shock (26). Interestingly, very low levels of SNF1 activity suffice for resistance to some stresses, and in some cases phosphorylation of Thr210 is not essential (24-26). Different stresses have specific effects on SNF1 signaling. For example, SNF1 is regulated differently during adaptation of cells to alkaline pH and NaCl with respect to the time course of activation, and SNF1 becomes enriched in the nucleus in response to alkaline pH but not salt stress (26). Most of the known roles of SNF1 in stress responses involve transcriptional control; for example, the *snf1* mutant is defective in induction of *ENA1*, encoding Na^+-ATPase, by alkaline or sodium ion stress (123, 124).

SNF1 also appears to have a role in regulating protective mechanisms against stress in response to glucose starvation. Thus, glucose-depleted cells exhibit SNF1-dependent induction of *ENA1* (22), activation of HSF (106, 107), and phosphorylation of the stress-response transcription factor Msn2 (108, 109).

6.4. Roles in regulation of other cellular processes

SNF1 is also central to various nutrient-responsive cellular developmental processes. SNF1 is required for both early and late stages of meiosis (17); the role of SNF1 in entry into meiosis appears to be allowing high-level expression of *IME,* which encodes a master regulator of meiosis (125). SNF1 is a positive regulator of autophagy, a process for recycling organelles and macromolecules, possibly by regulating Apg1 and Apg13 (115). In addition to its role in diploid pseudohyphal growth, SNF1 is also required for haploid filamentous invasive growth in response to glucose limitation (19) or growth on various nonpreferred carbon sources (126); SNF1 regulates transcription of *FLO11,* which encodes a cell surface glycoprotein (flocculin) required for adherence, by antagonizing the repressors Nrg1 and Nrg2 (20), and it also affects the filamentation process (68). Finally, SNF1 appears to be involved in the connections between energy metabolism and aging; overexpression of Snf1 and the absence, or lack of N-myristoylation, of Sip2, promote aging, while the absence of Snf4 extends life span (18, 67, 69).

7. PERSPECTIVE

The SNF1 pathway is central to many aspects of cellular regulation in yeast, and genetic analysis of the pathway in this model organism has provided insights relevant to SNF1/AMPK pathways in other eukaryotes.

Structural analysis promises deeper understanding of mechanisms affecting function of this kinase, but genetic approaches will continue to be useful in addressing many of the remaining questions regarding its regulation. The identity of the glucose signal(s) is not yet clear, and it remains possible that AMP or ATP regulates SNF1 *in vivo*. Further studies are needed to determine whether the activity of the Snf1-activating kinases or the Reg1-Glc7 protein phosphatase 1, or both, is regulated, or whether signals to the kinase complex affect its accessibility to the kinases or phosphatase. Autoinhibition is also an important control point, involving alternate binding of the AIS to the Snf1 kinase domain or to Snf4, and the mechanism by which this is regulated is not understood. Moreover, the role of Snf4 in glucose inhibition of phosphorylation has only recently been recognized. The regulation of this kinase is clearly complex, and many more exciting discoveries await us.

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Abbreviations: AMPK: AMP-activated protein kinase, SNF1: Sucrose Nonfermenting 1, CaMKK: Ca^{2+}/calmodulin-dependent protein kinase kinase, TAK1: transforming growth factor-beta activated protein kinase, CBS: cystathionine-beta-synthase, TOR: target of rapamycin, AIS: autoinhibitory sequence, NES: nuclear export signal, GBD: glycogen binding domain, HSF: heat shock factor, TBP: TATA-binding protein, PKA: protein kinase A

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