The transcriptional program of yeast cells undergoes dramatic changes during the shift from fermentative growth to respiratory growth. A large part of this response is mediated by the stress responsive transcription factor Msn2. During glucose exhaustion, Msn2 is activated and concentrated in the nucleus. Simultaneously, Msn2 protein levels also drop significantly under this condition. Here we show that the decrease in Msn2 concentration is due to its increased degradation. Moreover, Msn2 levels are also reduced under chronic stress or low protein kinase A (PKA) activity, both conditions that cause a predominant nuclear localization of Msn2. Similar effects were found in msn5 mutant cells that block Msn2 nuclear export. To approximate the effect of low PKA activity on Msn2, we generated a mutant form with alanine substitutions in PKA phosphorylation sites. High expression of this Msn2 mutant is detrimental for growth, suggesting that the increased degradation of nuclear Msn2 might be necessary to adapt cells to low PKA conditions after the diauxic shift or to allow growth under chronic stress conditions.

In budding yeast, two redundant stress-inducible transcription factors, Msn2 and Msn4, play an important role in the response to environmental cues (1). Both of these highly related zinc finger transcription factors mediate the induction of stress protective genes whenever cells encounter a shift toward suboptimal growth conditions (2–4). Furthermore, Msn2 and Msn4 are activated during diauxic shift, a transition from fermentative growth on glucose to respiratory growth, causing a transient increase of stress inducible transcripts (5, 6). Activation of Msn2 usually occurs through a series of complex steps that increase its nuclear concentration and promoter recruitment. It is thought that Msn4 is mechanistically regulated in similar ways. Exposure to stress conditions such as high osmolality, rapamycin treatment, heat stress, short chain organic acids, alcohols, and membrane damage throttles Msn2 nuclear export and increases its binding to the stress response element (STRE) (7–10). However, in all these cases the nature of the signaling events leading to Msn2 activation is still largely obscure.

In contrast to our lack in understanding Msn2 activating signals, it is well established that protein kinase A (PKA)\(^1\) activity plays an overriding role in the negative regulation of Msn2 function. PKA activity imposes itself onto Msn2 nuclear import, nuclear export, and possibly DNA binding (9). Low PKA activity has been found to increase the expression level of Msn2-dependent genes, whereas high PKA activity has been described to cause the opposite effect (11). Consequently, the question emerged whether environmental stresses activate Msn2 function simply by antagonizing or modulating PKA signaling. In fact, genetic interactions have been described between the TOR (target of rapamycin) pathway and the Ras-PKA pathway that are consistent with this assumption (12). Moreover, TOR-mediated nutrient signals have been shown to affect Msn2 function (13). Other studies, however, rather support the conclusion that the Ras-PKA pathway does not mediate acute stress responses (14). Of all tested stress treatments only glucose starvation appeared to induce changes in the PKA-dependent phosphorylation of serine 620 in the Msn2 NLS (14), whereas all other acute stresses do not change a PKA-dependent modification of this site. These findings might reflect differences between short term and long term responses to environmental fluctuations. For example, during the post diauxic shift, Msn2 is probably mainly activated due to an extended drop in protein kinase A activity.

The distinct transcriptional response to acute stress exposure is usually transient, suggesting that adaptive systems are in operation that help a cell to resume its support for growth and cell division rather than invest into damage protection. Indeed, during stress relief, and during stress adaptation, Msn2 is rapidly relocated from the nucleus to the cytoplasm. The situation is different, however, when glucose is exhausted in a growing culture after the diauxic shift with constantly low PKA activity. Since genetic evidence has shown that under low PKA activity Msn2 becomes detrimental for growth, the question arises by which mechanism Msn2 is inactivated after the diauxic shift to avoid growth arrest. Here we provide evidence that this adaptive effect might be achieved through different degradation rates of nuclear versus cytoplasmic Msn2. Further analysis showed that Msn2 protein levels are always reduced under conditions that cause prolonged nuclear localization of the protein. In comparison to other transcription factors, for which degradation has been shown as a means of regulation, the overall rates of Msn2 degradation are relatively slow in all cases. This observation might

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\(^1\) The abbreviations used are: PKA, protein kinase A; GFP, green fluorescent protein; PKI, protein kinase A inhibitor.
suggest that protein degradation is unimportant for Msn2 regulation under short term fluctuating conditions. As shown through the use of a hyperactive allele of Msn2, however, nuclear degradation of the factor might be important for avoiding prolonged growth arrest under sustained stress conditions.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains, Media, and Growth Conditions**—All strains are derivatives of W303-1A (MAT a, ura3-1, leu2-3, his3-11, trp1-1, ade2-1, can1-1000) (Table I). W303msnΔΔmsn4ΔΔ-msn5Δ was generated by isoenic crossing of W303msnΔΔmsn4ΔΔ with W303msn4ΔΔ (9). The correct genotype of the resulting strain was verified by PCR. W303cdc35Δade2Δ was generated by introducing the cdc35-kanMX disruption cassette, which was recovered from the EUROSCARF collection, into W303ade2Δ (14). W303erg6Δ was a kind gift from K. Kuchler (Vienna BioCenter).

Standard yeast culture methods were used as described by Görner et al. (9). Yeast cells were grown in YPD (1% yeast extract, 2% bactopeptide, 2% glucose) or synthetic medium at 30 °C to an A600 of 1 and either used immediately, exposed to stress (7% EtOH, 0.4 M NaCl, or 37 °C heat shock) or further grown through stationary phase. For cAMP depletion cultures of W303cdc35Δade2Δ were grown at 30 °C in selective medium containing 3 mM cAMP to A600 = 0.8, washed and resuspended in cAMP-free medium and further incubated at 30 °C. For heat shock, 2% glucose) or synthetic medium at 30 °C to an A600 of 1. MG132 (Sigma) was added to a final concentration of 600 μg of total protein for each sample was loaded on a 7% SDS-PAGE and separated. Proteins were transferred to nitrocellulose membranes, and detected with rabbit anti-Msn2 antiserum (kindly provided by F. Esch, Valencia, Spain), Kar2 with rabbit anti-Kar2 antiserum (a gift from F. Estruch, Valencia, Spain), and trich, 2% glucose) or synthetic medium at 30 °C to an A600 of 1.

**TABLE I**

| Yeast strains and plasmids used in this study | Genotype | Source |
|---------------------------------------------|----------|--------|
| W303-1A                                      | a ura3 leu2 his3 trp1 ade2 can1 | K. Nasmyth (Vienna) |
| W303-msnΔΔmsn4ΔΔ                             | a msn4Δ::TRP1 msn4Δ::HIS3 | Ref. 9 |
| W303-msn5Δ                                  | a msn5Δ::HIS3 | Ref. 9 |
| W303erg6Δ                                   | a erg6Δ::LEU2 | K. Kuchler (Vienna) |
| W303msnΔΔmsn4ΔΔ-msn5Δ                       | a msn4Δ::TRP1 msn4Δ::HIS3 msn5Δ::HIS3 | This study |
| W303cdc35Δade2Δ                             | a ade2Δ::Trp1 cdc35Δ::KanMX6 | This study |

**TABLE II**

| Oligonucleotides used in this study | Source |
|------------------------------------|--------|
| EGFPseq-rev: 5′-AGGCCGATCAGGTCGCTGCTG-3′ | Ref. 9 |
| Msn2Sal1: 5′-CTAAATAGCCGAGACCATG-3′ | Ref. 9 |
| Msn2 × 128I-rev: 5′-AACCTTAATCTGATCGATCGATCTCGATGAGTCTATGTGTCG-3′ | Ref. 9 |
| IPP1-fwd: 5′-CCCTTTGATGCCGACAGG-3′ | Ref. 9 |
| IPP1-rev: 5′-GCTCCAGCCGAGACC-3′ | Ref. 9 |
| SacII-Cup1_fwd: 5′-GATCCCGGGGGATATTACGCGACACCCGAG-3′ | Ref. 9 |
| Sall-Cup1_rev: 5′-ATGGCTGACCGCTCTTATGATGTTGATTGATTGATG-3′ | Ref. 9 |
| PKI_NES fwd: 5′-ATGAGGTCACAGATGTTGATGATTGATTGATG-3′ | Ref. 9 |
| Sall-Cup1-PKINES_rev: 5′-GCTCCAGCCGACACCCGAG-3′ | Ref. 9 |
| BamHI-ΔNES-fwd: 5′-CTACTTACGCGTAAGGAGGGACACAGCT-3′ | Ref. 9 |
| BamHI-ΔNES-rev: 5′-CTACTTACGCGTAAGGAGGGACACAGCT-3′ | Ref. 9 |

**Plasmids**

| Plasmids | Relevant inserts | Source |
|----------|-----------------|--------|
| pADH1-MSN2 | ADH1-MSN2 | Ref. 9 |
| pADH1-MSN2-GFP | ADH1-MSN2-GFP | Ref. 9 |
| pYMSN2-myc | MYC-NAT | Ref. 9 |
| pYMSN2-GFP | MSN2-GFP | Ref. 9 |
| pCUP1MSN2 | CUP1 Promoter-MSN2 | This study |
| pCUP1MSN2-GFP | CUP1 Promoter-MSN2-GFP | This study |
| pCUP1MSN2ΔNES | CUP1 Promoter-MSN2ΔNES-GFP | This study |
| PCUP1MSN2ΔNES-GFP | CUP1 Promoter-MSN2ΔNES-GFP | This study |
| pCUP1MSN2A5 | CUP1 Promoter-MSN2A5ΔNES-GFP | This study |
| pCUP1MSN2A5-GFP | CUP1 Promoter-MSN2A5ΔNES-GFP | This study |
| pCUP1PKMSN2 | CUP1 Promoter-PKMSN2 | This study |
| pCUP1PKMSN2-GFP | CUP1 Promoter-PKMSN2-GFP | This study |
monoclonal 9E10 antibody. Northern blot analysis was carried out as described (16). CTTT was detected with a probe derived from the EcoRI fragment from plasmid pBR322–5109 (17) and for IP1 we used a PCR fragment generated with the oligonucleotides IP1-fwd and IP1-rev.

Catalase activity was determined in crude extracts prepared by breaking 20 A_{600} equivalents of cells in 100 μl breaking buffer (50 mM Tris pH 7, 10% glycerol) with glass beads at 4°C. 10 μl of crude extract (protein concentration usually between 5 and 10 mg/ml) was added to 3 ml of catalase buffer (50 mM Na2HPO4, pH 7, 0.1% Triton X-100, 200 mM H2O2) and mixed immediately, and the disappearance of H2O2 at 240 nm was followed for up to 3 min. Catalase activity was calculated in micromolar H2O2 per minute per mg total protein (ε = 43.75).

**RESULTS**

**Msn2 Levels Are Diminished after the Diauxic Shift**—Under logarithmic growth conditions yeast cells maintain a balanced level of Msn2 protein that does not change under acute stress conditions (9). Similarly, according to Chi et al. (18) adaptation to heat shock had no effect on Msn2 levels, leading to the proposal that an increase in the nuclear export rate of Msn2 was the main stress adaptive feature. However, an analysis of Msn2 behavior during the diauxic shift made us re-evaluate the possibility of Msn2 regulation by protein degradation. A Western blot analysis showed that an epitope tagged version of Msn2, which was expressed from a centromeric plasmid under the control of the native promoter (pYMSN2-myc), revealed a dramatic reduction of Msn2 protein levels at later growth stages between A_{600} of 6 and 8 (Fig. 1A). Surprisingly this effect was not accompanied by a similar reduction in MSN2 transcript levels. Moreover, similar results were obtained with native Msn2 and with Msn2 expressed from a heterologous promoter. Since Msn2 disappeared within a short time in the early diauxic shift, it was most likely that Msn2 was degraded at higher rates during this period of growth. The localization of Msn2 during the diauxic shift was followed by fluorescence microscopy of a Msn2-GFP fusion driven by the ADH1 promoter. Msn2-GFP accumulates in the nucleus when glucose is exhausted in the culture (Fig. 1B). However, we noticed reduction of the GFP signal at later growth stages, an impression that conformed with our Western blot analysis. This observation also suggested a connection between Msn2 localization and protein stability.

**Cells under Chronic Stress Exhibit a Reduction in Msn2 Levels**—To address the question whether it is nuclear localization per se or stress and starvation signals that caused enhanced Msn2 degradation, we analyzed different conditions known to relocate Msn2 to the nucleus. Exponentially growing cells were exposed to prolonged treatments with ethanol, mild osmotic stress, and heat shock. Msn2 protein levels decreased noticeably during growth at high temperature and after the addition of stressful amounts of ethanol to the culture (Fig. 2A). In contrast, osmotic stress had no effect on Msn2 levels. Analysis of the localization pattern of Msn2-GFP during stress treatments indicated that both chronic ethanol and chronic heat stress lead to the permanent nuclear localization of Msn2-GFP. On the contrary, osmotic stress caused only transient nuclear localization of Msn2-GFP, and Msn2-GFP began to

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2 E. Durchschlag, W. Reiter, G. Ammerer, and C. Schüller, unpublished data.
reappear in the cytosol after 1 h (Fig. 2B). These observations support the hypothesis that prolonged nuclear accumulation of Msn2-GFP leads to a reduction of Msn2 protein levels.

Reduction of Protein Kinase A Causes a Decrease in Msn2 Protein Levels—Since PKA is a potent modulator of Msn2 localization and activity, we tested whether low PKA activity has a similar effect as glucose depletion and stress on Msn2 stability (9, 14). To manipulate PKA activity we used a strain lacking both the adenylylcyclase gene (CDC35) and the high affinity phosphodiesterase gene (PDE2), causing a prolonged stability of cAMP in the cells, a requirement for manipulation of the intracellular cAMP pool. After cAMP removal, Msn2 protein levels dropped significantly over a period of 2 h (Fig. 3A). To verify effective Msn2 activation under these conditions, we analyzed nuclear localization of Msn2-GFP (Fig. 3B) and transcriptional induction of the Msn2-dependent CTT1 gene (Fig. 3C) (16). The kinetics of the induction of the CTT1 gene and the slow kinetics of nuclear concentration of Msn2 after cAMP removal might be due to the pde2 deletion causing a delayed drop in intracellular cAMP levels (19). These data demonstrate that in unstressed cells, and in the presence of glucose, reduced cAMP levels, presumably through a drop of PKA activity, are sufficient to cause a drop of Msn2 levels.

Nuclear Accumulation Destabilizes Msn2—The common denominator of the conditions causing a drop in Msn2 protein levels is its nuclear accumulation. Nuclear export of Msn2 is dependent on the nuclear export factor Msn5, which has been implicated in a wide variety of signaling systems (20–23). To monitor the stability of Msn2 in the nuclear compartment, we determined Msn2 protein levels in msn5/J9004 strains by Western blotting. We observed that msn5/J9004 strains exhibit a 3-fold reduced steady state level of Msn2 protein (Fig. 4A). As shown in Fig. 4B, msn5/J cells exhibit a permanently enhanced nuclear signal from a Msn2-GFP fusion. Interestingly, despite the unusual, constitutive nuclear localization of Msn2, environmental stress response gene transcription is still under proper stress regulation (Fig. 4C), suggesting that an increase in nuclear localization of Msn2 alone is normally not sufficient for the induction of its target genes. We used catalase activity as a readout system for CTT1 expression (24). Both the basal CTT1 expression as well as the temperature-induced expression are at comparable levels. The relatively normal basal CTT1 expres-
Nuclear Msn2 Degradation

To exclude interference with the de novo synthesis of Msn2, the open reading frame of the MSN2 gene was placed under the control of a conditional promoter. We chose the CUP1 promoter because it is inducible by low (50 μM) amounts of Cu²⁺ ions, which do not activate any genes known to be under Msn2 control. The CUP1 promoter controlled Msn2 construct was introduced on a centromeric plasmid (pCUP1/MSN2) in the strains W303msn2Δmsn4Δ and W303msn2Δmsn4Δmsn5Δ. Msn2 synthesis was induced for 30 min followed by removing Cu²⁺ from the medium. We followed the Msn2 protein levels on Western blots and found a loss of detectable Msn2 protein within 90 min. In contrast, in the wild type strain no such decrease was visible (Figs. 4D and 5A), thus indicating that the reduced Msn2 levels in the msn5Δ mutant are most probably due to enhanced turnover. To avoid the pleiotropic effects of the msn5Δ mutant, we also studied the behavior of Msn2 mutants that show increased nuclear accumulation. To permanently localize Msn2 in the nucleus, we used a mutant version of Msn2 that lacked a sequence required for efficient cytoplasmic localization. This sequence most likely included a Msn5-dependent nuclear export signal (amino acids 246–325) and was therefore designated Msn2 NES. Msn2NES was placed under the control of the CUP1 promoter (pCUP1/MSN2NES) and expressed in the strain W303msn2Δmsn4Δ. As shown in Fig. 5A, the levels of the mutant protein clearly declined more rapidly than the wild-type product. Northern blot analysis indicated that this effect is not due to influences on mRNA stability. In both cases, MSN2 mRNA levels rise sharply after Cu²⁺ addition, only to fall to their previous levels within minutes after removal of Cu²⁺. The decline in Msn2 protein levels obviously occurs at a much slower rate. Fluorescence microscopy with a CUP1-regulated Msn2NES-GFP expression system confirms the predominant nuclear localization of the mutant protein. Moreover, after its synthesis is triggered by the presence of Cu²⁺, the signal is lost more rapidly after removal of Cu²⁺ than its wild type control (Fig. 5B). We also considered the possibility that the internal deletion of Msn2NES might affect the stability of the mutant protein. Therefore, we investigated the behavior of another Msn2 mutant form, which is also constitutively accumulated in the nucleus. This mutant carried simultaneous point mutations in five putative PKA phosphorylation sites substituting serine 288, 582, 620, 625, and 633 to alanine (Msn2A5) (Fig. 5A) but still contained the sequence deleted in Msn2NES. Expression analysis of a CUP1-regulated Msn2A5 mutant revealed that after CUP1 promoter shut-off, the drop in Msn2A5 protein level was comparable with what we observed with the Msn2NES mutant (Fig. 5, A and C). Quantification of the band intensities shows that the half-life of nuclear Msn2 is between 60 and 90 min (Fig. 5C). It is important to note that this increase in degradation occurred even in the absence of stress. To further investigate the possibility of an intrinsic instability of the Msn2NES mutant, we compared its protein levels to nuclear Msn2. To achieve comparable conditions the stability of both proteins was determined after CUP1 promoter shut-off under 7% ethanol stress (Fig. 5A). Quantification indicates a similar stability of both Msn2 and Msn2NES in the nucleus (Fig. 5C), thus excluding a large impact of the internal deletion on Msn2 stability. Taken together, these data therefore suggest that nuclear localization of Msn2 could be causally linked with lower Msn2 protein stability.

Subcellular Localization but Not Stress Is the Major Determinant for Msn2 Stability—If degradation of Msn2 is exclusively dependent on nuclear localization, then Msn2 levels observed in the msn5Δ might be explained by the reduction in Msn2 protein levels.

Fig. 5. Subcellular localization determines Msn2 stability. A, Msn2 stability in the nucleus. Plasmids expressing MSN2, Msn2NES, and Msn2A5 under the control of the CUP1 promoter were introduced into strain W303msn2Δmsn4Δ. Transformants were grown overnight, diluted to A₆₀₀ 0.1 and grown to logarithmic phase until A₆₀₀ 0.8. 50 μM CuSO₄ was added. After 30-min incubation cells were washed and resuspended in Cu²⁺-free medium (0 min). Samples were taken after 1, 2, and 3 h. Msn2, Msn2NES, and Msn2A5 levels were analyzed on Western blots with an antiserum directed against Msn2. Kar2 levels served as a loading control. B, fluorescence microscopy visualizing of Msn2NES-GFP and Msn2A5-GFP. Both GFP fusion genes were under the control of the CUP1 promoter and were inspected after 1 h induction with 50 μM Cu²⁺. C, quantification of Msn2 protein levels. Blots were scanned with ImageQuant. Msn2 bands were normalized with the Kar2 signal. The Msn2 level after copper induction was set to one for each series.
should be stable in the cytosol even under stress or starvation conditions. To test this possibility we fused a short nuclear export signal derived from the mammalian PKI (25) to the N terminus of Msn2. This construct was expressed under the control of the CUP1 promoter (pCUP1MSN2). Synthesis of both, Msn2 and PKIMsn2, was induced for 30 min by addition of copper in exponentially growing cultures. After removal of copper, cells were stressed with 7% ethanol, and Msn2 as well as PKIMsn2 protein levels were analyzed by Western blots. While Msn2 levels dropped significantly, PKIMsn2 protein levels remained constant over a period of three hours (Fig. 6A). The subcellular localization of both proteins under these conditions was verified using GFP fusions. As shown in Fig. 6B, Msn2-GFP accumulates in the nucleus in ethanol-stressed cells; however, PKI-Msn2-GFP remained in the cytosol with a slight accumulation at the outer nuclear rim. These data supported the notion that it is not stress per se but the localization of the protein that determines the stability of Msn2.

The Proteasome Inhibitor MG132 Inhibits Degradation of Nuclear Msn2—Most short lived proteins are degraded via the 26 S proteasomal pathway. If Msn2 degradation in the nucleus is mediated through this route, inhibition of the proteasome should lead to accumulation of Msn2. To test this we used a strain lacking the ERG6 gene (W303 erg6/H9004), a mutation facilitating the uptake of the proteasome inhibitor MG132 (26). We used two concentrations of MG132 dissolved in Me2SO and found increased levels of Msn2 protein in a dose dependent manner (Fig. 7A) relative to the Me2SO control. The Me2SO concentration was adjusted to 0.1% in both treated cultures. Quantification shows a 2–3-fold increase of the Msn2 protein level within a relatively short time (30 min) of incubation. To verify the intracellular localization of Msn2 under the used conditions, we determined the localization of a Msn2-GFP fusion protein. Exposure to 0.1% Me2SO causes rapid nuclear accumulation of Msn2-GFP (Fig. 7B). Prolonged treatment with 0.1% Me2SO results in decreasing levels of Msn2 similar to other stress conditions (Fig. 7C). Taken together, these data suggest intranuclear degradation of Msn2 by the proteasome.

Constitutive Activation of Msn2 Is Detrimental for Growth—To assess whether degradation of Msn2 has a physi-
FIG. 8. Forced expression of active Msn2 is detrimental for growth. A, protein kinase A consensus sites in Msn2. Positions of serine residues changed to alanine are indicated. B, W303Δmsn2Δmsn4A cells transformed with plasmids carrying pCUP1MSN2A5 and the wild type plasmid pCUP1MSN2 were spotted in serial dilutions on YPD and on YPD containing 0.25 mM Cu²⁺. Growth was recorded after incubation for 48 h at 30 °C. C, Msn2A5 is a hyperactive allele. W303Δmsn2Δmsn4A cells carrying pCUP1MSN2A5 and pCUP1MSN2 were grown to logarithmic phase and CuSO₄ was added to 50 μM final concentration. Northern blot showing the mRNA level of the Msn2-dependent catalase T (CTT1) gene before and after induction of Msn2A5 and Msn2 by the copper promoter.

The simple assumption of different, but constant, degradation rates between nuclear and cytoplasmic Msn2 could not explain the data. However, such a conclusion would make it difficult to explain the effects observed under low PKA kinase activity. Since prolonged nuclear accumulation currently remains the only common denominator of all the conditions causing low Msn2 levels, we believe that nuclear location of Msn2 per se is the crucial parameter. The fact that enforced nuclear export suppresses an increase in Msn2 degradation under chronic stress clearly supports this contention. The same data also invalidate any model according to which PKA-dependent modification sites should not be relevant. We propose that the degradation is rather a consequence of nuclear occupation rates than stress or starvation-dependent modification signals. In the two best studied examples of SCFcdc4 mediated degradation pathways, namely Sic1 and Gcn4, phosphorylation plays an essential part in raising the affinity between the E3 complex and its substrates (31, 32). So far, similar modifications have not been identified for Msn2. It is clear, however, that PKA-dependent modification sites should contribute to the degradation rate of Msn2. Moreover, not all conditions that cause low levels of Msn2 lead to a prolonged decrease in PKA-dependent Msn2 phosphorylation. So, if there are targets in Msn2 that direct signal-induced increases in ubiquitination and degradation, they are likely to differ from the PKA motifs. However, such a conclusion would make it difficult to explain the effects observed under low PKA kinase activity.
as they are derived from promoter shut-off analysis followed by Western blot assays. The half-life, as estimated by us, for nuclear Msn2 correlates well with the half-life mentioned by Chi et al. (18), who used metabolic labeling. One has to take into account that the growth conditions necessary, and routinely used, for effective metabolic labeling experiments constitute a poor growth environment. Therefore, the values for the Msn2 half-life of about 1 h in stressed or starved cells is likely to be correct. A similar value was obtained for cells under optimal growth conditions but with enforced nuclear accumulation of Man2. This contrasts with our estimates in normal cells at optimal growth conditions, in which the Msn2 half-life should approach 3 h. We are aware that values between the indicated levels are difficult to measure and that subtle differences cannot be quantified and therefore interpreted with any confidence. Nevertheless, we assume that the differences documented here are indeed noticeable. In this regard it should be noted that Chi et al. (18) claimed to find no difference in Msn2 stability between msn5Δ and wild type cells. We assume that this is not due to technical reasons but that the differences might have been missed if wild type cells and mutant cells were compared at late growth stages (e.g. post-diauxic shift).

To what extent could different degradation rates of nuclear versus cytoplasmic Msn2 be useful for a yeast cell? Acute stress situations, during otherwise optimal growth conditions, will normally cause a transient growth arrest and elicit dramatic changes in the transcriptional program (2, 33). Msn2 plays an important role in this response. Upon stress relief, it should be advantageous to resume growth as quickly as possible. Indeed, the rapid return of stress-specific transcripts to normal levels is reflected in the dynamic localization pattern of Msn2 (9, 16). Control of the nucleo-cytoplasmic shuttling of Msn2 could therefore easily lead to the required rapid redistribution of Msn2 without impairing the capacity of a cell for subsequent responses. The nuclear half-life of Msn2 would still exceed the time frame of the response (as actually observed here during osmotic shock), and its contribution toward adaptation would thus be negligible. Chronic stress might require a different strategy from acute stress since cells will undergo long term changes in their physiology. The diauxic shift, during which cells switch from fermentative growth to respiratory growth, might serve as an example for such a chronic condition. The drop in glucose concentration leads to lower PKA activity, which in turn activates Msn2 (14). If glucose is permanently depleted, Msn2 receives a constitutive signal for nuclear accumulation and activation. Under these conditions, higher nuclear degradation rates should be sufficient to prevent, over time, an inappropriate high activation of stress specific genes. Similar arguments could be made for permanent stress situations. A relatively modest difference in degradation rates between nuclear and cytoplasmic Msn2 may promote Msn2 inactivation under such chronic stress or starvation conditions but might also preserve an ample supply of the factor when responding to rapidly changing conditions.

There is previous evidence that active Msn2 could become detrimental for growth. First, absence of PKA activity, which causes dephosphorylation and activation of Msn2, leads to growth arrest, which is suppressed by the absence of Msn2 and Msn4 (34). Here we provide a second example, as a largely unregulated form of Msn2 can be mimicked by serine to alanine replacements in five PKA consensus sites. This Msn2A5 mutant protein is constitutively localized in the nucleus. It activates Msn2-dependent genes and is detrimental for growth when expressed at high levels. The exact reason for the growth arrest caused by active Msn2 is currently not known but could be a cumulative effect of the up-regulation of whole environmental stress response cluster or the specific effects of a few regulatory genes.

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