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SUI1/p16 Is Required for the Activity of Eukaryotic Translation Initiation Factor 3 in *Saccharomyces cerevisiae*

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A genetic reversion analysis at the *HIS4* locus in *Saccharomyces cerevisiae* has identified SUI1 as a component of the translation initiation complex which plays an important role in ribosomal recognition of the initiator codon. SUI1 is an essential protein of 12.3 kDa that is required in vivo for the initiation of protein synthesis. Here we present evidence that SUI1 is identical to the smallest subunit, p16, of eukaryotic translation initiation factor 3 (eIF3) in *S. cerevisiae*. SUI1 and eIF3-p16 comigrate upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis and cross-react with anti-SUI1 and anti-eIF3 antisera. Anti-SUI1 antisera immunoprecipitate all of the subunits of eIF3, whereas antisera against the eIF3 complex and the individual PRT1 and GCD10 subunits of eIF3 immunoprecipitate SUI1. Finally, the N-terminal amino acid sequence of a truncated form of eIF3-p16 matches the sequence of SUI1. eIF3 isolated from a *sui1*^{ts} strain at 37°C lacks SUI1 and fails to exhibit eIF3 activity in the in vitro assay for methionyl-puromycin synthesis. A free form of SUI1 separate from the eIF3 complex is found in *S. cerevisiae* but lacks activity in the in vitro assay. The results, together with prior genetic experiments, indicate that SUI1 is essential for eIF3 activity and functions as part of eIF3 and in concert with eIF2 to promote eIF2–GTP–Met-tRNA$_i$ ternary complex recognition of the initiator codon.

Initiation of protein synthesis in eukaryotic cells involves the binding of the initiator methionyl-tRNA$_i$ (Met-tRNA$_i$) and mRNA to the 40S ribosomal subunit, followed by junction of the 60S ribosomal subunit to form an 80S initiation complex. These reactions are promoted by 10 or more proteins, called initiation factors (reviewed in references 13 and 17). A prominent feature of the initiation pathway is a process called scanning, whereby a 40S ribosomal subunit carrying Met-tRNA$_i$ and initiation factors eIF2, eIF3, and eIF1A first binds to the m^{7}G-cap region at the 5′ terminus of the mRNA. The 40S ribosome then scans along the mRNA in a 3′ direction, dependent on the unwinding of secondary structure by the RNA helicase activities of initiation factors eIF4B and eIF4F (a complex of eIF4E, eIF4A, and eIF4G). The initiation codon, usually the 5′-proximal AUG, is selected through an interaction with the anticodon of the bound Met-tRNA$_i$ and by recognition of the sequence context of the AUG by an as yet uncharacterized mechanism.

Historically, eukaryotic initiation factors have been identified by purification of proteins from mammalian cells followed by in vitro studies of how they interact to promote the initiation pathway. More recently, genetic approaches using the yeast *Saccharomyces cerevisiae* have enabled studies of the mechanism of protein synthesis in vivo. Two genetic systems in particular have provided insight into the role of the initiation factors in translation. (i) Regulation of the synthesis of transcription factor GCN4 involves reinitiation following translation of a small upstream open reading frame by a mechanism that is sensitive to the rate of binding of Met-tRNA$_i$ to the reinitiating ribosome (14). Some of the mutant strains that are constitutively derepressed for GCN4 synthesis are altered in the genes that encode the subunits of initiation factors involved in Met-tRNA$_i$ binding, eIF2 and eIF2B. (ii) Extragenic suppressors of a His$^-$ mutant strain, in which the AUG initiation codon for *HIS4* was mutated, were characterized as mutations in genes whose products affect initiation codon selection. Two of the suppressors, called *sui2* (6) and *SUI3* (7), encode subunits of eIF2, as judged by their sequence homology with the corresponding mammalian proteins. A third suppressor, a temperature-sensitive mutant gene called *sui1* (22), encodes a 12.3-kDa protein of unknown function, although the protein appears to act during the initiation phase of protein synthesis since a substantial reduction in polysomes occurs upon a shift to the nonpermissive temperature.

The yeast homologs of most of the known mammalian initiation factors have been identified, and their genes have been cloned and sequenced. Identification of the functional role of yeast proteins that are implicated in translation but that do not correspond to known mammalian homologs has been difficult because of the lack of a biochemical characterization of yeast initiation factors and the initiation pathway. The major gap in our knowledge concerns eIF3, an initiation factor that comprises eight or more subunits in mammalian cells. We recently embarked on a biochemical approach to isolating yeast eIF3 and have purified and characterized a protein complex that stimulates methionyl-puromycin (Met-PM) synthesis in a heterologous mammalian assay system lacking mammalian eIF3 (18). Yeast eIF3 comprises eight subunits ranging in apparent mass from 16 to 135 kDa. Two of the subunits, p90 and p62, are encoded by previously known yeast genes implicated in the initiation pathway, namely, *PRT1* (18) and *GCD10* (10), respectively. It is noteworthy that (i) the mutant *prt1-1* reduces Met-tRNA$_i$ binding to the 40S ribosomal subunit (8) and (ii) mutations in *GCD10* derepress GCN4 synthesis (10), thereby implicating eIF3 in Met-tRNA$_i$ binding in vivo.

The apparent mass of the smallest subunit of yeast eIF3 is only 16 kDa when analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), a size comparable to that reported for SUI1 (although only a 12.3-kDa protein as calculated from its sequence, it behaves like a 17-kDa

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protein upon SDS-PAGE in the gel system employed by Yoon and Donahue [22]). Since SUI1 is implicated in the initiation phase of protein synthesis, and since eIF3 affects Met-tRNA$_i$ binding to 40S subunits, it is reasonable to suspect that SUI1 is a subunit of eIF3. We therefore characterized the p16 subunit of eIF3 and describe experiments showing that this protein indeed is encoded by *SUI1*.

MATERIALS AND METHODS

Yeast strains, cell growth, and lysis. The following *S. cerevisiae* strains were used in the work reported here: W303, *MAT***a** *leu2-3,112 his3-11 ade2-1 trp1-1 can1-100* (19); BC64, *MAT*α *his4-401 ura3-52 leu2-3,112* (22); BC61, *MAT*α *his4-401 ura3-52 leu2-3,112* [YEp24-*SUI1*] (22); 138-7B, *MAT***a** *sui1-17 his4-301*(*ACG*) *ura3-52* (22). Strain W303 was used for purification of eIF3, in immunoprecipitation experiments, and as a reference for eIF3 activity when other mutant strains were assayed. The *sui1^{ts}* suppressor strain (138-7B) has a temperature-sensitive phenotype for growth at 37°C (22). For analyses, duplicate cultures of W303 and *sui1^{ts}* were grown at 23°C, and then one culture each was shifted to 37°C for 3 h. Strain BC64 contains a single copy of *SUI1*, whereas the isogenic strain BC61 carries *SUI1* on the high-copy-number vector YEp24 (22). Total cell extracts were prepared from all cultures as previously described (19).

Subcellular fractionation and purification of eIF3. The preparation of the ribosomal high-salt wash (HSW) was performed as previously described (18). Briefly, cells were grown to an optical density at 600 nm of 1.6, digested with lyticase, homogenized on ice with a Dounce homogenizer, and clarified by centrifugation, and the ribosomes were pelleted from the lysate by centrifugation at 46,000 rpm (Beckman Ti65 rotor). The HSW, enriched in initiation factors, was obtained by suspending the pelleted ribosomes in buffer containing 0.5 M potassium acetate (KOAc), centrifuging again, and removing the supernatant.

The HSW was further fractionated in buffer containing 0.3 M KCl on a Superdex 200 gel filtration column of a fast protein liquid chromatography system (FPLC; Pharmacia LKB Biotechnology Inc.). The fractions were analyzed for the presence of eIF3 and SUI1 by immunoblotting as described below. The eIF3 activity was determined by the Met-PM synthesis assay, a reaction that mimics formation of the first peptide bond in vitro (18). Further purification of eIF3 on a MonoS 5/5 column (FPLC; Pharmacia LKB Biotechnology Inc.) was performed for protein sequencing of the p16 subunit, as previously described (18).

Immunoprecipitations. The rabbit antisera used in the analyses have been described previously: anti-eIF3 (18), anti-PRT1 (5), anti-GCD10 (10), and anti-SUI1 (22). Affinity columns were prepared by cross-linking of bound antibodies to GammaBind-G Sepharose beads (Pharmacia) via the bifunctional coupling reagent dimethylpimelimidate (12). Approximately 2 mg of eIF3 and PRT1 and 4 mg of GCD10 and SUI1 antiserum protein were bound per milliliter of beads. Prior to mixing with the antigen, the beads were equilibrated in buffer A [30 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), pH 8.0, 100 mM KOAc, 3 mM $Mg(OAc)_2$, 1 mM dithiothreitol, 0.1% SDS, 1% Nonidet P-40, 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF)]. Samples containing 50 μg of total HSW proteins were adjusted to 100 mM KOAc with buffer A in a final volume of 100 μl and were incubated for 30 min at 4°C with 25 μl of a 1:1 slurry of un-cross-linked GammaBind-G Sepharose beads in buffer A. The beads were collected by brief centrifugation and discarded to remove antigens that bind nonspecifically to the beads. The supernatants were mixed with 30 μl of a 1:1 slurry of the protein G beads containing cross-linked antibodies, and the mixture was incubated for 3 to 5 h at 4°C in a rotating mixer. Samples were briefly centrifuged, and the supernatants were removed. The beads were washed four times with 400 μl of buffer A and once with 400 μl of 62.5 mM Tris-HCl, pH 6.8. The washed beads were incubated with 50 μl of SDS gel sample buffer (16) and heated at 90°C for 3 min to solubilize the eluted proteins, and the samples were analyzed by SDS-PAGE and Western blot (immunoblot) analysis as described below. In initial experiments, equivalent proportions of the bead supernatants and immunoprecipitates (pelleted beads) were analyzed; the efficiency of the immunoprecipitations was estimated at between 50 and 70%. Although antibodies were cross-linked to the protein G beads, the presence of the immunoglobulin G heavy chain could still be observed on Western blots. The presence of this band can be explained by an efficiency of coupling of about 60%.

SDS-PAGE and immunoblotting. Because of the broad range of SDS mobilities between the largest subunits of eIF3 and SUI1, all samples were analyzed in duplicate: SDS–9% PAGE was used for analysis of the higher-molecular-weight subunits of eIF3, and SDS–15% PAGE was used for detection of SUI1 and the smaller subunits of eIF3. Following SDS-PAGE (16), proteins were transferred to an Immobilon polyvinylidene difluoride membrane (Millipore) with a 10 mM CAPS [3-(cyclohexylamino)-1-propanesulfonic acid], pH 11, buffer containing 10 or 20% (vol/vol) methanol to ensure the proper transfer of eIF3 subunits or SUI1, respectively. 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 either a 1:1,000 dilution of rabbit polyclonal antiserum directed against eIF3 (18), a 1:5,000 dilution of rabbit polyclonal antiserum directed against PRT1 (5), or a 1:200 dilution of rabbit polyclonal antiserum directed against the SUI1 gene product generated from a TrpE-SUI1 fusion protein (22). Subsequently, the blots were incubated with alkaline phosphatase-conjugated anti-rabbit immunoglobulin G antibody and visualized colorimetrically with the alkaline phosphatase substrates Nitro Blue Tetrazolium–5-bromo-4-chloro-3-indolylphosphate toluidinium as previously described (18).

Peptide sequencing. For partial sequencing of p16, purified and active eIF3 complex was fractionated by SDS–15% PAGE and subunit proteins were transferred to a PRO-blot membrane (Applied Biosystems Inc.) as described above for the detection of SUI1. The membrane was stained according to the manufacturer's recommendation, and the two bands (ca. 16 kDa) corresponding to proteins immunoreactive to anti-SUI1 antibodies were individually excised. Each was subjected to automated Edman degradation by using an Applied Biosystems amino acid analyzer ABI 470D in the Protein Structure Laboratory at the University of California, Davis.

RESULTS

SUI1 is the p16 subunit of eIF3. From biochemical characterization of mammalian eIF3 activity, it is known that eIF3 stabilizes the binding of Met-tRNA$_i$ to 40S ribosomes (3, 20). Genetic studies with *S. cerevisiae* implicate SUI1 in the recognition of the initiation codon, a function that might well involve eIF3 in addition to eIF2. Since the apparent mass of SUI1 as exhibited by SDS-PAGE is reported to be 17 kDa (22) and since yeast eIF3 possesses a 16-kDa subunit, it was appropriate to investigate whether this subunit is encoded by *SUI1*. Rabbit antiserum raised against a TrpE-SUI1 fusion protein (22) was used to probe a Western blot of crude ribosomal HSW and purified yeast eIF3. As shown in Fig. 1A, the antiserum recognizes two proteins in the HSW (lane 1) which correspond to the previously characterized SUI1 protein (22). The antibodies also recognize two closely migrating proteins in purified eIF3 that correspond to the p16 subunit (lane 2). With the samples analyzed in Fig. 1A, the faster-migrating band is more abundant than the one which migrates more slowly, but in some cases the slower band is the major immunoreactive substance (data not shown; see Fig. 4). The ratio of the two bands varies from preparation to preparation, suggesting that the faster protein may be a partial proteolytic fragment of the larger.

To obtain additional evidence that p16 and SUI1 may be the same protein, we attempted to obtain a partial amino acid sequence of the p16 subunit. eIF3 was purified from *S. cerevisiae* W303 as described previously (18), and the purified, active factor was subjected to SDS–15% PAGE. After transfer of the proteins to a membrane, the two protein bands corresponding to p16 were excised and sequenced as described in Materials and Methods. Amino acid sequence analysis of the more slowly migrating protein revealed that the protein contained a blocked amino terminus. Sequencing of the faster-migrating protein generated an amino acid sequence (Fig. 1B) which showed near identity to a region in SUI1. The sequenced amino acids correspond to residues 10 to 19 of the SUI1 protein, with only residue 17 failing to match. Since analysis of the last four residues gave low yields and uncertain identifications, the mismatch likely represents an error in the peptide sequence. The sequencing result demonstrates that the faster band corresponds to a fragment of SUI1 that is truncated at its N terminus by 9 amino acid residues. It is therefore likely that the more slowly migrating band that cross-reacts with the anti-SUI1 serum is the intact SUI1 protein.

Coimmunoprecipitation of SUI1 and eIF3 polypeptides. Having established that p16 corresponds to SUI1, we next wished to determine if this protein is an integral part of the eIF3 complex. In previous studies, polyclonal antibodies raised against either GCD10, PRT1, or purified yeast eIF3 were used to precipitate the entire eIF3 complex of eight subunits (10, 18). To obtain evidence for the presence of SUI1 in eIF3 complexes, a ribosomal HSW was prepared as described in Materials and Methods and was used for the immunoprecipita-

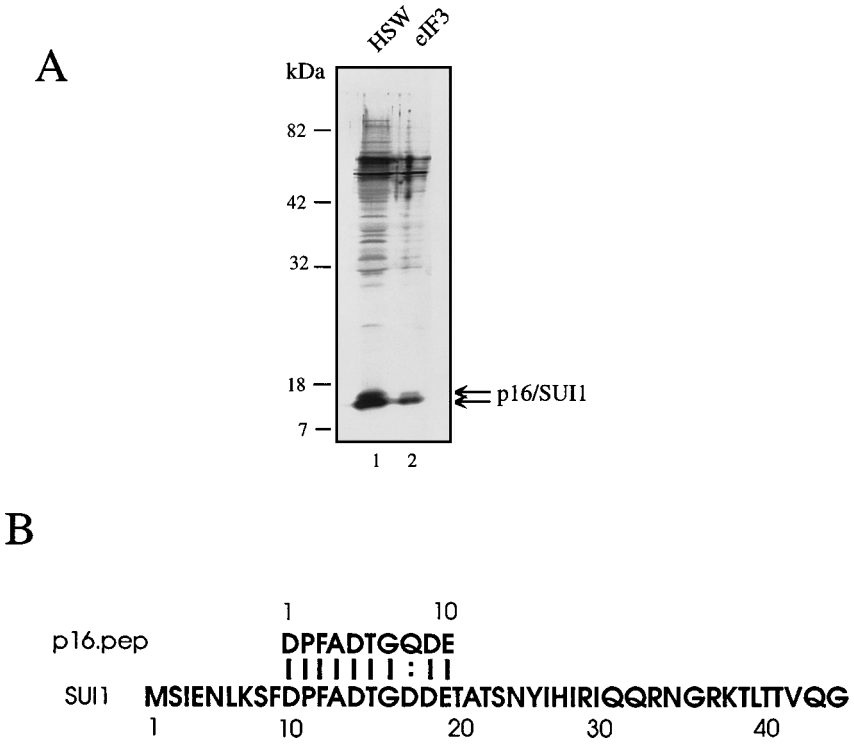

FIG. 1. Western immunoblotting analysis of eIF3 with anti-SUI1 antibody and amino acid determination. (A) Samples were subjected to SDS–15% PAGE and immunoblotting with anti-SUI1 antibody as described in Materials and Methods. Lane 1, 25 μg of total proteins in the HSW fraction; lane 2, 100 ng of purified eIF3. The migration positions of molecular mass markers are shown on the left. The arrows on the right indicate the migration positions of SUI1. (B) Purified eIF3 was fractionated by SDS–15% PAGE, and the proteins corresponding to the p16 subunit were sequenced as described in Materials and Methods. Shown are the peptide sequence (p16.pep) in the upper line and the sequence of the first 44 amino acids of SUI1 in the lower line. Identical amino acid residues are connected by vertical dashes.

tion experiments with antibodies against SUI1, eIF3, GCD10, and PRT1. The resulting immune complexes were fractionated by SDS-PAGE and probed by immunoblot analysis for SUI1 and the other subunits of eIF3 by using the various antisera. Probing of the immune complexes with antibodies against eIF3 (Fig. 2A) shows that each of the four antisera immunoprecipitates similar quantities of eIF3 subunits from p33 to p135 (lanes 2 to 5). In a control experiment, a preimmune serum (eIF3,PI) failed to precipitate these proteins (Fig. 2A, lane 1). Confirmation that the immunopurified complexes correspond to eIF3 was obtained by probing the immunoprecipitates with antibody to PRT1, a known component of eIF3 (Fig. 2B). Analysis of the smaller proteins in the immunoprecipitates with anti-SUI1 (Fig. 2C) shows clearly that SUI1 is present in all of the immunoprecipitates (lanes 2 to 5) except that generated by the preimmune serum (lane 1). The facts that anti-SUI1 precipitates the larger subunits of eIF3 and that SUI1 is present in all immune complexes strongly indicate that SUI1 is part of the same protein complex as PRT1 and GCD10, rather than simply a contaminant in eIF3 preparations.

SUI1 is important for eIF3 activity. A *sui1*^{ts} strain that generates smaller polysomes at the restrictive temperature was isolated, suggesting that SUI1 performs an essential function during the translation initiation process (22). The question of whether the translational defect involves the action of eIF3 arises. In preliminary experiments we used a cell-free translation system derived from the *sui1*^{ts} strain. Preincubation of such an extract at 37°C shows a deficiency in translation (data not shown). The addition of purified yeast eIF3 stimulates translation in such an extract, whereas preincubation of eIF3 with anti-SUI1 or anti-eIF3 polyclonal antisera prevents the stimulation (data not shown). These experiments suggested that an in vitro approach might be suitable for analyzing the *sui1*^{ts} defect.

An assay system in which yeast proteins functionally substitute for mammalian eIF3 in Met-PM synthesis, a reaction that requires formation of the 80S initiation complex, had been constructed earlier (18). We have employed this in vitro assay to test the activity of yeast eIF3 present in the HSW fractions obtained from wild-type and *sui1*^{ts} strains grown at permissive and nonpermissive temperatures. Duplicate cultures of the *sui1*^{ts} and wild-type strains were grown at 23°C, and then one culture of each was shifted to 37°C for 3 h. Ribosomal HSWs were prepared and tested for activity in the Met-PM synthesis assay. Wild-type and *sui1*^{ts} strains grown at the nonrestrictive temperature showed comparable stimulations (two- to three-fold) of Met-PM synthesis (Fig. 3A). These values are close to the maximum stimulation (3.7-fold) obtained with HeLa eIF3. A similar stimulation of 2.7-fold was seen when the wild-type strain was shifted to the restrictive temperature for 3 h (Fig. 3A). In contrast, little or no activity was obtained when an HSW from the *sui1*^{ts} strain that had been shifted to 37°C was used (Fig. 3A). Clearly, eIF3 activity in this preparation is lacking.

Western immunoblot analysis of the four HSWs revealed the presence of all of the larger subunits of eIF3 from p135 to p21 (data not shown). Incubation of the blot with anti-SUI1 serum showed differences in the abundance of SUI1 in the HSW samples. A doublet (as described above) representing the two SUI1 forms was detected in the wild-type and *sui1*^{ts} strains

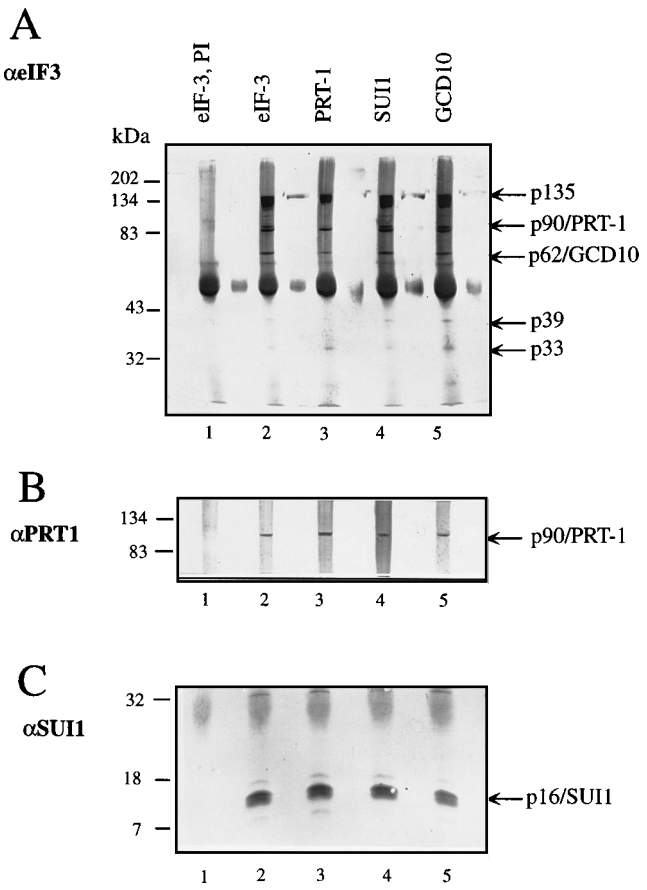

FIG. 2. Coimmunoprecipitation of SUI1 with the subunits of eIF3. Approximately 50 μg of total proteins from the ribosomal HSW was adsorbed to polyclonal antibodies against eIF3 (lane 2), PRT1 (lane 3), SUI1 (lane 4), or GCD10 (lane 5) or to preimmune sera (lane 1). The immune complexes were separated by SDS–9% PAGE (A and B) or SDS–15% PAGE (C), transferred to a polyvinylidene difluoride membrane, and probed with polyclonal antibodies against eIF3 (A), PRT1 (B), or SUI1 (C) as described in Materials and Methods. The migration positions of molecular mass markers are indicated on the left, and the migration positions of the subunits of eIF3, which are named according to their apparent masses, are shown on the right. (A) The immunoreactive bands seen between the numbered lanes are due to leakage of material from the adjoining lanes.

grown at the nonrestrictive temperature (Fig. 3B, lanes 1 and 3). However, when the *sui1*^{ts} strain was shifted to the restrictive temperature, significantly less SUI1 was detected in the HSW (Fig. 3B, lane 4) compared with that for the wild-type strain (lane 2). Since the other subunits of eIF3 are present in the HSW samples analyzed in Fig. 3B, lane 4, the absence of SUI1 indicates either that the protein has left the eIF3 complex and distributes into a different cell fraction or that SUI1 is degraded. Because little or no SUI1 is detected when total cell extracts are analyzed (data not shown), it appears that the level of SUI1 is strongly reduced in the *sui1*^{ts} strain upon shift to the nonpermissive temperature. This is consistent with the previous report (22) that SUI1 is thermolabile in the *sui1*^{ts} strain. Given that the HSW from the *sui1*^{ts} strain grown at 37°C lacks Met-PM synthesis activity yet contains all of the subunits of eIF3 except SUI1, it appears that SUI1 is required for eIF3 activity in vitro.

SUI1 is present in cells both as a component of eIF3 and as a free protein. It was reported recently that a human homolog of SUI1, named sui1iso1, is identical to the mammalian initiation factor eIF1 (15). eIF1 is one of the least-characterized initiation factors; it only weakly promotes initiation of protein synthesis with highly purified components (21). However, mammalian eIF1 is not detected in preparations of eIF3, nor does it stimulate the Met-PM synthesis assay (3). Identification of SUI1 in *S. cerevisiae* as a component of eIF3 raises the question of whether SUI1 might serve in two capacities, one as an essential component of eIF3 and another as a free protein that functions like mammalian eIF1.

To address this question, we have fractionated by gel filtration the HSW fractions from strains expressing either a single copy or multiple copies of the *SUI1* gene. Chromatographic fractions from a Superdex 200 FPLC column were analyzed by SDS–9% PAGE and SDS–15% PAGE and by Western blot analysis for the presence of eIF3 and SUI1, respectively. The larger polypeptides constituting the yeast eIF3 complex (p135, p90, p62, p39, p33, and p29) elute from the gel filtration column at a position corresponding to a molecular mass of about 550 kDa (Fig. 4A and C, fractions 19 and 20), as has been reported previously (18). Some additional antigenic bands detected on the same blot are probably due to proteolytic cleavage of the larger subunits. The same chromatographic fractions

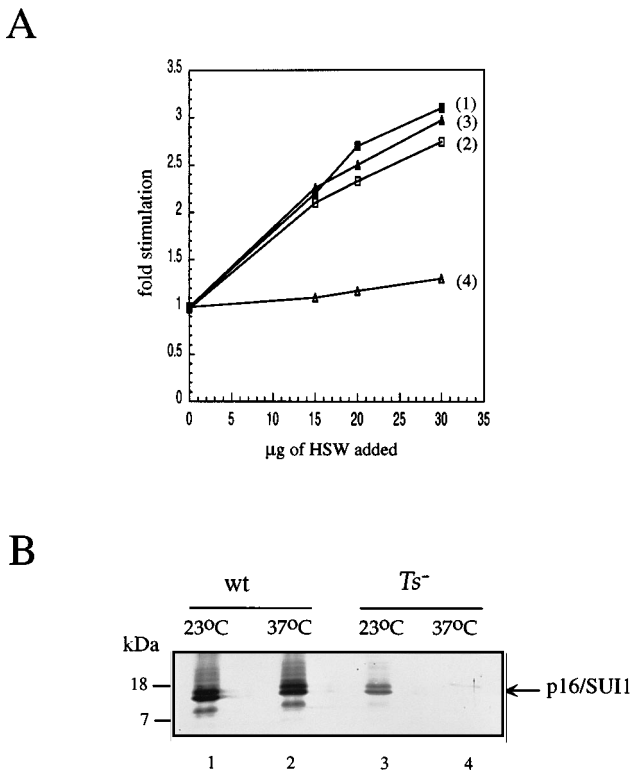

FIG. 3. SUI1 stimulation of the Met-PM synthesis assay for eIF3 activity. (A) Assay of eIF3 biochemical activity. The Met-PM synthesis assay (18) was carried out with the indicated amounts of total proteins present in ribosomal HSW fractions. HSWs were prepared from wild-type and *sui1*^{ts} strains, each grown at 23 and 37°C, and eIF3 activity is reported as fold stimulation over that observed when eIF3 was omitted. A background value (4,294 cpm) obtained with the charged tRNA alone was subtracted. The 100% value obtained with HeLa eIF3 is 19,310 cpm. Curves: 1, wild type at 23°C; 2, wild type at 37°C; 3, *sui1*^{ts} at 23°C; 4, *sui1*^{ts} at 37°C. (B) Immunoblot analysis of SUI1. Total proteins (20 μg) from each of the four HSW preparations used for panel A were analyzed by SDS–15% PAGE and immunoblotting with rabbit polyclonal antiserum against SUI1, and immune proteins were visualized with alkaline phosphatase-conjugated antibodies against rabbit immunoglobulin G (see Materials and Methods). Lane 1, wild type (wt) at 23°C; lane 2, wild type at 37°C; lane 3, *sui1*^{ts} at 23°C; lane 4, *sui1*^{ts} at 37°C. The arrow on the right indicates the migration position of SUI1.

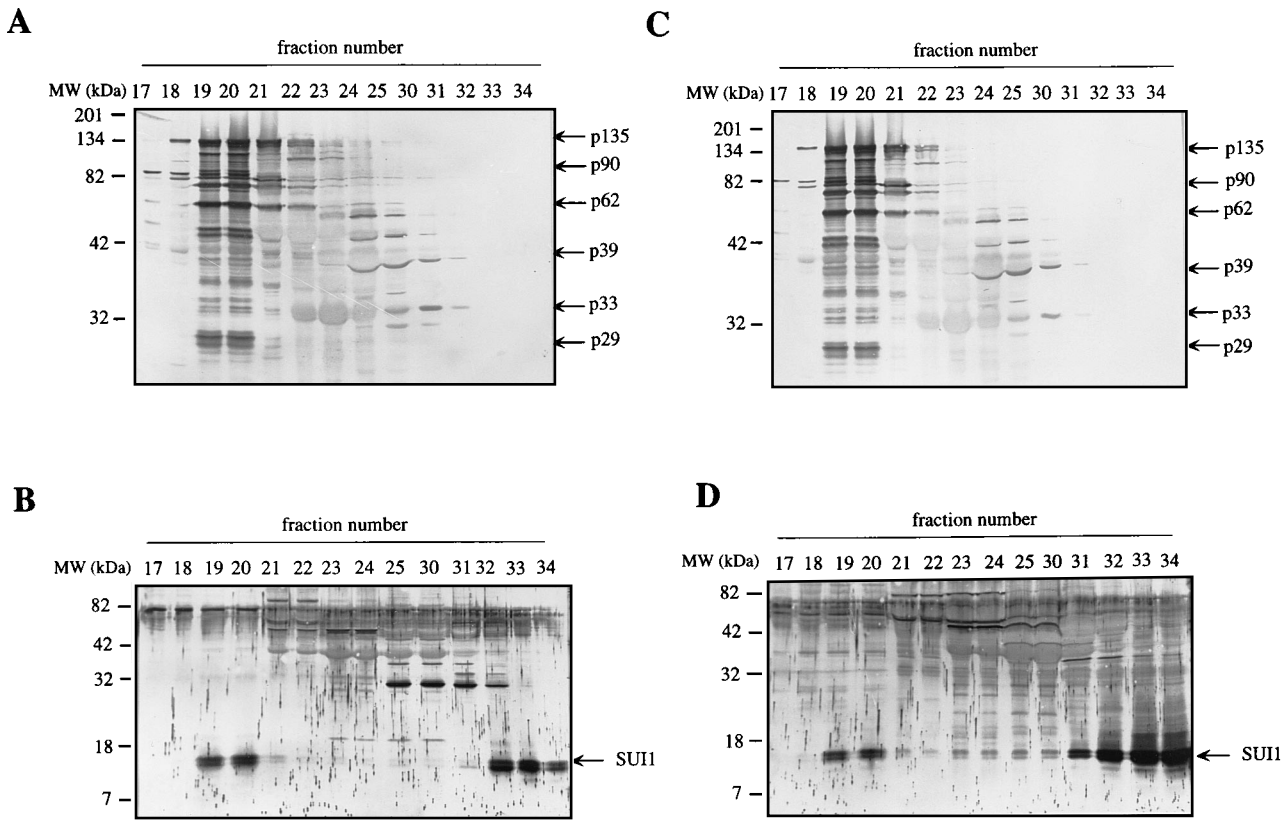

FIG. 4. Detection of SUI1 and eIF3 in a size-fractionated ribosomal HSW. Ribosomal HSWs were prepared from the single-copy *SUI1* wild-type strain BC64 (A and B) and from the multicopy *SUI1* wild-type strain BC61 (C and D). Each was fractionated on a Superdex 200 gel filtration column as described in Materials and Methods. Protein fractions were separated by SDS–9% PAGE and analyzed for the presence of eIF3 subunits (A and C) by immunoblotting with anti-eIF3 serum or separated by SDS–15% PAGE and analyzed by immunoblotting with anti-SUI1 serum (B and D) as described in the legend to Fig. 2. The column fraction numbers are indicated at the top of each blot; note that analyses of fractions 26 to 29 are not shown. Molecular mass protein markers (not shown) eluted from the column at the following positions: 670 kDa, fraction 17; 440 kDa, fraction 22; 67 kDa, fraction 30; 17 kDa, fraction 35. The migration positions of molecular mass markers in the SDS gels and their masses are shown on the left. The arrows on the right identify where the subunits of eIF3 and SUI1 migrate.

were tested for the presence of SUI1 (Fig. 4B and D). The immunoblots show that in the strain containing a single copy of *SUI1*, the SUI1 protein elutes at two different positions (Fig. 4B), corresponding to molecular masses of 550 kDa (fractions 19 and 20) and 20 kDa (fractions 32 to 34). About 30% of the SUI1 appears in the larger eIF3 complex, whereas 70% of the SUI1 is much smaller, with a size that is in agreement with the predicted or SDS mobility mass of free SUI1 (22). The two forms of SUI1 could be due to an excess of SUI1 over the other subunits of eIF3 or could involve a stoichiometric amount of SUI1 relative to eIF3, but with a portion of SUI1 dissociated from eIF3 because of low affinity for the complex.

When the same fractionation procedure was performed with the strain containing *SUI1* on a high-copy-number plasmid, comparable amounts of eIF3 subunits (Fig. 4C) and SUI1 (Fig. 4D) were detected in the high-molecular-weight complex (fractions 19 and 20), whereas the abundance of the free form increased significantly (fractions 31 to 34). Thus, overexpression of SUI1 does not affect the assembly, apparent size, or composition of the eIF3 complex (compare single-copy strain in Fig. 4A with high-copy-number strain in Fig. 4C), suggesting that SUI1 is not limiting for eIF3 biogenesis in the single-copy strain. Furthermore, the result militates against the argument that in wild-type cells SUI1 readily dissociates from eIF3, leaving a SUI1-deficient complex and a free form. If this were so, the high level of free SUI1 in the high-copy-number plasmid strain would cause an increased amount of SUI1 in eIF3 by mass action, which in fact is not seen here.

The free form of SUI1 (fractions 32 to 34) was analyzed by immunoprecipitation with antisera against eIF3, PRT1, SUI1, and GCD10 (Fig. 5). Anti-SUI1 antibody precipitated about 60% of the SUI1, whereas anti-eIF3 precipitated about 20%. The other antibodies failed to precipitate SUI1 as expected, since the free form of SUI1 is not in complexes with those proteins. The less efficient immunoprecipitation by anti-eIF3 likely is due to the low titer of antibodies that recognize the free form of SUI1. The more efficient immunoprecipitation of SUI1 by the anti-eIF3 antiserum seen in Fig. 2 is readily explained by the high titers of antibodies against the larger subunits of eIF3.

The free form of SUI1 does not stimulate Met-PM synthesis. The question of which form of SUI1 is responsible for its activity in initiation arises. To answer this question, we have performed the Met-PM synthesis assay on fractions obtained by gel filtration chromatography of the HSW from the single-copy *SUI1* strain (Fig. 4A). Fractions 19 and 20 which contained the eIF3 complex stimulated the Met-PM assay by over 3-fold (Table 1). In contrast, fractions 32, 33, and 34 that contain the free form of SUI1 gave a much lower, but reproducibly detectable, 1.6- to 1.7-fold stimulation. It is possible that the low stimulatory activity is due not to SUI1 but rather to initiation factors eIF1A and/or eIF5A, whose sizes are sim-

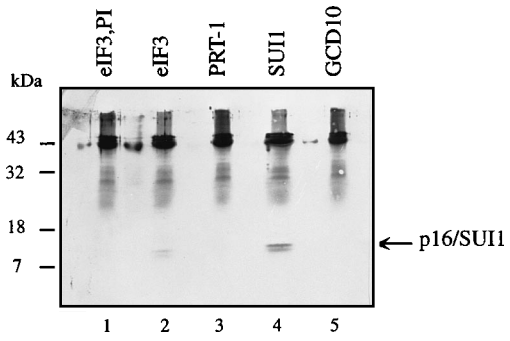

FIG. 5. Analysis of the free form of SUI1. Approximately 100 μl of Superdex 200 column fraction 33 (Fig. 4B) was adsorbed to polyclonal antibodies against eIF3 (lane 2), PRT1 (lane 3), SUI1 (lane 4), or GCD10 (lane 5) or to preimmune serum (lane 1). The immune complexes were separated by SDS–15% PAGE, transferred to a polyvinylidene difluoride membrane, and probed with polyclonal antibodies against SUI1 as described in Materials and Methods. The migration positions of molecular mass markers are indicated on the left, and the migration position of SUI1 is shown on the right.

ilar to that of SUI1 and whose presence was detected in these fractions by immunoblotting (results not shown). Both factors are routinely added to the assay system but may not have been saturating. In order to resolve this question, pooled Superdex 200 fractions 32 to 34 from the experiment whose results are shown in Fig. 4B and Table 1 were applied to a MonoS 5/5 column and eluted with a linear 100 to 450 mM KCl gradient as previously described (18). Fractions containing eIF1A, eIF5A, and SUI1 were identified by Western immunoblotting (results not shown). Peak fractions (9 μl each) containing the three separated initiation factors were tested for eIF3 activity in the Met-PM synthesis assay as described in the legend to Fig. 3. SUI1 is not active in the Met-PM synthesis assay, although eIF1A and eIF5A each stimulates weakly (ca. 1.4-fold). These data, together with the evidence that eIF3 lacking SUI1 is inactive, strongly indicate that SUI1 functions in the Met-PM assay as the p16 subunit of eIF3. However, we cannot rule out the possibility that the lack of activity with the more highly fractionated SUI1 is due to inactivation during purification.

DISCUSSION

Mutations in *SUI1* were identified originally by their ability to restore the expression of *HIS4* whose AUG initiation codon was altered (4). The basic assumption is that the *sui1* suppressor locus contains mutations in a gene that encodes a *trans*-acting factor that alters translation start site selection and therefore functions in mediating ribosomal recognition of the initiator region during the scanning process. It was suggested that *SUI1* encodes an additional factor that functions in concert with eIF2 to enable Met-tRNA$_i$ to recognize the AUG initiator codon (22). Here we present numerous lines of evidence showing that *SUI1* encodes the p16 subunit of eIF3: (i) the apparent masses of SUI1 (17 kDa) and p16 (16 kDa) estimated independently from SDS-PAGE are similar; (ii) anti-SUI1 antibody prepared from a recombinant TrpE-SUI1 fusion protein specifically recognizes the p16 subunit of purified eIF3; (iii) the N-terminal sequence of truncated p16 matches 9 of 10 amino acids of SUI1 beginning at residue 10; (iv) anti-SUI1 antibodies adsorb a protein complex that contains essentially all of the subunits of eIF3, whereas anti-eIF3, anti-PRT1, and anti-GCD10 each adsorb the eIF3 complex which contains SUI1; and (v) a portion of SUI1 coelutes from a gel filtration column as a high-molecular-weight complex with the other subunits of eIF3. We conclude that *SUI1* encodes the p16 subunit and that this subunit is an integral part of the eIF3 complex.

Analyses of polysomes from the *sui1^{ts}* strain shifted to the nonpermissive temperature of 37°C indicate that SUI1 is required for efficient initiation of protein synthesis, since polysomes are diminished and the quantity of 80S ribosomes increases (22). It was recently reported that a human protein, first identified as sui1iso1 because it shares 57% sequence identity with yeast SUI1 (9), corresponds to eIF1 (15). It is therefore plausible that SUI1 has two functions: to serve as a component of eIF3 and to function separately as eIF1. Unfortunately, the role of mammalian eIF1 in the initiation pathway is not well established. It is not found in active preparations of mammalian eIF3, but when added to a fractionated translation assay system with highly purified initiation factors (including eIF3), it stimulates globin synthesis modestly, up to about twofold (2, 20). It cannot be ruled out that mammalian eIF1 associates weakly with mammalian eIF3, is readily removed during purification of the initiation factors, yet fulfills its function in vivo as part of the eIF3 complex. In the results reported here, we show that SUI1 is essential for the activity of yeast eIF3 in the assay for Met-PM synthesis. Furthermore, the free form of SUI1 does not replace eIF3 in this assay (see above). Whether SUI1 also plays a role as a free form comparable to eIF1 remains to be elucidated. There already is a precedent for an initiation factor functioning in two forms: eIF4A is an abundant initiation factor which is found in part in a complex with eIF4E and eIF4G, called eIF4F. In in vitro assays that are saturated for eIF4F, eIF4A nevertheless stimulates protein synthesis (1), suggesting two functional roles for the factor.

The *sui1^{ts}* suppressor strain results in initiation at a UUG codon in the early *HIS4* coding region. The fact that Met-tRNA$_i$ is used to initiate HIS4 synthesis suggests that the usual translational machinery and mechanism are involved in the suppressor phenotype (22). *SUI3* (encoding a mutant form of eIF2β) suppression events also utilize Met-tRNA$_i$ to initiate at UUG (7), suggesting that the mechanism of suppression is common to *sui1* and *SUI3*. Therefore, SUI1 appears to have a function related to recognition of the start codon by eIF2. It is highly likely that this function occurs through the action of eIF3. Results from work with mammalian systems show that eIF3 is bound to the 40S ribosomal subunit during the scanning process and stabilizes the binding of Met-tRNA$_i$ to the 40S

TABLE 1. eIF3 activity in HeLa and Superdex 200 column fractions[a]

Fraction tested	Fold stimulation
HeLa eIF3	3.7
Superdex 200	
17	1.1
19	3.3
20	3.4
22	1.0
24	1.0
32	1.6
33	1.6
34	1.7

[a] SUI1 in the ribosomal HSW of strain W303 was fractionated on a Superdex 200 FPLC column as described in the legend to Fig. 4, and the indicated fractions were analyzed for eIF3 activity in the Met-PM synthesis assay as described in the legend to Fig. 3.

subunit (3, 21). It is possible that eIF3 interacts with eIF2 on the 40S subunit, since eIF3 stabilizes ternary complexes of eIF2–GTP–Met-tRNA$_i$ in the absence of ribosomes (11), thereby indicating a direct interaction of the two initiation factors. Results from the derepression of GCN4 synthesis in *S. cerevisiae* also implicate eIF3 in Met-tRNA$_i$ binding. Mutations in *GCD10*, the gene encoding the p62 subunit of eIF3, reduce the apparent rate or stability of Met-tRNA$_i$ binding to scanning 40S ribosomal subunits and adversely affect the initiation process (10).

eIF3 stands in the center of the 40S initiation complex and plays a vital role in the initiation pathway. It is an RNA-binding protein that also binds to the 40S ribosomal subunit, interacts with eIF2 and eIF4G, and promotes the binding of both Met-tRNA$_i$ and mRNA. It may be possible to attribute a different, specific role to each of its subunits. Although eIF3 currently is one of the least characterized of the initiation factors, a combination of genetic and biochemical approaches with *S. cerevisiae* may identify and elucidate the different functions of the subunits of eIF3.

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