Carbon Source-dependent Assembly of the Snf1p Kinase Complex in Candida albicans

Carsten Corvey¶§§, Peter Koetter¶§§, Tobias Beckhaus‡, Jeremy Hack‡, Sandra Hofmann‡, Martin Hampel¶, Torsten Stein¶, Michael Karas‡, and Karl-Dieter Entian‡

From the Institutes for Microbiology and Pharmaceutical Chemistry, Johann Wolfgang Goethe-University of Frankfurt, Marie-Curie-Strasse 9, D-60439 Frankfurt, Germany

Received for publication, April 5, 2005, and in revised form, May 4, 2005
Published, JBC Papers in Press, May 12, 2005, DOI 10.1074/jbc.M503719200

The Snf1p/AMP-activated kinases are involved in transcriptional, metabolic, and developmental regulation in response to stress. In Saccharomyces cerevisiae, Snf1p (Cat1p) is one of the key regulators of carbohydrate metabolism, and cat1 (snf1) mutants fail to grow with non-fermentable carbon sources. In Candida albicans, Snf1p is an essential protein and cells depend on a functional Snf1 kinase even with glucose as carbon source. We investigated the CaSnf1p complex after tandem affinity purification and mass spectrometric analysis and show that the complex composition changes with the carbon source provided. Three subunits were identified, one of which was named CaSnf4p because of its homology to the ScSnf4 protein and the respective CaSnf4 gene could complement a S. cerevisiae snf4 mutant. The other two proteins revealed similarities to the S. cerevisiae kinase β subunits ScGal83p, ScSip2p, and ScSip1p. Both genes complemented the scaffold function in a S. cerevisiae gal83, sip1, sip2 triple deletion mutant and were named according to their scaffold function as CaKIS1p and CaKIS2p. Matrix-assisted laser desorption ionization peptide mass fingerprint analysis indicated that CaKis2p is N-terminal myristoylated and the incorporation of CaKis2p in the Snf1p complex was reduced when compared with cells grown with glucose as a carbon source. To verify the different complex assemblies, a stable isotope labeling technique (iTraq™) was employed, confirming a 3-fold decrease of CaKis2p with ethanol. Yeast two-hybrid analysis confirmed the interaction partners, and the results showed an activator domain for the CaKis2 protein that has not been reported for S. cerevisiae scaffold subunits.

Candida albicans is a widely distributed commensal fungus that is carried as a part of the human microbial flora. However, it is also an opportunistic pathogen that can cause serious infections, particularly in immunocompromised individuals (1). In C. albicans, Snf1p (CaSnf1p, NCBI accession number 46437276) seems to be essential for growth; moreover, CaSnf1p has the ability to complement the snf1 mutant of Saccharomyces cerevisiae (2, 3). In Candida tropicalis, a close relationship of CaSnf1p (86% identity to CaSnf1p) mRNA levels and cell growth was demonstrated (4) that may relate CaSnf1p to more fundamental cellular processes, but to date no direct interaction partners of the essential CaSnf1p have been identified.

In the model yeast S. cerevisiae, the ScSnf1p kinase is a homologue to the highly conserved AMP-activated serine/threonine kinases that are found in plants, Drosophila, Caenorhabditis elegans, mammals, and fungi (for review see Ref. 5)). These kinases seem to be essential components of cascades that function as metabolic sensors in eukaryotic cells and are activated under conditions of nutrient stress. Additionally, they were reported to be involved in pathogenesis and the treatment of several human diseases, including type 2 diabetes, obesity, heart disease, and cancer (6).

The importance of ScSNF1 (ScCAT1) was first identified genetically after the isolation of cat1 (snf1) mutants. This gene was identified as a key element for the regulation of glucose repression because of the failure of cat1 mutants to grow with non-fermentable carbon sources and to derepress glucose-repressible genes (7). The mutation of the regulatory subunit cat3 had a similar phenotype (8) and was later also identified as snf4 (9). ScSnf1p assists cells to adapt to glucose-limited conditions, regulating the transcription of metabolic genes and the activity of metabolic enzymes (11, 12), meiosis and sporulation (13, 14), pseudohyphal and invasive growth (15–17), and life span and aging (18, 19). The ScSnf1p kinase activity correlates with increased concentrations of AMP, but the enzyme does not exhibit allosteric regulation by AMP in vitro (20). ScSnf1p down-regulates ATP-consuming enzymes such as acetyl-CoA carboxylase (21) and phosphorylates transcription factors like ScMig1p, causing its translocation from the nucleus to the cytoplasm and derepressed gene expression (22).

In S. cerevisiae, the ScSnf1p kinase complex contains a catalytic α subunit encoded by ScSNF1 (23) comprising 633 amino acids with a catalytic N-terminal serine/threonine kinase domain. The first half of the regulatory domain in the remaining part down-regulates the kinase activity under nutrient-rich conditions (24), whereas the second half is needed for the interaction with the β subunits (25). The γ subunit encoded by ScSNF4 plays a key regulatory role here (26, 27). It associates with the β subunit by a consecutive interaction, and it binds to the autoinhibitory domain of ScSnf1p (as previously mentioned), releasing its catalytic domain. Under glucose-limited conditions, phosphorylation of Thr210 in the ScSnf1p C-terminal loop promotes kinase activity (28). The upstream activating Snf1p kinases in S. cerevisiae were recently identified as ScPak1p, ScTos3p, and ScElm1p (29). The β subunits encoded either by ScSip1p, ScSip2p, or ScGal83p mediate heterotrimer
formation between ScSnf1p and ScSnf4p, and hence three distinct forms exist in vivo (30). The β subunits are also responsible for the interaction with downstream targets and regulate the subcellular localization of the kinase (31, 32). The C terminus contains an internal kinase association (KIS)² domain associated with the α subunit and a conserved 80-residue ASC (association with the Snf1p kinase complex) domain that is associated with the γ subunit (25).

The transmission of a wide range of biological signals depends upon direct physical interaction of specific cellular proteins (33). To study these interactions we used the tandem affinity purification (TAP) method (34) to purify the Snf1p kinase complex from the human pathogen C. albicans. A strain expressing only the TAP-tagged fusion protein was chosen, which allowed the test for functionality to be close to physiological concentrations by forming a complex with endogenous components. Because the deletion of CaSnf1p is lethal, it was interesting whether the TAP tag would interfere with the physiological function. After deletion of the second copy, the cells showed no phenotypic change on either glucose or ethanol. The combination of two different affinity tags greatly reduced the binding of nonspecific proteins to the complex. We could identify the ScSnf4 homologue protein from C. albicans and two scaffold subunits, which we named CaKis1p and CaKis2p. The protein interactions within the Snf1p kinase complex were also confirmed using yeast two-hybrid analysis. In addition, a transcriptional activator domain in CaSip2p could be identified.

MATERIALS AND METHODS

Unless otherwise noted, all chemicals were of analytical grade.

Plasmid Constructs and Genetic Manipulation—Established protocols for molecular biology were followed (35). Transformation of yeast strains utilized the lithium acetate (36) procedure. The CaURA3 open reading frame including 421 nucleotides of upstream 5′-sequence and 121 nucleotides of downstream 3′-sequence was amplified by PCR using primers CaURA3-ApaI/CaURA3-PstI and SC5314 genomic DNA as a template. The 1361-bp PCR product was digested with ApaI/PstI and primers CaURA3-ApaI/CaURA3-PstI and SC5314 genomic DNA as a selection marker. The CaSNF1−TAP cassette and the deletion from genomic DNA was prepared as described (38).

Subunit Analysis of the C. albicans Snf1 Kinase Complex

The transmission of a wide range of biological signals depends upon direct physical interaction of specific cellular proteins (33). To study these interactions we used the tandem affinity purification (TAP) method (34) to purify the Snf1p kinase complex from the human pathogen C. albicans. A strain expressing only the TAP-tagged fusion protein was chosen, which allowed the test for functionality to be close to physiological concentrations by forming a complex with endogenous components. Because the deletion of CaSnf1p is lethal, it was interesting whether the TAP tag would interfere with the physiological function. After deletion of the second copy, the cells showed no phenotypic change on either glucose or ethanol. The combination of two different affinity tags greatly reduced the binding of nonspecific proteins to the complex. We could identify the ScSnf4 homologue protein from C. albicans and two scaffold subunits, which we named CaKis1p and CaKis2p. The protein interactions within the Snf1p kinase complex were also confirmed using yeast two-hybrid analysis. In addition, a transcriptional activator domain in CaSip2p could be identified.

The TAP tag would interfere with the physiological function. After deletion of the second copy, the cells showed no phenotypic change on either glucose or ethanol. The combination of two different affinity tags greatly reduced the binding of nonspecific proteins to the complex. We could identify the ScSnf4 homologue protein from C. albicans and two scaffold subunits, which we named CaKis1p and CaKis2p. The protein interactions within the Snf1p kinase complex were also confirmed using yeast two-hybrid analysis. In addition, a transcriptional activator domain in CaSip2p could be identified.
chromatography the sample was dried.

**Strong Cation Exchange Chromatography**—The combined mixture was separated by strong cation exchange chromatography on an HP 1100 HPLC system (Hewlett-Packard, Waldbronn, Germany) using a PolySulfoethyl A column (4.6 \( / \) 11003 100 mm, 5 \( / \) 9262 m, 300 Å). Sample was dissolved in 1 ml of strong cation exchange loading buffer (25% (v/v) ACN, 10 mM KH2PO4 adjusted to pH 3 with phosphoric acid), loaded, and washed for 20 min at 1 ml/min. Peptides were eluted in a two-step gradient (0–500 mM KCl (25% (v/v) ACN, 10 mM KH2PO4 adjusted to pH 3 with phosphoric acid)) over 15 min at a flow rate of 1 ml/min with fractions collected from 20 min onward.

**HPLC MS**—All LC MS/MS experiments were performed in positive ion mode using either an XCT ion trap (Agilent Technologies) or a QTrap 2000 hybrid tandem mass spectrometer (ABI MDS Sciex, Ontario, Canada) connected to a nano-LC (Agilent Technologies). A linear gradient was applied ranging from 3–97% (v/v) ACN/0.1% (v/v) FA. Samples were trapped on a Zorbax 300 SB precolumn (0.3 \( / \) 11003 5 mm, 5 \( / \) 9262 m, Agilent Technologies) with a flow rate of 20 \( / \) 9262 l/min and a solvent composition of H2O/ACN/FA (97/3/0.1 v/v/v). Separation was performed on a Zorbax C18 column (75 \( / \) 11003 150 mm, Agilent Technologies) using 0.1% (v/v) FA in water as solvent A and 0.1% (v/v) FA in ACN as solvent B at a flow rate of 300 nl/min.

**Yeast Two-hybrid Analysis**—Cells were harvested and washed once with KPP (potassium-phosphate) buffer (pH 6.5, 4 °C). After washing, cells were resuspended in 500 \( / \) 9262 l of KPP buffer, glass beads were added, and the mixture was vortexed for 90 s at 4 °C. 1 ml of cold KPP was added, and cell debris were separated by centrifugation (4000 rpm, 10 min). Protein concentration was determined using a microbiuret assay. \( / \)-Galactosidase was assayed according to Ref. 46.

**RESULTS**

**Construction of C. albicans SNF1-tagged Strain**—For the analysis of the CaSnf1p kinase complex assembly, a TAP tag cassette was chromosomally fused to the C terminus of CaSnf1p (Fig. 1A). To show the functionality of the CaSNF1-TAP construct and to avoid any influence of CaSnf1p, the remaining wild-type gene CaSNF1 was additionally deleted, resulting in strain CPK141–28 (supplemental Table I, Fig. 1B). Correct integration of the TAP cassette and the deletion of the second allele were shown using Southern hybridization (Fig. 2A). Strain CPK141–28 showed wild-type growth on glucose and on ethanol, which proved that the recombinant tagged CaSNF1 gene, transcribed from its native promoter, was fully functional (data not shown). Western blot analysis following SDS-PAGE separation (using the peroxidase-anti-peroxidase antibody directed against the protein A part of the TAP tag) (Fig. 2B) showed an apparent molecular mass of the fusion protein of 90 kDa. The observed value fits well with the calculated value of CaSnf1p (69 kDa) fused to the calmodulin-binding peptide (5 kDa) and protein A (16 kDa) separated by a TEV cleavage site.

**Tandem Affinity Purification of the CaSnf1p Kinase Complex**—To identify the interaction partners of CaSnf1p we performed tandem affinity purifications of the TAP-tagged protein.

**FIG. 1.** Plasmid map of pPK335 and CaSnf1p TAP tagging. A, vector pPK335 used for the TAP tagging consists of a protein A domain from Staphylococcus aureus, separated by a tobacco etch virus (TEV) cleavage site, and a calmodulin-binding peptide (CBP), and CaURA3 as a selective marker. B, schematic diagram illustrating the introduction of the TAP cassette using the CaURA3 gene as a selection marker. The second allele was deleted using the CaHIS1 gene.

**FIG. 2.** Southern and Western blot analysis of CaSNF1 TAP-tagged strains. A, genomic DNA of the corresponding strains was digested with PvuII and, following gel electrophoresis, was transferred to a nylon membrane. 586-bp CaSNF1 DNA fragment was amplified by PCR (primers CaSNF1-P1/-P2) and used as a probe after labeling with \[\text{PdCTP}\]. Lane 1, RM1000 (CaSNF1/CaSNF1); lane 2, CPK140–6 (CaSNF1::TAP-CaURA3/CaSNF1); lane 3, CPK141–28, (CaSnf1::TAP-CaURA3/CaHIS1). B, Western blotting was carried out using the peroxidase-conjugated anti-peroxidase antibody against the protein A domain of the fusion protein. Extracts from RM1000 and CPK141–28 were separated.
from cells grown on different carbon sources. Following TAP, the protein complexes were separated via SDS-PAGE, and the corresponding band patterns were compared (Fig. 3A). Subsequently, all gels were entirely sliced and subjected to an in-gel digestion protocol. Proteins were identified via PMF and nano-LC MS/MS. When cells were grown on glucose, the complex displayed three intense bands and one faint band (Fig. 3A, lane 1), whereas cells grown on ethanol displayed three intense bands (Fig. 3A, lane 2). In both experiments we could identify the most intense band (band 2) as the bait (CaSnflp) fused to the calmodulin-binding protein (Figs. 3B and 4A). The protein had an apparent molecular mass of 74 kDa, which fits well to the calculated value of CaSnflp (69 kDa) fused to the calmodulin binding peptide (5 kDa).

Using both PMF and nano-LC MS/MS analysis, all four bands could be unambiguously assigned to C. albicans proteins. Band 4 could be assigned to hypothetical protein CaO19.13191 during SDS-PAGE fits to the calculated molecular mass of 41 kDa (Fig. 3A, band 4). After PMF, 45% of the amino acid sequence could be covered, and subsequent LC MS/MS analyses enhanced the sequence coverage to 56% with well assigned peptide sequences. As a representative example, the MS/MS spectrum of a very hydrophobic peptide (1672.99 Da), which elutes at 56 min (42% (v/v) ACN), is shown (Fig. 4B). From the nearly complete y- and b-ion fragmentation series of the parent ion, the peptide sequence LINVYEAVDILALVK could be determined with a Spectrum Mill score >15 and a scored peak intensity >70%, (scores >15 lie within the category of highest confidence). The identified hypothetical protein CaO19.13191 shows 64% identity to the γ subunit Snf4p from S. cerevisiae and is now named CaSnf4p (Fig. 5).

Protein band 3 (Fig. 3A) with an apparent molecular mass of 50 kDa could be identified as CaO19.4084 (NCBI accession number 46435808) with a calculated molecular mass of 46 kDa. Using PMF, 24% of the amino acid sequence was covered, which was enhanced to 29% using high quality MS/MS data (Fig. 4C). This protein shows striking similarities to the S. cerevisiae β subunits ScGal83p and ScSip2p, having 49 and 45% homology, respectively (Fig. 5C). With this degree of similarity it was not possible to clearly attribute CaO19.4084 to one of the S. cerevisiae proteins. Furthermore, CaO19.4084 also showed homologies to the ASC and KIS domains of the third S. cerevisiae β subunit, ScSip1p, being 27% identical and 43% similar in the respective regions.

Protein band 1 (Fig. 3A) migrating at 100 kDa was identified as CaO19.12464 (NCBI accession number 46444282) with a calculated molecular mass of 81 kDa. Using PMF, 33% of the amino acid sequence was covered, which was increased to 38% when LC MS/MS fragmentation data of two additional peptides were considered. To substantiate this result the MS/MS spectrum of the peptide LLVIYNDILAVK is shown (Fig. 4D). Using homology prediction, the protein shows similarities to the ASC domains of the S. cerevisiae β subunits, ScSip2p having 42% identity and 65% similarity, ScGal83p with 64% identity and 80% similarity, and ScSip1p with 45% identity and 78% similarity (Fig. 5D). In comparison, CaO19.12464 possesses only a partial KIS domain, having 33% identity to ScSip2p, 36% iden-
tity to ScGal83p, and 40% identity to ScSip1p within the conserved region. CaO19.12464 co-purified from ethanol-grown cells was not visible using SDS-PAGE but could be identified using PMF. We were able to match two peptides (one with one missed cleavage site) within 25 ppm mass accuracy to a myristoylated N-terminal tryptic peptide of CaO19.12464 (Fig. 6). The leader sequence already indicates a high probability for N-terminal myristoylation, which was also shown for the *S. cerevisiae* subunits ScSnf1p (47) and ScSip1p (48).

These results showed that CaO19.4084 and CaO19.12464 are homologous to the *S. cerevisiae* scaffold proteins of the ScSnf1 kinase complex. Homology alone, however, does not relate the newly identified *C. albicans* Snf1 kinase subunits to any of the *S. cerevisiae* proteins. Therefore, we assigned the new names **CaKIS1** (encoding CaO19.4084) and **CaKIS2** (encoding CaO19.12464) for the CaSnf1p subunits according to their KIS homologous domains and their function as kinase scaffold proteins.

**Functional Analysis of CaKIS1 and CaKIS2**—Proof was required that CaKIS1 and CaKIS2 actually encode the γ subunits of the CaSnf1 kinase complex. To genetically verify the scaffold function in *S. cerevisiae* we used the triple mutant
gal83, sip1, sip2; the genes encoding all scaffold proteins were deleted. This mutant cannot grow with ethanol as the sole carbon source. Independent transformation of \( \text{CaKIS1} \) and \( \text{CaKIS2} \) recovered the ability of the triple deletion mutant to grow on ethanol (data not shown). This clearly confirmed that \( \text{CaKis1p} \) and \( \text{CaKis2p} \) have scaffold functionality.

Additionally, the physical interactions of the identified \( \text{C. albicans} \) proteins were measured with yeast two-hybrid analysis (see Table I). \( \text{CaKis1p} \) interacted with \( \text{CaSnf4p} \) in both directions of the assay, whereas an interaction with \( \text{CaSnf1p} \) was only found when \( \text{CaKis1p} \) was fused to the binding domain. Interestingly, the interaction of \( \text{CaSnf4p} \) with the scaffold proteins, in comparison to \( \text{CaSnf1p} \), was severalfold increased on both carbon sources. This analysis confirmed the interactions of \( \text{CaSnf1p} \) with \( \text{CaKis1p} \) and \( \text{CaKis2p} \), respectively. As already reported for \( \text{S. cerevisiae} \) (49), the interaction of \( \text{CaSnf4p} \) and \( \text{CaSnf1p} \) was only weak after growth on ethanol but increased 5-fold under glucose repression conditions. Surprisingly \( \text{CaKis2p} \) fused to a DNA-binding domain itself shows strong activity in the yeast two-hybrid assay (pPK343, Table I). This suggests strong transcriptional activation without the presence of an activation domain, which was so far not reported for any of the \( \beta \) subunits from \( \text{S. cerevisiae} \).

Carbon Source-dependent Assembly of \( \text{C. albicans} \) Scaffold Subunits Using iTRAQ™ Quantification—Because \( \text{ScSnf1} \) is involved in cell signaling upon starvation, we followed the complex composition when cells were grown on different carbon sources. Using SDS-PAGE, we analyzed the banding pattern. The ratios of intensities changed, with a high reproducibility, depending on the carbon source provided. Grown on glucose, four bands became visible after Coomassie Blue staining, of which \( \text{CaSnf1p} \) was the most intense. Grown on ethanol, only three bands were visible, all with similar intensities. The amount of \( \text{CaKis2p} \) incorporated in the complex decreased when cells were grown on ethanol. To study the varying protein abundance, we used iTRAQ™ (ABI, Sciex), a multiplexed peptide quantification method. This amine-specific labeling reagent enabled us to per-

---

**Table I**

Yeast two-hybrid analysis of the \( \text{CaSnfl} \) complex

| Activation/ binding domain | pGBT9 | pPK337 | pPK339 | pPK341 | pPK343 |
|----------------------------|-------|--------|--------|--------|--------|
| \( \text{CaSnf1} \)       | 3     | 4      | 5      | 6      | 111    |
| \( \text{CaSnf4} \)       | 10    | 8      | 20     | 13     | 416    |
| \( \text{CaKis1} \)       | 10    | 10     | 54     | 33     | 542    |
| \( \text{CaKis2} \)       | 3     | 10     | 3      | 33     | 423    |
| \( \text{CaKis1} \)       | 8     | 9      | 16     | 90     | 702    |
| \( \text{CaKis2} \)       | 2     | 6      | 3      | 55     | 369    |
| \( \text{CaKis1} \)       | 10    | 9      | 181    | 15     | 443    |
| \( \text{CaKis2} \)       | 11    | 19     | 330    | 16     | 518    |

Yeast two-hybrid analysis of intact \( \text{C. albicans} \) proteins. Values show an average \( \beta \) galactosidase activity in Miller units (nmol \( \beta \)-galactosidase activity/min/mg 1) for two transformants each measured at least twice. S.E. were less than 10%. The values obtained were found from cells grown in liquid SC-trp-leu medium containing either 4% glucose (upper case) or 3% ethanol (lower values).
Relative protein quantification using iTRAQ™ and nano-LC MS/MS analysis. Counts represent the number of MS/MS experiments used to quantify the ratios. Changes in protein abundance are described as a n-fold change with respect to cells grown on glucose. S.D. is the standard deviation calculated from corresponding MS/MS experiments.

| Protein name | Count | Fold change | S.D. |
|--------------|-------|-------------|------|
| CaKis2p      | 5     | 2.75        | 0.54 |
| CaSnfl       | 12    | 1.00        | 0.2  |
| CaKis1p      | 14    | 1.04        | 0.1  |
| CaSnflp      | 4     | 0.85        | 0.15 |

DISCUSSION

Because of its specificity and sensitivity, tandem affinity purification combined with mass spectrometry analysis provides an excellent technique for studying protein complexes (34). Here we describe an approach to genomically tag C. albicans proteins at their C terminus, so that the fusion construct is still expressed from its native promoter, whereas the other allele was deleted. To avoid any artificial changes in subunit composition the unchanged expression rate of the respective bait is of major importance.

Here we investigated the CaSnfl protein complex, which functions as a metabolic sensor upon metabolic changes in various yeasts. In S. cerevisiae three distinct forms of the Snfl kinase complex exist in vivo (30). The ScSnfl kinase is associated with the γ subunit ScSnflp and one of the three β subunits, ScGal83p, ScSip1p, and ScSip2p.

As C. albicans is a diploid organism, we deleted the remaining wild-type allele to exclude its possible influence on the complex composition (Fig. 1). Cells showed a normal growth behavior after the genomic integration of a TAP cassette at the C terminus of the CaSnfl protein and after deletion of the remaining wild-type CaSNFI gene. This clearly proved the functionality of the CaSnfl-TAP fusion protein. Using our adapted tandem affinity purification protocol for C. albicans, we were able to show the interaction of CaSnflp with its predicted γ subunit, CaSnflp (Fig. 3). Transposon mutagenesis in a large scale loss-of-function genetic screening identified CaSnflp as being involved in filamentous growth (51). Furthermore, we identified two β subunits of the CaSnfl kinase complex. Both proteins showed homologies to the three known S. cerevisiae β subunits, Gal83p, Sip1p, and Sip2p. Because of their homology they could not be clearly assigned to any of the S. cerevisiae proteins and were named CaKis1p and CaKis2p. CaKis1p was homologous to all three S. cerevisiae β subunits. The primary structure of the CaKis2 protein is different from all previously described β subunits of the Snfl kinase complexes. Although in all currently known β subunits the KIS and ASC domains are located adjacent to each other, these two domains are separated in CaKis2p by a spacer region of ~200 amino acid residues. Furthermore, the CaKis2p ASC domain is also interrupted by a spacer region of 73 amino acid residues (Fig. 5).

The migration behavior of CaKis2p in a denaturing gel does not fit to the calculated molecular mass, suggesting possible posttranslational modification of the protein (Fig. 3). From the PFM data we were able to match, with a tolerance better than 25 ppm, two peptides (one with a missed cleavage site) that correspond to a fatty acid attached to the glycine residue following the removal of the N-terminal methionine (Fig. 6). These data support our hypothesis that CaKis2p is N-terminal myristoylated. The CaKis2p protein is the first Snfl β subunit for which the myristoylation has been shown biochemically. Such myristoylations were also suggested for the S. cerevisiae scaffold proteins Sip1 (48) and Sip2 (47), and the myristoyl moiety seems to direct ScSip2p to the plasma membrane (18) and ScSip1p to the vacuole (48). In this case, myristoylation may function as an anchor to retain the kinase complex in the cytoplasm.

The newly identified C. albicans Kis1p and Kis2p were tested for functionality as β subunits in S. cerevisiae. After transformation of the respective genes into a gal83Δ sip1Δ sip2Δ triple deletion mutant, both genes could complement the growth failure of this mutant on ethanol. This clearly shows that CaKis1p and CaKis2p both function as scaffold proteins in the ScSnfl kinase complex although the primary structure of CaKis2p is significantly different as compared with the other known β subunits.

Interaction studies on different carbon sources using the yeast two-hybrid system showed that the CaSnflp interacts with the two β subunits CaKis1p and CaKis2p (Table I). We show that CaSnflp weakly interacts with CaSnflp in the presence of ethanol as it is described for S. cerevisiae (49). Surprisingly, CaSnflp fused to the DNA-binding domain demonstrated a very strong activity. We could map the activation domain to the middle region of the protein between amino acid residues 265–584 using N- and C-terminal deletions (data not shown). To date, no such transcriptional activation has been shown for any other known Snfl β subunits, and its possible physiological relevance needs further investigation.

Because ScSnflp is one key regulator when cells are starved for glucose, we purified the complex from cells grown in the presence of ethanol as a non-fermentable carbon source. Using the iTRAQ™ reagents, we were able to monitor relative protein abundance in the complex purified from cells grown on different carbon sources. The β subunit composition changed upon glucose starvation with a 3-fold decrease of CaKis2p on ethanol. In S. cerevisiae, the Snfl kinase complex is localized in the cytosol in the presence of glucose. In contrast, under derepressing conditions such as on ethanol, the Snfl kinase is needed in the nucleus. Because CaKis2p is myristoylated, it may function as an anchor and therefore retain the complex in the cytosol as described for S. cerevisiae (18, 48).

The C. albicans Snfl kinase complex differs from that of S. cerevisiae in many respects. Snfl is an essential protein in C. albicans, but not in S. cerevisiae. The scaffold proteins, especially CaKis2p, differ in their primary sequences. In addition, the C. albicans Snfl1 composition is less complex when compared with the Snfl complex of S. cerevisiae where six associated proteins could be identified. Therefore, the investigation of the C. albicans Snfl1 kinase complex is of particular interest for

---

4 C. Corvey, T. Stein, P. Koetter, K.-D. Entian, and M. Karas, manuscript in preparation.
understanding its essential role in the regulation of cellular metabolism. The TAP method presented here is applicable to virtually any protein in _C. albicans_ and provides an efficient way to focus research on metabolic pathogenicity factors in the protein networks.

Acknowledgments—We thank Svenja Hempel and Stefanie Lambert for technical support, Hamilton Robotics for providing the Microclab® Star digestion robot, Agilent Technologies for providing the XTC ion trap, and Applied Biosystems MDS Scieix (especially Jianru Stahl-Zeng) for support and help using the iTRAQ™ system.

REFERENCES

1. Odds, F. (1988) _Candida and Candidosis_, 2nd Ed., pp. 279–313, Leicester University Press, Leicester, UK
2. Petter, R., Chang, Y. C., and Kwon-Chung, K. J. (1997) _Infect. Immun._ **65**, 4909–4917
3. Enloe, B., Diamond, A., and Mitchell, A. P. (2000) _J. Bacteriol._ **182**, 5730–5736
4. Kanai, T., Ogawa, K., Ueda, M., and Tanaka, A. (1999) _Arch. Microbiol._ **172**, 256–263
5. Hardie, D. G., Carling, D., and Carlson, M. (1998) _Annu. Rev. Biochem._ **67**, 821–855
6. Kemp, B. E., Stapleton, D., Campbell, D. J., Chen, Z. P., Murthy, S., Walter, M., Gupta, A., Adams, J. J., Katsis, F., Van Denderen, B., Jennings, I. G., Iseli, T., Michell, B. J., and Witters, L. A. (2003) _Biochem. Soc. Trans._ **31**, Pt. 1, 162–168
7. Powell, N. E., Zarnowski, J. T., and Carlson, M. (1999) _J. Biol. Chem._ **274**, 25330–25334
8. Ashrafi, K., Lin, S. S., Manchester, J. K., and Gordon, J. I. (2000) _J. Biol. Chem._ **275**, 35347–35354
9. Jiang, R., and Carlson, M. (1997) _Mol. Cell. Biol._ **17**, 2099–2106
10. Schuller, H. J., and Entian, K. D. (1988) _Genes Dev._ **2**, 257–258
11. Celenza, J. L., Eng, P. J., and Carlson, M. (1999) _Mol. Cell. Biol._ **9**, 5045–5054
12. Gancedo, J. M. (1998) _Mol. Cell. Biol._ **18**, 850–857
13. Shevchenko, A., Wilm, M., Vorm, O., and Mann, M. (1996) _Anal. Chem._ **68**, 850–858
14. Ausubel, F. M., Brent, R., Kingston R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1989) _Current Protocols in Molecular Biology_, John Wiley and Sons Inc., New York
15. Messer, F. N., and Weatherall, D. J. (1999) _J. Biol. Chem._ **274**, 25330–25334
16. Vyas, V. K., Kuchin, S., Berkey, C. D., and Carlson, M. (2003) _Curr. Opin. Microbiol._ **6**, 75–80
17. Perny, J., and Carlson, M. (1997) _J. Biol. Chem._ **272**, 3017–3024
18. Ashrafi, K., Lin, S., and Carlson, M. (1996) _Biochem. Soc. Trans._ **23**, Pt. 1, 175–177
19. Cullen, P. J., and Sprague, G. F., Jr. (2000) _Proc. Natl. Acad. Sci. U. S. A._ **97**, 13619–13624
20. Ross, P. L., Huang, Y. N., Marchese, J. N., Williamson, B., Parker, K., Hattan, S., Khainovski, N., Pillai, S., Dey, S., Daniels, S., Purkayastha, S., Juhasz, P., Martin, S., Bartlett-Jones, M., He, F., Jacobson, A., and Pappin, D. J. (2004) _Mol. Cell. Proteomics_ **3**, 1154–1169
21. Uhl, M. A., Biery, M., Craig, N., and Johnson, A. D. (2003) _EMBO J._ **22**, 2668–2678
22. Doerr, S., and Fohlman, J. (1984) _Biomed. Mass. Spectrom._ **11**, 601
Carbon Source-dependent Assembly of the Snf1p Kinase Complex in Candida albicans
Carsten Corvey, Peter Koetter, Tobias Beckhaus, Jeremy Hack, Sandra Hofmann, Martin Hampel, Torsten Stein, Michael Karas and Karl-Dieter Entian

J. Biol. Chem. 2005, 280:25323-25330.
doi: 10.1074/jbc.M503719200 originally published online May 12, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M503719200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2005/05/13/M503719200.DC1

This article cites 48 references, 24 of which can be accessed free at http://www.jbc.org/content/280/27/25323.full.html#ref-list-1