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The P1/P2 Protein Heterodimers Assemble to the Ribosomal Stalk at the Moment When the Ribosome Is Committed to Translation but Not to the Native 60S Ribosomal Subunit in *Saccharomyces cerevisiae*

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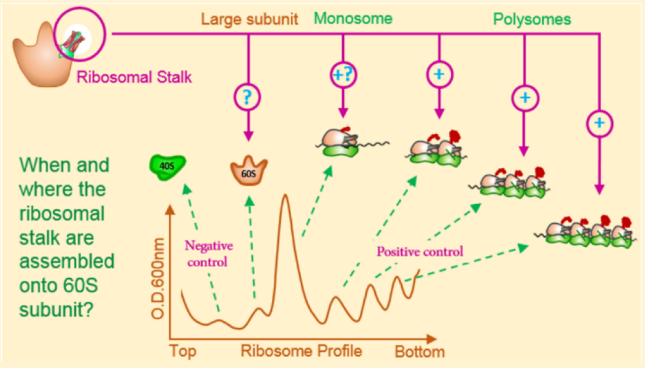
ABSTRACT: The four structural acidic ribosomal proteins that dissociate from P1A/P2B and P1B/P2A heterodimers of *Saccharomyces cerevisiae* were searched in the 60S ribosomal subunit, the 80S monosome, and the polysomal fractions after ribosome profile centrifugation in sucrose gradients in TMN buffer, and after dissociation of monosomes and polysomes to small and large ribosomal subunits in LMS buffer. Analysis by isoelectric focusing, sodium dodecyl sulfate−polyacrylamide gel electrophoresis, and Western blotting of these fractions or the purified acidic protein samples showed eight bands that correspond to the acidic ribosomal proteins in the 60S dissociated subunits of the 80S monosome and polysomes. After samples had been radiolabeled with ^{32}P, four bands were shown to correspond to the phosphorylated form of the acidic ribosomal proteins located in the 80S monosome and the polysomes. Surprisingly, native 60S subunits have no acidic ribosomal proteins. Altogether, these findings indicate that P1/P2 heterodimers bind to P0 when both ribosomal subunits are joined and committed to translation, and they detached from the stalk, just after the small and large ribosomal subunits were separated from the mRNA. Evidence that the phosphorylated and unphosphorylated P1 and P2 acidic ribosomal proteins are part of the functional stalk is also presented.



All living cells have a lateral flexible stalk structure in the large ribosomal subunit. This structure influences and regulates the activity of soluble factors involved in protein synthesis all along translation.[1,2] In the yeast *Saccharomyces cerevisiae*, the canonical arrangement of the five ribosomal proteins in the stalk is [P2β/P1α]-P0-[P1β/P2α].[1−3] They are designated as P proteins because they are substrates of several protein kinases like CK2,[4] PK60,[5] and RAP I, II, and III.[6,7] It is known that the structure of ribosomal protein P0 acts as a scaffold where the other four acidic ribosomal proteins bind. For the P0 (33.7 kDa, pI 4.56) protein, different functions for its N-terminal and C-terminal regions have been described. P0 interacts through its N-terminal domain with the highly conserved 25S rRNA GTPase-associated region (GAR) located close to ribosomal protein L12.[8] The C-terminal domain has two short amino acid regions located at positions A (amino acid residues 199−230; 197−231 in humans) and B (amino acid residues 231−258; 232−268 in humans) that independently bind P1α−P2β and P1β−P2α heterodimers, respectively.[8−10] The C-terminal domain is also known as the P domain, because it is more homologous with P1/P2 proteins.[8] The acidic ribosomal P2 (α and β) proteins are located at the periphery of the stalk complex acting like a "shield" for the innermost acidic ribosomal P1 (α and β) proteins.[10,11] The P1 proteins (α and β) are the only ones that make contact with P0.[12,13] It should be noted that the key element for stalk formation is the P1α−P2β heterodimer, and the P1β−P2α heterodimer has been implicated in regulatory stalk functions.[8,14] On the other hand, nonphosphorylated P1/P2 acidic ribosomal proteins, obtained after mutation of their respective serine residues, have been described also to form a nonphosphorylated stalk that selectively translates mRNAs like those of osmotic sensitive strains.[15]

The P1 (α and β) and P2 (α and β) proteins also form heterodimers through their N-terminal domains[16,17] and bind to sites A and B of P0 by means of their bilateral hydrophobic zipperlike structure identified through evolution.[10,12,14,17,18] The C-terminal domains of all five structural stalk proteins interact with translation elongation factors[19] and bind to ribosome-inactivating proteins, such as trichosanthin, ricin A-chain, and Shiga-like toxin 1.[10] It has been demonstrated that proper folding of protein P0 is achieved only in the presence of the P1α−P2β heterodimer, and if the P1 pair is absent, the P2 complex binds weakly to the ribosome.[13,14] This fact might explain why heterodimer combinations other than the canonical ones have different metabolic implications in yeast cells.[9]

P0 binds to the 60S subunit irreversibly, and it is an indispensable protein for ribosome structure and function

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because its deletion is lethal for the cell.[13] In contrast, P1/P2 heterodimers are dispensable (except in *Schizosaccharomyces pombe*) for ribosome function and bind to P0 reversibly.[13,20] P0 remains attached to the ribosome even after treatment with NH_4Cl and ethanol, whereas P1 and P2 do not, demonstrating the different binding stabilities of these proteins with respect to the ribosome.[13,21]

In *S. cerevisiae*, the P1/P2 acidic ribosomal proteins are detected in the cytoplasm in two chemical states: phosphorylated when they are bound to the ribosome or to the cell wall[22] and dephosphorylated when they are free in a cytoplasmic pool.[23] Their exchange between the pool and the ribosome[24] during translation has been described.[25] It has been shown that there is only one phosphorylation site for each acidic ribosomal protein, located at Ser-96 for P1α, P1β, and P2α and Ser-100 for P2β.[15] The P0 phosphorylation residue located at its C-terminal domain is Ser-258.[15,18] For the five stalk proteins, the amino acid sequence in the C-terminal end is EESDDDMGFGLFD, which is highly conserved throughout evolution.[18] It is known that P0 binds to the 60S ribosomal subunit in the cytoplasm after Yvh1 phosphatase replacement of Mrt4,[26,27] and it has been assumed that the stalk binds as a preformed whole complex.[27]

We report in this work that the acidic ribosomal proteins do not bind to the 60S-P0 (free) ribosomal subunit but do bind and stay attached to 80S ribosomes and polysomes, just when ribosomes are joined. Also, we present direct evidence of the phosphorylated and unphosphorylated acidic P proteins in the functional stalk.

EXPERIMENTAL PROCEDURES

Yeast Strain. *S. cerevisiae* W303-1B (MATα, *leu2-3,112 ura3-1, trp1-1 his3-11, 15, ade2-1, can1-100*) was used. This strain was grown in YPD liquid medium at 23 °C up to stationary phase and served as a stock culture that was renewed monthly.

Ribosomal Profiles. Yeast cultures were incubated in YPD medium at 23 °C up to midlog phase (0.8 OD_{660} unit). Cycloheximide was added to a final concentration of 100 μg/mL to stop protein synthesis and incubation continued for an additional 2 min. The cultures were chilled at 4 °C for 10 min to stop cell metabolism abruptly. Cells were harvested by centrifugation at 3500*g* for 10 min, and the pellet was washed twice with LHB buffer [10 mM Tris (pH 7.4), 100 mM NaCl, 30 mM $MgCl_2$, 50 μg/mL cycloheximide, and 200 μg/mL heparin]. Cells were broken up (0.1 g of 0.5 mm cell beads/g of cells) by five rounds of vigorous vortexing for 30 s and 30 s in an ice/water mixture. The lysed yeasts were resuspended in 1 mL of LHB and centrifuged twice at 10000*g* for 10 min, and the supernatants were recovered and quantified at 260 nm. Ninety OD_{260} units was loaded on top of 10 to 35% sucrose gradients in TMN buffer [50 mM acetyl-Tris (pH 7.0), 50 mM NH_4Cl, and 12 mM $MgCl_2$] and centrifuged for 5.5 h at 4 °C and 25000 rpm in the SW28 rotor (Beckman). The gradients were scanned at 254 nm through a UA-5 model fraction collector (ISCO), and the 40S and 60S ribosomal subunits, the monosomes, and the polysomes were isolated as described by Zhao and colleagues.[28]

Obtaining 40S and 60S Ribosomal Subunits. Ribosomes and polysomes obtained as described above were centrifuged at 50000 rpm for 3 h at 4 °C in a 55.2 Ti rotor (Beckman) to wash out sucrose residues. Pellets were rinsed with LMS buffer [0.1 M NaCl, 0.01 M Tris (pH 7.4), and 10^{-5} M $MgCl_2$], and the samples (50−90 OD_{260} units) were resuspended in LMS buffer, loaded onto 10 to 30% sucrose gradients in the same buffer, and centrifuged for 5.5 h at 4 °C and 25000 rpm (Beckman).[24]

Low-Phosphate Medium. It was essentially prepared as described by Rubin.[29] Inorganic phosphate was precipitated from YPD by adding 10 mL of 1 M Mg_2SO_4 and 10 mL of concentrated aqueous ammonia per liter. The phosphates were allowed to precipitate at room temperature for 30 min, and the precipitate was removed by filtration through Whatman No. 1 filter paper. The filtrate was adjusted to pH 5.8 with HCl and autoclaved.

rRNA Electrophoresis. The procedure for rRNA electrophoresis was adapted from that of Schmitt.[30] Ribosomes were centrifuged at 50000 rpm for 3 h at 4 °C, and the pellets were resuspended in 400 μL of buffer AE [50 mM $NaCH_4COO^-$ (pH 5.3)] and 10 mM EDTA (pH 8.0). Samples were transferred to Eppendorf tubes, and 40 μL of 10% SDS was added and immediately mixed in the vortex. An equal volume of phenol, previously equilibrated in AE buffer, was added, mixed in the vortex, and incubated at 65 °C for 4 min, and the mixtures were centrifuged again at 15000 rpm for 2 min. The aqueous phase was recovered, transferred to a new Eppendorf tube, and re-extracted with a phenol/chloroform mixture for 5 min at room temperature. The aqueous phase was recovered and adjusted to 0.3 M $NaCH_3COO^-$ with 40 μL of a stock solution of 3 M $NaCH_3COO^-$ (pH 5.3). Traces of phenol were eliminated by washing the samples twice with 1 volume of chloroform at room temperature; 2.5 volumes of ice-cold ethanol was added to the samples, mixed gently, and centrifuged at 15000 rpm. The pellets were washed with 80% ethanol and dried. Samples were resuspended in 20 μL of DEPC-treated bidistilled water and resolved by electrophoresis at 80 V for 2 h on 2% agarose gels in TAE buffer (0.04 M Tris-acetate and 0.001 M EDTA) and stained with 0.5 μg/mL EtBr.[31]

Obtaining Acidic Ribosomal Proteins. The method was performed as previously described with some modifications.[17] Samples dissolved in buffer I [20 mM Tris-HCl (pH 7.4), 100 mM $MgCl_2$, 500 mM $NH_4CH_3COO^-$, and 5 mM β-mercaptoethanol] were centrifuged at 10000*g* for 15 min at 4 °C. The supernatants were recovered and centrifuged at 50000 rpm for 14 h at 4 °C through 8 mL of 20% sucrose and 12 mL of 40% sucrose discontinuous gradients in buffer I. The pellets were dissolved in SP1 buffer [10 mM Tris-HCl (pH 7.4), 20 mM $MgCl_2$, 1 M NH_4Cl, and 3 mM β-mercaptoethanol], adjusted to 250 OD_{260} units/mL, and stirred for 20 min in ice-cold water; 0.5 volume of ethanol at 4 °C was added and stirred for 5 min followed by another addition of 0.5 volume of ice-cold ethanol and stirring for 5 min, and the sample was incubated for an additional 15 min and then centrifuged at 9500*g* for 15 min at 4 °C. To the supernatants was added 2.25 volumes of acetone at −20 °C, and the sample was mixed gently and incubated overnight at −20 °C. The pellets were centrifuged at 10000*g* for 20 min at 4 °C and vacuum-dried. The samples were dissolved in 8 M urea and stored at −20 °C.

Isoelectric Focusing. Acidic ribosomal proteins were resuspended in charging buffer [1.6 M urea, 5% ampholytes (pH 2.5−5), and 0.02% bromophenol blue] and loaded on a plate gel (5% acrylamide and 2% bisacrylamide). Focusing of the samples was achieved by using 0.03 N NaOH (cathode) and 0.37 N H_2SO_4 (anode). The run started at 50 V with a constant current (~6 mA) and a stepwise 100 V rise to 600 V.

Electrophoresis ended when the current dropped to 2 mA. The gels were stained with Coomassie blue or silver depending on the amount of sample.

Antibodies. The antibodies used in this work against P0 and the acidic ribosomal proteins were obtained from Vilella.[32]

Polyacrylamide Gel Electrophoresis of Ribosomal Proteins. Sodium dodecyl sulfate (SDS)−polyacrylamide gels (15%) were prepared as described previously.[31] Protein samples (25 μg) were loaded in the corresponding well and run at 90 V for ∼3 h. The gels were stained with Coomassie blue according to the Sambrook specifications.[31]

Western Blot Analysis. The method was described in ref 33. Salts were washed out from the gel with distilled water. The transfer of samples to nitrocellulose membrane sheets was conducted at 100 V for 30 min and 4 °C. The membrane was washed with 0.1% PBS-Tween [50 mM Tris-HCl (pH 7.6) and 150 mM NaCl]. It was washed again with 1% TBS-Tween for 5 min at room temperature and blocked with 5% milk in TBS-Tween for 60 min at room temperature. The membrane was rinsed with TBS-Tween and incubated overnight with the primary antibody at 4 °C. The nitrocellulose membranes were washed twice for 5 min with TBS-Tween at room temperature and incubated with the goat anti-mouse secondary antibody (1:5000 dilution) for 1.5 h. Secondary antibody excess was removed by washing twice with TBS-Tween. The membranes were revealed by 0.06 g of 4-chloro-naftol dissolved in 20 mL of recently made H_2O_2 in cold methanol with Tween-TBS, poured over the same membrane, or by incubation with chemiluminescence and autoradiographed.

RESULTS

To clarify the stage of the ribosomal biogenesis route during which and the cellular compartment in which the complete stalk assembles onto the 60S ribosomal subunit, it was necessary to obtain pure cytoplasmic 60S ribosomal subunits, free from any overlapping 80S fraction after sucrose gradient centrifugation. Experimental evidence of the presence of the stalk structure in functional ribosomes has been widely reported, but in contrast, its presence in the 60S ribosomal subunits remains unclear. To avoid contamination by the 80S ribosomal particles given that we could not achieve a complete separation among the 60S and 80S ribosomal fractions, only the first half of the 60S ribosomal subunit fraction was collected and subjected to analysis (Figure 1A). As shown in Figure 1B, the absence of 18S rRNA in the 60S ribosomal subunit fraction supports its purity.

The acidic ribosomal proteins from each ribosomal subunit fraction were purified (see Experimental Procedures) and resolved by pH 2.5−5.0 isoelectric focusing. After the gels had been stained with Coomassie blue, eight bands that correspond to the phosphorylated and the nonphosphorylated acidic P proteins were clearly detected, present in both the 80S and polysomal fractions. These bands were not detected in the 60S ribosomal subunit fraction even when it was 10 times more concentrated than the other ribosomal fractions (Figure 2). To eliminate the possibility that the buffer treatment combined with the repetitive high-speed centrifugation was responsible for the detachment of the 60S ribosomal subunit acidic ribosomal proteins from the stalk, the ribosomal subunits were obtained through an alternative procedure. The ribosomal pellets obtained after sucrose gradient ultracentifugation in TMN buffer were rinsed with LMS buffer to eliminate any traces of TMN buffer and were resuspended in the same low-magnesium buffer. The monosomal and polysomal particles were separated into ribosomal subunits in LMS buffer as described in Experimental Procedures. The 40S- and 60S-derived ribosomal particles are shown in Figure 3A.

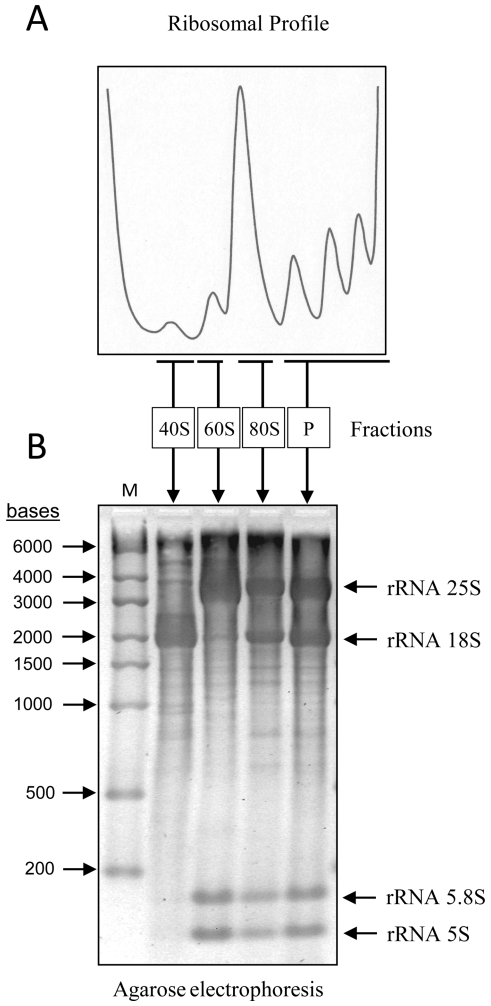

Figure 1. Profile of ribosomal particles and their RNAs. *S. cerevisiae* W303-1B was grown to midlog phase (0.8 OD_{660} unit) in YPD medium. (A) Ninety OD_{254} units of total cell extract was centrifuged for 5.5 h at 4 °C through 10 to 35% sucrose gradients in TMN buffer. To avoid 80S ribosomal particle contamination, only the ascendant portion of the 60S ribosomal subunit peak was collected. (B) The rRNA extracted from the 40S and 60S ribosomal subunits and the 80S monosomes and polysomes were displayed via 2% agarose gel electrophoresis. The absence of 18S rRNA in the lane of the 60S ribosomal subunit assures the purity of the 60S ribosomal subunit fraction. The rRNAs from the 40S ribosomal subunit and the 80S monosomes and polysomes were included as negative and positive controls, respectively.

The total ribosomal proteins from the 40S, 60S, 80S, and polysomal fractions obtained by the TMN buffer method and the 60S fractions derived from 80S monosomes and polysomes by the LMS buffer method, after ultracentrifugation, were obtained and subjected to narrow range isoelectric focusing (pH 2.5−5.0). The results of silver staining (Figure 3B) showed, again, the absence of acidic ribosomal proteins in native 60S ribosomal subunits, but they are clearly visible in the 80S monosomes, polysomes, and 60S ribosomal subunits derived from 80S monosomes and polysomes after treatment with LMS buffer. Note the protein band that focused near pH

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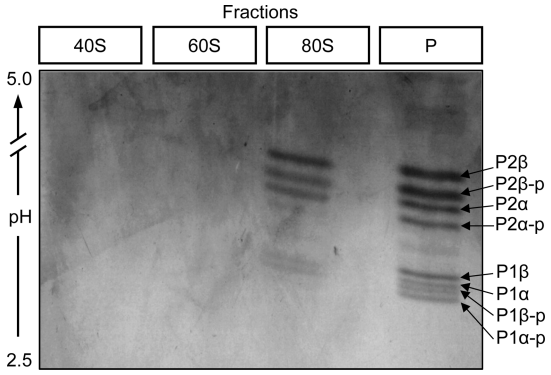

Figure 2. Acidic ribosomal proteins extracted from purified 40S and 60S ribosomal subunits, 80S monosomes, and polysomes. The ribosomal particles from the ribosomal profiles were purified from the 10 to 30% sucrose gradients in TMN buffer. The acidic ribosomal proteins were extracted from each ribosomal fraction, resolved by narrow range (2.5−5.0) isoelectric focusing techniques (Experimental Procedures), and stained with Coomassie blue. Ten times more protein was loaded in the slots of 40S and 60S ribosomal proteins than in the 80S monosomes and polysomes. Only the part of the gel that focused on the acidic proteins is presented.

5.0 could be ribosomal protein P0, which can be detected in all the samples. These results support the notion that the acidic ribosomal proteins are bound only to cytoplasmic monosomes and polysomes.

Immunodetection of the Stalk Proteins. It has been reported that at early stages of 60S ribosomal biogenesis, the nuclear paralogue of P0, Mrt4, is displaced by Yvh1-mediated P0.[26,27] P0 binds firmly and irreversibly to the cytoplasmic 60S ribosomal subunit, and it has been assumed that it binds to the acidic P protein heterodimers as a pentameric preassembled complex.[27] The results described above support the notion that P0 is attached to the 60S subunit but not with the heterodimers. To confirm that the P1/P2 heterodimers were not attached to the 60S−P0 ribosomal subunit particle and to search these heterodimers in dissociated 60S subunits isolated from the 80S monosomes and polysomes, we resolved total proteins of each fraction from the TMN buffer sucrose profile and the dissociated 60S fractions from the LMS buffer pellet by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) and transferred them to nitrocellulose membranes for Western blot analysis. After being transferred to the nitrocellulose membrane, the samples were exposed to two different monoclonal antibodies: 3BH5, which recognizes the C-terminal end of all five ribosomal P proteins, and P1A, directed against the P1A acidic P protein. As expected, through the use of the 3BH5 antibody, the chromogenic substrate revealed a well-defined band for P0 in all protein samples, except for those that had the negative control 40S ribosomal subunit proteins (Figure 4A). Because of the small difference in size, the four acidic P proteins were not resolved as sharp, separated bands via conventional SDS−PAGE. Except for the negative control (40S fractions), the P1/P2 proteins were detected in all samples as faint widespread bands of ∼11 kDa, including the two 60S-dissociated samples. However, once again, they were not detected in the samples containing the native 60S subunits (Figure 4A). The Western blot assay using the P1A antibody revealed two bands that correspond to the nonphosphorylated and phosphorylated forms of P1A. Similar results were obtained with the chromogenic substrate (Figure

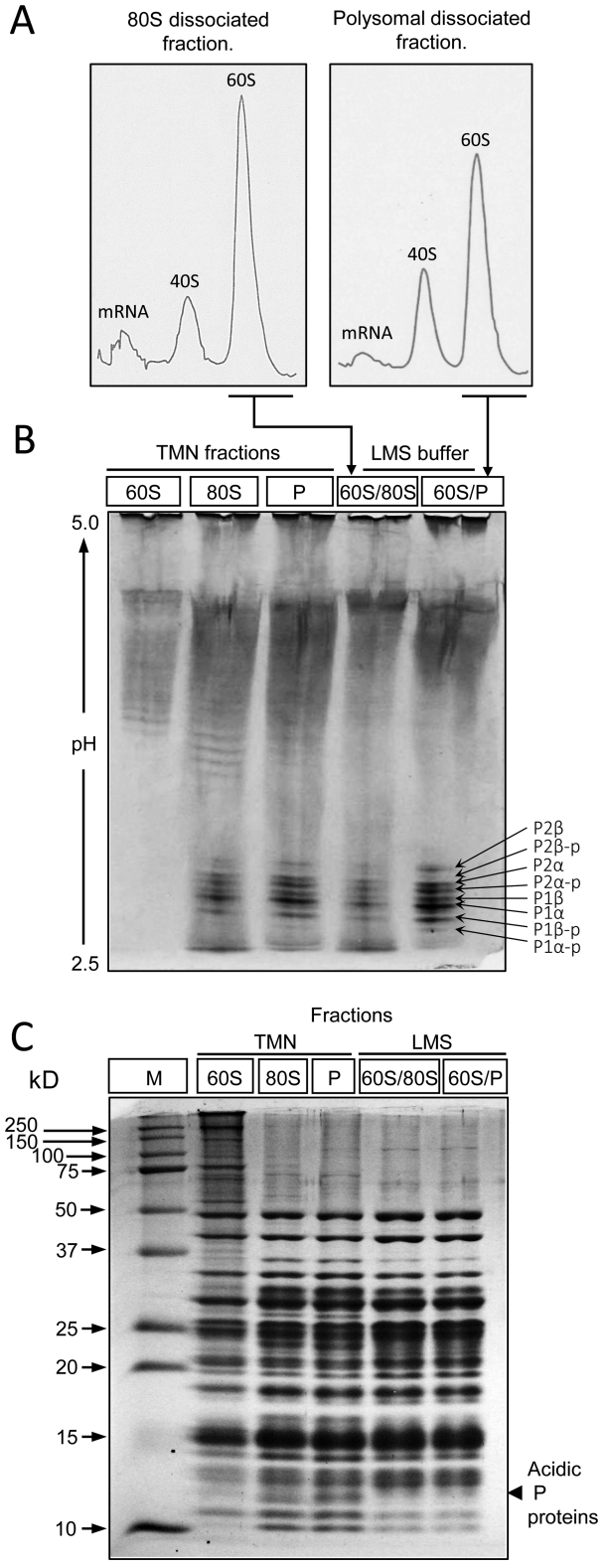

Figure 3. Isoelectric focusing of the total ribosomal extract from each fraction of the ribosomal profile and the dissociated 60S fractions from monosomes and polysomes. The 80S ribosomes and polysomes from the ribosomal profile in TMN buffer were isolated and centrifuged at 50000 rpm for 3 h at 4 °C. Both pellets were rinsed with LMS buffer to discard traces of salts from the TMN buffer. The ribosomal pellets were resuspended in LMS, and 90 OD_{254} samples were centrifuged through 10 to 30% sucrose gradients in LMS. (A) Derived small and large ribosomal subunits were collected and plotted with the aid of an

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Figure 3. continued

absorbance monitor and fraction collector. The total amounts of protein from each fraction of the sucrose gradients in TMN buffer and from the dissociated 60S subunit in LMS buffer were obtained by ultracentrifugation, except for that of the 40S ribosomal subunit fraction. (B) Each sample (0.25 mg) was resolved by isoelectric focusing. Silver staining produced eight bands whose pI values corresponded to the acidic ribosomal proteins. (C) Control. Protein (25 μg) from each fraction from TMN and LMS buffers was resolved by a 15% polyacrylamide gel electrophoresis assay and stained with Coomassie blue.

4B). To rule out any minor P1/P2 protein signal in the native 60S fraction, the proteins in the Western blot were revealed with a more sensitive chemiluminescence assay. In the autoradiograms, no signal was detected in the native 60S sample or with the 3BH5 (Figure 4C) or P1A (Figure 4D) antibody. The rest of the fractions had a pattern similar to that described for the chromogenic assay.

Uptake of Radioactive Phosphate by the Acidic Ribosomal Proteins. The presence of phosphorylated and nonphosphorylated acidic ribosomal proteins in ribosomes extracted from the cytoplasm has been documented,[15] but the ribosomal particles in which they are found have not been identified. To demonstrate that from the eight bands unveiled by isoelectric focusing (Figures 2 and 3B) four correspond to the phosphorylated forms of the acidic P proteins (and, therefore, the other four correspond to the nonphosphorylated forms), radiolabeling with the ^{32}P isotope was conducted. To that end, yeast cells were incubated in low-phosphate medium (Experimental Procedures) to label them for 60 min with ^{32}P, and the cytoplasmic protein extracts were resolved by one-dimensional SDS−PAGE. The autoradiograph in Figure 5B shows the incorporation of the isotope into a low-molecular weight band, compatible with the size of the acidic ribosomal proteins, on the 80S monosome, and the light (PI in Figure 5B) and heavy polysomal fractions (PII in Figure 5B). Neither 40S nor 60S ribosomal subunits were radioactively labeled under these experimental conditions.

To determinate if the phosphorylated form of each of the four acidic P proteins was present, the radiolabeled samples described above were separated by narrow range pH 2.0−4.0 isoelectric focusing. The results in Figure 5C show the presence of the four phosphorylated acidic ribosomal proteins in monosomes and polysomes but not in native 60S ribosomal subunits.

■ DISCUSSION

In this work, we have focused on finding, in the complex pathway of ribosomal biogenesis, the accurate step at which the acidic P protein heterodimers are loaded into the ribosomal 60S−P0 particles and assemble the completely structured and functional stalk. Lo and co-workers[27] demonstrated that the activity of phosphatase Yvh1 promotes the displacement of the Mrt4 nucleolar protein by P0 in the cytoplasm, under the assumption that it was bound as a preformed P0−[P1/P2]$_2$ complex.[27] When we analyzed the *S. cerevisiae* ribosomal stalk with either sample, acidic ribosomal protein or total ribosomal protein, from each profile fraction, purified with sucrose gradients in either TMN or LMS buffer, our results showed that the native 60S subunit does not have acidic ribosomal proteins (Figures 2 and 3B).

The 60S fraction isolated from the cytoplasm consists of *de novo* particles exported from the nucleus and "recycled"

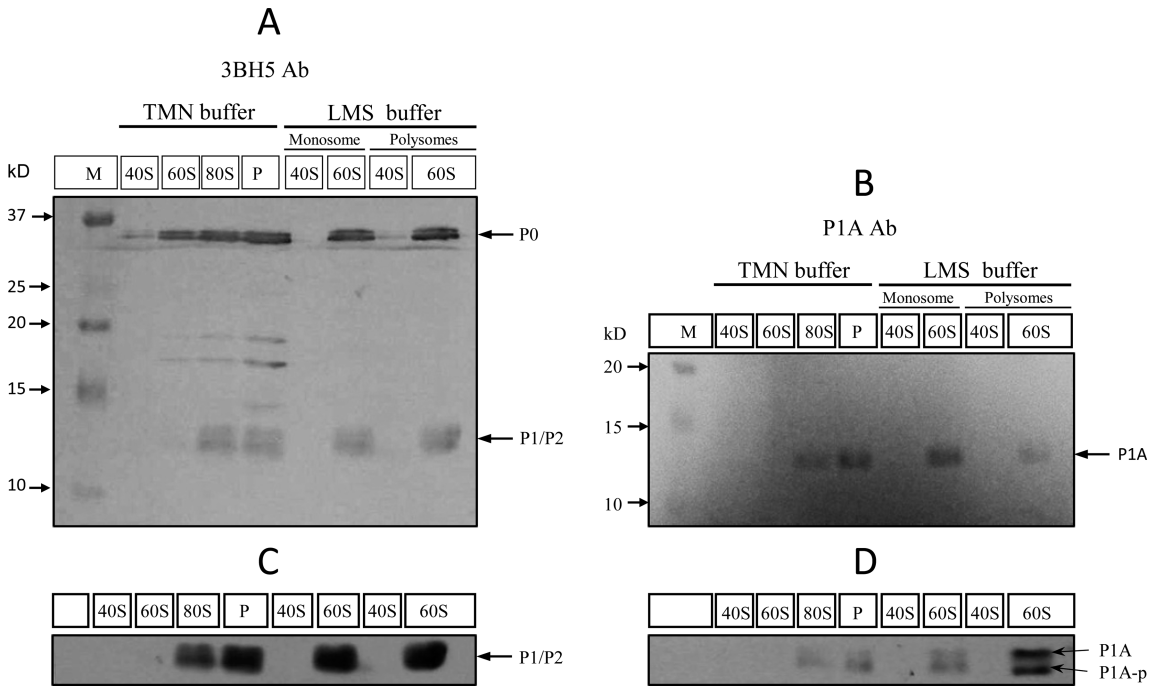

Figure 4. Western blot analysis of total ribosomal proteins. The 40S and 60S ribosomal subunits, the 80S monosome, and the polysomes from the ribosomal profiles in TMN buffer and the 40S and 60S ribosomal subunits dissociated with LMS buffer were analyzed. The following antibodies were used: 3BH5, directed against the C-terminal end of the stalk proteins, or P1A, against P1α protein. (A) Chromogenic detection assay of ribosomal protein P0 and ribosomal proteins P1 and P2 using the 3BH5 antibody. (B) Chromogenic detection using the P1A antibody. (C) Chemiluminescent detection of the P1/P2 heterodimer using the 3BH5 antibody. (D) Enhanced chemiluminiscence assay using the P1A antibody.

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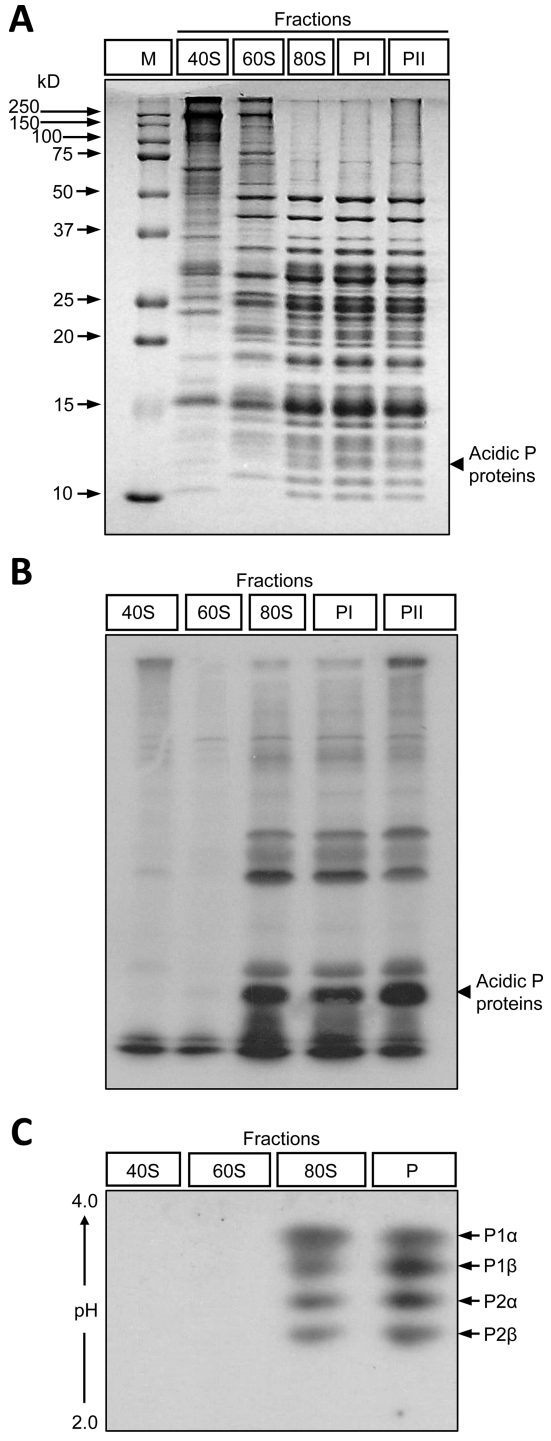

Figure 5. Autoradiogram of ^{32}P-labeled ribosomal proteins. (A) Total ribosomal proteins (25 μg) from each gradient fraction were assessed by one-dimensional SDS–PAGE. (B) By autoradiogram, the acidic ribosomal proteins were detected only in the lanes of 80S monosomes, trisomes (PI), and heavier (PII) polysomes. (C) Narrow range (pH 2–4) isoelectric focusing of the acidic ribosomal proteins. Each of the four phosphorylated acidic ribosomal proteins was resolved. The 40S ribosomal subunit fraction was included as a negative control.

particles involved in translation rounds. Therefore, if the recycled 60S particles (naturally dissociated) could retain the complete stalk, the acidic P protein most probably would have been misestimated, giving weaker signals *via* chromogenic detection. Nevertheless, the results presented here ruled out this possibility as demonstrated by both the chemiluminescence (Figure 4C, D) and the radiolabeling detection assays (Figure 5). It is known that the ribosomal stalk of prokaryotes is more stable and less flexible than the eukaryotic one[21] and that the latter can be detached from ribosomes by increasing the ionic concentration of the medium, therefore disassembling the P1/P2 acidic ribosomal protein heterodimers.[13] Also, a bilateral hydrophobic zipper has been described, weaker than the leucine zipper, which holds the P2β–P1α/P1β–P2α heterodimers together, and in turn attached to P0.[18,10] This fact might explain the cytoplasmic exchange of heterodimers, and their stronger susceptibility to detachment from the 60S ribosomal subunit during their isolation and purification, explaining their absence from the large ribosomal subunit. However, this possibility was ruled out in our experiments, because handling of the particles was identical and the complete stalk was present in each of the 80S monosome and polysomal particles purified from the ribosomal profiles. Moreover, to eliminate other possible ways of detachment of the heterodimers from the large ribosomal subunit, as a result of harsh handling, analysis of the 60S–80S and 60–polysome dissociated fractions that went through an additional run of ultracentrifugation showed a complete set of structural proteins in the stalk (Figures 3 and 4).

In previous reports,[18,25] it had been suggested that phosphorylation controls both the binding of the ribosomal acidic P proteins to the ribosome and their exchange with the cytoplasmic pool. Hence, only the phosphorylated form of these proteins would be present in the ribosomal stalk.[25] More recently, in 1997, Zambrano and co-workers demonstrated that the mutated nonphosphorylated P proteins associate to ribosomes, but they did not specify to which ribosomal particle.[15] As shown by immunodetection, radiolabeling, and isoelectric focusing, the results of this work demonstrate that from the eight bands shown in Figures 2 and 3B, four are phosphorylated acidic P proteins (Figures 2, 3, and 5) and prove for the first time the specific structural presence of the stalk on the phosphorylated and unphosphorylated P1/P2 protein heterodimers in ribosomes, but not in the native 60S subunits. These results are important, because it has been reported that phosphorylation of ribosomal proteins would select the mRNAs to be translated.[15]

The results of this work support the conclusion that the P1/P2 heterodimers do not interact with P0 in an independent way, on the pre-60S or 60S subunit, but do interact with P0 and conform the stalk just before commitment of the 80S particle to translation, by a mechanism that has to be clarified. P1 and P2 enter the cytoplasmic pool probably as heterodimers once the ribosome dissociates, in contrast to P0 that remains bound to the 60S ribosomal subunit (Scheme 1).

Some questions about our finding of the absence of complete ribosomal stalk are as follows: Why are the five acidic P proteins not imported into the nucleus like the other structural ribosomal proteins to be exported to the cytoplasm as a complete mature 60S ribosomal subunits? When ribosomes dissociate after translation rounds, why is the release of the stalk P1/P2 heterodimers necessary? A complex mechanism has been developed by the eukaryotic cell to prevent the mature large ribosomal subunits from being present inside the nucleus. P0 and its nuclear paralogue, Mrt4, provide such evidence.

Our experimental data strongly suggest that the complete stalk is somehow toxic in the cytoplasm and should be partially disassembled from the 60S subunit.

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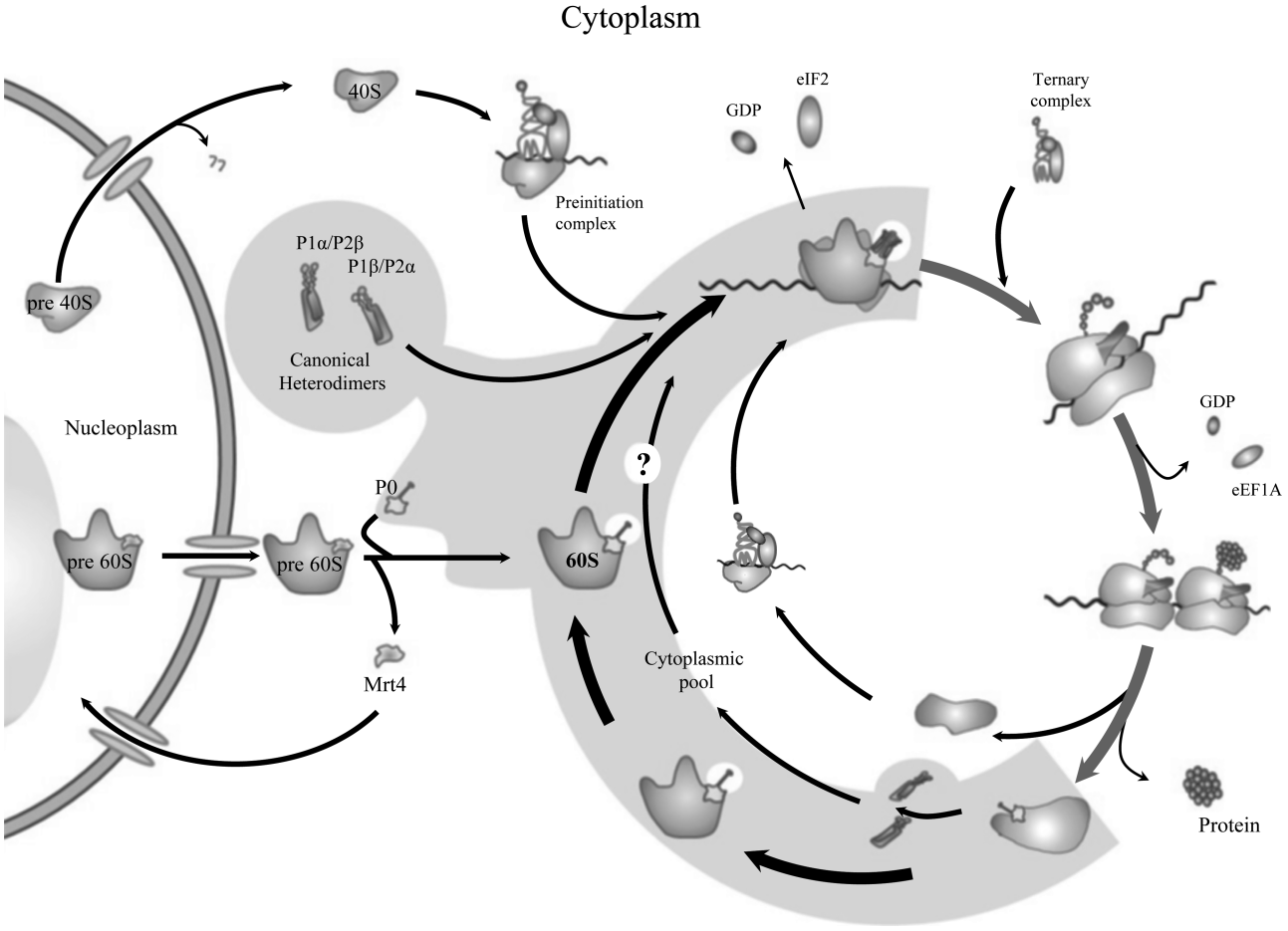
Scheme 1. Model of the Cytoplasmic Interaction and Exchange for the Stalk Acidic Ribosomal Proteins[a]



[a]The pre-60S ribosomal subunits are exported to the cytoplasm where the bound Mrt4 protein is replaced by P0. The protein heterodimers, recruited by an unknown mechanism, form the stalk on the 80S monosome but do not as part of the native 60S subunit. Just when ribosomes dissociate from the mRNA, the P1/P2 heterodimers disassemble. Acidic ribosomal proteins P1 and P2 enter a cytoplasmic pool and are probably recycled. The experimental data from this work are highlighted with a gray background.

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Notes

The authors declare no competing financial interest.

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