Convergence of the Target of Rapamycin and the Snf1 Protein Kinase Pathways in the Regulation of the Subcellular Localization of Msn2, a Transcriptional Activator of STRE (Stress Response Element)-regulated Genes*

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The subcellular localization of Msn2, a transcriptional activator of STRE (stress response element)-regulated genes, is modulated by carbon source availability. In cells growing in glucose, Msn2 is located mainly in the cytosol, whereas in carbon source-starved cells, Msn2 is located largely inside the nucleus. However, in cells lacking Reg1 (the regulatory subunit of the Reg1/Glc7 protein phosphatase complex), the regulation of subcellular distribution is absent, Msn2 being constitutively present in the cytosol. The localization defect in these mutants is specific for carbon starvation stress, and it is because of the presence of an abnormally active Snf1 protein kinase that inhibits the nuclear localization of Msn2 upon carbon starvation. Active Snf1 kinase is also able to avoid the effects of rapamycin, a drug that by inhibiting the TOR kinase pathway leads to a nuclear localization of Msn2 in wild type cells. Therefore, active Snf1 and the TOR kinase pathway may affect similar cytosolic steps in the regulation of the subcellular localization of Msn2.

The yeast Saccharomyces cerevisiae regulates metabolism, gene expression, and growth in response to carbon source availability. In the presence of glucose, the expression of a large number of genes, including those involved in the utilization of alternative carbon sources, gluconeogenesis, and respiration, is repressed at the level of transcription by a process known as glucose repression (see Refs. 1–4 for review). Biochemical and genetic studies have identified several crucial players in this pathway. Snf1 (Cat1) is a serine-threonine protein kinase that regulates transcription by inhibiting transcriptional repressors (e.g. Mig1) or by stimulating transcriptional activators (e.g. Cat8 and Sip4). The Snf1 protein kinase is found in complexes containing the activating subunit Snf4 (Cat3) and members of the Sip1/Sip2/Gal83 family (5), and its activity is regulated negatively by glucose (6). The Reg1/Glc7 protein phosphatase complex is involved in the regulation of the activity of the Snf1 kinase complex. GLC7 (CID1) is an essential gene that encodes the catalytic subunit of the yeast protein phosphatase type 1 (PP1) (7). It is involved in glucose repression and also in the regulation of different processes including glyogen metabolism, translation initiation, sporation, chromosome segregation, and cell cycle progression (7–12). Glc7 participates in the regulation of these processes by binding to specific regulatory subunits that target the phosphatase to the corresponding substrates (13–16). Reg1 (Hex2) is one of these regulatory subunits and targets Glc7 to substrates involved in the glucose repression pathway and other processes (15, 17–19). In response to a glucose signal, Glc7 (targeted by Reg1) dephosphorylates Snf1 kinase and inactivates the complex (20, 21). In the absence of Reg1, Glc7 cannot perform its function, so the Snf1 kinase complex is constitutively active, even in the presence of glucose (20, 21). Another crucial component of the glucose repression pathway is hexokinase PII (Hk2). This protein also participates in the regulation of the Snf1 kinase complex by regulating the phosphorylation status of Reg1 (21).

Another process that controls growth and metabolism in response to nutrient availability is the TOR kinase pathway (22–25). The key players in this pathway are the TOR protein kinases, Tor1 and Tor2, members of the phosphatidylinositol-related kinase family. Activation of this pathway leads to several physiological events including activation of protein translation. This pathway is inhibited by nitrogen starvation and by the drug rapamycin, a macrolide antibiotic with anti proliferative and immunosuppressive effects. Recently, it has been proposed that the TOR pathway also regulates the transcriptional response related to changes in both nitrogen and carbon source availability. As an example of the latter case, the TOR pathway affects the subcellular localization of Msn2, a transcriptional activator of genes regulated by stress (STRE-regulated genes), by regulating its interaction with Bmh2, a yeast member of the 14-3-3 protein family, which acts as a cytosolic anchor (22).

In cells growing in glucose, cAMP-dependent protein kinase A phosphatases nuclear Msn2 and allows its transport out of the nucleus (26, 27). Once in the cytosol, phosphorylated Msn2 binds to Bmh2 and forms a stable complex (22). In this study we describe a convergence of the TOR and the Snf1 kinase pathways in the regulation of the subcellular localization of Msn2, ensuring a proper adaptation to carbon source availability.

EXPERIMENTAL PROCEDURES

Strains and Genetic Methods—S. cerevisiae strains used in this study are listed in Table I. To construct strains reg1Δ snf1Δ and reg1Δ gal83Δ, a BamHI fragment of pUC-snf1::KanMX4 (see below) and a Xhol/SpeI fragment from plasmid pOV1 (28) were used to introduce, respectively, snf1::KanMX4 and gal83::TRP1 mutated alleles by gene disruption

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PAGE using 7% polyacrylamide gels and analyzed by immunoblotting using anti-Msn2 antiseraum (26). Antibodies were detected by enhanced chemiluminescence with ECL or ECL Plus reagents (Amersham Biosciences).

RESULTS

Active Snf1 Protein Kinase Prevents Nuclear Localization of Msn2 in Response to Carbon Starvation—As described in the Introduction, the subcellular localization of Msn2, a transcriptional activator of STRE-regulated genes, is modulated by carbon source availability. In cells growing in glucose, Msn2 is localized mainly in the cytosol, whereas after carbon starvation, it is located inside the nucleus (22, 26, 27). Because the Snf1 protein kinase pathway is one of the main regulators of glucose signaling, we decided to determine whether components involved in the Snf1 pathway were also involved in the regulation of the subcellular localization of Msn2 in response to glucose. To this end, we used an in frame fusion of Msn2 to the green fluorescent protein (plasmid pMSN2-GFP) (26). This construct contains the MNS2-GFP gene fusion under the control of the ADH1 promoter in a low copy centromeric plasmid and complements the stress response defects of msn2Δ strains (40). As shown in Fig. 1, in the wild type strain, Msn2-GFP was transported into the nucleus upon carbon starvation, as described previously (26). However, in the regΔ mutant, a GFP tag was inserted at the 3′ end of the chromosomal MNS2 gene. This was carried out in several steps. First, an XbaI/HindIII fragment from plasmid pMSN2-GFP, containing most of the MNS2-GFP coding region was subcloned into pRS306 (31). This plasmid was digested with EcoRI, which released around 500 bp from the central part of the MNS2 coding region. Flanking regions were then used to direct the integration of the linear plasmid into the chromosomal MNS2 locus by gene disruption. In this way, we placed the expression of the MNS2-GFP fusion under the control of the original MNS2 promoter. Integrative transformants were confirmed by PCR analysis. To construct YEpACT-HA-Gal83, a fragment from pHA-Gal83 (15) was introduced in YEpACT vector (32), a YEplac195 (33) derivative containing the promoter of the ACT1 gene from −540 to −46, with respect to (+1)ATG. New restriction sites are underlined.

To construct the SNF1 disruption cassette containing KanMX4 as a selection marker, we first subcloned an EcoRI/SalI fragment from plasmid pRS55 (LexA-Snf1; see Ref. 6) in pUC18 to give pUC-Snf1. This plasmid was then digested with NcoI, blunt-ended with Klenow DNA polymerase, and digested with RglI. The RglI/EcoRV fragment from pFA6a-KanMX4 was then introduced in the latter (34), generating plasmid pUC-snfΔ::KanMX4, where an inner fragment of 537 bp of the SNF1 gene was replaced by the KanMX4 selection marker.

Microscopic Observations—Logarithmic cultures were used to visualize GFP fusions. Aliquots (2 μl) of the cultures were put on microscope slides and covered with 18 × 18-mm coverslips. Cells were then viewed using a Zeiss Axioskop II fluorescence microscope. Images were scanned with a SPOT digital camera (Diagnostic Instruments Inc.) and processed through Adobe Photoshop 5.0 software.

Invertase Assay—Invertase activity was assayed in whole cells as described previously (6).

Northern blot Analysis—Northern blot analysis was performed as described previously (35).

Preparation of Cell Extracts—Cells corresponding to 1 A600 unit were collected by rapid centrifugation (14,000 rpm, 1 min), resuspended in 100 μl of Lаemmlı sample buffer (36), and boiled for 3 min. Glass beads (0.3 g, 450-μm-diameter) were added to the suspension and then cells were vortexed at full speed for 30 s. The suspension was boiled again for 3 min and centrifuged at 14,000 rpm for 1 min. 10 μl of the supernatant was subjected to SDS-PAGE and immunoblotting.

Immunoblot Analysis—Protein samples were separated by SDS-
mutant and observed a similar nuclear localization of the protein upon carbon starvation as compared with reg1Δ snf1Δ mutant or wild type (Fig. 1). These results confirmed that the abnormal localization of Msn2-GFP was because of the action of an activated Snf1 protein kinase that required Gal83 to perform its function.

Because it has been described that Reg2 (another Glc7 regulatory subunit; see Ref. 13) functions with Reg1 and Snf1 protein kinase to regulate growth, we checked the localization pattern of Msn2-GFP in reg2Δ mutants and found that it was similar to wild type (Table II). Double reg1Δ reg2Δ and triple reg1Δ reg2Δ snf1Δ mutants showed the same phenotype as reg1Δ and reg1Δ snf1Δ mutants, respectively (Table II). Therefore, Reg2 was not involved in the regulation of the localization of Msn2.

The abnormal localization of Msn2-GFP in reg1Δ mutants was also suppressed by overexpressing Glc7 (Fig. 1). However, this overexpression also alleviated the glucose repression defect observed in the reg1Δ mutant (30 units of invertase in the overexpressing transformants vs. 97 units in the reg1Δ mutant, in cells growing in 4% glucose (wild type cells showed less than 1 unit of invertase under these conditions)). So, the increase in the Glc7 dosage could either (8) be directly responsible for the suppression of the abnormal phenotype, or it could act indirectly by partially inactivating the Snf1 protein kinase.

The Alteration in the Localization of Msn2 in reg1Δ Mutants Is Specific for Carbon Starvation Stress—In addition to nutrient stress, Msn2 is able to respond to other forms of stress (osmotic, heat shock, etc.) (38). To test whether the abnormal localization pattern of Msn2-GFP observed in reg1Δ and hxk1hxk2Δ mutants was specific for carbon starvation conditions, we subjected these cells to osmotic stress (0.4 M NaCl) and checked the localization of the fusion protein. After 10 min of the treatment, Msn2-GFP was localized mainly in the nucleus in all the strains, including the wild type (Fig. 2). Similar results were obtained when the cells were heat-shocked at 42 °C for 10 min (data not shown). Therefore, the phenotype observed in reg1Δ mutant was specific for carbon starvation stress.

The alteration in the intracellular localization pattern of Msn2 in reg1Δ mutants was confirmed by transcriptional analysis of target genes. As shown in Fig. 3, reg1Δ cells failed to...
activate the expression of the \textit{CTT1} and \textit{HSP26} genes (STRE-regulated genes whose expression in response to carbon starvation is dependent on Msn2 and Msn4; see Ref. 38). However, the same mutant was also able to activate the expression of the same genes in response to osmotic stress (0.4 M NaCl), confirming that the observed activation defect was specific for carbon starvation conditions. In the double \textit{reg1}Δ \textit{snf1}Δ mutant, the activation of \textit{CTT1} and \textit{HSP26} upon carbon starvation was kinetically similar to \textit{snf1}Δ or wild type (Fig. 3), confirming that the activation defect was because of an activated Snf1 kinase. In the case of the double \textit{hxk1}Δ \textit{hxk2}Δ mutant, it failed to activate the \textit{CTT1} gene upon carbon starvation, but it could activate the expression of \textit{HSP26} (Fig. 3). This would be in agreement with our observation that this mutant showed a less severe phenotype than \textit{reg1}Δ mutant (see above). However, the same mutant was also able to activate both \textit{CTT1} and \textit{HSP26} upon osmotic stress (Fig. 3). In the triple \textit{hxk1}Δ \textit{hxk2}Δ \textit{snf1}Δ mutant we observed an activation of \textit{CTT1} and an improvement in the activation of \textit{HSP26} upon carbon starvation. Fig. 3 also shows that the activation of the reporter genes was always improved in any of the \textit{snf1}Δ derivatives (\textit{snf1}Δ, \textit{reg1}Δ \textit{snf1}Δ, and \textit{hxk1}Δ \textit{hxk2}Δ \textit{snf1}Δ), indicating that in the absence of Snf1, Msn2 enhanced its activating properties. So, there was an indirect correlation between the activity of Snf1 and the activity of Msn2 as a transcriptional activator.

\textbf{Activation of Snf1 Inhibits Nuclear Localization of Msn2-GFP Triggered by Rapamycin.—}It has been described recently that rapamycin, a drug that inhibits the TOR kinase pathway, causes the nuclear localization of Msn2 in wild type cells growing in glucose (see Ref. 22 and Fig. 1). To know whether this nuclear localization was also altered in \textit{reg1}Δ mutants, we treated these cells with this drug. As shown in Fig. 1, \textit{reg1}Δ cells treated with rapamycin still localized Msn2-GFP in the cytosol, whereas the double \textit{reg1}Δ \textit{snf1}Δ mutant imported the fusion into the nucleus. Similar results were obtained after rapamycin treatment of double \textit{hxk1}Δ \textit{hxk2}Δ and triple \textit{hxk1}Δ \textit{hxk2}Δ \textit{snf1}Δ mutants, respectively (Fig. 1). Therefore, active Snf1 may counteract the effects of the inhibition of the TOR kinase pathway, suggesting that it may affect similar cytosolic steps in the regulation of the subcellular localization of Msn2.

\textbf{Targets of the Action of Activated Snf1—}To study how the presence of an active Snf1 altered the localization of Msn2-GFP in \textit{reg1}Δ mutants upon carbon starvation, we analyzed Msn2 to see whether any differential post-translational modification had occurred under these conditions. We found that wild type, \textit{reg1}Δ, \textit{hxk1}Δ \textit{hxk2}Δ, and \textit{snf1}Δ mutants showed a similar pattern of post-translational modifications of the Msn2-GFP protein (Fig. 4A). Upon carbon starvation, Msn2-GFP was hyperphosphorylated in all the strains, in agreement with previous results (39). If a pulse of glucose was then added to the cells, a decrease in the phosphorylation status of the protein was also observed in all the cases. As described above, Msn2-GFP remained mainly in the cytosol in the \textit{reg1}Δ mutants (see above), suggesting that the hyperphosphorylation occurred before the protein was transported to the nucleus. Similar hyperphosphorylated forms were observed when \textit{reg1}Δ cells were treated with rapamycin or were subjected to either osmotic stress (0.4 M NaCl) or heat shock (42 °C for 10 min) (data not shown).

Another possibility to explain the abnormal localization of Msn2-GFP in \textit{reg1}Δ mutants could be that active Snf1 modified Bmh2, the cytosolic anchor of Msn2 (22). To test this possibility we analyzed the electrophoretic mobility of a hemagglutinin epitope-tagged version of Bmh2 in wild type, \textit{reg1}Δ, and \textit{snf1}Δ mutants, but no change in the electrophoretic mobility of hemagglutinin-Bmh2 was observed in either cells growing in glucose or shifted to carbon starvation conditions for 15 min, in any strain (data not shown).

\textit{Bmh2, the Cytosolic Anchor of Msn2, Plays a Minor Role in the Regulation of the Subcellular Localization of Msn2 in Response to Glucose—}When cells are growing in glucose, Msn2 is located in the cytosol where it binds to its anchoring protein Bmh2 (22). We wanted to test the importance of the anchoring protein in the regulation of the subcellular localization of Msn2. As Bmh2 has a very close homologue (Bmh1) that is functionally redundant, we analyzed the localization pattern of Msn2-GFP in a double \textit{bmh1}Δ\textit{bmh2}Δ mutant. It is known that a double \textit{bmh1}Δ\textit{bmh2}Δ mutation is lethal in most yeast genetic backgrounds, but it is still viable in the \textit{Σ1278b} background (40). We tried to transform the \textit{Σ1278b} \textit{bmh1}Δ\textit{bmh2}Δ double mutant with the pMSN2-GFP construct, but we were unable to recover any viable transformant, suggesting that perhaps the overexpression of Msn2 was lethal in this double mutant. So, we integrated a GFP tag at the 3' end of the chromosomal \textit{MSN2} gene to allow the expression of the gene fusion under the control of the original \textit{MSN2} promoter (see “Experimental Procedures”). Although the expression of the fusion protein was much lower than in the other cases, we observed that the double mutant localized Msn2-GFP inside the nucleus upon carbon starvation (Fig. 5A). This nuclear localization of Msn2-GFP correlated with an increase in the expression of \textit{CTT1}, an STRE-regulated gene (Fig. 5B). However, the kinetics of \textit{CTT1} induction were slower than in the corresponding wild type, suggesting a positive role of Bmh1 and Bmh2 in the regulation of the subcellular localization of Msn2. These results suggest that the anchoring proteins play only a minor role on the regulation of the subcellular localization of Msn2.

We also analyzed the phosphorylation status of Msn2 in the double \textit{bmh1}Δ\textit{bmh2}Δ mutant. Because the transformation of the \textit{Σ1278b} \textit{bmh1}Δ\textit{bmh2}Δ double mutant with the pMSN2-GFP construct was not possible (see above), we studied the phosphorylation status of endogenous Msn2. As observed in Fig. 4B, in wild type cells endogenous Msn2 underwent a hyperphosphorylation in glucose starvation conditions that was reversible.
FIG. 3. Transcriptional activation of CTT1 and HSP26 under different conditions. The indicated strains were grown in YPD medium at 30 °C until they reached an A600 nm of 0.3–0.5. Cells were harvested by centrifugation, washed twice with sterile water, and resuspended in SC medium without any carbon source. After 15 and 30 min of incubation at 30 °C, aliquots were taken and processed for Northern analysis. Aliquots of YPD growing cells were made 0.4 M NaCl, and after 10 min of incubation at 30 °C cells were processed for Northern analysis. Northern blots were hybridized with radiolabeled CTT1 and HSP26 probes (35). A picture of total RNA stained with ethidium bromide was taken and used as loading control.

FIG. 4. Electrophoretic mobility of Msn2 under different conditions. A, different strains expressing Msn2-GFP fusion protein from plasmid pMSN2-GFP were grown exponentially in 4% glucose (lane 1). Aliquots of these cells were harvested, washed twice with sterile water, and resuspended in SC medium without any carbon source. After 15 min at 30 °C (lane 2). Then, glucose (2% final concentration) was added, and the cultures were incubated at 30 °C for 15 min (lane 3). Cell extracts (fast boiling method; see “Experimental Procedures”) were prepared under the different conditions. 10-μl aliquots of these extracts were immunodetected with anti-Msn2 polyclonal antibody. B, untransformed Σ1278b wild type and bmh1bmh2 cells were grown and treated as above. 30-μl aliquots of these extracts were immunodetected with anti-Msn2 polyclonal antibodies. Size standards are indicated in kilodaltons.
upon glucose readdition to the culture. However, in \textit{bmh1bmh2}\textDelta mutants the hyperphosphorylation of Msn2 was absent, indicating that the presence of Bmh2 was completely necessary for this modification. As Msn2-GFP was localized inside the nucleus upon carbon starvation of the \textit{bmh1bmh2}\textDelta mutants (see above), this may indicate that Msn2 hyperphosphorylation was dispensable for its import to the nucleus.

**DISCUSSION**

Stress factors influence cellular metabolism, gene expression, and growth rate by a “general stress response” system that regulates the coordinated induction of many stress genes through a common \textit{cis} element in their promoter, the stress response element STRE (38). This element is regulated by two functionally redundant \textit{trans}-activating factors named Msn2 and Msn4. The activity of these transcription factors is modulated by two non-exclusive mechanisms: 1) regulated nuclear localization, and 2) regulated DNA binding, controlled inside the nucleus (38). Msn2 and Msn4 are able to respond to different kinds of stress conditions (osmotic, heat shock, carbon starvation, etc.), and although all of these stresses result in the activation of STRE-regulated genes, it seems that each kind of stress is able to trigger the activation of Msn2 and Msn4 in a different manner (38). The components that are involved in the activation of Msn2 by salt or heat shock stress are still defined poorly; only recently was it reported that PP2A phosphatases may be involved in the activation of Msn2 by salt stress (41).

More is known about the components involved in the activation of Msn2 by carbon starvation. In this case, two regulatory pathways have been implicated in the regulation of the subcellular localization of Msn2. The first is the cAMP-dependent protein kinase A pathway. An inverse correlation has been found between cAMP-dependent protein kinase A activity and nuclear localization of Msn2. In fact, mutations of the potential cAMP-dependent protein kinase A modification sites of Msn2 lead to constitutive nuclear localization of the protein (26). Msn2 contains a typical nuclear localization signal and also a functional nuclear export signal (26), and it has been shown recently that the Msn2 nuclear localization signal is inhibited by phosphorylation driven by cAMP-dependent protein kinase A and activated by dephosphorylation by a still unknown phosphatase. The Msn2 nuclear localization signal phosphorylation status is highly sensitive to carbohydrate fluctuations during fermentative growth. After glucose withdrawal, dephosphorylation occurs in less than 5 min, and the effect is reversed rapidly by refeeding with glucose (42). Recent results also indicate that Sit4 (a PP2A-related phosphatase) or its regulatory subunit Tap42 is not involved in the regulation of the localization of Msn2 (22).

The second regulatory pathway is the one defined by the TOR protein kinases. It has been described recently that rapamycin, a drug that inhibits the TOR kinase pathway, induces the nuclear accumulation of Msn2 by releasing the binding of Msn2 to its cytosolic protein anchor Bmh2, a yeast 14-3-3 protein family homologue (22). We have tested the participation of Bmh2 on the nuclear localization of Msn2. In a double \textit{bmh1bmh2}\textDelta mutant, Msn2-GFP could still be localized inside the nucleus upon carbon starvation, indicating a minor role of the anchoring proteins in the regulation of the subcellular localization of Msn2.

In this study we present strong evidence that the Snf1 protein kinase glucose repression pathway participates actively in the regulation of the subcellular localization of Msn2 in response to glucose. An active Snf1 protein kinase was able to affect the regulation of the localization of Msn2 and made it insensitive to carbon starvation. As a result, activation of STRE-regulated genes was prevented. We also present evidence that the action of Snf1 in this process was mediated via Gal83 (an Snf1 complex \(\beta\)-subunit), because active Snf1 was not able to perform its inhibitory function in the absence of Gal83.

The absence of nuclear localization of Msn2-GFP and activation of STRE-regulated genes observed in strains having an activated Snf1 kinase was specific for carbon starvation stress. Other stress conditions such as osmotic or heat shock stresses were able to localize Msn2-GFP in the nucleus and activate the STRE-regulated genes. These results support the hypothesis that cells may contain alternative mechanisms to cope with different stress conditions.

We also present evidence that active Snf1 is able to counteract the effects of rapamycin on the subcellular localization of Msn2. These results may indicate that TOR and Snf1 protein kinases would inactivate similar steps in the regulation of Msn2, probably by inactivating the same or related components.
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in the pathway. Because the action of Snf1 is located in the cytosol, this describes alternative functions of the kinase different from its role in nuclear transcriptional control.

Does Snf1 play a physiological role in wild type cells in terms of Msn2 regulation? In wild type cells growing in glucose, conditions in which the Snf1 complex is inactive, TOR kinases are active and would inhibit the nuclear import of Msn2 as a "nutrient sensing response." Upon a shift to poor carbon source medium or carbon starvation, TOR kinases would become inactivated, resulting in an activation of the nuclear import of Msn2, probably by dephosphorylation of Msn2, which would release it from binding to its cytosolic anchor, Bmh2. At the same time, Snf1 would become active, inhibiting the nuclear import of Msn2 ("reset response"). In this way the cell would be prepared for the arrival of phosphorylated Msn2 (driven by cAMP-dependent protein kinase A) that would be produced when glucose was sensed again. In the presence of glucose, Snf1 would be inactivated and the TOR kinases activated, maintaining Msn2 in the cytosolic compartment. Therefore there would be a clear convergence of the TOR and the Snf1 kinase pathways in the regulation of the response to carbon source availability. This hypothesis is in agreement with our observation that in mutants lacking Snf1 more activation of the STRE-regulated genes was detected, probably because more Msn2 could be imported to the nucleus, as it could have more time to be activated.

What is the component inactivated by Snf1 and TOR kinases? We cannot tell at present which components might be inactivated by Snf1 and TOR kinases, although Msn2 is an attractive candidate. Analysis of the phosphorylation status of Msn2 is very complicated, because it has been demonstrated that this protein suffers hyperphosphorylation upon different stress conditions (39). We checked the phosphorylation status of Msn2 in different mutants and found no apparent differences. In all the strains Msn2 was hyperphosphorylated upon carbon starvation. However, this result may not reflect the actual post-translational modification of Msn2 in each case, because in terms of electrophoretic mobility this hyperphosphorylation could mask the dephosphorylation of some of the phosphates added by cAMP-dependent protein kinase A, and perhaps the removal of these phosphates would be necessary to allow the interaction of the protein with specific importins to be transported into the nucleus (39).

The convergence of the TOR and the Snf1 kinase pathways in the subcellular localization of Msn2 is a good example of the cross-talk that exits between different pathways regulated by the same effector, glucose in this case. Very recently it has been described that both pathways also converge into the regulation of Gln3, a GATA-type transcription factor involved in the activation of nitrogen catabolite-repressible genes (43). Because both the TOR and the Snf1 kinase pathways are conserved from yeast to mammalian cells (44, 45), one could speculate that AMPK (AMP-activated protein kinase; the mammalian homologue of Snf1 kinase) could also participate in the regulation of the subcellular localization of transcriptional factors related to carbon or nitrogen starvation stress in mammalian cells.

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