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The tRNA Modification Complex Elongator Regulates the Cdc42-Dependent Mitogen-Activated Protein Kinase Pathway That Controls Filamentous Growth in Yeast[▽]†

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Signal transduction pathways control multiple aspects of cellular behavior, including global changes to the cell cycle, cell polarity, and gene expression, which can result in the formation of a new cell type. In the budding yeast *Saccharomyces cerevisiae*, the mitogen-activated protein kinase (MAPK) pathway that controls filamentous growth induces a dimorphic foraging response under nutrient-limiting conditions. How nutritional cues feed into MAPK activation remains an open question. Here we report a functional connection between the elongator tRNA modification complex (*ELP* genes) and activity of the filamentous growth pathway. Elongator was required for filamentous growth pathway signaling, and *elp* mutants were defective for invasive growth, cell polarization, and MAPK-dependent mat formation. Genetic suppression analysis showed that elongator functions at the level of Msb2p, the signaling mucin that operates at the head of the pathway, which led to the finding that elongator regulates the starvation-dependent expression of the *MSB2* gene. The Elp complex was not required for activation of related pathways (pheromone response or high osmolarity glycerol response) that share components with the filamentous growth pathway. Because protein translation provides a rough metric of cellular nutritional status, elongator may convey nutritional information to the filamentous growth pathway at the level of *MSB2* expression.

In response to nutrient limitation, *Saccharomyces cerevisiae* undergoes filamentous (invasive/pseudohyphal) growth, a cellular differentiation response to nutrient limitation (28, 66). Filamentous cells have a distinct morphology and display enhanced cell-cell adhesion and cell surface properties that result from the expression of adhesion molecules such as the flocculin Flo11p (31, 49). Yeast cells also expand in connected mats or biofilms (77–79). In fungal pathogens, filamentous growth and biofilm formation are required for virulence (48, 62). Budding yeast therefore represents an attractive system to study the genetic basis for some fungal-specific behaviors. Indeed, many regulatory elements that control dimorphism are conserved across species (46, 61).

Among the pathways that control filamentous growth in yeast are the RAS-protein kinase A-cyclic AMP pathway (27, 57, 58), the target of rapamycin pathway (97), and the Cdc42p-dependent mitogen-activated protein kinase (MAPK) pathway commonly referred to as the filamentous growth pathway (10, 47, 81). The filamentous growth pathway controls Flo11p-dependent adhesion (31), enhanced cell elongation by a delay in the G_2 phase of the cell cycle (42), and the reorganization of cell polarity by changes in bud site selection (14, 91). The filamentous growth pathway is also required for mat formation (78).

At the head of the filamentous growth pathway, the cell surface proteins Msb2p (10) and Sho1p (54, 55, 101) connect to the polarity control GTPase Cdc42p (37, 67). GTP-bound Cdc42p interacts with the p21-activated kinase Ste20p (43, 68) to induce a canonical MAPK pathway composed of the Ste11p, Ste7p, and Kss1p protein kinases (53, 81). Although limiting glucose and fixed nitrogen are potent inducers of filamentous growth (12, 28), it is not clear whether the cell surface proteins sense nutrient levels directly. One connection between nutrient levels and MAPK signaling is that expression of the *MSB2* gene and the gene that encodes its cognate protease, *YPS1*, which processes Msb2p in its extracellular domain, are induced under nutrient-limiting conditions (96). The CDK Srb10p/Cdk8p has been shown to phosphorylate the transcription factor Ste12p (60), which may also connect nutritional status to filamentous growth pathway activity.

In this report, we describe an unexpected connection between a tRNA modification complex (elongator or Elp complex) (90) and the activity of the filamentous growth pathway. Elongator was required for activation of the filamentous growth pathway and functioned at the level of *MSB2* expression. This regulatory feed may connect general cellular nutrition (at the level of protein translation) to MAPK signaling. Orthologs of elongator have been shown to regulate signaling networks in mammalian cells (3, 9), and our results extend the governance of tRNA modification enzymes to the regulation of MAPK pathways.

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MATERIALS AND METHODS

Strains, plasmids, and microbiological techniques. The yeast strains used in this study are listed in Table 1. Yeast and bacterial strains were manipulated by standard methods (82, 84). Gene disruptions and *GAL1* promoter fusions were made by PCR-based methods (2, 50), including the use of antibiotic resistance markers (30) and epitope fusions (86). Integrations were confirmed by PCR

TABLE 1. Yeast strains used in this study

Strain	Genotype	Source
PC313	*MAT***a** *ura3-52*	47
PC538[a]	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52*	10
PC986[b]	*MAT***a** *ura3-52 leu2 his3 trp1*	80
PC999	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 MSB2-HA*	10
PC586	*MAT*α *ura3-52 leu2*	10
PC1531	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 sho1*::*HYG*	10
PC948	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 msb2*::*KanMX6*	10
PC1083	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 GAL-MSB2-HA*::*KanMX6*	10
PC1516	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 MSB2*$^{\Delta 100\text{-}818}$	96
PC2053	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 pbs2*::*KanMX6*	72
PC1519	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 pgu1*::*KanMX6*	72
PC539	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 ste12*::*URA3*	72
PC2061	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 ssk1*::*KanMX6 ste11*::*URA3*	72
PC1029	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 flo11*::*KanMX6*	S. Karauranthi et al., submitted
PC1523	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 ssk1*::*NAT*	72
PC2043	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA*::*KanMX6*	S. Karauranthi et al., submitted
PC1894	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 leu2*::*HYG*	C. Chavel et al., submitted
PC611	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 ste11*::*URA3*	This study
PC2977	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 MSB2*$^{\Delta 100\text{-}818}$ *elp2*::*KlURA3*	This study
PC2986	*MATa ura3-52 leu2 elp2*::*KlURA3*	This study
PC2976	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 MSB2-HA elp2*::*KlURA3*	This study
PC2991	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 ssk1*::*NAT elp2*::*KlURA3*	This study
PC2983	*MATa ura3-52 elp2*::*KlURA3*	This study
PC2980	*MAT***a** *ura3-52 elp2*::*KlURA3*	This study
PC2989	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 MSB2-HA elp2*::*NAT*	This study
PC2978	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 GAL-MSB2-HA*::*KanMX6 elp2*::*URA3*	This study
PC3192	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 leu2*::*HYG elp2*::*KlURA3*	This study
PC3517	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 MSB2-HA urm1*::*KlURA3*	This study
PC3518	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 MSB2-HA ncs2*::*KlURA3*	This study
PC3520	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 MSB2-HA kti11*::*KlURA3*	This study
PC3524	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 MSB2-HA elp6*::*KlURA3*	This study
PC2979	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA*::*KanMX6 elp2*::*URA3*	This study
PC2763	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 elp2*::*URA3*	This study

[a] All strains are isogenic derivatives from the Σ1278b background unless otherwise indicated.
[b] S288c background ordered deletion collection control strain. Deletion strains from the *MAT***a** ordered deletion collection (24) in the S288c background were also used in this study.

analysis and phenotype. The Msb2p-GFP plasmid (GFP is green fluorescent protein) was used to measure the localization of Msb2p in live cells (96). The plasmid containing Msb2p-HA (HA is hemagglutinin) was used to examine the levels and secretion of the Msb2p protein (96). Plasmids containing filamentous growth pathway targets fused to *lacZ* were provided by C. Boone (80) and H. Madhani (53). Overexpression constructs were obtained from an ordered collection obtained from Open Biosystems (23). The *FUS1-lacZ* reporter was used to evaluate the activity of the filamentous growth pathway, which in Σ1278b strains lacking an intact mating pathway (*ste4*Δ) exhibits Msb2p- and Sho1p-dependent expression (10). The *FUS1-HIS3* reporter was used to confirm *FUS1-lacZ* reporter data and was measured by spotting equal amounts of cells onto synthetic medium lacking histidine and containing the competitive inhibitor of the His3p enzyme 3-amino-1,2,4-triazole (40, 88). β-Galactosidase assays were performed as described previously (11), and the results shown represent at least two independent trials. All experiments were carried out at 30°C unless otherwise indicated.

Cell biological assays. Budding pattern was based on established methodology (5) and was confirmed for some experiments by visual inspection of connected cells. The single-cell invasive growth assay (12) and plate-washing assays (81) were performed to evaluate filamentous growth. Halo assays and the evaluation of shmoo morphologies were performed as described previously (13). Assays to evaluate mat form growth were performed as described previously (78), on low-agar yeast extract-peptone-dextrose (YEPD) medium (0.3% agar). Pectinase assays were performed based on reference 52 and are described in reference 72.

Msb2p-HA secretion by colony blot analysis. A detailed analysis of the screen will be described elsewhere (C. Chavel, H. M. Dionne, B. Birkaya, and P. J. Cullen, submitted for publication). Briefly, haploid (*MAT***a**) mutants from an ordered deletion collection (24) were transformed with a plasmid carrying a functional epitope-tagged *MSB2* gene (pMSB2-HA) by using a high-throughput microtiter plate transformation protocol (25). Transformants were pinned to synthetic defined medium without uracil (SD-URA) and, once grown, transferred to a 96-well plate containing 100 μl of water, pinned to SD-URA overlaid with a nitrocellulose disc filter (0.4 μm; HAHY08550; Millipore), and incubated for 48 h at 30°C. Filters were rinsed in distilled water to remove cells and probed with antibodies to the HA epitope (12CA5.16.4). Process/function and Gene Ontology annotations for proteins were obtained from public databases, including the *Saccharomyces* Genome Database (http://www.yeastgenome.org/) (7, 33). The codon adaptation index was determined according to previously published procedures (4). Codon bias in *S. cerevisiae* was obtained from reference 39.

Immunoblot analysis. Immunoblot analysis was performed as described previously (96). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 10% precast gels (Bio-Rad, Hercules, CA) and transferred to nitrocellulose membranes (Protran BA85). Membranes were incubated in blocking buffer (5% nonfat dry milk, 10 mM Tris-HCl [pH 8], 150 mM NaCl, 0.05% Tween 20) for 1 h at 25°C. ECL Plus immunoblotting reagents were used to detect secondary antibodies. Nitrocellulose membranes were incubated for 18 h at 4°C in blocking buffer containing a rabbit polyclonal immunoglobulin G antibody against the HA epitope.

Microscopy and protein localization. Differential interference contrast microscopy and fluorescence microscopy were performed with an Axioplan 2 fluorescence microscope (Zeiss) with a PLAN-APOCHROMAT 100×/1.4 (oil) objective (numerical aperture 0.17). Digital images were obtained with an Axiocam MRm camera (Zeiss). Axiovision 4.4 software (Zeiss) was used for image acquisition and analysis. For protein localization experiments, cells were grown to saturation in selective medium to maintain plasmids harboring *MSB2-GFP* fusions. Cells were harvested by centrifugation and resuspended in YEPD medium for 4.5 h at 30°C. Cells were washed three times in water and visualized by fluorescence microscopy at a magnification of ×100.

mRNA level determination by quantitative PCR. Total RNA was isolated from 25-ml cultures grown in yeast extract-peptone-galactose (YEP-GAL) medium

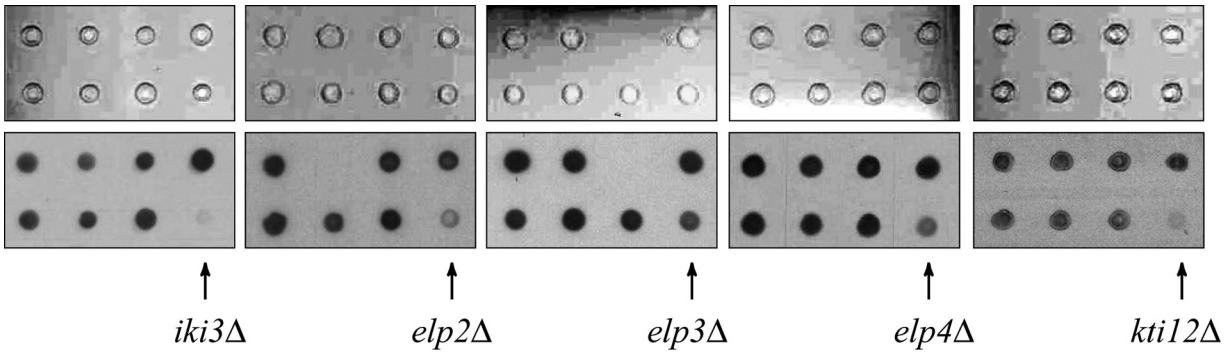

FIG. 1. Elongator contributes to shedding of Msb2p-HA. Colony immunoblot assays are shown in which ordered deletions (24) transformed with plasmid pMSB2-HA were pinned onto SD-URA medium overlaid with a nitrocellulose filter (top panels). Colonies were incubated for 2 days at 30°C. Colonies were washed off the filters, and secreted proteins were examined by immunoblot analysis. Each *elp* mutant is indicated by an arrow in the lower right corner.

for 8 h by hot acid phenol extraction. cDNA synthesis was carried out with 1 μg RNA and the iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer's instructions. One-tenth of the synthesized cDNA was used as the template for real-time PCR. Twenty-five-microliter real-time PCRs were performed on a Bio-Rad MyiQ Cycler with iQ SYBR green Supermix (Bio-Rad). Quantitative reverse transcription (RT)-PCR was performed by initial denaturation for 8 min at 95°C, followed by 35 × cycle 2 (denaturation for 15 s at 95°C and annealing for 1 min at 60°C). Melting curve data collection was enabled by decreasing the set point temperature after cycle 2 by 0.5°C. The specificity of amplicons was confirmed by generating the melting curve profile of all amplified products. *ACT1* was used as the reference gene for normalization of mRNA levels. Gene expression was quantified as described previously (71). The primers used were based on reference 98 and were *FLO11* forward (5′-GTTCAACCAGTCCAAGCGAAA-3′) and reverse (5′-GTAGTTACAGGTGTGGTAGGTGAAGTG-3′), *MSB2* forward (5′-TGACCAAACTTCGACTGCTGG-3′) and reverse (5′-AGCTGCTGAT GCAGTGGTAA-3′), and *ACT1* forward (5′-GGCTTCTTTGACTACCTTCCAA CA-3′) and reverse (5′-GATGGACCACTTTCGTCGTATTC-3′).

RESULTS

Identification of elongator in a screen for mutants defective for *Msb2p* secretion. Components of the elongator (Elp) protein complex were identified in a screen for mutants defective for the secretion of Msb2p (Chavel et al., submitted). Elp proteins constitute an evolutionarily conserved complex (6, 32) composed of six proteins (Iki3p/Elp1p, Elp2p, Elp3p, Elp4p, Elp5p, and Elp6p) that exist in different subcomplexes (41, 45, 70, 89, 99). The *iki3*Δ, *elp2*Δ, *elp3*Δ, and *elp4*Δ mutants were identified in the screen as secretion defective (Fig. 1; Table 2). The *elp5*Δ mutant is inviable (41) and was not tested (Table 2), and the *elp6*Δ mutant was not identified, although direct testing confirmed a role for Epl6p in Msb2p regulation (data not

TABLE 2. Components of elongator and other tRNA modification proteins that influence the secretion of Msb2p-HA or MAPK activity

ORF	Phenotype[i]	tRNA	*MSB2-lacZ*[k]	Secretion[j]		Localization	Process	Function
				24 h	48 h			
Wild type[a]	Wild type	NA	48 ± 4.88[d]	23.45 ± 1.96[e]	28.93 ± 2.53[e]	Wild type	NA[g]	NA
IKI3/ELP1	Undersecretion	GLN, LYS	32.06	29.4	20.17	Diffuse	tRNA modification	Subunit of elongator[b]
ELP2	Undersecretion	GLN, LYS	37.16	20.99	13.54	Wild type	tRNA modification	Subunit of elongator
ELP3	Undersecretion	GLN, LYS	33.94	18.25	20.9	Wild type	tRNA modification	Subunit of elongator
ELP4	Undersecretion	GLN, LYS	40.51	27.73	14.97	Wild type	tRNA modification	Subunit of elongator
ELP5	NA	GLN, LYS	NA	NA	NA	NA	tRNA modification	Subunit of elongator
ELP6	Wild type	GLN, LYS	ND[h]	ND	28.71	ND	tRNA modification	Subunit of elongator
KTI12	Undersecretion	GLN, LYS	41.91	18.59	15.97	Wild type	tRNA modification	Elongator-associated protein
TRM1	Undersecretion	ARG, GLU	48.6	51.08	ND	Wild type	tRNA modification	tRNA methyltransferase[c]
TRM9	Undersecretion	ARG, GLU	32.7	20.82	19.64	Diffuse	tRNA modification	tRNA methyltransferase[f]
URM1	Undersecretion	LYS, GLU	32.9	18.78	11.31	Brighter	tRNA modification	Ubiquitin-like modifier

[a] PC986 was used as the S288c isogenic wild-type control strain.
[b] Complex modifies 5-methoxycarbonylmethyl and 5-carbamoylmethyl groups.
[c] N^2,N^2-Dimethylguanosine.
[d] The standard deviation was less than ±5 for the mutants shown.
[e] The standard deviation was less than ±3 for the mutants shown.
[f] 5-Methylcarbonylmethyluridine in tRNA(Arg3) and 5-methylcarbonylmethyl-2-thiouridine in tRNA(Glu).
[g] NA, not applicable.
[h] ND, not determined.
[i] Phenotype refers to the secretion of Msb2-HA determined by colony immunoblot analysis.
[j] Secretion levels represent intensity measurements and were determined by Imagequant relative to those of wild-type controls. Each value is shown for the corresponding deletion for Msb2-HA secretion by colony immunoblot analysis.
[k] β-Galactosidase assays for the corresponding deletions were performed in duplicate. The values shown are in Miller units.

shown). Kti12p associates with the Elp complex and is required for Elp function (69). The *kti12*Δ mutant was also identified in the screen (Fig. 1; Table 2). The *elp* mutants were among those that displayed the strongest defect in the screen (~4,800 mutants tested). The Msb2p-HA secretion defect was roughly equivalent between *elp* mutants (Fig. 1A; Table 2), which is consistent with the idea that the Elp proteins work together as a complex (20). Differences in Msb2p-HA secretion between *elp* mutants in Fig. 1 may be due to slight nutritional differences resulting from differences in processing time and the positions of the colonies on the plates (see below).

Elongator modifies 5-methoxycarbonylmethyl and 5-carbamoylmethyl groups at the wobble positions of uridine tRNAs (35), which represents one of many different tRNA modifications (34). Components of the Trm tRNA methyltransferase complex, including Trm1p (17) and the Urm1p ubiquitin-related modifier, which modifies some tRNA species (44, 85), were also identified in the screen (Table 2). Most of the genes that function in tRNA modification were not defective for Msb2p secretion (see Table S1 in the supplemental material), which indicates that a general defect in tRNA regulation does not underlie the secretion defect of the Msb2p protein. Similarly, many genes that function in protein translation were not identified in the screen, further suggesting a specific function for elongator in Msb2p regulation.

The Elp complex is a positive regulator of the filamentous growth pathway. The secretion of Msb2p is tied to its function because processing and release of the N-terminal inhibitory domain of the Msb2p protein are necessary for filamentous growth pathway activity (96). Because Msb2p functions at the head of the MAPK pathway that controls filamentous growth (10), we investigated whether elongator regulates the filamentous growth pathway. The gene that encodes one of the components of elongator (Elp2p) was disrupted because loss of any member of the complex is thought to render the complex inactive (20). The *ELP2* gene was disrupted in strains of the Σ1278b background, and the *elp2*Δ mutant was evaluated for filamentous growth and MAPK signaling. The *elp2*Δ mutant was defective for invasive growth (Fig. 2A), as determined by the plate-washing assay (81). The invasive growth defect of the *elp2*Δ mutant was comparable to that of other filamentous growth pathway *msb2*Δ, *sho1*Δ, and *ste11*Δ mutants (Fig. 2A) and less severe than that of the *ste12*Δ mutant (Fig. 2A), which lacks the transcription factor for the pathway, rendering the pathway inoperative (51). The *elp2*Δ mutant colony had a smooth appearance (Fig. 2A), indicative of a defect in MAPK- and Flo11p-dependent cell-cell adhesion. The *elp2*Δ mutant also exhibited cell elongation and distal-pole budding defects. As assessed by the single-cell invasive growth assay (12), the *elp2*Δ mutant failed to exhibit the elongated cell morphology characteristic of wild-type cells (Fig. 2B, black arrows). The *elp2*Δ mutant was similarly defective in distal pole budding, and cells of the *elp2*Δ mutant were observed budding back toward their parents (Fig. 2B, white arrow and data not shown). The polarity defect of the *elp2*Δ mutant was equivalent to that of other filamentous growth pathway components (Fig. 2B). The *elp2*Δ mutant was also defective for production and release of the polygalacturonidase Pgu1p (Fig. 2C), which is also regulated by the filamentous growth pathway (52).

The *elp2*Δ mutant was defective for MAPK activity based on the expression of the *FUS1-lacZ* reporter (Fig. 2D) and showed a phenotype similar to that of the *msb2*Δ, *ste11*Δ, and *ste12*Δ mutants (Fig. 2D). The expression of other filamentous growth pathway target genes, particularly the *FLO11* gene (83), was also reduced in the *elp2*Δ mutant (Fig. 2E). Direct testing confirmed an equivalent role for Elp6p in FG pathway signaling, and although not present in the deletion collection, the *KTI11* gene, which functions with elongator (21), was also required for pathway signaling (data not shown). Urm1p and Ncs2p constitute a ubiquitin-like modification system for tRNA species (44, 85) and are required for filamentous growth (29). Direct testing showed that Urm1p and Ncs2p also had a positive role in FG pathway signaling, although not to the same degree as the Elp complex (data not shown). To summarize, we conclude that elongator is required for activation of the filamentous growth pathway.

Elongator contributes to mat formation. The filamentous growth pathway contributes to the expansion of cells in a microbial mat that occurs in a MAPK- and Flo11p-dependent manner (78, 79). Our Σ1278b strains showed a particularly strong dependency on the filamentous growth pathway for mat form growth. We tested whether the Elp complex is required for the expansion of microbial mats. The *elp2*Δ mutant was defective in mat expansion (Fig. 3A) and exhibited a phenotype similar to but less severe than that of the *flo11*Δ and *ste12*Δ mutants (Fig. 3A). The *elp2* mutant also showed a reduction in the characteristic ruffling pattern of microbial mats (Fig. 3B). The contribution of elongator to microbial mat expansion corroborates the conclusion that elongator is a positive regulator of the filamentous growth pathway.

Elongator functions at the level of Msb2p. Genetic suppression (epistasis) analysis can provide information as to where in a regulatory cascade a given protein functions. To determine at which point in the MAPK pathway elongator functions, genetic analysis was performed by examining double-mutation combinations between *elp2*Δ and mutations that hyperactivate the filamentous growth pathway. Overexpression of *MSB2* stimulates the filamentous growth pathway (10). Overexpression of *MSB2* partially bypassed the agar invasion defect of the *elp2*Δ mutant (Fig. 4A). Likewise, overexpression of *MSB2* partially bypassed the signaling defect of the *elp2*Δ mutant. Specifically, the activity of the growth reporter *FUS1-HIS3* was partially restored in the *GAL-MSB2 elp2*Δ double mutant (Fig. 4A). β-Galactosidase assays confirmed that *FUS1-lacZ* expression was partially restored in the *GAL-MSB2 elp2*Δ mutant (Fig. 4B). The cell elongation defect of the *elp2*Δ mutant was not suppressed by overexpression of *MSB2* (Fig. 4C), indicative of partial suppression. Because *MSB2* can partially bypass the defect in elongator function, elongator may function at the head of the filamentous growth pathway.

Genetic suppression analysis with a strong activated allele of *MSB2* (96) bore out these findings. The hyperactive allele $MSB2^{\Delta 100\text{-}818}$ effectively bypassed the agar invasion defect of the *elp2*Δ mutant (Fig. 4D). The signaling and cell elongation defects were similarly suppressed by $MSB2^{\Delta 100\text{-}818}$ (Fig. 4E and F). Activated alleles of *SHO1* and *STE11* (87, 96), which function below Msb2p in the pathway, also suppressed the signaling defect associated with loss of Elp function (data not shown). Together, the genetic analysis results indicate that elongator

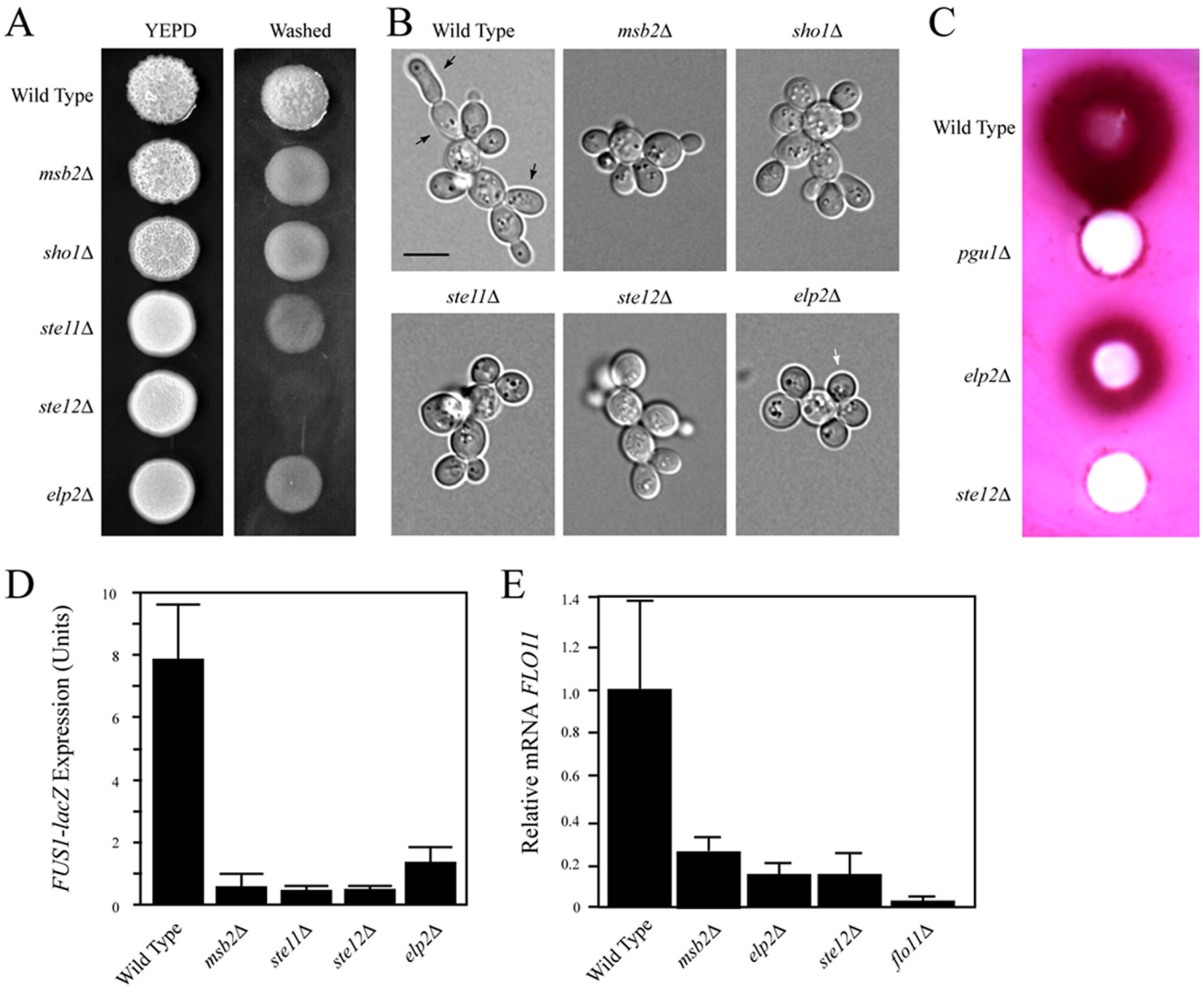

FIG. 2. The *elp2* mutant is defective for filamentous growth and MAPK pathway activation. (A) Plate-washing assay showing the agar invasion defect of the *msb2*Δ (PC948), *sho1*Δ (PC1531), *ste11*Δ (PC611), and *ste12*Δ (PC539) filamentous growth pathway mutants alongside the *elp2*Δ mutant (PC2763). (B) Single-cell invasive growth assay. The strains in panel A were used. The black arrows designate the cell elongation phenotype of filamentous cells. The white arrow designates a proximal bud formed in the *elp2*Δ mutant. Bar, 5 μm. (C) Pgu1p activity in wild-type (PC538) and *elp2*Δ (PC2763), *pgu1*Δ (PC1519), and *ste12*Δ (PC539) mutant cells. (D) *FUS1-lacZ* expression of the strains described in panel A. β-Galactosidase assays were performed in duplicate, and error bars represent standard deviations. (E) *FLO11* expression in wild-type cells, the *elp2*Δ mutant, and control strains as determined by quantitative real-time RT-PCR analysis.

functions at or above the level of Msb2p. Because MAPK activity was not fully restored, we cannot exclude the possibility that elongator also functions at another point in the signaling cascade.

Elongator regulates starvation-dependent expression of the *MSB2* gene. Based on the fact that elongator functions at or above Msb2p in the filamentous growth pathway, we investigated different aspects of Msb2p regulation in elongator mutants. The Elp complex contributes to multiple cellular processes (90) and might potentially regulate the expression or translation of the *MSB2* gene or the glycosylation, cell surface delivery, localization, processing, or shedding of the Msb2p protein. We examined the localization pattern of an Msb2p-GFP fusion protein in wild-type cells and in the *elp2*Δ mutant. Msb2p-GFP exhibits a diffuse localization pattern, with several punctate sites distributed throughout mother and daughter cells (Fig. 5A). The localization pattern of Msb2p-GFP was indistinguishable between wild-type and *elp2*Δ mutant cells (Fig. 5A). Other *elp* mutants also showed a wild-type Msb2p-GFP localization pattern (Table 2), which indicates that elongator does not regulate Msb2p localization.

Elongator might regulate the delivery of Msb2p to the cell surface, particularly because elongator has an established function in exocytosis (74, 75), which involves the targeted delivery of secretory vesicles to polarized sites (22, 63, 95, 102). The role of elongator in exocytosis, however, appears to be inhibitory (74, 75), and therefore elongator mutants might be expected to show elevated secretion of Msb2p. Immunoblot anal-

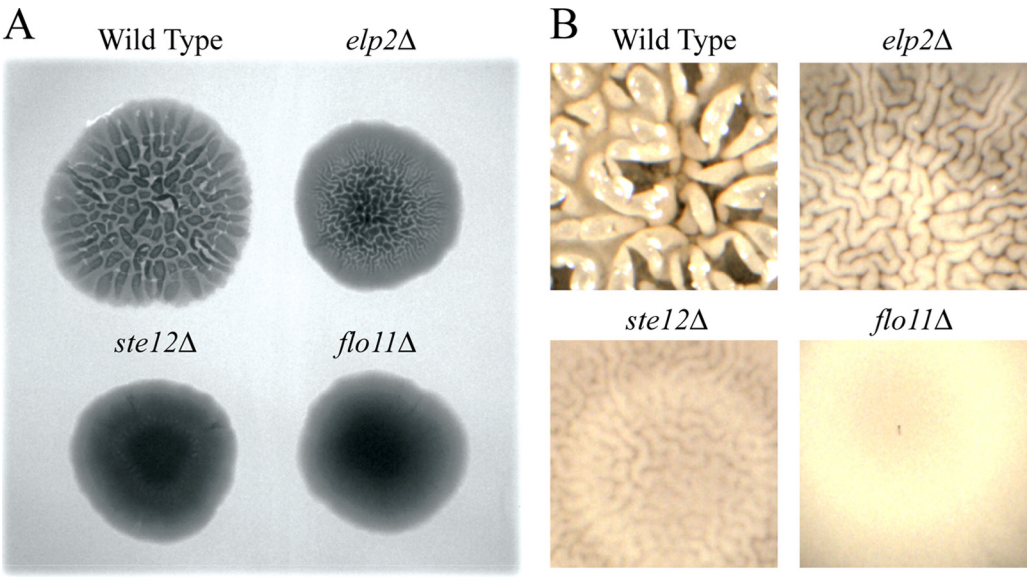

FIG. 3. Elp2p plays a role in mat expansion and patterning. (A) Wild-type (PC538, upper left) and *elp2*Δ (PC2763, upper right), *ste12*Δ (PC539, lower left), and *flo11*Δ (PC1029, lower right) mutant cells were spotted onto medium permissive for mat formation (YEPD medium plus 0.3% agar atop a nitrocellulose filter) for 3 days at 30°C. The mats were photographed with transmitted light to reveal the contoured morphology. (B) Close-up of mats in panel A showing Flo11p-dependent and Ste12p-dependent contours. The *elp2*Δ mutant exhibits a partial defect in mat patterning.

ysis demonstrated that the Msb2p-HA protein was produced, glycosylated, and secreted in the *elp2*Δ mutant in a manner equivalent to that observed in wild-type cells, which indicates that elongator does not function in the glycosylation, trafficking, exocytosis, or processing of Msb2p (Fig. 5B).

Immunoblot analysis did show that the overall level of the Msb2p-HA protein was reduced in the *elp2*Δ mutant (Fig. 5B). We therefore tested whether elongator contributes to the expression of the *MSB2* gene. The expression of an *MSB2-lacZ* reporter was examined in wild-type cells and the *elp2*Δ mutant. *MSB2-lacZ* expression was reduced in the *elp2*Δ mutant (Fig. 5C). This effect was subtle but was more pronounced under nutrient-limiting conditions, under which *MSB2* expression is induced (S-GAL medium compared to SD medium, Fig. 5C). Other *elp* mutants also showed a reduction in *MSB2-lacZ* expression (Table 2). Consistent with this result, the Msb2p-HA secretion defect in *elp* mutants was more pronounced after 2 days (Fig. 5D; Table 2), presumably when environmental nutrients had become depleted. The fact that elongator acts at the level of *MSB2* expression is consistent with the genetic analysis, in that overexpression or hyperactivation of Msb2p can partially bypass the signaling defect of *elp* mutants.

Elongator modifies tRNAs that code for glutamine (GLN) and lysine (LYS) (19, 35). We also tested whether the wobble positions of GLN and LYS codons were biased in the *MSB2* gene, which might result in a translation defect of the Msb2p protein in *elp* mutants. The GLN codon frequency in *MSB2* (73.5% CAA, 26.5% CAG) was in close agreement with the overall codon bias observed throughout the yeast genome (69% CAA, 31% CAG) (39). Similarly, the LYS codon bias in *MSB2* (56% AAA, 44% AAG) resembled the overall bias throughout the genome (58% AAA, 42% AAG) (39). Thus, codon bias does not appear to account for the decrease in Msb2p protein levels in *elp* mutants. The fact that the expression of the *MSB2* gene, not translation of the *MSB2* message, is affected in elongator mutants was further supported by quantitative PCR analysis (Fig. 5E). Therefore, elongator appears to function at the level of expression of the *MSB2* gene.

Elongator is not required for pheromone response or high-osmolarity glycerol response (HOG) pathway activation. The filamentous growth pathway shares components with the pheromone response pathway (1), including the p21-activated kinase Ste20p, the adaptor protein Ste50p, the MAPK kinase kinase Ste11p, the MAPK kinase Ste7p, and transcription factor Ste12p (15, 16, 18). Msb2p does not appear to be required for activation of the pheromone response pathway (10). To determine whether elongator is required for activation of the pheromone response pathway, the *elp2*Δ mutant was examined for defects in pheromone response. A haploid *MAT***a** strain lacking the *ELP2* gene showed sensitivity to the mating pheromone α-factor equivalent to that of the wild-type *MAT***a** strain (Fig. 6A). Specifically, the initial response to α-factor, assessed by shmoo formation, was equivalent between the two strains (Fig. 6A). Both the number of cells that formed shmoos and the degree of polarization of shmoo tips were similar between wild-type and *elp2*Δ mutant cells (Fig. 6A). In addition, α-factor-induced cell cycle arrest was equivalent between the two strains, as determined by halo assay (Fig. 6A, right panels). Pheromone response pathway mutants did not exhibit morphological characteristics of shmoos or halo formation in response to α-factor (data not shown). Therefore, elongator is not required for pheromone response pathway activation.

The filamentous growth pathway also shares components with the Sho1p branch of the HOG pathway (64, 65, 94). The Ste11p branch of the HOG pathway functions redundantly with the Sln1p branch (76, 92), so we tested for a role for elongator in mutants defective for the Sln1p branch of the HOG pathway (*ssk1*Δ). Cells lacking the MAPK kinase for the

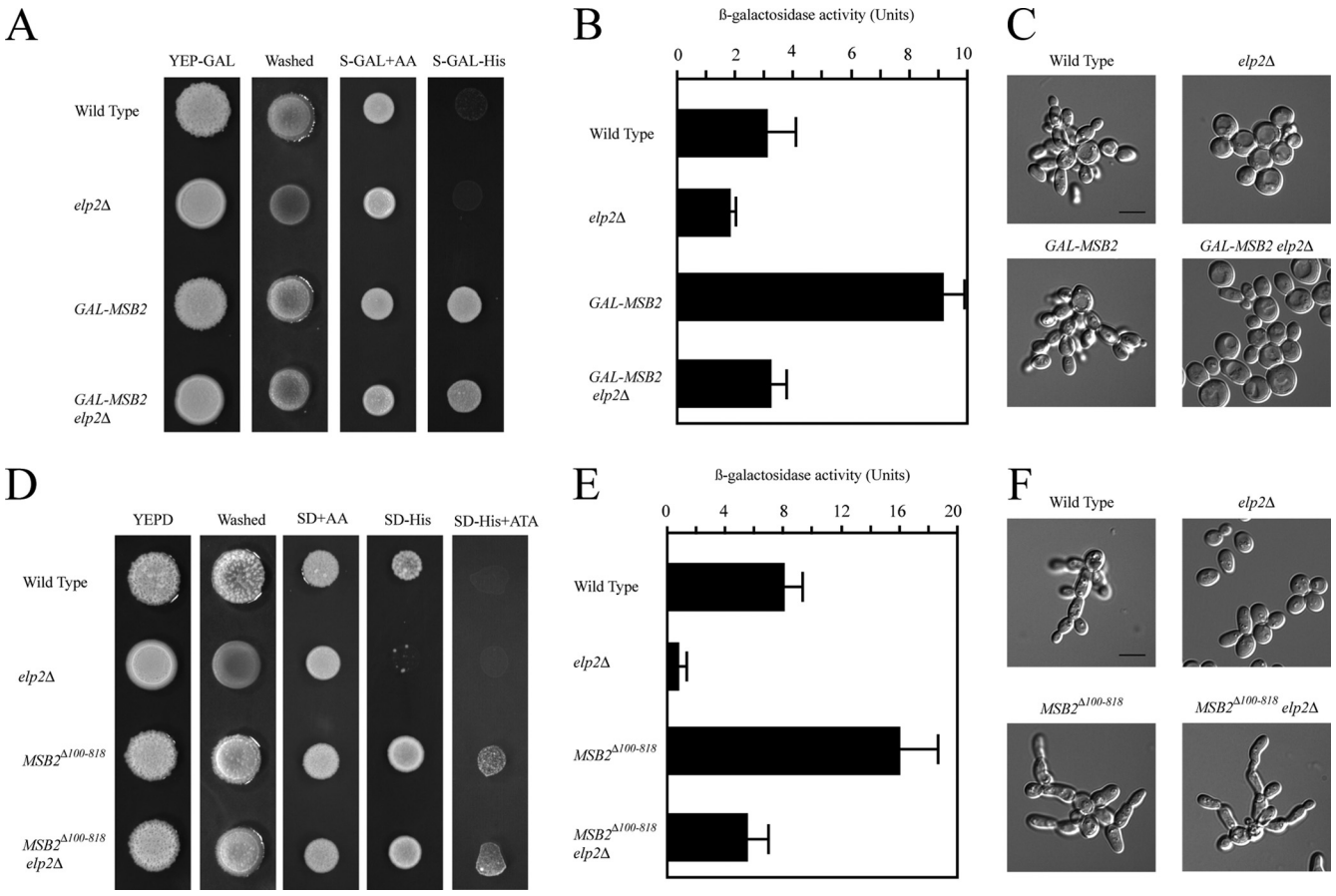

FIG. 4. Bypass of *elp2* by overexpression or hyperactivation of *MSB2*. (A to C) Partial suppression of the *elp2*Δ mutant defects in cells overexpressing *MSB2*. (A) Equal concentrations of cells were spotted onto plates. The wild type (PC538) and the *elp2*Δ (PC2763), *GAL-MSB2* (PC1083), and *GAL-MSB2 elp2*Δ (PC2978) mutants were tested. For the left two panels, cells were spotted onto YEP-GAL medium. The plate was incubated for 2 days, photographed (leftmost panel), washed, and photographed again (second panel from the left). For the right two panels, cells were spotted onto S-GAL plus amino acids (S-GAL+AA) and S-GAL without histidine (S-GAL-HIS) to examine the activity of the growth-dependent *FUS1-HIS3* reporter. (B) β-Galactosidase activity of strains in panel A carrying an integrated *FUS1-lacZ* reporter. Assays were performed in duplicate, and error bars represent standard deviations. (C) Examples of cell morphologies of the strains examined in panel A. Bar, 5 μm. (D to F) Suppression of the *elp2*Δ mutant defects in cells carrying an activated version of Msb2p, Msb2p$^{\Delta 100\text{-}818}$. (D) Equal concentrations of cells were spotted onto YEPD medium. Wild-type (PC538) and *elp2*Δ (PC2763), *MSB2*$^{\Delta 100\text{-}818}$ (PC1516), and *MSB2*$^{\Delta 100\text{-}818}$ *elp2*Δ (PC2977) mutant cells were compared. The plate was incubated for 2 days, photographed (leftmost panel), washed, and photographed again (second panel from left). At the right, cells were spotted onto SD medium plus amino acids (SD+AA) and SD medium without histidine (SD-HIS) containing 5 mM 3-amino-1,2,4-triazole. (E) *FUS1-lacZ* expression of the strains from panel D. Cells were grown for 16 h in SD medium plus amino acids. Assays were performed in duplicate, and error bars represent standard deviations. (F) Examples of cell morphologies of the strains examined in panel D. Bar, 5 μm.

HOG pathway (*pbs2*Δ) or both upstream regulatory branches (*ste11*Δ *ssk1*Δ) were sensitive to the osmolite KCl (Fig. 6B). In contrast, the *elp2*Δ *ssk1*Δ double mutant showed the same level of resistance to KCl as wild-type cells (Fig. 6B). The *ssk1* (PC1523), *ste11* (PC611), and *elp2* (PC2980) single mutants did not exhibit sensitivity to KCl (data not shown). This result demonstrates that elongator is not required for activation of the Ste11p branch of the HOG pathway. Evaluation of Elp2p function in the pheromone, filamentous growth, and HOG pathways was performed in the same background (Σ1278b). The fact that elongator regulates *MSB2* expression is consistent with its failure to influence the mating and HOG pathways, given that Msb2p does not function in the mating pathway (10) and functions in the HOG pathway redundantly with the cell surface protein Hkr1p (93).

DISCUSSION

We report here a new regulatory input to the filamentous growth pathway by the tRNA modification enzyme complex elongator. Elongator is required for filamentous growth pathway activity and contributes to the established outputs of the pathway, including invasive growth, the reorganization of cell polarity, and Flo11p-dependent cell-cell adhesion of filamentous cells (Fig. 2) and expanding mats (Fig. 3). A precedent connecting tRNA metabolism to filamentous growth has been established by a GLN tRNA (tRNA-CUG), which signals nitrogen status and regulates filamentous growth (59). In that case, tRNA-CUG did not appear to function through the filamentous growth pathway. Our results support the overall notion that tRNA levels serve as an intracellular metric for

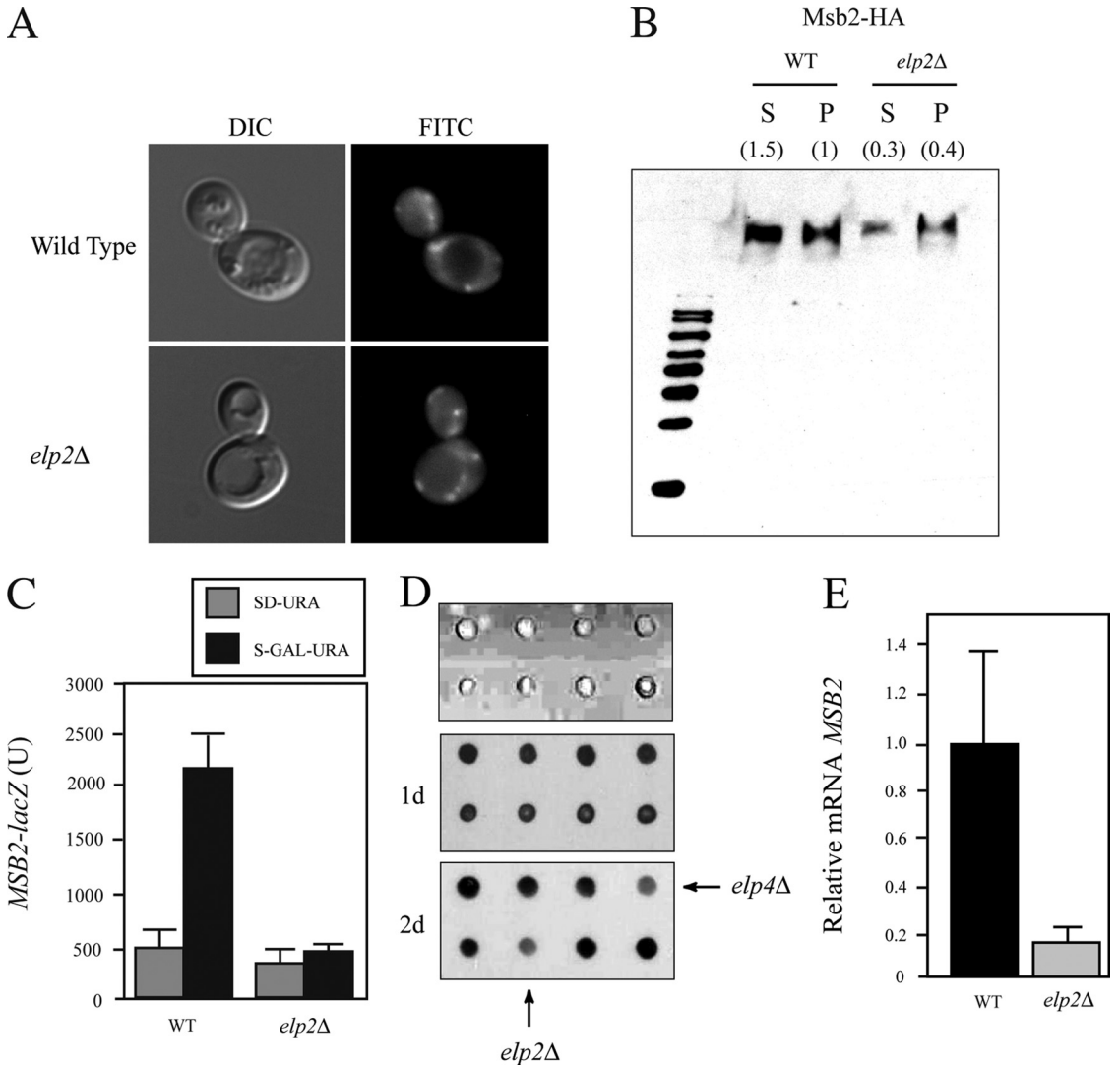

FIG. 5. Elongator promotes starvation-dependent expression of the *MSB2* gene but does not influence the localization or maturation of the protein. (A) Localization of Msb2p-GFP in wild-type and *elp2*Δ mutant strains. DIC, differential interference contrast; FITC, fluorescein isothiocyanate. (B) Immunoblot analysis of Msb2p-HA levels in extracts derived from wild-type (WT; PC999) and *elp2*Δ mutant (PC2976) cells. Cells (10 ml) were grown in YEPD medium for 16 h at 30°C. Equal numbers of cells, as determined by A_{600}, were harvested by centrifugation at 13,000 rpm to separate supernatants (S) from cell pellets (P). Cells were disrupted by addition of 200 μl "Thorner" lysis buffer (8 M urea, 5% sodium dodecyl sulfate, 40 mM Tris-HCl [pH 6.8], 0.1 M EDTA, 0.4 mg/ml bromophenol blue, 1% β-mercaptoethanol) and glass beads, followed by vortexing for 5 min at the highest setting and boiling for 5 min. Supernatants were examined by boiling in 1.5 volumes of lysis buffer for 5 min. Quantitation in parentheses was performed with the ImageJ plug-in Imagequant. (C) *MSB2-lacZ* expression in wild-type cells and the *elp2* mutant. The light band represents *MSB2-lacZ* expression in mid-log-phase cells (SD-URA). The dark band represents *MSB2-lacZ* expression in synthetic medium containing 2% galactose and lacking uracil (S-GAL-URA). (D) The Msb2p-HA secretion defect of the *elp2*Δ and *elp4*Δ mutants is evident after prolonged incubation. Transformants were pinned onto SD-URA, overlaid with nitrocellulose, and incubated for 1 or 2 days (the top panel shows the 2-day incubation). Colonies were washed off of the filters (middle and lower panels), which were probed by immunoblot analysis. (E) Quantitative mRNA analysis of the *MSB2* transcript by real-time RT-PCR analysis. Cells were grown for 8 h in YEP-GAL medium. RNA was harvested and evaluated by real-time RT-PCR as described in Materials and Methods.

cellular nutrient status by impacting the overall rate of protein translation. In this way, protein translation rate may feed into signaling networks that regulate cellular behaviors. The specific advance made by this study is the connection between nutrient regulation (at the level of tRNA modification) and the activity of a MAPK pathway that functions primarily under conditions of starvation. This discovery may begin to explain how nutrient levels feed into MAPK regulation.

We show that elongator contributes to the expression of the *MSB2* gene. The *MSB2* gene is induced under nutrient-limiting conditions (96), and elongator contributes to starvation-dependent induction of *MSB2* expression (Fig. 5). Elongator may influence *MSB2* expression through several different mechanisms. Elongator is a component of the RNA polymerase II holoenzyme and contributes to transcription elongation (26, 38) through a mechanism that is not entirely clear (73). Elongator also functions in chromatin remodeling (36) and has roles that overlap those of the SAGA histone acetyltransferase

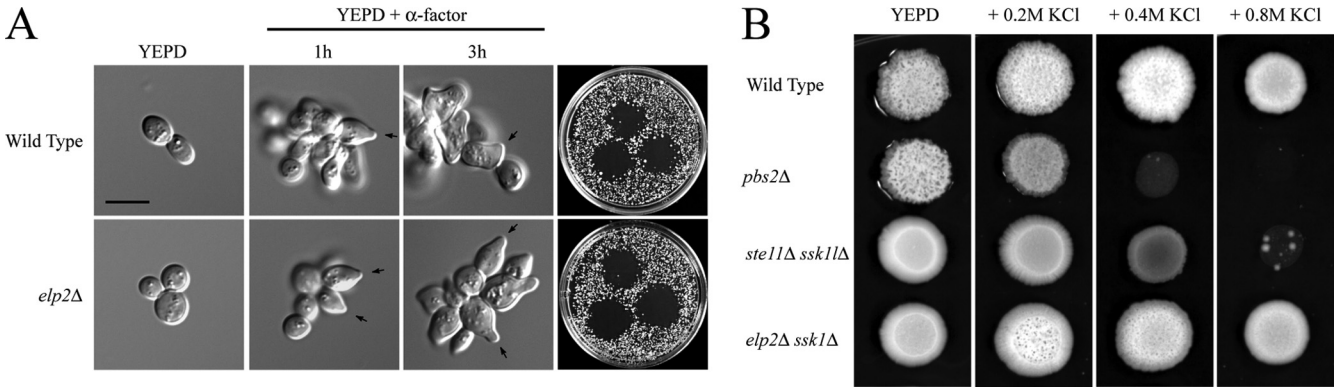

FIG. 6. Elp2p is not required for pheromone response or HOG pathway function. (A) Shmoo formation over time. Wild-type (PC313; upper panel) and *elp2*Δ mutant (PC2980, lower panel) cells were grown to mid-log phase in YEPD medium, washed, and resuspended in YEPD medium containing 30 μM α-factor for 1 or 3 h. Cells were harvested by centrifugation and examined by microscopy at a magnification of ×100. Bar, 5 μm. Right panels, halo assay. Equal amounts of wild-type and *elp2*Δ mutant cells were spread onto YEPD medium, and 2, 4, or 6 μl of 590 μM α-factor was applied to the plates. Plates were incubated for 48 h at 30°C and photographed. (B) Role of Elp2p in HOG pathway activation. Equal concentrations of wild-type (PC538) and *pbs2*Δ (PC2053), *ssk1*Δ *ste11*Δ (PC2061), and *ssk1*Δ *elp2*Δ (PC2991) mutant cells were spotted onto YEPD medium supplemented with the indicated concentrations of KCl. The plates were incubated for 4 days at 30°C and photographed.

complex (100). It is possible that expression of the *MSB2* gene is regulated by either transcription elongation or chromatin remodeling proteins or a combination of these mechanisms. Elongator may also contribute to the translation of the *MSB2* mRNA. However, a specific codon bias was not detected in the coding region of the *MSB2* open reading frame, which argues against this possibility. It is likely that the effect of elongator on *MSB2* expression is mediated indirectly as a result of the tRNA modification function of the enzyme complex (19).

Mammalian components of elongator have been shown to regulate signal transduction networks. For example, IKAP, the mammalian homolog of Iki3p/Elp1p, has been shown to associate with the JNK kinase (56) and with IκK, the IκB kinase (3, 8, 56). StIP1, the mammalian homolog of Elp2p, binds to multiple STAT proteins and Janus kinases (9). Like Elp2p, StIP1 is thought to act as a scaffold for signaling complex assembly by virtue of its WD-40 domain. Based on the genetic suppression analysis, we cannot exclude the possibility that Elp proteins directly interact with components of the filamentous growth pathway to modulate their function. The salient finding from our study is the regulation of a MAPK pathway by a tRNA modification system through regulation of expression of the gene that functions at the pathway's head. This regulatory mechanism may occur in other systems to control signaling networks by tRNA modification enzymes.

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REFERENCES

1. **Bardwell, L.** 2006. Mechanisms of MAPK signalling specificity. Biochem. Soc. Trans. **34:**837–841.
2. **Baudin, A., O. Ozier-Kalogeropoulos, A. Denouel, F. Lacroute, and C. Cullin.** 1993. A simple and efficient method for direct gene deletion in Saccharomyces cerevisiae. Nucleic Acids Res. **21:**3329–3330.
3. **Bouwmeester, T., A. Bauch, H. Ruffner, P. O. Angrand, G. Bergamini, K. Croughton, C. Cruciat, D. Eberhard, J. Gagneur, S. Ghidelli, C. Hopf, B. Huhse, R. Mangano, A. M. Michon, M. Schirle, J. Schlegl, M. Schwab, M. A. Stein, A. Bauer, G. Casari, G. Drewes, A. C. Gavin, D. B. Jackson, G. Joberty, G. Neubauer, J. Rick, B. Kuster, and G. Superti-Furga.** 2004. A physical and functional map of the human TNF-α/NF-κB signal transduction pathway. Nat. Cell Biol. **6:**97–105.
4. **Carbone, A., A. Zinovyev, and F. Kepes.** 2003. Codon adaptation index as a measure of dominating codon bias. Bioinformatics **19:**2005–2015.
5. **Chant, J., and J. R. Pringle.** 1995. Patterns of bud-site selection in the yeast Saccharomyces cerevisiae. J. Cell Biol. **129:**751–765.
6. **Chen, Z., H. Zhang, D. Jablonowski, X. Zhou, X. Ren, X. Hong, R. Schaffrath, J.-K. Zhu, and Z. Gong.** 2006. Mutations in ABO1/ELO2, a subunit of holo-Elongator, increase abscisic acid sensitivity and drought tolerance in *Arabidopsis thaliana*. Mol. Cell. Biol. **26:**6902–6912.
7. **Cherry, J. M., C. Adler, C. Ball, S. A. Chervitz, S. S. Dwight, E. T. Hester, Y. Jia, G. Juvik, T. Roe, M. Schroeder, S. Weng, and D. Botstein.** 1998. SGD: *Saccharomyces* Genome Database. Nucleic Acids Res. **26:**73–79.
8. **Cohen, L., W. J. Henzel, and P. A. Baeuerle.** 1998. IKAP is a scaffold protein of the IκB kinase complex. Nature **395:**292–296.
9. **Collum, R. G., S. Brutsaert, G. Lee, and C. Schindler.** 2000. A Stat3-interacting protein (StIP1) regulates cytokine signal transduction. Proc. Natl. Acad. Sci. USA **97:**10120–10125.
10. **Cullen, P. J., W. Sabbagh, Jr., E. Graham, M. M. Irick, E. K. van Olden, C. Neal, J. Delrow, L. Bardwell, and G. F. Sprague, Jr.** 2004. A signaling mucin at the head of the Cdc42- and MAPK-dependent filamentous growth pathway in yeast. Genes Dev. **18:**1695–1708.
11. **Cullen, P. J., J. Schultz, J. Horecka, B. J. Stevenson, Y. Jigami, and G. F. Sprague, Jr.** 2000. Defects in protein glycosylation cause SHO1-dependent activation of a STE12 signaling pathway in yeast. Genetics **155:**1005–1018.
12. **Cullen, P. J., and G. F. Sprague, Jr.** 2000. Glucose depletion causes haploid invasive growth in yeast. Proc. Natl. Acad. Sci. USA **97:**13619–13624.
13. **Cullen, P. J., and G. F. Sprague, Jr.** 2002. The Glc7p-interacting protein Bud14p attenuates polarized growth, pheromone response, and filamentous growth in *Saccharomyces cerevisiae*. Eukaryot. Cell **1:**884–894.
14. **Cullen, P. J., and G. F. Sprague, Jr.** 2002. The roles of bud-site-selection proteins during haploid invasive growth in yeast. Mol. Biol. Cell **13:**2990–3004.
15. **Dohlman, H. G., and J. E. Slessareva.** 2006. Pheromone signaling pathways in yeast. Sci. STKE **2006:**cm6.
16. **Elion, E. A.** 2000. Pheromone response, mating and cell biology. Curr. Opin. Microbiol. **3:**573–581.
17. **Ellis, S. R., M. J. Morales, J.-M. Li, A. K. Hopper, and N. C. Martin.** 1986. Isolation and characterization of the *TRM1* locus, a gene essential for the N^2,N^2-dimethylguanosine modification of both mitochondrial and cytoplasmic tRNA in *Saccharomyces cerevisiae*. J. Biol. Chem. **261:**9703–9709.
18. **Errede, B., R. M. Cade, B. M. Yashar, Y. Kamada, D. E. Levin, K. Irie, and K. Matsumoto.** 1995. Dynamics and organization of MAP kinase signal pathways. Mol. Reprod Dev. **42:**477–485.
19. **Esberg, A., B. Huang, M. J. Johansson, and A. S. Bystrom.** 2006. Elevated levels of two tRNA species bypass the requirement for elongator complex in transcription and exocytosis. Mol. Cell **24:**139–148.
20. **Fellows, J., H. Erdjument-Bromage, P. Tempst, and J. Q. Svejstrup.** 2000.

The Elp2 subunit of elongator and elongating RNA polymerase II holoenzyme is a WD40 repeat protein. J. Biol. Chem. **275:**12896–12899.

21. **Fichtner, L., D. Jablonowski, A. Schierhorn, H. K. Kitamoto, M. J. Stark, and R. Schaffrath.** 2003. Elongator's toxin-target (TOT) function is nuclear localization sequence dependent and suppressed by post-translational modification. Mol. Microbiol. **49:**1297–1307.
22. **Finger, F. P., T. E. Hughes, and P. Novick.** 1998. Sec3p is a spatial landmark for polarized secretion in budding yeast. Cell **92:**559–571.
23. **Gelperin, D. M., M. A. White, M. L. Wilkinson, Y. Kon, L. A. Kung, K. J. Wise, N. Lopez-Hoyo, L. Jiang, S. Piccirillo, H. Yu, M. Gerstein, M. E. Dumont, E. M. Phizicky, M. Snyder, and E. J. Grayhack.** 2005. Biochemical and genetic analysis of the yeast proteome with a movable ORF collection. Genes Dev. **19:**2816–2826.
24. **Giaever, G., A. M. Chu, L. Ni, C. Connelly, L. Riles, S. Veronneau, S. Dow, A. Lucau-Danila, K. Anderson, B. Andre, A. P. Arkin, A. Astromoff, M. El-Bakkoury, R. Bangham, R. Benito, S. Brachat, S. Campanaro, M. Curtiss, K. Davis, A. Deutschbauer, K. D. Entian, P. Flaherty, F. Foury, D. J. Garfinkel, M. Gerstein, D. Gotte, U. Guldener, J. H. Hegemann, S. Hempel, Z. Herman, D. F. Jaramillo, D. E. Kelly, S. L. Kelly, P. Kotter, D. LaBonte, D. C. Lamb, N. Lan, H. Liang, H. Liao, L. Liu, C. Luo, M. Lussier, R. Mao, P. Menard, S. L. Ooi, J. L. Revuelta, C. J. Roberts, M. Rose, P. Ross-Macdonald, B. Scherens, G. Schimmack, B. Shafer, D. D. Shoemaker, S. Sookhai-Mahadeo, R. K. Storms, J. N. Strathern, G. Valle, M. Voet, G. Volckaert, C. Y. Wang, T. R. Ward, J. Wilhelmy, E. A. Winzeler, Y. Yang, G. Yen, E. Youngman, K. Yu, H. Bussey, J. D. Boeke, M. Snyder, P. Philippsen, R. W. Davis, and M. Johnston.** 2002. Functional profiling of the Saccharomyces cerevisiae genome. Nature **418:**387–391.
25. **Gietz, R. D., and R. H. Schiestl.** 2007. Microtiter plate transformation using the LiAc/SS carrier DNA/PEG method. Nat. Protoc. **2:**5–8.
26. **Gilbert, C., A. Kristjuhan, G. S. Winkler, and J. Q. Svejstrup.** 2004. Elongator interactions with nascent mRNA revealed by RNA immunoprecipitation. Mol. Cell **14:**457–464.
27. **Gimeno, C. J., and G. R. Fink.** 1994. Induction of pseudohyphal growth by overexpression of *PHD1*, a *Saccharomyces cerevisiae* gene related to transcriptional regulators of fungal development. Mol. Cell. Biol. **14:**2100–2112.
28. **Gimeno, C. J., P. O. Ljungdahl, C. A. Styles, and G. R. Fink.** 1992. Unipolar cell divisions in the yeast S. cerevisiae lead to filamentous growth: regulation by starvation and RAS. Cell **68:**1077–1090.
29. **Goehring, A. S., D. M. Rivers, and G. F. Sprague, Jr.** 2003. Urmylation: a ubiquitin-like pathway that functions during invasive growth and budding in yeast. Mol. Biol. Cell **14:**4329–4341.
30. **Goldstein, A. L., and J. H. McCusker.** 1999. Three new dominant drug resistance cassettes for gene disruption in Saccharomyces cerevisiae. Yeast **15:**1541–1553.
31. **Guo, B., C. A. Styles, Q. Feng, and G. R. Fink.** 2000. A Saccharomyces gene family involved in invasive growth, cell-cell adhesion, and mating. Proc. Natl. Acad. Sci. USA **97:**12158–12163.
32. **Hawkes, N. A., G. Otero, G. S. Winkler, N. Marshall, M. E. Dahmus, D. Krappmann, C. Scheidereit, C. L. Thomas, G. Schiavo, H. Erdjument-Bromage, P. Tempst, and J. Q. Svejstrup.** 2002. Purification and characterization of the human elongator complex. J. Biol. Chem. **277:**3047–3052.
33. **Hong, E. L., R. Balakrishnan, Q. Dong, K. R. Christie, J. Park, G. Binkley, M. C. Costanzo, S. S. Dwight, S. R. Engel, D. G. Fisk, J. E. Hirschman, B. C. Hitz, C. J. Krieger, M. S. Livstone, S. R. Miyasato, R. S. Nash, R. Oughtred, M. S. Skrzypek, S. Weng, E. D. Wong, K. K. Zhu, K. Dolinski, D. Botstein, and J. M. Cherry.** 2008. Gene ontology annotations at SGD: new data sources and annotation methods. Nucleic Acids Res. **36:**D577–D581.
34. **Hopper, A. K., and E. M. Phizicky.** 2003. tRNA transfers to the limelight. Genes Dev. **17:**162–180.
35. **Huang, B., M. J. Johansson, and A. S. Bystrom.** 2005. An early step in wobble uridine tRNA modification requires the Elongator complex. RNA **11:**424–436.
36. **Jablonowski, D., A. R. Butler, L. Fichtner, D. Gardiner, R. Schaffrath, and M. J. Stark.** 2001. Sit4p protein phosphatase is required for sensitivity of Saccharomyces cerevisiae to Kluyveromyces lactis zymocin. Genetics **159:**1479–1489.
37. **Johnson, D. I.** 1999. Cdc42: an essential Rho-type GTPase controlling eukaryotic cell polarity. Microbiol. Mol. Biol. Rev. **63:**54–105.
38. **Jona, G., B. O. Wittschieben, J. Q. Svejstrup, and O. Gileadi.** 2001. Involvement of yeast carboxy-terminal domain kinase I (CTDK-I) in transcription elongation in vivo. Gene **267:**31–36.
39. **Kliman, R. M., N. Irving, and M. Santiago.** 2003. Selection conflicts, gene expression, and codon usage trends in yeast. J. Mol. Evol. **57:**98–109.
40. **Klopotowski, T., and A. Wiater.** 1965. Synergism of aminotriazole and phosphate on the inhibition of yeast imidazole glycerol phosphate dehydratase. Arch. Biochem. Biophys. **112:**562–566.
41. **Krogan, N. J., and J. F. Greenblatt.** 2001. Characterization of a six-subunit Holo-Elongator complex required for the regulated expression of a group of genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **21:**8203–8212.
42. **Kron, S. J., C. A. Styles, and G. R. Fink.** 1994. Symmetric cell division in pseudohyphae of the yeast Saccharomyces cerevisiae. Mol. Biol. Cell **5:**1003–1022.
43. **Leberer, E., C. Wu, T. Leeuw, A. Fourest-Lieuvin, J. E. Segall, and D. Y. Thomas.** 1997. Functional characterization of the Cdc42p binding domain of yeast Ste20p protein kinase. EMBO J. **16:**83–97.
44. **Leidel, S., P. G. Pedrioli, T. Bucher, R. Brost, M. Costanzo, A. Schmidt, R. Aebersold, C. Boone, K. Hofmann, and M. Peter.** 2009. Ubiquitin-related modifier Urm1 acts as a sulphur carrier in thiolation of eukaryotic transfer RNA. Nature **458:**228–232.
45. **Li, Y., Y. Takagi, Y. Jiang, M. Tokunaga, H. Erdjument-Bromage, P. Tempst, and R. D. Kornberg.** 2001. A multiprotein complex that interacts with RNA polymerase II elongator. J. Biol. Chem. **276:**29628–29631.
46. **Liu, H.** 2001. Transcriptional control of dimorphism in Candida albicans. Curr. Opin. Microbiol. **4:**728–735.
47. **Liu, H., C. A. Styles, and G. R. Fink.** 1993. Elements of the yeast pheromone response pathway required for filamentous growth of diploids. Science **262:**1741–1744.
48. **Lo, H. J., J. R. Kohler, B. DiDomenico, D. Loebenberg, A. Cacciapuoti, and G. R. Fink.** 1997. Nonfilamentous C. albicans mutants are avirulent. Cell **90:**939–949.
49. **Lo, W.-S., and A. M. Dranginis.** 1996. *FLO11*, a yeast gene related to the *STA* genes, encodes a novel cell surface flocculin. J. Bacteriol. **178:**7144–7151.
50. **Longtine, M. S., A. McKenzie III, D. J. Demarini, N. G. Shah, A. Wach, A. Brachat, P. Philippsen, and J. R. Pringle.** 1998. Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. Yeast **14:**953–961.
51. **Madhani, H. D., and G. R. Fink.** 1997. Combinatorial control required for the specificity of yeast MAPK signaling. Science **275:**1314–1317.
52. **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:**12530–12535.
53. **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:**673–684.
54. **Maecker, H. T., S. C. Todd, and S. Levy.** 1997. The tetraspanin superfamily: molecular facilitators. FASEB J. **11:**428–442.
55. **Maeda, T., M. Takekawa, and H. Saito.** 1995. Activation of yeast PBS2 MAPKK by MAPKKKs or by binding of an SH3-containing osmosensor. Science **269:**554–558.
56. **Mercurio, F., B. W. Murray, A. Shevchenko, B. L. Bennett, D. B. Young, J. W. Li, G. Pascual, A. Motiwala, H. Zhu, M. Mann, and A. M. Manning.** 1999. IκB kinase (IKK)-associated protein 1, a common component of the heterogeneous IKK complex. Mol. Cell. Biol. **19:**1526–1538.
57. **Mösch, H. U., E. Kubler, S. Krappmann, G. R. Fink, and G. H. Braus.** 1999. Crosstalk between the Ras2p-controlled mitogen-activated protein kinase and cAMP pathways during invasive growth of Saccharomyces cerevisiae. Mol. Biol. Cell **10:**1325–1335.
58. **Mösch, H. U., R. L. Roberts, and G. R. Fink.** 1996. Ras2 signals via the Cdc42/Ste20/mitogen-activated protein kinase module to induce filamentous growth in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA **93:**5352–5356.
59. **Murray, L. E., N. Rowley, I. W. Dawes, G. C. Johnston, and R. A. Singer.** 1998. A yeast glutamine tRNA signals nitrogen status for regulation of dimorphic growth and sporulation. Proc. Natl. Acad. Sci. USA **95:**8619–8624.
60. **Nelson, C., S. Goto, K. Lund, W. Hung, and I. Sadowski.** 2003. Srb10/Cdk8 regulates yeast filamentous growth by phosphorylating the transcription factor Ste12. Nature **421:**187–190.
61. **Nemecek, J. C., M. Wuthrich, and B. S. Klein.** 2006. Global control of dimorphism and virulence in fungi. Science **312:**583–588.
62. **Nobile, C. J., and A. P. Mitchell.** 2006. Genetics and genomics of Candida albicans biofilm formation. Cell. Microbiol. **8:**1382–1391.
63. **Novick, P., M. Medkova, G. Dong, A. Hutagalung, K. Reinisch, and B. Grosshans.** 2006. Interactions between Rabs, tethers, SNAREs and their regulators in exocytosis. Biochem. Soc. Trans. **34:**683–686.
64. **O'Rourke, S. M., and I. Herskowitz.** 1998. The Hog1 MAPK prevents cross talk between the HOG and pheromone response MAPK pathways in Saccharomyces cerevisiae. Genes Dev. **12:**2874–2886.
65. **O'Rourke, S. M., I. Herskowitz, and E. K. O'Shea.** 2002. Yeast go the whole HOG for the hyperosmotic response. Trends Genet. **18:**405–412.
66. **Pan, X., T. Harashima, and J. Heitman.** 2000. Signal transduction cascades regulating pseudohyphal differentiation of Saccharomyces cerevisiae. Curr. Opin. Microbiol. **3:**567–572.
67. **Park, H. O., and E. Bi.** 2007. Central roles of small GTPases in the development of cell polarity in yeast and beyond. Microbiol. Mol. Biol. Rev. **71:**48–96.
68. **Peter, M., A. M. Neiman, H. O. Park, M. van Lohuizen, and I. Herskowitz.** 1996. Functional analysis of the interaction between the small GTP binding protein Cdc42 and the Ste20 protein kinase in yeast. EMBO J. **15:**7046–7059.

69. **Petrakis, T. G., T. M. Sogaard, H. Erdjument-Bromage, P. Tempst, and J. Q. Svejstrup.** 2005. Physical and functional interaction between Elongator and the chromatin-associated Kti12 protein. J. Biol. Chem. **280:**19454–19460.
70. **Petrakis, T. G., B. O. Wittschieben, and J. Q. Svejstrup.** 2004. Molecular architecture, structure-function relationship, and importance of the Elp3 subunit for the RNA binding of holo-elongator. J. Biol. Chem. **279:**32087–32092.
71. **Pfaffl, M. W.** 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. **29:**e45.
72. **Pitoniak, A., B. Birkaya, H. S. Dionne, N. Vadiae, and P. J. Cullen.** 2009. The signaling mucins Msb2 and Hkr1 differentially regulate the filamentation mitogen-activated protein kinase pathway and contribute to a multimodal response. Mol. Biol. Cell **20:**3101–3114.
73. **Pokholok, D. K., N. M. Hannett, and R. A. Young.** 2002. Exchange of RNA polymerase II initiation and elongation factors during gene expression in vivo. Mol. Cell **9:**799–809.
74. **Rahl, P. B., C. Z. Chen, and R. N. Collins.** 2005. Elp1p, the yeast homolog of the FD disease syndrome protein, negatively regulates exocytosis independently of transcriptional elongation. Mol. Cell **17:**841–853.
75. **Rahl, P. B., and R. N. Collins.** 2008. Analysis of the Elp complex and its role in regulating exocytosis. Methods Enzymol. **439:**315–325.
76. **Reiser, V., D. C. Raitt, and H. Saito.** 2003. Yeast osmosensor Sln1 and plant cytokinin receptor Cre1 respond to changes in turgor pressure. J. Cell Biol. **161:**1035–1040.
77. **Reynolds, T. B.** 2006. The Opi1p transcription factor affects expression of *FLO11*, mat formation, and invasive growth in *Saccharomyces cerevisiae*. Eukaryot. Cell **5:**1266–1275.
78. **Reynolds, T. B., and G. R. Fink.** 2001. Bakers' yeast, a model for fungal biofilm formation. Science **291:**878–881.
79. **Reynolds, T. B., A. Jansen, X. Peng, and G. R. Fink.** 2008. Mat formation in *Saccharomyces cerevisiae* requires nutrient and pH gradients. Eukaryot. Cell **7:**122–130.
80. **Roberts, C. J., B. Nelson, M. J. Marton, R. Stoughton, M. R. Meyer, H. A. Bennett, Y. D. He, H. Dai, W. L. Walker, T. R. Hughes, M. Tyers, C. Boone, and S. H. Friend.** 2000. Signaling and circuitry of multiple MAPK pathways revealed by a matrix of global gene expression profiles. Science **287:**873–880.
81. **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.
82. **Rose, M. D., F. Winston, and P. Hieter.** 1990. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
83. **Rupp, S., E. Summers, H. J. Lo, H. Madhani, and G. 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.
84. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
85. **Schlieker, C. D., A. G. Van der Veen, J. R. Damon, E. Spooner, and H. L. Ploegh.** 2008. A functional proteomics approach links the ubiquitin-related modifier Urm1 to a tRNA modification pathway. Proc. Natl. Acad. Sci. USA **105:**18255–18260.
86. **Schneider, B. L., W. Seufert, B. Steiner, Q. H. Yang, and A. B. Futcher.** 1995. Use of polymerase chain reaction epitope tagging for protein tagging in Saccharomyces cerevisiae. Yeast **11:**1265–1274.
87. **Stevenson, B. J., N. Rhodes, B. Errede, and G. F. Sprague, Jr.** 1992. Constitutive mutants of the protein kinase STE11 activate the yeast pheromone response pathway in the absence of the G protein. Genes Dev. **6:**1293–1304.
88. **Struhl, K., and R. W. Davis.** 1977. Production of a functional eukaryotic enzyme in *Escherichia coli*: cloning and expression of the yeast structural gene for imidazole-glycerolphosphate dehydratase (*his*3). Proc. Natl. Acad. Sci. USA **74:**5255–5259.
89. **Sun, J., J. Zhang, F. Wu, C. Xu, S. Li, W. Zhao, Z. Wu, J. Wu, C. Z. Zhou, and Y. Shi.** 2005. Solution structure of Kti11p from Saccharomyces cerevisiae reveals a novel zinc-binding module. Biochemistry **44:**8801–8809.
90. **Svejstrup, J. Q.** 2007. Elongator complex: how many roles does it play? Curr. Opin. Cell Biol. **19:**331–336.
91. **Taheri, N., T. Kohler, G. H. Braus, and H. U. Mösch.** 2000. Asymmetrically localized Bud8p and Bud9p proteins control yeast cell polarity and development. EMBO J. **19:**6686–6696.
92. **Tatebayashi, K., M. Takekawa, and H. Saito.** 2003. A docking site determining specificity of Pbs2 MAPKK for Ssk2/Ssk22 MAPKKKs in the yeast HOG pathway. EMBO J. **22:**3624–3634.
93. **Tatebayashi, K., K. Tanaka, H. Y. Yang, K. Yamamoto, Y. Matsushita, T. Tomida, M. Imai, and H. Saito.** 2007. Transmembrane mucins Hkr1 and Msb2 are putative osmosensors in the SHO1 branch of yeast HOG pathway. EMBO J. **26:**3521–3533.
94. **Tatebayashi, K., K. Yamamoto, K. Tanaka, T. Tomida, T. Maruoka, E. Kasukawa, and H. Saito.** 2006. Adaptor functions of Cdc42, Ste50, and Sho1 in the yeast osmoregulatory HOG MAPK pathway. EMBO J. **25:**3033–3044.
95. **TerBush, D. R., T. Maurice, D. Roth, and P. Novick.** 1996. The Exocyst is a multiprotein complex required for exocytosis in Saccharomyces cerevisiae. EMBO J. **15:**6483–6494.
96. **Vadaie, N., H. Dionne, D. S. Akajagbor, S. R. Nickerson, D. J. Krysan, and P. J. Cullen.** 2008. Cleavage of the signaling mucin Msb2 by the aspartyl protease Yps1 is required for MAPK activation in yeast. J. Cell Biol. **181:**1073–1081.
97. **Vinod, P. K., N. Sengupta, P. J. Bhat, and K. V. Venkatesh.** 2008. Integration of global signaling pathways, cAMP-PKA, MAPK and TOR in the regulation of FLO11. PLoS ONE **3:**e1663.
98. **Voynov, V., K. J. Verstrepen, A. Jansen, V. M. Runner, S. Buratowski, and G. R. Fink.** 2006. Genes with internal repeats require the THO complex for transcription. Proc. Natl. Acad. Sci. USA **103:**14423–14428.
99. **Winkler, G. S., T. G. Petrakis, S. Ethelberg, M. Tokunaga, H. Erdjument-Bromage, P. Tempst, and J. Q. Svejstrup.** 2001. RNA polymerase II elongator holoenzyme is composed of two discrete subcomplexes. J. Biol. Chem. **276:**32743–32749.
100. **Wittschieben, B. O., J. Fellows, W. Du, D. J. Stillman, and J. Q. Svejstrup.** 2000. Overlapping roles for the histone acetyltransferase activities of SAGA and elongator in vivo. EMBO J. **19:**3060–3068.
101. **Zarrinpar, A., R. P. Bhattacharyya, M. P. Nittler, and W. A. Lim.** 2004. Sho1 and Pbs2 act as coscaffolds linking components in the yeast high osmolarity MAP kinase pathway. Mol. Cell **14:**825–832.
102. **Zhang, X., E. Bi, P. Novick, L. Du, K. G. Kozminski, J. H. Lipschutz, and W. Guo.** 2001. Cdc42 interacts with the exocyst and regulates polarized secretion. J. Biol. Chem. **276:**46745–46750.