

Nucleotide Exchange Factor for the Yeast Hsp70 Molecular Chaperone Ssa1p

Mehdi Kabani,¹ Jean-Marie Beckerich,² and Jeffrey L. Brodsky^{1*}

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260,¹ and Laboratoire de Génétique Moléculaire et Cellulaire, INRA, INAPG, CNRS, 78850 Thiverval-Grignon, France²

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We report on the identification of Fes1p (yBR101cp) as a cytosolic homologue of Sls1p, an endoplasmic reticulum (ER) protein previously shown to act as a nucleotide exchange factor for yeast BiP (M. Kabani, J.-M. Beckerich, and C. Gaillardin, *Mol. Cell. Biol.* 20:6923-6934, 2000). We found that Fes1p associates preferentially to the ADP-bound form of the cytosolic Hsp70 molecular chaperone Ssa1p and promotes nucleotide release. Fes1p activity was shown to be compartment and species specific since Sls1p and *Escherichia coli* GrpE could not substitute for Fes1p. Surprisingly, whereas Sls1p stimulated the ATPase activity of BiP in cooperation with luminal J proteins, Fes1p was shown to inhibit the Ydj1p-mediated activation of Ssa1p ATPase activity in steady-state and single-turnover assays. Disruption of *FES1* in several wild-type backgrounds conferred a strong thermosensitive phenotype but partially rescued *ydj1-151* thermosensitivity. The Δ *fes1* strain was proficient for posttranslational protein translocation, as well as for the ER-associated degradation of two substrates. However, the Δ *fes1* mutant showed increased cycloheximide sensitivity and a general translational defect, suggesting that Fes1p acts during protein translation, a process in which Ssa1p and Ydj1p are known to be involved. In support of this hypothesis, Fes1p was found to be associated with ribosomes.

The 70-kDa heat shock proteins (Hsp70s) are a ubiquitous family of molecular chaperones found in all organisms and cellular compartments, and they are known to facilitate protein synthesis, folding, translocation, and degradation (2, 15, 22). This functional diversity relies on the intrinsic properties of these proteins as well as on their interaction with specific regulatory cofactors. Hsp70s are composed of a highly conserved N-terminal 44-kDa ATPase domain, an 18-kDa peptide-binding domain, and a C-terminal 10-kDa variable domain. In the ATP-bound state, Hsp70s display fast on and off rates of peptide binding, whereas in the ADP-bound state these constants are slowed (43, 57). The modulation of the intrinsic affinity for peptides is attributed to a cross talk between the ATPase domain and the C-terminal domain. Indeed, ATP hydrolysis triggers a conformational change in the C-terminal domain that is predicted to form a lid over the peptide-binding pocket and stabilizes the interaction with the substrate (74).

The weak ATPase activity of Hsp70s is stimulated by members of the DnaJ family (also known as Hsp40s), which share a common 70-amino-acid J domain required for binding and activation of Hsp70s (34). In *Escherichia coli*, the ATPase activity of the Hsp70 homologue DnaK is stimulated by the DnaJ chaperone which results in stable binding with polypeptide substrates (23, 61, 62). In addition, upon binding to the ATPase domain, the GrpE protein acts as a nucleotide exchange factor that promotes ADP release (9, 27, 42). Subsequently, ATP rebinding catalyzes the dissociation of the DnaK-substrate complex. Cycles of association and dissociation

prevent aggregation of the polypeptide substrate and facilitate folding. Whereas members of the DnaJ family are found in all organisms and in all cellular compartments (34), homologues of GrpE are found only in prokaryotes and archaea, or in organelles of prokaryotic origin such as mitochondria and chloroplasts. However, the antiapoptotic Bcl2-associated athanogene 1 (Bag-1) protein binds to Hsp70 and appears to be a nucleotide exchange factor for mammalian cytosolic Hsp70s and Hsc70s (9, 10, 29, 59, 64). Whereas GrpE exchanges both ATP and ADP from DnaK, Bag-1 promotes only ADP dissociation from Hsc70 (9), but the importance of the nucleotide exchange activity of Bag-1 in vivo is still subject to controversy (64). Moreover, Bag-1 was shown to either negatively or positively regulate Hsp70 and Hsc70 refolding activity depending on Bag-1:Hsp70 stoichiometry (24). Finally, there exist other cofactors that modulate the activity of Hsp70s chaperones in a manner different from that of J proteins or nucleotide exchange factors (4, 28, 30, 54). Overall, these data suggest that the regulation of eukaryotic Hsp70s is more complex than for bacterial DnaK homologues.

At least 10 members of the Hsp70 family are found in the yeast *Saccharomyces cerevisiae* (22). Ssa1p is the most extensively studied cytosolic Hsp70, as it plays essential functions in protein folding and translocation across the endoplasmic reticulum (ER) and mitochondrial membranes in combination with its J-domain partner Ydj1p and facilitates ER-associated degradation (ERAD) (21). Recently, a function for Ssa1p in translation initiation has been described (31). Ssa1p was found to be associated with translating ribosomes, and it associates with Pab1p, the poly(A) binding protein, and Sis1p, a DnaJ homologue. Depletion of Ssa1p results in a general translational defect as well as changes in the polysome profile (31). Moreover, efficient translation of heterologous proteins such as green fluorescent protein (GFP) or firefly luciferase de-

* Corresponding author. Mailing address: Department of Biological Sciences, 267 Crawford Hall, University of Pittsburgh, Pittsburgh, PA 15260. Phone: (412) 624-4831. Fax: (412) 624-4759. E-mail: jbrodsky@pitt.edu.

TABLE 1. Yeast strains used in this study

Strain	Genotype	Reference
RSY801	<i>MATa ade2-101 leu2-3,113 ura3-52</i>	56
W3031b	<i>MATα ade2-101 his3 leu2-3,113 ura3-52 trp1 can1-100</i>	16
<i>ydj1-151</i> (ACY17b)	W3031b <i>ydj1-2::HIS3 LEU2::ydj1-151</i>	16
RSY801, Δ <i>fes1</i>	RSY801 <i>fes1::KANMX4</i>	This study
W3031b, Δ <i>fes1</i>	W3031b <i>fes1::KANMX4</i>	This study
<i>ydj1-151</i> , Δ <i>fes1</i>	<i>ydj1-151 fes1::KANMX4</i>	This study
W3031b, <i>GFP-FES1</i>	W3031b <i>HIS3MX6-GAL1-GFP-FES1</i>	This study

pendes upon functional Ydj1p (13), suggesting an involvement of the Ssa1p-Ydj1p pair in this vital cellular process. The Ssb1p and Ssb2p Hsp70s are also found to be associated with ribosomes, play a role in the elongation step of protein translation (49, 52), and interact with the Hsp40 homologues Sis1p and zuo1in to facilitate translation (70, 73).

Another well-studied Hsp70 in yeast is Kar2p, the ER luminal homologue of mammalian BiP (26). Kar2p is also involved in protein translocation across the ER membrane, protein folding, and ERAD (21). Although three luminal J proteins mediate the recruitment and the regulation of Kar2p for specific functions (50), a GrpE or Bag-1 analogue was unknown until we showed that the ER localized protein Sls1p (yOL031cp; also called Sil1p [66] or Per100p [65]), identified as a homologue of a protein required for efficient cotranslational protein translocation in the yeast *Yarrowia lipolytica* (6), binds to the ATPase domain of the luminal Hsp70 BiP (Kar2p) and catalyzes nucleotide exchange (7, 32, 33). Sls1p is also involved in protein translocation and ERAD (32, 65, 66).

Because a nucleotide exchange factor for the yeast cytosolic Hsp70s had not been defined, we screened the yeast genome database for homologues of Sls1p and report here on Fes1p, the product of the *yBR101c* open reading frame (ORF), as a likely candidate. Fes1p binds to Ssa1p in an ATP-dependent manner and promotes nucleotide exchange. The *FES1* gene is required for growth at high temperature, but its disruption suppresses the *ydj1-151* thermosensitive phenotype, suggesting that Fes1p acts antagonistically to Ydj1p. Several assays excluded a function of Fes1p in protein translocation, ERAD, or folding, but instead suggest that this protein may be involved in protein translation.

MATERIALS AND METHODS

Strains and media. *E. coli* strains used were DH5 α (*endA1 hsdR17 supE44 thi-1 recA1 gyrA relA1 Δ[*lacZYA-argF*] U169 deoR [φ80 *dlacΔ lacZ M15*]*) and BL21 (F⁻ *ompT hsdS [r_B⁻ m_B⁻] gal*). Bacteria were grown in Luria-Bertani (LB) medium supplemented with ampicillin (50 μg/ml) for plasmid selection (3). Yeast strains used in this study are described in Table 1 and were grown on yeast extract-peptone-dextrose (YPD) medium or YNB-N0 lacking amino acids (Difco) but supplemented with the appropriate nutrients (1).

DNA manipulation techniques. Standard techniques were used (3), and restriction enzymes were used according to the manufacturer's instructions. Ready-To-Go PCR beads (Pharmacia Biotech) or *Taq* DNA polymerase (Gibco BRL) and Crocodile III Thermocycler (Appligene Oncor) were used for PCRs.

To overexpress Fes1p for protein purification (see below), the *yBR101c* ORF was amplified by PCR of genomic DNA. The oligonucleotides used were MK1 (5'-CGCGGATCCGTGAAAAGCTATTACAGTGGT-3') and MK2 (5'-CGCGGATCCTAATACATACTTTACGGC-3'), which allowed cloning into pBlue-script SK- vector (Stratagene) via the underlined *Bam*HI sites. The cloned PCR product was verified by DNA sequence analysis as described previously (33). The plasmid for glutathione *S*-transferase (GST)-Fes1p fusion protein expression

was obtained by in-frame cloning of the *yBR101c* ORF into the *Bam*HI site of pGEX-5X-1 (Amersham Pharmacia Biotech).

The GST-Fes1p coding sequence was amplified by PCR with pGEX-5X-1-*yBR101c* as a template. The oligonucleotides used were MK3 (5'-GCGCGCAC TAGTATGTCCCCTATACTAGGTTATTGG-3') and MK4 (5'-CCCATCGA TTCATAATACATACTTTACGGCTAAATAATC-3'), which allowed cloning into pGPD425 (47) via the underlined *Spe*I and *Cla*I sites, respectively. This construct was transformed into the strains from which *yBR101c* had been deleted (see below) and fully complemented the thermosensitive phenotype of these mutants (see Results).

A *yBR101c* disruption cassette was obtained by amplifying pRS400 (8) with primers MK5 (5'-AACGAAAGAGTAAAATAGAAAAAAAACACATACAT AACTCTGTGCGGTATTTACACCG-3') and MK6 (5'-AAATGGTGAATGT AATATCATTTTATTTCTACGGACGTAAAGATTGTACTGAGAGTGCAC -3'), which contain a 40-bp sequence homologous to the promoter and terminator portions of *yBR101c*, respectively, along with a 20-bp region of homology with the KanMX4 cassette in pRS400. Disrupted mutants were then obtained by transforming the resulting PCR fragment into yeast and selecting for G418-resistant colonies at a final concentration of 200 μg/ml. Disruption of the *yBR101c* ORF was confirmed in resistant colonies by using primers MK7 (5'-CTCATCGTCAGTCAGAAAGC -3') and MK8 (5'-ATAAATGTTTCGAGATTCCGG-3'), which flank the *yBR101c* ORF. In order to exclude possible mutations arising from the transformation procedure, the phenotype (see Results) of the *yBR101c* disruption in each strain was analyzed in three independent clones.

Localization of a GFP-tagged Fes1p. A GFP-tagged version of Fes1p was obtained by using the method described by Longine et al. (40). A HIS3MX6-GAL1-GFP cassette was obtained by PCR amplification using oligonucleotides JMB59 (5'-CCCTGAATTGCAATAGACCACTGTAATAGCTTTTCTTTGTATAGTTC ATCCATGC-3') and JMB68 (5'-GGTAAAGCTTTTCAGCTACTGGTTGGTCA AGTTGGGCCCTATTAAGGCGAGCTCGTTTAACTGGATGG-3'). The resulting cassette bears regions of homology to *yBR101c* (underlined) that allow integration into the yeast genome by double recombination. The W3031b yeast strain was transformed with this construct, and integrants expressing GFP-Fes1p under the control of the GAL1 promoter were selected on minimal medium lacking histidine and checked by PCR and Southern blot analysis. This strain was grown on raffinose-containing medium and then transferred into galactose-containing medium for 8 h, and the cells were observed with an Olympus BX 51 fluorescence microscope.

Cellular fractionation was performed using 20 *A*₂₅₄ U of the lysate prepared for ribosome fractionation (see below). The lysate volume was brought up to 1 ml with polysome lysis buffer (PLB; see below) and centrifuged at 16,000 × *g* at 4°C for 15 min. The pellet was resuspended in 500 μl of PLB, and half of the supernatant was centrifuged at 150,000 × *g* at 4°C for 1 h. The pellet was resuspended in 500 μl of PLB. A 10-μl aliquot of each sample was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by Western blotting using anti-GFP (a gift from S. Subramani, University of California, San Diego), anti-Sec61p (60), and anti-Sse1p antibodies (a gift from J. Goeckeler, University of Pittsburgh). Western blots were developed using chemiluminescence and ECL reagents (Pierce) according to the manufacturer's instructions.

Ribosome fractionation. The yeast strains were grown in 200 to 400 ml of YPD (RSY801 and RSY801; Δ *fes1*) or YP-galactose (W303; *GFP-FES1*) at 30°C to log phase (*A*₆₀₀ = ~1) and, where indicated, the cells were shifted for 30 min at 37°C. Cycloheximide was added at a final concentration of 50 μg/ml, and cells were pelleted and washed with ice-cold PLB (20 mM HEPES [pH 7.4], 100 mM NaCl, 20 mM MgCl₂, 100 μg of cycloheximide/ml) containing 0.2 μl of diethylpyrocarbonate (Sigma) per ml. The cells were resuspended in 2 ml of the same buffer, and lysates were prepared by beating the cells with glass beads eight times for 15 s, with a 30-s period of cooling on ice between each vortexing step. The

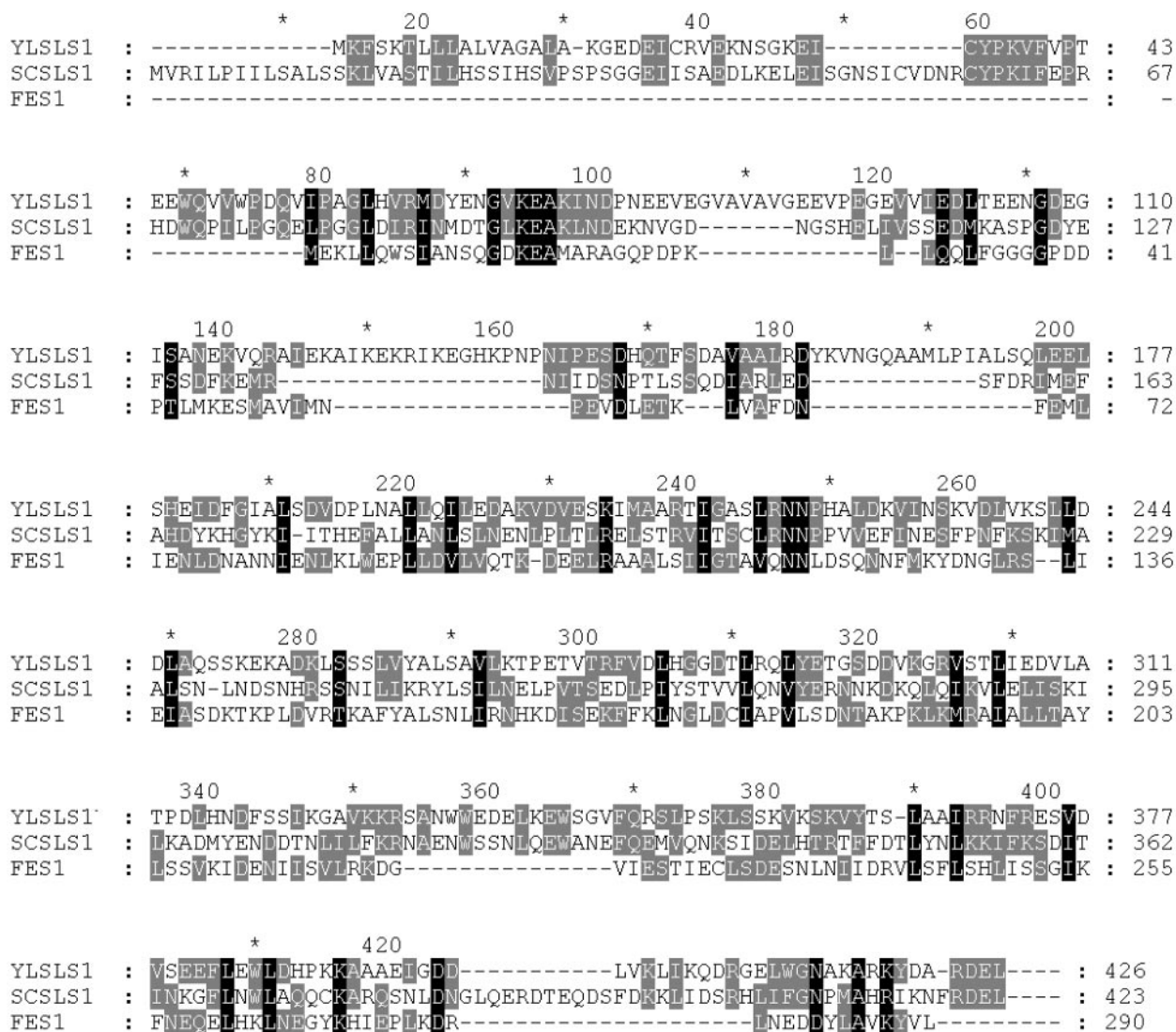


FIG. 1. Alignment of Fes1p with the amino acid sequences encoded by the *Y. lipolytica* and *S. cerevisiae* *SLS1* genes. Black boxes represent conserved residues; shaded boxes represent similar residues. Amino acids 1 to 17 (6) and 1 to 19 correspond to the putative signal sequences of *Y. lipolytica* Sls1p and *S. cerevisiae* Sls1p, respectively. Neither signal sequences nor C-terminal ER retention motifs are found in Fes1p.

lysate was cleared by two centrifugation steps, each at 5,000 rpm for 5 min in a microcentrifuge at 4°C. A fraction of the supernatant corresponding to 50 to 100 *A*₂₅₄ U was layered over a 30-ml linear sucrose gradient (7 to 47%, wt/vol) prepared in PLB. The sucrose gradient was centrifuged at 27,000 rpm for 4.5 h

at 4°C in a Beckman SW28.1 rotor. Gradients were collected with continuous monitoring at 254 nm.

To determine the association of GFP-Fes1p and Ssa1p on ribosomes and polysomes, fractions representing the load, the cytosol (top of the gradient), fractions after the cytosol but before ribosome-containing fractions, the 80S ribosomes, and polysomes were collected and proteins were resolved by SDS-PAGE and analyzed by Western blotting using anti-GFP, anti-Ssa1p (a gift from C. Koehler, University of California, Los Angeles), and anti-L3 (a gift from J. Warner, Albert Einstein College of Medicine, New York, N.Y.) antisera.

Protein purification. The GST-Fes1p protein was purified from *E. coli* strain BL21 transformed with the expression plasmid described above. A 50-ml culture in LB containing 50 µg of ampicillin per ml was grown overnight at 26°C and diluted into 2 liters of the same medium. After 2 h at 28°C, IPTG (isopropyl-β-D-thiogalactopyranoside) was added to a final concentration of 0.5 mM, and cells were grown for an additional 4 h. Cells were harvested and washed once in water and once in phosphate-buffered saline (pH 7.4)–2 mM EDTA before the cell pellet was snap-frozen in liquid nitrogen and stored at –70°C. The cell pellet was thawed and resuspended in ~20 ml of buffer A (phosphate-buffered saline [pH 7.4], 5 mM EDTA, 1 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 1 µg of leupeptin per ml, 1 µg of pepstatin A per ml), and the cells were

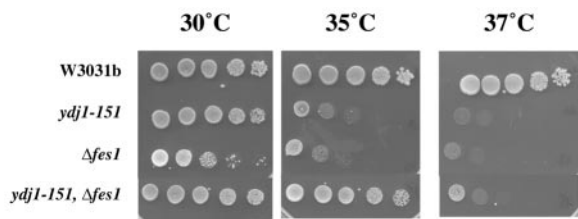


FIG. 2. Genetic effects of the disruption of the *FES1* gene. Serial dilutions of overnight cultures of the wild-type and indicated mutant strains were inoculated on YPD plates and incubated at the indicated temperatures for 3 days.

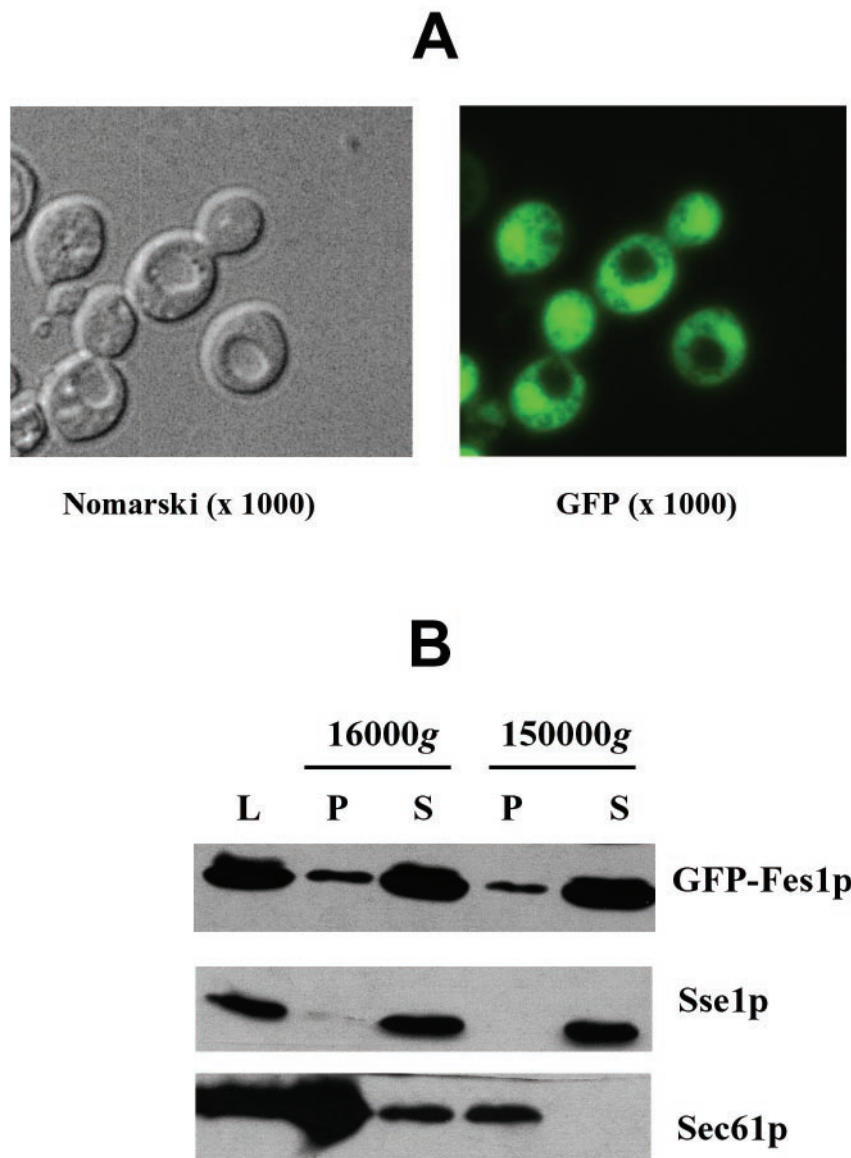


FIG. 3. Subcellular localization of Fes1p. (A) A strain bearing a GFP-tagged version of Fes1p under the control of the *GALI* promoter was obtained as described in Materials and Methods. This strain was grown on raffinose-containing medium and then for 8 h in galactose-containing medium. The cells were then observed directly with a fluorescence microscope, and both Nomarski and GFP fluorescence images are shown (magnification, $\times 1,000$). (B) Cell lysate (L) was subjected to $16,000 \times g$ and $150,000 \times g$ centrifugations. Equal amounts of proteins from pellet (P) and supernatant (S) fractions were resolved by SDS-PAGE and analyzed by Western blotting with anti-GFP (Fes1p), anti-Sse1p (cytosolic protein), and anti-Sec61p (integral membrane protein) antibodies.

disrupted by sonication for 30 s six times at the highest setting with 1 min on ice between sonications. The broken cells were centrifuged at 13,000 rpm in a Sorvall SA600 rotor for 15 min, and the resulting supernatant was further centrifuged at 20,000 rpm in a Sorvall SA600 rotor for 20 min to obtain a cleared lysate. This lysate was loaded onto a 2-ml glutathione Sepharose 4B column (Pharmacia Biotech) equilibrated in buffer A containing 1% Triton X-100. The column was washed sequentially with 30 ml of the following: (i) buffer A containing 5% glycerol; (ii) buffer A containing 1 M NaCl and 5% glycerol; (iii) 50 mM Tris-Cl (pH 7.5), 10 mM magnesium acetate, 200 mM potassium acetate, 5 mM ATP, 5% glycerol; and (iv) buffer A containing 5% glycerol. GST-Fes1p was eluted with 10 ml of elution buffer (50 mM Tris-Cl [pH 8.0], 50 mM NaCl, 20 mM reduced glutathione, 5% glycerol), and 1-ml fractions were collected and analyzed by SDS-PAGE and Coomassie brilliant blue staining. Peak fractions were pooled, dialyzed against dialysis buffer (10 mM HEPES [pH 7.0], 50 mM NaCl, 10 mM dithiothreitol [DTT], 10% glycerol), and frozen in small aliquots in liquid

nitrogen and stored at -70°C . GST-Fes1p was purified to near homogeneity as assessed by SDS-PAGE and Coomassie brilliant blue staining.

GST-Sls1p and Kar2p were purified as described before (32, 45), and purified Ssa1p and Ydj1p, prepared as published (63), were a kind gift from S. W. Fewell (University of Pittsburgh). Purified DnaK and GrpE proteins were obtained from StressGen.

Protein interaction assays. GST pull-down assays were performed essentially as described in reference 32, with minor modifications. A total of $4 \mu\text{g}$ of GST-Fes1p or GST-Sls1p was incubated with $2 \mu\text{g}$ of either Ssa1p or Kar2p in the presence of 1 mM ATP or ADP in GST-binding buffer (20 mM HEPES [pH 6.8], 100 mM KCl, 5 mM MgCl_2 , 0.1% NP-40, 2% glycerol, 1 mM DTT, 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride) for 1 h at 4°C . A $20\text{-}\mu\text{l}$ aliquot of glutathione-Sepharose 4B (50% slurry) (Amersham Pharmacia Biotech) was added, and reaction mixtures were rotated for 30 min at 4°C . Reaction mixtures were centrifuged for 2 min at 13,000 rpm in a microcentrifuge, the supernatant

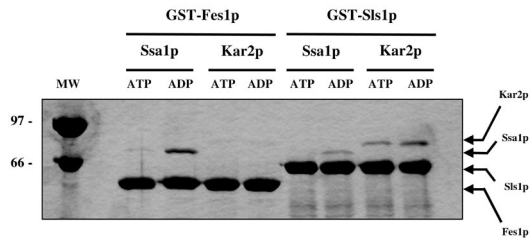


FIG. 4. Fes1p interacts with Ssa1p but not Kar2p. GST-Fes1p, GST-Sls1p, Ssa1p, and Kar2p were purified as described in Materials and Methods. GST-Fes1p or GST-Sls1p was incubated with Ssa1p or Kar2p in the presence of 1 mM nucleotide (ATP or ADP) and incubated at 4°C for 1 h. Glutathione-Sepharose 4B beads were added, and reactions were rotated for 30 min at 4°C. Proteins bound to the glutathione matrix were resolved by SDS-PAGE and visualized by Coomassie brilliant blue staining. Molecular weight standards (in thousands) are shown at the left.

was removed, and the pellet was washed three times with 100 μ l of GST-binding buffer. The pellet fraction was then analyzed by SDS-PAGE using 8% polyacrylamide gels followed by Coomassie brilliant blue staining.

Nucleotide exchange and ATPase assays. An Hsp70- $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ complex (with Ssa1p or DnaK) was obtained as described previously (63). In brief, 25 μ g of Hsp70 was incubated in complex buffer (25 mM HEPES-KOH [pH 7.5], 100 mM KCl, 11 mM magnesium acetate) containing 25 μ M ATP and 100 μ Ci of $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ (3,000 Ci/mmol; NEN) for 30 min on ice. Complexes were purified from free nucleotide by gel filtration on G-50 Nick Columns (Amersham Pharmacia Biotech). Peak complex fractions were pooled, the glycerol concentration was adjusted to 10%, and the samples were frozen in small aliquots in liquid nitrogen. For nucleotide exchange assays, 25 μ l of complex was quickly thawed and mixed with 25 μ l of complex buffer with or without the indicated cofactors (at a threefold molar excess over Ssa1p) at 30°C. At the specified time points, a 15- μ l aliquot was removed and purified from free nucleotide on G-50 microspin columns (Amersham Pharmacia Biotech), and the eluate was mixed with 5 μ l of stop buffer (36 mM ATP, 2 M LiCl, 4 M formic acid). The amount of remaining nucleotide was measured by scintillation counting and analyzed by thin-layer chromatography as described previously (58).

Single-turnover ATPase assays were performed essentially as described elsewhere (63). In brief, 25 μ l of Ssa1p- $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ complex (see above) was mixed with 25 μ l of complex buffer containing or lacking the indicated cofactors (at a threefold molar excess over Ssa1p) at 30°C. At the specified time points, a 6- μ l aliquot of the reaction mixture was removed and added to 2 μ l of stop buffer on ice. Triplicate 2- μ l aliquots of this mixture were spotted on a thin-layer chromatography plate and developed (63).

For steady-state ATPase assays, 4 μ g of Ssa1p was mixed with a twofold molar excess of Ydj1p in ATPase buffer (50 mM HEPES [pH 7.4], 50 mM NaCl, 10 mM DTT, 2 mM MgCl_2) containing 50 μ M ATP and 0.2 μ Ci of $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ in a total volume of 20 μ l. After 10 min at 30°C, a 4- μ l aliquot was stopped with 4 μ l of stop buffer, and 5- μ l aliquots were added to 15 μ l of buffer with or without the specified amount of cofactor (GST-Fes1p or GST-Sls1p). At the indicated time points, 4- μ l aliquots were taken and added to 4 μ l of stop buffer on ice. Triplicate 1- μ l samples were analyzed by thin-layer chromatography as described above.

RESULTS

Identification of *yBR101c* as a homologue of SLS1 (*yOL031c*). The identification of Sls1p as a nucleotide exchange factor for yeast BiP (6, 7, 32) raised the possibility for the existence of a similar factor for cytosolic Hsp70s. In order to address this question, we used the Psi-BLAST 2.0 software (<http://www.ncbi.nlm.nih.gov>) to identify Sls1p homologues in the yeast genome. The only gene uncovered in this search was *yBR101c* (Fig. 1), which encodes a predicted 290-amino-acid protein. The protein encoded by this gene, which we call Fes1p (mnemonic for factor exchange for Ssa1p), is significantly shorter than Sls1p and lacks a signal sequence, ER retention

motif, nuclear localization sequence, and core glycosylation consensus sites, consistent with a cytosolic localization of Fes1p. Although the Sls1p homologues that we have found in the databases so far are poorly conserved, they share several clusters of amino acids that are present in Fes1p (Fig. 1).

The Δ *fes1* mutant is thermosensitive. As a first step toward investigating the function of Fes1p, the encoding ORF was disrupted by using a kanamycin resistance cassette (8) in two unique wild-type genetic backgrounds (see Materials and Methods) (Table 1). In both cases, the disruption of *FES1* did not affect viability but strongly impaired growth at high temperature (Fig. 2).

We also sought genetic interactions between Δ *fes1* and *ydj1-151*, a thermosensitive mutant of the Ydj1p J DnaJ homologue that is defective in its ability to activate Ssa1p (16). Genetic interactions were previously found between Δ *sls1* and a thermosensitive *sec63* allele (32) which encodes a mutated form of the ER J protein Sec63p (55). Disruption of *FES1* in the *ydj1-151* strain partially suppressed the Δ *fes1* and *ydj1-151* thermosensitive phenotypes (Fig. 2). Since several independent transformants were analyzed, we excluded the possibility that this result is due to a third mutation resulting from the transformation procedure. The observed genetic interactions suggest that Fes1p and Ydj1p may collaborate to facilitate a cellular pathway and/or coregulate another factor.

Fes1p is a soluble protein located in the cytosol. In order to determine the subcellular localization of Fes1p, the protein was tagged at the N terminus with GFP. GFP-Fes1p is expressed under the control of the *GAL1* promoter, and the corresponding strain grew as well as the isogenic wild type at 37°C (data not shown), suggesting that the GFP tag does not affect the function of Fes1p.

We used fluorescence microscopy to assess the localization of GFP-Fes1p after inducing its expression for 8 h in galactose-containing medium. As shown in Fig. 3A, the protein was found primarily in the cytosol, as expected from the primary sequence analysis (Fig. 1). The GFP-Fes1p protein appeared enriched at the nucleus (Fig. 3A), but whether this is physiologically relevant or whether this is an effect due to the overexpression of the fusion protein is not known. Indeed, the nuclear colocalization was more pronounced when the expression of the protein was induced for longer times (data not shown). In any event, GFP-Fes1p was excluded from the vacuole (Fig. 3A).

To confirm the cytosolic localization of the protein, subcellular fractionation was performed on crude lysates as described in Materials and Methods. As shown in Fig. 3B, GFP-Fes1p was found in soluble fractions, as was Sse1p, a cytosolic Hsp110 (14) (Fig. 3B; supernatant fractions), and relatively minor amounts of GFP-Fes1p were found in the crude membrane fraction (Fig. 3B; 16,000 \times g pellet), where Sec61p, the ER translocation pore protein, is detected. It should be noted that Ssa1p is also detected in both supernatant and pellet fractions (14). We conclude that Fes1p is a soluble cytosolic protein.

Fes1p interacts with Ssa1p in an ATP-dependent manner. To determine if Fes1p, like Sls1p (32), binds to Hsp70, the protein was overexpressed as a GST fusion protein in *E. coli* and purified to near homogeneity by affinity chromatography (see Materials and Methods). The GST-Fes1p fusion protein fully complemented the thermosensitive phenotype of the

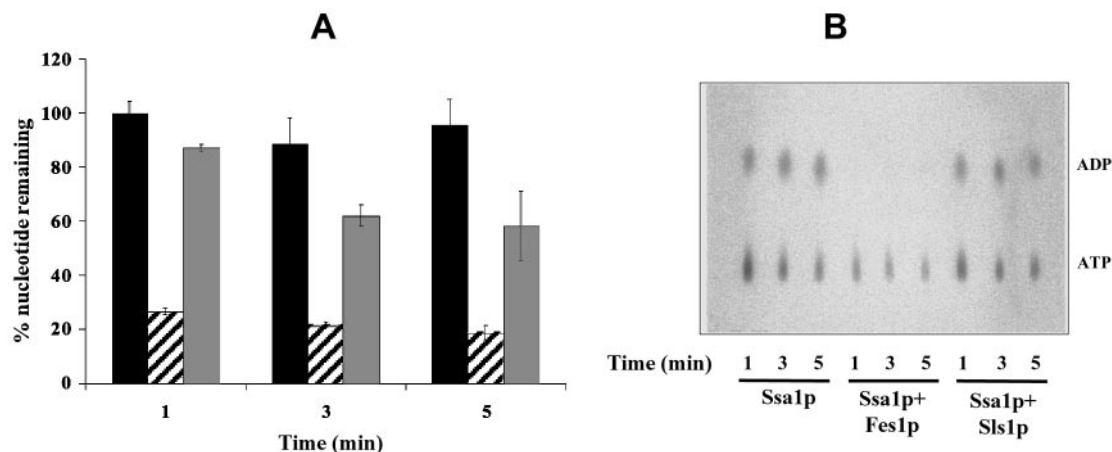


FIG. 5. Fes1p is a nucleotide exchange factor for Ssa1p. The $[\alpha\text{-}^{32}\text{P}]\text{ATP-Ssa1p}$ complex was incubated with buffer (black bars), or a threefold molar excess of GST-Fes1p (striped bars) or GST-Sls1p (gray bars) at 30°C. At the indicated time points, an aliquot was taken and free nucleotide was removed on microspin G-50 columns. Further reaction in the eluate was stopped, and the amount of Ssa1p-bound nucleotide was determined by scintillation counting (A) or analyzed on thin-layer chromatography plates (B) as described previously (32). Error bars represent the standard deviations from three independent experiments.

Δfes1 strain when placed under the control of a strong constitutive promoter (see Materials and Methods), suggesting that the GST tag does not affect the function of Fes1p.

Although *S. cerevisiae* possesses six cytosolic Hsp70s (Ssa1 to Ssa4, Ssb1, and Ssb2; [22]), we first examined the interaction between Fes1p and the cytosolic Hsp70, Ssa1p, because of its defined roles in protein translation, translocation, folding, and ERAD (21). We also purified GST-Sls1p and Kar2p (32, 45) to provide negative and specificity controls for the binding reactions. Binding of GST-Fes1p and GST-Sls1p to the Hsp70s was assessed by GST pull-down assays in either 1 mM ATP or 1 mM ADP (see Materials and Methods). After 1 h at 4°C, the mixtures were centrifuged and washed, and the pellet fractions were analyzed by SDS-PAGE and Coomassie blue staining. As shown in Fig. 4, Fes1p binds to Ssa1p, but not to Kar2p, and conversely Sls1p binds preferentially to Kar2p, as shown previously (32), al-

though some nonspecific binding was also observed between Sls1p and Ssa1p (Fig. 4); however, the Sls1p-Ssa1p binding is ~10-fold lower than the interaction between Sls1p and Kar2p. Also previously described for the Sls1p-Kar2p interaction (32), Fes1p binds more efficiently to the ADP-bound form of Ssa1p, suggesting that the interaction is specific. It should be noted that the binding experiments shown in Fig. 4 were performed at pH 7.4 (with similar results at pH 8.0; data not shown) and under these conditions there is some interaction between Sls1p and the ATP-bound form of Ssa1p, consistent with the ability of Sls1p to release ATP from Kar2p (32). The possibility that the Hsp70s recognize a population of misfolded GST fusion proteins is excluded by the specificity of interaction between each chaperone and either Fes1p or Sls1p and by control experiments using purified GST to which neither factor was fused (data not shown). Overall, the preferred interactions between Kar2p

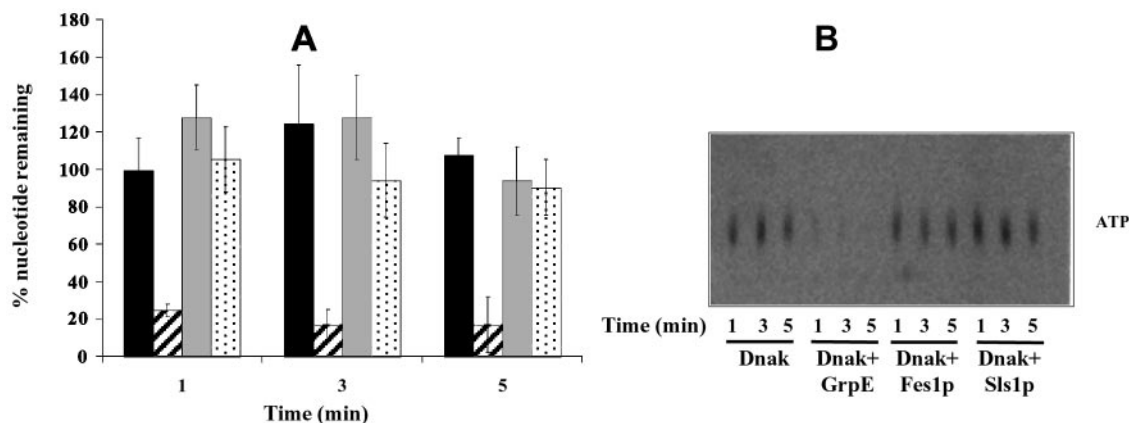


FIG. 6. Fes1p and Sls1p cannot substitute for GrpE as nucleotide exchange factors for DnaK. Nucleotide exchange reactions with *E. coli* DnaK were performed as described for Fig. 5. The $[\alpha\text{-}^{32}\text{P}]\text{ATP-DnaK}$ complex was incubated with buffer (black bars) or a threefold molar excess of GrpE (dashed bars), GST-Fes1p (gray bars), or GST-Sls1p (dotted bars) at 30°C. At the indicated time points, an aliquot was taken and free nucleotide was removed on microspin G-50 columns. Reactions in the eluates were stopped, and radioactivity was analyzed by scintillation counting (A) and thin-layer chromatography (B). Error bars represent the standard deviations from three independent experiments.

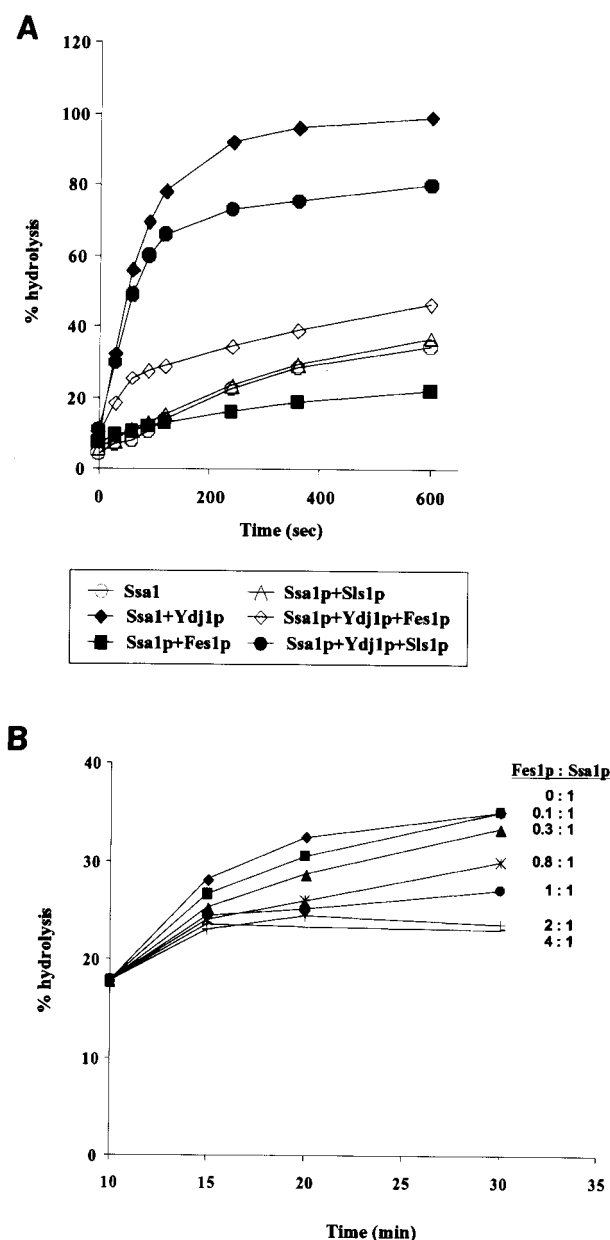


FIG. 7. (A) Effects of Fes1p and Ydj1p on single-turnover ATPase activity. Single-turnover experiments were performed as described previously (63). The Ssa1p- $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ complex (as in Fig. 5) was mixed with complex buffer with or without the indicated cofactors (at a threefold molar excess over Ssa1p) at 30°C. At the specified times, an aliquot was removed from the reaction mixture and added to stop buffer on ice. Triplicate aliquots of this mixture were analyzed on thin-layer chromatography plates. A representative experiment is shown. (B) Fes1p inhibits Ssa1p steady-state ATPase activity. Ssa1p was mixed with a twofold molar excess of Ydj1p in a reaction mixture containing 50 μM ATP and 0.2 μCi of $[\alpha\text{-}^{32}\text{P}]\text{ATP}$. After 10 min at 30°C, the indicated amount of Fes1p was added, and an aliquot of the reaction mixture was analyzed at the indicated times as described in Materials and Methods.

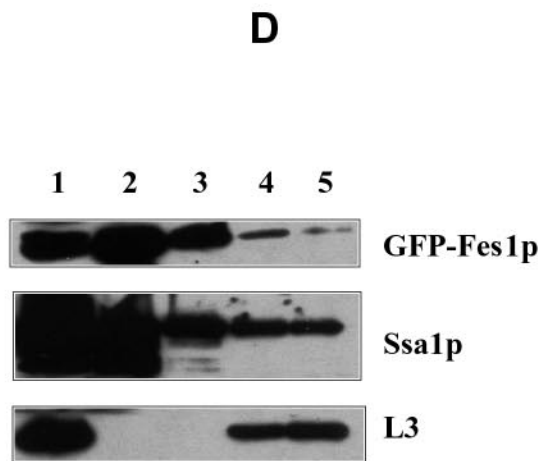
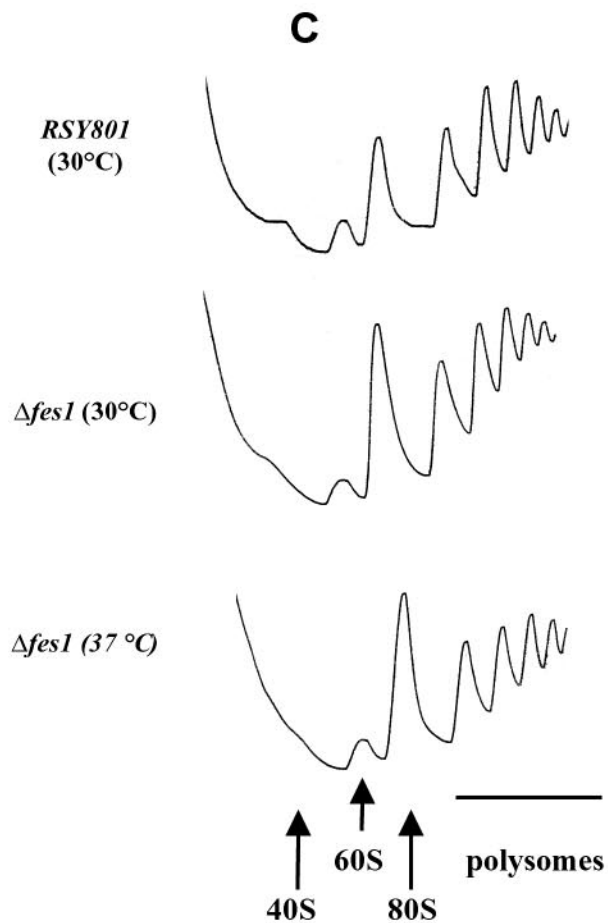
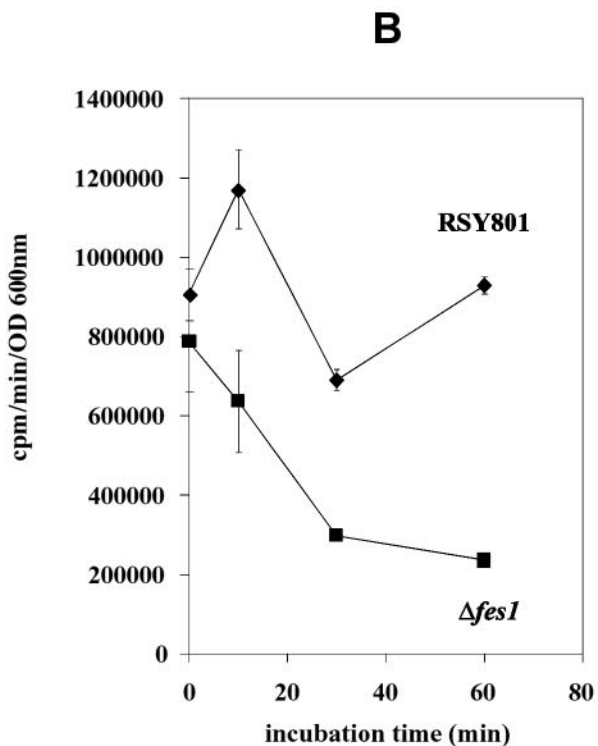
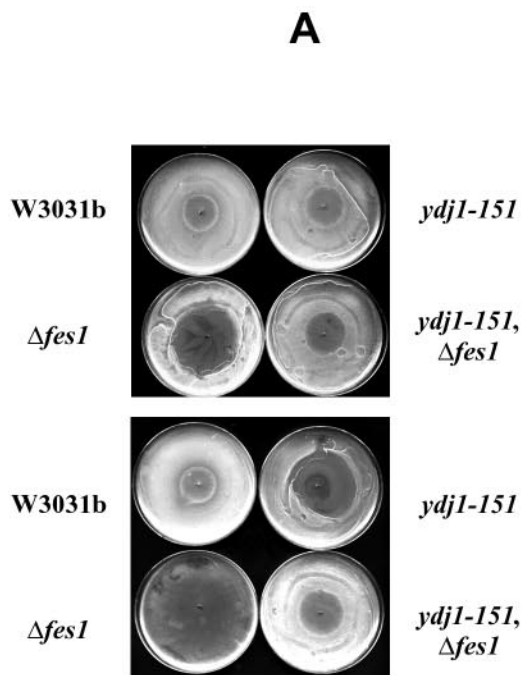
and Sls1p and between Fes1p and Ssa1p are consistent with the compartmentalization of these chaperone pairs in the ER lumen and cytoplasm, respectively.

Fes1p is a nucleotide exchange factor for Ssa1p. To determine whether Fes1p promotes nucleotide release upon binding

to Ssa1p, we prepared an Ssa1p- $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ complex (63) and performed nucleotide exchange assays (see Materials and Methods). The Ssa1p- $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ complex was incubated at 30°C in the presence of buffer, GST-Fes1p, or GST-Sls1p, aliquots were removed over time, and Ssa1p-bound and free nucleotide were resolved by gel filtration chromatography. The amount of bound nucleotide was assessed by scintillation counting (Fig. 5A) and analyzed by thin-layer chromatography (Fig. 5B). In the presence of GST-Fes1p a rapid decrease (within 1 min) of bound nucleotide was observed compared to that of the buffer control. The thin-layer chromatography plate (Fig. 5B) shows that both ADP and, to a lesser extent, ATP were stripped by GST-Fes1p. Importantly, GST-Sls1p had very little effect on nucleotide release from Ssa1p (Fig. 5), a result which was consistent with the GST pull-down assays (Fig. 4). Also, the Fes1p-stimulated nucleotide release was most likely not due to its ability to act as a peptide substrate, which was previously shown to enhance nucleotide release from Ssa1p (75), because GST-Sls1p had less effect in this assay (Fig. 5A; compare striped bars to grey bars). These results show that Fes1p is a nucleotide exchange factor for Ssa1p.

In order to validate our results and to gain more insight into the specificity of these cofactors, we performed similar assays with the *E. coli* DnaK protein and its nucleotide exchange factor GrpE, which lacks homology to Sls1p and Fes1p. We prepared a DnaK- $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ complex and performed nucleotide exchange assays as described for Ssa1p. As shown in Fig. 6A, GrpE induced the rapid release of nucleotide, consistent with previously published data (38, 42, 43), whereas Sls1p and Fes1p had no effect on the amount of nucleotide bound to DnaK (Fig. 6A; compare striped bars to grey and dotted bars). The thin-layer chromatography plate shows that GrpE quantitatively released ATP from DnaK (Fig. 6B). This result is consistent with previous findings that GrpE can release both ATP and ADP (9, 38, 42), although our preparation of the DnaK- $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ complex had insufficient specific activity that $[\alpha\text{-}^{32}\text{P}]\text{ADP}$ was not detected by thin-layer chromatography. As described previously (37), we also found that GrpE was unable to trigger nucleotide release from Ssa1p (data not shown). These and previous results (32) suggest that the Sls1p and Fes1p class of exchange factors, like GrpE, bind preferentially to the ADP-bound form of Hsp70s, but can release both ADP and ATP from their respective chaperones. Further experiments will be required to determine if one nucleotide is more efficiently and/or rapidly released than the other by Fes1p and/or Sls1p.

Effects of Fes1p on the ATPase activity of Ssa1p. We then examined the effects of the Fes1p and Sls1p nucleotide exchange factors on Ssa1p single-turnover ATPase activity (63). The Ssa1p- $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ complex was incubated at 30°C in the presence or absence of GST-Fes1p, GST-Sls1p, and Ydj1p, which enhances ATP hydrolysis (16, 18, 19, 44, 45). The extent of ATP hydrolyzed over time was assessed by thin-layer chromatography and PhosphorImager analysis (63), and the results are shown in Fig. 7A. Ssa1p alone displayed a weak ATPase activity, which was efficiently stimulated by Ydj1p. Interestingly, Fes1p significantly inhibited the ATPase activity of Ssa1p both in the presence and in the absence of Ydj1p, whereas Sls1p had only a limited effect, probably due to its poor interaction with Ssa1p (Fig. 4). As discussed in another study (39),



the observed inhibition of ATPase activity in single-turnover conditions is expected for a nucleotide exchange factor, since stripping the bound nucleotide will decrease the apparent rate of hydrolysis. Therefore, this result further supports the previous data showing that Fes1p is a nucleotide exchange factor for Ssa1p.

We next assessed the effect of Fes1p on the ATPase activity of Ssa1p under steady-state conditions, as it was previously shown that both GrpE and Sis1p stimulate the ATPase activity of their respective Hsp70s synergistically with J proteins (32, 38). Ssa1p was incubated for 10 min at 30°C in the presence of [α - 32 P]ATP and Ydj1p to allow ATPase activation. Then, following addition of either buffer or the indicated amounts of GST-Fes1p, ATP hydrolysis was monitored over time by thin-layer chromatography and PhosphorImager analysis (Fig. 7B). Surprisingly, Fes1p inhibited the steady-state ATPase activity of Ssa1p in a concentration-specific manner. Control experiments showed no effect of purified GST, to which no factor was fused, on the ATPase activity of Ssa1p (data not shown). It is important to state that although our nucleotide exchange experiments demonstrate that Fes1p is able to induce nucleotide dissociation from Ssa1p, they do not exclude the possibility that Fes1p also prevents nucleotide binding per se. In fact, inhibition of nucleotide binding by Fes1p could give rise to the observed effects on Ssa1p ATPase activity. For example, mammalian HspBP1 inhibits Hsp70 ATPase activity by preventing ATP binding (54). Regardless, we conclude that Fes1p has a negative effect on both steady-state and single-turnover Ssa1p ATPase activity.

Fes1p is dispensable for posttranslational protein translocation, ERAD, and luciferase refolding. Since Ssa1p is required for posttranslational protein translocation (5, 17, 20, 44), ERAD of the cystic fibrosis transmembrane conductance regulator in yeast (72), and protein folding (14, 36, 37), we examined whether Fes1p is involved in the same pathways using well-established *in vivo* and *in vitro* assays.

Mutations in *SSA1* affect the posttranslational translocation of pre-pro- α -factor (pp α F) (5, 17, 20, 44); therefore, we examined the Δ *fes1* strain for accumulation of pp α F. We also performed *in vitro* translocation assays (12) using microsomes purified from wild-type and Δ *fes1* strains. However, in neither case was a translocation defect apparent (data not shown). Next, cystic fibrosis transmembrane conductance regulator degradation was monitored in the Δ *fes1* strain by cycloheximide-chase analysis as described previously (71, 72), but no difference in the degradation rates of the Δ *fes1* mutant and of the wild type was observed (data not shown). We then performed

in vitro ERAD assays using purified microsomes and cytosol from both wild-type and mutant strains (46, 68). In these experiments, \sim 70% of the translocated Δ Gp α F was degraded in a cytosol- and ATP-dependent manner, regardless of the origin of the microsomes or cytosol (data not shown). Finally, we failed to detect any defects in Ssa1p-dependent refolding activity of luciferase *in vitro* when using wild-type or Δ *fes1* mutant cytosol, or upon addition of excess purified Fes1p (reference 14 and data not shown).

From these data, we conclude that Fes1p is not a cofactor for every aspect of Ssa1p function but might be involved in another specific pathway. By analogy, function-specific cofactors for Hsp70s have been observed with DnaJ homologues (50, 67, 70, 73).

Evidence for a function of Fes1p in protein translation. Many reports indicate the importance of molecular chaperones in protein translation. The Ssb1p and Ssb2p Hsp70s (49, 52) and Hsp40 family members (Sis1p, zuotin, Ydj1p [13, 70, 73]) associate with ribosomes and have been implicated in efficient protein synthesis or translation initiation (22). Similarly, Ssa1p was shown recently to be associated with ribosomes through its interaction with Sis1p, a cytosolic J protein, and Pab1p, the poly(A) binding protein (31). The thermosensitive *ssa1-45* mutant is defective for protein translation at high temperature by affecting the interaction of Pab1p with the initiation factor eIF4G (31).

To determine if deletion of *FES1* affected protein translation, we first assessed the sensitivity of wild-type and mutant strains to cycloheximide, an inhibitor of translation elongation, in a halo assay (for an example, see reference 51). As shown in Fig. 8A, the Δ *fes1* strain was more sensitive to cycloheximide than the wild-type control, as revealed by the formation of a larger halo of inhibition at 30°C. Interestingly, while the *ydj1-151* strain showed increased cycloheximide sensitivity at 35°C, as expected from former studies (13), the *ydj1-151* Δ *fes1* double mutant was almost indistinguishable from the wild-type strain at both temperatures, which might explain the suppressive effect of the *FES1* disruption on the *ydj1-151* thermosensitive growth (Fig. 2). It should be noted that the severe growth inhibition of the Δ *fes1* strain at 35°C probably results from both thermosensitivity and cycloheximide sensitivity. We were unable to observe significant differences in sensitivity between the wild-type and Δ *fes1* strains for anisomycin (data not shown), an inhibitor of peptidyltransferase activity, suggesting the specificity of Fes1p action in translation.

It was previously shown that the depletion of active Ssa1p affected protein translation, as the incubation of the *ssa1-45*

FIG. 8. Fes1p plays a role in protein translation. (A) The indicated strains were grown overnight in YPD at 26°C and then plated on the same media and grown for 8 h at 26°C. Cycloheximide (20 μ g) was inoculated in the middle of plates that were then incubated overnight at 30°C (top) or 35°C (bottom). (B) The indicated strains were grown at 26°C to mid-log phase and shifted to 37°C. At 0, 10, 30, and 60 min after incubation at 37°C, samples were taken and labeled with [35 S]-Express Labeling mix for 2 min at 30°C. Proteins were trichloroacetic acid precipitated and assayed by scintillation counting. Error bars represent the standard deviations from three independent experiments. (C) The indicated yeast strains were grown at 30°C and, when indicated, shifted for 30 min at 37°C. Cycloheximide was added to a final concentration of 50 μ g/ml, and cell lysates were prepared. Equal amounts of lysate (\sim 70 A_{254} U) were loaded onto 7 to 47% linear sucrose gradients and analyzed as described in Materials and Methods. The gradients were collected with continuous monitoring of A_{254} . The positions of the 40S, 60S, 80S, and polysome peaks are shown. (D) Cells expressing GFP-Fes1p were grown in galactose-containing medium until log phase, and cell lysates were prepared as described for panel C. Approximately 100 A_{254} U were loaded onto 7 to 47% linear sucrose gradients and processed as described for panel C. Aliquots corresponding to the lysate (lane 1), the cytosol (top of the gradient; lane 2), ribosome-free fractions (lane 3), 80S ribosomes (lane 4), and the polysomes (lane 5) were resolved by SDS-PAGE and analyzed by Western blotting using anti-GFP, anti-Ssa1p, and anti-L3 antisera.

mutant at various times at the nonpermissive temperature of 37°C progressively reduced the ability of this strain to incorporate [³⁵S]methionine into newly synthesized proteins (31). We recapitulated this experiment by measuring the incorporation of [³⁵S]methionine-cysteine into newly translated proteins following a 2-min pulse at 0, 10, 30, and 60 min after incubation of the wild-type and $\Delta fes1$ strains at 37°C. Whereas the wild-type strain displayed little change in [³⁵S]methionine-cysteine incorporation between 10 and 30 min, the translation rate decreased in the $\Delta fes1$ strain (Fig. 8B), similarly to what was observed for the *ssa1-45* mutant (31). To verify that this effect was not a result of cell death of the $\Delta fes1$ mutant at 37°C, aliquots were taken at each time point, plated on complete media, and incubated at the permissive temperature of 26°C. Approximately equal numbers of colonies were obtained with wild-type and mutant strains at each time point; moreover, growth curves at 37°C showed that the $\Delta fes1$ mutant kept dividing after 2 h of incubation at 37°C, although at this time point a reduced rate compared to that of the wild-type strain was observed. However, until this time, similar growth rates were apparent (data not shown). Therefore, we conclude that the observed decrease in translation rate is due to a defect associated with the disruption of the *FES1* gene.

Defects in protein translation may lead to changes in the ratio of free ribosomes to polysomes. The *ssa1-45* strain showed an increase of 80S ribosomes concomitant with a decrease in polysomes when shifted to the restrictive temperature (31). To determine whether deletion of *FES1* affected polysome profile, linear sucrose gradients (7 to 47%) were used to assess ribosome distribution in the wild-type and $\Delta fes1$ strains at 30°C and after a 30-min incubation at 37°C. As shown in Fig. 8C, the $\Delta fes1$ strain showed a slight increase in the 80S ribosomes relative to polysomes compared to the wild type at 30°C. At 37°C, the increase in 80S ribosomes was more pronounced (Fig. 8C; compare the 80S peak to the first polysome peak). The polysome profile for the wild-type strain after 30 min of incubation at 37°C was undistinguishable from that obtained at 30°C (data not shown).

To examine whether Fes1p is polysome associated, lysates prepared from the *GFP-FES1* strain were fractionated through the sucrose gradient and proteins corresponding to the lysate (Fig. 8D, lane 1), the nonribosomal cytosolic fraction (top of the gradient, lane 2), the position between cytosol- and ribosome-containing fractions (lane 3), the 80S ribosomes (lane 4), and polysomes (lane 5) were separated by SDS-PAGE and analyzed by Western blotting. As expected, the L3 large subunit ribosomal protein was found in the lysate and ribosomal fractions and was excluded from soluble cytosolic and preribosomal fractions. Also, as published previously, Ssa1p was found in the 80S and polysome fractions (31). Finally, we identified Fes1p in these same fractions. These data are consistent with a role of Fes1p in protein translation and with its interaction with Ssa1p.

DISCUSSION

The work described here establishes that Fes1p, encoded by yBR101c, a previously uncharacterized yeast ORF, functions as a nucleotide exchange factor for the Hsp70 molecular chaperone Ssa1p. This protein was identified based on its homology

to Sls1p, an ER protein previously shown to act as a nucleotide exchange factor for yeast BiP (32). Even though Fes1p is shorter than Sls1p, which is in part due to the absence of any targeting sequence or retention motif, it shares important features with the latter. The regions that are conserved between *S. cerevisiae* and *Y. lipolytica* Sls1p proteins are also found in Fes1p, whereas regions of weak homology are absent or are not conserved in Fes1p (Fig. 1). These proteins are also similar in the way they interact with their respective Hsp70 partner. Indeed, Sls1p was shown to preferentially interact with the ADP-bound form of Kar2p (32) and, using GST pull-down assays, we show here that Fes1p also preferentially interacts with the ADP-bound form of Ssa1p (Fig. 4). Both proteins release bound nucleotide from their respective Hsp70 chaperone partners specifically (Fig. 5) (32), as Sls1p could not substitute for Fes1p in the exchange assays. Moreover, whereas GrpE efficiently promoted nucleotide exchange on DnaK (38), thereby validating our experimental model, Fes1p and Sls1p were inactive (Fig. 6). Conversely, GrpE was unable to promote nucleotide release from Ssa1p (reference 37 and data not shown). Such specificity between chaperones and their cofactors has been observed for J protein-Hsp70 interactions (5, 11, 18, 35, 41, 45, 69). Because Hsp70 can be classified in several subclasses depending on the presence or absence of specific structural features within the highly conserved ATPase domain (9), it is reasonable to think that at least in some cases each chaperone and its cofactor(s) have evolved to fit one another. Structural analysis of several exchange factor-Hsp70 pairs would afford insights into this question and in defining the different mechanisms of nucleotide exchange, as GrpE, Bag-1, and Sls1p and Fes1p are unrelated.

The nucleotide exchange factors of the GrpE family can stimulate synergistically the ATPase activity of Hsp70s in cooperation with J proteins. For example, *E. coli* GrpE and DnaJ each stimulate the DnaK ATPase activity ~2-fold and ~3- to 13-fold, respectively (37, 38, 43), but up to ~50-fold when added together in steady-state assays (38). Similarly, we showed that Sls1p synergistically stimulates the ATPase activity of Kar2p in the presence of Sec63p (32) and Jem1p (our unpublished results). Surprisingly, we found that Fes1p inhibited the steady-state ATPase activity of Ssa1p in a concentration-dependent manner (Fig. 7B). The GrpE protein was shown to act negatively on DnaK when added in excess (53), and recently, it was shown that Bag-1 positively or negatively modulates Hsp70 and Hsc70 refolding activity depending on its concentration (24). However, the inhibition of Ssa1p ATPase activity by Fes1p was observed even at substoichiometric concentrations of the cofactor, suggesting that Fes1p has evolved as a negative regulator of the Hsp70 chaperone. Similar negative regulation was observed with HspBP1, which inhibits ATP binding to Hsc70 (54). In addition, the Hsp70-interacting protein, HiP, regulates Hsp70 ATPase activity by stabilizing the ADP-Hsp70-peptide complex, which in turn promotes efficient folding of the substrate (30).

The $\Delta fes1$ mutant was viable at the permissive temperature but not at high temperature (Fig. 2), suggesting that this protein is important under conditions of elevated metabolism and stress. Using a functional GFP-tagged version of Fes1p, fluorescence microscopy, and subcellular fraction-

ation, we were able to show that Fes1p is a soluble cytosolic protein (Fig. 3).

The $\Delta fes1$ mutant was proficient for protein translocation, ERAD, and protein folding, which are established Ssa1p-dependent processes (14, 17, 20, 36, 41, 44). Instead, Fes1p facilitates protein translation, a process in which both Ssa1p and Ydj1p have been shown to play a role (13, 31). Indeed, increased cycloheximide sensitivity is observed upon disruption of *FES1* (Fig. 8A) and, as described for Ssa1p (31), a general translational defect is observed when the $\Delta fes1$ mutant is incubated at high temperature (Fig. 8B). Interestingly, the cycloheximide sensitivity is suppressed in the *ydj1-151* $\Delta fes1$ double mutant, which is consistent with the suppression of the thermosensitive defect (Fig. 2). This suggests that Fes1p and Ydj1p play antagonist functions during translation, consistent with their respective negative and positive regulation of Ssa1p ATPase activity. Another important finding was that the absence of Fes1p leads to a slight decrease in polysomes and an increase in 80S ribosomes, a defect enhanced at high temperature (Fig. 8C). A similar phenotype was also observed with the *ssa1-45* strain (31). Furthermore, Fes1p was found to be associated with 80S and polysome fractions, as was Ssa1p (Fig. 8D). Also, in support of a role of Fes1p in translation, the recent construction of a yeast proteome-wide interaction map indicated that Fes1p interacted with Tif1p, the yeast homologue of mammalian eIF4A, and with Hsp42, a small heat shock protein (25). Finally, Mutka and Walter found that the expression of *FES1* was transiently activated during the cellular adaptation to the loss of the signal recognition particle (see Fig. 4 in reference 48). Since Fes1p does not seem to be involved in posttranslational protein translocation, its induction may alter the translation rate that is observed during the cellular adaptation to loss of cotranslational translocation (48). Although the deletion of Ydj1p did not significantly affect the adaptation (48), it will be interesting to examine if Fes1p and Ydj1p compete for binding to Ssa1p and/or if another protein (Sis1p or Pab1p, perhaps) or substrate regulates the temporal interaction of Ssa1p with its many partners. For example, Ssa1p is necessary for the association between Pab1p and initiation factor eIF4G, an association that is thought to be important for cap-dependent translation initiation (31). Although the sequential interaction of Ssa1p with Sis1p and Pab1p, as well as yet-unidentified substrates on translating ribosomes, is not well understood, an attractive hypothesis would be that Fes1p participates in the regulation and proper coordination of these different interactions.

Several homologues of Sls1p and Fes1p have been identified in the genome databases, with homologues in the human, mouse, *Drosophila melanogaster*, *Schizosaccharomyces pombe*, and *Candida albicans* genomes (M. Kabani, J.-M. Beckerich, and J. L. Brodsky, unpublished data). These proteins can be separated into two subclasses, one corresponding to ER Sls1p-related proteins, and the other to the cytosolic Fes1p-related proteins; each subclass displays the similarities and differences that are found between Sls1p and Fes1p (see above). While these proteins share features that might be important for binding with the same domain of their Hsp70 counterpart and promote nucleotide release, structural differences might be

responsible for the different effects observed in steady-state ATPase assays. Clearly, much more work is warranted on this new class of chaperone modulators.

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