An Intragenic Recombination Event Generates a Snf4-Independent Form of the Essential Protein Kinase Snf1 in *Candida albicans*

Austin Mottola, a Joachim Morschhäuser

a Institut für Molekulare Infektionsbiologie, Universität Würzburg, Würzburg, Germany

**ABSTRACT** The heterotrimeric protein kinase SNF1 plays a key role in the metabolic adaptation of the pathogenic yeast *Candida albicans*. It consists of the essential catalytic α-subunit Snf1, the γ-subunit Snf4, and one of the two β-subunits Kis1 and Kis2. Snf4 is required to release the N-terminal catalytic domain of Snf1 from autoinhibition by the C-terminal regulatory domain, and *snf4*Δ mutants cannot grow on carbon sources other than glucose. In a screen for suppressor mutations that restore growth of a *snf4*Δ mutant on alternative carbon sources, we isolated a mutant in which six amino acids between the N-terminal kinase domain and the C-terminal regulatory domain of Snf1 were deleted. The deletion was caused by an intragenic recombination event between two 8-bp direct repeats flanking six intervening codons. In contrast to truncated forms of Snf1 that contain only the kinase domain, the Snf4-independent Snf1Δ311–316 was fully functional and could replace wild-type Snf1 for normal growth, because it retained the ability to interact with the Kis1 and Kis2 β-subunits via its C-terminal domain. Indeed, the Snf4-independent Snf1Δ311–316 still required the β-subunits of the SNF1 complex to perform its functions and did not rescue the growth defects of *kis1*Δ mutants. Our results demonstrate that a preprogrammed in-frame deletion event within the *SNF1* coding region can generate a mutated form of this essential kinase which abolishes autoinhibition and thereby overcomes growth deficiencies caused by a defect in the γ-subunit Snf4.

**IMPORTANCE** Genomic alterations, including different types of recombination events, facilitate the generation of genetically altered variants and enable the pathogenic yeast *Candida albicans* to adapt to stressful conditions encountered in its human host. Here, we show that a specific recombination event between two 8-bp direct repeats within the coding sequence of the *SNF1* gene results in the deletion of six amino acids between the N-terminal kinase domain and the C-terminal regulatory domain and relieves this essential kinase from autoinhibition. This preprogrammed deletion allowed *C. albicans* to overcome growth defects caused by the absence of the regulatory subunit Snf4 and represents a built-in mechanism for the generation of a Snf4-independent Snf1 kinase.

**KEYWORDS** AMP-activated kinases, *Candida albicans*, genetic recombination, metabolic adaptation, suppressor mutation
a member of the AMP-activated protein kinase (AMPK) family, which is highly conserved in eukaryotic organisms and has been studied in great detail in the model yeast \textit{Saccharomyces cerevisiae} (7). In \textit{S. cerevisiae}, the SNF1 complex consists of the catalytic \textit{α}-subunit Snf1, the \textit{γ}-subunit Snf4, and one of three alternative \textit{β}-subunits, Sip1, Sip2, and Gal83. Snf4 binds to the C-terminal regulatory domain of Snf1, thereby releasing the N-terminal catalytic domain from autoinhibition under inducing conditions (8). The \textit{β}-subunits, which also bind to a C-terminal region in Snf1, are responsible for the interaction with target proteins and regulate the subcellular localization of the kinase (9–11). Snf1 is activated in response to glucose limitation and other stresses via phosphorylation at Thr210 in its activation loop by the three partially redundant upstream activating kinases Elm1, Sak1, and Tos3 (12–14). It is dephosphorylated at Thr210 and thereby inactivated by Reg1-Glc7 protein phosphatase1 (15, 16). In \textit{C. albicans}, the SNF1 complex consists of the \textit{α}-subunit Snf1, the \textit{γ}-subunit Snf4, and one of the two \textit{β}-subunits Kis1 and Kis2 (17).

Despite the structural and functional conservation of the SNF1 complex, there are some notable differences between \textit{S. cerevisiae} and \textit{C. albicans}. In \textit{S. cerevisiae}, Snf1 is constitutively phosphorylated at Thr208 (corresponding to Thr210 of ScSnf1) in the activation loop (18), and this phosphorylation is mediated by a single upstream activating kinase, Sak1 (19). Remarkably, while \textit{S. cerevisiae} \textit{snf1Δ} mutants are viable, \textit{SNF1} seems to be an essential gene in \textit{C. albicans} (19–22). In contrast, the genes encoding the upstream kinase Sak1 or the \textit{β}-subunits Kis1 and Kis2 and the \textit{γ}-subunit Snf4 of the SNF1 complex can be deleted. \textit{C. albicans sak1Δ} and \textit{snf4Δ} mutants have growth defects on alternative carbon sources and are highly sensitive to cell wall/membrane stress but are viable (19). This observation indicates that Snf1 retains some basal function that is essential for viability even in its unphosphorylated state or when other subunits of the SNF1 complex are absent.

Although the function of Snf1 in \textit{C. albicans} is similar to that of its homologs in \textit{S. cerevisiae} and other organisms, it has not been established how the kinase regulates metabolic activities in this pathogenic yeast. Several cases of transcriptional rewiring of metabolic pathways have been described where the expression of functionally related genes is controlled by different transcription factors in \textit{S. cerevisiae} and \textit{C. albicans} (23–25). In \textit{C. albicans}, the transcriptional repressor Mig1, whose activity is regulated by Snf1-dependent phosphorylation in \textit{S. cerevisiae}, does not contain a consensus recognition sequence for Snf1, suggesting that the target proteins of Snf1 might differ in the two species (26). Insight into how the Snf1 kinase enables metabolic adaptation of \textit{C. albicans} might be gained from the isolation of suppressor mutations that restore growth in mutants with a defective SNF1 complex. We have used this approach with \textit{snf4Δ} mutants, which (in contrast to \textit{snf1Δ} mutants) are viable but have stronger growth defects than \textit{sak1Δ} mutants and cannot grow at all on alternative carbon sources. Here, we report the isolation and characterization of a suppressor mutation in the Snf1 kinase itself, which arose by an intragenic recombination event that resulted in the deletion of 6 internal amino acids and rendered the SNF1 complex functional in the absence of the \textit{γ}-subunit Snf4.

\textbf{RESULTS}

\textbf{An in-frame deletion in SNF1 suppresses snf4Δ mutant phenotypes.} \textit{C. albicans} mutants lacking the \textit{γ}-subunit Snf4 of the SNF1 complex are unable to grow on alternative carbon sources and also exhibit reduced growth on glucose (19). We hypothesized that cultivation of \textit{snf4Δ} mutants in glucose-containing media would enrich for faster-growing spontaneous mutants and allow the isolation of suppressor mutations that bypass the dependence on Snf4 for growth on alternative carbon sources. Therefore, a \textit{snf4Δ} mutant was passaged twice in yeast extract-peptone-dextrose (YPD) medium, and the cultures were plated on YNB minimal medium containing sucrose as the sole carbon source. By this procedure, we were able to isolate two mutants, designated \textit{SCΔsnf4SupB} and \textit{SCΔsnf4SupC}, which appeared as single colonies on YNB + sucrose plates after the first and second YPD subcultures, respec-
Dilution spot assays confirmed that both suppressor mutants had regained the ability to utilize sucrose despite the absence of Snf4 and also exhibited improved growth on glucose (Fig. 1A).

A candidate for snf4Δ suppressor mutations is the gene encoding the catalytic Snf1 of the SNF1 complex, because mutations in Snf1 that reduce its dependence on Snf4 have been identified in S. cerevisiae (27–29). We therefore amplified the SNF1 alleles of the suppressor mutants and directly sequenced the PCR products. The two SNF1 alleles of C. albicans strain SC5314, the wild-type parent of our mutants, differ slightly. In comparison with allele A (the reference sequence), allele B lacks one of the 10 histidine codons in the N-terminal polyhistidine tract and contains the silent nucleotide exchanges A1443G, T1620C, and A1809G (http://www.candidagenome.org). No mutations were found in the SNF1 alleles of strain SCΔsnf4SupC, and the basis of the suppressor phenotype of this strain will be the subject of future investigations. In contrast, strain SCΔsnf4SupB contained an in-frame deletion of codons 311 to 316 in one of the SNF1 alleles. As illustrated in Fig. 1B, this deletion most likely occurred by an intragenic recombination event between two 8-bp direct repeats bordering the deleted region. Using primers that bind within the deleted sequence, we determined that the deletion had occurred in allele A whereas allele B was unaltered.

To verify that the deletion of six amino acids within Snf1 was the reason for the improved growth of strain SCΔsnf4SupB, we introduced the same 18-bp deletion into one of the endogenous SNF1 alleles in two independently generated snf4Δ mutants. For comparison, we also introduced an L181I mutation, which corresponds to an L183I snf4Δ suppressor mutation in Snf1 of S. cerevisiae (29) and which we had previously

**FIG 1** An in-frame deletion in SNF1 suppresses the growth defect of a snf4Δ mutant on sucrose as the sole carbon source. (A) Growth of wild-type strain SC5314, snf4Δ mutant SC5314Δsnf4M4A, and two spontaneous suppressor mutants (SC5314Δsnf4SupB and SC5314Δsnf4SupC) on YP agar plates (top panels) and YNB agar plates containing 2% glucose or 2% sucrose as the carbon source. YPD overnight cultures of the strains were adjusted to an optical density (OD600) of 2.0 and serial 10-fold dilutions plated and incubated for 4 days at 30°C. (B) Partial nucleotide and deduced amino acid sequences of the SNF1 alleles of wild-type strain SC5314 (top; positions within the SNF1 coding sequence and Snf1 protein sequence are indicated) and of the mutated SNF1 allele of suppressor mutant SC5314Δsnf4SupB (bottom). The 8-bp direct repeat sequence in the wild-type allele is underlined, and the amino acids deleted in Snf1Δ311–316 are highlighted in bold.
found to partially suppress the growth defects of sak1Δ mutants (19), into the snf4Δ mutants. As illustrated in Fig. 2, the two mutations were equally efficient in restoring growth of cells lacking Snf4 on different carbon sources. Growth on glucose was improved, and growth on sucrose was also restored to nearly wild-type levels. The growth defect on acetate was partially rescued, while no growth improvement on glycerol was observable. These results show that the deletion of amino acids 311 to 316 in Snf1 rendered the protein partially independent of the presence of the regulatory γ-subunit Snf4.

In a previous study, we had found that a C-terminally truncated Snf1 containing only the first 340 amino acids, which corresponds to a hyperactive form of mammalian AMPK, did not remediate the growth defects of C. albicans snf4Δ mutants (19). Interestingly, the internal deletion in SnfΔ311/H11002316 is located near the end of a C-terminally truncated Snf1 of S. cerevisiae that exhibited reduced dependence on Snf4 (27). We therefore hypothesized that a complete C-terminal truncation of Snf1 after amino acid 310 instead of amino acid 340, resulting in a protein that contains only the kinase domain (amino acids 53 to 304), might also suppress snf4Δ mutant phenotypes. However, similarly to SNF1ΔC340, the SNF1ΔC310 allele did not improve growth of the snf4Δ mutants (Fig. 2). This may be due to the fact that the C-terminal truncations removed not only the autoinhibitory domain of Snf1 but also the interaction domain with β-subunits Kis1 and Kis2, thereby impairing the functionality of the kinase, in line with results previously obtained in S. cerevisiae (29). In contrast, the internal deletion of only six amino acids directly behind the kinase domain probably abolished its interaction with the inhibitory domain, such that Snf4 was no longer required to prevent this interaction, while it may still allow binding of Kis1 and Kis2 to Snf1 and formation of a functional SNF1 complex.

**Homozgyosity augments the suppressor activity of mutated SNF1 alleles.**

Gain-of-function mutations in several transcription factors of C. albicans that confer increased fluconazole resistance have a stronger effect when they occur in both alleles of the respective genes (30). We therefore reasoned that the growth of snf4Δ mutants would be further improved if they were homozygous for suppressor mutations in SNF1. To test this possibility, we introduced the Δ311–316 and L181I mutations into both endogenous SNF1 alleles of the snf4Δ mutants and compared the growth of heterozygous and homozygous strains on different carbon sources (Fig. 3A). Indeed, the two suppressor mutations further augmented growth on acetate in the homozygous strains, and growth on glycerol was now also partially restored. These phenotypes were similar to those of sak1Δ mutants lacking the upstream Snf1-activating kinase, which have weaker growth defects than snf4Δ mutants.

**FIG 2** Growth of wild-type strain SC5314, snf4Δ mutants (SCSNF4M4A and SCSNF4M4B), and derivatives in which one endogenous SNF1 allele was replaced by the indicated mutated allele on different carbon sources. YPD overnight cultures of the strains were adjusted to an optical density (OD₆₀₀) of 2.0 and serial 10-fold dilutions spotted on YNB agar plates containing 2% glucose, sucrose, acetate, or glycerol as the sole carbon source. Plates were incubated for 4 days at 30°C. Both independently generated series of mutants are shown. Strains in the top and bottom panels were grown on the same plate, and the photographs are arranged accordingly for clarity of presentation.
The SNF1<sup>Δ311–316</sup> allele suppresses other snf4Δ mutant phenotypes. *C. albi-
cans* sak1Δ and snf4Δ mutants are also hypersensitive to cell wall/membrane stress (19). We therefore tested if the suppressor mutations in Snf1 could increase the resistance of snf4Δ mutants to agents causing cell wall/membrane stress. As can be seen in Fig. 3B, both mutated forms of Snf1 improved the growth of snf4Δ mutants on plates containing Congo red, caffeine, or SDS. Although resistance to Congo red and SDS was not restored to wild-type levels, the suppressor mutants grew better in the presence of these inhibitors than sak1Δ mutants lacking the upstream activating kinase Sak1. These results demonstrate that the deletion of amino acids 311 to 316 in Snf1, like the L181I mutation, partially restores different functions of the SNF1 complex in the absence of the regulatory γ-subunit.

The Δ311–316 mutation does not increase Snf1 phosphorylation. In *C. albi-
cans* mutants lacking the γ-subunit Snf4 of the SNF1 complex, Snf1 is still phosphorylated at Thr208 by the upstream activating kinase Sak1, but with lower efficiency, which may contribute to the growth defects of snf4Δ mutants (19). We therefore tested if the L181I and Δ311–316 mutations resulted in improved Snf1 phosphorylation in the absence of Snf4. However, no increased Snf1 phosphorylation was observed in snf4Δ mutants containing the suppressor mutations in all tested growth media (Fig. 4). In a previous study, we had observed that the L181I mutation also relieved the growth defects of sak1Δ mutants, in which Snf1 phosphorylation is abolished (19). Taken together, these results support the idea that the suppressor mutations partially restore the functionality of Snf1 in the absence of Snf4 by preventing autoinhibition independently of the Snf1 phosphorylation status.
Snf1 activity requires the β-subunits of the SNF1 complex. As discussed above, the ability of the SNF1Δ311 − 316 allele, but not the C-terminally truncated SNF1Δ310 allele, to rescue growth defects of a snf4Δ mutant might have been due to functional interactions with the β-subunits Kis1 and Kis2. Mutants lacking Kis1 have growth defects on alternative carbon sources similar to those of sak1Δ mutants, while kis2Δ mutants grow as well as the wild type under these conditions. We therefore tested if the SNF1Δ311 − 316 allele could revert kis1Δ mutant phenotypes. As can be seen in Fig. 5, replacement of one or both wild-type SNF1 alleles in kis1Δ mutants by the SNF1Δ311 − 316 allele did not markedly improve the growth of the mutants on alternative carbon sources or in the presence of cell wall stress. These results demonstrate that the Snf4-independent form of Snf1 still requires the β-subunits of the SNF1 complex to perform its functions.

Functionality of Snf1Δ311 − 316 in a wild-type background. Finally, we investigated if the Δ311 − 316 mutation only diminished the dependence of Snf1 on Snf4 or if it resulted in a hyperactive form of the kinase. With this aim, we replaced one or both endogenous SNF1 alleles by the SNF1Δ311 − 316 allele in the wild-type strain SC5314. As
can be seen in Fig. 6A and B, the homozygous mutants grew as well as the wild-type strain under various tested conditions, except for a slight hypersusceptibility to Congo red, indicating that the internal deletion had little effect on the functionality of Snf1.

We reasoned that cells containing a hyperactive Snf1 might more rapidly adapt to changed growth conditions than wild-type cells and might exhibit a shorter lag phase after a switch from glucose to an alternative carbon source. To test if Snf1Δ311/H11002 provides such a growth advantage, overnight cultures of the strains grown in YNB/glucose medium were inoculated into fresh YNB medium with glucose or sucrose as the carbon source and growth was monitored by measuring the optical densities of the cultures over time. As can be seen in Fig. 6C, all strains grew more slowly on sucrose than on glucose, but no differences were observed between the wild type and the mutants in either medium, indicating that the mutated Snf1 did not enable the cells to adapt more quickly to the change in the carbon source.

**DISCUSSION**

In this study, we identified a novel type of mutation in Snf1 that restores the functionality of the kinase in the absence of the normally required γ-subunit Snf4. Studies in *S. cerevisiae* have shown that Snf4 binds to the C-terminal part of Snf1 and (under inducing conditions) relieves the N-terminal catalytic domain from inhibition by the autoinhibitory domain (8). Screenings for suppressor mutations have revealed...
various point mutations in the catalytic domain that render Snf1 function independent
of Snf4, indicating that autoinhibition is abolished in the mutated proteins (27–29).
Unlike these mutations, the snf4Δ suppressor mutation described here is not located
within the catalytic domain of Snf1; instead, the mutation resulted in the deletion of six
amino acids between the N-terminal and C-terminal domains of the kinase. Internal
deletions that bypass the requirement for Snf4 have also been engineered in Snf1 of
S. cerevisiae; however, these deletions destroyed the autoinhibitory domain (29). In
contrast, the small internal deletion of amino acids 311 to 316 directly behind the
kinase domain left the inhibitory domain intact but most likely prevented its interaction
with the kinase domain, thereby bypassing the dependence of Snf1 functionality on
Snf4. The retention of the C-terminal domain enabled Snf1Δ311–316 to exert its normal
functions in C. albicans, in contrast to the C-terminally truncated versions, which cannot
interact with the β-subunits Kis1 and Kis2. Indeed, Snf1Δ311–316 still depended on the
presence of the β-subunits to enable growth on alternative carbon sources, because it
could not rescue the growth defect of kis1Δ mutants. The functions of Kis1 and Kis2 in
C. albicans seem to be partially overlapping, as mutants lacking only one of them have
comparatively mild growth defects (19). So far, we were unable to construct double
mutants lacking both KIS1 and KIS2 (A. Mottola and J. Morschhäuser, unpublished
results), suggesting that C. albicans requires at least one of the two β-subunits of the
SNF1 complex for growth.

Another interesting aspect of our study is the mechanism by which the mutated
SNF1 allele arose. Unlike previously isolated suppressor mutations in SNF1 of S. cerevi-
siae, which were point mutations resulting in amino acid exchanges or C-terminal
deletions (27–29), the deletion of six codons in SNF1Δ311–316 occurred by a recombi-
nation event involving two flanking direct repeats. In contrast to point mutations, such
a deletion is not a totally random event but can be regarded as a built-in mechanism
that could involve recombination between the two repeats of one allele or unequal
reciprocal recombination between the two homologous alleles, the latter resulting in
hybrid alleles. The fact that allele-specific polymorphisms were retained on both sides
of the deleted region in the mutated allele A and in the homologous wild-type allele
B indicates that the deletion had occurred by an intra-allelic recombination event in our
mutant.

As in other organisms, nucleotide sequence repeats are a rich source of genetic
variation in C. albicans. In protein-encoding genes, these are usually tandem repeats
that allow rapid expansion and shortening of amino acid repeat tracts, which may
modify the functionality of the proteins (31). In contrast, the two 8-bp repeats in SNF1
flank an intervening sequence, and recombination between these repeats leads to a
precise in-frame deletion of six codons. In principle, the result of this recombination
event is an irreversible deletion, which at first sight makes it unclear how the retention
of the ability to undergo this event should be selected for. However, since C. albicans
is a diploid organism, heterozygous mutants could revert to the original state by gene
conversion using the wild-type allele as the template. In addition, even homozygous
mutants might retain a wild-type SNF1 allele by mating. Whether recombination
between the two 8-bp repeats in SNF1 is indeed used by C. albicans as a mechanism to
generate a mutated form of Snf1 remains speculative. The experiments that we
performed so far did not provide evidence that a Snf4-independent form of Snf1 would
 confer an advantage also in a wild-type background, but this might possibly be the case
under some conditions encountered within the human host. In any case, the genera-
tion of a mutated form of SNF1 by a preprogrammed intragenic recombination event
is a new example of how the genomic flexibility of C. albicans can generate phenotypic
variation and adaptation.

MATERIALS AND METHODS

Strains and growth conditions. The C. albicans strains used in this study are listed in Table 1. All
strains were stored as frozen stocks with 17.2% glycerol at −80°C and subcultured on YPD agar plates
(10 g yeast extract, 20 g peptone, 20 g glucose, 15 g agar per liter) at 30°C. Strains were routinely grown
in YPD liquid medium at 30°C in a shaking incubator. For selection of nourseothricin-resistant transfor-
TABLE 1 C. albicans strains used in this study

| Strain | Parent | Relevant characteristics or genotype | Reference or source |
|--------|--------|--------------------------------------|---------------------|
| SC5314 | SC5314 | Wild-type reference strain           | 34                  |
| SC3404MA and -B | SC5314 | sak1Δ::FRT/sak1Δ::FRT               | 19                  |
| SC5F4MA and -B | SC5314 | snf4Δ::FRT/snf4Δ::FRT               | 19                  |
| SC51S14MA and -B | SC5314 | kis1Δ::FRT/kis1Δ::FRT               | 19                  |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M1A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |
| SC5S1F118A M2A | SC5N4MA | SNF1Δ311 - SAT1-FLIP               | This study          |

*SAT1-FLIP denotes the SAT1 flipper cassette; FRT is the FLP recombination target sequence, one copy of which remains in the genome after recycling of the SAT1 flipper cassette.

mnants, 200 μg/ml nourseothricin (Werner Bioagents, Jena, Germany) was added to YPD agar plates. To obtain nourseothricin-sensitive derivatives in which the SAT1 flipper cassette was excised by FLP-mediated recombination, transformants were grown overnight in YCB-BSA-YE medium (23.4 g yeast base [YCB], 4 g bovine serum albumin [BSA], 2 g yeast extract [YE] per liter, pH 4.0) without selective pressure to induce the SAP2 promoter controlling caFLP expression. Appropriate dilutions were plated on YPD agar plates and grown for 2 days at 30°C. Individual colonies were picked and streaked on YPD plates as well as on YPD plates with 100 μg/ml nourseothricin to confirm nourseothricin sensitivity.

**Sequencing of the SNF1 alleles.** The SNF1 alleles of the suppressor mutants SCAnS1F118A and SCAnS1F118B were amplified with primers SNF1.01 and SNF1.04, which bind in the SNF1 upstream and downstream regions, respectively, and the PCR products directly sequenced using additional internal primers. All oligonucleotide primers used in this study are listed in Table 2.

**Plasmid constructions.** A SNF1 allele lacking codons 311 to 316 was generated by PCR in the following way. The upstream region and the 5’ part of the SNF1 coding region were amplified with primers SNF1.01 and SNF1d311-316.03, and the 3’ part of the SNF1 coding region and downstream sequence with primers SNF1d311-316.04 and SNF1.05. Primers SNF1d311-316.03 and SNF1d311-316.04 are complementary and lack SNF1 codons 311 to 316. The gel-purified PCR products were then used as the templates in a fusion PCR with primers SNF1.01 and SNF1.05, and the SacI/SacI-digested PCR product was substituted for the SNF1 upstream region in the SNF1 deletion construct.
TABLE 2 Primers used in this study

| Primer | Sequence (5’-3’)* |
|--------|-------------------|
| SNF1.01 | ATCCGAAGCTCACAAAAAGACAAAGAC |
| SNF1.04 | AGTGCGGCCCATTTAATTGCTGATTATAAG |
| SNF1.05 | AGTCTCGGGGATTAATTTGCTTAGATTTATAG |
| SNF1.09 | GCGGATATTTTGGTACGCGC |
| SNF1.10 | GCAGTTAACAAATAATCAG |
| SNF1d311-316.01 | TATCCGAGGTATTTACGAAATTAGACAGAG |
| SNF1d311-316.02 | GATATCGGAGATCTGTTTAAACCATTTGAC |
| SNF1d311-316.03 | GTTCTAAACAGATAGTTGCGAATTCTCATATCTTCAC |
| SNF1d311-316.04 | CTITTGGATTTCTTGTTGATATTCAAATCTGGAATCTTGTATTATAAC |
| SNF1d311.03 | CCTAATCTTATCTTAATCAGATACATCTGCAATAGATTT |
| SNF1d311.04 | CATATCGCAGATATAAAAGTTAAGTACGTATCTTTGATATTGATG |
| SNF1G1SR.01 | AGAATAGACGGTATCAATCTCTTACAGCAAT |
| SNF1-GFP.01 | TCTTGAAGATCACGATCTGAAATCAATGAGATAT |
| SNF1n-seq.01 | GCCATCTGTTGATATCCTC |
| SNF1n-seq.02 | GCCAAATCTTACAGCAAT |

*The added SacI and SacII restriction sites are underlined; the stop codon introduced after SNF1 codon 310 is highlighted in bold.
*Primer used for sequencing the SNF1 alleles of snf4Δ suppressor mutants.

pSNF1M1 (19) to obtain pSNF1Δ311 – 316. To generate the SNF1Δ310 allele, the upstream region and a part of the SNF1 coding region were amplified with primers SNF1.01 and SNF1dC311.03, which introduced a TAA stop codon instead of the Tyr311 codon. The SNF1 downstream region was amplified with primers SNF1dC311.04 and SNF1.05. The gel-purified PCR products served as the templates in a fusion PCR with primers SNF1.01 and SNF1.05, and the SacI/SacII-digested PCR product was substituted for the SNF1 upstream region in the SNF1 deletion construct, yielding pSNF1Δ310.

Strain constructions. C. albicans strains were transformed by electroporation (32) with the gel-purified insertions from plasmids pSNF1Δ311 – 316, pSNF1Δ310, pSNF1Δ310 (19), and pSNF1L181I (19). The correct genomic integration of all constructs and excision of the SAT1 flipper cassette were confirmed by Southern hybridization using the flanking sequences as probes.

Isolation of genomic DNA and Southern hybridization. Genomic DNA from C. albicans strains was isolated as described previously (33). The DNA was digested with appropriate restriction enzymes, separated on a 1% agarose gel, transferred by vacuum blotting onto a nylon membrane, and fixed by UV cross-linking. Southern hybridization with enhanced chemiluminescence-labeled probes was performed with an Amersham ECL direct nucleic acid labeling and detection system (GE Healthcare UK Limited, Little Chalfont Buckinghamshire, United Kingdom) according to the instructions of the manufacturer.

Phenotypic assays. Growth on different carbon sources and resistance to cell wall/membrane stress were tested by dilution spot assays as described in the legends to the figures. Carbon source utilization was tested on YNB agar plates (0.67% yeast nitrogen base with ammonium sulfate, 2% agar) containing 2% glucose, sucrose, potassium acetate, or glycerol. Growth on sucrose was also tested on YPD plates containing 2% sucrose instead of glucose (YPD). Cell wall/membrane stress resistance was tested on YPD plates containing 50 μg/ml Congo red, 15 mM caffeine, or 0.04% SDS. Growth curves in liquid media were obtained by cultivating the strains in microtiter plates and measuring the OD of the cultures every 10 min in a Tecan Infinite F200 PRO plate reader.

Western blotting. Overnight cultures of the strains were diluted 10−2 in 50 ml fresh YPD medium and grown for 3 h at 30°C. For growth on alternative carbon sources, the strains were incubated for only 1 h at 30°C, washed in sterile water, resuspended in 50 ml of YP medium supplemented with 2% of the different carbon sources, and grown for additional 2 h. Cells were collected by centrifugation, washed in 50 ml H2O, and resuspended in 500 μl breaking buffer (100 mM triethylammonium bicarbonate buffer [TEAB], 150 mM NaCl, 1% SDS, cComplete EDTA-free Protease Inhibitor Cocktail, and PhosStop Phosphatase Inhibitor Cocktail [Roche Diagnostics GmbH, Mannheim, Germany]) supplemented with protease and phosphatase inhibitors. Equal volumes of 0.5-mm-diameter acid-washed glass beads were added to all tubes. Cells were mechanically disrupted on a FastPrep-24 cell homogenizer (MP Biomedicals, Santa Ana, CA, USA) with three 40-s pulses, with 5 min on ice between pulses. Samples were centrifuged at 13,000 rpm for 15 min at 4°C, the supernatant was collected, and the protein concentration was quantified using the Bradford protein assay. Equal amounts of protein of each sample were mixed with 1 volume of 2× Laemmli buffer, heated for 5 min at 95°C, and separated on an SDS-9% polyacrylamide gel. Separated proteins were transferred onto a nitrocellulose membrane with a mini-Protein system (Bio-Rad, Munich, Germany) and stained with Ponceau S to control for equal loading. To detect T208 phosphorylation of Snf1, membranes were blocked in 5% BSA–TBST (5% bovine serum albumin–Tris-buffered saline with Tween 20) at room temperature for 1 h and subsequently incubated overnight at 4°C with Phospho-AMPKα (Thr172) antibody (catalog no. 2531; Cell Signaling Technology, Danvers, MA, USA). Membranes were washed in TBST and incubated at room temperature for 1 h with anti-rabbit horseradish peroxidase (HRP) G-21234 antibody (Invitrogen GmbH, Darmstadt, Germany).
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