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SSB, Encoding a Ribosome-Associated Chaperone, Is Coordinately Regulated with Ribosomal Protein Genes

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Genes encoding ribosomal proteins and other components of the translational apparatus are coregulated to efficiently adjust the protein synthetic capacity of the cell. Ssb, a *Saccharomyces cerevisiae* Hsp70 cytosolic molecular chaperone, is associated with the ribosome-nascent chain complex. To determine whether this chaperone is coregulated with ribosomal proteins, we studied the mRNA regulation of *SSB* under several environmental conditions. Ssb and the ribosomal protein rpL5 mRNAs were up-regulated upon carbon upshift and down-regulated upon amino acid limitation, unlike the mRNA of another cytosolic Hsp70, Ssa. Ribosomal protein and Ssb mRNAs, like many mRNAs, are down-regulated upon a rapid temperature upshift. The mRNA reduction of several ribosomal protein genes and Ssb was delayed by the presence of an allele, *EXA3-1*, of the gene encoding the heat shock factor (HSF). However, upon a heat shock the *EXA3-1* mutation did not significantly alter the reduction in the mRNA levels of two genes encoding proteins unrelated to the translational apparatus. Analysis of gene fusions indicated that the transcribed region, but not the promoter of *SSB*, is sufficient for this HSF-dependent regulation. Our studies suggest that Ssb is regulated like a core component of the ribosome and that HSF is required for proper regulation of *SSB* and ribosomal mRNA after a temperature upshift.

Molecular chaperones of the heat shock protein 70-kDa (Hsp70) class are highly conserved proteins that bind unfolded polypeptides, preventing nonproductive interactions that can lead to misfolding or protein aggregation. Hsp70s are composed of three domains: a conserved 44-kDa ATPase segment, an 18-kDa domain which is the binding site for unfolded polypeptides, and a 10-kDa variable region at the C terminus. A variety of cellular processes such as protein synthesis, protein folding, and polypeptide translocation across organellar membranes are assisted by these molecular chaperones (5).

The budding yeast *Saccharomyces cerevisiae* contains two major classes of cytosolic Hsp70 chaperones, Ssa and Ssb (3). This report focuses on the ribosome-associated chaperone, Ssb. The *SSB* Hsp70 family is composed of two genes, *SSB1* and *SSB2*. In contrast to *SSA* genes, *SSB* genes are not heat inducible; in fact, their expression is reduced after a heat shock. The *SSB*-encoded proteins have greater than 99% identity. In this paper, both *SSB* genes will be collectively referred to as *SSB* unless specified otherwise. Strains containing gene disruptions for both *SSB* genes are hypersensitive to certain translation inhibitors and are cold sensitive (24). These two phenotypes are completely suppressed by one copy of either of the *SSB* genes but not by a constitutive overexpression of the heat-inducible *SSA* genes (4). Ssb associates with translating ribosomes and can be cross-linked to the nascent polypeptide chain (24, 28). Its association with translating ribosomes is resistant to treatment with high concentrations of salt, implying that Ssb associates with the ribosome like a core component of this apparatus.

Genes encoding many components of the translational machinery have a coordinated regulation in response to environmental changes even though they are dispersed throughout the genome (9). Cells increase or decrease their ribosomal protein (RP) mRNA pools based on growth conditions to accommodate their needs for protein synthetic capacity. For example, upon a carbon upshift (i.e., when glucose is added to a culture growing on a poor carbon source such as ethanol or glycerol) the mRNA levels for RP genes increase. Upon amino acid limitation, cells elicit a response known as stringent control which induces transcription of amino acid biosynthetic genes and reduces the mRNA levels of RP genes (37). A promoter sequence found in some RP genes, known as the RPG box, is required to regulate transcription of RP genes upon a carbon upshift and amino acid limitation. This promoter sequence is the binding site for the transcriptional regulator Rap1 (6, 13, 22, 25).

While expression of RP genes, *SSB*, and many other genes is decreased, a set of genes called heat shock genes is induced upon temperature upshift (21). It has been reported that both transcription and mRNA stability of RP genes are reduced after a heat shock (12, 16), but sequences required for this regulation have not been identified. In contrast, the induction of heat shock genes has been well characterized. The transcriptional activator of many heat-inducible genes, the heat shock factor (HSF), is a homotrimer that binds sequences known as heat shock elements (HSEs) in the promoters of heat-inducible genes (23). Upon an increase in temperature, HSF is activated, resulting in augmentation of transcription from HSE-containing promoters. The yeast HSF monomer is composed of four domains: an N-terminal activation domain, a DNA binding domain, an oligomerization domain, and a C-terminal activation domain. One HSF allele, *EXA3-1*, has a single base substitution in the DNA binding domain that changes the proline at position 214 to glutamine (10). This mutation reduces the ability of HSF to bind to the HSE, resulting in a delay in the induction of heat shock genes upon a temperature upshift.

Unlike heat-inducible proteins, RPs are coordinately expressed to allow the cell to vary ribosome abundance depending on its needs for protein synthesis. Ssb, while a member of a heat shock family of proteins, is a component of translating

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ribosomes. To better understand Ssb's regulation, we analyzed *SSB* mRNA levels under three different growth conditions (carbon upshift, amino acid starvation, and heat shock) known to affect RP gene expression. We report that *SSB* and RP genes are regulated in a coordinated manner. In addition, to elucidate components of the heat shock regulation of *SSB* and RP mRNA, we analyzed mRNA levels in cells containing a defective heat shock response due to the presence of the *EXA3-1* mutation. Our results indicate that the negative regulation of RP and *SSB* mRNAs after a heat shock is HSF dependent.

MATERIALS AND METHODS

S. cerevisiae* strains.** With the exception of F113 (21), yeast strains used in this study have the following genotype: *ura3-52 lys1 lys2 trp1-Δ1 his3-11,15 leu2-3,112*. The F113 genotype is *MATa** *can1 ino1-13 ura3-52* (22). The wild-type strains were DS10 (*MAT***a**) and JH27A (*MAT*α). The following strains carry the additional alleles in parentheses: NL113 (*ssb1:LEU2* and *ssb2:HIS3*), MH297 (*EXA3-1*) (10), and NL95 (*ssb1:LEU2 ssb2:HIS3 EXA3-1 URA3*). The *EXA3-1* allele was introduced into cells containing *ssb1 ssb2* disrupted by mating an *ssb1:LEU2 ssb2:HIS3* strain (JN208) (24) with an *EXA3-1* strain (MH297) in which the *EXA3-1* allele was genetically marked by the *URA3* gene. The resulting haploids (NL95 and NL113) were confirmed by marker segregation and by using Northern blot analysis to measure the transcript levels of an internal control *RPL11* (previously *RPL16A*) in a wild-type strain and the *EXA3-1* strain after a heat shock.

Bacterial strains, transformations, plasmids, and gene fusion constructions. DH5α was the preferred *Escherichia coli* strain for general cloning procedures [genotype: ϕ*80dlacZΔM15 endA1 recA1 hsdR17* (r_K^- m_K^+) *supE44 thi-1* λ$^-$ *gyrA relA1* F$^-$ Δ*lacZYA-argF*]. *E. coli* cells were transformed by $CaCl_2$ procedures (20), and yeast strains were transformed by the lithium acetate procedure described elsewhere (8). Plasmids used in this study include pRS313-U2 (generously provided by Warren Heideman [27]), YEpCUP1-HSE-M-lacZ (a gift from Dennis Thiele [35]), pSSB-URA3, pEC302, pJHSSB1P, and pCUP-SSB1. To construct pSSB-URA3, the 5′ untranslated region and promoter of the *SSB1* gene were isolated from the plasmid pEC302 as a 617-bp *Eco*RI-*Xba*I fragment. This fragment includes DNA sequences from the polylinker which encode the restriction sites for *Xba*I and *Sal*I. The fragment was cloned directionally into pUC18 digested with *Eco*RI and *Xba*I to create pJHSSB1P. The 962-bp *Pst*I-*Hin*dIII fragment from YEp24 containing the *URA3* coding region and 19 nucleotides from the 5′-end untranslated region was inserted into pJHSSB1P digested with *Pst*I and *Hin*dIII to create pJHSSB1P-URA3. This construct contained the *SSB1* promoter fused to the coding region of the *URA3* gene. The *SSB-URA* fusion gene was cloned into the yeast centromeric pRS314 by removing the insert from pJHSSB1P-URA3 by *Hin*dIII and *Eco*RI digestion. This fragment was inserted directionally into pRS314 digested with *Eco*RI and *Bam*HI (filled in) to create pSSB-URA3.

To construct pCUP1-SSB, a modified version of the *CUP1* promoter designated *CUP1* hse-m and the first half of the *SSB1* gene were amplified by two separate PCRs. The *CUP1* hse-m promoter was amplified from plasmid YEpCUP1-HSE-M-lacZ with two oligonucleotides: CupSSBa (TTT TCT CGA GCG AGA TGA AAT GAA TAG C) and CupSSBb (CTG TAA TGA TCC TAT ATG ATA TTG CAC TAA C). The amplified first half of the *SSB1* gene started from the first guanine nucleotide in the transcript and ended at the *Bgl*II site in the middle of the gene. This *SSB1* fragment was amplified with the following oligonucleotides: CupSSBc (GCA ATA TCA TAT AGG ATC ATT ACA GTA TTT TAA TTG) and CupSSBd (CTT CGT CGA TTT GAG AC). The two PCR products were fused to each other by PCR-mediated overlapping extension (14), and the resulting 2.6-kb PCR fragment placed the first guanine nucleotide of the *SSB1* transcript immediately after the first start of transcription found in the *CUP1* hse-m promoter. The 2.6-kb PCR fragment has two restriction sites at the ends (*Xho*I and *Bgl*II) that were used to digest and subclone it into a 6.5-kb *Xho*I-*Bgl*II-opened pRS314-SSB1 vector. This 6.5-kb *Xho*I-*Bgl*II pRS314-SSB1 fragment provided the other half of the *SSB1* gene to the Cup1-SSB1 fusion after the *Bgl*II site. This plasmid was used to transform NL113 (*ssb1 ssb2*) and NL95 (*ssb1 ssb2 EXA3-1*) strains. This gene fusion was functional, as it completely suppressed the *ssb1 ssb2* mutant phenotypes of cold sensitivity and hypersensitivity to translation inhibitors. In addition, transcript levels from this gene fusion increased upon addition of copper (data not shown). The analysis of this gene fusion did not require addition of copper to the medium due to appropriate basal expression under our growth conditions.

Chemicals. Yeast extract, peptone, and yeast nitrogen base without amino acids were from Difco Laboratories (Detroit, Mich.); dextrose and 3-amino-1,2,4-triazole (3AT) were from Sigma Chemical Co. (St. Louis, Mo.); SeaKem agarose was from FMC Corp. (Rockland, Maine).

Growth conditions. The carbon upshift was performed by growing cells in glycerol-based medium (YPG [1% yeast extract, 2% peptone, 5% glycerol]) to exponential growth (A_{600} between 0.4 and 0.8) and then adding glucose to the culture to a final concentration of 2%. For amino acid starvation, 10 mM 3AT was added to exponentially growing cells cultured in SD minimal medium (29). The heat shock response was elicited by growing cells on YPD (1% yeast extract, 2% peptone, 2% dextrose) rich medium at 23°C and then shifting the medium to 39°C. The temperature upshift for stable messages (i.e., 50% reduction of basal levels takes longer than 5 min) was done by moving a 25-ml aliquot of the cell suspension to a 39°C prewarmed 250-ml flask. For short-half-life mRNAs such as that for *URA3*, the temperature upshift was done by mixing approximately 10 ml of prewarmed YPD medium at 68°C into a 15-ml culture growing at 23°C and placing it in a 39°C water bath. In each condition, aliquots of the cell suspension were taken at times indicated and total RNA was prepared from pelleted cells by the heat-freeze method (31).

Northern (RNA) blot analysis and primer extension analysis. Northern blot analysis was done by separating 4 to 10 μg of total RNA in a 1% agarose-formaldehyde gel, transferring the gel to a nylon membrane, and hybridizing it with radiolabeled probes (specific activity ≈ 10^7 cpm/μg) made by random priming (2). Probes were generated with [α-^{32}P]dCTP (3,000 Ci/mmol) (DuPont NEN). After stringent washes, filters were exposed to a detection screen with a PhosphorImager, and the signal was quantified with the ImageQuant software package (Molecular Dynamics). Differences in RNA loading on Northern blots were normalized with indicated loading controls on each experiment. rRNA was used to normalize loaded amounts of RNA in heat shock experiments since even the levels of relatively stable transcripts decrease significantly during the time course of the experiment, making quantitative analysis difficult. The validity of this method has been confirmed elsewhere (10).

For primer extension analysis, a 20-mer oligonucleotide (5′-GAT AGC ACC TTG GAA AAC AC-3′) complementary to the 5′ ends of the *SSB1* and *SSB2* coding regions was used to prime cDNA synthesis. To normalize amounts of RNA used per reaction, a primer with sequence complementary to the small nuclear RNA (snRNA) U4 (CGG ACG AAT CCT CAC TGA TAT GC) was included on each reaction. Gel-purified oligonucleotides were radiolabeled at the 5′ end with T4 polynucleotide kinase and [γ-^{32}P]ATP (3,000 Ci/mmol) (DuPont NEN) (2). Radiolabeled primers were hybridized to 20 to 30 μg of total RNA at 90°C for 3 min and then quickly chilled on ice. Annealed primers were extended with avian myeloblastosis virus reverse transcriptase (Promega, Madison, Wis.), and primer extension products were separated with 6% polyacrylamide gels. Signals were visualized with a PhosphorImager and quantified with the ImageQuant software package.

RP nomenclature. RPs of *S. cerevisiae* have been named in this work according to the new nomenclature described in the work of Mager et al. (18).

RESULTS

***SSB* mRNA levels increase upon a carbon upshift.** Most RP genes possess a transcriptional regulatory sequence designated an RPG box (CCC ATA CAT CT) (39, 40) and a T-rich region important for constitutive expression (30). Analysis of the *SSB* promoter sequence indicates that both genes possess a T-rich region; the *SSB1* promoter also has a putative RPG box centered at 261 bases upstream of the adenine of the start codon with the sequence CCC ATA CAC CG. These common structural features and the fact that both are components of ribosomes suggested to us that *SSB* and RP gene regulation may be coordinated.

It has been observed that mRNA levels of RPs rapidly increase by more than twofold upon a carbon upshift (13, 15). We analyzed *SSB* mRNA levels by Northern blot analysis after supplying glucose to wild-type cells growing in glycerol-based medium. As shown in Fig. 1, *SSB* mRNA levels increased by more than twofold within 30 min of carbon upshift, similar to the mRNA increase of the RP gene *RPL5* (previously called L1a). In contrast, the mRNA levels of genes encoding another cytosolic chaperone, Ssa, did not increase but rather transiently decreased upon glucose addition.

The *SSB* chaperone family is composed of two genes, *SSB1* and *SSB2*, which are more than 90% identical. To determine whether both *SSB* transcripts increase upon a carbon upshift, we analyzed the mRNA levels of both genes in wild-type cells. Primer extension analysis was used to distinguish between the two transcripts. We designed an oligonucleotide complementary to the 5′ end of the *SSB* coding sequence. Due to the complete sequence identity of *SSB1* and *SSB2* in this region, both *SSB* transcripts are able to efficiently serve as templates during a primer extension reaction. As shown in Fig. 2A, the

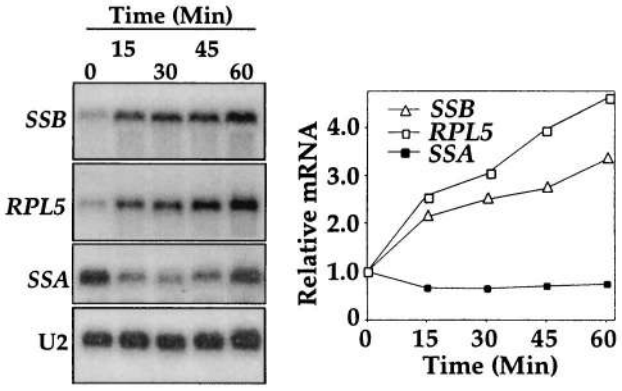

FIG. 1. mRNA levels of *SSB* genes upon a carbon upshift. At time zero, glucose was added to wild-type cells growing in glycerol-based medium. At left, signals from Northern blotting of several transcripts upon a carbon upshift are shown. The graph at right shows quantification of the signals after normalization to snRNA U2 levels.

analysis of the *SSB* mRNA species in a wild-type strain resulted in two predominant bands (lane 2) representing products extending 29 and 22 bases beyond the initiation codon ATG. It seemed likely that each band represented the transcript from a single *SSB* gene, but it was unclear which band was the extension product of which mRNA. We reasoned that increasing the gene dosage of either *SSB1* or *SSB2* would result in higher amounts of the respective mRNA than that expressed from a single chromosomal copy. Consequently, we overexpressed *SSB1* or *SSB2* by supplying wild-type cells with a high-copy-number plasmid containing either *SSB1* or *SSB2*. Overexpression of *SSB1* resulted in enhanced levels of the upper band, while overexpression of *SSB2* caused an increase in the signal of the lower band (Fig. 2A, lanes 1 and 3, respectively). Interestingly, overexpression of the *SSB1* gene appears to decrease *SSB2* mRNA levels, although the reverse does not appear to be true. We conclude that the upper band is an extension of the *SSB1* gene and that the lower band is an extension of the *SSB2* gene. This experiment localized the 5′ end of the *SSB1* and *SSB2* genes to 29 and 22 nucleotides, respectively, upstream from the A of the initiating ATG codon (Fig. 2B).

The individual increase in mRNA levels of *SSB1* and *SSB2* after a carbon upshift was studied by primer extension analysis with the same oligonucleotide that was used to localize the start site of transcription of these RNAs. We also included in the reactions a primer that complements sequences of the U4 snRNA to normalize amounts of RNA used in reactions. Both *SSB1* and *SSB2* mRNA species increased upon a carbon upshift, although *SSB1* mRNA increased to a greater extent than did *SSB2* mRNA (Fig. 2C).

The stringent control response results in a coordinated reduction in the mRNA levels of *SSB* and RP genes. Amino acid starvation elicits a cellular response known as stringent control resulting in the transcriptional activation of amino acid biosynthetic genes and reduction in the expression of RP genes (37). We hypothesized that if Ssb is a component of the ribosome it would have a pattern of regulation similar to that of RP genes not only under carbon upshift but under other nutritional conditions as well. We expanded the analysis of *SSB* mRNA regulation by studying its mRNA levels after amino acid starvation. The stringent response was induced by adding 3AT, a competitive inhibitor of an enzyme required for histidine biosynthesis (22), to cells growing in minimal medium. Total RNA was prepared from aliquots of the culture taken at various times after 3AT addition. Northern blot analysis was used to quantify mRNA levels of *SSB* and the RP gene *RPL5*. As negative controls, we analyzed mRNAs of two genes whose products do not function in translation: *SSA*, which encodes another cytosolic Hsp70 chaperone, and *HHO1*, the histone

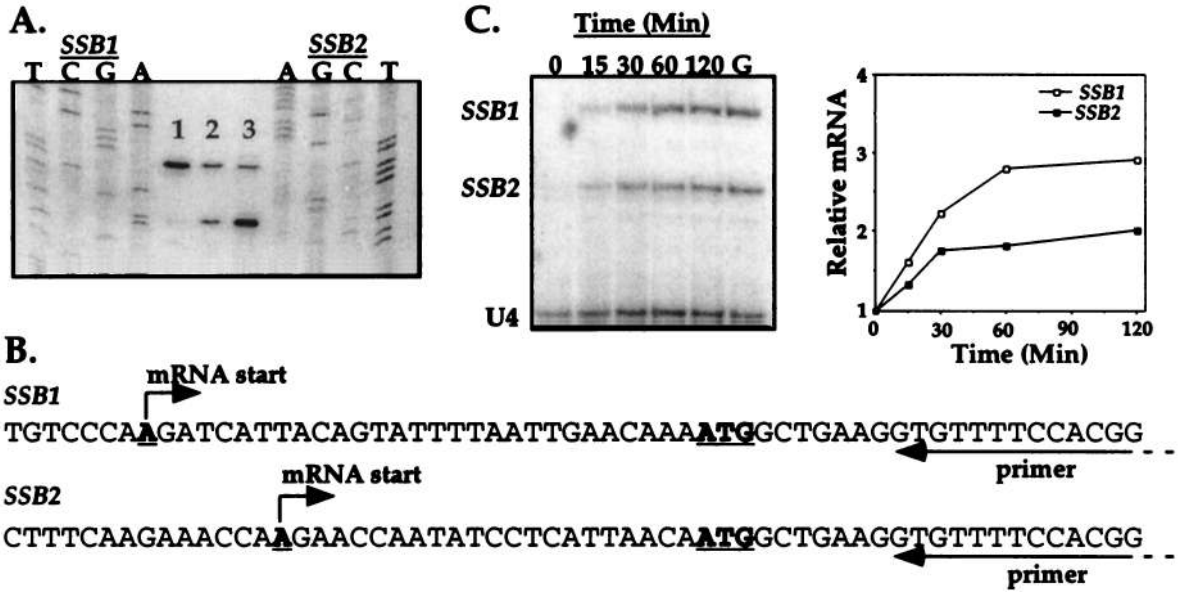

FIG. 2. Localization of the 5′ ends of the *SSB1* and *SSB2* genes and analysis of their mRNA levels upon a carbon upshift. (A) Primer extension reactions are shown in the center: lane 1, strain overexpressing the *SSB1* gene; lane 2, wild-type cells; lane 3, strain overexpressing the *SSB2* gene. Sequencing reactions are shown on the left for the *SSB1* gene and on the right for the *SSB2* gene. (B) Sequences from the *SSB1* and *SSB2* genes showing the start site of transcription relative to the initiation ATG codon. Sequences complementary to the primer are located above the arrow labeled as primer. (C) mRNA levels of *SSB1* and *SSB2* genes after a carbon upshift. Samples of the culture were collected at the indicated times after glucose addition. At left is shown a sequencing gel which separates the primer extension products. Lane G shows results from cells grown on glucose-based medium. The graph shows the quantification of the signal obtained after normalization to snRNA U4.

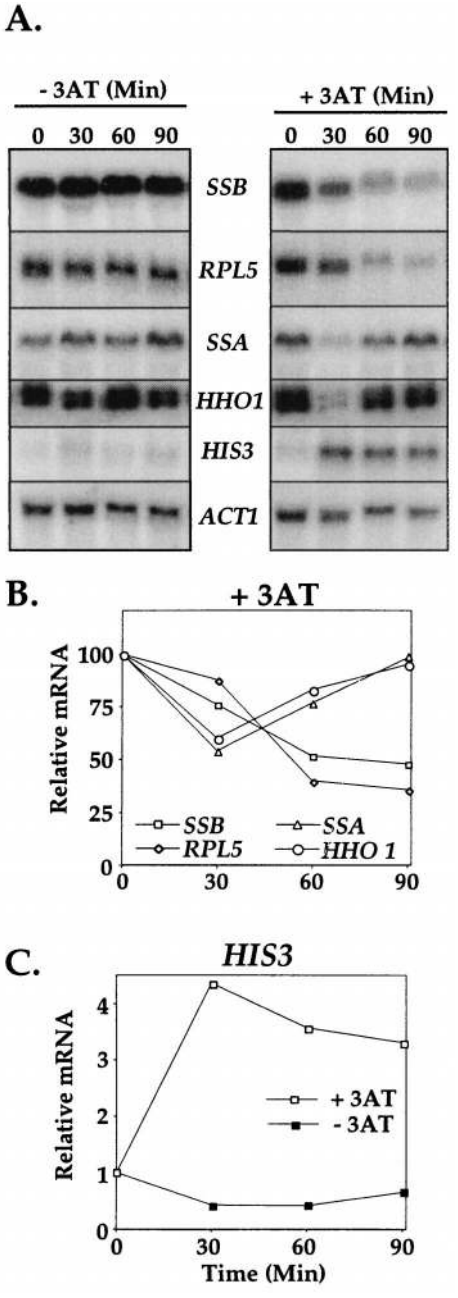

FIG. 3. mRNA levels of various transcripts after amino acid limitation. 3AT was added to cells (strain F113) growing in SD minimal medium at time zero. RNA samples were analyzed by Northern blotting. (A) (Left) Control from cells that had no 3AT (−3AT) treatment. (Right) Signals obtained from cells treated with 3AT. Genes analyzed were *SSB*, *RPL5* (RP), *SSA* (a yeast cytosolic Hsp70), and *HHO1* (histone H1). *HIS3* was used as a control to detect the efficiency of the treatment. (B and C) Quantification of the mRNA levels in the presence of 3AT (+3AT) and of *HIS3* mRNA with (+3AT) and without (−3AT) 3AT, as shown in panel A, after normalization to actin transcript levels.

H1 gene. *RPL5* and *SSB* mRNA levels gradually decreased, dropping below 50% of the control levels by 90 min after addition of 3AT. We observed a rapid and transient decrease of *SSA* and histone H1 mRNAs 30 min after 3AT addition with a return to basal levels by 90 min (Fig. 3). To examine the effectiveness of the 3AT treatment, we measured the mRNA levels of *HIS3*, a histidine biosynthetic gene. As we expected, after amino acid limitation the mRNA levels of *HIS3* increased up to fourfold. Thus, we conclude that *SSB* is regulated in coordination with RP genes during amino acid starvation, while the gene encoding the other cytosolic Hsp70, Ssa, is not.

***EXA3-1*, an allele of HSF1 which encodes the yeast HSF, causes a delay in mRNA reduction of *SSB* and RP genes upon a heat shock.** As shown previously, the mRNA levels of RP genes and *SSB* decrease drastically upon a heat shock in a wild-type strain (16, 38). However, the cause of the negative regulation is not known. Since induction of many heat shock genes is dependent on the transcriptional activator HSF, we asked whether HSF activity has an effect on the negative regulation of *SSB* and RP genes. Therefore, we analyzed the heat shock regulation of several mRNAs in a mutant strain (MH297) containing the *EXA3-1* HSF allele. Cells were grown in rich medium to mid-log phase at 23°C and then shifted to 39°C. Aliquots of the culture were taken at the indicated times, and mRNA levels were quantified by Northern blot analysis. The *SSB* mRNA reduction was much less rapid in MH297 than in a wild-type strain (Fig. 4). For example, after 20 min of a heat shock the *SSB* mRNA levels in wild type were reduced by greater than 75% while the levels in the MH297 strain were reduced by less than 20%. A similar delay in the reduction of mRNA levels was seen for the RP mRNAs of *RPL5*, *RPL11*, *RPS14* (previously known as *CRY1* and *CRY2*), and *RPS17* (formerly known as *RP51*) (Fig. 4 and data not shown).

It is known that most mRNA species are reduced in abundance upon a heat shock. To determine whether *EXA3-1* has a global effect on the negative regulation of genes after a heat shock, we tested the effect of the mutation on the mRNA levels of two genes whose products are unrelated to the protein synthesis process. *URA3* encodes a biosynthetic enzyme required for uracil synthesis; *MAT*α encodes the mating pheromone α-factor. Little or no difference was observed in the rate of decrease of either of these two mRNAs upon a heat shock (Fig. 5), indicating that *EXA3-1* does not have a global effect on the regulation of all mRNAs upon temperature upshift.

The *SSB* promoter is not sufficient to decrease its mRNA levels in an HSF-dependent manner. To aid in understanding the regulation of mRNA levels by HSF, we wanted to determine the sequences in the *SSB* gene essential for this regulation. To test the sufficiency of the *SSB1* promoter, we utilized an *SSB1:URA3* engineered fusion to analyze the *SSB1* promoter contribution to the *URA3* mRNA regulation in the wild type and the MH297 mutant strain. This gene fusion expressed a functional protein that allowed growth of cells lacking a functional genomic *URA3* gene in medium lacking uracil (data not shown). Since the *ura3-52* genomic allele in this strain background does not encode detectable transcript (data not shown), we were assured that *URA3* mRNA detected in our experiments was expressed from the *SSB1* promoter. *URA3* was appropriate for this analysis because the decrease of its mRNA after heat shock is unaffected by *EXA3-1* mutation (Fig. 5B).

Wild-type and MH297 strains were transformed with pSSB-URA. As shown in Fig. 6, the *EXA3-1* mutation in MH297 did not have an obvious effect on the reduction of *URA3* mRNA produced from the *SSB1:URA3* gene fusion after a temperature upshift. In contrast, the internal control of the RP transcript encoded by the chromosomal copy of *RPL11* showed a significant delay in its rapid reduction in the MH297 strain. These results indicate that the *SSB* promoter is not sufficient to regulate its mRNA after heat shock in an HSF-dependent manner.

Sequences within the transcribed region of *SSB* are sufficient to regulate *SSB* transcript levels after a heat shock in an HSF-dependent manner. Since the promoter of *SSB1* was not

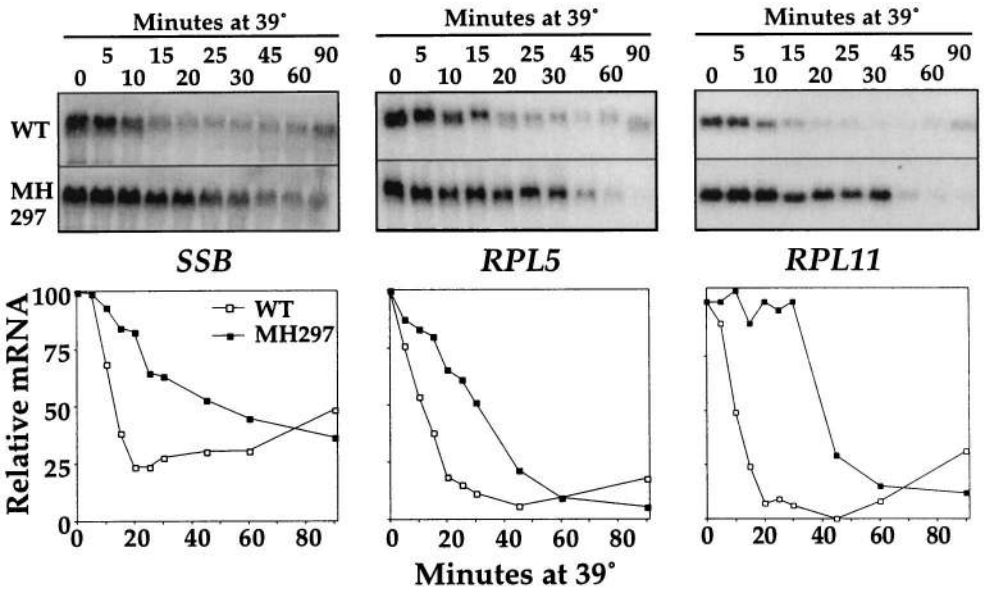

FIG. 4. mRNA levels of ribosomal components upon a heat shock in the wild-type (WT) strain and in cells containing the *EXA3-1* mutation. JH27A (wild-type) and MH297 (*EXA3-1*) cells growing in YPD medium at 23°C were rapidly shifted to 39°C. Aliquots of the culture were collected at the times indicated after the temperature upshift, and extracted RNA was subjected to Northern blot analysis. (Top) Northern blot images. (Bottom) Quantification after normalization to rRNA (see Materials and Methods).

sufficient for the HSF-dependent *SSB* mRNA regulation upon a heat shock, we tested whether the transcribed region of the *SSB1* gene possesses sequences sufficient for such regulation. We designed a gene fusion that contained the entire *SSB1* transcribed region downstream of the *CUP1* promoter (Fig. 7). Since the native *CUP1* promoter is induced by a heat shock through the action of HSF, we used a *CUP1* hse-m promoter which has no functional interaction with HSF (*Cup1* hse-m) (35). Therefore, transcription from this promoter is not heat induced (data not shown). The fusion was created in such a way that the start of transcription of *CUP1* hse-m is the first nucleotide in the *SSB1* transcript. This *CUP1:SSB1* fusion suppressed both phenotypes (cold sensitivity and hypersensitivity

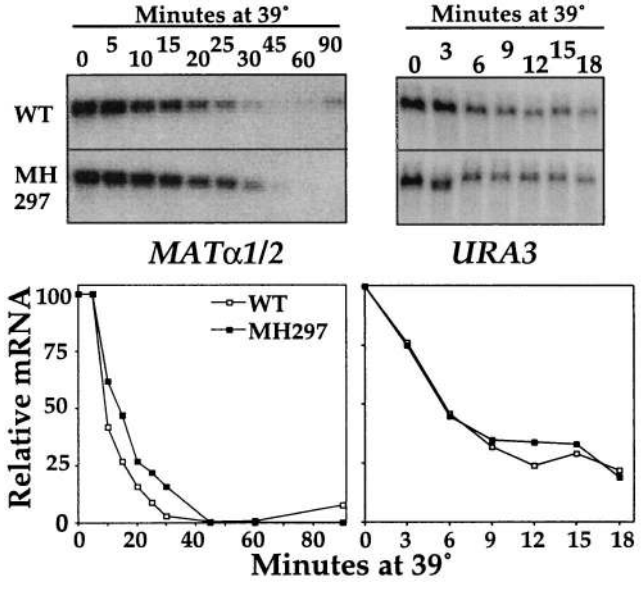

FIG. 5. Levels of *MATα1/2* and *URA3* transcripts upon a heat shock in the wild-type (WT) (JH27A) strain and in cells containing the *EXA3-1* mutation (MH297). Experiments were performed as described for Fig. 4. In the case of the short-half-life message *URA3*, samples were taken at 0 to 18 min after a temperature upshift (see Materials and Methods). (Top) Northern blot images. (bottom) Graphs showing the quantification of signal after normalization to rRNA.

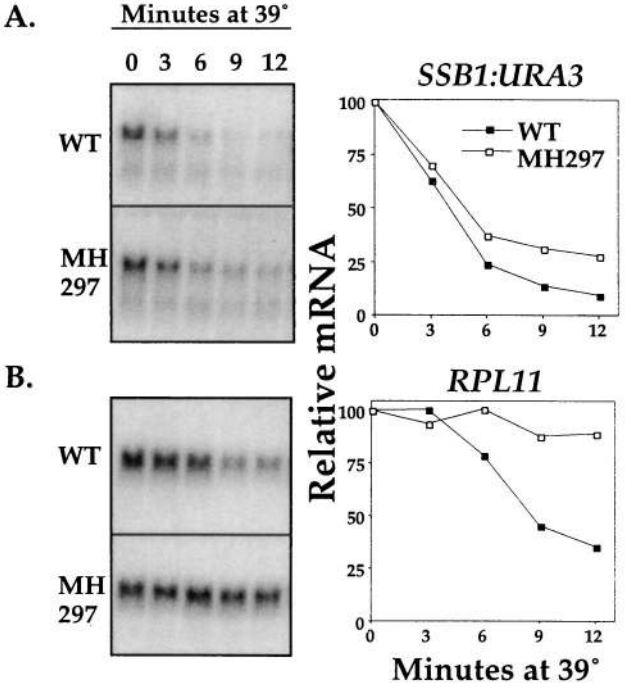

FIG. 6. mRNA levels of an *SSB1:URA3* fusion in wild-type (WT) and mutant MH297 cells after a heat shock. The experiment was performed as described for *URA3* in Materials and Methods. Northern blot images of each transcript are shown at left. (A) *SSB1:URA3* fusion; (B) *RPL11*. Quantification of the signals detected in panels A and B is shown on the right. Signals were normalized to rRNA levels.

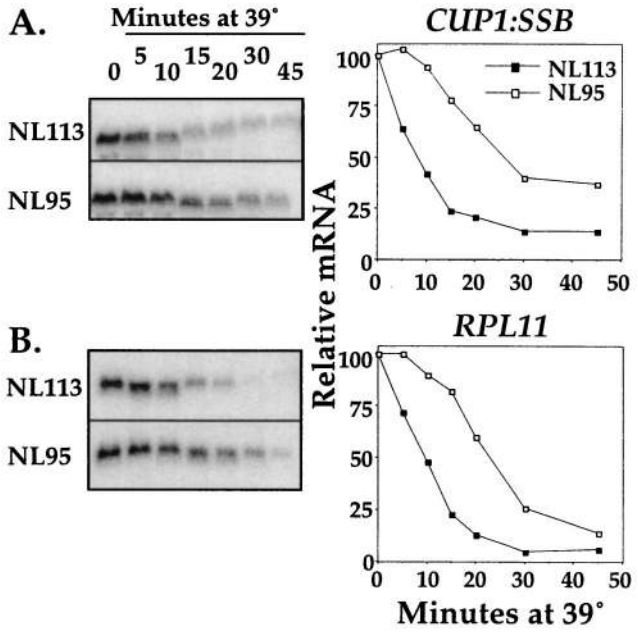

FIG. 7. Influence of the *EXA3-1* mutation on the transcript levels of a *CUP1*:*SSB1* fusion. The experiment was done as described in the legend to Fig. 5. At left, Northern blot images for *CUP1:SSB1* fusion (A) and *RPL11* (B) are shown. At right is shown quantification of the signals after a heat shock with rRNA as a loading control.

to translation inhibitors) of a mutant strain containing deletions of both *SSB* genes. In addition, primer extension analysis showed that the 5′ end of the *CUP1:SSB1* transcript is similar in size to that of the native *SSB1* transcript (data not shown).

This *CUP1:SSB1* fusion was used to transform two strains that have the following genotypes: *ssb1 ssb2 EXA3-1* (NL95) and *ssb1 ssb2* (NL113). Analysis of the *CUP1:SSB1* fusion showed rapid reduction of its mRNA levels in NL113. This reduction was retarded in the presence of the *EXA3-1* allele. The delay in the heat shock regulation of the *CUP1:SSB1* transcript in NL95 is comparable to what was observed for the internal control, the genomic RP gene *RPL11*. After 20 min of the temperature upshift, the mRNA levels of both *RPL11* and *CUP1:SSB1* were about threefold higher in NL95 than in NL113. Therefore, the sequences included in the *SSB1* transcribed region are sufficient to provide a heat shock mRNA regulation dependent on HSF.

DISCUSSION

It has been established that cells regulate components of the ribosome according to their growth rates and stress conditions that compromise protein synthesis (19, 39). Here we have demonstrated that mRNA levels of the Hsp70 molecular chaperone Ssb are coregulated with RP mRNA levels under three different growth conditions: carbon upshift, amino acid starvation, and heat shock. Other data indicate a relationship of Ssb with ribosomes. Ssb, which is present within the cell in a two- to three-times molar excess over ribosomes (28), is associated with the ribosome-nascent chain complex in a salt-resistant manner (28). In addition, strains lacking Ssb are more sensitive to certain translation inhibitors (24). Hence, the regulation reported here coupled with previous results supports the hypothesis that Ssb should be considered an important component of the translational apparatus.

The regulation of *SSB* and *SSA*, which encode two major classes of cytosolic Hsp70s, is strikingly different. These two Hsp70s have evolved with similar protein structures as evidenced by the fact that they are more than 60% identical in sequence, but their regulation is different, presumably because of their different functional niches within the cell. Ssa has been implicated in the refolding of proteins partially denatured upon exposure to increased temperatures and in regulation of the heat shock response. Therefore, it is not surprising that *SSA* expression is induced by a heat shock to cope with the protein damage generated at high temperatures. In contrast, expression of Ssb, as well as of RPs, is repressed after a temperature upshift. Apparently, it is advantageous for cells not to expend energy in translation under conditions in which proteins will be jeopardized by denaturation conditions and subjected to aggregation. Also, both *SSB* and RP gene mRNA levels are regulated according to the growth rates of the cell. For example, we observed that mRNA levels of *SSB* and RP genes decline upon starvation for amino acids when the level of protein synthesis is dropping. Moreover, *SSB* mRNA levels increase rapidly upon addition of a rich carbon source when the rates of protein synthesis in a cell are rising. Therefore, the synthesis of Ssb and structural RPs is carefully regulated by the cell, presumably to minimize the energy used in forming the translational apparatus, which comprises about 16 to 18% of cellular protein.

The mechanism of down-regulation of RP mRNA levels upon a heat shock is not well understood. Regulation at the level of transcription and mRNA degradation have been suggested (12, 16). We found the negative heat shock regulation of both *SSB* and four RP genes analyzed to be dependent on HSF. This conclusion is based on our observation that a strain containing the HSF allele *EXA3-1* has a delay in the reduction of mRNA levels of *SSB* and RP genes upon a heat shock. Moreover, the *EXA3-1* mutation did not affect the negative heat shock mRNA regulation of two proteins whose function is not related to the translation machinery, the mating α-factor gene *MATα1/2* and the *URA3* gene transcripts. The rate of reduction for these two mRNAs after a temperature upshift was similar in wild type and in cells containing the *EXA3-1* mutation. These data suggest that two mechanisms are responsible for reducing mRNA species upon a heat shock: a global mechanism that is HSF independent and a specific mechanism for RP mRNAs that requires HSF.

Considering the results reported here, it is somewhat surprising that another HSF allele (*hsf1-m3*/*mas3*) was reported to have no effect on the mRNA regulation of RP genes upon a heat shock (7). This HSF allele was thought to abolish its heat activation, reducing drastically the induction of heat-inducible genes (34). Recently, it was found that a mutant strain containing the *hsf1-m3* allele did not have an obvious decrease in the induction of several heat shock proteins (17, 36), including Hsp104. However, we have observed that the *EXA3-1* mutation appears to retard the heat induction of Hsp104 as well as that of other Hsp proteins (10). Consistent with this result, the *EXA3-1* mutation also delays the reduction of *SSB* and RP transcripts. It is possible that the *hsf1-m3* mutation does not significantly affect the activity important in the up-regulation of Hsp104 as well as the down-regulation of genes encoding ribosomal components.

We can envision two general ways in which HSF may be acting in the regulation of *SSB* and RP genes: (i) HSF may act as a transcriptional repressor of *SSB* and RP genes or (ii) HSF is a transcriptional activator of a heat-inducible regulatory factor needed for the down-regulation of RP and *SSB* genes. We favor the second idea, that the *EXA3-1* mutation causes a

delay in the expression of a heat-inducible factor, for several reasons. If HSF is a repressor, it must be binding to cryptic sequences since neither *SSB* gene contains a canonical HSE. There is a precedent for the binding of a transcriptional activator or repressor to variant sequences in different modes of regulation (32). However, alignment of *SSB* and the RP genes studied here did not reveal a conserved sequence present in these genes which might act as a novel binding site for HSF acting as a repressor. In addition, our results show that the transcribed region of *SSB* is sufficient to regulate its mRNA levels after a heat shock in an HSF-dependent manner, while the promoter is not. This result does not preclude HSF acting directly as a repressor since binding of repressors to the coding region has been found previously (11, 33). However, such cases appear to be rare, particularly in yeast. Finally, previous experiments suggest that a factor needs to be synthesized de novo for the appropriate mRNA regulation of RP genes upon a heat shock (12, 26). Together, these observations suggest that HSF acts as a transcriptional activator of a heat-inducible factor, not as a transcriptional repressor of *SSB* and RP genes.

What might the function of such a heat-inducible factor be? Such a factor could be or could activate a heat-inducible transcriptional repressor or an RNase activity specific for these genes or mRNAs. Unfortunately, it is very difficult to determine directly whether the differences in *SSB* regulation in wild-type and HSF mutant cells are at the transcriptional or the posttranscriptional level due to unique problems in studying RNA regulation during the heat shock response. The methods currently available to measure RNA stability are inadequate under these conditions. The addition of drugs such as 1,10-phenanthroline and thiolutin, which inhibit the vast majority of mRNA synthesis, induces the synthesis of heat shock mRNAs (1). The temperature-sensitive mutation in the RNA polymerase subunit *RPB1* commonly used (26) requires a "heat shock" to inhibit RNA synthesis, but the temperature inactivation of the *RPB1* subunit is too slow to eliminate induction of a heat-inducible factor (data not shown). In the absence of additional experimental data, we favor the simplest idea, that HSF is required for induction of an RNase or of a factor required for activation of an RNase. This RNase or factor may recognize an RNA secondary structure common to both *SSB* and RP mRNAs which is not recognizable at the primary sequence level.

The experiments reported here emphasize an HSF-dependent mode of regulation of mRNA levels upon a temperature upshift. This mode of regulation does not affect all genes, as the rates of decrease of *MAT*α*1/2* and *URA3* mRNAs were not significantly affected by the *EXA3-1* mutation. There is certainly a second mechanism of regulation, as both these mRNAs, as well as many others, decrease in levels upon a heat shock. The regulatory mechanism unaffected by the *EXA3-1* mutation which controls the expression of many genes, including *SSB* and RP genes, may be a general cessation of transcription of non-heat-inducible genes, as pulse-labeling experiments indicate that transcription of RP genes in *S. cerevisiae* is reduced after a heat shock (16). However, this regulatory pathway is overlaid by an HSF-dependent mechanism that down-regulates *SSB* and RP mRNAs, perhaps by hastening the degradation of existing mRNA molecules. This HSF-dependent mechanism is an extremely efficient way for the cell to couple the activation of heat-inducible genes with the negative regulation of the translational apparatus, by linking both modes of regulation to the same regulatory molecules. This coupling implies that the down-regulation of the translational apparatus is an important component of the regulatory pathways that the cell has evolved to cope with environmental stress.

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