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

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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 G2 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.

### 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  | MATα ura3-52 | 47 |
| PC538a | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 | 10 |
| PC986b | MATα ura3-52 leu2 | 80 |
| PC999  | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 MSB2-HA | 10 |
| PC586  | MATα ura3-52 leu2 | 10 |
| PC1531 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 sho1::HYG | 10 |
| PC945  | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 msb2::KanMX6 | 10 |
| PC1083 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 GAL-MSB2-HA::KanMX6 | 10 |
| PC1516 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 MSB2::100::SIS | 96 |
| PC2053 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 pbs2::KanMX6 | 72 |
| PC1519 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 pgel1::KanMX6 | 72 |
| PC539  | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 ste12::URA3 | 72 |
| PC2061 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 His3p | 72 |
| PC1029 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 fio1::KanMX6 | 72 |
| PC1523 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 ssk1::NAT | 72 |
| PC2043 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 FLO11-HA::KanMX6 | S. Karauranthi et al., submitted |
| PC1894 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 leu2::HYG | S. Karauranthi et al., submitted |
| PC611  | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 ste12::URA3 | C. Chavel et al., submitted |
| PC2977 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 MSB2::100::SIS, elp2::URA3 | This study |
| PC2986 | MATα ura3-52 leu2 elp2::URA3 | This study |
| PC2976 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 MSB2-HA elp2::URA3 | This study |
| PC2991 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 ssk1::NAT, elp2::URA3 | This study |
| PC2983 | MATα ura3-52 elp2::URA3 | This study |
| PC2980 | MATα ura3-52 elp2::URA3 | This study |
| PC2989 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 MSB2-HA elp2::NAT | This study |
| PC2978 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 GAL-MSB2-HA::KanMX6 elp2::URA3 | This study |
| PC3102 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 leu2::HYG elp2::URA3 | This study |
| PC3517 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 MSB2-HA urm1::KURA3 | This study |
| PC3518 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 MSB2-HA ncs2::KURA3 | This study |
| PC3520 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 MSB2-HA kti11::KURA3 | This study |
| PC3524 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 MSB2-HA elp6::KURA3 | This study |
| PC2979 | MATα ste4 FUS1-lacZ, FUS1-HIS3 ura3-52 FLO11-HA::KanMX6 elp2::URA3 | This study |
| PC2763 | MATα 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 MATα 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. M. Dionne (53). Overexpression constructs were obtained from an ordered collection obtained from Open Biosystems (23). The FUS1-lacZ reporter was used to examine activity of the filamentous growth pathway in this Σ1278b strains lacking an intact mating pathway (steΔ) 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 (YPD) 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. Boone, H. M. Dionne, B. Birkaya, and P. J. Cullen, submitted for publication). Briefly, haploid (MATα) 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 pipetted to
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 Myq Q 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 cycles: 95°C for 1 min 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'-GTCAGCTGATGGCCTTTGTG-3') and reverse (5'-AGCTGCTGATGGCTTTGTG-3'), FLO12 forward (5'-GATGGACCACTTTCGTCGTATTC-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 iki3Δ, elp2Δ, elp3Δ, and elp4Δ mutants were identified in the screen as secretion defective (Fig. 1; Table 2). The elp5Δ mutant is inviable (41) and was not tested (Table 2), and the elp6Δ mutant was not identified, although direct testing confirmed a role for Epl6p in Msb2p regulation (data not shown).

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

| ORF | Phenotype/ | tRNA | MSB2-lacZ | Secretion/ | Localization | Process | Function |
|-----|------------|------|-----------|------------|-------------|---------|----------|
|     |            |      |           | 24 h       | 48 h        |         |         |
| Wild type  | Wild type | NA   | 48 ± 4.88 | 23.45 ± 1.96 | 28.93 ± 2.53 | Wild type | NA | NA |
| Iki3/Elp1 | Undersecretion | GLN, LYS | 32.06 | 29.4 | 20.17 | Wild type | tRNA modification | Subunit of elongator |
| 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 | Wild type | NA | 41.91 | 18.39 | 15.97 | Wild type | tRNA modification | Elongator-associated protein |
| Elp6 | Wild type | GLN, LYS | ND | ND | 28.71 | ND | tRNA modification | Subunit of elongator |
| K112 | Undersecretion | GLN, LYS | 32.9 | 18.78 | 11.31 | Wild type | tRNA modification | Ubiquitin-like modifier |
| TRM1 | Undersecretion | ARG, GLU | 48.6 | 51.08 | ND | Wild type | tRNA modification | TRNA methyltransferase |
| TRM9 | Undersecretion | ARG, GLU | 32.7 | 20.82 | 19.64 | Diffuse | tRNA modification | TRNA methyltransferase |
| URM1 | Undersecretion | LYS | 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** N2,N2-Dimethylguanosine.

**d** The standard deviation was less than ±5 for the mutants shown.

**e** The standard deviation was less than ±5 for the mutants shown.

**f** 5-Methylcarbonylmethyluridine in tRNA(Asp3) 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 kti12Δ 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 elp2Δ mutant was evaluated for filamentous growth and MAPK signaling. The elp2Δ mutant was defective for invasive growth (Fig. 2A), as determined by the plate-washing assay (81). The invasive growth defect of the elp2Δ mutant was comparable to that of other filamentous growth pathway msb2Δ, sho1Δ, and ste11Δ mutants (Fig. 2A) and less severe than that of the ste12Δ mutant (Fig. 2A), which lacks the transcription factor for the pathway, rendering the pathway inoperative (51). The elp2Δ mutant colony had a smooth appearance (Fig. 2A), indicative of a defect in MAPK- and Flo1p-dependent cell-cell adhesion. The elp2Δ mutant also exhibited cell elongation and distal-pole budding defects. As assessed by the single-cell invasive growth assay (12), the elp2Δ mutant failed to exhibit the elongated cell morphology characteristic of wild-type cells (Fig. 2B, black arrows). The elp2Δ mutant was similarly defective in distal pole budding, and cells of the elp2Δ mutant were observed budding back toward their parents (Fig. 2B, white arrow and data not shown). The polarity defect of the elp2Δ mutant was equivalent to that of other filamentous growth pathway components (Fig. 2B). The elp2Δ 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 elp2Δ 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 msb2Δ, ste11Δ, and ste12Δ mutants (Fig. 2D). The expression of other filamentous growth pathway target genes, particularly the FLO11 gene (83), was also reduced in the elp2Δ mutant (Fig. 2E). Direct testing confirmed an equivalent role for Elp6p in FG pathway signaling, and although not present in the deletion collection, the KTH11 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 Flo1p-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 elp2Δ mutant was defective in mat expansion (Fig. 3A) and exhibited a phenotype similar to but less severe than that of the flo11Δ and ste12Δ mutants (Fig. 3A). The elp2Δ 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 elp2Δ 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 elp2Δ mutant (Fig. 4A). Likewise, overexpression of MSB2 partially bypassed the signaling defect of the elp2Δ mutant. Specifically, the activity of the growth reporter FUS1-HIS3 was partially restored in the GAL-MSB2 elp2Δ double mutant (Fig. 4A). β-Galactosidase assays confirmed that FUS1-lacZ expression was partially restored in the GAL-MSB2 elp2Δ mutant (Fig. 4B). The cell elongation defect of the elp2Δ 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 MSB2Δ100-818 effectively bypassed the agar invasion defect of the elp2Δ mutant (Fig. 4D). The signaling and cell elongation defects were similarly suppressed by MSB2Δ100-818 (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
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 elp2Δ 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 elp2Δ 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-

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**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 msb2Δ (PC948), sho1Δ (PC1531), ste11Δ (PC611), and ste12Δ (PC539) filamentous growth pathway mutants alongside the elp2Δ 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 elp2Δ mutant. Bar, 5 μm. (C) Pgu1p activity in wild-type (PC538) and elp2Δ (PC2763), pgu1Δ (PC1519), and ste12Δ (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 elp2Δ mutant, and control strains as determined by quantitative real-time RT-PCR analysis.
y demonstrated that the Msb2p-HA protein was produced, glycosylated, and secreted in the elp2Δ 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 elp2Δ 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 elp2Δ mutant. MSB2-lacZ expression was reduced in the elp2Δ 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 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 elp2Δ mutant was examined for defects in pheromone response. A haploid MATa strain lacking the ELP2 gene showed sensitivity to the mating pheromone α-factor equivalent to that of the wild-type MATa 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 elp2Δ 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 Sin1p branch (76, 92), so we tested for a role for elongator in mutants defective for the Sin1p branch of the HOG pathway (ssk1Δ). Cells lacking the MAPK kinase for the
HOG pathway (pb2Δ) or both upstream regulatory branches (ste11Δ ssk1/Δ) were sensitive to the osmolite KCl (Fig. 6B). In contrast, the elp2Δ 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.

**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...
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
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 Ik3p/Elp1p, has been shown to associate with the JNK kinase (56) and with IκB, the IκB kinase (3, 8, 56). Stf1p, the mammalian homolog of Elp2p, binds to multiple STAT proteins and Janus kinases (9). Like Elp2p, Stf1p 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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FIG. 6. Elp2p is not required for pheromone response or HOG pathway function. (A) Shmoo formation over time. Wild-type (PC313; upper panel) and elp2α 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 elp2α 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 pbs2Δ (PC2053), ssk1Δ ste11Δ (PC2061), and ssk1Δ elp2Δ (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.
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