Yeast Proteins Related to the p40/Laminin Receptor Precursor Are Essential Components of the 40 S Ribosomal Subunit*

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We report here the isolation of two genes from the yeast, Saccharomyces cerevisiae, that encode proteins closely related to mammalian p40/laminin receptor precursors (LRPs). The yeast genes, designated YST1 and YST2, encode proteins with over 95% amino acid sequence identity with one another and over 60% identity with the human p40/laminin receptor precursor. The Yst/p40/37-LRP proteins are also more distantly related to the S2 family of ribosomal proteins. Analysis of the distribution of Yst1 tagged with the c-myc epitope revealed that the Yst proteins are components of the 40 S ribosomal subunit. Disruption of either YST1 or YST2 causes a significant reduction in growth rate, while disruption of both genes is lethal. Compared to wild type, polysome profiles in strains lacking either YST1 or YST2 show a pronounced shift from larger to smaller polysomes. This shift is accompanied by a substantial increase in free 60 S subunits and reduced levels of 40 S subunits. We conclude that the Yst proteins are required for translation and contribute to the assembly and/or stability of the 40 S ribosomal subunit.

A cDNA originally reported to encode the 67-kDa high affinity laminin receptor has also been implicated in the production of an abundant intracellular protein of approximately 37 kDa that is highly conserved in a wide spectrum of eukaryotic cells (1–3). The relationship between these two proteins is unclear but it has been proposed that a fraction of the intracellular pool of the 37-kDa protein may serve as a precursor for the 67-kDa laminin receptor (4, 5). The cDNA encoding the 37-kDa laminin receptor precursor (37-LRP)1 is virtually identical to a cDNA encoding a mouse protein, p40, initially identified in a screen for mRNAs under translational control in ascites tumors (6, 7). A cDNA encoding p40 was also shown to encode an antigen that shows regional specificity in developing mice retinas (8). This antigen appears to be a conformational isomer of intracellular p40 that has been proposed to play a role in defining the dorsal/ventral axis in developing retinas (9). Finally, a gene encoding a Drosophila homolog of p40 was shown to complement mutations at the subbarista locus (10). Certain mutant alleles of Drosophila p40 are zygotic lethals that have been shown to affect oogenesis and imaginal disc development. Together, these data indicate that the 67-kDa laminin receptor may be derived from an abundant intracellular protein, p40/37-LRP, and that p40/37-LRP proteins, apart from their potential role as precursors for laminin receptors, may play important roles in early stages of metazoan development.

Several lines of evidence suggest that p40/37-LRP proteins are components of the protein synthetic machinery. Mammalian, Arabidopsis, and Urechis caupo p40 proteins are polysome associated and appear to be preferentially associated with 40 S ribosomal subunits (3, 11–13). The distribution of p40 proteins between free and polysome-associated states has been shown to depend on the age, growth stage, or developmental state of the cells examined (3, 11, 13). In addition to the physical association of p40 with polysomes, genetic studies in Drosophila suggest that p40 may be a component of the translational machinery. Phenotypes associated with certain alleles of the Drosophila p40 locus are similar to minute phenotypes that are often associated with genes encoding ribosomal components (10). Finally, Davis et al. (14) showed that p40/37-LRP proteins are structurally related to the S2 family of ribosomal proteins. This relationship has been further strengthened by the identification of an archaeabacterial member of this family whose sequence is approximately equidistant in terms of similarity between the eubacterial/organellar S2 proteins and the eukaryotic p40/37-LRP proteins (15).

We report here the isolation of two genes from the yeast, Saccharomyces cerevisiae, that encode proteins closely related to the p40/37-LRP family of proteins. The yeast genes, designated YST1 and YST2, encode proteins that exhibit over 95% sequence identity with each other, over 60% sequence identity with mammalian p40/37-LRP proteins, and approximately 30% sequence identity with members of the S2 family of ribosomal proteins. Epitope-tagged Yst1 cosediments with 40 S ribosomal subunits, 80 S monosomes, and polysomes during sucrose gradients centrifugation indicating that the Yst proteins are small subunit ribosomal proteins. Disruption of either YST1 or YST2 shows a reduction in growth rate compared to wild type. Cells disrupted in both YST1 and YST2 fail to germinate indicating that Yst function is essential. Relative to wild type, polysome distributions in cells lacking one or the other of the Yst genes show a pronounced shift from larger to smaller polysomes. This shift to smaller polysomes in mutant extracts is accompanied by a substantial increase in free 40 S ribosomal subunits and a reduction in the level of free 40 S subunits. The Yst proteins are therefore required for translation and contribute to the assembly and/or stability of 40 S ribosomal subunits.

MATERIALS AND METHODS

Yeast and Bacterial Strains—The yeast strains used in this study were W303 [MATa MATαΔa-1 ade2-1 ade2-1, can1-100/can1-100, his3-11, 15/ his3-11, 15, ura3-1/ura3-1, leu2-3, 112/leu2-3, 112, trp1-

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1 The nucleotide sequences reported in this paper have been submitted to the GenBankTM/EMBL Data Bank with accession number(s) M88277 (YST1) and U33756 (YST2).

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3 The abbreviations used are: 37-LRP, 37-kDa laminin receptor precursor; bp, base pair(s); kb, kilobase pair(s).
The clear upper phase containing proteins was dialyzed two times.

The Yst Proteins Are Essential Components of 40 S Subunits

Isolation of DNA-binding Proteins—DNA-binding proteins were isolated from a crude nuclear fraction of the yeast S. cerevisiae and used to prepare antibodies. A 2-liter culture of strain 7208-12 was grown to 107/ml in YM-1, and the cells were collected by centrifugation (10 min at 6,000 × g). The 6.4-g cell pellet was suspended in 20 ml of Tris-HCl (pH 9.1), 20 mM Na3EDTA, 1 mM NaCl, 1 mM 2-mercaptoethanol, and incubated for 10 min at room temperature. The cells were collected by centrifugation (5 min at 4,000 × g), and suspended in 40 ml of 40 mM glycerol, 1 mM KH2PO4, 34 mM sodium citrate (pH 5.8). The cells were collected again, suspended in 40 ml of glusulase buffer (10% (w/v) glycerol, 1 mM sorbitol, 42 mM KH2PO4, 8 mM sodium citrate (pH 5.8), collected, and suspended in 10 ml of glusulase buffer. 0.3 ml of glusulase solution (105,000 units/ml glucuronidase, 11,500 units/ml sulfatase; DuPont NEN) was added to the suspension, and the mixture was incubated 1 h at 30 °C. The cells were collected by centrifugation for 5 min at 2,000 × g, gently resuspended in 20 ml of glusulase buffer, and collected again. The washed spheroplasts were suspended in 20 ml of 2 mM MgCl2, 0.2% (v/v) Triton X-100, 40 mM Tris-HCl, pH 7.4, 1 mM 2-mercaptoethanol, 5% (w/v) Ficoll 400, 0.5 mM leupeptin, 0.7 μg/ml pepstatin, 0.5 μM phenylmethylsulfonyl fluoride) and lysed with five strokes in a Dounce homogenizer on ice. The suspension was centrifuged for 10 min at 11,000 × g; the pellet consisted of cellular debris including nuclei and other organelles. This material was extracted with 10 ml of 50 mM Tris-HCl, pH 7.4, 2 mM Na3EDTA, 1 mM 2-mercaptoethanol, 2.5 mM NaCl, 0.5 μg/ml leupeptin, 0.7 μg/ml pepstatin, and 0.5 μM phenylmethylsulfonyl fluoride, then disrupted with 10 strokes in a Dounce homogenizer on ice. The suspension was centrifuged for 20 min at 16,000 × g. 1.75 g of NaCl, 0.66 g of polyethylene glycol 8000, and 0.44 g of dextran 500 were added to 12.5 ml of the supernatant and mixed gently for 1 h at 4 °C. The mixture was centrifuged for 10 min at 11,000 × g to separate the phases containing protein and nucleic acids. The clear upper phase containing proteins was dialyzed two times against 2 liters of each time of DC0.05, then used to inoculate rabbits (two to three injections with 0.3–0.5 mg each injection). Antisera were collected and used to screen a library of yeast genomic sequences in phagemid (provided by M. Snyder, Yale University). The clone containing the YST1 gene was recovered and used to purify the epitope-tagged Yst1 protein is functional. The epitope-

Cloning and Sequencing the Yeast YST1 and YST2 Genes—The DNA-binding protein fraction was dialyzed into DC0.05, then used to inoculate rabbits (two to three injections with 0.3–0.5 mg each injection). Antisera were collected and used to screen a library of yeast genomic sequences in phagemid (provided by M. Snyder, Yale University). The clone containing the YST1 gene was recovered and used to purify the epitope-tagged Yst1 protein.

To examine the effect of the disruption of both YST1 and YST2, the diploid strain heterozygous for yst1Δ::URA3 (HIS3) was transformed with the yst1Δ::URA3 DNA fragment derived from pJ10. Transformants were selected by uracil prototrophy and sporulated, and the resulting tetrads were dissected by micromanipulation. Spores were germinated on YPD medium and analyzed for the YST1 disruption by Southern blot hybridization.

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Mapping YST1 and YST2—The YST1 gene was shown by meiotic analysis to be linked to the ADE3 gene on the left arm of chromosome VII (56 tetrads; ADE3::C-Mser2::L-mCYT2). Hybridization of yeast genomic clone grids confirmed this location and indicated that YST2 is on chromosome XII near SPT8 (YST1 hybridizes to ATCC clone 70361, YST2 hybridizes to clone 70582).

Polysome Analyses—Polysomes were prepared from yeast cell extracts and fractionated on a 7–47% sucrose gradient as described by Baim et al. (22). Centrifugation was for either 5 or 12 h. The longer time was necessary to resolve S ribosomal subunits from soluble components at the top of the gradient. Conditions used to prepare yeast cell extracts under high salt or low magnesium conditions were described by Foiani et al. (23). Macronuclei in the polysome fractions were probed with 32P-labeled DNA containing the 10-amino acid sequence EQKLISEEDL recognized by the monoclonal antibody 9E10 (25). A 2.7-kb BamHI/SalI fragment containing the YST1-c-myc gene was cloned into the yeast shuttle vector pRS315 (26). This construct complements phenotypes linked to the disruption of either YST1, YST2, or both genes indicating that sufficient 5′- and 3′-flanking regions from the YST1 reading frame were included for expression in yeast and that the epitope-tagged Yst1 protein is functional. The epitope-

The YST2 gene was isolated using a radiolabeled fragment of the YST1 gene to probe a yeast genomic library (American Type Culture Collection no. 37323). The YST2 gene was subcloned into Bluescript II KS in two fragments: a 1,500-bp HindIII/HindIII 5′ fragment and a 2,100-bp HindIII/SalI 3′ fragment creating plasmid pMD12. The junction between these two fragments was sequenced in the original clone to assure that they were contiguous. The YST2 gene was subcloned for sequencing by creating nested sets of deletions using an Exo III/mung bean nuclease restriction enzyme kit from SPT8. This gene was subcloned through selecting restriction enzyme sites. Sequencing was carried out by the chain termination method of Sanger et al. (19). This sequence has been deposited in GenBankTM originally under the name NAB1B and has since been changed to YST2 (accession no. U33756).

Disruption of YST1 and YST2—The YST1 gene located on a 2.7-kb BglII fragment of yeast genomic DNA was subcloned into Bluescript II KS at the unique EcoRI site. The plasmid with the 5′ end of the YST1 gene oriented toward the Sad site in Bluescript KS was designated pJ8 and the reverse orientation, pJ9. The pJ8 plasmid was digested with BsmI and BstEII, liberating a fragment of 951 bp containing 88% of the YST1 open reading frame. This fragment was replaced by a HindIII fragment containing the yeast URA3 gene after filling in to produce blunt ends on all fragments (designated pJ10). This construct produces an allele denoted yst1Δ-1::URA3. pJ10 was digested with EcoRI and SnaBI, liberating a fragment of approximately 3.0 kb. The fragment released contains the URA3 gene flanked by approximately 1,000 bp of YST1 sequence at its 5′ end and 413 bp at its 3′ end. The EcoRI/SnaBI fragment was used to transform the diploid strain W303-1A prototrophic for uracil or histidine and confirmed when possible by Southern hybridization.
Isolation and Characterization of Yeast YST Genes—In an effort to identify proteins involved in DNA metabolism in the yeast *S. cerevisiae*, a general class of DNA-binding proteins was isolated and used as a heterogeneous group of antigens. The antibodies produced were used to screen an expression library to identify the genes encoding the DNA-binding proteins. One of these genes, which we originally called *NAB1* (Nucleic Acid Binding protein 1) and subsequently changed to *YST1* (Yeast S Two) was found to encode a 30-kDa protein that was enriched in the low speed centrifugation pellet after hypotonic lysis of spheroplasts. This fraction contains large, insoluble portions of cells, including cell walls, nuclei, and mitochondria. Under the gentle extraction conditions used for the preparation of nuclei, a substantial number of ribosomes are detected in the crude nuclear fraction as judged by the presence of a large number of small basic proteins observed by two-dimensional gel electrophoresis (data not shown).

The nucleotide sequence of *YST1* revealed two adjacent open reading frames separated by a region containing consensus 5'-donor, lariat, and 3'-acceptor sequences, suggesting a gene interrupted by a single intron (Fig. 1, top). The inferred spliced message encodes a protein of 252 amino acids with a predicted molecular mass of 28 kDa. A deletion of the second exon of the *YST1* gene was constructed and introduced into diploid yeast cells. This mutation is marked with the *URA3* gene and should prevent expression of 88% of *YST1* open reading frame (Fig. 1, top). A fragment containing the disrupted *YST1* gene was transformed into the diploid strain W303 and transformants selected by uracil prototrophy. Transformants were sporulated and the resulting tetrads dissected. Spores prototrophic for uracil grew more slowly than the uracil auxotrophs indicating that disruption of the yeast *YST1* gene conferred a modest reduction in growth rate (data not shown, but see Figs. 3 and 5).

DNA and RNA hybridization analyses indicated that *YST1* is not unique and suggested that cells with the disrupted *YST1* allele were expressing Yst protein from a second locus (data not shown, but see Fig. 4). Using a *YST1* fragment as a hybridization probe, we cloned a second gene closely related to *YST1*. The second gene also has an open reading frame capable of coding for a protein of 252 amino acids that appears to be interrupted with a single intron located at the same relative position as the intron in the *YST1* gene (Fig. 1, bottom). The two genes show over 90% sequence identity at the nucleotide level and 95% identity in deduced amino acid sequence (Fig. 2).

We have named the second locus *YST2*. A search of the GenBank™ data base indicated that the Yst proteins are closely related to the p40/37-LRP family of proteins (27). The Yst proteins are also more distantly related to the S2 family of ribosomal proteins found in eubacteria and organelles, suggesting that Yst/p40/37-LRP proteins are eukaryotic members of the S2 family of proteins. Members of the S2 family have also been identified in archaeabacteria, indicating that the S2 family of proteins evolved prior to the divergence of the three major lines of descent (15). Fig. 2 shows the alignment of the Yst proteins with the human p40/37-LRP protein and the yeast mitochondrial ribosomal protein Mrp4. The Yst proteins show over 60% sequence identity with human p40/37-LRP over 201 amino acids spanning the bulk of the three proteins (Fig. 2). The Yst and p40/37-LRP proteins diverge at their amino and carboxyl termini where the human protein also has a carboxyl-terminal extension of 42 amino acids. Fig. 2 also shows the alignment of the Yst/p40/37-LRP proteins with Mrp4, a member of the S2 family of ribosomal proteins (14). The Mrp4 protein has approximately 30% sequence identity with at least one of the other proteins shown in Fig. 2. Overall, the four proteins have 23% sequence identity. This level of identity is similar to comparisons between other homologous ribosomal proteins found in both eubacteria and eukaryotes (28).

We have disrupted *YST2* alone and in combination with the *YST1* disruption. The bulk of the *YST2* reading frame was deleted and replaced by the *HIS3* gene. A fragment containing the disrupted *YST2* gene was transformed into the diploid yeast strain W303 and transformants were selected by histidine prototrophy. Transformants were sporulated and the resulting tetrads dissected. Histidine prototrophy segregated with a slow growth phenotype indicating that just as for *YST1*, disruption of *YST2* is tolerated, but has an impact on growth rate (data not shown, but see Figs. 3 and 5).

The diploid yeast strain heterozygous for the *YST2* disruption was transformed with a disrupted copy of *YST1* to assess the effects of the disruption of both *YST* genes. Transformants...
were sporulated and tetrads dissected. Representative results from the growth of individual spores on YPD are shown in Fig. 3. Examination of colony size in spores from tetrads 2, 5, 6, and 7 suggests four distinct growth rates. The fastest growing spores were auxotrophic for both uracil and histidine, suggesting that they contained wild-type \( YST1/2 \) alleles. The two intermediate sized colonies were prototrophic for either uracil or histidine. The larger colony was prototrophic for uracil and auxotrophic for histidine, indicating that it was disrupted in \( YST1 \) but wild-type for \( YST2 \). The smaller colony was prototrophic for histidine and auxotrophic for uracil indicating that it contained a disrupted allele of \( YST2 \) and a wild-type allele of \( YST1 \). The fourth spore in each of these tetrads did not germinate. The inviable spores were presumably disrupted in both \( YST1 \) and \( YST2 \), suggesting that disruption of both \( YST \) genes is lethal. Tetrad 4, on the other hand, gave two colonies and two nonviable spores. In this tetrad, the cells that grew were both auxotrophic for uracil and histidine, indicating they had wild-type alleles of \( YST1/2 \). We assume that the nonviable spores were disrupted in both \( YST \) genes. Tetrad 1 and 3 showed four spores with intermediate growth rates. In tetrad 1 the four viable spores were auxotrophic for either uracil or histidine, indicating that they were disrupted in one or the other \( YST \) gene. One of the colonies derived from tetrad 3 was prototrophic for both uracil and histidine, but was eventually shown to be a mixed population of cells. Overall, the examination of 124 spores revealed no viable double mutants, whereas 31 would have been expected by chance.

The segregation pattern of the \( YST1 \) and \( YST2 \) alleles in Fig. 3 suggested that the two genes were unlinked. This was confirmed by mapping the \( YST1 \) and \( YST2 \) genes either genetically or by hybridization to a genomic clone grid library. \( YST1 \) hybridized to ATCC clone 70361 and maps near \( ADE3 \) on the left arm of chromosome VII, whereas \( YST2 \) hybridized to ATCC clone 70582 and maps near \( SPT8 \) on chromosome XII (data not shown).

Disruption of the \( YST \) genes was confirmed by Northern hybridization analysis. Northern analysis revealed that the two \( YST \) genes encode mRNAs that differ in size by approximately 150 bases with the \( YST2 \) gene encoding the larger of the two mRNAs (Fig. 4). The observation that in wild-type cells the hybridization signals for the two mRNAs are comparable even though the probe was derived from \( YST1 \) suggests that the steady-state levels of \( YST2 \) mRNAs might be somewhat higher than for the \( YST1 \) mRNA. This might explain why the disruption of \( YST2 \) causes a more severe reduction in growth rate than does the disruption of \( YST1 \); the Yst proteins are functionally equivalent, but \( YST2 \) makes a greater contribution to the pool of Yst molecules. Alternatively, since the two Yst proteins differ in primary structure in several positions it is possible that the two proteins may only partially overlap in function or both proteins may also have unique functional characteristics that contribute differentially to growth rate.

To examine the extent to which the functions of the two Yst proteins overlap, we asked whether each of the \( YST \) genes were sporulated and tetrads dissected. Representative results from the growth of individual spores on YPD are shown in Fig. 3. Examination of colony size in spores from tetrads 2, 5, 6, and 7 suggests four distinct growth rates. The fastest growing spores were auxotrophic for both uracil and histidine, indicating that they were disrupted in one or the other \( YST \) gene. One of the colonies derived from tetrad 3 was prototrophic for both uracil and histidine, but was eventually shown to be a mixed population of cells. Overall, the examination of 124 spores revealed no viable double mutants, whereas 31 would have been expected by chance.

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could complement phenotypes linked to the disruption of the other. Strains harboring disrupted alleles of either YST1 or YST2 were transformed with wild-type copies of either YST1 or YST2 cloned into the low copy number vector pRS315 (26). Fig. 5 shows that plasmid-borne copies of YST1 or YST2 are able to complement the growth defects associated with disruptions in either gene. These data suggest that the two Yst proteins have largely overlapping functions during growth on rich media. However, we cannot rule out the possibility that the two proteins may have distinct functions under growth conditions that were not examined here.

Distribution of Epitope-tagged Yst1 in Cell Extracts Fractionated by Sucrose Gradient Centrifugation—Mammalian, Arabidopsis, and U. caupo p40/37-LRP proteins have been shown to be polysome-associated and preferentially associated with 40 S ribosomal subunits (3, 11–13). To determine if the Yst proteins had a similar distribution, we examined the distribution of epitope-tagged Yst1 in cell extracts fractionated by sucrose gradient centrifugation. Yst1 was tagged with the human c-myc epitope, which is recognized by the monoclonal antibody 9E10, as described under “Materials and Methods.” The epitope-tagged Yst1 complements phenotypes linked to the disruption of either or both YST genes indicating that it is functional (data not shown). Fig. 6, lane 2, shows that the 9E10 antibody recognizes a protein with an apparent molecular mass of approximately 30 kDa in YST1-disrupted cells transformed with epitope-tagged YST1 on a low copy number plasmid. This protein is of the size predicted for epitope-tagged Yst1. A weaker signal in this size range is found in extracts from cells transformed with YST1 alone (Fig. 6, lane 1). This weak signal corresponds to an abundant protein in whole cell extracts that cross-reacts with either the primary or secondary antibody. When cell extracts are fractionated by differential centrifugation, virtually all of the epitope-tagged Yst1 is found in the ribosomal pellet (lane 4), whereas the abundant cross-reacting protein is localized to the soluble fraction (lane 3).

To analyze the distribution of epitope-tagged Yst1 among ribosomal components, extracts were fractionated by sucrose gradient centrifugation. Fig. 7, panel A, shows that the epitope-tagged Yst1 is found in several regions of the gradient. The bulk of the epitope-tagged Yst1 is distributed in a broad peak that coincides with polysomes. Epitope-tagged Yst1 is also found in a peak that coincides with 80 S monosomes. Finally, there appears to be a small amount of epitope-tagged Yst1 in a region of the gradient that coincides with 40 S subunits. In this gradient 40 S subunits appear as a shoulder to the main absorbance peak which corresponds to the soluble fraction. To better evaluate the apparent association of epitope-tagged Yst1 with 40 S subunits, gradients were run for a longer time to separate the small ribosomal subunits from the soluble fraction. Fig. 7, panel B, shows after the longer centrifugation time 40 S subunits are clearly resolved from the soluble fraction and that the epitope-tagged Yst1 protein cosediments with 40 S subunits. Signals found in soluble fractions appear to be from the abundant cross-reacting proteins although we cannot rule out a small amount of the epitope-tagged Yst1 protein in this region of the gradient.

Loss of Yst Proteins Alter Polysome Profiles—While several studies have reported that p40/37-LRP proteins are associated with ribosomal components, none of these studies addressed
whether they are necessary for protein synthesis. To determine if the loss of yeast Yst protein influences translation rates we examined polysome distributions in strains disrupted in either YST1 or YST2. Fig. 8, panel A, shows that polysome profiles from the strain disrupted in YST2 differs substantially from wild type. Relative to wild type, there is a shift from larger to smaller polysomes. Similar results were obtained for strains disrupted in YST1 but they were less pronounced than with YST2 (data not shown). In addition to the shift in polysomes, extracts from the disrupted strains also have higher steady-state levels of 60 S ribosomal subunits. Assessment of 40 S subunit levels in these gradients is complicated since they run as a shoulder to the major absorbance peak at the top of the gradient. Therefore, gradients were run for longer periods of time to resolve 40 S subunits. Fig. 8, panel B, shows that the amount of free 40 S subunits are reduced in extracts from the strain disrupted in YST2 relative to wild type. However, the 60 – 80 S region of this gradient shows a rather unusual shape with a shoulder between the 60 and 80 S peaks. This shoulder may be the consequence of the sedimentation-induced dissociation of 80 S monosomes (29). Foiani et al. (23) have shown that inactive 80 S couples can be distinguished from 80 S couples engaged in translation by their sensitivity to dissociation by high salt. Fig. 8, panel C, shows gradients run for extended periods of time in high salt. Under these conditions, 40 S subunits can be detected in extracts from disrupted strains but are still reduced in amount relative to wild-type extracts. To more specifically address the overall reduction in 40 S subunits in the mutant strains, extracts were prepared and gradients run under conditions of low magnesium ion concentration where polysomes and 80 S monosomes dissociate into 40 and 60 S subunits (23). Fig. 8, panel D, shows that relative to wild type 40 S subunits in extracts from strains disrupted in YST2 are reduced by 20 to 35%.

DISCUSSION

We report here the isolation and characterization of two yeast genes, YST1 and YST2, that encode members of the S2 family of ribosomal proteins and are homologous to genes encoding p40/37-LRP proteins in a number of eukaryotic organisms. Yst/p40/37-LRP proteins are highly conserved, exhibiting over 60% sequence identity between yeast and humans. The mammalian p40/37-LRP proteins have been implicated in diverse processes, from playing a role in defining the dorsal/ventral axis in the developing mouse retina (9) to serving as a precursor for the 67-kDa laminin receptor (4). Despite their importance, little is known regarding the specific function of p40/37-LRP proteins in these processes. The results reported here demonstrate that the yeast Yst proteins are essential components of 40 S ribosomal subunits. In addition, we show that these proteins are required for translation and contribute to the assembly and/or stability of the 40 S subunit.

Based largely on observations that p40/37-LRP proteins co-sediment with ribosomal components during fractionation through sucrose gradients, several studies suggested that p40/37-LRP proteins from mammals and plants were components
of the translational machinery (3, 11–13). In these experiments, the p40/37-LRP proteins were found in fractions containing polysomes and in earlier regions of gradients containing monosomes and individual ribosomal subunits. In addition, a significant amount of the total p40/37-LRP protein was found in fractions containing soluble proteins. Treatments that disrupted polysomes in Arabidopsis and mammalian extracts led to somewhat different conclusions regarding localization of p40/37-LRP protein. The Arabidopsis p40 protein appeared to be preferentially associated with 40 S subunits after polysome disruption, while the mammalian p40 protein appeared to be distributed in particles larger and more heterogeneous than 40 S subunits. While the nature of these larger particles was unclear, at least a fraction of the mammalian p40 protein appears to be associated with 40S subunits, since Tohgo et al. (12) showed that purified preparations of mammalian 40 S subunits contained p40/37-LRP protein.

Consistent with the view that members of the Yst/p40/37-LRP family of proteins are ribosomal components is the observation that the YST genes share two properties with genes known to encode ribosomal proteins in yeast. First, the yeast genome contains two virtually identical YST genes. While redundant genes are generally uncommon in S. cerevisiae, almost half of the ribosomal proteins are encoded by duplicated genes (30). Second, the YST genes each appear to contain an intron, another phenomenon that is relatively rare in yeast but prevalent in genes encoding ribosomal proteins. While these characteristics are not unique to genes encoding ribosomal proteins, they are consistent with a role in translation (31).

Both physical and functional properties of the Yst proteins also indicate that they are ribosomal components. Garrels et al. (32) found that Yst proteins are abundant in yeast whole cell extracts. Moreover, they reported that the Yst proteins were physically associated with ribosomes. Our data confirm this association and extend it by showing that the Yst proteins are components of the 40 S ribosomal subunit. Furthermore, our studies are the first to demonstrate that the association of a member of the Yst/p40/37-LRP with ribosomes is of functional importance rather than a fortuitous association. Disruption of either of the YST genes has a pronounced effect on polysome profiles. Relative to wild type, strains lacking one or the other of the Yst proteins have fewer 40 S subunits and polysomes but show a pronounced increase in the level of free 60 S subunits. Similar effects on the relative amount and distribution of ribosomal subunits have been reported for disruptions in genes coding for other small subunit ribosomal proteins in yeast (33–35). The reduction in 40 S subunits and polysomes seen in strains disrupted in either YST1 or YST2 is physiologically relevant, since these strains have decreased growth rates relative to wild type and cells lacking both YST genes are inviable.

García-Hernández et al. (3) have pointed out that the Arabidopsis p40/37-LRP protein has certain characteristics in common with the acidic class of ribosomal proteins. These properties include an acidic isoelectric point, distribution between ribosome-associated and soluble states, and physiological and developmental control over the distribution of p40/37-LRP protein between these two states. Similar properties have also been reported for the mouse and U. caupo p40 proteins (11, 13). Like their counterparts, the Yst proteins have acidic isoelectric points: Yst1 = 4.67, Yst2 = 4.7 (32). In contrast to the results reported for mammalian, U. caupo, and Arabidopsis p40/37-LRP proteins, there did not appear to be a significant fraction of soluble Yst proteins in yeast cell extracts. However, we examined the distribution of Yst proteins only in extracts from log phase cells, so it is possible that, under other growth conditions, the amount of soluble Yst protein may be different. In this regard it is worth noting that the distribution of Arabidopsis p40/37-LRP protein between soluble and ribosome-associated states has been reported to be influenced by growth parameters; young, actively growing cell cultures contain...
relatively low amounts of soluble protein compared with older cultures (3). Clearly, more studies are necessary in yeast cells before a definitive statement can be made regarding the distribution of Yst proteins between soluble and ribosome-associated states.

Members of the acidic class of ribosomal proteins are widely distributed in nature and are found in multiple copies in large ribosomal subunits. These proteins have been linked to the ribosomal GTPase center and are important for the association of soluble factors with ribosomes (36, 37). Some of these proteins, while not absolutely required for protein synthesis, have been shown to promote optimal ribosome function both in vivo and in vitro (37). The P0 acidic protein, in contrast, is essential (38). P0 appears to mediate the interaction of the other members of the acidic class of ribosomal proteins with subunits and may also be necessary for other aspects of 60 S function.

While the Yst/p40/37-LRP proteins have certain characteristics in common with the acidic class of ribosomal proteins, there are also substantial differences. In contrast to the acidic proteins of the 60 S subunit, the Yst/p40/37-LRP proteins are components of the 40 S subunit. Moreover, unlike the acidic proteins of the 60 S subunit that play a key role in elongation, our results indicate that the Yst proteins likely influence initiation rather than elongation rates, since we see a dramatic shift to smaller polysomes in strains disrupted in the YST genes. While part of this decrease in initiation is likely the result of a reduction in 40 S subunits, 40 S subunits that are not polysome-associated in the disrupted strains have distinctive properties. These properties may be a consequence of sedimentation induced dissociation of 80 S complexes. The results reported here showing that the Yst proteins bind to a DNA cellulose column are consistent with the possibility that they may be nucleic acid binding proteins, lending support to the hypothesis that members of the p40/37-LRP family of proteins may contribute to the function of the 67-kDa laminin receptor via their ability to interact with nucleic acids. Recent observations indicate that the recruitment of members of the Yst/p40/37-LRP family to the cell surface to function as laminin receptors is not restricted to mammals and that this may be an important route by which pathogenic fungi such as Candida albicans and Pneumocystis carinii interact with basement membranes in their hosts (42, 43).

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Yst Proteins Are Essential Components of 40 S Subunits

11391

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