Identification of Translational Regulation Target Genes during Filamentous Growth in *Saccharomyces cerevisiae*: Regulatory Role of Caf20 and Dhh1

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The dimorphic transition of yeast to the hyphal form is regulated by the mitogen-activated protein kinase and cyclic AMP-dependent protein kinase A pathways in *Saccharomyces cerevisiae*. Signaling pathway-responsive transcription factors such as Ste12, Tec1, and Flo8 are known to mediate filamentation-specific transcription. We were interested in investigating the translational regulation of specific mRNAs during the yeast-to-hyphal-form transition. Using polyribosome fractionation and RT-PCR analysis, we identified *STE12*, *GPA2*, and *CLN1* as translation regulation target genes during filamentous growth. The transcript levels for these genes did not change, but their mRNAs were preferentially associated with polyribosomes during the hyphal transition. The intracellular levels of Ste12, Gpa2, and Cln1 proteins increased under hyphal-growth conditions. The increase in Ste12 protein level was partially blocked by mutations in the *CAF20* and *DHH1* genes, which encode an eIF4E inhibitor and a decapping activator, respectively. In addition, the caf20 and dhh1 mutations resulted in defects in filamentous growth. The filamentation defects caused by caf20 and dhh1 mutations were suppressed by *STE12* overexpression. These results suggest that Caf20 and Dhh1 control yeast filamentation by regulating *STE12* translation.

The cellular morphology of diploid *Saccharomyces cerevisiae* frequently switches between the yeast and filamentous forms depending on nutritional signals (16). Several signal transduction modules, including the mitogen-activated protein kinase (MAPK) cascade and the cyclic AMP-dependent protein kinase A (PKA) pathway, are known to participate in this switch (14, 21, 31, 36). The MAPK cascade involves Ste20, Ste11, Ste7, Kss1, and the transcription factors Ste12 and Tec1 (15, 21, 29, 30). The PKA pathway involves Gpr1, Gpa2, Ras2, Tpk2, and the transcription factors Flo8 and Sfl1 (22, 26, 31, 32). These signaling pathways control the transcription of a number of filamentation-specific genes, including *FLO11* (19, 23, 29).

Although the signaling pathways and transcriptional regulation of yeast filamentous growth have been studied in considerable detail, little is known about translational regulation related to the transition from the yeast to the filamentous form. In this study, we searched for specific mRNAs that are preferentially translated during the yeast-to-hyphal-form transition. Genome-wide analysis of mRNA translation profiles indicates that the loading of ribosomes onto individual mRNA species varies broadly (20, 28). The association of mRNA transcripts in polyribosomes reflects the rate of synthesis of their corresponding proteins (3, 45). By purifying polyribosome fractions and employing RT-PCR analysis, we found that the mRNA transcripts of *STE12*, *GPA2*, and *CLN1* were preferentially recruited to polyribosomes during filamentation compared to during normal vegetative growth, even though their levels in the total cell extracts were not changed. Consistently, the protein levels of Ste12, Gpa2, and Cln1 also increased during filamentation. The up-regulation of *STE12* mRNA translation during filamentous growth appeared to be partly dependent on *CAF20* and *DHH1*, which encode an eIF4E (the cap-binding protein) inhibitor and an mRNA decapping activator, respectively. Both *CAF20* and *DHH1* were shown to be important for filamentous growth in yeast.

**MATERIALS AND METHODS**

**Strains, plasmids, and growth conditions.** The *S. cerevisiae* strains and plasmids used in this study are listed in Table 1. Standard yeast media were prepared using the established procedure (1). Synthetic low-ammonium medium (SLAD) was prepared as described previously (16). 5-FOA (5-fluoro-orotic acid) medium was composed of 0.67% yeast nitrogen without amino acid, 2% dextrose, and 0.1% 5-FOA (1). Standard methods of yeast transformation and genetic crosses were used for the constructions of all strains.

**Cell lysis and polyribosome fractionation.** Yeast cells were grown at 30°C in YEPD (yeast extract-peptone-dextrose) or SLAD to an *A*<sub>600</sub> of 0.8 to 1.0. Prior to cell collection, cycloheximide was added to a final concentration of 50 μg/ml. Cells were pelleted by centrifugation and washed with 1/30 culture volume of breaking buffer A (BBA; 20 mM Tris-Cl [pH 7.5], 50 mM NaCl, 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 50 μg/ml cycloheximide, and 20 μg/ml heparin) on ice (34). Cells were resuspended in 1.5 cell volumes of BBA and lysed by vortexing in the presence of 1 volume of glass beads. Lysates were centrifuged by centrifugation at 4,200 rpm for 5 min, and the supernatants were centrifuged at 13,000 rpm for 20 min. Twenty-five *A*<sub>260</sub> units of lysates were fractionated on 5- to 45% sucrose gradients as described previously (12). Gradients were centrifuged at 40,000 rpm for 3 h, and the pellets were resuspended in 0.8 to 1.0. Prior to cell collection, cycloheximide was added to a final concentration of 50 μg/ml. Cells were pelleted by centrifugation and washed with 1/30 culture volume of breaking buffer A (BBA; 20 mM Tris-Cl [pH 7.5], 50 mM NaCl, 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 50 μg/ml cycloheximide, and 20 μg/ml heparin) on ice (34). Cells were resuspended in 1.5 cell volumes of BBA and lysed by vortexing in the presence of 1 volume of glass beads. Lysates were centrifuged by centrifugation at 4,200 rpm for 5 min, and the supernatants were centrifuged at 13,000 rpm for 20 min. Twenty-five *A*<sub>260</sub> units of lysates were fractionated on 5- to 45% sucrose gradients as described previously (12). Gradients were centrifuged at 35,000 rpm in an SW41 rotor (Beckman) at 4°C for 3.5 h and were then fractionated with monitoring of *A*<sub>260</sub>.

**RNA analysis and RT-PCR.** Total RNA was isolated from each fraction with an RNeasy kit (QIAGEN). cDNA synthesis was performed using 20 μg/ml RNA in 10 μg/ml oligo(dT), 50 mM Tris-Cl (pH 8.3), 75 mM KCl, 3 mM MgCl<sub>2</sub>, 10 mM dithiothreitol, 2.5 mM deoxynucleoside triphosphates (dNTPs), and Moloney murine leukemia virus reverse transcriptase (Gibco BRL). Reactions were car-
TABLE 1. Strains and plasmids used in this study

| Strain or plasmid | Genotype | Source or reference |
|-------------------|----------|---------------------|
| Strains<sup>a</sup> |          |                     |
| 10500-2B          | MAT<sup>a</sup> ura3-52 his3::hisG leu2::hisG | G. R. Fink         |
| 10500-5B          | MAT<sup>a</sup> ura3-52 trp1::hisG leu2::hisG | G. R. Fink         |
| JK353             | MAT<sup>a</sup> MAT<sup>a</sup> ura3-52 ura3-52 his3::hisG<sup>+</sup> trp1::hisG<sup>+</sup> leu2::hisG/leu2::hisG | This laboratory |
| JK354             | MAT<sup>a</sup> MAT<sup>a</sup> ura3-52 ura3-52 his3::hisG<sup>+</sup> trp1::hisG<sup>+</sup> leu2::hisG/leu2::hisG | This laboratory |
| JK371             | MAT<sup>a</sup> ura3-52 his3::hisG leu2::hisG FLO11::HA | This work          |
| JK372             | MAT<sup>a</sup> ura3-52 his3::hisG leu2::hisG GPA2::HA | This work          |
| JK373             | MAT<sup>a</sup> ura3-52 his3::hisG leu2::hisG STE12::HA | This work          |
| JK374             | MAT<sup>a</sup> ura3-52 his3::hisG leu2::hisG CLN1::HA | This work          |
| JK375             | MAT<sup>a</sup> ura3-52 his3::hisG leu2::hisG FLO8::HA | This work          |
| JK376             | MAT<sup>a</sup> MAT<sup>a</sup> ura3-52 ura3-52 his3::hisG<sup>+</sup> trp1::hisG<sup>+</sup> leu2::hisG/leu2::hisG FLO11/FLO11::HA | This work          |
| JK377             | MAT<sup>a</sup> MAT<sup>a</sup> ura3-52 ura3-52 his3::hisG<sup>+</sup> trp1::hisG<sup>+</sup> leu2::hisG/leu2::hisG GPA2::HA | This work          |
| JK378             | MAT<sup>a</sup> MAT<sup>a</sup> ura3-52 ura3-52 his3::hisG<sup>+</sup> trp1::hisG<sup>+</sup> leu2::hisG/leu2::hisG STE12/STE12::HA | This work          |
| JK379             | MAT<sup>a</sup> MAT<sup>a</sup> ura3-52 ura3-52 his3::hisG<sup>+</sup> trp1::hisG<sup>+</sup> leu2::hisG/leu2::hisG CLN1/CLN1::HA | This work          |
| JK380             | MAT<sup>a</sup> MAT<sup>a</sup> ura3-52 ura3-52 his3::hisG<sup>+</sup> trp1::hisG<sup>+</sup> leu2::hisG/leu2::hisG FLO8/FLO8::HA | This work          |
| JK381             | MAT<sup>a</sup> ura3-52 his3::hisG leu2::hisG caf20::LEU2 | This work          |
| JK382             | MAT<sup>a</sup> MAT<sup>a</sup> ura3-52 ura3-52 his3::hisG<sup>+</sup> trp1::hisG<sup>+</sup> leu2::hisG/leu2::hisG caf20::LEU2/caf20::LEU2 | This work          |
| JK384             | MAT<sup>a</sup> ura3-52 ura3-52 his3::hisG leu2::hisG tfi1::LEU2 | This work          |
| JK386             | MAT<sup>a</sup> MAT<sup>a</sup> ura3-52 ura3-52 his3::hisG<sup>+</sup> trp1::hisG<sup>+</sup> leu2::hisG/leu2::hisG tfi1::LEU2/tfi1::LEU2 | This work          |
| JK387             | MAT<sup>a</sup> ura3-52 his3::hisG leu2::hisG dhhl1::LEU2 | This work          |
| JK389             | MAT<sup>a</sup> MAT<sup>a</sup> ura3-52 ura3-52 his3::hisG<sup>+</sup> trp1::hisG<sup>+</sup> leu2::hisG/leu2::hisG dhhl1::LEU2/dhhl1::LEU2 | This work          |
| Plasmids          |          |                     |
| pJ1255            | STE12-HA URA3 CEN | This work          |
| pJ1256            | CLN1-HA URA3 CEN | This work          |
| pJ1257            | FLO8-HA URA3 CEN | This work          |
| pJ1274            | STE12-HA 2µ ARS URA3 | This work          |
| pJ1276            | CAF20 URA3 CEN | This work          |
| pJ1277            | DIH1 URA3 CEN | This work          |
| YEp355-FLO11::lacZ | FLO11::lacZ URA3 2µ | This work          |
| pRS426            | 2µ ARS URA3 | 37               |
| pRS316            | CEN ARS URA3 | 42               |

<sup>a</sup> All yeast strains are derived from the Σ1278b background.

RESULTS

STE12, GPA2, and CLN1 mRNA transcripts are preferentially recruited to polyribosomes under filamentous-growth conditions. Little is known about the translational regulation of specific mRNAs during the yeast-to-hyphal-form transition. Based on the finding that actively translated mRNAs are as-

... through a 10% formaldehyde gel and was subsequently transferred to a Nitran membrane (Hoefer). Blotting was performed as described elsewhere (38). The PCR products of FLO11 (ORF 3541 to 4074), GPA2 (408 to 1164), STE12 (1095 to 1759), CLN1 (428 to 1165), FLO8 (1081 to 1797), and ACT1 (49 to 770) were used as probes. Probes were labeled with a Random Prime labeling system (Amersham).

Construction of deletion mutants. Deletion mutations of CAF20, DHH1, and TFI1 were constructed using PCR-based gene disruption methods (27, 44). The disruption marker LEU2 was PCR amplified using primers containing a 51-bp sequence homologous to the target gene and an 18-bp sequence from the LEU2 marker. The PCR products were transformed into a haploid strain of the a or a mating type. Integration of LEU2 at each gene was confirmed by PCR analysis of genomic DNA from each transformant.

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associated with polyribosomes, we analyzed the polyribosomal mRNAs and searched for genes actively translated during hyphal induction. A diploid yeast strain was cultured in either YEPD medium (yeast form) or SLAD (pseudohyphal form) at 30°C for 8 h. At this time point, cells in SLAD are in the early stage of the hyphal transition. This is sufficient to induce hyphal-form-specific gene expression. Total cellular mRNAs were fractionated through a 5-to-45% sucrose gradient, and the abundance of target mRNAs in polyribosomal fractions was analyzed by RT-PCR using gene-specific primers (Fig. 1).

The specific mRNA molecules examined include those of two protein kinases (Ste20 and Ste11), five transcription factors (Ste12, Tec1, Flo8, Msn1, and Ms11), a cyclin (Cln1), two membrane-bound signaling molecules (Mep2 and Gpa2), and the cell surface protein Flo11 (13, 14, 24, 26). As shown previously (23), the levels of FLO11 total RNAs were higher under conditions promoting hyphal growth than conditions promoting yeast growth (Fig. 1A). Accordingly, polyribosomal FLO11 mRNAs were more abundant in the hyphal culture than the yeast culture (Fig. 1C). Importantly, we found that even though the mRNA levels of STE12, GPA2, and CLN1 were not induced under nitrogen starvation conditions (Fig. 1A), these transcripts were enriched in the polyribosomal fractions (Fig. 1C). Total and polysomal levels of FLO8 mRNA did not change during the yeast-to-hyphal-form transition. Similarly, the transcripts of six other genes (STE20, STE11, TEC1, MSN1, MSS11, and MEP2) were not enriched in the polyribosomal fractions (data not shown). These results suggest that the mRNA of STE12, GPA2, and CLN1 were preferentially recruited to polyribosomes for translation during the yeast pseudohyphal differentiation.

Ste12, Gpa2, and Cln1 protein levels increase during filamentous growth. To determine whether the levels of Ste12, Gpa2, and Cln1 proteins increase during the yeast-to-hyphal-form transition, we inserted the HA epitope into the chromosome copy of FLO11, STE12, GPA2, CLN1, and FLO8 genes in a haploid strain (see Materials and Methods). All of the HA-tagged genes except GPA2 appeared to be functional in the filamentous phenotype, as assayed by a haploid invasive-growth test (Fig. 2). Diploid strains, which were constructed by mating the HA-tagged strains with the opposite mating type, behaved like a wild-type strain in a pseudohyphal-growth test (data not shown).

HA-tagged diploid strains were grown to the late exponential phase. The cultures were then shifted to filamentation-inducing medium (SLAD), and total protein was isolated after 2, 4, and 8 h. At the 4- and 8-h time points, the level of Flo11-HA protein in the hyphal culture was higher than in the
of translation initiation, the cap-binding protein eIF4E binds to m’G caps at the 5’ termini of mRNA and subsequently associates with eIF4G (33, 43). Caf20 is known to compete with eIF4G for binding to eIF4E and to inhibit cap-dependent translation (2, 9, 35). The RNA helicase eIF4A is another binding partner of eIF4G and is thought to unwind the 5’ secondary structure of mRNA. Two duplicate genes, TIFI and TIF2, encode eIF4A, and disruption of both is lethal to the cell (39). As shown in Fig. 4A, caf20/caf20 and tif1/tif1 diploid mutant strains showed lower levels of Ste12-HAp than the wild type under filamentous-growth conditions. The relative levels of Ste12p were 0.53 for caf20 and 0.74 for tif1 (Fig. 4B). The effects of the caf20 or tif1 mutations did not appear to be due to general translational repression in the filamentation-inducing medium, because these mutations did not affect the level of Cln1-HAp or Flo8-HAp.

The regulation of translation initiation and the stability of mRNAs are intimately linked (41). We asked whether mutations in mRNA decapping or degradation enzymes affect the expression of STE12. Previously, our group reported that the deletion mutation of KEM1/XRN1, which encodes a major cytoplasmic 5’-3’ exoribonuclease, causes a defect in haploid invasive and diploid filamentous growth (18). DHH1 encodes a DEAD box RNA helicase and has been reported to be an activator of decapping (8, 11). DHH1 and KEM1/XRN1 have been shown to be the components of the mRNA processing bodies (5, 25). Recent results suggest that Dhh1 also functions as a repressor of translation (7). The level of Ste12 protein was examined in the dhh1/dhh1 and kem1/kem1 mutant strains. As shown in Fig. 4A, the kem1 mutation did not affect the level of Ste12 protein under filamentous-growth conditions. These results rule out the possibility that the kem1 mutation affects the stability of STE12 mRNAs and thus alters STE12 expression. In the dhh1/dhh1 mutant strains, the Ste12p level did not increase during filamentation. The level of Ste12p in dhh1 mutant cells was 0.14 relative to that in the wild type (Fig. 4B). Northern blotting showed that the caf20, dhh1, and tif1 mutations have no effect on STE12/H4 transcripts (Fig. 4C). Our results suggest that CAF20, DHH1, and TIFI are required for STE12 expression at the protein level during filamentous growth.

caf20/caf20 and dhh1/dhh1 mutants show defects in filamentous growth. The transcription factor Ste12 is essential for activation of filamentation-specific genes. We investigated whether the low levels of Ste12 protein in caf20/caf20, dhh1/dhh1, and tif1/tif1 mutant cells result in defects in filamentous growth. Pseudohyphal phenotypes of these mutant strains were tested. The colony morphologies on SLAD were examined before and after washing (Fig. 5A). The caf20/caf20 mutants exhibited pseudohyphal colony morphology with an unusual colony edge. The differences from the wild type were more evident in the agar invasion phenotype. The dhh1/dhh1 mutants were defective in the pseudohyphal colony morphologies and agar invasion phenotypes. The tif1/tif1 mutants, however, did not show any defects in the pseudohyphal phenotypes.

We next examined the cellular morphologies on the SLAD plates by light microscopy (Fig. 5B). After 10 h, the colony-forming cells of the wild-type strain were elongated and formed pseudohyphae, whereas caf20/caf20 and dhh1/dhh1 cells were in the yeast form. These results indicate that caf20

FIG. 3. Increased levels of Gpa2, Ste12, and Cln1 proteins during the pseudohyphal transition. (A) Western blots of Flo11-HA (JK376), Gpa2-HA (JK377), Ste12-HA (JK378), Cln1-HA (JK379), and Flo8-HA (JK380) strains in YEPD medium or SLAD. Tubulins, commonly used as a loading control, showed an increase in the protein level in SLAD. Flo8-HA showed a constant level of proteins. (B) Northern blots of Flo11-HA, Gpa2-HA, Ste12-HA, Cln1-HA, and Flo8-HA strains in YEPD medium or SLAD. Act1 is a loading control.
FIG. 5. Pseudohyphal defects of caf20/caf20 and dhh1/dhh1 mutant strains. (A) Colony morphologies. Wild-type strain JK353 and mutant strains JK383 (caf20/caf20), JK386 (tif1/tif1), and JK389 (dhh1/dhh1) were tested on SLAD plates. After 5 days of incubation, the colonies were photographed before (top) and after (bottom) cells were washed off the agar plate. (B) Enlarged view of the cells on the SLAD plate after 10 h of incubation.

FIG. 4. Effects of caf20, dhh1, and tif1 mutations on the induction of Ste12-HA protein during the pseudohyphal transition. (A) Western blots of Ste12-HA, Cln1-HA, and Flo8-HA. Plasmid pJ1255 (STE12-HA), pJ1256 (CLN1-HA), or pJ1257 (FLO8-HA) was introduced into wild-type strain JK353 and mutant diploid strains JK383 (caf20/caf20), JK386 (tif1/tif1), JK354 (kem1/kem1), and JK389 (dhh1/dhh1). After growth for 8 h in YEPD medium or SLAD, total proteins were analyzed by Western blotting. (B) Relative levels of Ste12-HA protein in SLAD. The results are averages for three independent Western experiments. (C) Northern blots of STE12-HA in SLAD.
and dhhl mutations show defects in pseudohyphal development.

Invasive growth of the haploid mutant strains was tested on YEPD medium (Fig. 6A). The caf20 and dhhl mutants were markedly defective in invasive growth. The invasive growth of the tif1 mutant, however, was similar to that of the wild type. The invasive-growth phenotypes of the mutant strains were in good correlation with FLO11-lacZ expression in the mutant cells (Fig. 6B). These results indicate that Caf20 and Dhh1 play critical roles in both haploid invasive growth and diploid pseudohyphal development.

**Filamentous growth defects caused by caf20/caf20 and dhhl/dhhl mutations are suppressed by overexpression of STE12.**

To determine whether the overexpression of STE12 suppresses the filamentation defects caused by caf20 and dhhl mutations, we introduced a 2μ-based plasmid carrying STE12 with its own promoter into diploid mutant strains. As shown in Fig. 7A, STE12 overexpression restored both the filamentous colony morphology and the agar invasion phenotype to the caf20/caf20 and dhhl/dhhl mutant strains. The level of Ste12p in each overexpressing strain, which was analyzed with the STE12-HA allele, was consistent with its suppression phenotype (Fig. 7B). STE12 overexpression enhanced filamentation in the caf20/caf20 strain to nearly the same extent as in the wild-type strain. STE12 overexpression in the dhhl/dhhl strain, by contrast, only slightly enhanced filamentation and resulted in a reduced colony size. On the basis of these results, we propose that the filamentation phenotypes in the caf20/caf20 and dhhl/dhhl mutant strains are closely related to their low levels of Ste12p.

**DISCUSSION**

The signaling pathways and the transcriptional regulations associated with filamentous growth of S. cerevisiae have been analyzed in considerable detail, but understanding of the regulation at the protein level is limited (14, 19, 21). Here, we identified three genes, STE12, GPA2, and CLN1, that are up-regulated at the protein level during the yeast-to-pseudohyphal-form transition. The increased levels of these proteins could be due to increased translation or greater protein stability. On the basis of our data, it is likely that these regulations are at the translational level. Polyribosomal mRNAs for STE12, GPA2, and CLN1 were abundant under hyphal-culture conditions, indicating that they were actively translated. We also showed that Caf20, which is a cap-dependent translation inhibitor, is involved in the up-regulation of Ste12 protein during filamentous growth.

Our findings suggest for the first time that CAFA0 and DHH1 participate in filamentous growth. The 4E-BPs, which were the first IF4E-inhibitory proteins discovered, modulate eIF4E-eIF4G interaction by sequestering available eIF4E (35). In S. cerevisiae, Caf20 was found to be equivalent to 4E-BPs (2). Deletion of CAFA0 increases the growth rate in rich media and partially suppresses the effects of mutations in translation initiation factors (2, 9). In vitro translation assays show that p20 inhibits the translation of capped reporter mRNAs (2). There have been fewer studies on the significance of Caf20 as a cap-dependent translation repressor in S. cerevisiae than in cells of higher eukaryotes. Dhh1 was previously reported as a decapping activator but was recently shown also to function as a translational repressor (7, 8, 11). Our finding that the level of the Ste12 protein does not increase in the caf20/caf20 or dhhl/dhhl mutant cells implies that Caf20 and Dhh1, previously known as general translational repressors, play positive roles in the up-regulation of Ste12 protein under filamentous-growth conditions.

The low level of Ste12p in caf20/caf20 and dhhl/dhhl mutant cells appeared to be the main reason for the filamentation defects, because overexpression of STE12 in caf20/caf20 and dhhl/dhhl mutant cells restored the filamentation phenotypes. Each of these mutants, however, has a different phenotype. The caf20/caf20 mutant strain showed a reduced invasiveness, whereas the dhhl/dhhl mutant strain had a more severe defect in filamentation. In addition, overexpression of STE12 in the dhhl/dhhl mutant strain resulted in a synthetic phenotype (i.e., reduced colony size) (Fig. 7A). In the present study, we observed mainly a reduced level of Ste12p in the caf20/caf20 or dhhl/dhhl mutant strains, but it remains possible that the caf20 or dhhl mutation could affect expression of other filamentation-associated genes, such as SFL1, TEC1, etc. (29, 32). In addition, DHH1 has been implicated in a number of cellular processes, including mRNA decapping, deadenylation, tran-
scription, and G1/S cell cycle arrest (4, 8). Further analysis of the role of CAF20 and DHH1 in filamentous growth and STE12 expression, therefore, should help clarifying their roles in yeast cells.

Three genes, STE12, GPA2, and CLN1, were identified in our screening as genes that are up-regulated at the protein level during filamentous growth. We observed that the caf20 and dhh1 mutations did not affect the level of Cln1p. These results imply that the up-regulation of CLN1 mRNA translation is independent of CAF20 and DHH1. The Cln1p level appeared to be further increased by the STE11-4 hyperactive allele under the filamentous-growth conditions, whereas the Ste12p level was not affected by this allele (data not shown).

These results suggest that different mechanisms regulate CLN1 and STE12 expression. We are currently investigating other components of the translation initiation and mRNA decay pathways that appear to participate in translational regulation during filamentation.

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REFERENCES

1. Adams, A. D., E. Gottschling, C. A. Kaiser, and T. Stearn. 1997. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

2. Altman, G. L., K. S. Bickel, V. L. MacKay, and D. R. Morris. 2003. General translation of mRNA in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA 100:3889–3894.

3. Bergkes, M., and C. Kaiser. 2004. An essential role for the Saccharomyces cerevisiae DEAD-box helicase Dhh1 in G1/S DNA-damage checkpoint recovery. Genetics 167:21–33.

4. Brengues, M., D. Teixeira, and R. Parker. 2005. Movement of eukaryotic mRNAs between polysomes and cytoplasmic processing bodies. Science 310:486–489.

5. Christianson, T. W., R. S. Sikorski, M. Dante, J. H. Shero, and P. Hieter. 1999. A novel inhibitor of retrovirus RNA. Proc. Natl. Acad. Sci. USA 96:5301–5306.

6. Elder, R. T., E. Y. Loh, and R. W. Davis. 1997. The p20 and Ded1 proteins have antagonistic roles in eIF4E-dependent translation in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA 94:5201–5206.

7. Lo, W. S., and A. M. Dranginis. 1998. The cell surface flocculin Flo11 is required for pseudohyphal formation and invasion by Saccharomyces cerevisiae. Mol. Biol. Cell 9:161–171.

8. Loeb, J. D., T. A. Kerentseva, T. Pan, M. Sepulveda-Beccera, and H. Liu. 1996. A novel RNA-dependent RNA polymerase involved in pseudohyphal growth and infection. Science 271:2005–2012.

9. Lorent, M. C., and J. Heitman. 1997. Yeast pseudohyphal growth is regulated by GPA4, a G protein alpha homolog. EMBO J. 16:7008–7018.

10. MacKay, V. L., X. Li, M. R. Flory, E. Turcott, G. L. Law, K. A. Serikawa, X. L. Xu, H. Lee, D. R. Goodlett, R. Abeskind, L. P. Zhao, and D. R. Morris. 2004. Gene expression analyzed by high-resolution state array analysis and quantitative proteomics: response of yeast to mating pheromone. Mol. Cell. Proteomics 3:478–489.

11. Madhani, H. D., T. Galitski, E. S. Lander, and G. R. Fink. 1999. Effectors of a developmental mitogen-activated protein kinase cascade revealed by expression signatures of signaling mutants. Proc. Natl. Acad. Sci. USA 96:12350–12355.

12. Madhani, H. D., C. A. Styles, and G. R. Fink. 1997. MAP kinases with distinct inhibitory functions impart signaling specificity during yeast differentiation. Cell 91:673–686.

13. Pan, X., and J. Heitman. 1999. Cyclic AMP-dependent protein kinase regulates pseudohyphal differentiation in Saccharomyces cerevisiae. Mol. Cell. Biol. 19:4874–4887.

14. Rupp, S., E. Summers, H. J. Lo, H. Madhani, and G. R. Fink. 1999. MAP kinase and cAMP filamentation signaling pathways converge on the unusually large promoter of the yeast FLO11 gene. EMBO J. 18:1257–1269.

15. Rupp, S., E. Summers, H. J. Lo, H. Madhani, and G. R. Fink. 1999. A novel inhibitor of retrovirus RNA. Proc. Natl. Acad. Sci. USA 96:5301–5306.

16. Richter, J. D., and N. Sonenberg. 2005. Regulation of cap-dependent translation initiation by eIF4E inhibitory proteins. Nature 433:477–480.

17. Roberts, R. L., and G. R. Fink. 1994. Elements of a single MAP kinase cascade in Saccharomyces cerevisiae mediate two developmental programs in the same cell type: mating and invasive growth. Genes Dev. 8:2974–2985.

18. Rupp, S., E. Summers, H. J. Lo, H. Madhani, and G. R. Fink. 1999. MAP kinase and cAMP filamentation signaling pathways converge on the unusually large promoter of the yeast FLO11 gene. EMBO J. 18:1257–1269.

19. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

20. Schmid, S. R., and P. Linder. 1991. Translation initiation factor 4A from Saccharomyces cerevisiae: analysis of residues conserved in the D-E-A-D family of RNA helicases. Mol. Cell. Biol. 11:3463–3471.

21. Schneider, S., M. Bucher, and C. M. Hovens. 1996. An in vitro assay of beta-galactosidase from yeast. BioTechniques 20:960–962.

22. Sheth, U., and R. Parker. 2003. Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. Science 300:805–808.

23. Sikorski, R. S., and P. Hieter. 1998. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics 158:1613–1626.

24. von der Haar, T., and J. E. McCarthy. 2002. Intracellular translation initiation factor levels in Saccharomyces cerevisiae and their role in cap-complex function. Mol. Microbiol. 46:531–544.

25. Wach, A., A. Brachat, R. Pohlmann, and P. Philippsen. 1994. New heterologous modules for classical or PCR-based gene disruptions in Saccharomyces cerevisiae. Yeast 10:1793–1808.

26. Zong, Q., M. Schummer, I. Hood, and D. R. Morris. 1999. Messenger RNA translation state: the second dimension of high-throughput expression screening. Proc. Natl. Acad. Sci. USA 96:10652–10636.