Protein P0 interacts with proteins P1α, P1β, P2α, and P2β, and forms the Saccharomyces cerevisiae ribosomal stalk. The capacity of RPP0 genes from Aspergillus fumigatus, Dictyostelium discoideum, Rattus norvegicus, Homo sapiens, and Leishmania infantum to complement the absence of the homologous gene has been tested. In S. cerevisiae W303dGP0, a strain containing standard amounts of the four P1/P2 protein types, all heterologous genes were functional except the one from L. infantum, some of them inducing an osmosensitive phenotype at 37 °C. The polymerizing activity and the elongation factor-dependent functions but not the peptide bond formation capacity is affected in the heterologous P0 containing ribosomes. The heterologous P0 proteins bind to the yeast ribosomes but the composition of the ribosomal stalk is altered. Only proteins P1α and P2β are found in ribosomes carrying the A. fumigatus, R. norvegicus, and H. sapiens proteins. When the heterologous genes are expressed in a conditional null-P0 mutant whose ribosomes are totally deprived of P1/P2 proteins, none of the heterologous P0 proteins complemented the conditional phenotype. In contrast, chimeric P0 proteins made of different amino-terminal fragments from mammalian origin and the complementary carboxyl-terminal fragments from yeast allow W303dGP0 and D67dGP0 growth at restrictive conditions. These results indicate that while the P0 protein RNA-binding domain is functionally conserved in eukaryotes, the regions involved in protein-protein interactions with either the other stalk proteins or the elongation factors have notably evolved.

The ribosomal stalk is an important structural element of the large subunit, which has been proposed to have a role in the translocation step of protein synthesis (1, 2). It was shown to be involved in the activity of the elongation factors in bacteria (3) as well as in eukaryotes (4, 5), and a direct interaction with EF-Tu and EF-G has recently been confirmed by electron microscopy (6, 7). Moreover, the stalk may also have a regulatory role for the activity of the eukaryotic ribosome (8).

The bacterial stalk is formed by a pentamer made of two dimers of protein L7/L12 and protein L10, that interacts with one of the most highly conserved regions of the large rRNA, the so-called GTPase center (9, 10). The similar eukaryotic pentameric complex is made of four 12-kDa acidic proteins and the 34-kDa protein P0, which are equivalents to L7/L12 and L10, respectively. In eukaryotes having two types of acidic proteins, called P1 and P2, they seem to be present as dimers in the stalk (11). The acidic proteins interact through their NH2-terminal domain (12) with P0 in a region close to the carboxyl end (13). The whole protein complex binds to the rRNA GTPase center through the NH2-terminal domain of protein P0 (13, 14). The COOH-terminal end of the proteins is exposed to the medium and interacts with the elongation factors (6, 7).

In quite a few eukaryotic species there are more than one protein of the P1 and P2 types. A third protein type, P3, has even been reported in plants (15). In Saccharomyces cerevisiae there are two P1 proteins, P1α and P1β, and two P2 proteins, P2α and P2β. The four yeast stalk proteins share the same COOH-terminal peptide, EESDDDMGFLFD, which is also present in protein P0, but show substantial differences in the rest of the amino acid sequence. Thus, the overall sequence similarity in the two proteins of the same type is only close to 80% and their function is not totally equivalent (8).

As an average, there are about four 12-kDa acidic proteins per yeast ribosome (16). Therefore, if the yeast acidic proteins are present as dimers there cannot be two copies of each protein per particle, and, consequently, the ribosome population should be heterogeneous regarding the stalk composition. There is, nevertheless, evidence suggesting that, contrary to mammals, the acidic proteins can be as monomers in the yeast ribosome (17). However, further experimental data are required to totally resolve this question. In any case, independently of the copy number, the presence of at least one protein of the P1 type and one of the P2 type is required to form a complex with protein P0. Thus, in S. cerevisiae D67, a mutant in which the two genes encoding the P1α/P1β proteins have been disrupted, the P2α/P2β proteins accumulate in the cytoplasm but are not found bound to the ribosome (18).

Although the bacterial and eukaryotic stalk proteins seem to play a similar role with respect to their basic function in the ribosome, they have substantial structural differences (19, 20, 13, 21). Protein P0 shows a higher degree of structural and functional complexity than the bacterial protein L10 (20, 13). The eukaryotic protein has a carboxyl end extension of around 100 amino acids that resembles the amino acid sequence of proteins P1 and P2 (19). This extension plays an important role in the interaction with the P1 and P2 proteins and, therefore, in the formation of the pentameric complex (13). In addition, it is able to perform the functions of the complete stalk in the absence of the other components (22). Protein P0 by itself constitutes the minimal stalk sufficient to support accurate protein synthesis although at a lower rate than the complete stalk (23). The binding of P1 and P2 proteins, which are present also in an exchangeable cytoplasmic pool, increases the effi-
ciency of the ribosome. This evolutionary peculiarity of the eukaryotic P0 protein seems to have provided the eukaryotic ribosome a way to regulate the translation process that is missing in the prokaryotic organisms (see Ref. 8, for review).

Protein P0 also binds to the GTPase site in the eukaryotic large RNA. This highly conserved region in the RNA molecule has been shown to be interchangeable between bacteria and eukaryotes (24, 25). However, the bacterial and eukaryotic proteins differ in respect to the RNA binding since, while protein L10 is easily removed from the ribosome by washing with ammonium-ethanol buffers (26), the eukaryotic polypeptide resists this treatment and remains tightly bound to the ribosome (27).

Two additional functional domains can be clearly defined in the P0 protein in addition to the RNA-binding one, the region involved in the formation of the pentameric complex with P1 and P2, and the region connected to the interaction with the elongation factors, both probably located in the COOH-terminal part.

Data on the capacity of P0 protein from different organisms to complement the different functions of the endogenous protein in S. cerevisiae, together with a comparative analysis of the respective amino acid sequences can provide information relative to the functional role of the conserved and non-conserved regions and can help to define more precisely the protein active domains. This type of study has been carried out expressing the RPP0 gene from five different species in a conditional P0 null mutant of S. cerevisiae that carries the genomic RPP0 gene under the control of the inducible GAL1 promoter.

MATERIALS AND METHODS

Yeast and Bacterial Strains and Growth Media

S. cerevisiae W303dGFP (MAT a, leu2-3, 112, ura3-1, trp1-1, his3-11, 15, ade2-1, can1-100, RPP0::URA3-GAL1-RPP0) and S. cerevisiae D67dGFP (MAT a, leu2-3, 112, ura3-1, trp1-1, his3-11, 15, ade2-1, can1-100, rpy1+::LEU2, rpy1+::TRP1, RPP0::URA3-GAL1-RPP0) were derived from S. cerevisiae W303 and D67, respectively, by integration through homologous recombination in the RPP0 locus of a construction carrying the P0 coding region fused to the GAL1 promoter (23).

Yeasts were grown in either YEP medium (1% yeast extract, 2% peptone) or minimal YNB medium, supplemented with the necessary nutritional requirements. In both cases, the carbon source was either 2% glucose or 2% galactose as indicated. Stock cultures of all yeast strains were maintained in galactose medium. When required, cells were shifted to glucose medium and allowed to grow for at least 20 generations to reach steady state growth conditions. Escherichia coli DH5α was used as a host for the routine maintenance and preparation of plasmids and was grown in LB medium.

Enzymes and Reagents

Restriction endonucleases were purchased from Roche Molecular Biochemicals, MBI Fermentas, New England Biolabs, and Amersham Pharmacia Biotech, and were used as recommended by the suppliers. T4 DNA ligase, calf intestinal alkaline phosphatase, and the DNA polymerase I Klenow fragment were from Roche Molecular Biochemicals. DNA manipulations were done basically as described in Ref. 28. PCR was carried out using PFU DNA polymerase from Perkin Elmer and custom made oligonucleotides from IsoGen, following the recommendations of Dieffenbach and Dveksler (29).

Plasmids

pFL37 was derived from pFL38 (30) by removing the URA3 marker with BglII and introducing in the same position a BamHI fragment carrying the HIS3 marker. BSPO was obtained by inserting a 2.8-kilobase pair EcoRI-AvaII fragment containing the yeast RPP0 gene in the MCS of Bluescript (13). BSPOSc(NdeI) was derived from BSPO by introducing a NdeI restriction site (CATATG) at the initiation ATG in the RPP0 gene by heteroduplex mutagenesis (31). pUC-P0Dd resulted from the insertion into the MCS of pUC19 of a 1-kilobase pair EcoRI cDNA fragment from a positive λ phage, containing 10 bases of the 5′-UTR, the coding region and 42 bases of the 3′-UTR of the Dictyostelium discoideum RPP0 gene. The positive phage was cloned from an expression library using antibodies specific to the D. discoideum protein (32).

pFL37-P0Sc—A Smal-Xhol fragment, containing the 5′-UTR, the coding region and the 3′-UTR of S. cerevisiae RPP0 gene from plasmid BSPO, was inserted in the corresponding sites of the pFL37 vector.

pFL37-P0Dd—The D. discoideum RPP0 gene as a Klenow-filled 1-kilobase pair EcoRI fragment from a pUC-P0Dd (32), was subcloned in the blunt-ended NdeI-EcoRI sites of pBSPOSc(NdeI), substituting the coding region of the S. cerevisiae gene and yielding the plasmid BSPODd. When the D. discoideum gene in BSPODd was under control of the yeast RPP0 promoter. Afterwards, the Smal-Xhol fragment from BSPODd was introduced in the corresponding sites of pFL37.

pFL37-P0Rn, pFL37-P0Af, pFL37-P0Li, and pFL37-P0Hs—in the four cases a similar strategy was followed. Using appropriate oligonucleotides, the coding region of the P0 protein gene obtained by PCR from either a cDNA library (Aminigatus fumigatus) or previously prepared cDNA clones (Homo sapiens, Rattus norvegicus, and Leishmania infantum). The oligonucleotides were designed to introduce a NdeI site at the initiation end and either an EcoRI site (R. norvegicus and L. infantum) or a NehI site (A. fumigatus and H. sapiens) at the termination end of the coding region. The PCR product was digested with NdeI and either EcoRI or NehI and introduced in the corresponding sites of BSPOSc(NdeI), obtaining plasmids with the heterologous gene under the control of the yeast P0 promoter. The fragment containing the human gene was subcloned into pFL37.

pFL37-P0HHY—Using a conserved EcoRV restriction site present at an equivalent position in both S. cerevisiae and H. sapiens RPP0 genes, plasmids pFL37-P0Sc and pFL37-P0Hs were treated with EcoRV and EcoRI and the fragment 0.7-kilobase pair fragment derived from the yeast gene, containing the COOH-terminal region of the protein, was subcloned into the pFL37-P0Hs. The resulting pFL37-P0HHY plasmid contains a chimeric that encodes a 314-amino acid long protein composed by the first 203 amino acids and the last 108 amino acids from the human and the yeast polypeptide, respectively.

pFL37-P0HYY—This plasmid was prepared taking advantage of a 30-nucleotide long stretch starting around position 400 that is identical in the H. sapiens and S. cerevisiae genes. Using complementary oligonucleotides to both strands of this region as well as the universal primers at the other end, two PCR fragments were obtained from plasmid BSPOHs and BSPOSc. The fragment comprising the 5′ and 3′ regions were derived from the human and yeast genes, respectively. These two fragments together with the universal primers were used for an overlap extension PCR (33) to obtain a chimeric P0HYY gene, which was subcloned as a Xhol-EcoRI fragment into the pFL37 vector. The plasmid was checked by restriction analysis and by DNA sequencing. The protein encoded by the P0HYY gene contains the first 138 amino acids from H. sapiens and the last 176 amino acids from S. cerevisiae.

Cell Transformations

Bacterial transformations were performed according to the procedure of Hanahan (34). Yeasts were transformed using the lithium acetate method as described (35).

Cell Fractionation and Ribosome Preparation

Yeasts were grown exponentially in rich YEP medium up to A600 = 1 and cells were collected by centrifugation and washed with buffer 1 (100 mM Tris-HCl, pH 7.4, 20 mM KCl, 12.5 mM MgCl2, 5 mM β-mercaptoethanol). Cells in buffer 1 were supplemented with protease inhibitors (0.5 μmol of phenylmethylsulfonyl fluoride, 1.25 μg of leupeptin, aprotonin, and pepstatin per gram of cells), and broken with glass beads. The extract was centrifuged in a Beckman SW-54 rotor (12,000 rpm, 15 min, 4 °C), yielding the supernatant 30 fraction which was afterward sub-
Ribosomal proteins were analyzed either by 15% SDS-PAGE or isoelectrofocusing. Isoelectrofocusing was carried out as described previously (36). Particles were pretreated with RNase A (10 μg/mg ribosomes) on ice for 30–45 min. After lyophilization, the samples containing 0.5 mg of ribosomes were resuspended in loading buffer (6% ampholytes, 8 M urea) and directly loaded into a standard vertical gel (5% acrylamide, 0.2% bis-acrylamide, 6 M urea, 6% pH 2.5–5.0 ampholytes). As cathode and anode solutions 30 mM NaOH and 180 mM H₂SO₄ were used at the upper and bottom part of the gel, respectively. Isoelectrofocusing was run in the cold room at 6 mA constant current until the voltage reached 600 V and then at 250 V for 16 h.

Proteins were usually detected by standard silver staining. Alternatively, gels were stained in a solution containing 0.25% Coomassie Blue R-250 (Sigma) dissolved in 45% ethanol, 10% acetic acid. After 20 min, gel was destained using the same solution but without Coomassie Blue. Scanning of the bands in the gels was performed using a Molecular Dynamics computing densitometer model 300A. Protein sequencing was performed by Edman degradation using an Applied Biosystems 447 automatic peptide sequenator at the Centro de Biología Molecular “Severo Ochoa” Protein Sequencing Service.

Western Blotting

Proteins in gels were transferred to membranes by electrophoresis in a semi-dry system using Novablot LKB buffer. The membranes were treated with 5% non-fat milk dissolved in TBS (10 mM Tris-HCl, pH 7.4, 200 mM NaCl) for 30 min and afterward they were incubated for 1 h with the antibody diluted in the same buffer. Subsequently, the membranes were washed 15 min in TBS containing 5% non-fat milk and 0.1% Tween 20. Then, the second antibody (Rabbit or Goat, RRPO) diluted in the former buffer, was added and the membranes were incubated for 30 min. Finally, they were washed 15 min with 0.1% Tween 20 in TBS. Bound antibodies were located by detecting peroxidase activity using the ECL system (Amersham Pharmacia Biotech) and then exposed to film.

Activity Tests

Polyphenylalanine Synthesis—The reaction was performed in 50-μl samples containing 10 pmol of 80S ribosomes, 5 μL of S-100, 0.5 μmol trNA, 0.3 mg/ml polyuridylic acid, 40 μM [3H]phenylalanine (120 cpn/pmol), 0.5 μM GTP, 1 μM ATP, 2 μM phosphocreatine, and 40 μg/ml creatine phosphokinase in 50 mM Tris-HCl, pH 7.6, 15 mM MgCl₂, 90 mM KCl, 5 mM β-mercaptoethanol. After incubation at 30 °C for 30 min, samples were precipitated with 10% trichloroacetic acid, boiled for 10 min, and filtered through glass fiber filters. Radioactivity incorporated in bound polyphenylalanine was assayed by incubating the ribosomal fraction with the antibody diluted in the same buffer. Subsequently, the membranes were incubated for 30 min. Finally, they were washed 15 min with 0.1% Tween 20 in TBS. Bound antibodies were located by detecting peroxidase activity using the ECL system (Amersham Pharmacia Biotech) and then exposed to film.

RESULTS

Complementation of a Conditional P0 Null Phenotype by Heterologous Proteins—S. cerevisiae W303dGP0 carries the essential RRPO gene under the control of the GAL1 promoter and it is viable only when grown in galactose as a carbon source (20). This strain has been transformed with the plasmids pFL37-P0Af, pFL37-P0Dd, pFL37-P0Rn, and pFL37-P0Li which, respectively, carry the coding sequence of the RRPO genes from A. fumigatus, D. discoideum, R. norvegicus, and L. infantum under the control of the 5′ regulatory region of the yeast RRPO gene to assure the same level of expression for all of them. As a positive control, the strain was also transformed with the plasmid pFL37-P0Sc, carrying the S. cerevisiae RRPO gene. The capacity of the transformed strains to grow in glucose media will define the ability of the heterologous P0 proteins to functionally substitute for the endogenous polypeptide.

Due to the existence of a large pool of active ribosomes carrying the yeast P0 protein, the transformants growing in galactose are able to grow in glucose for some time, and usually they have to be transferred to a second glucose plate to clearly detect the effect of the heterologous protein expression. In addition to the strain transformed with the homologous RRPO gene, cells transformed with pFL37-P0Af, pFL37-P0Dd, and pFL37-P0Rn were able to continuously grow at 30 °C on glucose plates. On the contrary, plasmid pFL37-P0Li did not support the growth of the transformed strain in glucose (Fig. 1A).

To test the complementation capacity of the heterologous P0 proteins in the absence of the P1/P2 proteins in the stalk, the S. cerevisiae D67dGP0 strain was used. This strain, like W303dGP0, carries the genomic RRPO gene under the control of the GAL1 promoter but, as commented in the Introduction, its ribosomes are deprived of acidic P1/P2 proteins (18, 13). None of the S. cerevisiae D67dGP0 transformed strains, except the control expressing the homologous P0, was able to grow in glucose (Fig. 1B). It seems, therefore, that the complementing effect of the P0 proteins from A. fumigatus, D. discoideum, and R. norvegicus requires the presence of the 12-kDa P1/P2 proteins in the ribosome.

Effect on the Ribosomal Stalk Composition—To test alterations in the stalk composition by the presence of the rat, A. fumigatus, and D. discoideum proteins, washed ribosomes from transformed S. cerevisiae W303dGP0 strains growing in glucose for more than 20 generations were fractionated by SDS-PAGE electrophoresis and the P0 proteins detected by Western using monoclonal antibody 3BH5, specific to the carboxyl end of the yeast P0 (Fig. 2A). The intensity of the yeast and rat P0 protein was similar. The ribosomes carrying the A. fumigatus yielded the same results (not shown).

The D. discoideum P0 protein was not recognized by the monoclonal 3BH5 antibody since no band is detected in the corresponding sample (Fig. 2A). These results confirm differences in the sequence of the COOH-terminal peptide of D. discoideum P proteins (32), and, in addition, clearly indicate that the endogenous yeast P0 protein from the galactose culture is not present in the glucose-grown cells.

**Fig. 1.** S. cerevisiae W303dGP0 (A) and D67dGP0 (B) transformed plasmid pFL37 carrying the gene encoding protein P0 from S. cerevisiae (S.c.), A. fumigatus (A.f.), D. discoideum (D.d.), L. infantum (L.i.), and R. norvegicus (R.n.) were plated in medium YNB supplemented with the corresponding carbon source as indicated and incubated at 30 °C.
P0 was detected in this case using a rabbit antiserum raised against the slime mold protein (32). The rabbit antiserum, which does not cross-react with the yeast and rat proteins, shows that the D. discoideum P0 is present in the corresponding ribosomes in a similar proportion than in the control D. discoideum ribosomes (Fig. 2B).

The results showing similar amounts of P0 in all the ribosomes suggest that the expression level of the heterologous proteins is similar in all transformed strains. This is not surprising since the heterologous genes are under the control of the yeast P0 5′-UTR in all the plasmids. However, to exclude the formation of a large cytoplasmic pool of heterologous proteins due to overexpression or poor incorporation into the ribosome, a Western analysis of the cell supernatant S100 extracts was performed using the monoclonal antibody 3BH5. The results confirmed the absence of free P0 in the cytoplasm of either the wild-type cells or the transformed yeasts, as shown in Fig. 3 for cell expressing the R. norvegicus protein.

The 12-kDa acidic proteins in washed ribosomes from the transformed strains were analyzed by isoelectrofocusing, and a densitometric estimation of the acidic protein bands in each sample was performed (Table 1). The results showed that while the control ribosomes from cells expressing the yeast P0 protein have standard amounts of the four proteins, P1α, P1β, P2α, and P2β, in the particles from cells expressing the rat and A. fumigatus P0, only proteins YP1α and YP2β are detected, and the amount is smaller than in the control. The ribosomes containing the D. discoideum P0 protein show almost standard amounts of YP1α and YP2β but YP1β and P2α are notably reduced.

Expression of L. infantum Protein P0—The protozoan P0 did not complement the absence of the yeast P0, but if it is expressed and binds to the yeast ribosome, it should be detected in the particles at short times after shifting the cells to a glucose medium. Unfortunately, the COOH-terminal end of the L. infantum P0, like the one from D. discoideum, is not recognized by the yeast monoclonal antibody, and there was no specific antibody available. However, the protozoan protein is larger than the yeast polypeptide and a new band at the expected position was detected in the gel from ribosomes of transformed cells growing in a glucose medium (Fig. 4).

The proteins in the band were transferred to a polyvinylidene difluoride membrane, treated with trypsin, and the peptides resolved by high-performance liquid chromatography. Edman degradation sequencing confirmed the presence of specific L. infantum P0 peptides in the band.

Effect of Heterologous P0 Proteins on Cell Growth—The S. cerevisiae W303dGP0 strains containing the D. discoideum, A. fumigatus, or R. norvegicus genes, kept permanently in glucose plates, showed a doubling time of 125, 165, and 190 min, respectively, when growing in liquid YEP glucose medium at 30 °C. The cells expressing the homologous RPP0 gene duplicated every time of 95 min in the same conditions. On the other hand, the growth rate of the D67dGP0 transformants growing in YEP-galactose liquid medium declined rapidly upon transferring to glucose medium, and they stopped growing completely after a few generations.

The capacity of the heterologous P0 proteins to support growth in stress conditions was tested by growing W303dGP0 transformants in high salt at 37 °C. The strain expressing the RPP0 gene from R. norvegicus did not grow in the presence of either NaCl or sorbitol at 30 and 37 °C. The cells expressing the A. fumigatus protein grew at high ionic conditions only at 30 °C but not at 37 °C (results not shown). Similar phenotypes have been related to other stalk protein mutations (36, 39), suggesting that the translation of proteins involved in the cellular stress response is affected by changes in the ribosomal stalk.

In Vitro Activity of Ribosomes Carrying Heterologous Proteins—Ribosomes from the different S. cerevisiae W303dGP0 strains were tested in different in vitro assays trying to char-
acetylation of the ribosomes, and the interaction with substrates in a non-enzymatic peptide bond formation capacity by a modified fragment reaction, poly(U)-dependent polyphenylalanine synthesis assay, the pep-
The in vitro polymerizing capacity of the ribosomes containing complementing heterologous P0 proteins is reduced, and there is a relationship between the ribosome activity and the amount of bound yeast 12-kDa acidic proteins. It seems, therefore, that the capacity of the heterologous P0 proteins to support cell growth depends on their potential to bind the yeast acidic proteins in the ribosomal stalk. This conclusion was totally confirmed when the heterologous genes were tested in the \textit{S. cerevisiae} D67dGP0 mutant. In this strain, whose ribosomes are totally deprived of 12-kDa proteins, none of the heterologous proteins was able to support cell growth in glucose. Moreover, the substitution of the carboxyl-terminal domain in the mammalian P0 protein by its yeast equivalent fully activate the capacity of the chimera to complement the growth of \textit{S. cerevisiae} W303dGP0 at the restrictive conditions. Similarly, the chimeric P0 allows the binding of standard amounts of the 12-kDa P1/P2 proteins to the stalk, confirming the importance of the last 110 amino acids for the formation of the ribosomal stalk, as previously reported (13).

Altogether, the reported results indicate that while the rRNA-binding domain of eukaryotic P0 proteins has been functionally conserved, the regions involved in protein-protein interactions have faster diverged. The RNA-binding domain is included in the NH2-terminal region comprising about 200 amino acids (13). A comparison of the P0 protein sequences indicates the existence of three amino acid clusters in this region, showing a conservation ranging from 50 to 75\%, which might be involved in the interaction of these proteins with the rRNA GTPase region (Fig. 6). The highly conserved RNA binding capacity of P0 is not surprising since, as commented previously, the corresponding nucleic acid moiety of the GTPase center is also among the most conserved regions in the rRNA (43). In contrast, the region from approximately position 200 to position 275, involved in the interaction with the acidic P1/P2 proteins, has notably evolved as indicated by the different capacity of the heterologous P0 to bind the yeast 12-kDa proteins.

A third functional domain in protein P0 is defined by its interaction with the elongation factors during translation. P0 shares a highly conserved COOH-terminal peptide with the 12-kDa P1/P2, linked to the rest of the molecule through an alanine-rich hinge (19). This domain is essential for the protein activity, and it is proposed to have a relevant role in the interaction with the supernatant factors (13). Nevertheless, the presence of this common structural element is not sufficient for the heterologous P0 protein complementarity. Thus, although the carboxyl end is almost identical in yeast, \textit{A. fumigatus}, \textit{R. norvegicus}, and \textit{H. sapiens} P0, none of them complement the absence of the yeast protein in D67dGP0 when the 12-kDa proteins are not present in the ribosome. It seems that other structural elements in P0, besides the conserved COOH-terminal peptide, play a role in determining a functional interaction of the elongation factor with the ribosome. These elements seem to be, at least in part, located in the amino-terminal domain since the chimeric protein P0HYY, carrying the first 138 amino acids from the \textit{H. sapiens} sequence, only partially complement the absence of the native yeast P0 in the \textit{S. cerevisiae} D67dGP0.

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