Derepression of RNA Polymerase III Transcription by Phosphorylation and Nuclear Export of Its Negative Regulator, Maf1*

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Maf1 is the global repressor of RNA polymerase III (Pol III) in yeast Saccharomyces cerevisiae. Transcription regulation by Maf1 is important under stress conditions and during the switch between fermentation and respiration. Under repressive conditions on nonfermentable carbon sources, Maf1 is dephosphorylated and located predominantly in the nucleus. When cells were shifted to glucose medium, Maf1 became phosphorylated and concomitantly relocated to the cytoplasm. This relocation was dependent on Msn5, a carrier responsible for export of several other phosphoproteins out of the nucleus. Using coimmunoprecipitation, Maf1 was found to interact with Msn5. When msn5-Δ cells were transferred to glucose, Maf1 remained in the nucleus. Remarkably, despite constitutive presence in the nucleus, Maf1 was dephosphorylated and phosphorylated normally in the msn5-Δ mutant, and Pol III was under proper regulation. That phosphorylation of Maf1 and Pol III derepression are tightly linked was shown by studying tRNA transcription in Maf1 mutants with an altered pattern of phosphorylation. In summary, we conclude that phosphorylation of Maf1 inside the nucleus acts both directly by decreasing of Maf1-mediated repression of Pol III and indirectly by stimulation of Msn5 binding and export of nuclear Maf1 to the cytoplasm.

Transcription in yeast is regulated by global nutrient-sensing signal transduction cascades. A central component of the eukaryotic growth regulatory network is the TOR (target of rapamycin) cascade, which controls all three polymerase systems involved in ribosome biogenesis (1). How the different environmental signals are integrated by the yeast transcription factor Maf1, a general and global repressor of RNA polymerase III (Pol III), is of considerable interest (2–4). Maf1 was found to mediate several signaling pathways by repressing Pol III transcription in response to diverse stresses such as nutrient deprivation, rapamycin treatment, secretary defects, or DNA damage (5–7). Cells depleted of Maf1 remain alive under stress conditions but, surprisingly, Maf1-mediated regulation is essential during the transition from fermentative to glycerol-based respiratory growth. This observation underscored the critical function of Maf1 in the coupling of Pol III transcription to metabolic processes and/or energy production dependent on the carbon source (8).

The molecular mechanisms that trigger Maf1 activity in response to different signaling pathways are only partially understood. Maf1 is a phosphoprotein harboring two nuclear localization signals (2, 9). Differentially phosphorylated forms of Maf1 can be resolved, and it is the least phosphorylated form that binds Pol III (3, 4). In cells growing exponentially in rich glucose medium, Maf1 is highly phosphorylated and localized mostly in the cytoplasm. When cells are shifted to repressive conditions, Maf1 is dephosphorylated and relocated to the nucleus (3, 4, 8, 9). Protein phosphatase 2A, a component of the TOR signaling pathway, is required for Maf1 dephosphorylation, nuclear accumulation, and Pol III repression in response to rapamycin (3). On the other hand, Pol III repression is blocked by artificially increased activity of cAMP-dependent protein kinase A (8, 9), possibly via inhibition of Maf1 import to the nucleus (9, 10).

The simplest hypothesis accommodating these observations would be that Maf1 activity is regulated via its phosphorylation state-dependent cellular localization (9). Alternatively, both states, phosphorylation and cellular localization, might contribute in synergy to the regulation of Maf1 activity. At this point, the factor(s) involved in Maf1 shuttling as well as the cellular site(s) of Maf1 phosphorylation-dephosphorylation reactions remain(s) to be identified. In addition, the link between the Maf1 phosphorylation state and its cellular location remains to be established to clarify the key determinant controlling Maf1 activity.

Here we identify Msn5 as the nuclear export receptor for Maf1. In the presence of Msn5, phosphorylated Maf1 is relocated out of the nucleus. However, in msn5-Δ cells the cellular localization of Maf1 does not correlate with its phosphorylation state. We show that not Maf1 nuclear export but, rather, Maf1 phosphorylation is necessary for derepression of Pol III transcription.

EXPERIMENTAL PROCEDURES

Strains, Plasmids, and Medium—Yeast strains used in this study included BY4741 (MATα his3-Δ1 leu2-Δ0 lys2-Δ0 ura3-
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Δ0); BY4743 (MATa/MATα his3-Δ1/his3-Δ1 leu2-Δ0/leu2-Δ0 lys2-Δ0/LYS2 MET15/met15-Δ0 ura3-Δ0/ura3-Δ0); YPH500 maf1-Δ, derivative of YPH500 (MATα ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52) (8); msn5-Δ, derivative of BY4741 (YDR335w:kanMX4, Euroscarf); and Y800 MSN5-HA, derivative of BY4743 (MSN5-HA/MSN5, Open Biosystems). The multicopy plasmid YEp24-MSN5 was kindly provided by Mark Johnston.

Rich medium contained 1% yeast extract, 2% peptone, and 2% glucose (YPD) or 2% glycerol (YPGly). The minimal medium (synthetic complete) contained 2% glucose and 0.67% yeast nitrogen base without amino acids (11). Solid medium contained 2% agar. All reagents were from Difco.

Protein Extraction and Immunoblotting—To ensure that kinases or phosphatases were not functional during cell harvesting and protein extraction, yeast cells were rapidly harvested by centrifugation at 4°C, and 20% trichloroacetic acid was added to the cell pellet as described earlier (8). Cells were broken with acid-washed glass beads, the supernatant was retained, and trichloroacetic acid-precipitated proteins were broken with acid-washed glass beads, the supernatant was harvested by centrifugation at 4°C, and 20% trichloroacetic acid was prehybridized in phosphate buffer (7% SDS, 0.5M sodium phosphate, pH 7.2, 1 mM EDTA, pH 7.0, and 1% bovine serum albumin) and hybridized at 37°C in the same solution with oligonucleotide probes labeled with [γ-32P]ATP and T4 polynucleotide kinase (New England Biolabs). The probes used were 5′-CGAGTCGAACGCCCGAT-3′ for tRNA3′(GUA), 5′-TATTCCTACCAGTAAAATCGG-3′ for tRNA1′(GCU), 5′-CCTCCAGATGACTTGACCG-3′ for tRNA5′(GAU), and 5′-GAATGCGGACCAAGCTAA-3′ for U3 small nuclear RNA.

After hybridization, blots were washed 2×10 min with 1× SSC, 1% SDS, and 3×10 min with 0.5× SSC, 0.1% SDS at 37°C, and exposed to film or to a phosphorimager screen (Molecular Dynamics). RNA was quantified using a Storm 820 phosphorimager (Molecular Dynamics). Band intensities were quantified using ImageQuant version 6.2 software.

Generation of Maf1 Mutants—Mutations in plasmid-borne MAF1 were created using a rapid method for localized mutagenesis (12). A fragment of the MAF1 gene was amplified with primers 5′-CGAGTGGTCTTGGTCAATCAGG-3′ and 5′-CTGCTACTGCTCTTTCTTCT-3′ under low-fidelity conditions using a Diversify PCR random mutagenesis kit (Clontech). The pRS315 (LEU2, CEN) plasmid carrying MAF1 was digested with BclI and BsgI. The gapped linear plasmid and product of the low-fidelity PCR were cotransformed into the YPH500 maf1-Δ strain. Transformants, selected on minimal medium lacking leucine, were subsequently tested for Maf1 activity by replica plating on YPGly and incubating at 37°C for 3 days. Colonies unable to grow were expected to have compromised Maf1 function. Plasmids with mutated alleles encoding Maf1 were sequenced.

RESULTS

Maf1 Is Exported Out of the Nucleus in Response to Glucose—Variations in glucose availability in its natural environment force yeast to undergo frequent physiological transitions between fermentation and respiratory growth by using nonfer-
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Maf1 Shuttling and Phosphorylation State—Subsequent studies of msn5Δ cells showed that in these cells Maf1 was retained in the nucleus upon transition from respiratory conditions to glucose, and incubation was prolonged for up to 3 h (Fig. 4A), thus confirming the requirement of Msn5 for Maf1 export. Most remarkably, in the msn5Δ mutant, the Maf1 localization and phosphorylation state were totally uncoupled as Maf1 was dephosphorylated and phosphorylated normally. During the transitions from glucose medium to a nonfermentable carbon source and back to glucose (compare Figs. 1 and 4A), both phosphorylation and dephosphorylation reactions and identified by immunoblotting as a function of time after culture transfer from YPGly back to YPD (Fig. 1, bottom panel). As reported previously, Maf1 was dephosphorylated upon transition from glucose to glycerol medium (compare YPD and YPGly lanes), and the opposite transition resulted in gradual Maf1 phosphorylation (compare YPGly and YPD lanes; 5 min to 1 h). Remarkably, relocation of Maf1 to the cytoplasm in response to glucose appeared to be concomitant with Maf1 hyperphosphorylation, suggesting a link between the Maf1 phosphorylation state and its cellular location.

Msn5 Is Required for Export of Maf1 from the Nucleus—To identify the factors involved in Maf1 shuttling, we examined yeast strains carrying individual deletions of known importin or exportin genes (13). Nuclear transport receptors such as Mtr10, Kap114, Pdr6, Yrb4, Nmd5, Pse1, Srp1, and Sxm1 appeared to play no role in Maf1 transport (results not shown). In contrast, the Msn5 carrier was found to be required for cytoplasmic localization of Maf1. As shown in Fig. 2, msn5Δ mutant cells growing in a glucose-rich medium retained Maf1 in the nucleus, in contrast to the wild type. To test whether the expression of Msn5 was sufficient to relocate Maf1 to the cytoplasm, we transformed the msn5Δ mutant with a multicopy plasmid carrying the MSN5 gene. Indeed, under these conditions Maf1 was relocated to the cytoplasm. We therefore conclude that Msn5 is an exportin for Maf1.

Because exportins have been shown to bind directly to their target proteins, we used communoprecipitation experiments to test whether Msn5 and Maf1 were able to interact with each other. Crude extracts were prepared from Msn5-HA-tagged cells and control wild-type cells with untagged Msn5. Cells were grown in glycerol medium, transferred to prewarmed glucose medium, and harvested after 30 min. HA-tagged Msn5 was immunoprecipitated from cell extracts with anti-HA 12CA5 antibodies, and the immunoprecipitates were examined for the presence of Maf1 by immunoblotting. As shown in Fig. 3, Maf1 was selectively coimmunoprecipitated with HA-tagged Msn5 (compare lane 2 with control lane 4). This result indicates that Maf1 and Msn5 interact with each other.

Maf1 Shuttling and Phosphorylation State—Subsequent studies of msn5Δ cells showed that in these cells Maf1 was retained in the nucleus upon transition from respiratory conditions to glucose, and incubation was prolonged for up to 3 h (Fig. 4A), thus confirming the requirement of Msn5 for Maf1 export. Most remarkably, in the msn5Δ mutant, the Maf1 localization and phosphorylation state were totally uncoupled as Maf1 was dephosphorylated and phosphorylated normally. During the transitions from glucose medium to a nonfermentable carbon source and back to glucose (compare Figs. 1 and 4A), both phosphorylation and dephosphorylation reactions...
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did occur while Maf1 was predominantly retained inside the nucleus.

Msn5 overproduction did not perturb the nuclear accumulation of Maf1 under respiratory conditions (Fig. 4B, YPGly lane), although the Maf1 nuclear import rate was slightly decreased (data not shown). Conversely, Msn5 overproduction increased to some extent the Maf1 nuclear export rate. Thirty minutes after the shift from glycerol to glucose medium, the Maf1 signal became uniformly distributed throughout the cell overproducing Msn5, in contrast to the wild-type cell, which needed 1 h to achieve the similar state (compare Figs. 1 and 4B, top panels). The phosphorylated form of Maf1 appeared concomitantly with its relocation out of the nucleus, 30 min after the shift of cells overproducing Msn5 from glycerol to glucose medium (Fig. 4B, bottom panel), suggesting that export and phosphorylation are linked.

Maf1 Nuclear Export Is Not Required for Derepression of Transcription—Knowing that nuclear export of Maf1 repressor was mediated by Msn5, we wondered whether the constitutive nuclear location of Maf1 in msn5-Δ cells might result in constitutive repression of Pol III transcription. We therefore compared the derepression of Pol III transcription in wild-type and msn5-Δ cells upon transition from respiratory conditions to glucose. The msn5-Δ mutant and isogenic wild-type control cells were grown at 30 °C to exponential phase in glucose medium (YPD), transferred to prewarmed glycerol medium (YPGly) for 1 h, and then transferred back to YPD. RNA was isolated from cells harvested from the glycerol medium or after 2 or 3 h of growth in glucose-rich medium and analyzed by Northern blotting. To estimate the amounts of newly synthesized tRNAs, we used intron probes complementary to pre-tRNA\textsubscript{Tyr}, pre-tRNA\textsubscript{Leu}, and pre-tRNA\textsubscript{Phe} (Fig. 5A). Quantification of the intron-containing tRNA precursors revealed a similar repression of Pol III activity in both wild-type and msn5-Δ strains upon transition of glucose-grown cells to glycerol and a similar derepression level upon transition back to glucose medium (Fig. 5B). Clearly, Maf1 nuclear export was not essential for the derepression of Pol III transcription upon growth on a fermentable carbon source.

Derepression of Pol III Is Correlated with Phosphorylated State of Maf1—To better understand the relationship between Pol III derepression and the presence of phosphorylated Maf1, we selected Maf1 mutants defective in Pol III regulation and examined their phosphorylation status. A fragment of the MAF1 gene was PCR-amplified under mutagenic conditions and transformed to maf1-Δ/H9004 cells together with the gapped Maf1-encoding single copy plasmid pRS315-MAF1. Transformants selected as unable to grow at 37 °C on YPGly, likely to influence Maf1 phosphorylation, were checked by sequencing for MAF1 mutations and then examined by Western blotting.

This revealed two mutant strains, pAG23 (Phe\textsuperscript{32} → Ser, Ile\textsuperscript{54} → Thr, Ans\textsuperscript{142} → Asp) and
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DISCUSSION

RNA Pol III produces essential components of the protein biosynthesis machinery, and therefore its activity is tightly coupled to cell growth and metabolism. The mechanism for this coordination is provided by Maf1, a general negative regulator conserved from yeast to man (2, 14, 15). Maf1 of Saccharomyces cerevisiae contains 15.7% of serines, which provide nearly 30 potential phosphorylation sites specific for several kinases (data according to Swiss-Prot PROSITE). Thus, phosphorylation of different sites may be used to regulate Maf1 function under various conditions. However, so far there has been no proof that Maf1 activity is controlled by phosphorylation, although indirect indications were obvious. First, protein phosphatase 2A was shown to be required for Maf1-generated repression of Pol III and for nuclear accumulation of Maf1 in response to rapamycin (3, 4). Second, it was shown that under conditions of favorable growth, Maf1 was largely phosphorylated and located mostly in the cytoplasm, although a low level of phosphorylated and nuclearily localized Maf1 was also observed (8, 9). In this report we present evidence that both phosphorylation and dephosphorylation of Maf1 may occur inside the nucleus. Concomitantly with phosphorylation, Maf1 is exported to the cytoplasm by the Msn5 carrier. However, it is not the export but the phosphorylation of Maf1 that is essential for the derepression of Pol III transcription in response to favorable growth conditions.

As shown previously, Maf1 is relocated out of the nucleus in response to a shift of respiratory growing culture to a medium with glucose (8). We used this observation to examine yeast mutants defective in various exportins and found constitutive nuclear localization of Maf1 in msn5-Δ cells. Two lines of evidence show that Msn5 functions as an exportin for Maf1. When the msn5-Δ cells are transformed with an Msn5-encoding plasmid, relocation of Maf1 to the cytoplasm is observed again. Furthermore, using coimmunoprecipitation we were able to show that Maf1 interacts with Msn5.

It is worth mentioning that the Msn5 carrier, nuclearily localized under standard conditions in glucose, becomes redistributed to the cytoplasm upon transfer to a medium with a non-fermentable carbon source (16). It was shown that whenever Msn5 was detected in the cytoplasm, Saa4 protein, another Msn5 cargo, was concentrated in the nucleus (16). It was suggested that when Msn5 was sequestered in the cytoplasm, it was unable to ferry its cargo from the nucleus. The location of Msn5 may affect distribution of Maf1 in a similar manner.

It has been suggested previously that Msn5 specifically exports phosphoproteins because several Msn5 cargoes, such as Pho4, Mig1, Crz1, Aft1, Cdh1, and HO, have to be phosphorylated to be relocated out of the nucleus (17–23). However, binding of Msn5 to other cargoes, such as Far1, does not depend on the cargo phosphorylation state (24).
The good synchronization of phosphorylation and export to the cytoplasm observed for Maf1 in wild-type cells suggests that both processes might be interdependent. This relation becomes more understandable when studied in strains with altered Msn5 levels. Examination of msn5-Δ cells shows that export to the cytoplasm is not necessary for Maf1 phosphorylation. This experiment clearly documents that Maf1 can be dephosphorylated and phosphorylated in the nucleus, suggesting that phosphorylation of Maf1 precedes its export. This is not questioned by examination of a strain overproducing Msn5. Although the rate of Maf1 export was enhanced with no change in the kinetics of its phosphorylation, a slight amount of the phosphorylated form appeared at the same time when relocation to the cytoplasm became visible. Most likely, the excess of Msn5 titrates phosphorylated Maf1 more quickly. In summary, we concluded that during the transfer of cells from a nonfermentable carbon source to glucose, nuclear Maf1 becomes phosphorylated and subsequently exported to the cytoplasm by Msn5. Thus, Maf1 belongs to the major category of Msn5 substrates, which are bound by Msn5 as phosphoproteins. Similar to the other Msn5 cargoes, Maf1 does not contain an obvious leucine-rich nuclear export signal. A comparative bioinformatic analysis of all Msn5 cargoes showed no common motifs or domains, thus suggesting Msn5 binding to a specific secondary structure containing phosphate residues.

The kinase that phosphorylates Maf1 upon transferring cells from a nonfermentable carbon source to glucose has not been identified yet. The nuclear localization of this kinase has been suggested by examination of Maf1 phosphorylation in msn5-Δ cells. However, it cannot be excluded that the unknown kinase is also Msn5 cargo. In this case, the kinase would likewise be retained in the nucleus of msn5-Δ cells but transported from the nucleus to the cytoplasm in cells that do express Msn5. Thus, it remains to be shown whether Maf1 can be phosphorylated within the nucleus of wild-type yeast.

Maf1 was previously found as a substrate of protein kinase A kinase (9, 25). However, our previous data indicated that the protein kinase A-directed phosphorylation of Maf1 was not involved in the coupling of Pol III regulation to the carbon source (8). Moreover, protein kinase A is considered, rather, a cytoplasmic kinase, although its nuclear localization is also possible (26). Previously, Moir et al. (9) reported that protein kinase A-directed phosphorylation of Maf1 prohibited its nuclear import under favorable growth conditions. It may be that phosphorylation of protein kinase A sites in Maf1 prevents its entry to the nucleus, whereas phosphorylation of other sites by an unknown nuclear kinase works against interaction of Maf1 with Pol III.

Interestingly, despite the unusual, constitutively nuclear localization of Maf1 in msn5-Δ cells, Pol III-transcribed genes are under proper regulation. This indicates that phosphorylation of nuclear Maf1 causes Pol III derepression in glucose-grown cells. This hypothesis is further supported by our finding of a direct link between constant Maf1 phosphorylation and the defect in Pol III regulation observed in pAG23 and pAG49 mutants. Thus, we conclude that phosphorylation of Maf1 by an unknown nuclear kinase is a key part of the mechanism controlling Pol III (3). The detailed mechanism of this regulation remains to be elucidated.

A study of the Msn5-dependent cytoplasmic accumulation of Maf1 allowed us to learn important aspects of Maf1 function; a proposed scheme is presented in Fig. 7. Following a transfer of cells to favorable growth conditions, nuclear Maf1 is phosphorylated by an unknown kinase, which prevents its interaction with Pol III and leads to derepression of transcription. The phosphorylated Maf1 is relocated out of the nucleus by the Msn5 exportin. In parallel, protein kinase A-directed phosphorylation at different sites prevents nuclear import of the newly synthesized cytoplasmic Maf1 by interference with its nuclear localization signal sequence (9). This means that Maf1 phosphorylation operates at several levels. Apart from decreasing direct Maf1 binding to Pol III (3), phosphorylations act both to relocate the nuclear pool of Maf1 and to prevent import of cytoplasmic Maf1 to the nucleus.

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REFERENCES
1. Tsang, C. K., and Zheng, X. F. (2007) Cell Cycle 6, 25–29
2. Pluta, K., Lefebvre, O., Martin, N. C., Smagowicz, W. J., Stanford, D. R., Ellis, S. R., Hopper, A. K., Sentenac, A., and Boguta, M. (2001) Mol. Cell. Biol. 21, 5031–5040
3. Oficjalska-Pham, D., Harismendy, O., Smagowicz, W. J., Gonzalez de Peredo, A., Boguta, M., Sentenac, A., and Lefebvre, O. (2006) Mol. Cell 22, 623–632
4. Roberts, D. N., Wilson, B., Huff, J. T., Stewart, A. J., and Cairns, B. R. (2006) Mol. Cell 22, 633–644
5. Li, Y., Moir, R. D., Sethy-Coraci, I. K., Warner, J. R., and Willis, I. M. (2000) Mol. Cell. Biol. 20, 3843–3851
6. Upadhyya, R., Lee, J., and Willis, I. M. (2002) Mol. Cell 10, 1489–1494
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7. Desai, N., Lee, J., Upadhya, R., Chu, Y., Moir, R. D., and Willis, I. M. (2005) *J. Biol. Chem.* **280**, 6455–6462
8. Ciesla, M., Towpik, J., Graczyk, D., Oficjalska-Pham, D., Harismendy, O., Suleau, A., Balicki, K., Conesa, C., Lefebvre, O., and Boguta, M. (2007) *Mol. Cell. Biol.* **27**, 7693–7702
9. Moir, R. D., Lee, J., Haeusler, R. A., Desai, N., Engelke, D. R., and Willis, I. M. (2006) *Proc. Natl. Acad. Sci. U. S. A.* **103**, 15044–15049
10. Willis, I. M., and Moir, R. D. (2007) *Trends Biochem. Sci.* **32**, 51–53
11. Sherman, F. (2002) *Methods Enzymol.* **350**, 3–41
12. Muhlrad, D., Hunter, R., and Parker, R. (1992) *Yeast* **8**, 79–82
13. Mosammaparast, N., and Pemberton, L. F. (2004) *Trends Cell Biol.* **14**, 547–556
14. Geiduschek, E. P., and Kassavetis, G. A. (2006) *Curr. Biol.* **16**, R849–R851
15. Reina, J. H., Azzouz, T. N., and Hernandez, N. (2006) *PLoS ONE* **1**, e134
16. Quan, X., Tsoulos, P., Kuritzky, A., Zhang, R., and Stochaj, U. (2006) *Mol. Microbiol.* **62**, 592–609
17. Kaffman, A., Rank, N. M., O’Neill, E. M., Huang, L. S., and O’Shea, E. K. (1998) *Nature* **396**, 482–486
18. DeVit, M. J., and Johnston, M. (1999) *Curr. Biol.* **9**, 1231–1241
19. Boustany, L. M., and Cyert, M. S. (2002) *Genes Dev.* **16**, 608–619
20. Ueta, R., Fujiwara, N., Iwai, K., and Yamaguchi-Iwai, Y. (2007) *Mol. Biol. Cell* **18**, 2980–2990
21. Jaquenoud, M., van Drogen, F., and Peter, M. (2002) *EMBO J.* **21**, 6515–6526
22. Kaplun, L., Ivantsiv, Y., Bakhrat, A., and Raveh, D. (2003) *J. Biol. Chem.* **278**, 48727–48734
23. Hopper, A. K. (1999) *Curr. Biol.* **9**, R803–R806
24. Blondel, M., Alepuz, P. M., Huang, L. S., Shaham, S., Ammerer, G., and Peter, M. (1999) *Genes Dev.* **13**, 2284–2300
25. Budovskaya, Y. V., Stephan, J. S., Deminoff, S. I., and Herman, P. K. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102**, 13933–13938
26. Griffioen, G., and Thevelein, J. M. (2002) *Curr. Genet.* **41**, 199–207