Rpg1, the *Saccharomyces cerevisiae* Homologue of the Largest Subunit of Mammalian Translation Initiation Factor 3, Is Required for Translational Activity*

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Eukaryotic initiation factor 3 (eIF3) consists of at least eight subunits and plays a key role in the formation of the 43 S preinitiation complex by dissociating 40 and 60 S ribosomal subunits, stabilizing the ternary complex, and promoting mRNA binding to 40 S ribosomal subunits. The product of the *Saccharomyces cerevisiae* RPG1 gene has been described as encoding a protein required for passage through the G1 phase of the cell cycle and exhibiting significant sequence similarity to the largest subunit of human eIF3. Here we show that under nondenaturing conditions, Rpg1p copurifies with a known yeast eIF3 subunit, Prt1p. An anti-Rpg1p antibody co-immunoprecipitates Prt1p, and an antibody directed against the Myc tag of a tagged version of Prt1p co-immunoprecipitates Rpg1p, demonstrating that both proteins are present in the same complex. A cell-free translation system derived from the temperature-sensitive *rpg1–1* mutant strain becomes inactivated by incubation at 37 °C, and its activity can be restored by the addition of the Rpg1-containing protein complex. Finally, the *rpg1–1* temperature-sensitive mutant strain shows a dramatic reduction of the polysome/monosome ratio upon shift to the restrictive temperature. These data show that Rpg1p is an authentic eIF3 subunit and plays an important role in the initiation step of translation.

Initiation of protein synthesis is a complex process requiring the participation of several factors promoting (a) the association of initiator tRNA with the 40 S ribosomal subunit in order to form the 43 S preinitiation complex; (b) the melting of secondary mRNA structures; (c) binding of the mRNA to the 43 S preinitiation complex; and (d) the addition of the 60 S ribosomal subunit to assemble an 80 S ribosome at the initiation codon (reviewed in Ref. 1). At this point, the initiation step of translation is accomplished, and the ribosome is committed to the elongation process. Up to now at least 10 proteins called initiation factors have been implicated in the initiation of protein synthesis (2). Among these initiation factors (eIFs), 1 eIF3 represents the largest and the most complex one.

*eIF3 is thought to be involved in the dissociation of the 80 S ribosome into 40 and 60 S subunits, to bind to 40 S subunits (3–5), and thereby to act as an anti-association factor hand in hand with eIF6 (5–8). Furthermore, eIF3 prevents dissociation of the Met-tRNA(eIF2-GTP ternary complex caused by the addition of RNA (9), stabilizes the ternary complex by binding to the 40 S subunit (3, 5), and is required for mRNA binding to 40 and 80 S ribosomes (3, 5). eIF3 binds to eIF4F via the eIF4G subunit (10) and to eIF4B (11) and probably also interacts with eIF4A (12–14). eIF4P has been referred to as a protein complex composed of three subunits, eIF4G, eIF4A, and eIF4E, and was shown to be responsible for binding of the capped end of mRNA, melting of mRNA secondary structure, and binding to the 43 S preinitiation complex (1, 15, 16). This indicates that eIF3 may be the major factor orchestrating the accurate positioning of mRNA for binding to the 40 S subunit and subsequent recognition of the initiation AUG codon (6). Nevertheless, the timing of all translation initiation events as well as their coordination are only poorly understood. Thus, a characterization of not yet described proteins involved in that process can bring more light to our understanding of the mechanism of the initiation step of protein synthesis.

Mammalian initiation factor 3 is composed of at least 10 nonidentical subunits (17). It has been demonstrated that mammalian eIF3 directly interacts with the eIF4G subunit of eIF4F (10) and that the largest subunit of eIF3, p180, interacts with eIF4B (11). In addition, it has been proposed that p180 is necessary for binding and stabilization of the ternary complex (9). Thus, it seems that p180 plays a key role in all functions promoted by mammalian eIF3, ranging from the formation of the 43 S mRNA complex. Only recently, mammalian p180 has been cloned and characterized (6). It shares significant sequence similarity with *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and *Nicotiana tabacum* proteins, which were suggested to probably represent the corresponding subunits of eIF3 in these organisms.

Yeast eIF3 comprises eight subunits with apparent molecular masses ranging from 16 to 135 kDa (18). Up to now, only four yeast homologues of mammalian eIF3 subunits have been identified. These are Prt1p, which exhibits a defect in Met-tRNA binding to the 40 S ribosomal subunit in a corresponding conditional lethal mutant (19); Nip1p, originally identified as a protein required for efficient nuclear transport in yeast (20); and the participation of several factors promoting (a) the association of initiator tRNA with the 40 S ribosomal subunit in order to form the 43 S preinitiation complex; (b) the melting of secondary mRNA structures; (c) binding of the mRNA to the 43 S preinitiation complex; and (d) the addition of the 60 S ribosomal subunit to assemble an 80 S ribosome at the initiation codon (reviewed in Ref. 1). At this point, the initiation step of translation is accomplished, and the ribosome is committed to the elongation process. Up to now at least 10 proteins called initiation factors have been implicated in the initiation of protein synthesis (2). Among these initiation factors (eIFs), 1 eIF3 represents the largest and the most complex one.

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**TiEp1, a Yeast eIF3 Subunit**

DNA as a template was digested using PRT1 auxotrophic for uracil (YLV041). A strain was obtained that retained the 5-fluoroorotic acid. Following sequential streaking on four plates, a pra1-1 incorporated artificial sites (a 5′ SacI site of position 700 and a 3′ SacI site immediately following the stop codon) and ligated into SacI–SacI of pRS313 (29), producing pRSLPVPT1-N, which also contained a NotI restriction site inserted by PCR immediately in front of the stop codon of PRT1. The second step of plasmid construction consisted of an in frame insertion of a NorI-digested Myc tag into the NorI site of pRSLVPT1-N. The resulting pRSLPVPT1-Myc was used for transformation of the haploid yeast strain W303 to generate strain YLV10 expressing the Prt1-Myc protein. Strain P501–1C was constructed in order to test the function of the Myc-tagged PRT1 gene. A SacI–SacI fragment containing the corresponding construct derived from pRSLVPT1-Myc was inserted into YLV10 restriction sites of YCplac33 (28), resulting in YCLVPRT1-Myc. The plasmid was transformed into recipient strain P501–1, producing strain P501–1C.

**Assembly of the Full-length cDNA of p180**—To assemble the full-length sequence of p180, i.e. the cDNA encoding the human homologue of RGP1, a 4.75-kb MluI fragment containing the 3′ coding region of p180 (nucleotides 564–4148) extended by 250 nucleotides of the flanking sequence was isolated and cloned into pUC9 (23). This fragment was inserted into the MluI restriction site (at position 564 of the p180 nucleotide sequence) of pUC19–1 containing the 5′-end of the p180 sequence (nucleotides 1–574). The resulting plasmid carrying the full-length sequence of p180 was named pUCP180. Both pUC19–1 and -3 plasmids were kindly provided by Dr. Keith R. Johnson (University of Toledo, Toledo, OH).

The construction of YCpLV10, a 4.6-kb Cell–SnoBI fragment containing the full-length p180 from pUCP180 was blunt-ended and inserted into a blunted BamHI restriction site of YCpLV00 (25) producing the plasmid expressing p180 under control of the RGP1 promoter. A SacI–SacI fragment of YCpLV10 was ligated into SacI–SacI restriction sites of YCplac33 (28), resulting in YCLVPRT1, which differs from the former plasmid merely in the selective marker URA3 to replace his6. YCpLV00 was introduced into both YLPV10 and YLV41 strains, resulting in strains YLV3148 and YLV418, respectively.

For the construction of YCLP718, a PCR-generated 5′-coding region of p180 (nucleotides 1–600) using YCpLV10 as a DNA template was digested with BamHI (PCR-incorporated artificial site immediately in front of the p180 ATG) and NeoI (endogenous restriction site at position +441 of the p180 nucleotide sequence). Then both a 4.2-kb NeoI–SacI fragment containing the 3′ coding region of p180 (nucleotides 441–4148) extended by 455 nucleotides of the flanking sequence and the former PCR-generated fragment were ligated into BamHI–SacI restriction sites of the 6.8-kb plasmid K2082 (provided by Dr. K. Nasmyth), yielding YCpLV18, which expresses p180 under control of the GAL1 promoter. The plasmid was introduced into strains YLV314L and YLV414 yielding strains YLV3148G and YLV418G, respectively.

**Mating Efficiency and α-Pheromone Sensitivity Assays**—Quantitative mating assays were performed as follows. The cells were grown overnight at room temperature to mid-log phase, washed once with sterile water, and resuspended in YPD medium (pH 4.7). Cells (10⁷) of both wild-type and rpg1–1 mutant strains were mixed with an equal number of cells of mating tester strains JI-1A or JI-1C, and mixtures were brought to a final volume of 1 ml; and cells were gently propagated for 4 h at either permissive or restrictive temperature. Cells (10⁴) from prototype colonies were then mixed into molten soft agar containing 4% sterile water and 2% agarose to select for diploids and were incubated for 2 days. Subsequently, the plates were scored for single colonies.

To determine the ability of the rpg1–1 mutant to respond to an α-factor at the restrictive temperature, cultures of wild-type and rpg1–1 mutant cells were grown to an A₅₀₀ of 0.4 at room temperature and resuspended in prewarmed 25 or 37 °C medium supplemented with α-pheromone (60 µg/ml). After 2 h, cells were fixed in 2% formaldehyde and scored for shmoo formation by microscopic observation.

**[3H]Leucine Labeling Experiments**—These were performed as described previously (30). Briefly, mutant cells of strain YLV314U were pregrown to mid-log phase in SC medium containing a reduced amount (25 mg/liter) of leucine (SC− rLEU). The prewarmed (37 °C) and room temperature (25 °C) SC+ rLEU media were inoculated to a density of 3 × 10⁷ cells/ml and incubated at the permissive or restrictive temperature for 24 h. Then cells from both incubations were split into two parts. Cells from one part were resuspended at a density of 5 × 10⁷ cells/ml in 5 ml of prewarmed (37 °C) SC+ rLEU plus 10 µCi of [3H]leucine medium (hereafter called SC+ [3H]LEU) and assayed for incorporation of the labeled amino acid at 37 °C. The second sample was manipulated similarly but resuspended in 25 °C SC+ [3H]LEU medium and assayed for incorporation of the labeled amino acid at 25 °C. The third sample was manipulated similarly but after 2 h of incubation at 25 °C, shifted to restrictive temperature and assayed.

After incubation, samples were processed as follows. 10 µg/ml of cycloheximide was added to each sample; then cells were isolated by centrifugation, washed once with TE buffer (20 mM Tris-HCl, pH 7.5), mixed with 600 µl of glass beads (0.45–0.50-mm diameter), brought to...
of a final volume of 1 ml of TE buffer, and homogenized in a Braun homogenizer. After centrifugation at 10,000 × g, aliquots of clear supernatant were counted in a Packard scintillation counter. Radioactivity incorporated into protein was determined after applying equal aliquots onto Whatman DE81 filters, drying, incubating for 5 min in 20% trichloroacetic acid, and boiling for 5 min. Samples were counted in a scintillation counter using Evcoule (ICN) scintillation mixture after washing them twice with 20% trichloroacetic acid, washing once with 95% EtOH, washing once with pure acetone, and drying.

**Purification of the eIF3 Complex—**eIF3 was prepared from *S. cerevisiae* strain ABYS essentially as described previously (26).

**Western Blot Analysis—**Samples containing eIF3, crude cell extracts, or immunoprecipitates were fractionated by SDS-PAGE (31) using the Mini Protean system (Bio-Rad) and immobiloblotted to nitrocellulose (32). Immunodetection of proteins was carried out using either polyclonal (25) or monoclonal (33) antibodies directed against Rpg1p and either polyclonal rat anti-Prl1p antibody (26) or anti-Myc hybridoma supernatant antibody. As a secondary antibody, anti-rabbit, anti-rat, or anti-mouse IgG antibodies conjugated with alkaline phosphatase or with horseradish peroxidase, respectively, were used. Proteins were visualized either by using alkaline phosphatase substrates (nitro blue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate) in AP buffer (100 mM Tris-HCl, 100 mM NaCl, 5 mM MgCl2, pH 9.5) or as described in the ECL manual (Amersham Pharmacia Biotech). The preparation of crude cell extracts used is described below. For maximum sensitivity, the gels were stained using a silver staining protocol (34).

**Immunoprecipitations—**Human cells were prepared as follows. U937 promonocytes were cultured in RPMI medium containing 10% fetal serum and lysed in lysis buffer containing 10 mM Tris- HCl (pH 7.5), 50 mM NaCl, 30 mM NaF, 50 mM NaF, 2 mM EDTA, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM sodium vanadate, 1 μg/ml pepstatin, and 3 μg/ml aprotinin. Extracts were cleared by centrifugation at 4,000 × g at 4 °C for 8 min. In Vitro Translation—The following yeast strains were used for the preparation of cell-free translation systems: P501−1, YLV314L, and W303. Preparation of the extracts, cell-free translation, and the preparation of total yeast RNA were performed as described previously (35, 36).

**Polysome Profile Analysis—**To analyze polysome profiles, strains W303 and YLV314L were cultured overnight in YPD at room temperature to an A600 of 0.8. Samples of 20 ml were centrifuged at 950 × g for 3 min and resuspended in an equal volume of 0.5% SDS buffer. The cell lysates were centrifuged at 10,000 × g for 10 min. The supernatants were analyzed by SDS-PAGE and immunoblotted as described above. Equivalent portions of the supernatants and the pellet fractions were analyzed to estimate the fraction of the proteins bound in immune complexes.

**RESULTS**

**Mating Ability of rpg1−1 Mutant and Sensitivity to a-Factor—**We demonstrated recently that the *S. cerevisiae* essential gene Rpg1 is required for the passage through the G1 phase (25). There are three points in the yeast cell cycle where the further progression depends on active protein synthesis, of which the G1 phase is the most important (37). Mating of haploid *S. cerevisiae* cells of opposite mating types also requires a functional translational mechanism. When it appeared to us that the Rpg1 gene product could be a homologue of the marmalian p180 (6), a subunit of eIF3, we examined the mating ability of the *rpg1−1* ts mutant at permissive and restrictive temperature, to obtain further preliminary evidence for an impairment of translation in the absence of functional Rpg1p. YLV314U mutant cells as well as wild-type W303 cells were propagated overnight at 25 °C, and a mating assay was then performed at either 25 or 37 °C. As shown in Table I, the mating efficiency of the *rpg1−1* ts mutant is strikingly diminished (5-fold) even at the permissive temperature compared with wild-type cells. The ability to mate was completely lost in *rpg1−1* cells when mating was performed at the restrictive temperature. Mutant alleles of both mating types yielded virtually the same results, consistent with the assumption that protein synthesis of mutant cells might be affected.

To analyze this defect in more detail, we further investigated whether MATa *rpg1−1* cells respond to α-pheromone at the restrictive temperature. Treatment of cells with α-pheromone triggers a cascade of events resulting in the preparation of the cell for mating. The most remarkable event is the change of cell morphology, i.e. the projection of so called “shmoos” toward the mating partner, which also depends on active protein synthesis (38). While MATa *rpg1−1* cells responded fairly normally to α-pheromone at the permissive temperature, this response was lost virtually completely when mutant cells were treated at the restrictive temperature (Table II). When MATa *rpg1−1* ts cells were first arrested at the restrictive temperature for 4 h and subsequently transferred to the permissive temperature and treated with α-pheromone, we noticed that most cells regained their ability to form shmoos within 2–3 h (not shown). This demonstrates the reversibility of this defect and is also consistent with a block of protein synthesis as the cause of the arrest at the restrictive temperature.

**The rpg1−1 ts Mutation Causes a Block of Protein Synthesis in Vivo—**The mating ability experiments are consistent with the assumption that translation is blocked in *rpg1−1* cells at the restrictive temperature. To obtain more direct evidence, we examined the incorporation of radiolabeled amino acids into total protein of the *rpg1−1* mutant cells. As shown in Fig. 1A, *rpg1−1* mutant cells (strain YLV314U (25)) exhibit a severe block of leucine incorporation into protein upon shift to the restrictive temperature. We also tested leucine incorporation.
occurring in rpg1–1 cells incubated for 24 h at 37 °C and shifted back to 25 °C at time point 0 of the assay. This experiment revealed that the observed block of protein synthesis is reversible.

We further examined whether the block of protein synthesis occurred immediately upon the temperature shift. Mutant (rpg1–1) cells were incubated in [3H]leucine-containing medium for 2 h at the permissive temperature before being shifted to 37 °C and assayed. As demonstrated in Fig. 1B, an almost immediate drop in leucine incorporation was observed. In control experiments, the total uptake of [3H]leucine by the rpg1–1 ts cells was also assayed and shown to be negatively affected by the mutation (not shown), presumably by an indirect effect of the block in protein synthesis. In further control experiments, it was shown that in wild-type W303 cells protein synthesis as well as leucine uptake were not significantly affected by the changes of the temperature (data not shown). While these results indicate fairly convincingly the involvement of Rpg1p in protein synthesis, we could not rigorously exclude the possibility that the rpg1–1 mutation directly affects some other cell process, for instance the uptake of amino acids, and that the protein synthesis defect was a secondary effect. Thus, a more detailed analysis was needed to elucidate the real nature of this involvement.

Rpg1p Comigrates with Prt1p on Sucrose Gradients—Rpg1p exhibits significant similarity at the amino acid level to the recently characterized largest subunit of the human eIF3, p180 (6, 17, 25). To address the question of whether Rpg1p is indeed one of the subunits of yeast eIF3, we first attempted to detect Rpg1p in a fraction containing highly purified eIF3 (26). Prt1p was previously identified as the second largest subunit of yeast eIF3 (18) and was therefore used as a specific marker for the presence of the eIF3 complex, which was enriched in a four-step purification (26). A ribosomal salt wash fraction prepared from wild-type strain ABYS was fractionated by DEAE-cellulose and phosphocellulose chromatography and by sucrose density gradient centrifugation. The optical density profile of the sucrose gradient showed a major peak of rapidly sedimenting material in fractions 10–12 (not shown). Sucrose gradient fractions 8–14 were further analyzed by SDS-PAGE and subsequently by Western blotting. Silver staining of the gel revealed the copurification of five polypeptides in fractions 10–12 corresponding to five proteins with molecular masses of approximately 120, 80, 75, 40, and 30 kDa, previously identified as subunits of the yeast eIF3 (26) (Fig. 2A, arrows). As demonstrated by Western blotting (Fig. 2B), a polypeptide migrating as a protein of 120 kDa reacts with the monoclonal anti-Rpg1p antibody. The intensity of the signal obtained with the anti-Rpg1p monoclonal antibody, like the eIF3 subunit, peaks at fraction 11. Since the anti-Rpg1p antibody recognizes exclusively one band of about 120 kDa corresponding to Rpg1p (110 kDa calculated molecular mass) (25), the additional immuno-

Table II

| Strain     | Relevant genotype | Cells with shmoo-like morphology |
|------------|-------------------|----------------------------------|
|            | 25 °C treatment   | 37 °C treatment                  |
| W303       | MATa              | 97                               | 99                             |
| YLV314U    | MATa, rpg1–1      | 85                               | 1                              |

FIG. 1. [3H]leucine incorporation into rpg1–1 mutant cells. A, the rpg1–1 mutant (strain YLV314U) was propagated for 24 h in minimal medium containing a reduced amount of leucine at either 25 °C (squares) or 37 °C (diamonds). Then the cells from both incubations were resuspended in fresh medium, and 10 μCi of [3H]leucine per sample was added. Cells from the 25 °C incubation were divided into two parts, with cells from the first shifted to 37 °C (open squares) and from the other kept at 25 °C (filled squares). Cells from the 37 °C incubation were manipulated similarly, i.e., shifted to 25 °C (filled diamonds) and/or kept at 37 °C (open diamonds). B, the mutant cells were incubated in the medium containing [3H]leucine for 2 h at 25 °C and then (arrow) shifted to 37 °C (filled diamonds). As a control, the mutant cells were incubated at 25 °C (filled squares) and/or 37 °C (open squares). [3H]Labeled leucine incorporation into the protein of the cells was measured in all cases.

FIG. 2. SDS-PAGE and Western blot analysis of sucrose gradient fractions. A, silver-stained SDS-polyacrylamide gel. Lane PC, 5 μl of 300 ml phosphocellulose eluate; lanes 8–14, 20 μl of sucrose gradient fractions 8–14. B, alkaline phosphatase-stained Western blot decorated using monoclonal anti-Rpg1p antibody. Samples were the same as in A. C, alkaline phosphatase-stained Western blot (the same as in B) subsequently redecorated using polyclonal anti-Prt1p antibody.
reactive band detected as a 100-kDa protein (arrow) appears to represent a degradation product of Rpg1p, as was suggested already (26). The same probably applies to the 50-kDa immunoreactive band observed in the phosphocellulose (PC) fraction and fraction 11 of the sucrose gradient (Fig. 2B, arrowhead). Subsequent examination of the same blot using polyclonal rat anti-Prt1p antibody showed that Rpg1p and Prt1p exhibit virtually the same mass distribution over sucrose gradient fractions 9–14 (Fig. 2C). Since the sucrose density gradient fractions composed of five polyepitides with defined molecular weights (fractions 10–12 (Fig. 2A, arrows)) have been proposed to represent a core complex of yeast eIF3 (17), our results strongly suggest that Rpg1p is the large subunit of this core complex.

**Rpg1p Coimmunoprecipitates with Prt1p, a Known Subunit of eIF3**—To provide additional evidence for Rpg1p being a subunit of yeast eIF3, we asked whether it could be coimmunoprecipitated with the Prt1 protein (18, 26). Prt1p was already shown to coimmunoprecipitate with Gcd10p (23) and Sui1p (24) and to interact directly with Tif34p (22). A Myc-tagged version of Prt1p and anti-Myc antibodies were used for Prt1p immunodetection. The tagged protein was shown to complement the ts defect of the prt1–1 ts mutant strain P501–1, demonstrating that it is functional (result not shown). Crude extracts from strains expressing a tagged or an untagged version, respectively, of Prt1p were prepared and used for immuno-precipitation experiments with antibodies directed against Rpg1p or Prt1-Mycp. The resulting immunocomplexes were separated by SDS-PAGE and probed by immunoblot analysis using either anti-Rpg1p or anti-Myc antibodies. Both antibodies immunoprecipitated similar amounts of Rpg1p and Prt1-Myc fusion protein from the crude extracts as shown in Fig. 3, lanes 5 and 6. In control experiments, anti-Myc antibody immunoprecipitated detectable amounts of neither PRT1 nor RPG1 gene products of the Prt1p-untagged strain (Fig. 3, lane 8). Similarly, in an immunoprecipitate obtained with anti-Rpg1p antibody, untagged Prt1p was not detectable by anti-Myc antisemur (Fig. 3, lane 7). The finding that monoclonal anti-Rpg1p antibody coimmunoprecipitates Prt1p, a known subunit of yeast eIF3, and that anti-Prt1-Mycp antibody coimmunoprecipitates Rpg1p provides direct evidence that Rpg1p is an integral component of yeast translational machinery. We approached this question using an *in vitro* translation assay based on cell-free systems derived from ts strains deficient in translation initiation when the extract of cells is briefly preincubated at 37 °C (26). We first tested the Rpg1p-Prt1p-containing protein complex isolated as described above (Fig. 2) for biological activity in a cell-free system derived from strain P501–1. When the cell-free system derived from this *prt1–1* mutant was heated for 30 s at 37 °C, the methionine incorporation was dramatically diminished in the absence of endogenous RNA or after the addition of total yeast RNA compared with the nonheated extract (Fig. 4D versus A). The addition of the sucrose gradient fraction 11 (Fig. 2A) containing eIF3 restored the initiation of translation (about 6-fold stimulation) (Fig. 4D), while translation in the nonheated extract was stimulated only 1.5–2-fold (Fig. 4A). These results are consistent with results reported previously (26) and show that Rpg1p in the protein complex is active as a translation factor *in vitro*.

Having established that purified eIF3 containing Rpg1p stimulates translation in an *in vitro* system deficient in the activity of this factor, we next asked whether we could observe a similar stimulatory effect in a cell-free system derived from strain YLV314L (*rpg1–1*). The *rpg1–1* cell-free system was heated for 2 min, and the stimulatory effect of the addition of the sucrose gradient fraction 11 was assayed. The prolongation of the heating time was apparently necessary because of a higher heat resistance of the temperature-sensitive factor present in this system. Again, the activity of the system was blocked by preheating (Fig. 4, B versus E), and again, the addition of the eIF3-containing fraction stimulated the activity of the heated system to almost control levels (Fig. 4E). Neither heated nor nonheated cell-free systems derived from wild-type strain W303 were stimulated by the addition of eIF3. Accordingly, we did not observe any influence of the temperature shift on the translation efficiency (Fig. 4, C and F). These data confirm that Rpg1p, being one of the eIF3 subunits, has an essential translational activity *in vitro*.

When initiation is blocked, ribosomes finish elongation of mRNAs but do not efficiently reinitiate. This leads to a reduction in the polysome/monosome ratio and an accumulation of 80 S run-off ribosomes (21). To demonstrate a role of the *RPG1* gene product in translation initiation *in vivo*, we analyzed the polysome profile of the *rpg1–1* mutant at permissive and restrictive temperature (the YLV314L strain in this study) and compared it with that of the isogenic wild-type strain. We noticed no difference between polysome profiles of wild-type and mutant strains when polyosomes were isolated from cells grown at 25 °C (Fig. 5, A and C). While the wild-type cells grown at 25 and 37 °C also displayed polysome profiles (Fig. 5, B and D), the polysome profile of the mutant cells incubated at 37 °C for 1 h before harvesting showed a severe reduction in the
polysome/monosome ratio and remarkable accumulation of the 80 S ribosomes (Fig. 5D). Thus, an essential role of Rpg1p in the initiation of translation is supported by the in vitro translation assay and the polysome profiles.

**Human eIF3-p180 Cannot Replace Its Yeast Counterpart RPG1 in Vivo**—Many mammalian factors can substitute for their yeast counterparts (2), indicating functional homology between translational factors. Importantly, it has been shown that yeast eIF3 could substitute functionally for human eIF3 in a methionyl-puromycin synthesis assay with mammalian components, which mimics the formation of the first peptide bond (18). Only recently, a human cDNA homologue of the RPG1
gene has been cloned and named p180 (6). *S. cerevisiae* Rpg1p and human p180 share 29% sequence identity and 54% similarity. The N-terminal regions of both homologues show more similarity than C-terminal ones (6, 25). An investigation of the functional relatedness of both homologues could provide additional evidence for evolutionary conservatism of the translation process from the unicellular yeast to humans.

To investigate whether the human p180 cDNA can replace the yeast gene in vivo, we first had to assemble the 5.3-kb full-length p180 cDNA clone from two incomplete p180 cDNAs and place it behind either an endogenous *RPG1* or the *GAL1–10* promoter. Since *RPG1* is an essential gene, we were able to use the no-growth phenotype of both the Rpg1p deletion and the *rpg1–1* ts mutant strains for a complementation test. Human p180 cDNA driven from either the *RPG1* or the *GAL1–10* promoter failed to substitute for *RPG1* in the Rpg1p-depleted strains (YLV0418 and YLV0418g strains) and to rescue the growth of the *rpg1–1* ts mutant strains (YLV3148 and YLV3148g strains) at the restrictive temperature (data not shown). Northern blot analysis with the human p180 cDNA probe revealed that the human p180 is transcribed well (data not shown). The accumulation of human p180 in *S. cerevisiae* was tested by Western immunoblot analysis using an anti-p180 hybridoma supernatant antibody (a kind gift of Dr. Keith R. Johnson) (Fig. 6). Human p180 is expressed under control of the *GAL1–10* (lane 2) as well as the *RPG1* promoter (lane 4). No significant cross-reactivity was observed between the polyclonal anti-Rpg1p antibody and human p180 (not shown). Similarly, the monoclonal anti-p180 antibody did not recognize the yeast Rpg1p (Fig. 6). These data demonstrate that the lack of complementation of the *rpg1–1* mutation by human p180 is not caused by insufficient expression of this protein in yeast.

**DISCUSSION**

The essential *S. cerevisiae* gene *RPG1* has recently been shown to be required for the passage through the G1 phase of the cell cycle. Depletion of Rpg1p and/or inactivation of the ts Rpg1 mutant protein at the restrictive temperature resulted in cessation of cell growth and the accumulation of mutant cells in the G1 phase (25). By virtue of data base searches, *S. cerevisiae* Rpg1p has been shown to share significant sequence similarities with proteins from the nematode *C. elegans*, the plant *N. tabacum*, and the mammals *Mus musculus* and humans (6, 25). Just recently, the human cDNA homologue (p180) of the *RPG1* gene has been identified as the largest subunit of the eukaryotic translation initiation factor 3. Since *S. cerevisiae* Rpg1p and human p180 share 29% sequence identity and 54% similarity, it was suggested that yeast *RPG1* constitutes the corresponding subunit of yeast eIF3 (6). The failure of mutant cells lacking functional *RPG1* to pass through the G1 → S transition and the report on the human homologue of *RPG1* led us to examine the involvement of *RPG1* in translation. Here we present several lines of evidence showing that Rpg1p indeed represents a subunit of yeast translation initiation factor 3 required for the initiation of translation.

The passage through the regulatory step of the G1 phase called START requires efficient protein synthesis (37). Translational mechanisms have also been suggested to play a regulatory role in the genetic coordination of growth and cell division (41). Depending on growth conditions, eIF3 influences Cln3p synthesis (and probably not only Cln3p) and, thus, as a consequence, controls the G1 → S transition (41). Furthermore, protein synthesis has been shown to be essential for the progression of the mating pheromone pathway (42). We showed here that *rpg1–1* ts mutant cells are defective in incorporation of a labeled amino acid into protein and fail to mate and to respond to α-pheromone at the restrictive temperature.

These results are consistent with the previous finding that a lack of a functional Tif34p, another yeast eIF3 subunit, results in G1 arrest and in an inability to mate and to respond to α-factor (22). They further illustrate the dependence of the mating pheromone pathway on functional protein synthesis.

Our monoclonal antibody against Rpg1p cross-reacted with a polypeptide with an apparent molecular mass of 120 kDa, which comigrates on sucrose density gradients with Prt1p and three other subunits together referred to as the core complex of eIF3 (Fig. 2). We have shown that this fraction possesses the biochemical activity ascribed to eIF3, e.g., the restoration of translation in an extract in which an endogenous eIF3 subunit has been inactivated (Fig. 4) (26). It should be noted that the *RPG1* gene product was reported to be a protein ranging in molecular mass between 120 and 135 kDa (25, 26) whereas the calculated molecular mass derived from the amino acid sequence is 110 kDa. Likewise, the reported molecular mass of human p180 according to SDS-PAGE varies between 170 and 180 kDa (3, 6, 43) and also exceeds that deduced from its sequence (166 kDa).

The direct interaction between hPrt1 and the p180 subunit of mammalian eIF3 has already been demonstrated using Far Western analysis (40). p180 was also reported to be the subunit of mammalian eIF3 through which eIF3 interacts with eIF4B (6). We have shown here that Rpg1p could be specifically communoprecipitated with the Prt1p subunit of eIF3 using antibodies against Rpg1p and the Myc tag fused to Prt1p (Fig. 3). This indicates but does not prove that Rpg1p interacts directly with Prt1p. Furthermore, *S. cerevisiae* Rpg1p may possess more interacting partners than only Prt1p. The precise investigation of these interactions is needed to improve our understanding of the roles of particular eIF3 subunits in the initiation of translation.

Incubation of the *rpg1–1* ts mutant at the restrictive temperature rapidly blocks the initiation of protein synthesis as assessed from the polysome profile carried out 1 h after the upshift (Fig. 5). However, such an experiment cannot provide information on an exact role of *RPG1* in the initiation process; hence, this role still remains to be elucidated.

Many translation initiation factors have been tested for their ability to functionally replace the corresponding yeast factors (for a review, see Ref. 2). Up to now, only three translation components are known, namely eIF4A (2), bPrt1 (40), and human eIF3-p36 (21), which failed to substitute for their yeast counterparts. We show here that the human eIF3-p180 cDNA is also not able to replace its yeast homologue, *RPG1*, even
when the protein is overexpressed (Fig. 6). Since the exact roles of both proteins are still unclear, it is rather difficult to speculate about the reasons why human p180 is unable to fulfill functions of the yeast Rpg1p in vivo. One possible reason might be the inability of human p180 to incorporate efficiently into the yeast elf3 multiprotein complex. The failure of three human elf3 subunits to complement is somewhat surprising in light of the fact that yeast elf3 functions in a mammalian methionyl-puromycin assay system (18). This finding demonstrates the substantial diversity, apart from significant conservation, of translation initiation between these two evolutionary fairly distant species.

Only recently, mammalian elf3 has been purified from rabbit reticulocyte lysates using an assay that specifically measures its ability to stimulate the binding of the ternary complex to 40 S ribosomal subunits (8). Strikingly, these elf3 preparations lacked the 180-kDa polypeptide, which was present in mammalian elf3 preparations purified in other laboratories based on translation assays (3, 43). The finding that an elf3 preparation lacking p180 was fully competent to stimulate Met-RNAi binding to 40 S ribosomal subunits led the authors to speculate that the p180 subunit might not be a true subunit of mammalian elf3 but might rather associate with elf3 in a discrete step in the initiation process (8). Similarly, the yeast homologue of p180, Rpg1p, was not observed with elf3 in a discrete step in the initiation process (8).

A true subunit of mammalian elf3 but might rather associate

with elf3 in a discrete step in the initiation process (8).

is rather unstable under conditions of the elf3 complex preparation (44). The absence of Rpg1p in some of their elf3 preparations might mean that either Rpg1p/Tif32p

or their elf3 preparations might mean that either Rpg1p/Tif32p

functions of the yeast Rpg1p

ulate about the reasons why human p180 is unable to fulfill

putative function(s) outside of the elf3 complex and

the absence of hu-