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The 39-Kilodalton Subunit of Eukaryotic Translation Initiation Factor 3 Is Essential for the Complex's Integrity and for Cell Viability in *Saccharomyces cerevisiae*

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Eukaryotic translation initiation factor 3 (eIF3) in the yeast *Saccharomyces cerevisiae* comprises about eight polypeptides and plays a central role in the binding of methionyl-tRNA$_i$ and mRNA to the 40S ribosomal subunit. The fourth largest subunit, eIF3-p39, was gel purified, and a 12-amino-acid tryptic peptide was sequenced, enabling the cloning of the *TIF34* gene. *TIF34* encodes a 38,753-Da protein that corresponds to eIF3-p39 in size and antigenicity. Disruption of *TIF34* is lethal, and depletion of eIF3-p39 by glucose repression of *TIF34* expressed from a *GAL* promoter results in cessation of cell growth. As eIF3-p39 levels fall, polysomes become smaller, indicating a role for eIF3-p39 in the initiation phase of protein synthesis. Unexpectedly, depletion results in degradation of all of the subunit proteins of eIF3 at a rate much faster than the normal turnover rates of these proteins. eIF3-p39 has 46% sequence identity with the p36 subunit of human eIF3. Both proteins are members of the WD-repeat family of proteins, possessing five to seven repeat elements. Taken together, the results indicate that eIF3-p39 plays an important, although not necessarily direct, role in the initiation phase of protein synthesis and suggest that it may be required for the assembly and maintenance of the eIF3 complex in eukaryotic cells.

Initiation of protein synthesis is promoted by at least 10 proteins called initiation factors (reviewed in reference 14). The largest and most complex of these is eukaryotic translation initiation factor 3 (eIF3), a factor comprising at least eight subunits. eIF3 plays a central role in the initiation pathway in mammalian cells (1a, 26). It binds to 40S ribosomal subunits and is implicated in dissociating 80S ribosomes into 40S and 60S subunits (1a, 6). It prevents dissociation of the Met-tRNA$_i$ · eIF2 · GTP ternary complex caused by addition of RNA (7) and stabilizes ternary complex binding to 40S ribosomal subunits (1a). eIF3 is required for mRNA binding to 40S and 80S ribosomes (1a, 26), in part by binding the eIF4G subunit of the cap-binding complex, eIF4F (12, 13). Therefore, knowledge of the structure and function of eIF3 is essential for understanding the mechanism of the initiation phase of protein synthesis.

To better elucidate the function of eIF3 by the application of both biochemical and genetic methods, we have been studying the factor in the yeast *Saccharomyces cerevisiae*. Yeast eIF3 was initially isolated and purified by a biochemical approach that used an eIF3-dependent mammalian assay for the synthesis of methionyl-puromycin (Met-PM) (17). The resulting yeast complex comprised eight subunits with apparent masses of 16, 21, 29, 33, 39, 62, 90, and 135 kDa. The genes for three of the subunits had been identified previously, but it was not realized that they encode subunits of eIF3. The second-largest subunit, p90, is encoded by *PRT1*; the temperature-sensitive *prt1-1* mutant causes destabilization of Met-tRNA$_i$ binding to 40S ribosomal subunits (8, 15). *GCD10*, first characterized genetically as a gene involved in translational derepression of *GCN4* expression (10), was identified as the RNA-binding subunit of eIF3 (5). Since derepression is thought to be caused by slowing the rate of Met-tRNA$_i$ binding to 40S ribosomal subunits, the finding reinforces the notion that eIF3 is involved in this step of the initiation pathway. The *sui1* suppressor gene affects recognition of the initiation codon of *HIS4* by Met-tRNA$_i$ (28). The protein product of *SUI1* was characterized as the smallest subunit of eIF3, p16 (16). All three genetic studies therefore implicate yeast eIF3 in Met-tRNA$_i$ binding and function on the initiating 40S subunit.

Since the genes for the remaining five subunits of eIF3 have not yet been described, we have initiated a program to identify and clone them. Our approach takes advantage of possessing milligram quantities of purified yeast eIF3, with which partial amino acid sequences can be obtained for the separated protein subunits. Here we report the cloning and characterization of the *S. cerevisiae* gene for p39, which we name *TIF34* because it is the fourth-largest subunit of eIF3.

MATERIALS AND METHODS

Strains and genetic manipulations. The genotypes and sources of *S. cerevisiae* strains used or constructed in this work are described in Table 1. The diploid strain W303D was obtained by mating W303-1A and W303-1B (23). Construction of the strains carrying a disrupted *TIF34* gene is described below. Yeast cells were grown in YP or synthetic minimal (S) medium supplemented with the relevant amino acids and 2% glucose (D) or 2% galactose (G) as described previously (20). Cultures were grown at 30°C and were monitored by measuring the optical density at 600 nm (OD_{600}). For sporulation (20), cells were grown on YPD plates (containing 6% glucose) for 24 h and then were sporulated at room temperature on plates modified for strain W303 (27) containing 0.3% potassium acetate, 0.02% raffinose, and 10 μg of each amino acid per ml. Tetrad dissections and DNA transformations were carried out by using standard procedures (25). *Escherichia coli* HB101 (Bethesda Research Laboratories) was used for plasmid propagation.

Purification of the eIF3 complex. The yeast eIF3 complex was purified from strain W303D as previously described (17). Briefly, cells were grown to an OD_{600} of 1.8, digested with lyticase, and homogenized on ice with a Dounce homogenizer, and a ribosomal pellet was prepared from the S30 fraction by centrifugation at 46,000 rpm (Beckman Ti65 rotor). The ribosomal high-salt wash, enriched in initiation factors, was prepared and fractionated on a Superdex 200 gel filtration column of a fast protein liquid chromatography (FPLC) system (Pharmacia LKB Biotechnology Inc.). The eIF3 complex was purified further on a Mono S 5/5 FPLC column (Pharmacia LKB Biotechnology) with a linear 100 to 450 mM KCl gradient. Fractions were analyzed for the presence of eIF3 by immunoblotting as described below, and eIF3 activity was determined by stimulation of

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TABLE 1. Yeast strains used

Strain	Genotype	Reference
W303-1A	*MAT***a** *leu2-3,112 his3-11,15 ade2-1 trp1-1 ura3-1 can1-100*	9
W303-1B	*MAT*α *leu2-3,112 his3-11,15 ade2-1 trp1-1 ura301 can1-100*	9
W303D	*MAT***a**/α *leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 ade2-1/ade2-1 trp1-1/trp1-1 ura3-1/ura3-1 can1-100/can1-100*	23
TND1	*MAT***a**/α *leu2-3,112/leu2-3,112* his3-11,*15/his3-11,15 ade2-1/ade2-1 trp1-1/trp1-1 ura3-1/ura3-1 can1/100/can1-100 TIF34/tif34*::*HIS3*	This work
TND2	*MAT***a**/α *leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 ade2-1/ade2-1 trp1-1/trp1-1 urea3-1/ura3-1 can1-100/can1-100 TIF34/tif34*::*HIS3* [pTN1-Yp39]	This work
TND3	*MAT***a**/α*leu2-3,112/leu2-3,112 his3-11,15/*his3-11,*15 ade2-1/ade2-1 trp1-1/trp1-1 ura3-1/ura3-1 can1-100/can1-100 TIF34/tif34*::*HIS3* [pTN-Hp36]	This work
TND4	*MAT***a**/α *leu2-3,112/leu2-3,112 his3-11,15/*his3-11,*15 ade2-1/ade2-1 trp1-1/trp1-1 ura3-1/ura3-1 can1-100/can1-100* (pTN-Hp36]	This work
TN1	*MAT***a** *leu2-3,112 his3-11,15 ade2-1 trp1-1 ura3-1 can1-100 tif34*::*HIS3* [pTN1-Yp39]	This work
TN2	*MAT***a** *leu2-3,112 his3-11,15 ade2-1 trp1-1 ura3-1 can1-100 tif34*::*HIS3* [pTN2-Yp39]	This work

Met-PM synthesis an in vitro assay that mimics formation of the first peptide bond (17).

SDS-PAGE and immunoblotting. Samples containing eIF3 were fractionated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (10% gel) (11) and transferred to an Immobilon polyvinylidene difluoride membrane (Millipore) by using a 10 mM CAPS [3-(cyclohexylamino)-1-propanesulfonic acid] buffer (pH 11) containing either 10% (vol/vol) methanol (Fig. 4 and 5) or 20% (vol/vol) methanol (Fig. 2 and 8). Coomassie blue staining was used to confirm that the same quantity of protein was transferred in each lane on a parallel blot. The membrane was blocked in BLOTTO (0.5% [wt/vol] nonfat dry milk in TST [10 mM Tris-HCl {pH 7.4}, 150 mM NaCl, 0.075% {vol/vol} Tween 20]) and then probed with a 1:1,000 dilution of rabbit polyclonal antiserum directed against yeast eIF3 (17) or with a 1:100 dilution of goat antibody affinity purified against recombinant human eIF3-p36 (1). Subsequently, the blots were incubated with alkaline phosphatase-conjugated anti-rabbit or anti-goat immunoglobulin G (Cappel) and visualized colorimetrically with the alkaline phosphatase substrates nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate as described previously (17).

Peptide sequencing. Purified, active eIF3 was fractionated by SDS-PAGE (12% gel) and stained with Coomassie blue, and the bands corresponding to the p39 and p29 subunits were excised from the gel and submitted to the Protein Structure Laboratory at the University of California, Davis. p39 and p29 were digested with trypsin, and peptides were fractionated on an ABI model 172 high-pressure liquid chromatography apparatus with an Aquapore RP-300 microbore column (10 cm by 1 mm). Prominent, well-resolved peptides from p39 and p29 were subjected to 13 and 6 cycles, respectively, of automated Edman degradation with an ABI 470D amino acid analyzer. The N-terminal sequence of p29 was obtained directly following SDS-PAGE and blotting onto a membrane.

Cloning of the *TIF34* gene encoding p39. The *TIF34* gene was cloned from total genomic yeast DNA (24) by using PCR under standard conditions as recommended by the manufacturer (Perkin-Elmer Corp.). The upstream primer, 5′-GAGATGAGGATCCTTATTCAGCGGGTCTCTG-3′, and the downstream primer, 5′-CCCCTCAAGCTTAAAGTGACTCTCTATTAACG-3′, generate a single 1.6-kb DNA that includes about 400 bp flanking the 5′ end of the coding region (1,041 bp) and 200 bp flanking the 3′ end. The underlined regions in the primers identify *Bam*HI and *Hin*dIII restriction sites, respectively, which were introduced to facilitate subsequent cloning. The 1.6-kb DNA was gel purified and subcloned into the pNoTA vector by using a PCR cloning kit (5 Prime→3 Prime, Inc.) according to the manufacturer's recommendations. The resulting plasmid, pNoTA-TIF34, was digested with restriction enzymes to obtain the restriction map shown in Fig. 3A, and both strands of the 1.6-kb insert were sequenced (Sequenase 2.0 kit; Amersham). Comparisons with DNA and amino acid sequences in the data banks were carried out with the FASTA and TFASTA programs.

Disruption of *TIF34* in the chromosome. pNoTA-TIF34 was digested with *Sal*I and *Bgl*II to remove a 661-bp fragment with 64% of the *TIF34* coding region, and the ends of the remaining plasmid were filled in with Klenow DNA polymerase. A 1.75-kb *Bam*HI fragment carrying the entire *HIS3* gene was isolated from plasmid pHYH (23), and the ends were filled in with Klenow DNA polymerase. The two blunt-ended DNAs were ligated to generate pNoTA-*tif34*::*HIS3*. pNoTA-*tif34*::*HIS3* was digested with *Bam*HI (the downstream *Bam*HI site lies in the vector DNA) to generate a 2.56-kb fragment which contains 392 bp of the *TIF34* 5′ flanking region, 180 bp of *TIF34* coding the N terminus, the *HIS3* gene, 180 bp of the *TIF34* coding the C terminus, and 208 bp of downstream DNA. The fragment was transformed into the diploid yeast strain W303D to create a one-step gene deletion and disruption (21). Stable His^+ transformants called TND1 were selected, and disruption of one of the *TIF34* genes was confirmed by Southern blot analysis (results not shown).

Construction of plasmids for expressing *TIF34* and human eIF3-p36 cDNAs. Plasmid pTN1-Yp39 was constructed by ligating the centromeric vector pHSX3 (23) cleaved and blunted at the *Bam*HI site just downstream of the *GAL1* promoter with a 1.1-kb DNA fragment containing the *TIF34* coding region. The 1.1-kb DNA fragment was obtained by PCR amplification from plasmid pNoTA-TIF34 with an upstream primer (5′-GCACAGACAAGGCATATGAAGGCTA TCAAATTAACAGG-3′) and a downstream primer (5′-GGATCCTATACAT CAACATCAGCATC-3′). In addition to the coding region of *TIF34*, the amplified fragment includes 15 nucleotides upstream from the initiation codon (underlined in the upstream primer) and 56 nucleotides 3′ of the termination codon.

Plasmid pTN-Hp36 was constructed by ligating a 1.4-kb blunt-ended *Eco*RI fragment from pTZp36 (1 [accession number U39067]) and the yeast vector pHSX3 blunt ended at the *Bam*HI site just downstream from the *GAL1* promoter. The human cDNA insert (kindly provided by K. Asano) contains 8 bp upstream of the initiation codon for human eIF3-p36, the entire coding region, and 340 bp downstream from the stop codon. Inserts with the correct orientation were identified by restriction enzyme cleavage (results not shown).

Construction of haploid strains TN1 and TN2. Strain TND1 was transformed with pTN1-Yp39, and transformants were selected on SG-Trp plates and called TND2. TND2 was sporulated, and 20 asci were dissected. Spores that grew on galactose plates were screened on SG-His-Trp plates to identify *TIF34*-disrupted cells carrying pTN1-Yp39, which were named TN1. To overexpress yeast eIF3-p39, the 1.1-kb PCR fragment containing the *TIF34* coding sequence described above was subcloned into the blunt-ended *Sal*I site of YEp51 (2) just downstream from the *GAL10* promoter to yield pTN2-Yp39. Strain TN1 transformed with pTN2-Yp39 was grown in SG-His-Leu to allow the loss of pTN1-Yp39 by segregation. Isolated colonies were replica plated on SG-His-Trp and SG-His-Leu plates to screen for colonies that cannot grow in the absence of tryptophan due to the loss of pTN1-Yp39 but can grow in the absence of leucine due to the presence of pTN2-Yp39. The yeast cells from such colonies are called strain TN2.

Expression of eIF3-p39. Precultures of strains W303-1A and TN1 grown in YPG medium were used to inoculate YPD or YPG medium. Cell growth at 30°C was monitored by measuring the OD_{600} as indicated in Fig. 4. At various times, cells from 25 ml of the culture were pelleted and resuspended in buffer A [100 mM potassium acetate, 2 mM magnesium acetate, 30 mM HEPES (pH 7.4), 1 mM (2-aminoethyl) benzenesulfonyl fluoride, 10 μg each of aprotinin, leupeptin, and pepstatin per ml, 2 mM dithiothreitol]. Glass beads (0.5 ml) were added, the cells were disrupted by vortexing, and the lysates were centrifuged for 15 min at 12,000 × *g* at 4°C. Protein concentrations were determined by the Bradford assay (Bio-Rad, Inc.), and equal amounts of protein were analyzed by SDS-PAGE (10% gel) and immunoblotting as described above.

Polysome profile analysis. Precultures of strains W303-1A and TN1 were inoculated into YPG medium and grown to early log phase. The exponentially growing cultures were used to inoculate YPD medium at 30°C, and growth was monitored by measuring the OD_{600}. An hour after TN1 ceased to grow (OD = 0.6 for TN1 and 0.9 for W303-1A), 100 μg of cycloheximide per ml was added, followed by quick cooling by swirling the culture on ice. The cells were collected by centrifugation and washed with buffer B (10 mM Tris-HCl [pH 7.4], 100 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol) plus 100 μg of cycloheximide per ml. Pelleted cells were broken by vortexing with glass beads, and cell lysates were clarified by centrifugation at 10,000 × *g* for 10 min at 4°C. Extracts (5 A_{260} units) were layered onto 12.5-ml 10 to 45% (wt/vol) sucrose density gradients in buffer B prepared in a Gradient Master 105 (Biocomp) apparatus. The gradients were centrifuged in a Beckman SW40 rotor at 38,000 rpm for 100 min at 4°C and fractionated by upward displacement with an ISCO density gradient fractionator and UV monitor. Fractions were analyzed by SDS-PAGE (10% gel) and immunoblotting with an anti-eIF3 antiserum as described above.

Northern blot analysis. Total RNA from His^- Trp^+ and His^- Trp^- spore cultures obtained from TND3 was prepared as described previously (24). About 10 μg of RNA was fractionated on a 1.5% formaldehyde-agarose gel (22),

transferred to a Hybond-N (Amersham) membrane, and hybridized with a ^{32}P-labeled DNA probe. The probe consisted of the 1.4-kb *Eco*RI fragment containing the entire human eIF3-p36 coding sequence described above. The membrane was washed under conditions of high stringency (22) and exposed to X-ray film.

Nucleotide sequence accession number. The sequence shown in Fig. 1 has been assigned GenBank accession number U56937.

RESULTS

Identification and characterization of the *TIF34* gene. With the intent to clone DNA encoding the p39 subunit of eIF3, we performed partial protein sequencing of the subunit as described in Materials and Methods. Briefly, the eIF3 complex was purified to 90% homogeneity and fractionated by SDS-PAGE (12% gel), and the band corresponding to the p39 polypeptide was excised from the gel and submitted to the Protein Structure Laboratory at the University of California, Davis, for proteolytic cleavage and internal peptide sequencing. After 13 cycles of Edman degradation, the amino acid sequence was compared with known sequences in the GenBank, EMBL, and SWISS-PROT databases. The signal for the first residue gave multiple amino acids and therefore was not identified. Eleven of the next 12 residues matched a region in a hypothetical translation product from an open reading frame (ORF) in the *S. cerevisiae* genome that encodes a protein of 347 amino acids and lies in chromosome XIII. This DNA sequence was generated by the yeast genome project; the encoded protein was not identified, nor was it characterized in terms of function.

A 1.6-kb fragment of genomic DNA containing the putative coding region of p39 was amplified by PCR as described in Materials and Methods. The fragment was subcloned into the pNoTA vector (5 Prime→3 Prime) to yield pNoTA-p39, and both strands were sequenced (Fig. 1). Two bases (positions 674 and 1058) differ from the DNA sequence in the data banks (which report G and A, respectively), but the differences do not affect the encoded protein. Our sequence analysis confirms that the genomic 1.6-kb clone contains the peptide sequenced in this study as well as the large ORF identified in the database.

The first AUG in the large ORF occurs at position 390 and almost certainly is the initiation codon for the putative p39 polypeptide. It possesses the sequence 5′-AAUAUGA-3′ (initiation codon underlined), which resembles the yeast consensus context, A(A/U)AAUG (3). The genomic region upstream of this AUG contains three other AUGs beginning at base pair positions 76, 174, and 306. AUG-174 lies in a different reading frame from p39 and encodes a polypeptide of 26 amino acid residues. AUG-76 and AUG-306 lie in the same reading frame as p39, but both are followed by two termination codons and generate polypeptides of 13 and 8 residues, respectively. Thus, only AUG-390 is suitable for initiating the translation of the major ORF (p39), since the next in-frame downstream AUG does not occur until codon 116. The ORF terminates with a UAA codon at positions 1431 to 1433, followed by four additional stop codons. We have named the gene *TIF34* (the fourth subunit of translation initiation factor eIF3). It encodes a 347-residue protein with a calculated molecular weight of 38,753.

To investigate the size of the *TIF34* transcript, we performed Northern blot analysis of total yeast RNA, using as a probe the 0.72-kb *Ban*I/*Ava*II fragment (see Fig. 3) containing 70% of the *TIF34* coding sequence. This probe hybridizes with a single RNA transcript of ~1.3 kb (data not shown), consistent with the size of the *TIF34* coding region of 1,041 bp. The number of *TIF34* genes was investigated by Southern blot analysis of genomic DNA. Hybridization of the ^{32}P-labeled probe to a single band with each of eight different restriction enzyme digestions (results not shown) suggests the presence of a single

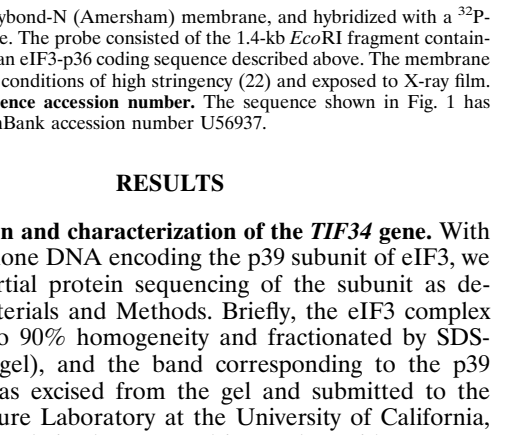

FIG. 1. Sequences of *TIF34* and eIF3-p39. The DNA sequence of the coding region of *TIF34* and its flanking sequences was determined as described in Materials and Methods. The sequence does not include the 5′ end of the PCR primers where restriction sites were introduced. The derived amino acid sequence for eIF3-p39 is aligned below; stop codons are indicated by asterisks. Residue numbers for nucleotides and amino acids are shown on both sides. The underlined region corresponds to the peptide whose amino acid sequence was determined; the double underline identifies the mismatched residue which is proline in the peptide sequence.

locus. A number of A/T-rich regions upstream from the initiation codon might serve as promoters; however, the structure and transcription of *TIF34* were not investigated further.

***TIF34* encodes the p39 subunit of eIF3.** The near identity between the peptide sequence determined from p39 and the sequence deduced from *TIF34*, together with the calculated mass (38.8 kDa) of the encoded protein, suggests that *TIF34* is indeed the gene that encodes the p39 subunit of eIF3. To better establish this, strains expressing a single copy (TN1) and multiple copies (TN2) of *TIF34* were constructed as described in Materials and Methods and in the text below. The relative amounts of p39 in the lysates from exponentially growing TN1 and TN2 were quantitated by SDS-PAGE followed by Western immunoblotting with antibodies against yeast eIF3 as described in the legend to Fig. 2. Purified eIF3 generates an immunoreactive band (Fig. 2, lane 2) with a migration rate identical to that of the p39 subunit in the Coomassie-stained gel (lane 1). A corresponding immunoreactive p39 band is detected in the lysate made from TN1 expressing a single *TIF34* gene (lane 3), whereas the lysate from TN2 with the high-copy-number plasmid generates a somewhat more intense p39 band (lane 4). The other bands in the blot represent both eIF3 subunits and other yeast proteins that react with the crude antiserum. The small increase in intensity of the p39

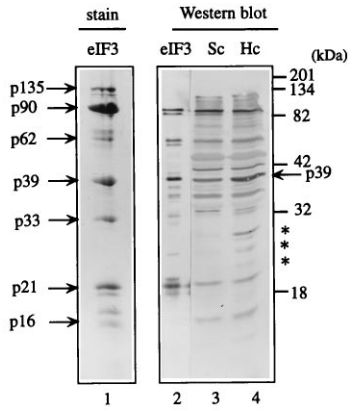

FIG. 2. Identification of eIF3-p39 expressed from *TIF34*. Strains TN1 and TN2 and their corresponding plasmids were constructed as described in Materials and Methods. TN1 expresses *TIF34* from the single-copy (Sc) plasmid pTN1-Yp39; TN2 expresses *TIF34* from the high-copy-number (Hc) plasmid pTN2-Yp39. Purified yeast eIF3 and yeast lysate proteins were fractionated by SDS-PAGE (10% gel), and the gel was stained with Coomassie blue (lane 1) or developed by Western immunoblotting (lanes 2 to 4) with a rabbit anti-yeast eIF3 antiserum as described in Materials and Methods. eIF3 subunits are identified on the left; positions of molecular weight markers are shown on the right. The migration position of p39 also is shown on the right by an arrow, and asterisks mark bands enhanced in the lysate of cells containing the high-copy-number plasmid (lane 4). Lanes 1 and 2, purified yeast eIF3; lane 3, lysate protein (20 μg) from strain TN1; lane 4, lysate protein (20 μg) from the p39-overproducing strain TN2.

band in TN2 relative to the intensities of the other eIF3 subunits supports the view that *TIF34* encodes the p39 subunit.

It is noteworthy that the eIF3 preparation analyzed in Fig. 2 appears to lack the p29 subunit detected in earlier preparations of the factor (17). It is therefore possible that the p29 subunit is not part of the native eIF3 complex. In the immunoblot analyses shown here, there are additional bands below p39 (marked by asterisks in Fig. 2) that are detected uniquely in the high-copy-number lysate. These likely represent partially degraded forms of p39. One of the bands corresponds in gel migration to the p29 subunit of eIF3, suggesting that p29 may be derived from p39. To test this, the p29 band from a suitable eIF3 preparation was excised from an SDS-polyacrylamide gel, and the N terminus and an internal peptide were subjected to amino acid sequencing. The N-terminal sequence XKAIKL (where X is undetermined) matches the sequence of the N terminus of p39; the internal peptide sequence ITHEGLDAA TVAG corresponds to residues 148 to 160 in the p39 sequence. The results indicate that p29 is derived from *TIF34* by proteolytic removal of a portion of the C terminus of p39. The apparent sensitivity of p39 to proteolysis may explain why only a modest overproduction of p39 is obtained in the high-copy-number strain; p39 produced in excess of the other eIF3 subunits may be especially unstable in the free, uncomplexed form.

***TIF34* is an essential gene.** At least three of the subunits of eIF3 are encoded by genes (*PRT1*, *GCD10*, and *SUI1*) that are essential for cell growth (5, 15, 28). To test for p39, we generated a null mutant strain as described in Materials and Methods. Briefly, pNoTA-*tif34*::*HIS3* contains a *HIS3*-disrupted *TIF34* gene with about 64% of the coding region deleted (Fig. 3A). After digestion with *Bam*HI, the resulting 2.56-kb *tif34*::*HIS3* fragment was transformed into the diploid strain W303D. Twenty His^+ transformants were isolated, and their genomic DNAs were purified and subjected to Southern blot analysis to screen for cells where one of the copies of *TIF34* is

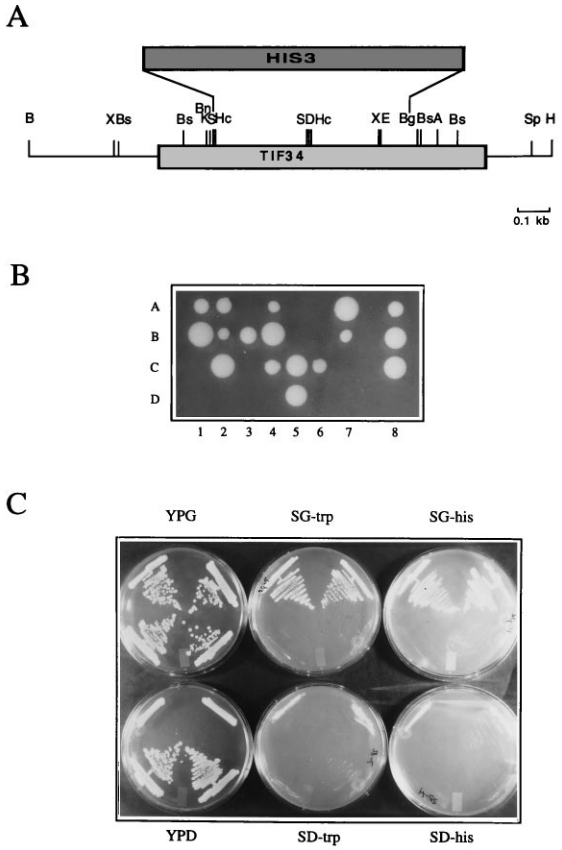

FIG. 3. Disruption of *TIF34*. (A) Restriction map of the *TIF34* region and a scheme of the gene disruption and replacement with *HIS3* as described in Materials and Methods. Restriction site abbreviations: A, *Ava*II; B, *Bam*HI; Bg, *Bgl*II; Bn, *Ban*I; Bs, *Bst*BI; D, *Dde*I; E, *Eco*RI; H, *Hin*dIII, Hc, *Hin*cII; K, *Kpn*I; S, *Sal*I; Sp, *Ssp*I; X, *Xmn*I. The *TIF34* coding region and the *HIS3* gene are shown as shaded rectangles. (B) Tetrad dissection of eight TND2 spores was carried out as described in Materials and Methods. Spores arranged vertically on a YPG plate as indicated by letters on the left were allowed to germinate and grow at 30°C for 48 h. A photograph of the plate is shown. (C) Strain TN1 (upper streaks) and W303-1A (lower streaks) were streaked onto galactose and glucose plates with and without histidine or tryptophan as indicated. The plates were incubated at 30°C for 4 days and photographed.

replaced with *tif34*::*HIS3* (results not shown). Transformants that carry a disrupted *TIF34* gene were named TND1. Tetrad analysis of two His^+ diploid transformants revealed that only two of the four spores in each of 20 asci formed colonies on rich medium, even after 7 days at 30°C. All viable spores were His^-, and Southern blot analysis of total DNA isolated from the viable spore colonies indicated the presence of wild-type *TIF34* (data not shown). This finding suggests that the phenotype of *tif34*::*HIS3* is lethal. The fact that the tetrad spores generate a $2^+:2^-$ segregation pattern and that no His^+ segregants are found indicates that *TIF34* is required for germination and/or cell growth.

To confirm that *TIF34* is the only gene whose function has been disrupted, the *TIF34* ORF was placed under control of the glucose-repressible *GAL1* promoter as described in Materials and Methods. The resulting plasmid, pTN1-Yp39, was transformed into the diploid strain TND1, and transformants were selected on SG-Trp plates. Transformants, named TND2, were subsequently sporulated followed by tetrad dissection on galactose plates. Due to random plasmid segregation, the ratio of viable to nonviable spores was 2:2, 3:1, or 4:0 (Fig. 3B and

results not shown). Spore colonies that were His^+ were also Trp^+ (therefore containing both the disrupted *tif34*::*HIS3* gene and the plasmid); one was selected and called TN1.

To investigate the effect of p39 depletion on cell growth and viability, we compared growth on galactose versus glucose medium of TN1 and the parental strain W303-1A after a 3-day incubation at 30°C (Fig. 3C). As expected, W303-1A (lower streaks) grows on both galactose and glucose but fails to grow when either histidine or tryptophan is omitted from the medium. The TN1 strain (upper streaks) grows in galactose medium supplemented with either tryptophan or histidine, indicating that the plasmid-borne *TIF34* is sufficient to complement the *tif34* null mutation on the chromosome. In contrast, when TN1 is streaked on glucose-containing plates, growth ceases, which indicates that *TIF34* is required for cell growth and viability. The steady-state concentrations of *TIF34* mRNA in the parental strain W303-1A and in TN1 expressed by the *GAL* promoter are similar during exponential growth (results not shown). Likewise, TN1 expresses almost the same level of p39 protein as do parental cells when analyzed by Western immunoblotting (results not shown).

Effect of *TIF34* depletion on eIF3 levels and activity. The results described above strongly suggest that the lack of growth on glucose-containing medium is caused by a failure to express *TIF34*. To examine the effects of depletion of p39 in TN1, growth was monitored by measuring the OD_{600} of liquid cultures. The parental strain W303-1A expressing endogenous *TIF34* grows equally well in glucose- or galactose-containing medium, with a doubling time of 1.9 h (Fig. 4A). The TN1 strain expressing p39 from pTN1-Yp39 shows slightly slower growth, with a doubling time of 2.5 h in galactose-containing medium. When TN1 is shifted from galactose to glucose medium, its growth is comparable to that of W303-1A for about three generations and then abruptly decreases in rate. After the fourth generation, TN1 cells cease to grow and completely arrest.

Following the shift of TN1 to glucose medium, transcription of *TIF34* from the *GAL* promoter is repressed and the level of p39 is expected to drop by protein turnover and/or by dilution due to cell division. Western immunoblot analysis of eIF3 in the lysates (Fig. 4B) shows that the level of p39 in TN1 is greatly diminished at 7 h, when growth begins to slow, and is nearly undetectable at 12 h following the shift to glucose medium. No such decrease in p39 is detected for TN1 grown in galactose or for the parental strain W303-1A grown in either galactose or glucose. At 12 h following the shift of TN1 to glucose, when cells were already in stationary phase for about 3 h, the depletion of p39 is accompanied by strongly reduced amounts of all of the other eIF3 subunits. Western blot analysis shows barely detectable amounts of eIF3 in such extracts, whereas nonspecific immunoreactive bands, for example, at about 43 kDa, are present with the same intensity as in the nondepleted TN1 and W303-1A cells. This unexpected result suggests an important role for p39 in maintaining the structure of eIF3, as discussed below.

The disappearance of most, if not all, eIF3 subunits following p39 depletion could be due a priori to a failure to synthesize these proteins and/or to rapid degradation. Synthesis is reduced for all proteins, as judged by the polysome profiles (see Fig. 6) and the reduced cell growth rate. Since yeast cells have divided only once between 7 and 12 h following the shift to glucose (Fig. 4A), dilution by cell division cannot explain the reduced level of eIF3. Instead, eIF3 depletion at 12 h must involve proteolytic degradation of the subunits. The question arises as to whether the rate of eIF3 subunit degradation is enhanced specifically due to p39 depletion or whether normal

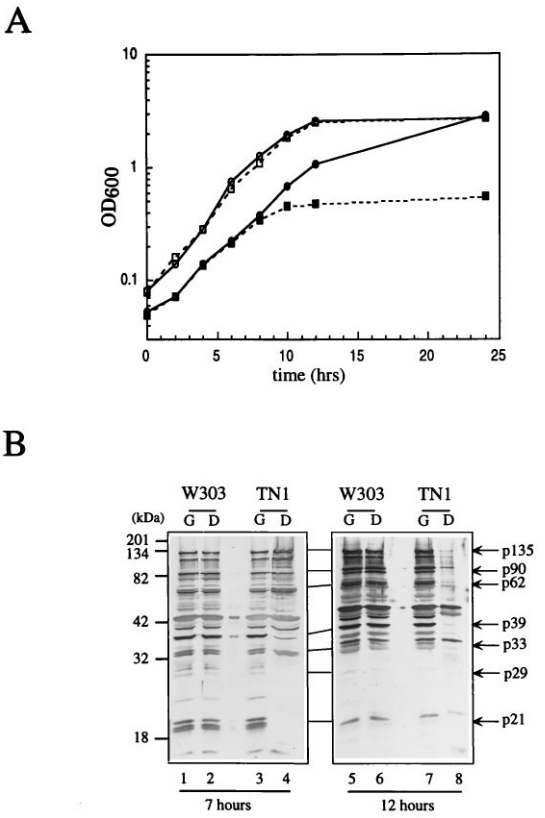

FIG. 4. Effects of p39 depletion on cell growth and eIF3 levels. (A) Exponential cultures of strain W303-1A (open symbols) and TN1 (closed symbols) were grown in YPG medium and then diluted to an OD_{600} of 0.06 to 0.08 in YPG (circles) or YPD (squares) medium. Growth was monitored by measuring the OD_{600}. (B) Western immunoblot analysis. Cell lysates were prepared from strains W303-1A and TN1 as described in Materials and Methods at 7 h (lanes 1 to 4) and 12 h (lanes 5 to 8) after dilution into galactose (G) or glucose (D) medium. Total lysate protein (20 μg) was fractionated by SDS-PAGE (10% gel), transferred to an Immobilon membrane, and treated with a polyclonal rabbit anti-eIF3 antibody as described in Materials and Methods. A photograph of the blot after color development is shown. Migration positions of the eIF3 subunits and molecular mass markers are indicated on the right and left, respectively. Different gels were run for analyses of the samples at time points 7 and 12 h. The lines between the panels connect the eIF3 subunit bands. The stained material between lanes 2 and 3 and lanes 6 and 7 is due to small amounts of spillover of adjacent lanes.

turnover rates for these proteins are sufficient to explain their disappearance. We measured the turnover rates in cells where translation was inhibited either by shifting a temperature-sensitive *prt1-1* strain to 37°C or by adding cycloheximide to the parental W303-1A strain. The *prt1-1* strain TR1-1-1 contains a mutation in the p90 subunit of eIF3 which results in rapid cessation of protein synthesis at 37°C (5, 15), whereas cycloheximide inhibits the elongation phase of translation. The relative levels of eIF3 subunits were measured up to 6 h after inhibition of protein synthesis by Western immunoblotting of SDS-gels (Fig. 5). Essentially no decrease in the abundance of the subunit proteins is detected over the 6-h period. Thus, the half-life for eIF3 subunits is greater than 12 h, ruling out normal turnover as an explanation for why eIF3 disappears following p39 depletion.

p39 depletion affects initiation of protein synthesis. Having established that p39 levels in TN1 are reduced after growth in glucose-containing medium, we used the strain to investigate the effect of p39 depletion on protein synthesis. The finding

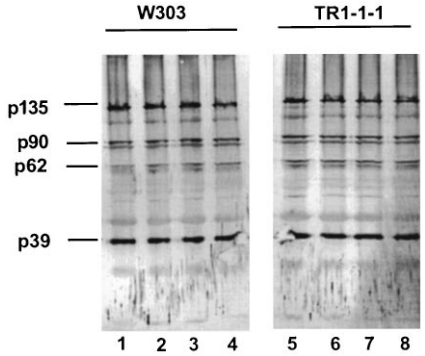

FIG. 5. eIF3 subunit turnover rates. Cycloheximide (100 μg/ml) was added to a culture of strain W303-1A, and aliquots were removed at 0, 2, 4, and 6 h (lanes 1 to 4, respectively). Strain TR1-1-1 (*prt1*^{ts}) carrying a temperature-sensitive mutation in *PRT1* was shifted from 25 to 37°C in YPD, and aliquots were removed at 0, 2, 4, and 6 h (lanes 5 to 8, respectively). Pelleted cells were lysed, and equal amounts of lysate protein (15 μg) were analyzed by SDS-PAGE (10% gel) and immunoblotting with a crude anti-eIF3 antiserum as described in Materials and Methods. A scan of the immunoblots developed colorimetrically is shown. eIF3 subunit bands are labeled on the left.

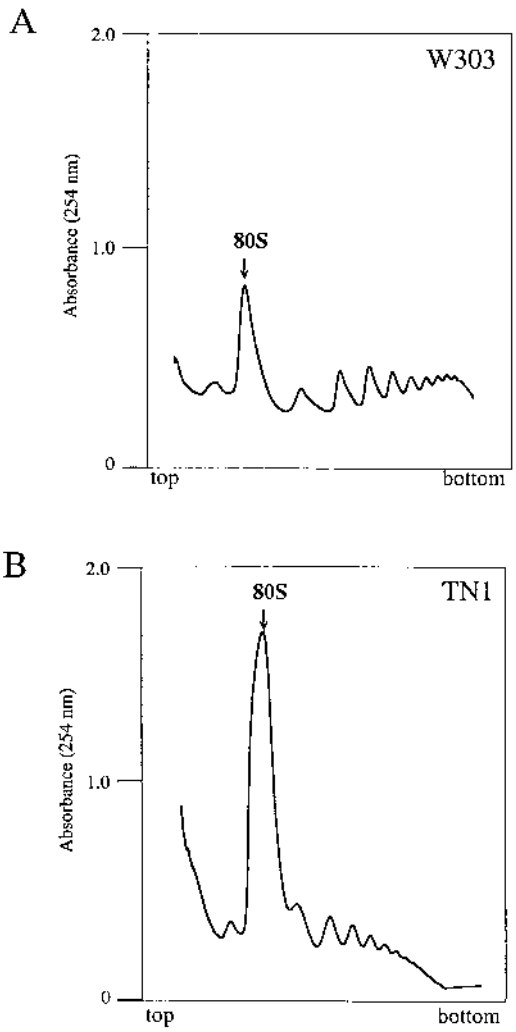

FIG. 6. Polysome analysis of p39-depleted cells. Exponential cultures of strains W303-1A (A) and TN1 (B) grown in YPG medium were diluted to an OD_{600} of 0.06 to 0.08 in YPD medium. At 9.5 h after the shift, the cells were harvested and lysed, and 5 A_{260} units from each extract was fractionated by centrifugation on 10 to 45% sucrose gradients as described in Materials and Methods. Gradients were collected from the top by using an ISCO density gradient fractionator and were scanned at A_{254} with an ISCO UA-5 detector. The resulting absorbance profiles are shown, with sedimentation from left to right. The positions of 80S ribosomal subunits are indicated by vertical arrows.

that *TIF34* is essential for cell growth, plus the fact that other eIF3 genes characterized thus far encode essential components of the yeast translational machinery (8, 28, 5), suggests that *TIF34* also may be required for initiation of translation. When initiation is slowed or blocked, ribosomes finish translating mRNA but do not efficiently reinitiate, leading to a reduction in the size of polysomes (number of ribosomes per mRNA), an accumulation of 80S ribosomes, and an increase of 40S and 60S ribosomal subunits. We therefore analyzed polysome profiles from p39-depleted and nondepleted cells.

To avoid indirect effects of prolonged depletion of p39 or stationary growth on protein synthesis, we analyzed a growth stage of the culture when the p39 level is strongly reduced yet the reduction in growth rate is just beginning. Therefore, exponential cultures of strains W303-1A (control) and TN1 were shifted to glucose medium, and extracts were prepared at 9.5 h after the shift. These lysates were fractionated by sucrose gradient centrifugation as described in Materials and Methods. Polysome profiles of the parental and depleted strains show clear differences in the monosome and polysome regions. The TN1 strain shows a marked reduction in the larger polysomes and a significant increase in 80S ribosomes (Fig. 6B) compared to the parental strain (Fig. 6A).

Immunoblot analysis of sucrose gradient fractions showed that in the parental strain, the majority of eIF3 is present in the region corresponding to free ribosomal subunits (data not shown). Under the condition of p39 depletion and polysome runoff, a significant overall reduction in the total amount of eIF3 is observed. The remaining subunits of eIF3 sediment more slowly than 40S particles (data not shown), suggesting that p39-depleted eIF3 no longer binds to 40S ribosomal subunits. The data imply an important role for the p39 subunit of eIF3 in the process of translation initiation. However, as discussed below, the reduction in amounts of all of the eIF3 subunits when p39 synthesis is repressed does not allow us to assign a direct, unambiguous role for p39 in initiation. Rather, the observed inhibition of initiation may be due entirely to loss of the whole eIF3 complex.

Human eIF3-p36 is a homolog of yeast eIF3-p39. Recently a human cDNA homolog of the *TIF34* gene has been cloned (GenBank accession number U39067) and characterized as encoding the p36 subunit of mammalian eIF3 (1). Human eIF3-p36 and *S. cerevisiae* eIF3-p39 have 46% sequence identity and 64% similarity (Fig. 7). The strong conservation of primary structure is apparent throughout the protein sequence and also is seen in hydrophobicity profiles (result not shown). Together with the fact that both yeast eIF3 and human eIF3 stimulate the in vitro Met-PM synthesis assay with mammalian components (17), these observations suggest that the yeast and human subunits may be functionally equivalent.

Since *TIF34* is essential for yeast cell growth, we are able to use the no-growth phenotype of the null strain to test if the human cDNA can replace the yeast gene in vivo. As shown in Fig. 8A, the human cDNA fails to support the germination and growth of spore colonies. Northern blot analysis with a human eIF3-p36 cDNA probe shows that the cDNA is transcribed well (Fig. 8B). Accumulation of human eIF3-p36 was tested directly by Western immunoblotting with antibodies to mammalian eIF3. After subcellular fractionation, a significant

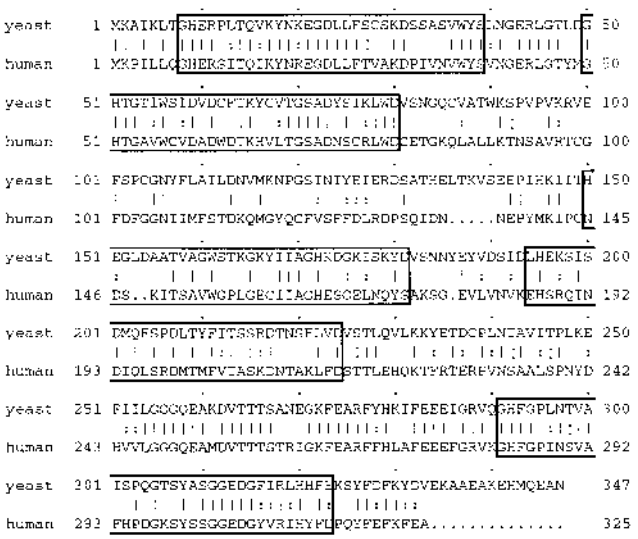

FIG. 7. Comparison of yeast and human sequences. Yeast eIF3-p39 and human eIF3-p36 sequences were aligned by using the program BESTFIT from the Genetics Computer Group software package on a VAX computer. Sequence identities are shown by vertical lines between the two sequences; similar residues, marked by two dots, are defined as VIL, DE, KR, NQ, FYW, and ST. The five regions bracketed by the consensus WD-repeat elements GH and WD in the two proteins are boxed. Every 10th yeast residue has a dot above it, whereas the first and the last residues in each row are numbered. The sequences exhibit 46% identity and 64% similarity.

amount of human eIF3-p36 was found in the high-salt wash of yeast ribosomes, a fraction enriched in yeast eIF3. Size exclusion chromatography of the ribosomal high-salt wash was performed to determine whether human eIF3-p36 is incorporated into the yeast eIF3 complex. Western blot analysis of the resulting column fractions shows that yeast eIF3 elutes as expected with an apparent molecular mass of ~550 kDa, in fractions 14 to 16 (Fig. 8C, upper panel). When the same fractions were analyzed by using affinity-purified antibodies specific for human eIF3-p36, a single strongly immunoreactive band corresponding to an apparent molecular mass of ~50 kDa was detected in fractions 42 and 43. No human eIF3-p36 was detected in fractions 14 to 16, where the yeast eIF3 complex elutes. We conclude that the human p36 subunit accumulates but is not stably incorporated into yeast eIF3. The inability to incorporate into yeast eIF3 may explain why it does not suppress the lethal phenotype of the *tif34*::*HIS3* allele and support cell growth. It is worth noting that no significant cross-reactivity is observed between anti-yeast eIF3 antiserum and the HeLa eIF3 subunits. Similarly, affinity-purified anti-human eIF3-p36 does not recognize the p39 subunit of yeast eIF3 (Fig. 8C, lower panel).

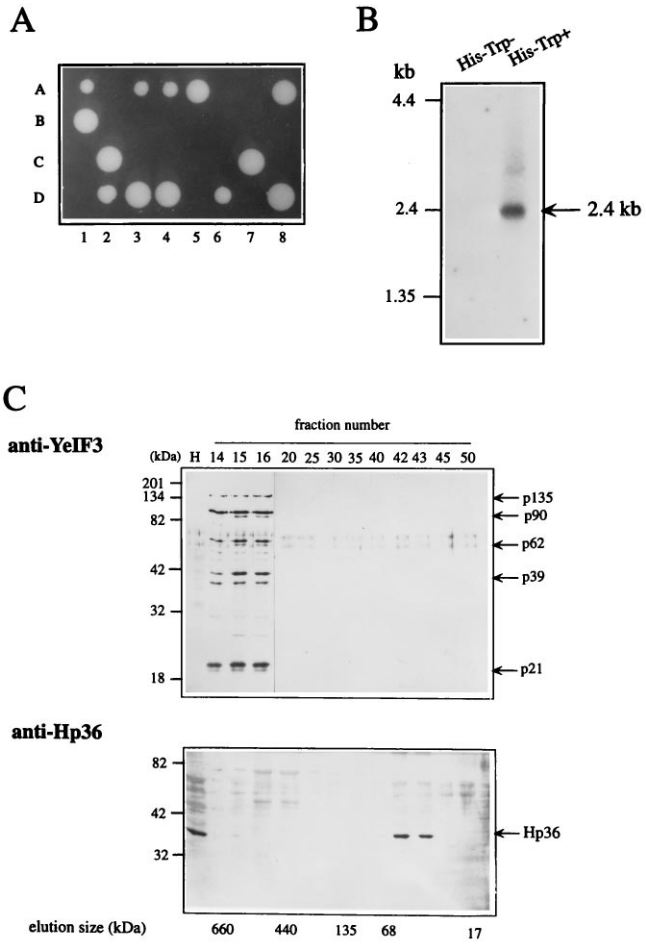

FIG. 8. Expression of human eIF3-p36. (A) Tetrad dissection of TND3 spores. Strain TND1 was transformed with pTN-Hp36, which carries the human eIF3-p36 cDNA under the *GAL1* promoter, to yield TND3. Spores from eight individual tetrads of TND3 were dissected, arranged vertically on a YPG plate as indicated by letters on the left, and allowed to germinate and grow at 30°C for 4 days. A photograph of the plate is shown. (B) Northern blot analysis. Spore colonies from panel A with a His^-Trp^- (lane 1) or His^-Trp^+ (lane 2) phenotype were selected and grown in YPG medium, and total RNA was extracted and analyzed by Northern blotting as described in Materials and Methods. A photograph of the autoradiogram is shown. Positions of molecular size markers are shown on the left. The human eIF3-p36 transcript is identified by the arrow on the right. (C) Western blot analysis. Strain TND3 was grown in 2 liters of YPG medium, the cells were lysed, and the standard procedure for yeast eIF3 purification was followed (17). The ribosomal high-salt wash enriched in initiation factors was prepared and fractionated on a Superdex 200 FPLC column. Column fractions (15-μl aliquots) as indicated at the top were separated by SDS-PAGE (10% gel) and analyzed by immunoblotting with a rabbit antiserum against yeast eIF3 (upper panel) as described in Materials and Methods. Protein (100-μl aliquots) from the same column fractions was trichloroacetic acid precipitated, separated by SDS-PAGE (10% gel), and analyzed by immunoblotting using affinity-purified goat antibodies against human eIF3-p36 (lower panel). Immune complexes were visualized by using alkaline phosphatase-conjugated antibodies against rabbit or goat immunoglobulin G. The first lane (H) contains purified HeLa eIF3 used as a standard. The migration positions of yeast eIF3 subunits and the p36 subunit of human eIF3 are labeled on the right. The migration positions of molecular mass markers and their masses are shown on the left. The fractions where different standard molecular mass proteins elute from the Superdex 200 column are shown at the bottom of the lower panel: 600 kDa, fractions 12 to 14; 440 kDa, fractions 17 to 19; 135 kDa, fractions 32 to 34; 68 kDa, 39 to 41; and 17 kDa, fractions 50 to 52.

DISCUSSION

eIF3 is a complex of at least eight polypeptides that plays a central role in the process of initiation of protein synthesis. Characterization of mammalian eIF3 indicates that the initiation factor is involved in the binding of both Met-$tRNA_i$ and mRNA. In yeast, the three genes previously identified as encoding eIF3 subunits, namely, *PRT1*, *GCD10*, and *SUI1*, are all essential, and their mutant forms are defective in promoting initiation. To understand the role played by eIF3 in the initiation process, the primary sequences of all of its subunits as well as three-dimensional structural information about its interactions on the ribosome are needed. We have embarked on a program to clone the cDNAs and genes encoding mammalian and yeast eIF3 subunits, since such clones provide the primary sequence of the proteins and can be used to generate tools for studying the structure and function of the eIF3 com-

plex. We report here our studies of the p39 subunit of yeast eIF3.

The conclusion that the cloned gene, called *TIF34*, encodes the p39 subunit of eIF3 is based on at least four lines of evidence. First, the encoded protein contains a region that matches 11 of the 12 residues of a sequenced peptide derived from p39 in purified eIF3 (Fig. 1). The mismatch at residue 9 (threonine instead of the peptide's proline) likely is due to an error in peptide sequencing, as the residue yields after the eighth amino acid were low and somewhat uncertain. Second, *TIF34* encodes a protein with a calculated mass of 38.8 kDa, matching closely the apparent mass of 39 kDa seen by SDS-PAGE. Third, overexpression of *TIF34* generates a somewhat higher level of a protein which reacts with anti-eIF3 antibodies and possesses the same gel mobility as p39 on SDS-PAGE (Fig. 2). The failure to obtain a high level of free p39 in excess of the eIF3 complex likely is due to proteolytic degradation of the free form of the polypeptide, as suggested by the detection of smaller immunoreactive proteins in the overproducing strain. Fourth, when *TIF34* expression under the *GAL1* promoter is repressed by glucose, not only p39 levels but also those of the higher-molecular-weight eIF3 polypeptides decrease (Fig. 4B). Taken together, these facts strongly support the conclusion that *TIF34* encodes the p39 subunit of eIF3.

Further evidence supporting p39 as a subunit of eIF3 comes from the observation that p39 is homologous (46% sequence identity) to the p36 subunit of mammalian eIF3. Their relatedness is similar to that of many other yeast and mammalian initiation factors, for example, eIF2α (58% identity), eIF2β (42%), eIF2γ (71%), eIF4A (65%), eIF4B (26%), eIF4E (33%), eIF4G (22%), and eIF5 (39%) (for a review, see reference 14). Of these initiation factor polypeptides, seven have been tested for the ability of the mammalian cDNA to function in place of the corresponding yeast gene, and six cDNAs scored positive (only eIF4A failed to complement). It was therefore surprising that the mammalian p36 cDNA does not replace *TIF34* in yeast strain TN1 (Fig. 8A). Mammalian eIF3-p36 is expressed and accumulates in yeast but is not incorporated stably into yeast eIF3 (Fig. 8C). Thus, the failure to complement may be due entirely to its inability to assemble into yeast eIF3. However, we cannot rule out the possibility that the exclusion of the human eIF3-p36 subunit from the yeast eIF3 complex is due to the presence of the endogenous yeast p39 subunit, which might outcompete the human p36 subunit for incorporation into the complex. Another explanation for the failure to complement is that human eIF3-p36 indeed incorporates into yeast eIF3 in the His^+ Trp^+ spores lacking yeast p39 but fails to function, leading to a no-growth phenotype. It will be interesting to test other homologous eIF3 subunits for their abilities to function across species as cloned cDNAs and genes become available.

It was found (1) that the human eIF3-p36 protein is a member of the WD-repeat family of proteins (18). Both yeast p39 and human p36 possess five putative WD-repeat elements (boxed regions in Fig. 7). The presence of WD-repeat elements in p39 provides a clue to its possible function. WD-repeat proteins such as the G-protein βγ subunits facilitate the formation of multiprotein complexes (19, 4). A role for p39 in forming the eIF3 complex is consistent with the depletion results discussed above.

It is important to demonstrate that all eight subunits of eIF3 are present in a homogeneous complex and that a collection of eIF3 complexes lacking one or more subunits does not exist. In previously reported work, we have shown that all eight subunits are present in purified preparations of eIF3 that are active in an assay for Met-PM synthesis (17). Furthermore, antibodies specific for PRT1 (p90), GCD10 (p62), and SUI1 (p16) immunoprecipitate eIF3 complexes containing all of the other subunits (5, 16). The p39 depletion studies provide further evidence that p39 resides in a complex with the seven other eIF3 subunits. Depletion of p39 (Fig. 4B) appears to lead to a rapid loss of the eIF3 subunits whose masses are smaller than p39 (lane 4, 7 h of growth in glucose) and a somewhat slower loss of the higher-molecular-weight subunits, since these proteins are present at rather high levels at 7 h yet are nearly absent at 12 h (compare lanes 4 and 8). However, it has not yet been possible to demonstrate clearly that the various subunits are lost at different rates, due to the variability of protein transfer on Western blotting. As argued above, the loss of eIF3 cannot be attributed to a specific inhibition of eIF3 subunit synthesis, since total protein synthesis is already strongly impaired. In addition, low levels through cell division cannot be invoked, since only a single division occurs between 7 and 12 h following the shift of the cells from galactose to glucose. Finally, the degradation of eIF3 is not due to normal rates of turnover, as the level of the entire complex is unchanged up to 6 h following inhibition of total protein synthesis (Fig. 5). The fact that all of the eIF3 subunits are degraded when p39 synthesis is specifically inhibited argues that p39 is essential for the stability of the entire complex. It follows that there must not be a sizable class of eIF3 complexes that lacks p39, as these complexes would not be expected to be affected by p39 depletion. Although apparently essential for eIF3 integrity, p39 appears not to be limiting for complex formation; overexpression of p39 does not result in increased levels of eIF3 (Fig. 2).

Depletion of p39 causes cessation of cell growth (Fig. 4A), consistent with the demonstration that deletion and disruption of *TIF34* result in failure of spores to germinate and grow (Fig. 3). Thus, *TIF34*, like *PRT1*, *GCD10*, and *SUI1*, is essential for cell viability. These results also are consistent with p39 being expressed from a single locus. Polysome analyses of p39-depleted cells show diminished polysomes and increased 80S ribosomes (Fig. 6), suggestive of a role in the initiation phase of protein synthesis. However, it has been impossible to attribute this effect directly to a defect in p39 activity, because the whole eIF3 complex is degraded. To demonstrate that p39 itself plays an essential role in eIF3 activity, it will be necessary to obtain mutant forms of p39 that can assemble into eIF3 and preserve the overall structure of the complex yet exhibit diminished function during protein synthesis. The availability of the *TIF34* clone should expedite the isolation of such mutants and provide tools for further studies of the assembly and activity of yeast eIF3.

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REFERENCES

1. **Asano, K., and J. W. B. Hershey.** Unpublished data.
1a. **Benne, R., and J. W. B. Hershey.** 1978. The mechanism of action of protein synthesis initiation factors from rabbit reticulocytes. J. Biol. Chem. **253:** 3078–3087.
2. **Broach, J. R., Y. Y. Li, L. C. C. Wu, and M. Jayarum.** 1983. Experimental manipulations of gene expression. Academic Press, New York, N.Y.
3. **Cigan, A. M., and T. F. Donahue.** 1987. Sequence and structural features associated with translational initiator regions in yeast. Gene (Amsterdam) **59:**1–18.
4. **Clapham, D. E., and E. J. Neer.** 1993. New roles for G-protein βγ-dimers in transmembrane signalling. Nature **365:**493–406.
5. **Garcia-Barrio, M. T., T. Naranda, C. R. Vazquez de Aldana, R. Cuesta, A. G.**

Hinnebusch, J. W. B. Hershey, and M. Tamame. 1995. GCD10, a translational repressor of *GCN4*, is the RNA-binding subunit of eukaryotic translation initiation factor-3. Genes Dev. **9:**1781–1796.
6. **Goumans, H., A. Thomas, A. Verhoeven, H. O. Voorma, and R. Benne.** 1980. The role of eIF-4C in protein synthesis initiation complex formation. Biochim. Biophys. Acta **608:**39–46.
7. **Gupta, N. K., A. L. Roy, M. K. Nag, T. G. Kinzy, S. MacMillan, R. E. Hileman, T. E. Dever, S. Wu, W. C. Merrick, and J. W. B. Hershey.** 1990. New insights into an old problem: ternary complex (Met-tRNAf-eIF2-GTP) formation in animal cells, p. 521–526. *In* J. E. G. McCarthy and M. F. Tuite (ed.), Springer-Verlag, Berlin, Germany.
8. **Hartwell, L. H., and C. S. McLaughlin.** 1968. A mutation of yeast apparently defective in the initiation of protein synthesis. Proc. Natl. Acad. Sci. USA **82:**468–474.
9. **Hill, J. E., A. M. Meyers, T. J. Koerner, and A. Tzagoloff.** 1986. Yeast/*E. coli* shuttle vectors with multiple unique restriction sites. Yeast **2:**163–167.
10. **Hinnebusch, A. G.** 1990. Involvement of an initiation factor and protein phosphorylation in translational control of *GCN4* mRNA. Trends Biochem. Sci. **15:**148–152.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
12. **Lamphear, B. J., R. Kirchweger, T. Skern, and R. E. Rhoads.** 1995. Mapping of functional domains in eIF4G with picornaviral proteases. Implications for cap-dependent and cap-independent translational initiation. J. Biol. Chem. **270:**21975–21983.
13. **Mader, S., H. Lee, A. Pause, and N. Sonenberg.** 1995. The translation initiation factor eIF-4E binds to a common motif shared by the translation factor eIF-4γ and the translational repressors, 4E-binding proteins. Mol. Cell. Biol. **15:**4990–4997.
14. **Merrick, W. C., and J. W. B. Hershey.** 1996. The pathway and mechanism of eukaryotic protein synthesis, p. 31–69. *In* J. W. B. Hershey, M. B. Mathews, and N. Sonenberg (ed.), Translational control. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
15. **Moldave, K., and C. S. McLaughlin.** 1988. The analysis of temperature-sensitive mutants of *Saccharomyces cerevisiae* altered in components required for protein synthesis, p. 271–281. *In* M. Tuite, F. Picard, and M. Bolotin-Fukuhara (ed.), Genetics of translation. Springer-Verlag, New York, N.Y.
16. **Naranda, T., S. E. MacMillan, T. F. Donahue, and J. W. B. Hershey.** 1996. SUI1/p16 subunit is required for the activity of translational initiation factor eIF3 in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:**2307–1613.
17. **Naranda, T., S. E. MacMillan, and J. W. B. Hershey.** 1994. Purified yeast translation factor eIF-3 is an RNA-binding protein complex that contains the PRT1 protein. J. Biol. Chem. **269:**32286–32292.
18. **Neer, E. J., C. J. Schmidt, R. Nambudripad, and T. F. Smith.** 1994. The ancient regulatory-protein family of WD-repeat proteins. Nature **371:**297–300.
19. **Pitcher, J. A.** 1992. Role of beta gamma subunits of G proteins in targeting the beta-adrenergic receptor kinase to membrane-bound receptors. Science **257:**1264–1267.
20. **Rose, M. D., F. Winston, and P. Hieter.** 1990. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
21. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101:**202–211.
22. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
23. **Schwelberger, H. G., H. A. Kang, and J. W. B. Hershey.** 1989. Translation initiation factor eIF-5A expressed from either of two yeast genes or from human cDNA. J. Biol. Chem. **268:**14018–14025.
24. **Sherman, F., G. R. Fink, and C. W. Lawrence.** 1972. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
25. **Struhl, K.** 1985. Nucleotide sequence and transcriptional mapping of the yeast pet56-his3-ded1 gene region. Nucleic Acids Res. **13:**8587–8601.
26. **Trachsel, H., and T. Staehelin.** 1979. Initiation of mammalian protein synthesis: The multiple functions of the initiation factor eIF-3. Biochim. Biophys. Acta **565:**305–314.
27. **Wei, C.-L., M. Kainuma, and J. W. B. Hershey.** 1995. Characterization of yeast translation initiation factor 1A and cloning of its essential gene. J. Biol. Chem. **270:**22788–22794.
28. **Yoon, H., and T. F. Donahue.** 1992. The *sui1* suppressor locus in *Saccharomyces cerevisiae* encodes a translation factor that functions during $tRNA_i^{Met}$ recognition of the start codon. Mol. Cell. Biol. **12:**248–260.