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Genetic and Biochemical Interactions Among Yar1, Ltv1 and RpS3 Define Novel Links Between Environmental Stress and Ribosome Biogenesis in *Saccharomyces cerevisiae*

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ABSTRACT

In the yeast *S. cerevisiae*, ribosome assembly is linked to environmental conditions by the coordinate transcriptional regulation of genes required for ribosome biogenesis. In this study we show that two nonessential stress-responsive genes, *YAR1* and *LTV1*, function in 40S subunit production. We provide genetic and biochemical evidence that Yar1, a small ankyrin-repeat protein, physically interacts with RpS3, a component of the 40S subunit, and with Ltv1, a protein recently identified as a substoichiometric component of a 43S preribosomal particle. We demonstrate that cells lacking *YAR1* or *LTV1* are hypersensitive to particular protein synthesis inhibitors and exhibit aberrant polysome profiles, with a reduced absolute number of 40S subunits and an excess of free 60S subunits. Surprisingly, both mutants are also hypersensitive to a variety of environmental stress conditions. Overexpression of *RPS3* suppresses both the stress sensitivity and the ribosome biogenesis defect of Δ*yar1* mutants, but does not suppress either defect in Δ*ltv1* mutants. We propose that *YAR1* and *LTV1* play distinct, nonessential roles in 40S subunit production. The stress-sensitive phenotypes of strains lacking these genes reveal a hitherto unknown link between ribosome biogenesis factors and environmental stress sensitivity.

ALL eukaryotic cells share the ability to sense and respond to a wide variety of environmental stress conditions. In the yeast *Saccharomyces cerevisiae*, screens for mutants hypersensitive to specific stress conditions have identified genes required for the detection and signaling of stress, as well as genes with functions in repair of stress-induced damage or in the establishment of a stress-tolerant state (Jamieson 1998; Estruch 2000; Hohmann 2002). More recently, genomic expression-profiling studies have revealed that a wide variety of stress conditions, ranging from starvation, heat shock, oxidization, or osmotic stress, all induce a large, stereotypical remodeling of gene expression (Gasch *et al.* 2000; Causton *et al.* 2001). About two-thirds of the ~900 genes included in this genomic "environmental stress response" are transiently repressed; the rest are transiently induced. Of the genes induced by multiple stresses, many have known functions in the stress response. However, not all genes that are induced/repressed in common by stress are thought to function directly to protect the cell from stress. Rather, many of the changes in gene expression may represent an adaptive adjustment in cellular metabolism under nonoptimal growth conditions.

One cluster of genes whose expression is coordinately and transiently repressed by multiple environmental stresses is composed almost entirely of genes that encode proteins with functions in ribosome structure, function, or biogenesis (Gasch *et al.* 2000; Causton *et al.* 2001). The ribosome is an enormous machine, assembled from four rRNAs and 80 ribosomal proteins. Ribosome biogenesis takes place primarily in the nucleolus where transcription of ~200 tandemly repeated rDNA genes produces 35S precursor rRNAs. The 35S pre-rRNA assembles with both ribosomal and nonribosomal proteins to form a 90S preribosome complex, which is subsequently processed into 66S and 43S preribosomal subunits. Further cleavage of the rRNA and maturation of the 66S preribosomes takes place in the nucleolus and nucleoplasm, with the final maturation of the 43S particle occurring in the cytoplasm (Kressler *et al.* 1999; Venema and Tollervey 1999; Fatica and Tollervey 2002; Fromont-Racine *et al.* 2003). Exponentially dividing yeast cells have been estimated to produce new ribosomes at a rate of almost 40/sec (Warner 1999). This makes ribosome synthesis a major cellu-

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lar biosynthetic activity, and the coordinate repression of the expression of these ribosomal components under adverse environmental conditions would liberate significant energy resources for other cellular processes.

We previously identified *YAR1* as a small gene required for a normal rate of proliferation, whose transcription is strongly and transiently repressed by heat shock (Lycan *et al.* 1996). Yar1p is composed almost entirely of two ankyrin repeats, which are conserved 33-amino-acid motifs that occur in tandem and fold to form L-shaped protein:protein interaction domains (Sedgwick and Smerdon 1999). While most ankyrin-repeat-containing proteins are large multidomain proteins with diverse cellular functions (Bork 1993), a more limited group of small ankyrin proteins, composed mostly or entirely of ankyrin repeats, functions simply by binding and hence regulating their nonankyrin partners. For example, the ankyrin-repeat protein IkBα regulates the cellular location of transcription factor NFkB by masking its nuclear localization signal (NLS) (Verma *et al.* 1995), while pINK4 inhibits the enzymatic activity of its partner, CDK4/6 (Serrano *et al.* 1993; Hannon and Beach 1994; Chan *et al.* 1995; Hirai *et al.* 1995; Guan *et al.* 1996). In this study, we identify a novel role for a small ankyrin-repeat protein in ribosome biogenesis. We provide genetic and biochemical evidence that Yar1 physically interacts with ribosomal protein S3 and with Ltv1, a protein recently copurified with a number of proteins implicated in 43S preribosome processing (Schafer *et al.* 2003). We demonstrate that both Δ*yar1* and Δ*ltv1* mutants are hypersensitive to certain protein synthesis inhibitors and exhibit aberrant polysome profiles with a reduced absolute number of 40S subunits and an excess of free 60S subunits, relative to wild-type cells. In addition, both mutants are hypersensitive to osmotic and oxidative stress, as well as to low- and high-temperature conditions. Overexpression of *RPS3* suppresses both the stress sensitivity and the ribosome biogenesis defect of Δ*yar1* mutants, but not that of Δ*ltv1* mutants. On the basis of these and other results, we propose that *YAR1* and *LTV1* play distinct, nonessential roles in 40S subunit production. The stress-sensitive phenotypes of strains lacking these genes reveal a hitherto unknown link between ribosome biogenesis factors and environmental stress.

MATERIALS AND METHODS

Yeast manipulation: Yeast were cultured and manipulated according to standard laboratory practices, which have been described previously (Guthrie and Fink 1991). Plates for stress and protein synthesis inhibitor assays were made by adding each drug (Sigma, St. Louis), dissolved in water (except anisomycin, which was dissolved in ethanol), to YPD agar cooled to 50°–55°, to the final concentration noted. Sorbitol plates were made by adding sorbitol to 1.5 m prior to autoclaving. Yeast transformations were performed using the lithium acetate method (Gietz *et al.* 1992).

All yeast strains used in this study are listed in Table 1. LY103 was constructed by one-step disruption of the *YAR1* locus by transformation of W303 with Ld6 (Lycan *et al.* 1996) cleaved with *Cla*I and *Kpn*I; disruption of *YAR1* was confirmed by Southern blot analysis. The heterozygous diploid was sporulated to produce LY103, -104, -105, and -106, four haploid segregants of one tetrad. LY124 was constructed by disruption of one *RPS3* allele by transformation of LY101 (Lycan *et al.* 1996) with pSK2 (Finken-Eigen *et al.* 1996) digested with *Xba*I and *Xho*I; the presence of one wild-type and one *rps3::HIS3* allele was confirmed by PCR. LY126 was generated similarly, except that W303 was used for transformation with pSK2. LY141 was constructed by disrupting the *YAR1* locus in LY139 with Ld6 cleaved with *Cla*I and *Kpn*I (confirmed by PCR). We sporulated this heterozygous diploid and the Δ*yar1::URA3* allele segregated 2:2 in seven tetrads. LY141 is a G418-resistant, Ura^+ haploid segregant from one of these seven tetrads. Strains LY150 and LY156 were generated by transforming W303 haploids with PCR-amplified pFA6a-13Myc-His3MX6 and pFA6a-3HA-kanMX6, respectively, as described (Longtine *et al.* 1998). Integration of the tag at the genomic locus in each strain was confirmed by PCR and production of the correct fusion protein was confirmed by Western blot analysis. LY161 was generated by mating LY150 and LY156, sporulating the diploid, and selecting for both G418 resistance and histidine prototrophy in random spores. LY161 was confirmed to be haploid by mating tests.

Construction of plasmids: We constructed p*YAR1*cen (Ld25) by amplifying the intergenic region between *YAR1* and *HSP82* using a forward primer that anneals upstream of HSE1 in the *HSP82* promoter and a reverse primer that anneals within the *YAR1* coding region. The PCR product was cleaved with *Bam*HI and *Nde*I and ligated into pRS314 (Sikorski and Hieter 1989) containing the *YAR1* ORF (Ld3). Plasmid structure was confirmed by restriction analysis. Transformation of LY103 with Ld25 confirmed that the centromere vector is able to fully complement the cold-sensitive, heat-sensitive, and slow-growth phenotypes of the Δ*yar1* strain.

To create pLexA-*YAR1* (Ld17), the *YAR1* ORF was amplified by PCR, ligated into pCRII-TA (Invitrogen, San Diego), and then subloned into *Sal*I/*Pst*I-digested pLEXA (gift of Stan Hollenberg). Ld17 retains the first codon for methionine of the *YAR1* ORF and adds two codons (for amino acids Pro and Asp) between the *Sal*I site and the *YAR1* ATG. The in-frame LexA-*YAR1* fusion junction was confirmed by DNA sequencing. Ld20 was derived from Ld17 by cleaving with *Afl*II and religating the vector to delete 1223 bp and the *Nde*I site from the *ADE2* gene. To create pGEX-*YAR1* (Ld19), we subcloned *YAR1* from Ld17 as a *Sal*I/*Not*I fragment into pGEX-6P-1 (Pharmacia). The presence of an in-frame fusion between *GST* and *YAR1* and the absence of any amino acid substitutions in the *YAR1* ORF were confirmed by DNA sequencing.

RPS3 and *LTV1* ORFs were each cloned into pCITE2a (Novagen) for *in vitro* transcription/translation as follows. The *LTV1* ORF was amplified from genomic W303 DNA using *Pfu* Turbo DNA polymerase (Stratagene, La Jolla, CA) and PCR primers. The primers create an *Nco*I site that includes the initiator codon (Met) for the protein at the 5′ end and an *Ava*I site immediately upstream of the stop codon at the 3′ end. The PCR product was cleaved with *Nco*I and *Ava*I and ligated into pCITE2a cleaved with the same enzymes. This creates an S2A substitution at position 2 in the *LTV1* sequence. The sequence of Ltv1 is otherwise unchanged except for the addition of LEHHHHHH at the C terminus. *RPS3* was cloned into pCITE2a similarly, except pSK1 (gift of M. Finken-Eigen) was used as a template for PCR instead of genomic DNA, and the forward primer creates an *Nco*I site without altering the amino acid sequence of the protein. Thus the *RPS3* sequence

TABLE 1

***S. cerevisiae* strains**

Strain	Genotype	Source
W303	*MAT***a**/*MAT*α *leu2*-3,112/*leu2*-3,112 *trp1*-1/*trp1*-1 *ura3*-1/*ura3*-1 *ade2*-1/*ade2*-1 *his3*-11,15/*his3*-11,15 *can1*-100/*can1*-100	L. Breeden
W303-1a	*MAT***a** *leu2*-3,112 *trp1*-1 *ura3*-1 *ade2*-1 *his3*-11,15 *can1*-100	R. Rothstein
SO607	*MAT***a** *leu2*-3,112 *trp1*-1 *ura3*-1 *ade2*-1 *his3*-11,15, *can1*-100 *pbs2::LEU2*	S. O'Rourke
SEY6210	MATα *leu2*-3, 112 *ura3*-52 *his3*-Δ200 *lys2*-801 *trp1*-Δ901 *suc2*-Δ9 Mel-	S. Moye-Rowley
SM13	SEY6210, *yap1*-Δ2::hisG	S. Moye-Rowley
LY101	W303, *yar1::URA3/yar1::URA3*	Lycan *et al.* (1996)
LY103	*MAT*α *leu2*-3,112 *trp1*-1 *ura3*-1 *ade2*-1 *his3*-11,15 *can1*-100 *yar1::URA3*	This study
LY106	*MAT*α *leu2*-3,112 *trp1*-1 *ura3*-1 *ade2*-1 *his3*-11,15 *can1*-100	This study
LY124	LY101, +/*rps3::HIS3*	This study
LY126	W303, +/*rps3::HIS3*	This study
LY134	*MAT*α *his3*D1, *leu2*DO, *ura3*DO, *lys2*DO	Research Genetics
LY135	*MAT***a** *his3*D1 *leu2*DO *ura3*DO *met15*DO *yar1*::kan^R	Research Genetics
LY136	*MAT*α, *his3*D1 *leu2*DO *ura3*DO *lys2*DO *ltv1*::kan^R	Research Genetics
LY141	*MAT***a** *his3*D1 *leu2*DO *ura3*DO *lys2*DO *yar1::URA3, ltv1*::kan^R	This study
LY150	W303α *LTV1::13Myc:HIS3MX6*	This study
LY156	W303**a** *YAR1::3HA:kanMX6*	This study
LY161	*MAT***a** *leu2*-3,112 *trp1*-1 *ura3*-1 *ade2*-1 *his3*-11,15 *can1*-100 *YAR1::3HA:kanMX6 LTV1::13MYC:His3MX6*	This study
L40	*MAT***a** *his3*Δ200 *leu2*-3-112 *trp1*-901 *ade2* LYS2:(lexAop)$_4$-HIS3 URA3::(lexAop)$_8$-lacZ GAL4	S. Hollenberg

is fully wild type except for the addition of LEHHHHHH at the C terminus. The sequence of all constructs was confirmed to be wild type, except for the changes expected, by DNA sequencing.

Site-directed mutagenesis: We mutated both K91 and K125 to alanine using a QuikChange site-directed mutagenesis kit following the protocol provided by the manufacturer (Stratagene). The mutagenic primer sets used to generate the K91A mutation were 5′-gttaatgaggt*gaatgca*acaggcaacacggctttacattg ggc-3′ and its complement, and for K125A, 5′-gcagacccctttatt agaa*acgcgt*tcggccacgatgc-3′ and its complement. Engineered *Bsm*I and *Mlu*I restriction sites are italicized. The presence of the intended mutations and the absence of other mutations were confirmed by DNA sequencing.

Two-hybrid screen: A two-hybrid screen for Yar1-interacting proteins was performed in yeast strain L40 harboring the bait plasmid pLexA-*YAR1* (LD17). L40 pLexA-*YAR1* was transformed with a yeast cDNA library fused to the Gal4 activation domain in pGAD24 (gift of S. Elledge). We screened 3.8×10^5 yeast Trp$^+$ transformants for *lacZ* expression using standard protocols (Vojtek and Hollenberg 1995) and picked 175 positives, of which 162 retested positive in a second assay of purified colonies. All of these were identified to be one of three genes by direct sequencing or by screening with *RPS3*-specific primers. For all three genes, we showed that loss of the bait plasmid correlated with loss of *lacZ* transcriptional activation and that each of the activating plasmids could again confer the His$^+$Lac$^+$ phenotype when reintroduced into cells containing the pLexA:*YAR1* bait plasmid.

β-Galactosidase activity assays: β-Galactosidase activity was assayed as described (Breeden and Nasmyth 1985) and normalized to protein levels using the following equation: $1000 \times (OD_{420}$/ml of supernatant)/protein concentration (mg/ml of supernatant) × reaction time (min). Individual single colonies were inoculated into 5 ml selective media and grown for 17 hr at 30° until the OD_{660} of the culture was between 0.7 and 1.3.

***In vitro* interaction assays:** GST pulldown assays were performed essentially as described (Ausubel *et al.* 1995). BL21 cells, transformed with the pGEX-6p-1 plasmid (Pharmacia) or with pGEX-*YAR1*, were induced for 2 hr with 0.4 mm isopropyl-1-thio-b-d-galactopyranoside (Fisher). Harvested cell pellets were lysed by sonication and cleared cell lysates (2 mg/ml total protein) were incubated with glutathione-linked agarose beads (Sigma) at room temperature for 5 min. The ratio of lysate volume to bead volume was between 5:1 and 10:1 in all experiments. After binding, beads were washed five times with five bed volumes of bead binding buffer.

Potential Yar1 partner proteins (cloned in pCITE2a vectors) were synthesized *in vitro* using an STP3 transcription/translation kit (Novagen) and 40 μCi [^{35}S]methionine (Amersham Pharmacia) according to the manufacturer's specifications. Radiolabeled protein was incubated with GST or GST-*YAR1*-loaded glutathione-agarose beads in the presence of 1–2 mg of BL21 extract for 1 hr at 4°. GST and GST-*YAR1* loaded beads were then washed three times and bound protein was eluted by boiling beads 5 min in 1× electrophoresis sample buffer. Eluted proteins were analyzed on 12% polyacrylamide gels. Proteins were visualized and quantified using phosphorimaging (Molecular Dynamics, Sunnyvale, CA).

Ribosome sedimentation and protein analysis: Yeast cell lysates were prepared as previously described (Nelson *et al.* 1992) from strains grown in YPD or minimal media to an OD_{600} of 0.4–0.7 at 30°. For polyribosome sedimentation, 10 OD_{260} units of lysate were loaded onto 10 ml continuous 15–50% sucrose gradients in CB buffer (20 mm HEPES-K, pH 7.5, 1 mm EDTA, 5 mm $MgCl_2$, 10 mm KCl, 10% (v/v) glycerol, 2 mm β-mercaptoethanol). For ribosome subunit analysis, 10 OD_{260} equivalents of cleared homogenate were loaded onto 15–50% sucrose gradients in CB buffer lacking $MgCl_2$ and including 10 mm EDTA. All gradients were centrifuged for 10 hr at 200,000 × *g* in an SW40 rotor (Beckman). The absorbance of gradient material at 254 nm was measured continuously and 0.5-ml fractions were harvested using an automated collector (ISCO). Where indicated, gradient fractions were precipitated with acetone and processed for SDS-PAGE and immunoblotting. After transfer to nitrocellulose membranes, individual proteins were detected using antibodies directed

against the Myc and HA epitopes (provided by C. Lingenfelter) or antibodies directed against the yeast ribosomal proteins S2 and L32 (provided by J. Warner) at 1:2000 dilutions. Primary antibodies were detected with HRP-coupled secondary antibodies at a 1:20,000 dilution and chemiluminescence detection reagent (Perkin-Elmer Life Sciences).

High-copy suppressor screen: Strain LY135 was transformed with a yeast centromere *GAL1*-regulated cDNA library (Liu *et al.* 1992; provided by L. Breeden) as described (Adams *et al.* 1998) with the following modifications: DMSO was added to 10% of the volume before the heat-shock step, and transformants were allowed to recover for 2 hr in YPD before being plated onto selective plates. We plated ~35,000 transformants onto selective plates containing 2% galactose and 5 mg/ml neomycin. Transformants with suppressing plasmids were patched onto selective galactose/neomycin plates, replica printed onto plates containing either glucose or galactose and neomycin, and scored relative to LY135 and LY134 strains. Plasmids were rescued from transformants that grew better than Δ*yar1* on galactose only and retransformed back into LY135. Plasmids that again conferred galactose-dependent suppression were characterized by restriction endonuclease digestion and sequenced from the 3′ end to identify the insert. The sequence of both strands of one of the *RPS3*-containing suppressing plasmids was determined to confirm that the cDNA was full length and without mutations. This plasmid, pGAL:*RPS3*, was used for all subsequent analysis.

RESULTS

Yar1 interacts with RpS3 and Ltv1: We previously reported that *YAR1* encodes a small ankyrin-repeat protein. Deletion of the gene is not lethal, but leads to a slow-growth phenotype that is especially pronounced when cells are grown at low or high temperature (Lycan *et al.* 1996). To further characterize *YAR1*, we undertook a yeast two-hybrid screen to identify interacting protein partners. *YAR1*, fused to the LexA DNA-binding domain, was the bait in a screen of a yeast cDNA library. Of 162 transformants that retested positive in a second assay, 158 were shown to contain *RPS3*, which encodes ribosomal protein S3, a component of the small subunit of the ribosome. Two other genes were recovered in this screen: *LTV1* and an uncharacterized ORF, *YOR021c*. Each was recovered twice as independent isolates from the library. While we find genetic evidence in support of Yar1:RpS3 and Yar1:Ltv1 protein:protein interactions (see below), we were not able to detect either an *in vitro* interaction between Yar1 and YOR021c or any genetic evidence in support of this interaction, and so this gene was not characterized further. RpS3 is a highly conserved, ribosomal protein that is part of the 40S subunit, with proposed roles in translation initiation (Westermann *et al.* 1981), decoding accuracy (Hendrick *et al.* 2001), and repair of oxidative DNA damage (Kim *et al.* 1995; Yacoub *et al.* 1996; Deutsch *et al.* 1997; Sandigursky *et al.* 1997). Ltv1 is a conserved protein of unknown function that was recently identified as a component of a purified preribosomal complex (Schafer *et al.* 2003).

To determine whether any of the *YAR1*-interacting proteins detected in our two-hybrid screen also interact with Yar1 *in vitro*, we cloned *YAR1* as an N-terminal GST fusion and expressed it in *Escherichia coli*. Each interacting protein was synthesized and labeled *in vitro* and then tested for retention by GST-*YAR1*-loaded glutathione agarose beads. Since the interacting proteins are synthesized in mammalian cell extracts, retention of labeled protein by GST-*YAR1* is evidence that the interaction does not require other yeast proteins. Labeled RpS3 was specifically retained by the GST-*YAR1* loaded beads and not by GST-loaded beads (Figure 1A). Quantitative analysis of the phosphorimager screen images indicate that ~10% of the input labeled RpS3 protein was retained by GST-*YAR1*, compared to 0.2% by the GST beads. In contrast, no significant amount of labeled Ltv1 was retained by the GST-*YAR1* beads over the levels retained nonspecifically by the GST beads alone (Figure 1B). These data indicate that the interaction between Yar1 and RpS3 can occur in the absence of other yeast proteins but that between Yar1 and Ltv1 must be indirect.

The Yar1 ankyrin repeats are required for the two-hybrid interaction between Yar1 and Ltv1: The co-crystal structures of a number of ankyrin-repeat proteins and their nonankyrin partners have identified variable residues at the tips of the β-hairpin loops of the ankyrin motif as frequent sites of specific protein:protein interaction (Gorina and Pavletich 1996; Brotherton *et al.* 1998; Russo *et al.* 1998). We aligned the amino acid sequence of Yar1 with the known structure of the CDK inhibitor, INK4p18. The optimal alignment of Yar1 is with the third and fourth ankyrin repeats of INK4p18 (Figure 2A). This alignment predicts that each β-hairpin loop in Yar1 has a positively charged lysine residue at the tip (highlighted in yellow).

We changed each of these lysines to alanine, both individually and in combination, by site-directed *in vitro* mutagenesis of *YAR1* in the p*LexA:YAR1* bait plasmid. These plasmids were then reintroduced into yeast carrying either *RPS3* or *LTV1* as activation-domain fusion plasmids. The resulting transformants were assayed for β-galactosidase activity as an indirect measure of their interaction. A representative experiment is shown in Figure 2, B and C. Mutation of lysine 91 reduces, and mutation of both lysines, eliminates the interaction between Yar1 and Ltv1 (Figure 2B). These data indicate that the tips of the ankyrin repeats are critical for the Yar1:Ltv1 two-hybrid interaction. Mutation of both lysines at the β-hairpin tips has essentially no effect on the interaction between Yar1 and RpS3 (Figure 2C). This suggests that Yar1 interacts with RpS3 and Ltv1 through different surfaces of the protein.

Stress-sensitive phenotypes of Δ*yar1* and Δ*ltv1* mutants: We previously reported that Δ*yar1* strains are viable but slow growing under either heat-shock (37°) or low temperature (25°) growth conditions (Lycan *et al.* 1996). As incubation at such temperatures is stressful for yeast, we asked whether Δ*yar1* cells exhibited any other stress-sensitive phenotypes. Specifically, we tested Δ*yar1* cells for sensitivity to oxidative stress, high osmolarity, and nutrient and amino acid starvation. Cells

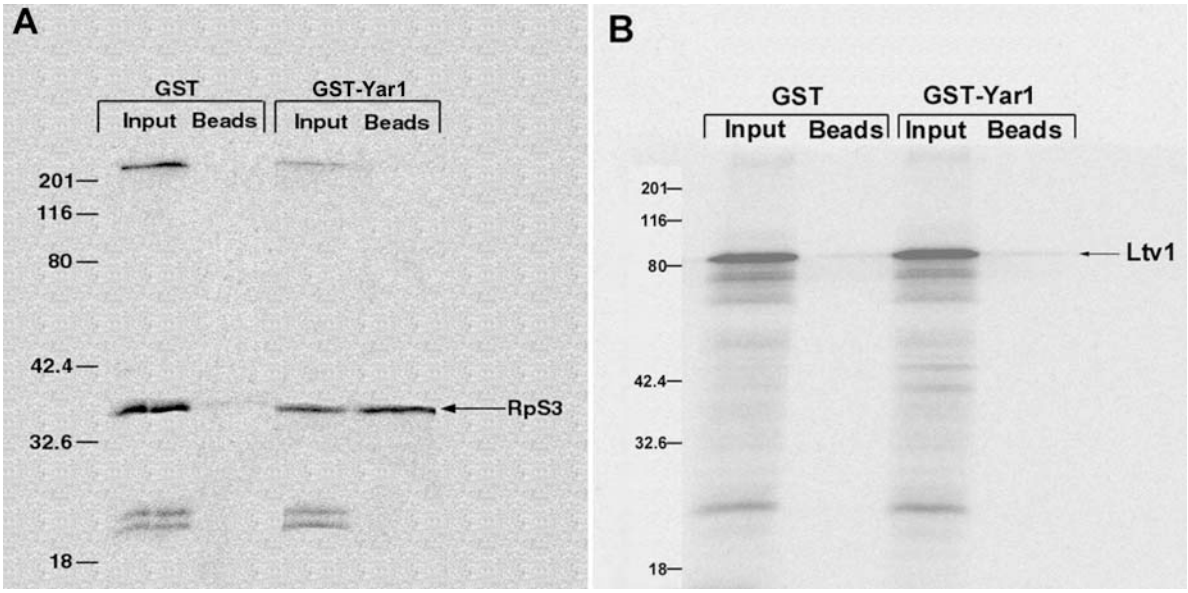

FIGURE 1.—Yar1 interacts with RpS3 *in vitro*. GST and GST-Yar1 expression was induced in BL21 cells and lysates incubated with glutathione agarose beads. (A) RpS3 (transcribed and translated *in vitro* in rabbit reticulocyte lysates in the presence of [^{35}S]methionine) was incubated with an excess of *E. coli* cell lysate and then added to glutathione agarose beads previously loaded with 4 μg of GST or GST-Yar1 protein. After extensive washing, the beads were boiled in 1× sample buffer and the supernatant analyzed by SDS-PAGE. Input lanes contain 5% of the reaction loaded onto the beads; bead lanes contain 90% of the supernatant boiled off the beads. (B) Ltv1, expressed and labeled as above, was incubated with GST- and GST-Yar1-loaded glutathione agarose beads as described in A.

lacking *YAR1* were sensitive to oxidative stress induced by treatment with diamide, at least as sensitive as cells lacking *YAP1*, the major oxidative stress-activated transcription factor (JAMIESON 1998; see Figure 3A). Diamide acts to deplete glutathione pools and oxidizes thiol groups (KOSOWER and KOSOWER 1987). The Δ*yar1* strain was also sensitive to osmotic stress induced by sorbitol, although not as sensitive as the Δ*pbs2* mutant. *PBS2* encodes the MAPKK that phosphorylates Hog1 in response to high-salt or sorbitol growth conditions (HOHMANN 2002). Thus *YAR1* is required for optimal long-term growth under both oxidative and osmotic stress conditions. However, the Δ*yar1* mutant showed no defects in viability after prolonged nitrogen deprivation (9 days). Mutant cells also accumulated storage carbohydrates comparable to wild-type cells, and the sporulation efficiency was essentially the same as in wild-type controls (data not shown). We also tested the phenotype of Δ*yar1* mutants constructed in the S288c background, in addition to our W303 background. Δ*yar1* cells exhibit the same phenotypes in both strain backgrounds (Figure 3B).

If Yar1 and Ltv1 physically interact *in vivo*, then we would predict that strains lacking either protein might share one or more phenotypes. We therefore asked whether strains lacking *LTV1* shared any of the stress-sensitive phenotypes of Δ*yar1* cells. We found that the Δ*ltv1* strain is hypersensitive to both osmotic and oxidative stress and is especially cold sensitive (Figure 3B). However, while *LTV1* stands for low-temperature viability, we find the Δ*ltv1* strain is slow growing but viable at all of the low temperatures that we tested (18°, 15°, 12°, and 10°; doubling time at 18° is ~7.5 hr). The Δ*ltv1* strain, like the Δ*yar1* strain, has a slight slow-growth phenotype at 30°. The fact that Δ*yar1* and Δ*ltv1* cells share multiple genetic phenotypes (as well as antibiotic hypersensitivity; see below) argues that the two-hybrid interaction between these two proteins reflects a common biological function *in vivo*.

Genetic interaction between *YAR1* and *RPS3*: Eukaryotic RpS3 is a highly conserved 36-kD protein located on the solvent side of the 40S subunit on the beak of the head region (SPAHN *et al.* 2001). Multiple functions have been proposed for this small protein. Mammalian RpS3 can be crosslinked to eIF3 (TOLAN *et al.* 1983) and eIF2 (WESTERMANN *et al.* 1979), the basis for its proposed role in translation initiation (WESTERMANN *et al.* 1981). In yeast, the missense allele of *RPS3*, *suf14-1*, is resistant to aminoglycosides and acts as an extragenic suppressor of +1 frameshift mutations, implicating RpS3 in translational decoding (HENDRICK *et al.* 2001). Finally, *in vitro* assays have implicated mammalian and Drosophila RpS3 in the repair of oxidative/UV DNA damage (KIM *et al.* 1995; YACOUB *et al.* 1996; DEUTSCH *et al.* 1997; SANDIGURSKY *et al.* 1997), and yeast RpS3 has been shown to have an endonuclease activity on apurinic DNA *in vitro* (JUNG *et al.* 2001).

To test the biological function of the detected two-hybrid and *in vitro* interactions between RpS3 and Yar1, we turned to genetic analysis. One way to uncover a genetic

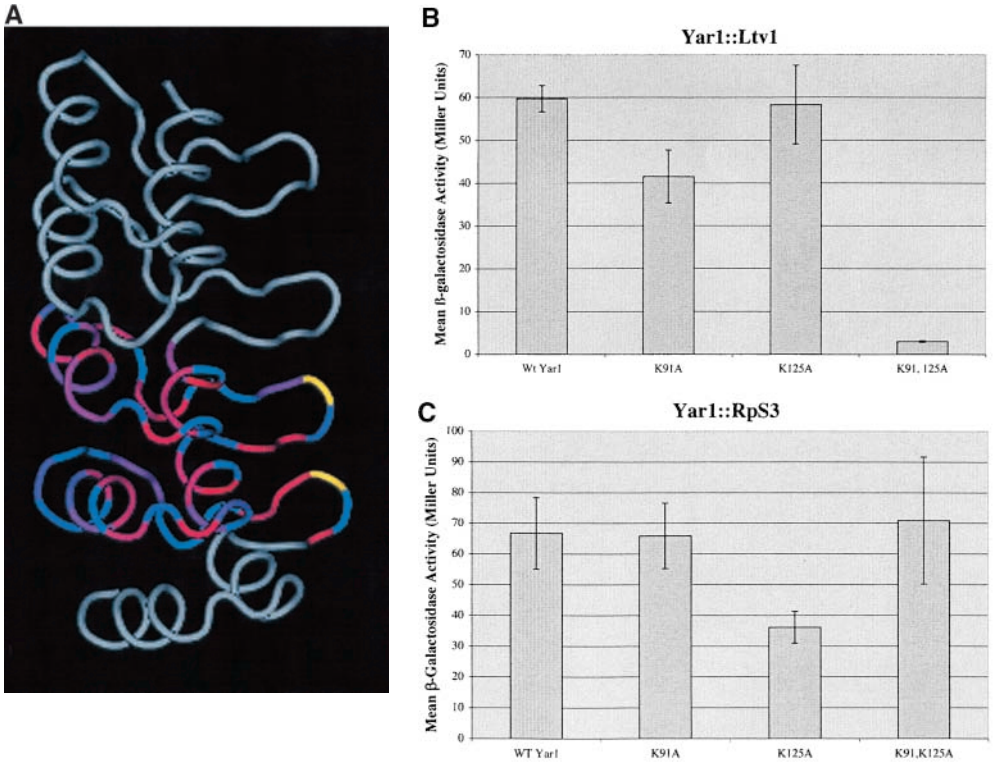

FIGURE 2.—The β-hairpin lysines in the ankyrin domain are critical for the Yar1:Ltv1 interaction. (A) The Cn3D program at NCBI was used to map the aligned (by gapped BLAST) amino acid sequence of the Yar1 ankyrin domain onto the 3D crystal structure of INK4p18. The program optimally aligned the Yar1 repeats with the third and fourth ankyrin repeats of INK4p18. In this representation, red represents identical residues; blue, nonconserved residues; and purple, conservative substitutions. The two positively charged lysines at the tip of each Yar1 loop have been highlighted in yellow. (B and C) Two-hybrid strains containing wild-type (WT Yar1) or mutant forms of Yar1 (K91A, K125A, or K91,125A), fused in frame with the LexA-DB and the Ltv1- or the RpS3-activation domain fusion plasmids, were grown at 30°. Three individual colonies were inoculated in each case and cell pellets were analyzed for β-galactosidase activity and protein content. Values shown represent the mean of three separate assays, with error bars representing 1 SD of the data.

interaction, when one of the two genes is essential, is to reduce the dosage of the essential protein in a background in which the nonessential protein is eliminated. We constructed a diploid strain, homozygous for Δ*yar1* and heterozygous for Δ*rps3*, and tested it for a synthetic growth defect under various stress conditions. The heterozygous Δ*rps3*/+ strain was indistinguishable from the wild-type parent under both optimal and all stress growth conditions that we tested (Figure 4a). The Δ*yar1*/Δ*yar1* diploid is slow growing at 23° and at 37°, but is less affected by temperature than the haploid strain. However, the Δ*yar1*/Δ*yar1*, Δ*rps3*/+ strain grew more slowly than either the Δ*yar1*/Δ*yar1* diploid or the Δ*rpS3*/+ strain at all temperatures, but especially at 23° and 37° (Figure 4a). In addition, Δ*yar1*/Δ*yar1*, Δ*rpS3*/+ is more sensitive to osmotic stress and oxidative stress than either the Δ*yar1*/Δ*yar1* strain or the Δ*rpS3*/+ strain (Figure 4b). The synthetic phenotype produced when the *RPS3* dosage is reduced in the Δ*yar1*/Δ*yar1* background is evidence of a genetic interaction between *YAR1* and *RPS3*. These data are consistent with the evidence of a physical interaction between the two proteins in the two-hybrid screen and *in vitro*.

***YAR1* and *LTV1* mutants are hypersensitive to protein synthesis inhibitors:** Identification of RpS3 and Ltv1 as interacting partners for Yar1 led us to look for further evidence that might link Yar1 to ribosome structure or function *in vivo*. Strains with mutations in genes involved in ribosome function or biogenesis often exhibit an altered sensitivity to drugs that affect protein synthesis (Lee *et al.* 1992; Nelson *et al.* 1992; Kressler *et al.* 1997; Yan *et al.* 1998; Liu and Thiele 2001). We tested four inhibitors of translational elongation. Aminoglycosides like paromomycin and neomycin bind to the A-site decoding region of the small subunit reducing translational accuracy (Carter *et al.* 2000; Ryu do *et al.* 2002; Lynch *et al.* 2003). Anisomycin binds the 28S rRNA in the 60S subunit and inhibits the peptidyl transferase reaction (Rodriguez-Fonseca *et al.* 1995). Cycloheximide inhibits translation by abrogating the translocation of the peptidyl tRNA from the A-site to the P-site (Pestka 1971). Cells lacking *YAR1* are hypersensitive to anisomycin, but are no more sensitive to cycloheximide than are wild-type cells (Figure 5B). They are also hypersensitive to both of the aminoglycosides tested, neomycin and paromomycin (Figure 5A). Strains lacking *LTV1* were sensitive to the same spectrum of protein synthesis inhibitors as are Δ*yar1* cells (Figure 5). These data further link Yar1 and Ltv1 function and suggest that both proteins have a role in ribosome biogenesis or function.

Yar1 and Ltv1 are required for normal ribosome biogenesis: Both biochemical interactions and mutant phenotypes link Yar1 and Ltv1 to RpS3 and to the ribosome. To test whether ribosome biogenesis is impaired in mutants lacking either of these proteins, we profiled cellular ribosomes from wild-type, Δ*yar1*, and Δ*ltv1* strains on sucrose density gradients. As shown in Figure 6, cells lacking *YAR1* or *LTV1* have aberrant polysome profiles

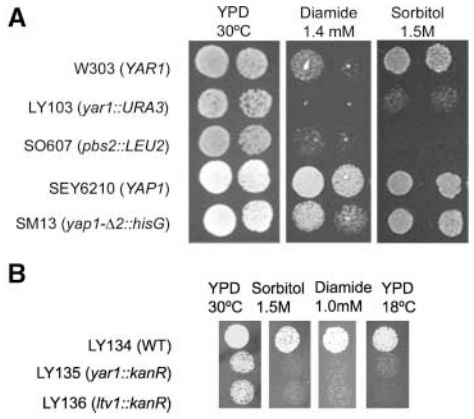

FIGURE 3.—Δ*yar1* strains and Δ*ltv1* cells exhibit stress-sensitive phenotypes. (A) Wild-type parental strain W303 and isogenic deletion derivatives Δ*yar1::URA3* and Δ*pbs::LEU2* were grown to midlog phase in YPD media, as were parental strain SEY6210 and its isogenic deletion derivative, Δ*yap1*-Δ*2*. Cell density was determined by hemocytometer and 5-μl aliquots containing 16,000 (left) and 4000 (right) cells were spotted onto YPD or YPD plus the indicated drugs. In this experiment, 1.3 times more Δ*yar1* and Δ*pbs2* cells were spotted at each position compared to the other strains to compensate for the slightly slower growth rate of these mutants under nonstress conditions. Plates were incubated at 30° and photographed after 2 days. The W303 strain background is more sensitive than the SEY6210 strain to diamide. (B) Parental strain LY134 (derived from S288c) and isogenic deletion derivatives LY135 and LY136 were grown as in A. Equal numbers of wild-type and mutant cells were spotted at each position. All plates were photographed after 2 days at 30° except the 18° plate, which was photographed after 3 days of incubation.

relative to wild-type cells. Both mutants have a significantly enlarged 60S peak relative to isogenic wild-type cells. In addition, the total number of 40S subunits, determined by dissociating monosomes and polyribosomes into free subunits, is significantly diminished in the mutants; the 40S:60S ratio is reduced by 52% in Δ*yar1* and by 58% in Δ*ltv1* (see insets in Figure 6). The reduction in total 40S subunits in both mutants is consistent with the excess of free 60S subunits in the polysome profile, and both pieces of data are consistent with a defect in 40S subunit biogenesis/maturation in both Δ*yar1* and Δ*ltv1* mutants.

One other aspect of the polysome profiles of Δ*yar1* and Δ*ltv1* mutants is worth noting. In both mutant profiles, there is a distinct shoulder on the 80S peak. This shoulder peak disappears if extracts are centrifuged under conditions that dissociate monosomes and polysomes into free 40S and 60S subunits. Similar shoulder peaks, called "half-mer" peaks, have been noted in numerous 60S subunit biogenesis mutants and in mutants with defects in subunit joining (WOOLFORD and WARNER 1991; KRESSLER *et al.* 1999) and are composed, in these mutants, of 80S monosomes plus a stalled 43S preinitiation complex attached to the same mRNA. However, in 60S mutants, there are also "half-mer" peaks associated with each of the polysome peaks, which we did not observe. In addition, gel analysis of RNA extracted from the shoulder peak fraction reveals that it is enriched for large subunit rRNA (the 25S:18S ratio in the shoulder peak is 2.8 compared to a ratio of 1.7 in the 80S peak). This ratio suggests an excess of 60S subunits, not an excess of small subunits. Since both the Δ*yar1* and Δ*ltv1* mutants have a deficiency in 40S subunits and an excess of free 60S subunits, the shoulder peak in these mutants is most likely dimers of free 60S subunits that form in the absence of sufficient 40S partners.

Ltv1 comigrates with ribosome subunits: The observed defects in 40S subunit biogenesis in Δ*yar1* and Δ*ltv1* mutants suggested that the interaction among

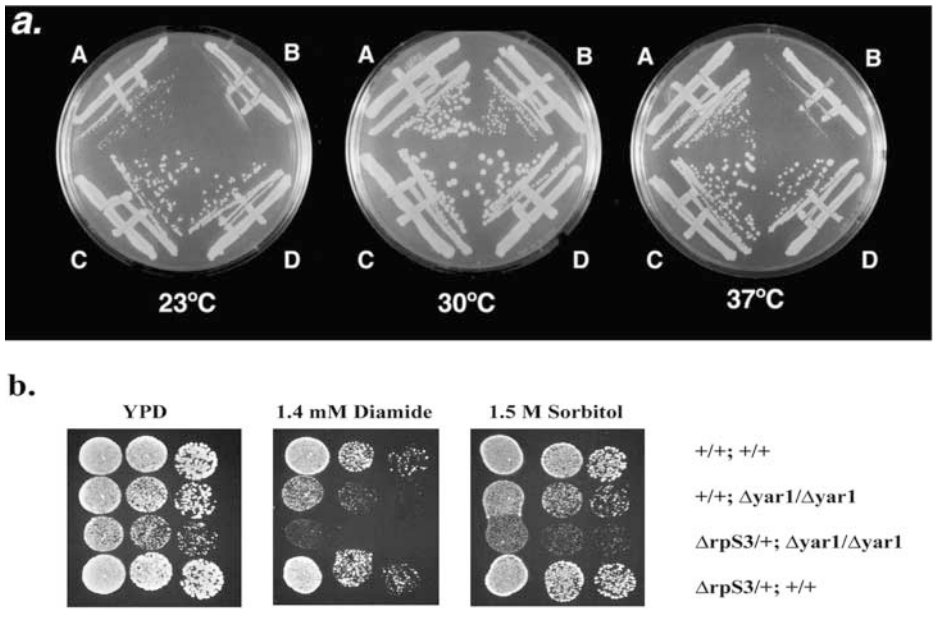

FIGURE 4.—Genetic interaction between *YAR1* and *RPS3*. (a) Diploid strains carrying the mutations indicated below were streaked for single colonies on YPD plates and incubated at 23°, 30°, or 37°. Homozygous Δ*yar1* diploids are not as sensitive to temperature stress as are the haploid strains. (A) +/+, Δ*yar1*/Δ*yar1*, (B) Δ*rps3*/+, Δ*yar1*/Δ*yar1*, (C) +/+, +/+, and (D) Δ*rps3*/+, +/+. (b) Diploid strains bearing the indicated mutations were grown as in Figure 1 and 16,000, 4000, and 1000 cells were spotted (left to right) onto YPD, YPD + diamide, and YPD + sorbitol plates. To compensate for the slower growth rate of the two homozygous Δ*yar1*/Δ*yar1* strains on YPD, 1.3 times more cells were spotted at each position for these two strains relative to the others. Plates were incubated at 30° and photographed after 25 hr of incubation.

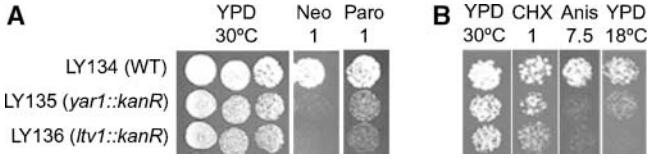

FIGURE 5.—Both Δ*yar1* and Δ*ltv1* strains are hypersensitive to protein synthesis inhibitors. The sensitivity of wild-type (WT) and isogenic Δ*yar1* and Δ*ltv1* cells to various protein synthesis inhibitors was tested by growing cells as described in Figure 1. (A) A total of 16,000, 4000, or 1000 cells (left to right) were spotted onto YPD plates with or without the denoted drugs. Only the 1000-cell column is shown for the neomycin (neo; 1 mg/ml) and paromomycin (paro; 1 mg/ml) plates. (B) A total of 400 cells of each strain were spotted onto YPD, YPD + cycloheximide (CHX; 1μg/ml), or YPD + anisomycin (Anis; 7.5 μg/ml) plates. Equal numbers of wild-type and mutant cells were spotted at each dilution. Plates were incubated at 30° unless otherwise indicated.

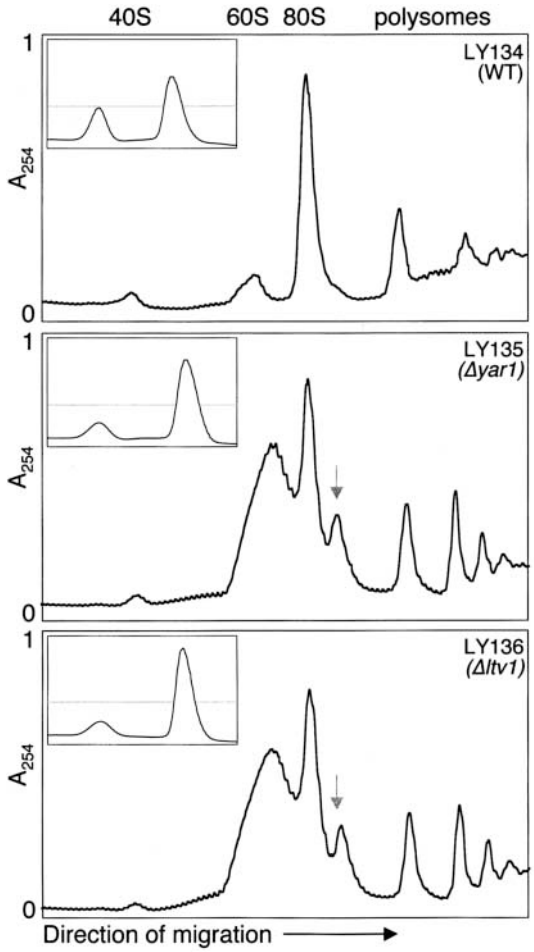

FIGURE 6.—Strains lacking *YAR1* and *LTV1* have altered polysome profiles. Strains containing chromosomal deletions of *YAR1* (LY135) and *LTV1* (LY136) and their isogenic wild-type parental strains were grown at 30° in rich medium and then fractionated by mechanical disruption and centrifugation. The postnuclear cytosolic homogenate was separated on continuous sucrose gradients either containing 5 mM $MgCl_2$ (full-size graphs) or lacking Mg^{2+} and containing 10 mM EDTA (insets). An RNA absorbance profile was determined using a continuous flow UV detector and is represented here in relative units. Only the portion of the gradient containing small subunits (40S), large subunits (60S), monomer ribosomes (80S), and small polysomes is shown. The arrow denotes the shoulder peak. The shaded line in the insets denotes the size of the wild-type small subunit peak.

Yar1 and Ltv1 and/or RpS3 might occur in the process of ribosome assembly. If so, we might expect Yar1 or Ltv1 to cosediment with ribosomes or preribosomal particles *in vivo*. To test this hypothesis, we epitope tagged each protein by integration of the epitope sequence at the appropriate chromosomal locus in different strains. Correct integration and synthesis of the fusion protein were confirmed in each strain by PCR and Western blot analysis. To generate a single strain (LY161) expressing both tagged proteins, we crossed the Yar1-HA strain to the Ltv1-Myc strain, sporulated the diploid, and selected for haploids with both fusion genes. We assessed the ability of each fusion protein to replace the function of the wild-type protein by testing these strains for slow growth and for stress sensitivity relative to wild-type and Δ*yar1* or Δ*ltv1* strains. The Yar1-HA and the Ltv1-Myc strains, as well as the LY161 strain with both tagged proteins, grew as well as wild-type cells at 30° and on plates containing sorbitol, indicating that the tag does not interfere with the function of either protein (data not shown).

The association of Yar1 and/or Ltv1 with ribosomal particles was assessed by sucrose gradient centrifugation and Western blotting (polysome blots) of extracts of the Yar1-HA Ltv1-Myc (LY161) strain. When whole-cell lysates are prepared under low-salt conditions, most of the Yar1-HA is present in the cytosolic fractions, although some Yar1 is also detected in denser fractions of the gradient in the 40S region (Figure 7). The opposite is true of Ltv1; while some Ltv1-Myc is detected at the top of the gradient, the majority comigrates with 40S ribosomal subunits (Figure 7). Intriguingly, Ltv1 does not comigrate with polysomes. This is in contrast to RpS2, which, as expected for a structural component of the mature small subunit, comigrates with both 40S particles and with translating ribosomes. These data suggest that Ltv1 associates with the 40S subunit, but is released some time prior to its incorporation into a translating ribosome.

***RPS3* is a high-copy suppressor of the Δ*yar1* phenotypes:** To identify other genes acting in the same pathway as *YAR1*, we carried out a screen for high-copy suppressors of the neomycin-sensitive (neo^S) phenotype of Δ*yar1* cells (see MATERIALS AND METHODS). We characterized 95 suppressors that exhibited galactose-dependent suppression of the neo^S phenotype, almost one-third of which turned out to be *RPS3*. *RPS3* was isolated 31 times in this screen and confirmed to be a full-length

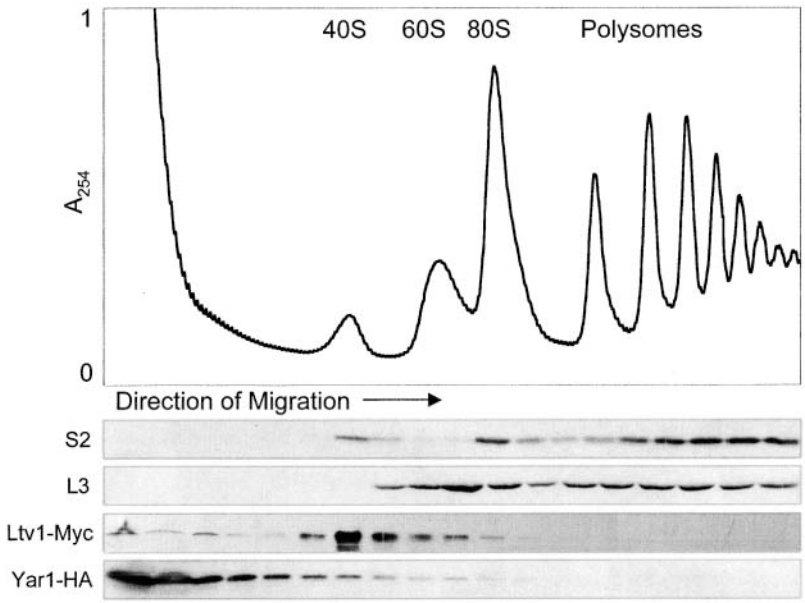

FIGURE 7.—Association of Yar1p and Ltv1p with ribosomal subunits. Wild-type cells expressing epitope-tagged Yar1 and Ltv1 fusion proteins (LY161) were cultured at 30° and fractionated to yield a postnuclear cytosolic homogenate. This was separated on a continuous sucrose gradient as in Figure 6. Individual gradient fractions were processed for SDS gel electrophoresis and Western blotting with antibodies to small (S2) and large (L3) ribosomal subunit proteins and the myc and hemagglutinin (HA) epitopes.

cDNA without mutations. This result supports the genetic interaction noted earlier between *YAR1* and *RPS3* and indicates that the translation defect in Δ*yar1* cells that is detected by aminoglycoside hypersensitivity can be alleviated by the overexpression of this ribosomal protein. This result places the function of the *YAR1* gene upstream of *RPS3*.

If the sensitivity of Δ*yar1* cells to protein synthesis inhibitors is a consequence of the defects noted in the 40S subunit assembly observed in mutant cells, then overexpression of RpS3 might be expected to alleviate the altered polysome profiles of Δ*yar1* mutants as well. To test this, we grew Δ*yar1* pGAL:*RPS3* cells in glucose or galactose selective media and analyzed the two cell lysates by sucrose gradient centrifugation. As shown in Figure 8A, Δ*yar1* cells grown on glucose exhibit a characteristically enlarged 60S peak and a large shoulder on the 80S monomer peak. Both of these aberrant peaks are missing in cells grown on galactose. Thus overexpression of *RPS3* suppresses both the neomycin sensitivity and the ribosome biogenesis defect of Δ*yar1* cells.

Cells lacking Yar1 are also hypersensitive to a number of environmental stress conditions. To explore further the link between the ribosome defects of Δ*yar1* mutants and their stress sensitivity, we tested whether overexpression of *RPS3* on galactose could suppress the sensitivity of Δ*yar1* cells to oxidative stress (Figure 8B) or osmotic stress (data not shown). Cells transformed with p*GAL:RPS3* grew as well as wild-type cells under all conditions. Suppression of the sensitivity in all cases was dependent on the presence of galactose. This surprising result suggests that it is unlikely that the multiple phenotypes of Δ*yar1* cells are due to multiple functions of Yar1. Rather, the ribosome defects and the stress-sensitive phenotypes are more likely directly linked, since overexpression of *RPS3* can alleviate both.

Because Δ*yar1* and Δ*ltv1* cells have very similar mutant phenotypes, we tested whether overexpression of pGAL:*RPS3* could also suppress the stress-sensitive phenotypes of Δ*ltv1*. In contrast to Δ*yar1* cells, overexpression of the pGAL:*RPS3* vector in Δ*ltv1* mutants did not alleviate the sensitivity of this strain to either neomycin or oxidative stress (Figure 8C). Furthermore, overexpression of *RPS3* did not suppress the polysome profile defect of Δ*ltv1* (Figure 8A). We also tested whether overexpression of *YAR1*, in cells transformed with a pGAL:YAR1cen vector that we constructed, could suppress the Δ*ltv1* stress-sensitive phenotypes. We did not observe any suppression (data not shown).

DISCUSSION

We characterize here a novel role for an ankyrin-repeat protein and uncover a putative new link between environmental stress and ribosome biogenesis. Yar1p interacts in a two-hybrid screen with two proteins associated with the small subunit of the ribosome, RpS3 and Ltv1. The Yar1:RpS3 interaction can be detected *in vitro* using proteins synthesized in *E. coli* or in mammalian cell extracts (Figure 1A), indicating that no other yeast proteins are required to mediate this interaction. While this suggests that the interaction is direct, we cannot exclude the possibility that a protein in the rabbit reticulocyte lysate used to synthesize RpS3 could be facilitating the interaction between Yar1 and RpS3. The biological relevance of the Yar1:RpS3 interaction is supported by evidence of genetic interaction. Reducing the *RPS3* gene dosage in diploids lacking both *YAR1* genes results in an enhanced stress-sensitive phenotype relative to Δ*yar1*/Δ*yar1* diploids or to heterozygous Δ*rpS3*/+ mutants (Figure 4). Furthermore, *RPS3* is a high-copy sup-

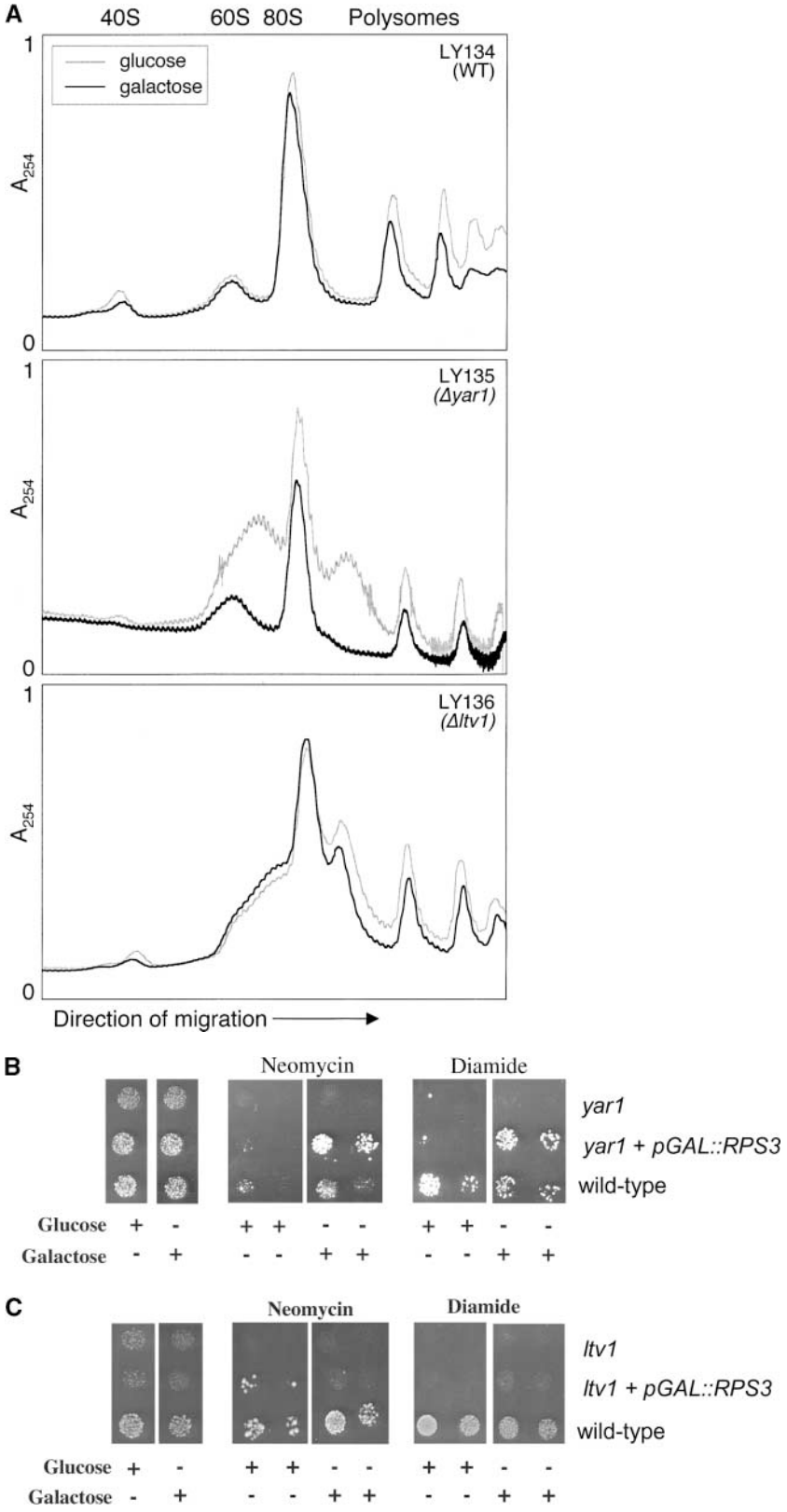

FIGURE 8.—Overexpression of *RPS3* suppresses the phenotypes of Δ*yar1* mutants. (A) Wild-type (LY134) cells transformed with pRS316 (SIKORSKI and HIETER 1989) and Δ*yar1* (LY135) and Δ*ltv1* (LY136) cells transformed with pGAL:*RPS3* were grown overnight at 30° in selective media including glucose and harvested by centrifugation. Half of the cells were resuspended in the same media, and half were resuspended in selective media including galactose. After a 7-hr incubation at 30°, cells were fractionated and the homogenate was centrifuged on continuous sucrose gradients, as described in Figure 6. The portion of the gradient containing small subunits (40S), large subunits (60S), monomer ribosomes (80S), and small polysomes is shown. Shaded lines indicate the glucose-grown samples, and solid lines indicate galactose-grown samples. (B) Δ*yar1* (LY-135) cells transformed with pGAL:*RPS3* or with pRS316 and wild-type (LY134) cells transformed with pRS316 were grown in selective medium to early log phase (OD_{660} 0.2–0.7). Equal numbers of each strain were plated in fourfold dilutions (left to right) on selective plates containing either glucose or galactose and neomycin (5 mg/ml) or diamide (1 mM). Plates were grown at 30° for 2–5 days and photographed. (C) Cells were grown and diluted as described in B except that Δ*ltv1* (LY136) cells transformed with pGAL:*RPS3* or pRS316 were used instead of Δ*yar1* cells.

pressor of both the neomycin hypersensitivity and the environmental stress sensitivity of Δ*yar1* cells (Figure 8).

The evidence that Ltv1 likely functions with Yar1 and RpS3 is several-fold. First, a two-hybrid interaction between Ltv1 and RpS3 has been reported by others (ITO *et al.* 2001), strengthening the link among these three proteins. Second, Δ*yar1* and Δ*ltv1* cells exhibit an almost identical spectrum of stress-sensitive phenotypes (Figure 3). Third, Δ*ltv1* is hypersensitive to the same spectrum of protein synthesis inhibitors as Δ*yar1* (Figure 5).

This links both *YAR1* and *LTV1* to ribosome function/assembly.

Both Δ*yar1* and Δ*ltv1* mutants exhibit clear defects in 40S-specific subunit production. The absolute number of 40S subunits is reduced in both mutants and the number of free 60S subunits is increased; the ratio of 40S:60S subunits declined by ~50% in both strains relative to wild-type strains. This decrease in the 40S:60S ratio is consistent with the fact that these deletion strains are viable. Two other viable ribosome biogenesis deletion mutants, Δ*mrt4* and Δ*loc1*, with defects in 60S subunit biogenesis, exhibit a similar (25–40%) decrease in the subunit ratio, in this case the 60S:40S ratio (Harnpicharnchai *et al.* 2001). The increase in free 60S subunits observed in Δ*yar1* and Δ*ltv1* cells has also been described for other 40S biogenesis mutants (Lee *et al.* 1992; Demianova *et al.* 1996; Baudin-Baillieu *et al.* 1997; Liu and Thiele 2001; Milkereit *et al.* 2003; Tabb-Massey *et al.* 2003) and has been ascribed to a paucity of functional small subunits with which large subunits may join. The hypersensitivity of Δ*ltv1* and Δ*yar1* mutants to protein synthesis inhibitors, including aminoglycoside antibiotics (Figure 5), is most easily understood as a consequence of the reduction in the number of functional ribosomes in these mutants; hypersensitivity to aminoglycosides has been previously noted in numerous other ribosome mutants, including three that are specifically defective in 40S subunit biogenesis (Lee *et al.* 1992; Kressler *et al.* 1997; Liu and Thiele 2001).

Ltv1 is a conserved protein, which was recently identified as a minor component copurifying with 40S preribosomal complexes purified by tandem affinity purification tagging three different nonribosomal proteins (Schafer *et al.* 2003). The association of Ltv1 with pre-40S complexes is consistent with our identification of Ltv1 in polysome blots in the 40S region of the gradient. It is noteworthy that we do not detect Ltv1 in the 80S or polysome region of the sucrose gradient, which suggests that the protein is released from the 40S subunit at some point prior to the initiation of translation. Rio2, a nonribosomal protein that is required for maturation of a pre-40S particle, exhibits a cosedimentation profile essentially identical to that of Ltv1 (Vanrobays *et al.* 2003).

Strains lacking *YAR1* exhibit the same ribosome biogenesis defects as cells lacking *LTV1*. Yar1 binds strongly with RpS3 in the two-hybrid and *in vitro*, yet it is only very weakly or transiently associated with 40S ribosomal subunits and has not been identified as a component of any preribosomal complex (Schafer *et al.* 2003). It may be that Yar1 interacts mostly with free RpS3, perhaps at a point prior to its incorporation into ribosomes. Since *RPS3* overexpression can suppress the ribosome biogenesis defect of Δ*yar1* mutants, perhaps *YAR1* functions as an RpS3 "chaperone," regulating its stability, nuclear import, or incorporation into ribosomes in the nucleolus. Whether RpS3 itself has any direct role in ribosome assembly will require further investigation. However, it is increasingly clear that ribosomal proteins have multiple functions and may figure in other aspects of ribosome function in addition to translation itself. For example, the RpS0 proteins and RpS14 have been shown to be required for the maturation of the 40S subunit (Ford *et al.* 1999; Jakovljevic *et al.* 2004) and RpS15 is required for the nuclear exit of 40S preribosomal complexes (Leger-Silvestre *et al.* 2004).

Strains lacking either Yar1 or Ltv1 are stress sensitive, as well as exhibiting defects in ribosome biogenesis. Since ribosome biogenesis is closely regulated by nutrient availability and by environmental stresses (Warner 1999; Gasch *et al.* 2000), one hypothesis is that Yar1 and Ltv1 function to link ribosome biogenesis to stress-response signaling. The Yar1 protein seems especially well suited to a regulatory role, being composed essentially of a protein interaction domain with a strong potential PEST element in the C terminus and two potential casein kinase II recognition sites (Lycan *et al.* 1996). However, an alternative hypothesis is that the stress-sensitive phenotypes of Δ*yar1* and Δ*ltv1* mutants are the consequence of a primary defect in ribosome biogenesis. At least for *YAR1*, the simplest interpretation of our data would support the latter interpretation. Overexpression of *RPS3* in Δ*yar1* mutants suppresses both the ribosome biogenesis phenotype and the stress-sensitive phenotypes. This suggests that Yar1 does not have multiple functions, but that it primarily affects the expression or function of RpS3. Whether ribosomes made in the absence of Yar1 cause cells to be especially sensitive to stress conditions, or whether it is simply the reduction in the number of functioning ribosomes that makes cells stress sensitive is unknown as yet. Many cold-sensitive strains in *E. coli* have defects in ribosome subunit assembly (Guthrie *et al.* 1969) and numerous yeast strains with mutations in genes for ribosome proteins or *trans*-acting factors involved in rRNA processing are cold sensitive (Venema and Tollervey 1999). Likewise many mutants in ribosome function/biogenesis are aminoglycoside sensitive and slow-growing under normal conditions (Kressler *et al.* 1999). However, Δ*yar1* and Δ*ltv1* strains display the additional phenotypes of sensitivity to osmotic and oxidative stress, which have not been reported for other ribosome biogenesis mutants. While viable knock-out strains with defects in ribosome biogenesis are rare, we did obtain two, Δ*mrt4* and Δ*loc1*, with fewer functional ribosomes similar to Δ*yar1* and Δ*ltv1*, but in this case due to a reduction in 60S subunit production (Harnpicharnchai *et al.* 2001). Both these mutants are slow growing at 30° and cold sensitive and sensitive to neomycin, but only one of the two was sensitive to osmotic stress or oxidative stress (our unpublished observations). This suggests that the link between ribosome biogenesis and oxidative/osmotic stress sensitivity is at least complex and deserves further investigation.

Our results clearly indicate that Yar1 and Ltv1 play a role in both the biogenesis of 40S ribosomal subunits and the ability of yeast cells to adapt to various stress conditions. We are currently exploring the molecular basis of the link between these two phenotypes.

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