

Feedback Control of Snf1 Protein and Its Phosphorylation Is Necessary for Adaptation to Environmental Stress*

Received for publication, January 20, 2015, and in revised form, May 5, 2015. Published, JBC Papers in Press, May 6, 2015, DOI 10.1074/jbc.M115.639443

Hsiang-En Hsu^{†§}, Tzu-Ning Liu[¶], Chung-Shu Yeh^{||**}, Tien-Hsien Chang^{||**1}, Yi-Chen Lo^{¶12}, and Cheng-Fu Kao^{†§3}

From the [†]Graduate Institute of Life Sciences, National Defense Medical Center, Taipei 11490, the [¶]Institute of Food Science and Technology, College of Bio-Resources and Agriculture, National Taiwan University, Taipei 10617, the ^{||}Institute of Biochemistry and Molecular Biology, National Yang Ming University, Taipei 11221, and the ^{**}Genomics Research Center and [§]Institute of Cellular and Organismic Biology, Academia Sinica, Taipei 11529, Taipei, Taiwan

Background: Snf1/AMPK activity plays a vital role in adaptation to environmental stress.

Results: Elevation of Snf1 phosphorylation restores Snf1 protein in a mutant with low Snf1 levels.

Conclusion: Snf1 activity is fine-tuned by a dynamic feedback loop between its protein level and phosphorylation status.

Significance: Our results reveal a previously unknown regulatory mechanism of Snf1 activity that is crucial for stress response and aging.

Snf1, a member of the AMP-activated protein kinase family, plays a critical role in metabolic energy control in yeast cells. Snf1 activity is activated by phosphorylation of Thr-210 on the activation loop of its catalytic subunit; following activation, Snf1 regulates stress-responsive transcription factors. Here, we report that the level of Snf1 protein is dramatically decreased in a *UBP8*- and *UBP10*-deleted yeast mutant (*ubp8Δ ubp10Δ*), and this is independent of transcriptional regulation and proteasome-mediated degradation. Surprisingly, most Snf1-mediated functions, including glucose limitation regulation, utilization of alternative carbon sources, stress responses, and aging, are unaffected in this strain. Snf1 phosphorylation in *ubp8Δ ubp10Δ* cells is hyperactivated upon stress, which may compensate for the loss of the Snf1 protein and protect cells against stress and aging. Furthermore, artificial elevation of Snf1 phosphorylation (accomplished through deletion of *REG1*, which encodes a protein that regulates Snf1 dephosphorylation) restored Snf1 protein levels and the regulation of Snf1 activity in *ubp8Δ ubp10Δ* cells. Our results reveal the existence of a feedback loop that controls Snf1 protein level and its phosphorylation, which is masked by Ubp8 and Ubp10 through an unknown mechanism. We propose that this dynamic modulation of Snf1 phosphorylation and its protein level may be important for adaptation to environmental stress.

AMP-activated serine/threonine protein kinase (AMPK)⁴ is highly conserved in all eukaryotes, in which it serves as a cellular energy sensor with a key role in energy homeostasis (1, 2). The AMPK homologue in budding yeast (*Saccharomyces cerevisiae*) is encoded by the gene *SNF1* (sucrose nonfermenting 1) (1). Snf1 protein kinase (SNF1) is required for several biological processes, including adaptation to limited glucose, the response to environmental and nutritional stresses, growth on alternative carbon sources, metabolic enzyme regulation, and aging (3–5). Members of the SNF1 protein kinase family are heterotrimers comprised of α , β , and γ subunits (1). Snf1 can be phosphorylated on the conserved activation loop of the catalytic Snf1/ α subunit at the Thr-210 residue (1), and this results in the activation of kinase activity (6). During glucose limitation, three β subunits (Gal83, Sip1, and Sip2) regulate the subcellular localization of SNF1 protein kinase (1, 7). The Snf4/ γ regulatory subunit binds with Snf1 α and β subunits, independent of glucose availability. Snf4 regulates the activity of SNF1 protein kinase by interacting with a sequence located in the α subunit, which has an autoinhibitory effect on the kinase domain (1, 3). SNF1 protein kinase plays a key role in adaptation of yeast cells to glucose limitation and utilization of alternative carbon sources, which occurs through activation of Snf1 via phosphorylation of Thr-210 on the activation loop of the catalytic subunit by upstream kinases (Sak1, Tos3, and Elm1) (8–10); the Reg1-Glc7 and Sit4 phosphatases reverse this modification (11–13).

In yeast, Ubp8 and Ubp10 are ubiquitin-specific proteases of monoubiquitylated histone H2B (H2Bub) (14–16). Several studies have suggested that these two proteases may deubiquitylate targets other than H2Bub (17–20). To identify additional targets of Ubp8 and Ubp10, we searched for genes that have been reported to interact with *UBP8* and *UBP10* via genetic or physical associations. We found that both Ubp8 and Ubp10 were reported to co-purify with Snf1, implying that Ubp8 and

* The authors declare that they have no conflicts of interest with the contents of this article.

¹ Supported by the Ministry of Science and Technology, Taiwan, Grants 101-2311-B-001-005 and 102-2311-B-001-029, Thematic Projects (Academia Sinica) Grants AS-99-TP-B20 and AS-103-TP-B12, and Academia Sinica.

² Supported by Ministry of Science and Technology, Taiwan, Grant 102-2313-B-002-057-MY3 and the College of Bioresources and Agriculture, National Taiwan University. To whom correspondence may be addressed: Institute of Food Science and Technology, Roosevelt Rd., Section 4, Taipei, Taiwan. Tel.: 886-2-3366-4123; E-mail: loyichen@ntu.edu.tw.

³ Supported by the Ministry of Science and Technology, Taiwan, Grants 103-2320-B-001-015 and 102-2320-B-001-020, Thematic Projects (Academia Sinica) Grants AS-99-TP-B20 and AS-103-TP-B02, and Academia Sinica. To whom correspondence may be addressed: Institute of Cellular and Organismic Biology, Academia Sinica 128, Academia Road, Section 2 Nanking, Taipei, 11529, Taiwan. Tel.: 886-2-27899513; Fax: 886-2-27858059; E-mail: ckao@gate.sinica.edu.tw.

⁴ The abbreviations used are: AMPK, AMP-activated serine/threonine protein kinase; IP, immunoprecipitation; RLS, replicative life span; SC, synthetic complete; TAP, tandem affinity purification; Ub, ubiquitin; CLS, chronological life span; G6PDH, glucose-6-phosphate dehydrogenase.

Ubp10 may deubiquitylate the Snf1 protein. Recent evidence suggests that Snf1 undergoes SUMOylation, which is triggered by Mms21; this modification promotes the recruitment of SUMO-targeted ubiquitin ligase, Slx5-Slx8, to Snf1. The resulting ubiquitylation of Snf1 results in its destruction (18, 21). However, the correlation between the control of Snf1 protein level and SNF1 protein kinase activity is still poorly understood.

Here, we report that Snf1 protein level is regulated by Ubp8 and Ubp10 through mechanisms independent of transcriptional control and ubiquitin-mediated degradation. We propose that the level of Snf1 may be controlled by an as yet unidentified mediator of protein synthesis, which is regulated by both Ubp8 and Ubp10. We report that despite the reduction of protein level of Snf1 caused by deletion of *UBP8* and *UBP10*, the remaining protein is still capable of mediating Snf1-mediated functions, such as the response to oxidative stresses and aging. Our findings reveal the existence of a feedback loop between Snf1 protein level and its phosphorylation status, which acts to fine-tune the activity of Snf1 for adaptation to environmental stress.

Experimental Procedures

Strains and Plasmids—Yeast strains used in this study were mainly derived from the following: FY406 (*MATa (hta1-htb1)Δ::LEU2 (hta2-htb2)Δ::TRP1 leu2Δ1 ura3-52 lys2-128δ his3Δ200 trp1Δ63-pZS145 (HTA1-Flag-HTB1 CEN HIS3)*); W303-1A (*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100*); MCY4908 (W303-1A *snf1Δ10*); and RDKY3615 (*MATa ura3-52 his3Δ200 leu2Δ1 trp1Δ63 lys2ΔBglI hom3-10 ade2Δ1 ade8 hxt13Δ::URA3*) genetic backgrounds (22–24). To delete *UBP8* or *UBP10*, we introduced the PCR-amplified fragments of *ubp8Δ::kanMX*, *ubp8Δ::natMX*, or *ubp10Δ::hphMX* into the individual strain backgrounds. To delete *SNF4*, we introduced the PCR-amplified fragments of *snf4Δ::hphMX* into the individual strain backgrounds. To delete *HSP82* or *HSC82*, we designed primers for PCR amplification of *hsp82::loxP-ble-loxP* or *hsc82::loxP-ble-loxP* fragments from pUG66 (25) and then transformed the fragments into the individual strain backgrounds. To delete *DHH1*, *PUF3*, or *XRN1*, we designed primers for PCR amplification of *dhh1::trp1*, *puf3::trp1*, or *xrn1::trp1* fragments from pRS304, and we then transformed the fragments into the individual strain backgrounds. One-step gene replacement was performed by transformation using standard techniques (26).

Preparation of Cell Extracts and Western Blot Analysis—For validation of the candidates identified to be targeted by Ubp8 and Ubp10 (Fig. 1B), cultures were grown to exponential phase (A_{600} from 0.2 to 0.6) in YP medium (1% bacto-yeast extract and 2% bacto-peptone) containing 2% glucose. Whole cell extracts ($\sim 1 \times 10^8$ cells) were prepared by vortexing cells with glass beads seven times (30 s/time) in cold lysis buffer (50 mM Tris-HCl, pH 7.5, 50 mM NaF, 5 mM sodium pyrophosphate, 1 mM EDTA, 0.5% Triton X-100, 10% glycerol, 1 mM DTT, complemented with PMSF (1 mM), and complete protease inhibitor mixture (Roche Applied Science)) (13). Proteins were separated by 8% SDS-PAGE, transferred to a PVDF membrane, and probed with anti-FLAG (Sigma) to detect H2B and ubiquitylated H2B or with P1291 antibody (Sigma) to detect Hsp82-

TAP, Hsc82-TAP, Doa1-TAP, Dbp2-TAP, and Snf1-TAP. Anti-G6PDH (Sigma) was used to detect G6PDH signals, as a loading control. The antibody signals were detected by enhanced chemiluminescence using ECL (Millipore). Band intensity was quantified on appropriate exposures using Bio-Spectrum Imaging System (UVP). For verification of Snf1 protein levels in the strain backgrounds shown in Fig. 1C, cells derived from RDKY3615 (S288C strain background) and MCY4908 (W303 strain background) were harvested, and extracts were prepared as described above. Proteins were separated by 8% SDS-PAGE and analyzed by immunoblotting using P1291 and G6PDH antibodies. The quantification ratios were calculated using ImageJ software (27) as intensities of Snf1-TAP or Snf1-3HA divided by those of G6PDH and then normalized to wild type. To measure the relative Snf1 protein level and its phosphorylation status (as shown in Figs. 1C, 2D, 3, A and G, 4, A, B, and F, and 5A), cultures were grown to exponential phase (A_{600} of 0.5) in synthetic complete (SC) medium containing 2% glucose. Cells were collected by centrifugation and immediately placed on ice. Western blotting was performed as described above. In brief, proteins were separated by 8% SDS-PAGE and analyzed by immunoblotting with P1291 antibody or anti-HA (3F10, Roche Applied Science) to detect Snf1-TAP or Snf1-HA, anti-phospho-Thr-172-AMPK (Cell Signaling Technologies) to detect Snf1 Thr-210 phosphorylation, and anti-G6PDH to detect G6PDH as a loading control. Signals were quantified using ImageJ software. To examine glucose regulation of Snf1 Thr-210 phosphorylation (Figs. 3B, 4C, and 5B), exponential phase cells were grown in 4% glucose, resuspended in 0.05% glucose for 15 min, and replenished with 2% glucose for 30 min, before being collected. Cell extracts were prepared as described above and analyzed by immunoblotting using anti-phospho-Thr-172-AMPK, P1291 antibody, anti-HA (3F10, Roche Applied Science), and anti-G6PDH (loading control). The quantification ratios were calculated (using ImageJ) as Snf1-T210-pho intensity divided by that of Snf1-TAP or Snf1-3HA.

RT-Quantitative PCR Analysis—Cells were harvested at exponential phase (A_{600} of 0.5) and immediately placed on ice. Transcripts for RT-quantitative PCR were extracted by the acid-phenol method, and reverse transcription reactions were performed using the SuperScript® III reverse transcriptase kit (Invitrogen); the resulting cDNA was used as template for quantitative PCR.

Detection of Hyperubiquitylated Snf1—Strains expressing galactose-inducible, FLAG-tagged Snf1 (FLAG-Snf1), and copper-inducible, His₆-myc-ubiquitin were constructed (as used in the experiments depicted in Fig. 2A). The *PDR5* gene was deleted to increase the sensitivity of cells to a variety of small molecules, such as MG132 (28). Overnight cultures grown in 10 ml of synthetic complete media lacking tryptophan and leucine (SC-TRP-LEU) with 2% galactose were diluted in 50 ml of SC-TRP-LEU containing 2% galactose from A_{600} 0.2 to 0.8 at 30 °C. MG132 (Sigma; final concentration of 25 μ M in DMSO) and CuSO₄ (final concentration of 500 μ M in DMSO) were added to each sample for 2 h, and the pellets were harvested by centrifugation. Samples were lysed in 500 μ l of IP buffer (50 mM Tris-HCl, pH 7.5, 300 mM NaCl, 2 mM MgCl₂, 0.1% Triton X-100,

10% glycerol, 1 mM β -mercaptoethanol, complemented with 20 mM deubiquitination inhibitor *N*-ethylmaleimide, 1 mM PMSF, and complete protease inhibitor mixture (Roche Applied Science)). Snf1-FLAG signals were purified from each lysate using anti FLAG-agarose beads (Sigma) for 2 h at 4 °C. After IP, samples were washed three times by inverting the mixtures 20 times using lysis buffer. Eluates were separated by SDS-PAGE and analyzed by immunoblotting with anti-ubiquitin (Cell Signaling Technologies) or anti-FLAG to detect Snf1-FLAG-Ub or Snf1-FLAG, respectively.

Snf1 Protein Stability Assay—Strains containing pMW18 (pGAL-FLAG-SNF1) (18) (as used in the experiment depicted in Fig. 2B) were grown overnight and then diluted in 50 ml of SC-LEU with 2% galactose to an A_{600} of 0.2 to 0.6 at 30 °C. To determine the effect of proteasome-mediated degradation, MG132 was added at each time point for 2 h before the start of the assay. Cultures were collected by centrifugation and resuspended in 15 ml of SC-LEU without sugar. Glucose (4%) and cycloheximide (Sigma; 200 μ g/ml) were added to the medium to terminate transcription and translation. Samples were harvested at the indicated time points (2 ml/each point) for immunoblotting using anti-FLAG and anti-G6PDH.

Replicative and Chronological Life Span—Replicative life span (RLS) assays were carried out as described previously (29). All experiments were carried out on YP plates plus 2% glucose. A dissection microscope MSM400 (Singer) was used to array the cells into the matrix; virgin mother cells were left in the located position, and then the numbers of daughter cells derived from each mother cell were recorded every 2–3 h. Survival curves show pooled data from multiple experiments with median RLS; the number of mother cells scored is shown in parentheses. Chronological life span assays were carried out as described previously, but with modifications (30). Briefly, strains from frozen stocks were used to inoculate YP plates plus 2% glucose; the resulting colonies were used to inoculate 5 ml of YP medium plus 2% glucose for overnight culture. After overnight culture, cells were diluted in 10 ml of fresh SC medium with 2% glucose to an A_{600} of 0.2 and then incubated at 30 °C with rotation at 200 rpm. to maintain the cells in suspension. Day 1 was considered to start after 24 h of culture. Cells ($\sim 1 \times 10^7$) were removed from the cultures and serially diluted 1:10 in sterile water in a 96-well plate; the dilutions were spotted onto YP plates plus 2% glucose from exponential phase (log phase) to day 4 or 5. The plates were incubated for 2–3 days to determine viability. Plate images were photographed using an imaging system (Alpha Innotech, Inc., San Leandro, CA).

Results

Level of Snf1 Protein Is Altered by Deletion of UBP8 and UBP10—To investigate potential substrates targeted by both Ubp8 and Ubp10, we searched the interaction data of Ubp8 and Ubp10 from BioGRID (31), which listed 285 and 165 interactions, respectively. We sorted the interaction results with Osprey, a Network Visualization System (31), identifying several individual biological processing factors that interact with both Ubp8 and Ubp10, including histone H2B, Hsc82, Hsp82, Doa1, Dbp2, and Snf1 (Fig. 1A). To determine whether or not these candidates are substrates of both Ubp8 and Ubp10, we

measured the levels of these candidate proteins fused to FLAG or TAP tags. Yeast cells were collected at exponential phase, and protein lysates were then extracted for use in Western blot. Consistent with the published results (14, 15, 32, 33), *ubp8 Δ ubp10 Δ* cells exhibited a higher level of H2B ubiquitylation (H2Bub) (Fig. 1B; *H2B*, arrow), although the heat shock-associated protein (Hsp82 and Hsc82), ubiquitin conjugation pathway protein (Doa1), and DEAD-box protein family (Dbp2) were unchanged as compared with controls (Fig. 1B). Intriguingly, Snf1 protein was reduced by about 5-fold in *ubp8 Δ ubp10 Δ* cells but unaffected in *ubp8 Δ* and *ubp10 Δ* single mutants (Fig. 1, B and C). A similar reduction in Snf1 was observed in a different genetic background bearing hemagglutinin (HA)-tagged Snf1 (Fig. 1C). Furthermore, *SNF1* mRNA was no different between wild type, *ubp8 Δ ubp10 Δ* , and *ubp8 Δ* and *ubp10 Δ* single mutants (Fig. 1D). This implied that the reduction of Snf1 protein level in *ubp8 Δ ubp10 Δ* cells may be due to post-transcriptional regulation or translational control.

Snf1 Is Not Targeted by Ubp8 and Ubp10 for Proteasomal Degradation—Both Ubp8 and Ubp10 are ubiquitin-specific proteases. We hypothesized that Snf1 may be targeted by Ubp8 and Ubp10 for deubiquitylation, and thus, in the absence of these proteases, Snf1 becomes highly ubiquitylated and degraded by the proteasome. To test this hypothesis, we examined whether ubiquitylated Snf1 (Snf1-Ub) is increased in *ubp8 Δ ubp10 Δ* cells. Both FLAG-tagged Snf1 (under the control of a galactose-inducible promoter) and His₆-myc epitope-tagged ubiquitin constructs were expressed in wild-type and *ubp8 Δ ubp10 Δ* cells. After immunoprecipitation using an antibody against the FLAG epitope, FLAG-bound proteins were resolved by SDS-PAGE, and Snf1-Ub was detected with anti-Ub. As expected, MG132-mediated inhibition of the proteasome significantly increased Snf1-Ub in wild type. Less Snf1-Ub was observed in the absence of both Ubp8 and Ubp10, with or without MG132 (Fig. 2A). To further confirm whether the stability of Snf1 in the *ubp8 Δ ubp10 Δ* cells was affected by the ubiquitin-proteasome pathway, we monitored the stability of Snf1 expressed under the control of a galactose-inducible promoter at various time points following a shift of cells to a repressing condition (the addition of glucose) in the presence of cycloheximide, thus blocking both transcription and translation of Snf1. Snf1 proteolysis occurred at the same level in both wild-type and *ubp8 Δ ubp10 Δ* cells, and MG132 treatment had no effect in either strain (Fig. 2B). However, there remains the possibility that Snf1 may be conjugated with polyubiquitin chains, which may cause proteasome-mediated degradation of Snf1 in the *ubp8 Δ ubp10 Δ* cells.

SUMOylation can also regulate the stability and function of Snf1 (21). To examine the role of ubiquitylation in the reduction of Snf1 in *ubp8 Δ ubp10 Δ* , we mutated the SUMOylation site (K549R) to inhibit SUMOylation-mediated recruitment of E3 ubiquitin ligases and subsequent degradation of Snf1. Intriguingly, Snf1-K549R protein levels were greater than those of Snf1 in both wild-type and *ubp8 Δ ubp10 Δ* cells; however, Snf1-K549R-3 $\times$ HA levels were still lower in *ubp8 Δ ubp10 Δ* than in wild type (Fig. 2C, lanes 3 and 6), indicating that Snf1 is regulated by other pathways in addition to SUMO-targeted ubiquitylation (possibly mRNA export or decay or translational

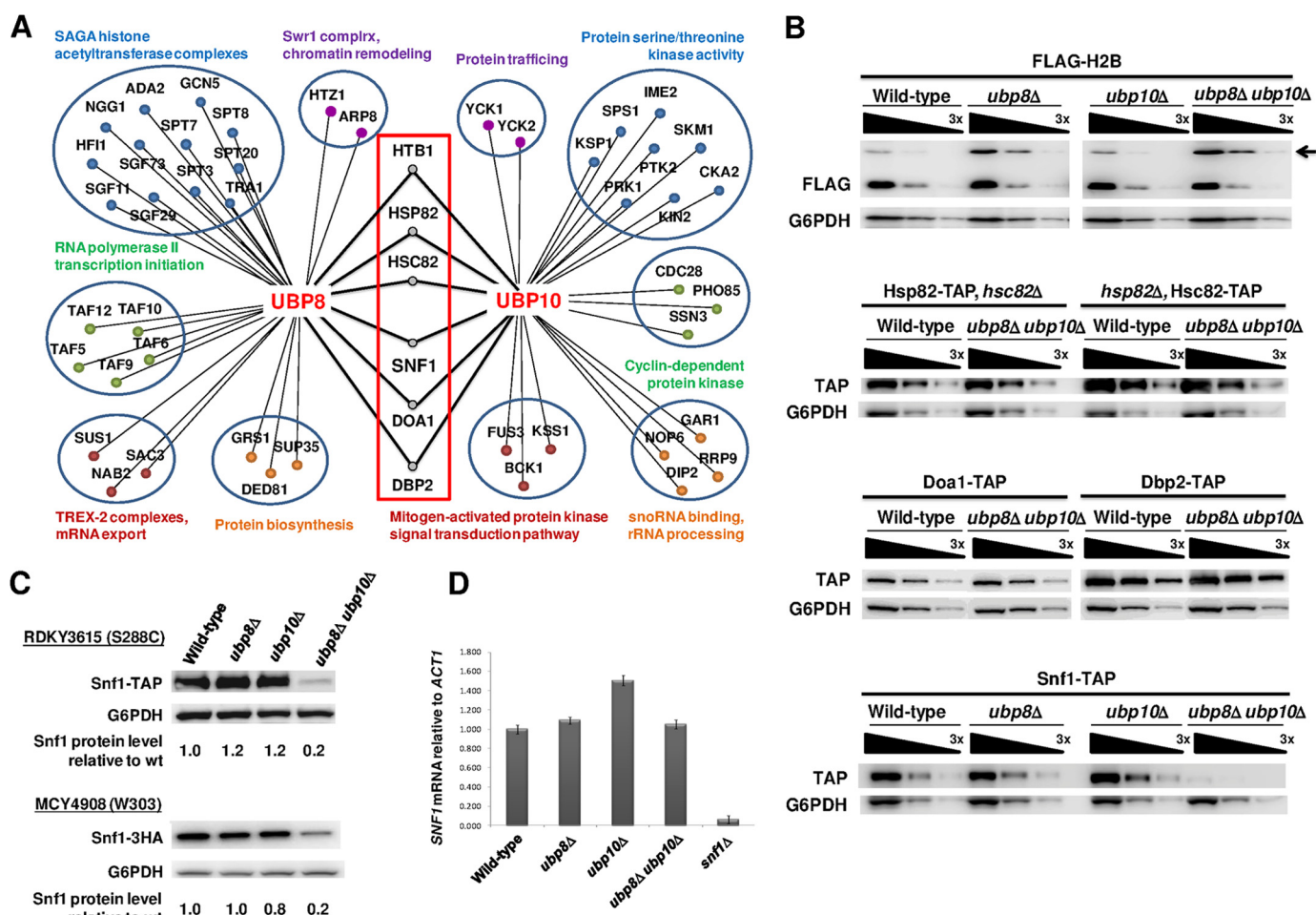


FIGURE 1. Snf1 interacts with both UBP8 and UBP10. *A*, candidate proteins that interact with UBP8 and UBP10. Lines connect UBP8 and UBP10 to genes that exhibit synthetic interactions (either positive or negative). The circles encompass genes that participate in similar processes. The red square indicates genes that interact with UBP8 and UBP10. *B*, Western blot analysis of FLAG-tagged H2B, TAP-tagged Hsp82, Hsc82, Doa1, Dbp2, and Snf1 levels in wild type and the indicated mutants. Serial 3-fold dilutions of each strain are shown. The arrow indicates H2B-ub signals. *C*, Western blot analysis of TAP-tagged Snf1 levels in the indicated RDKY3615 strains and HA-tagged Snf1 levels in the indicated MCY4908 strains at exponential phase. Values indicate relative intensity of Snf1-TAP or Snf1-HA divided by that of G6PDH, and then normalized to the equivalent ratio in wild type. *D*, quantitative RT-PCR analysis of *SNF1* mRNA expression normalized to *ACT1* mRNA in wild-type, *ubp8Δ*, *ubp10Δ*, *ubp8Δ ubp10Δ*, and *snf1Δ* strains ($n = 3$).

control). It is likely that Ubp8 and Ubp10 target other regulators that control Snf1 synthesis specifically, instead of targeting Snf1 directly. We tested three other candidates, Dhh1, Puf3, and Xrn1, which have been reported to be targets of Snf1 signaling involved in transcription-coupled mRNA decay (34–36). However, these factors did not participate in the regulation of Snf1 protein levels in *ubp8Δ ubp10Δ* cells (Fig. 2D). Therefore, at present it remains unclear how Ubp8 and Ubp10 specifically affect Snf1 mRNA.

Snf1 activation is required for utilization of alternative carbon sources (37). Therefore, we examined the growth of mutant cells in galactose compared with their growth in glucose. If the Snf1 protein level is correlated with its cellular functions, we predict that the function of Snf1 in *ubp8Δ ubp10Δ* cells would be impaired, and the increased protein level of Snf1 in *ubp8Δ ubp10Δ* cells would enhance its activity. We found that *ubp8Δ ubp10Δ* grew only slightly slower than wild-type cells on galactose. Surprisingly, introduction of the Snf1-K549R mutation into *ubp8Δ ubp10Δ* cells further reduced growth (Fig. 2E), despite Snf1 protein levels in the triple mutant being similar or higher than that in wild-type (*snf1Δ* with Snf1) cells (Fig. 2C,

lanes 2 and 6). Thus, these results suggest that the Snf1 in *ubp8Δ ubp10Δ* cells may be sufficient for Snf1-regulated processes.

Most Snf1-mediated Functions in ubp8Δ ubp10Δ Cells Are Unaffected, Despite the Reduction in Snf1 Protein—An earlier study reported that Snf1 protein level is also reduced in the *snf4Δ* mutant (38), which may be due to effects on formation of the Snf1 heterotrimer. To distinguish between the effects of reduced Snf1 levels in *snf4Δ* and *ubp8Δ ubp10Δ* cells, we measured the Snf1 protein level and the ratio of Snf1 phosphorylation in both mutant cells. Although the Snf1 protein level in *ubp8Δ ubp10Δ* cells was lower than that in *snf4Δ* cells, Snf1 phosphorylation was considerably reduced in *snf4Δ* cells as compared with that in *ubp8Δ ubp10Δ* cells, which exhibited levels comparable with wild type (Fig. 3A). Furthermore, the *ubp8Δ ubp10Δ* cells did not exhibit growth defects on different carbon sources (Fig. 3C), suggesting that the reduction of Snf1 protein in *ubp8Δ ubp10Δ* cells does not disrupt the function of the Snf1 complex. To further assess whether glucose-regulated Snf1 Thr-210 phosphorylation is affected by *ubp8Δ ubp10Δ*, exponential phase cells in 2% glucose were shifted to 0.05%

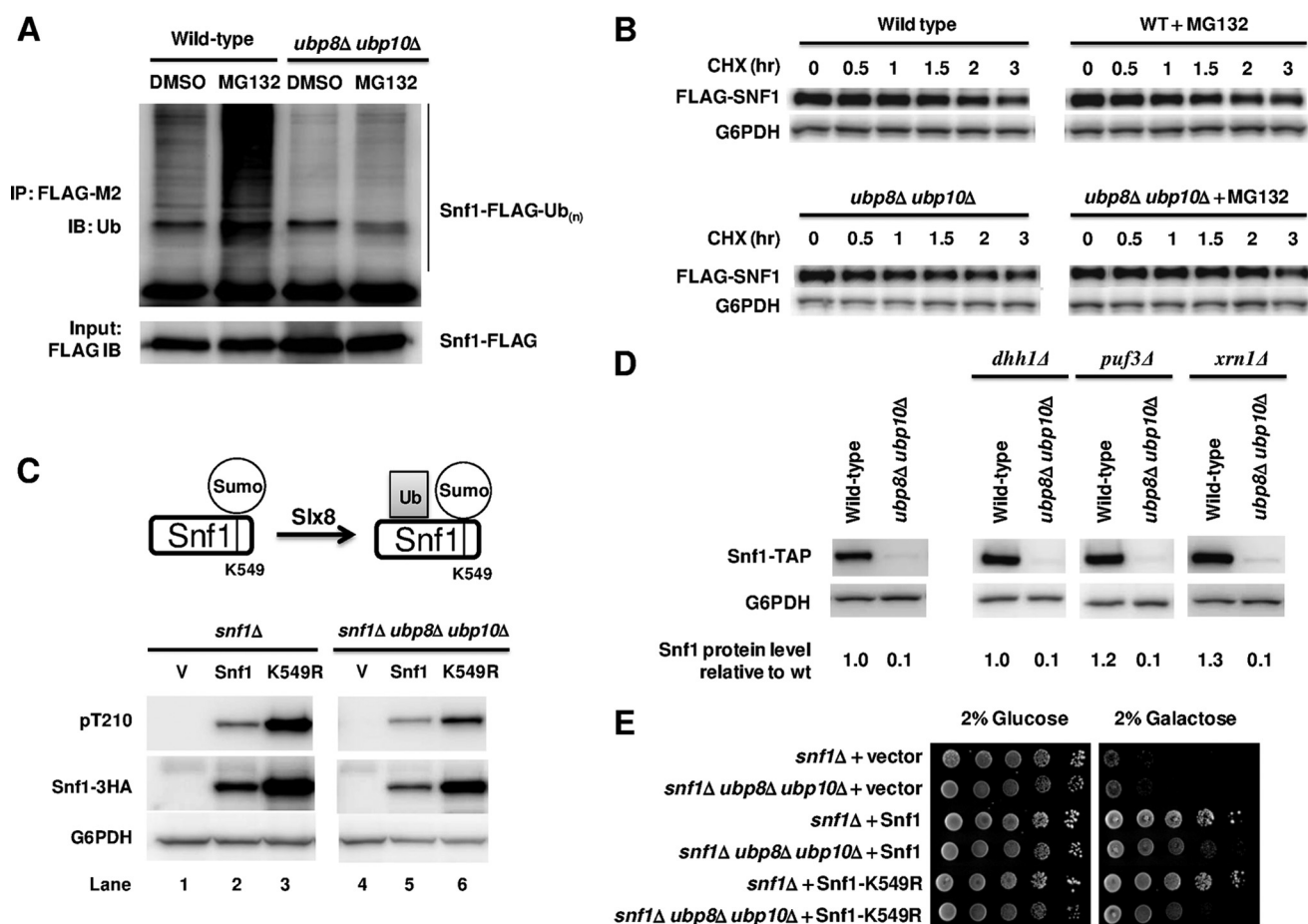


FIGURE 2. Reduction of Snf1 protein in *ubp8Δ ubp10Δ* is independent of proteasome-mediated degradation. A, Snf1-FLAG IP experiments using wild-type (*pdr5Δ*) and *ubp8Δ ubp10Δ* (*pdr5Δ ubp8Δ ubp10Δ*) strains. Polyubiquitinated Snf1-FLAG (Snf1-FLAG-Ub_(n)) was detected using anti-Ub antibody. IB, immunoblot. B, Western blot analysis of Snf1 stability in the indicated strains grown in the presence (right panel) or absence of MG132 (left panel) after release into cycloheximide (CHX). C, Western blot analysis of Snf1 in *snf1Δ* and *snf1Δ ubp8Δ ubp10Δ* cells with plasmids bearing vector only (v), wild-type SNF1 (Snf1), or SNF1 with a SUMOylation site mutation (K549R). D, Western blot of strains expressing TAP-tagged Snf1 and carrying the indicated mutations affecting transcription-coupled mRNA decay (*dhh1Δ*, *puf3Δ*, and *xrn1Δ*). E, growth of the indicated mutants on SC-LEU plates plus 2% glucose or 2% galactose; plates were photographed after 3 days of growth.

glucose for 15 min and then cultured in media with replenished glucose for 30 min (13). Despite the reduction of Snf1 in *ubp8Δ ubp10Δ*, the regulation of Snf1 Thr-210 phosphorylation was similar between wild-type and *ubp8Δ ubp10Δ* cells (Fig. 3B; a long exposure is shown to reveal the low levels of Snf1 in the mutant).

We further investigated whether the low level of Snf1 protein expression caused by the absence of both Ubp8 and Ubp10 influences the ability to grow on alternative carbon sources. Interestingly, we observed that *ubp8Δ ubp10Δ* and wild-type cells exhibited similar growth on galactose, raffinose, sucrose, and low glucose (0.5%). As expected, *snf1Δ* and *snf1Δ ubp8Δ ubp10Δ* cells could not grow on media containing alternative carbon sources or low glucose (Fig. 3C). Snf1 is also involved in the cellular responses to various environmental stresses, including sodium ion stress, alkaline pH, and oxidative stress, but not treatment with sorbitol (4). To determine whether the reduction in Snf1 caused by deletion of both *UBP8* and *UBP10* affected cellular resistance to various stressors, we performed several environmental stress tests using the indicated strains. We found that growth of *ubp8Δ ubp10Δ* cells was slightly impaired under 1 M NaCl and unaffected in alkaline pH,

whereas *snf1Δ* and *snf1Δ ubp8Δ ubp10Δ* triple mutant cells grew poorly under the same conditions (data not shown). Taken together, these findings suggest that Snf1 retains its activity despite its marked reduction upon deletion of both *UBP8* and *UBP10*, and it does not significantly compromise the tolerance of yeast cells to alternative carbon sources or environmental stresses.

Snf1 is also involved in the yeast aging process (5). Therefore, we speculated that Snf1 reduction caused by deletion of both *UBP8* and *UBP10* may affect the life span of yeast. To test this possibility, we measured the RLS of wild-type and mutant cells. As expected, the *sir2Δ* control cells exhibited a significantly shorter life span relative to the wild type (39). We further measured the RLS of *snf1Δ*, *ubp8Δ*, *ubp10Δ*, *ubp8Δ ubp10Δ*, and *snf1Δ ubp8Δ ubp10Δ* cells. The average RLS of wild type was 16.7 generations, whereas those of *ubp8Δ* and *ubp10Δ* cells were 27.2 and 21.2 generations, respectively (Fig. 3D). The RLS of *ubp8Δ* and *ubp10Δ* cells shared the same patterns as those observed in an earlier study (40). The average RLS of *ubp8Δ ubp10Δ* cells was 14.5 generations, that of *snf1Δ* cells was only 7.3 generations, and that of *snf1Δ ubp8Δ ubp10Δ* cells was 8.5 generations (Fig. 3D). Hence, reduced Snf1 in *ubp8Δ ubp10Δ*

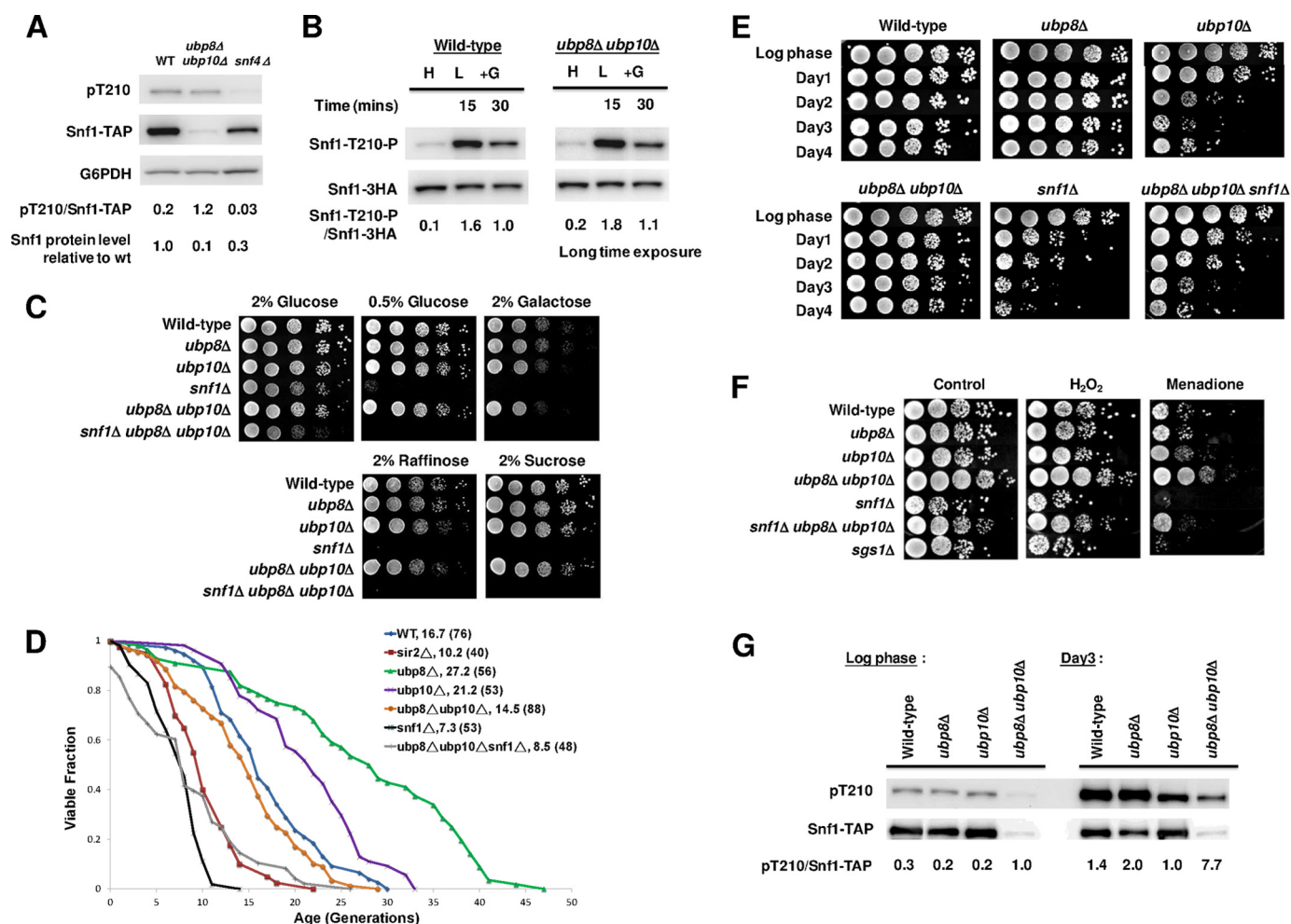


FIGURE 3. Snf1-mediated biological functions in a *ubp8Δ ubp10Δ* mutant. A, Western blot analysis of TAP-tagged Snf1 levels in the indicated strains. Upper values (pT210/Snf1-TAP) indicate the relative intensity of phosphorylated Snf1-Thr-210 divided by that of Snf1 protein. Lower values indicate relative intensity of Snf1-TAP (Snf1 protein level relative to WT), which was calculated by dividing the intensities of Snf1-TAP by the levels of G6PDH, and then normalizing the ratio to that of wild type. B, glucose regulation of Snf1 Thr-210 phosphorylation. Lanes are from the same blot, but longer exposures are shown for *ubp8Δ ubp10Δ* cells. Snf1-T210-P and Snf1-3HA were quantified using ImageJ. Values indicate the relative intensity of phosphorylated Snf1-Thr-210 divided by that of Snf1 protein. 4% glucose (H, high); 0.05% glucose (L, low); 2% glucose (+G, +glucose). C, utilization of alternative carbon sources by the indicated strains. Cells were adjusted to an A_{600} of 0.5, and 10-fold serial dilutions were spotted onto the indicated carbon sources. Plates were photographed after 3 days of growth. D, survival curve of the indicated strains, with median replicative life spans. Values in parentheses are the number of cells tested. E, chronological survival of the indicated strains expressing TAP-tagged Snf1. F, oxidative stress resistance of wild-type and the indicated mutants. The *sgs1Δ* mutant served as a positive control in this experiment. G, Western blot analysis of the indicated mutant strains at exponential phase (log phase) and stationary phase (day 3). The numbers indicate relative intensities of Snf1 phosphorylation, calculated as phosphorylation at Thr-210 of Snf1 divided by total Snf1 protein.

cells did not affect RLS in the same manner as deleting *SNF1*. Collectively, the above results demonstrate that the reduced level of Snf1 in *ubp8Δ ubp10Δ* cells retains sufficient activity to enable growth on alternative carbon sources and under environmental stress and to modulate RLS; this may be because regulation of Snf1 Thr-210 phosphorylation was unaffected in the mutant.

Cells Lacking Ubp8 and Ubp10 Exhibit a Higher Ratio of Active/Inactive Snf1 during Chronological Aging—Snf1 is implicated in chronological life span (CLS) regulation (41), and we thus hypothesized that Snf1 reduction in *ubp8Δ ubp10Δ* cells may affect CLS. We spotted cells at various days after glucose depletion and found that CLS was unaffected in *ubp8Δ ubp10Δ* cells (Fig. 3E). In contrast, *snf1Δ* and *snf1Δ ubp8Δ ubp10Δ* cells exhibited accelerated aging phenotypes (Fig. 3E), which are consistent with a previous report that *snf1Δ* cells possess a shorter CLS (41). The CLS results demonstrate that the low level of

Snf1 in *ubp8Δ ubp10Δ* cells is sufficient to protect against chronological aging. Yeast mutants with a premature chronological aging phenotype, such as *sgs1Δ* (42), are hypersensitive to heat and oxidative stress. As compared with wild-type cells, *snf1Δ* cells were observed to be more sensitive to extracellular reactive oxygen species damage induced by menadione or hydrogen peroxide, whereas *ubp8Δ ubp10Δ* cells were more resistant (Fig. 3F). To determine whether the resistance of *ubp8Δ ubp10Δ* cells to CLS and oxidative stress is linked to Snf1 activity, we analyzed Snf1 Thr-210 phosphorylation (indicating activation of Snf1) in cell extracts prepared at exponential phase (log phase) and stationary phase (day 3). Interestingly, we found that the ratio of active/inactive Snf1 was radically increased during chronological aging in *ubp8Δ ubp10Δ* cells as compared with the wild type (Fig. 3G); these results suggest that a feedback mechanism may stimulate Snf1 activation when a reduction in Snf1 protein is detected.

Novel Feedback Loop of Snf1 Activity in Yeast

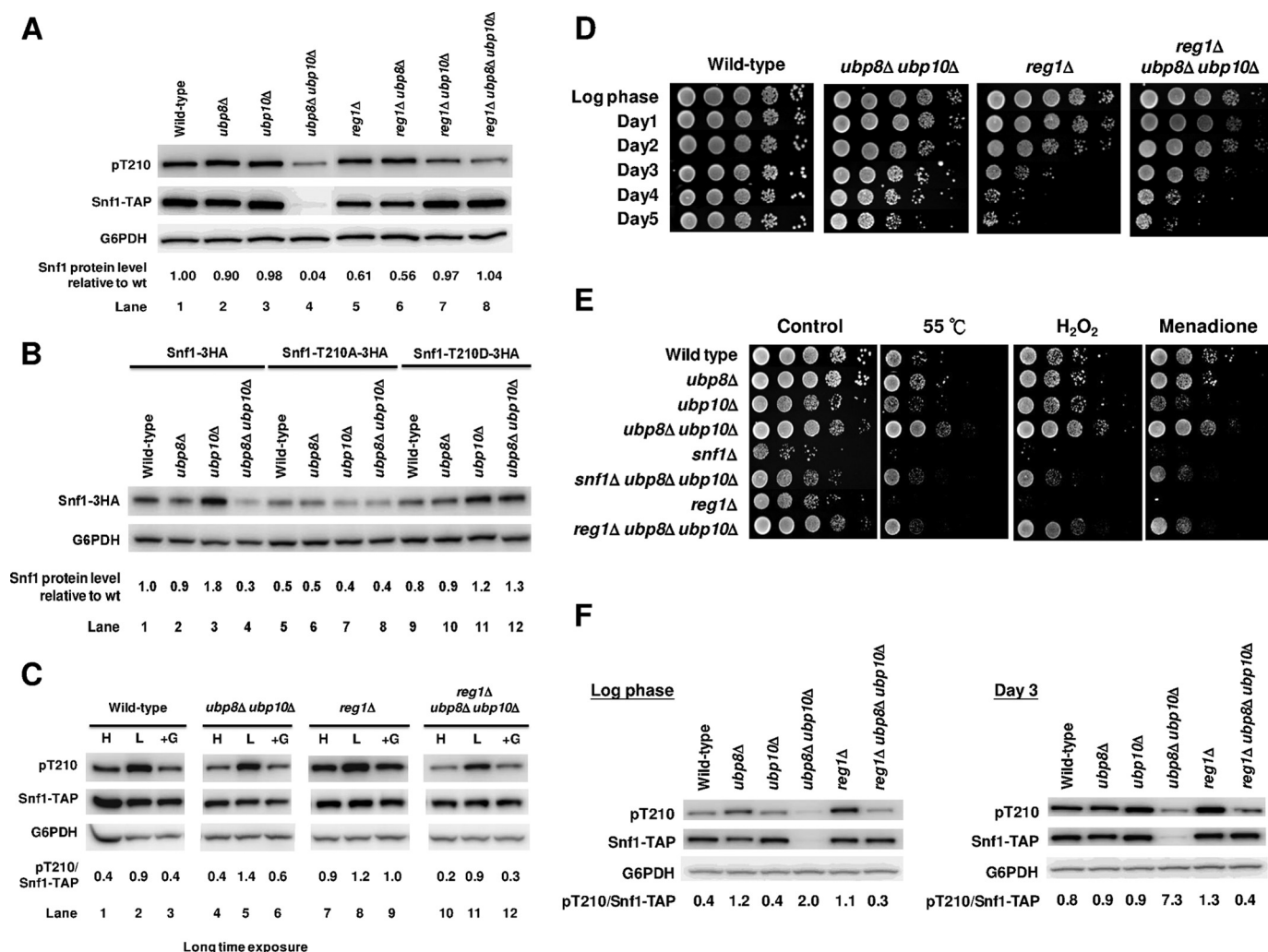


FIGURE 4. Snf1 protein level can be restored by modulation of its phosphorylation status in the *ubp8Δ ubp10Δ* mutant. A, Western blot of the indicated cells expressing TAP-tagged Snf1 and carrying *REG1* or *reg1Δ*. Relative Snf1 protein levels were quantified as described in Fig. 1C. B, Western blot analysis of the indicated strains expressing wild-type or phosphorylation-deficient alleles of Snf1 (Snf1, Snf1-T210A, or Snf1-T210D). Relative Snf1 protein levels were quantified as described in Fig. 1C. C, immunoblot analysis of the indicated strains, as described in Fig. 3B. 4% glucose (H, high); 0.05% glucose (L, low); 2% glucose (+G, +glucose). D, chronological survival of the indicated strains expressing TAP-tagged Snf1. Cells were photographed and arranged as in Fig. 3D. E, thermal and oxidative stress resistance of wild-type and the indicated mutants. F, Western blot analysis of the indicated wild-type and mutant strains at exponential phase (log phase) and stationary phase (day 3). The number indicates relative intensities of Snf1 phosphorylation as described in Fig. 3G.

Enhanced Phosphorylation of Snf1 Restores Its Protein Level in *ubp8Δ ubp10Δ* Cells and Overcomes Chronological Aging Stress—The above results suggest a novel feedback mechanism that may coordinate the activity of Snf1 with its cellular protein level (Fig. 3G). To test the hypothesis that Snf1 protein level can also be altered in response to its phosphorylation status, we deleted *REG1* to impair Snf1 dephosphorylation (12, 13, 43, 44). Surprisingly, although deletion of *REG1* (encoding a dominant phosphatase of Snf1) did not affect Snf1 phosphorylation in *ubp8Δ ubp10Δ* cells (Fig. 4A, lanes 4 and 8), it restored Snf1 protein to wild-type levels (Fig. 4A, lanes 4 and 8), although deletion of a different phosphatase, Sit4 (13), did not restore Snf1 (data not shown). In addition, a mutation in *SNF1* preventing phosphorylation at the activation loop (Snf1-T210A) inhibited the restoration of the Snf1 protein level, whereas a phosphorylation mimetic allele (Snf1-T210D) restored the Snf1 level in *ubp8Δ ubp10Δ* cells (Fig. 4B, lanes 4, 8 and 12). Furthermore, regulation of Snf1 Thr-210 phosphorylation in *reg1Δ*

ubp8Δ ubp10Δ cells was similar to wild type, unlike that in *reg1Δ* cells (Fig. 4C, lanes 7–12). Therefore, the Snf1 protein level and its phosphorylation status are interconnected and tightly regulated.

Subsequently, we investigated whether the restored level of Snf1 in *reg1Δ ubp8Δ ubp10Δ* cells affects CLS. Indeed, *REG1* deletion in *ubp8Δ ubp10Δ* cells extended the accelerated CLS in *reg1Δ* cells (Fig. 4D). Consistently, we also observed that *reg1Δ ubp8Δ ubp10Δ* cells were more resistant to extracellular reactive oxygen species damage than *reg1Δ* cells (Fig. 4E). Moreover, the activated form of Snf1 did not increase during chronological aging in *reg1Δ ubp8Δ ubp10Δ* cells as compared with *ubp8Δ ubp10Δ* cells, whereas *reg1Δ* cells, lacking Snf1 kinase regulation, exhibited defects in the stress response and CLS (Fig. 4, D–F). These results suggest that the restoration of Snf1 levels in *reg1Δ ubp8Δ ubp10Δ* cells may help overcome CLS and extracellular oxidative stress. This indicates that the newly identified feedback regulation of Snf1 activity may

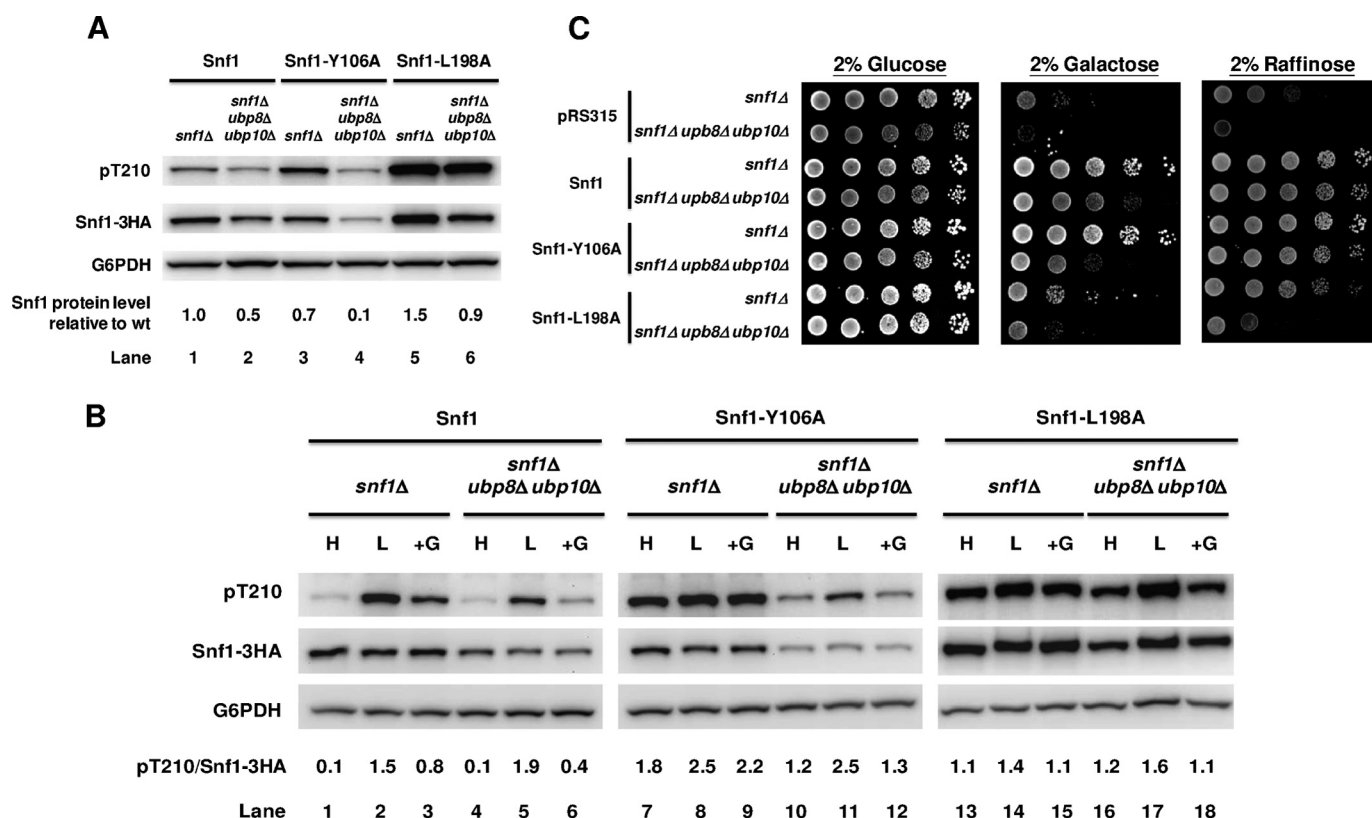


FIGURE 5. Feedback loop regulation between Snf1 protein level and its phosphorylation is blocked in the Snf1-L198A mutant, resulting in a decrease of Snf1 activity. A, Western blot analysis of the indicated strains expressing wild-type and mutant alleles of Snf1 (Snf1, Snf1-Y106A, and Snf1-L198A) with or without *UBP8* and *UBP10*. B, glucose regulation of Snf1 phosphorylation was examined in *snf1Δ* or *snf1Δ ubp8Δ ubp10Δ* cells expressing Snf1 or its mutant alleles (Y106A or L198A). 4% glucose (H, high); 0.05% glucose (L, low); 2% glucose (+G, +glucose). C, growth of the indicated mutants on SC-LEU plates plus 2% glucose, galactose, or raffinose; plates were photographed after 3 days of growth.

involve a single loop, and that dynamic regulation of both protein and phosphorylation status of Snf1 is required for effective running of the loop.

To further understand the role of Snf1 Thr-210 phosphorylation in feedback control of Snf1 activity, we took advantage of two Snf1 alleles, Tyr-106 mutant (Snf1-Y106A) and Leu-198 mutant (Snf1-L198A), both of which relieve glucose-mediated Snf1 Thr-210 dephosphorylation (22). As expected, both the Tyr-106 and Leu-198 mutations increased Snf1 Thr-210 phosphorylation in glucose (Fig. 5A, lanes 3 and 5). Of particular note, deletion of *UBP8* and *UBP10* reduced both protein levels and Thr-210 phosphorylation of the Tyr-106 mutant, but only the protein level of the Leu-198 mutant was reduced under glucose culture conditions. Moreover, the regulation of Snf1 Thr-210 phosphorylation remained defective in the Leu-198 mutant but was restored in the Tyr-106 mutant, when combined with deletion of *UBP8* and *UBP10* (Fig. 5B, lanes 7–18). We proceeded to examine whether restoration of Snf1 Thr-210 phosphorylation in a Y106A *ubp8Δ ubp10Δ* triple mutant affected growth on galactose or raffinose; as expected, the Tyr-106 mutant grew better than the Leu-198 mutant on both galactose and raffinose plates (Fig. 5C). These results are consistent with a previous report that Snf1 function is not affected by the Tyr-106 mutation but is disrupted by the Leu-198 mutation (22). Collectively, these results suggest that the levels of Snf1 protein and phosphorylation are reciprocally regulated and important for the adaptation of yeast to environmental stress.

Discussion

The regulation of Snf1 activity is a complex process (1). Previous evidence suggested that Snf1 can be SUMOylated, which promotes the conjugation of ubiquitin for degradation. This may in turn cause a decrease of Snf1 activity (18, 21). Our current findings reveal a novel layer of regulation of Snf1 activity, involving a feedback loop between Snf1 protein level and its phosphorylation. This finely tuned mechanism may help protect against different stress conditions (Fig. 6).

In wild-type cells, the presence of normal levels of Snf1 protein mediates an inhibitory effect on Snf1 phosphorylation, and its phosphorylation status in turn transmits a feedback signal for maintaining the expression of Snf1 through an as yet unidentified mechanism. We suggest that the Snf1 protein level and its phosphorylation status are reciprocally regulated and crucial for the adaptation of yeast to environmental stress (Fig. 6A). However, we observed that although the level of Snf1 protein is reduced in *ubp8Δ ubp10Δ* cells, Snf1 activity is only mildly affected. The reason for this surprising result is that the remaining Snf1 protein is hyper-phosphorylated, preserving Snf1 function for protection under different stress conditions (Figs. 3 and 6B). In addition, deletion of *REG1* (which encodes a phosphatase primarily responsible for Snf1 dephosphorylation) elevates Snf1 protein in cells lacking Ubp8 and Ubp10, indicating that enhanced phosphorylation of Snf1 plays an important role in feedback loop control of Snf1 protein level (Fig. 6C).

Novel Feedback Loop of Snf1 Activity in Yeast

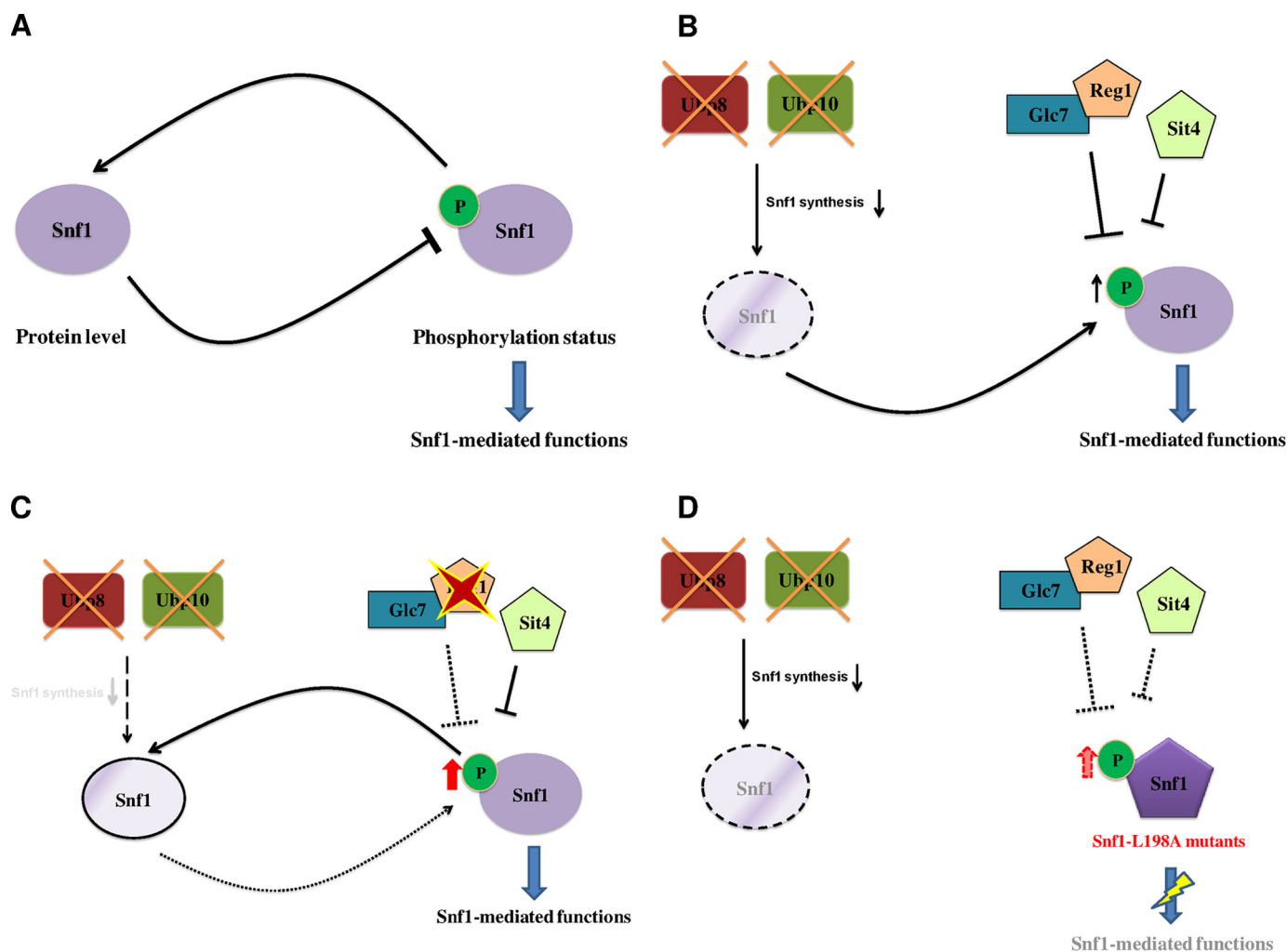


FIGURE 6. Models for feedback loop regulation between the protein level of Snf1 and its phosphorylation in *ubp8Δ ubp10Δ* mutants. A, working model for the interconnected regulation of Snf1 protein level and its phosphorylation status. In wild-type cells, normal levels of Snf1 protein exert an inhibitory effect on Snf1 phosphorylation, and its phosphorylation status in turn transmits a feedback signal for maintaining the expression of Snf1. B, in the absence of both Ubp8 and Ubp10, Snf1 synthesis is reduced. The reduced level of Snf1 promotes the hyperphosphorylation of Snf1 to counteract environmental stress. C, constitutive activation of Snf1 kinase caused by loss of a dephosphorylation regulation factor, Reg1 (*reg1Δ*), restores the protein level of Snf1 via a feedback loop mechanism; other phosphatases, such as Sit4, may facilitate regulation of Snf1 activity. D, dynamic feedback loop between Snf1 protein level and its phosphorylation status may be disrupted in the Snf1-L198A mutant.

Thus, the dominant dephosphorylation activity of Reg1 maintains a low level of Snf1 phosphorylation, preventing transmission of the feedback signal. However, inappropriately elevated Snf1 activity may be deleterious to cell growth (13, 44). Consistent with this idea, the *reg1Δ ubp8Δ ubp10Δ* mutant may sense elevated Snf1 activity and then transmit a signal to a Snf1 synthesis mediator to restore Snf1 protein levels (Figs. 4A and 6C). Once the Snf1 protein level is restored, Snf1 Thr-210 phosphorylation may be sustained by a subordinate phosphatase, Sit4 (13); thus, the triple mutant cells are more resistant to chronological aging and oxidative stresses than *reg1Δ* cells (Figs. 4, C–E, and 6C). In contrast, the Snf1-L198A allele may disrupt the accessibility of Thr-210 in the Snf1 catalytic domain to protein phosphatases, thereby blocking feedback loop control (Fig. 6D).

What is the post-transcriptional mechanism behind the decrease in Snf1 protein in *ubp8Δ ubp10Δ* cells? The transport of newly synthesized mRNA from nucleus to cytoplasm may involve the nuclear pore complex, which is conserved from

yeast to human (45, 46). Both Ubp8 and Ubp10 are primarily localized to the nucleus (47). We propose that Ubp8 and Ubp10 may control Snf1 protein synthesis by targeting factors that facilitate pre-mRNA processing and/or export. In *S. cerevisiae*, many such factors are reported to be ubiquitinated (48), including mRNA cap methyltransferase (Abd1), ATP-dependent RNA helicases (Dbp1, Dbp3, Ded1, Prp43, Sub2, and Tif2), mRNA transport factors (Gfd1 and Yra1), nuclear polyadenylated RNA-binding protein (Hrp1), pre-mRNA-processing factor (Prp39), and mRNA decay protein (Upf3). Ubp8 and Ubp10 may deubiquitinate some of these proteins, affecting mRNA processing and export. There may be additional factors that facilitate specific processing of Snf1 mRNA in response to cellular energy levels, connecting Snf1 activity with its protein synthesis.

Taken together, our findings reveal the existence of a single feedback loop connecting Snf1 protein level and its phosphorylation status. This provides new insight into the regulation of Snf1 Thr-210 phosphorylation, which is notably correlated

with Reg1-Glc7 protein phosphatases. This dynamic modulation of Snf1 is critical for adaptation to different environmental stresses.

Acknowledgments—We thank M. Carlson, S. Y. R. Dent, and J.-Y. Leu for yeast strains and plasmids. We also thank D. E. Wright for critical reading of the manuscript.

References

- Hedbacker, K., and Carlson, M. (2008) SNF1/AMPK pathways in yeast. *Front. Biosci.* **13**, 2408–2420
- Hardie, D. G. (2011) AMP-activated protein kinase: an energy sensor that regulates all aspects of cell function. *Genes Dev.* **25**, 1895–1908
- Jiang, R., and Carlson, M. (1996) Glucose regulates protein interactions within the yeast SNF1 protein kinase complex. *Genes Dev.* **10**, 3105–3115
- Hong, S. P., and Carlson, M. (2007) Regulation of snf1 protein kinase in response to environmental stress. *J. Biol. Chem.* **282**, 16838–16845
- Ashrafi, K., Lin, S. S., Manchester, J. K., and Gordon, J. I. (2000) Sip2p and its partner snf1p kinase affect aging in *S. cerevisiae*. *Genes Dev.* **14**, 1872–1885
- McCartney, R. R., and Schmidt, M. C. (2001) Regulation of Snf1 kinase. Activation requires phosphorylation of threonine 210 by an upstream kinase as well as a distinct step mediated by the Snf4 subunit. *J. Biol. Chem.* **276**, 36460–36466
- Vincent, O., Townley, R., Kuchin, S., and Carlson, M. (2001) Subcellular localization of the Snf1 kinase is regulated by specific β subunits and a novel glucose signaling mechanism. *Genes Dev.* **15**, 1104–1114
- Nath, N., McCartney, R. R., and Schmidt, M. C. (2003) Yeast Pak1 kinase associates with and activates Snf1. *Mol. Cell. Biol.* **23**, 3909–3917
- Sutherland, C. M., Hawley, S. A., McCartney, R. R., Leech, A., Stark, M. J., Schmidt, M. C., and Hardie, D. G. (2003) Elm1p is one of three upstream kinases for the *Saccharomyces cerevisiae* SNF1 complex. *Curr. Biol.* **13**, 1299–1305
- Hong, S. P., Leiper, F. C., Woods, A., Carling, D., and Carlson, M. (2003) Activation of yeast Snf1 and mammalian AMP-activated protein kinase by upstream kinases. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 8839–8843
- Tu, J., and Carlson, M. (1994) The GLC7 type 1 protein phosphatase is required for glucose repression in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **14**, 6789–6796
- Tu, J., and Carlson, M. (1995) REG1 binds to protein phosphatase type 1 and regulates glucose repression in *Saccharomyces cerevisiae*. *EMBO J.* **14**, 5939–5946
- Ruiz, A., Xu, X., and Carlson, M. (2011) Roles of two protein phosphatases, Reg1-Glc7 and Sit4, and glycogen synthesis in regulation of SNF1 protein kinase. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 6349–6354
- Henry, K. W., Wyce, A., Lo, W. S., Duggan, L. J., Emre, N. C., Kao, C. F., Pillus, L., Shilatifard, A., Osley, M. A., and Berger, S. L. (2003) Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. *Genes Dev.* **17**, 2648–2663
- Gardner, R. G., Nelson, Z. W., and Gottschling, D. E. (2005) Ubp10/Dot4p regulates the persistence of ubiquitinated histone H2B: distinct roles in telomeric silencing and general chromatin. *Mol. Cell. Biol.* **25**, 6123–6139
- Wright, D. E., Wang, C. Y., and Kao, C. F. (2011) Histone ubiquitylation and chromatin dynamics. *Front. Biosci.* **17**, 1051–1078
- Ptacek, J., Devgan, G., Michaud, G., Zhu, H., Zhu, X., Fasolo, J., Guo, H., Jona, G., Breitkreutz, A., Sopko, R., McCartney, R. R., Schmidt, M. C., Rachidi, N., Lee, S. J., Mah, A. S., et al. (2005) Global analysis of protein phosphorylation in yeast. *Nature* **438**, 679–684
- Wilson, M. A., Koutelou, E., Hirsch, C., Akdemir, K., Schibler, A., Barton, M. C., and Dent, S. Y. (2011) Ubp8 and SAGA regulate Snf1 AMP kinase activity. *Mol. Cell. Biol.* **31**, 3126–3135
- Richardson, L. A., Reed, B. J., Charette, J. M., Freed, E. F., Fredrickson, E. K., Locke, M. N., Baserga, S. J., and Gardner, R. G. (2012) A conserved deubiquitinating enzyme controls cell growth by regulating RNA polymerase I stability. *Cell Rep.* **2**, 372–385
- Gallego-Sánchez, A., Andrés, S., Conde, F., San-Segundo, P. A., and Bueno, A. (2012) Reversal of PCNA ubiquitylation by Ubp10 in *Saccharomyces cerevisiae*. *PLoS Genet.* **8**, e1002826
- Simpson-Lavy, K. J., and Johnston, M. (2013) SUMOylation regulates the SNF1 protein kinase. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 17432–17437
- Momcilovic, M., and Carlson, M. (2011) Alterations at dispersed sites cause phosphorylation and activation of SNF1 protein kinase during growth on high glucose. *J. Biol. Chem.* **286**, 23544–23551
- Hirschhorn, J. N., Bortvin, A. L., Ricupero-Hovasse, S. L., and Winston, F. (1995) A new class of histone H2A mutations in *Saccharomyces cerevisiae* causes specific transcriptional defects in vivo. *Mol. Cell. Biol.* **15**, 1999–2009
- Chen, C., and Kolodner, R. D. (1999) Gross chromosomal rearrangements in *Saccharomyces cerevisiae* replication and recombination defective mutants. *Nat. Genet.* **23**, 81–85
- Guedener, U., Heinisch, J., Koehler, G. J., Voss, D., and Hegemann, J. H. (2002) A second set of loxP marker cassettes for Cre-mediated multiple gene knockouts in budding yeast. *Nucleic Acids Res.* **30**, e23
- Woods, R. A., and Gietz, R. D. (2001) High-efficiency transformation of plasmid DNA into yeast. *Methods Mol. Biol.* **177**, 85–97
- Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **9**, 671–675
- Balzi, E., Wang, M., Leterme, S., Van Dyck, L., and Goffeau, A. (1994) PDR5, a novel yeast multidrug resistance conferring transporter controlled by the transcription regulator PDR1. *J. Biol. Chem.* **269**, 2206–2214
- Steffen, K. K., Kennedy, B. K., and Kaerberlein, M. (2009) Measuring replicative life span in the budding yeast. *J. Vis. Exp.* **28**, 1209
- Smith, D. L., Jr., McClure, J. M., Matecic, M., and Smith, J. S. (2007) Calorie restriction extends the chronological life span of *Saccharomyces cerevisiae* independently of the Sirtuins. *Aging Cell* **6**, 649–662
- Breitkreutz, B. J., Stark, C., Regul, T., Boucher, L., Breitkreutz, A., Livstone, M., Oughtred, R., Lackner, D. H., Bähler, J., Wood, V., Dolinski, K., and Tyers, M. (2008) The BioGRID Interaction Database: 2008 update. *Nucleic Acids Res.* **36**, D637–D640
- Emre, N. C., Ingvarsdottir, K., Wyce, A., Wood, A., Krogan, N. J., Henry, K. W., Li, K., Marmorstein, R., Greenblatt, J. F., Shilatifard, A., and Berger, S. L. (2005) Maintenance of low histone ubiquitylation by Ubp10 correlates with telomere-proximal Sir2 association and gene silencing. *Mol. Cell* **17**, 585–594
- Schulze, J. M., Hentrich, T., Nakanishi, S., Gupta, A., Emberly, E., Shilatifard, A., and Kobor, M. S. (2011) Splitting the task: Ubp8 and Ubp10 deubiquitinate different cellular pools of H2BK123. *Genes Dev.* **25**, 2242–2247
- Mitchell, S. F., Jain, S., She, M., and Parker, R. (2013) Global analysis of yeast mRNPs. *Nat. Struct. Mol. Biol.* **20**, 127–133
- Freeberg, M. A., Han, T., Moresco, J. J., Kong, A., Yang, Y. C., Lu, Z. J., Yates, J. R., and Kim, J. K. (2013) Pervasive and dynamic protein-binding sites of the mRNA transcriptome in *Saccharomyces cerevisiae*. *Genome Biol.* **14**, R13
- Braun, K. A., Vaga, S., Dombek, K. M., Fang, F., Palmisano, S., Aebersold, R., and Young, E. T. (2014) Phosphoproteomic analysis identifies proteins involved in transcription-coupled mRNA decay as targets of Snf1 signaling. *Sci. Signal.* **7**, ra64
- Carlson, M. (1999) Glucose repression in yeast. *Curr. Opin. Microbiol.* **2**, 202–207
- Ruiz, A., Liu, Y., Xu, X., and Carlson, M. (2012) Heterotrimer-independent regulation of activation-loop phosphorylation of Snf1 protein kinase involves two protein phosphatases. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 8652–8657
- Kaerberlein, M., McVey, M., and Guarente, L. (1999) The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. *Genes Dev.* **13**, 2570–2580
- Rhie, B. H., Song, Y. H., Ryu, H. Y., and Ahn, S. H. (2013) Cellular aging is associated with increased ubiquitylation of histone H2B in yeast telomeric heterochromatin. *Biochem. Biophys. Res. Commun.* **439**, 570–575
- Weinberger, M., Mesquita, A., Carroll, T., Marks, L., Yang, H., Zhang, Z., Ludovico, P., and Burhans, W. C. (2010) Growth signaling promotes chronological aging in budding yeast by inducing superoxide anions that

Novel Feedback Loop of Snf1 Activity in Yeast

- inhibit quiescence. *Aging* **2**, 709–726
42. Madaia, F., Gattazzo, C., Wei, M., Fabrizio, P., Burhans, W. C., Weinberger, M., Galbani, A., Smith, J. R., Nguyen, C., Huey, S., Comai, L., and Longo, V. D. (2008) Longevity mutation in SCH9 prevents recombination errors and premature genomic instability in a Werner/Bloom model system. *J. Cell Biol.* **180**, 67–81
43. Rubenstein, E. M., McCartney, R. R., Zhang, C., Shokat, K. M., Shirra, M. K., Arndt, K. M., and Schmidt, M. C. (2008) Access denied: Snf1 activation loop phosphorylation is controlled by availability of the phosphorylated threonine 210 to the PP1 phosphatase. *J. Biol. Chem.* **283**, 222–230
44. Ruiz, A., Xu, X., and Carlson, M. (2013) Ptc1 protein phosphatase 2C contributes to glucose regulation of SNF1/AMP-activated protein kinase (AMPK) in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **288**, 31052–31058
45. Pemberton, L. F., Blobel, G., and Rosenblum, J. S. (1998) Transport routes through the nuclear pore complex. *Curr. Opin. Cell Biol.* **10**, 392–399
46. Görlich, D., and Kutay, U. (1999) Transport between the cell nucleus and the cytoplasm. *Annu. Rev. Cell Dev. Biol.* **15**, 607–660
47. Huh, W. K., Falvo, J. V., Gerke, L. C., Carroll, A. S., Howson, R. W., Weissman, J. S., and O'Shea, E. K. (2003) Global analysis of protein localization in budding yeast. *Nature* **425**, 686–691
48. Lee, W. C., Lee, M., Jung, J. W., Kim, K. P., and Kim, D. (2008) SCUD: *Saccharomyces cerevisiae* ubiquitination database. *BMC Genomics* **9**, 440

**Feedback Control of Snf1 Protein and Its Phosphorylation Is Necessary for
Adaptation to Environmental Stress**

Hsiang-En Hsu, Tzu-Ning Liu, Chung-Shu Yeh, Tien-Hsien Chang, Yi-Chen Lo and
Cheng-Fu Kao

J. Biol. Chem. 2015, 290:16786-16796.

doi: 10.1074/jbc.M115.639443 originally published online May 6, 2015

Access the most updated version of this article at doi: [10.1074/jbc.M115.639443](https://doi.org/10.1074/jbc.M115.639443)

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

This article cites 48 references, 24 of which can be accessed free at
<http://www.jbc.org/content/290/27/16786.full.html#ref-list-1>