Functional Role of Acidic Ribosomal Proteins. Interchangeability of Proteins from Bacterial and Eukaryotic Cells†

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ABSTRACT: Core particles derived from yeast ribosomes by treatment with 50% ethanol and 0.4 M NH_4Cl ($P_{0.4}$ cores) are deprived of the acidic proteins L44/45 functionally equivalent to the bacterial proteins L7 and L12. These bacterial proteins are able to reconstitute the EF-2-dependent GDP binding capacity of the yeast cores but not their GTPase activity. On the other hand, yeast particles prepared in similar conditions but in the presence of 1 M NH_4Cl ($P_{1.0}$ cores) lose proteins L44/45, L15, and S31. These particles are able to reconstitute both activities by the bacterial proteins L7 and L12. Proteins L15 and S31 somehow affect the interaction of bacterial proteins L7 and L12 with the yeast particles. Indeed, in their presence only one dimer of L7 and L12 is bound to the $P_{0.4}$ cores, while in their absence ($P_{1.0}$ cores) the amount of bacterial proteins retained by the yeast particles is doubled. Elongation factor EF-2 seems to play an important role in the binding of the bacterial proteins to the yeast cores. Our results suggest that the two dimers of L7 and L12 normally present in the ribosomes might play a different functional role, one of the dimers being related to the binding of the substrate and the other one involved in the GTPase active center.

Since the function of ribosomes in prokaryotic and eukaryotic cells is very similar, it is highly possible that the ribosomal components involved in the different steps of protein synthesis correspond in both types of particles. It is clear that establishing this correspondence will facilitate the application of the numerous data now available on the structure of the bacterial ribosome for a better understanding of the eukaryotic particle. This correspondence and even interchangeability have been reported in the acidic proteins of the larger ribosomal subunit of *Escherichia coli* on the one hand and rat liver (Stoffler et al., 1974) and *Artemia saline* (Moller et al., 1975) on the other.

In *Saccharomyces cerevisiae*, Wool & Stöffler (1974) and Richter & Möller (1974) reported the reconstitution by bacterial acidic proteins L7 and L12 of the activity of $P_{1.0}$ particles prepared by treatment of yeast ribosomes with 1 M NH_4Cl and 50% ethanol (Hamel et al., 1972). On the basis of these data together with the immunological cross-reactivity detected between the proteins of *E. coli* and *S. cerevisiae* (Wool & Stöffler, 1974), the equivalence of these two types of proteins was defended. We were subsequently able to show that in the treatment conditions for preparing the $P_{1.0}$ cores, substantial amounts of ribosomal proteins other than the acidic L44 and L45 are released from the ribosomes. Also the NH_4Cl concentration of the washing procedure had to be lowered to 0.4 M ($P_{0.4}$ cores) in order to make the release of the acidic proteins specific. Using these $P_{0.4}$ core particles, we were unable to detect reconstitution of the partially lost EF-2 dependent GTP hydrolysis activity of the cores by the acidic bacterial proteins (Sánchez-Madrid et al., 1979a).

The discrepancy among results from different laboratories calls attention to the limitations of core preparation techniques. Characterization of the protein-deficient particles is important, and in this sense we must be aware that apparently small changes in the procedures and the state of the original ribosomes drastically affect the characteristics of the resulting cores (Sánchez-Madrid et al., 1979a). This is especially critical in the case of eukaryotic ribosomes which due to their higher structural complexity yield core particles that, lacking some of their ribosomal proteins, have considerable residual activity (Sánchez-Madrid et al., 1979a).

In spite of this, the technique is very useful, and, in fact, we have used it to clarify the above-mentioned apparent discrepancy, finding that, as in the case of $P_{1.0}$ cores, the bacterial L7 and L12 proteins are indeed able to interact with the $P_{0.4}$ core particle, although only the GDP binding capacity is reconstituted. The GTP hydrolysis activity of the particles is not. In addition, we have found that the nonacidic proteins released in the $P_{1.0}$ particles play an important role in the acidic protein binding site. The results of these experiments are reported in this communication.

Materials and Methods

Ribosomes were prepared from *Saccharomyces cerevisiae* grown in 2-L flasks with 1 L of YEPD medium to early exponential phase by sea sand grinding; they were then washed as described (Sánchez-Madrid et al., 1979a,b). Bacterial ribosomes were obtained from *Escherichia coli* MRE 600 following standard methods (Staehelin & Maglott, 1971). Acidic proteins were extracted from ribosomes by the ammonium–ethanol method (Hamel et al., 1972; Sánchez-Madrid et al., 1979a), the ammonium concentration in the treatment being either 0.4 or 1.0 M, depending on the type of particles desired. The proteins released will be referred to as $SP_{0.4}$ and $SP_{1.0}$ and the core particles as $P_{0.4}$ and $P_{1.0}$, respectively. In the case of *E. coli* $P_{1.0}$ particles, an additional treatment in the same conditions but at 37 °C was carried out, yielding a protein fraction referred to as SP_{0-37} (Highland & Howard, 1975). It must be noted that in order to obtain core particles susceptible to reconstitution, the ribosomes have to be prepared from early logarithmic phase cells. In our experience, ribosomes from later growing phases are very sensitive to ethanol precipitation at low salt concentration and were inactivated in conditions where there is no release of ribosomal proteins (Sánchez-Madrid et al., 1979a). Any ribosomal preparation, regardless of its original activity, that upon precipitation with 50% ethanol in the presence of 80 mM NH_4Cl loses more than

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30% of its polymerizing activity has not been used for core preparation.

The $SP_{0.4}$ fraction from yeast was resolved on a DEAE-cellulose column with a 10 mM to 1.5 M sodium acetate gradient as described (Sánchez-Madrid et al., 1979b). Eluted fractions were dialyzed against 1% acetic acid and lyophilized. The composition of the protein fractions was checked by sodium dodecyl sulfate ($NaDodSO_4$), two-dimensional gel electrophoresis (Kaltschmidt & Wittmann, 1970) and electrofocusing, and the yeast proteins were named according to the nomenclature of Kruiswijk & Planta (1975a,b). The results indicated that the preparation of protein L44/45 contained a contamination of 5–10% of protein A_x while the preparation of protein A_x was pure. The basic proteins fraction was composed of protein L15 (69%), protein S31 (13%), and three other unidentified proteins. [γ-^{32}P]GTP was prepared following the method described by Glynn & Chapell (1964). [^{3}H]GDP was obtained from the Radiochemical Center, Amersham, England. Highly purified EFG from *E. coli* B and EF-2 from rabbit reticulocytes were gifts from Dr. J. Modolell and Dr. A. González, respectively.

Activity Tests. EF-G and EF-2 dependent GTPase tests were carried out by using purified *E. coli* B (Parmeggiani et al., 1971) and reticulocytes elongation factors (Kemper & Merrick, 1979) as described (Sánchez-Madrid et al., 1979a).

The formation of ribosome–EF-2–GDP complex was tested in 50-μL samples containing 10 mM NH_4Cl, 20 mM tris-(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.4, 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 0.8 mg/mL ribosomes, 5 μM [^{3}H]GDP, and either 0.5 mg/mL of EF-2 or 0.09–0.15 mg/mL of EFG. Fusidic acid concentration, when required, was 1.50 mM. The samples, except GDP, were preincubated at 37 °C for 10 min. After addition of the nucleotide, the samples were kept at 37 °C for 15 min and then diluted with 2 mL of the same buffer and filtered through Millipore nitrocellulose filters. The filters after washing 3 times with 2 mL of buffer were dried, and the radioactivity was estimated.

L7 and L12 Binding Tests. Protein-deficient particles (100 pmol) from yeast and *E. coli* were incubated in 150–200 μL of 20 mM Tris-HCl, pH 7.4, 60 mM NH_4Cl, and 10 mM $MgCl_2$ with 80 μg of $SP_{1.0}$ proteins from *E. coli* at 30 °C for 15 min. When required, 0.5 mg/mL of EF-2 was also added. The mixtures were then applied to Sepharose 6B columns containing 5 mL of gel equilibrated with the same buffer but containing either 0.5 M NH_4Cl or 80 mM NH_4Cl. The column effluent was monitored at 260 nm, and the fractions containing the ribosomes were pooled. The amount of L7 and L12 present in the reconstituted particles was directly estimated by radial immunodiffusion (Mancini et al., 1965) by using a specific anti L7 and L12 serum obtained from rabbits immunized with the pure bacterial proteins, as indicated (Sánchez-Madrid et al., 1979a).

Results

Elongation Factor Dependent Activities of Yeast $P_{0.4}$ Core Particle. The treatment of *S. cerevisiae* ribosomes with 0.4 M NH_4Cl and 50% ethanol results in protein-deficient particles ($P_{0.4}$ cores) that are partially affected in elongation factor 2 (EF-2) dependent GTPase activity. The replacement of released protein fraction ($SP_{0.4}$) restores the lost GTPase activity to the cores (Sánchez-Madrid et al., 1979a).

As previously reported (Sánchez-Madrid et al., 1979b), chromatography on DEAE-cellulose of $SP_{0.4}$ resolves two protein fractions, A and A_x, in addition to the basic protein fraction not retained in the column. Fraction A_x is made up of one highly phosphorylated protein, while fraction A can be resolved into two proteins which correspond to the acidic proteins characterized by others either as L44/45 (Kruiswijk & Planta, 1975a,b) or L35/36 (Zinker & Warner, 1976). The unretained fraction, which accounts for about 10% of the extracted material, is mainly protein L15 with a smaller amount of protein S31 (Sánchez-Madrid et al., 1979a).

Table I: Activity of the Different Components of $SP_{0.4}$ Fraction[a]

particle	protein fraction	EF-2 dependent GTP hydrolysis: molecules/ribosome	EF-2 dependent GTP hydrolysis: %
control		121.0	100
cores $P_{0.4}$		44.7	37
cores $P_{0.4}$	L44/45 (5 μg)	87.1	72
cores $P_{0.4}$	A_x (4 μg)	42.3	35
cores $P_{0.4}$	L15, S31 (5 μg)	37.5	31

[a] 80S ribosomes precipitated with 50% ethanol in the presence of 80 mM NH_4Cl were used as controls in both types of tests. These particles contained standard amounts of acidic proteins (Sánchez-Madrid et al., 1979a). Reaction mixtures were preincubated at 37 °C for 15 min previous to initiation of the reaction by addition of [γ-^{32}P]GTP.

Table II: Effects of Split Acidic Proteins on the Activity of Core Particles[a]

particle	acidic protein added	elongation factor dependent: GTP hydrolysis molecules/ribosome	GTP hydrolysis %	GDP binding molecules/ribosome	GDP binding %
		Experiment I			
80S ribosomes		103	100	0.23	100
80S $P_{0.4}$ cores		35.6	34	0.10	47
80S $P_{0.4}$ cores	yeast $SP_{0.4}$, 7 μg	72.1	70	0.25	108
80S $P_{0.4}$ cores	*E. coli* $SP_{1.0}$, 7 μg	32.9	32	0.18	78
		Experiment II			
70S ribosomes		180	100	0.97	100
70S $P_{1.0}$ cores		18	10	0.39	41
70S $P_{1.0}$ cores	*E. coli* $SP_{1.0}$, 1 μg	132.5	74	0.96	99
70S $P_{1.0}$ cores	*E. coli* $SP_{1.0}$, 3 μg	160.2	89	1.10	115
70S $P_{1.0}$ cores	yeast $SP_{0.4}$, 1 μg	19.8	11	0.40	41
70S $P_{1.0}$ cores	yeast $SP_{0.4}$, 3 μg	16.2	9	0.39	41

[a] Reactions were carried out as indicated in Table I.

For determination of which component of the $SP_{0.4}$ fraction is responsible for GTPase stimulation of the $P_{0.4}$ cores, the activity of the treated particles supplemented by each of the column fractions was tested. Table I shows that only fraction A (proteins L44/45) has a stimulatory effect.

We have also previously shown that the GTPase activity of the $P_{0.4}$ cores was not stimulated by the bacterial acidic proteins L7 and L12, while the results of Richter & Möller (1974) indicated reconstitution of EF-2 dependent GDP binding of yeast $P_{1.0}$ core particles by using the bacterial proteins.

The results presented in Table II show, however, that the $P_{0.4}$ cores in the presence of *E. coli* $sP_{1.0}$ are also able to form

Table III: Reconstitution of GTPase Activity of $P_{1.0}$ Core Particles from Yeast by *E. coli* Ribosomal Proteins[a]

particle	protein added	hydrolyzed GTP (molecules/ ribosome)	activity (%)
80S ribosome		55.6	100
$P_{1.0}$ core		16.5	33
$P_{1.0}$ core	4 μg of SP_0 *E. coli*	30.1	54
$P_{1.0}$ core	8 μg of SP_0 *E. coli*	50.6	91
$P_{1.0}$ core	4 μg of SP_{0-37} *E. coli*	17.0	31
$P_{1.0}$ core	8 μg of SP_{0-37} *E. coli*	13.8	25

[a] Reaction carried out as in Table I.

the GDP–EF-2 complex in spite of being unable to carry out uncoupled EF-2 dependent GTP hydrolysis in the same conditions. On the other hand, contrary to previous results (Wool & Stöffler, 1974), the $SP_{0.4}$ protein fraction from yeast does not stimulate either the GTPase or the GDP binding capacity of the *E. coli* core particles.

Activity of $P_{1.0}$ Core Particles from Yeast Ribosomes. It was previously shown (Sánchez-Madrid et al., 1979) that treatment of yeast, or other eukaryotic ribosomes (Reyes et al., 1977; Lin et al., 1979), in the same conditions used for the removal of the bacterial proteins L7 and L12 from the ribosomes, namely 1 M NH_4Cl and 50% ethanol, yields particles that lack a large fraction of proteins L15 and S31 and completely lack the acidic proteins L44 and L45. These particles lose most of their original EF-2 dependent GTPase, which can be restored by addition of their own split proteins. They were also reactivated, however, by the *E. coli* acidic proteins (Table III).

The stimulation of the GTPase activity of the $P_{1.0}$ cores is due to the acidic bacterial proteins since the protein fraction SP_{0-37} that contains the possible contaminating proteins of the SP_0 fraction, namely L10, L11, L1, L5, and L25 (Bernabeu et al., 1976), has no stimulatory effect (Table III). In fact, polyacrylamide gel electrophoresis of SP_0 showed the presence of the acidic proteins L7 and L12 only.

Binding of E. coli Acidic Proteins to Yeast Core Particles. In order to check whether the different behavior of the yeast core particles $P_{0.4}$ and $P_{1.0}$ was due to their different capacity of interaction with the bacterial proteins, the amount of these proteins in the core particles after incubation in the appropriate conditions was tested by radial immunodiffusion. Table IV shows that the interaction of the bacterial proteins with the yeast cores is weak since no proteins are detected when the particles are washed with 0.5 M NH_4Cl after the binding. However, the interaction is clearly stabilized by elongation factor 2 (EF-2), and in optimal conditions, the $P_{1.0}$ cores bind twice as much L7 and L12 as the $P_{0.4}$ cores.

Inhibition of $P_{1.0}$ Core GTPase Reconstitution by Ribosomal Basic Proteins. $P_{1.0}$ cores lack about 60% of proteins L15 and S31 (Sánchez-Madrid et al., 1979a). These proteins, easily separated from the acidic proteins by ion-exchange chromatography, were used to reconstitute the $P_{1.0}$ particles. As shown in Figure 1, while the basic protein fraction has no effect on the residual GTPase of the particles, thus excluding the presence of some direct inhibitor of this activity, the incubation of the particles with the basic proteins previous to the addition of the bacterial acidic proteins totally inhibits the reconstitution of the GTPase activity of the cores by L7 and L12.

Table IV: Binding of Proteins L7 and L12 to Yeast Ribosomal Cores[a]

particle	protein added	proteins L7 and L12 present in particles washed with: 0.5 M NH_4Cl (mol of L7/L12 per mol of ribosomes)	80 mM NH_4Cl (mol of L7/L12 per mol of ribosomes) +EF-2	80 mM NH_4Cl (mol of L7/L12 per mol of ribosomes) −EF-2
$P_{1.0}$ *E. coli*		0.4		0.5
$P_{1.0}$ *E. coli*	+	3.4		3.5
$P_{0.5}$ yeast	+	0	1.5	0.7
$P_{1.0}$ yeast	+	0	3.0	1.7

[a] The particles were incubated in the presence of the proteins as described under Materials and Methods and then passed through a column of Sepharose 6B. The fractions containing the ribosomes were pooled, and the content of bacterial proteins was tested by radial immunodiffusion.

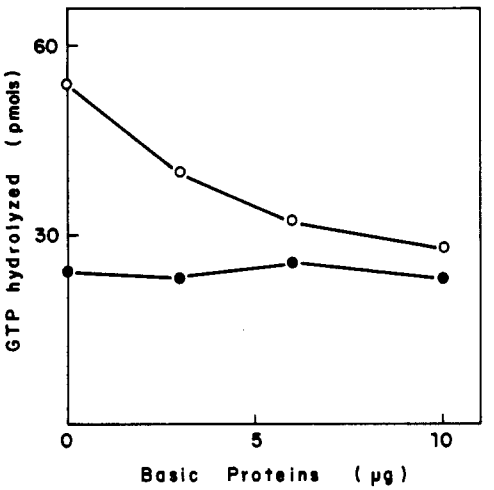

FIGURE 1: Reconstitution of EF-2-dependent GTP hydrolysis activity of $P_{1.0}$ core particles. $P_{1.0}$ cores (20 pmol; 100 μL) were incubated for 10 min in the standard ionic conditions for GTPase tests, in the presence of increasing concentrations of basic proteins obtained from the yeast $SP_{0.4}$ fraction. Aliquots of 50 μL were taken to test GTPase activity (filled circles), and the rest of the samples were supplemented with 6 μg of bacterial L7 and L12 and after additional incubation for 10 min tested for GTPase activity (open circles).

Discussion

The data presented in this report confirm previous results indicating a functional equivalence of bacterial proteins L7 and L12 and the yeast proteins L44 and L45 (Wool & Stöffler, 1974; Richter & Möller, 1974). Previously (Sánchez-Madrid et al., 1979a) we had questioned this equivalence mainly on the basis of results obtained by using $P_{0.4}$ core particles that, as it was then shown and now confirmed, are not reconstituted by the acidic bacterial proteins in their EF-2 dependent GTP hydrolysis activity. The results presented by other authors (Richter & Möller, 1974) showing reconstitution by bacterial proteins of the GDP-binding capacity of yeast particles also deprived of acidic proteins were interpreted in view of the different types of core particles used and/or possible contamination of the bacterial acidic proteins.

Our present data indicate that our previous interpretation was not correct, showing that the bacterial acidic proteins can interact with the yeast $P_{0.4}$ core particles. This interaction is only partially functional though, as it is able to induce formation of the ribosome–EF-2–GDP complex but not the hydrolysis of GTP, at least in a cyclic way. Our data do not exclude the possibility of a single round of GTP hydrolysis upon interaction of EF-2 and GTP with the ribosome, the EF-2

then being sequestered in the form of a ribosome–GDP–EF-2 stable complex. Unfortunately, the levels of endogenous GTPase of the yeast particles do not allow us to carry out experiments to test this possibility.

Our previous suggestion (Sánchez-Madrid et al., 1979a) that the type of core particle used in the experiments could affect the results of the functional tests is basically correct, as shown by the different behavior of the $P_{0.4}$ and the $P_{1.0}$ particles with respect to stimulation by the bacterial proteins. Contrary to what might be expected, the particles lacking more proteins are better reconstituted than those lacking only the acidic L44 and L45. In fact, our data clearly show that the basic yeast proteins released by the treatment, namely L15 and less probably S31, interfere somehow with the interaction of the bacterial proteins L7 and L12 when present in the particles. This interference affects the functionality of the interaction, strongly suggesting that protein L15 affects either directly or indirectly the binding site of the acidic proteins which is only partially recognized by L7 and L12 in the $P_{0.4}$ cores.

Nevertheless, since the structure of the acidic proteins binding site is not well-known even in the bacterial ribosome, little can be concluded on the actual role of protein L15. However, it is of interest to note that in *E. coli* ribosomes protein L10 seems to form a complex with proteins L7 and L12, suggesting an important role for this protein on the ribosomal binding site of the acidic proteins. The possible relationship between bacterial L10 and yeast L15 could then be a suggestive hypothesis, strengthened by the similar electrophoretic mobility and conditions for extraction of the two proteins. Previously we had also suggested the possibility of a structural relationship between yeast protein L15 and bacterial L11 (Sánchez-Madrid et al., 1979a).

It is also interesting to note that the presence of protein L15 in the $P_{0.4}$ cores allows the binding of approximately one dimer of proteins L7 and L12 (Table IV). This strongly suggests that the formation of the ribosome–EF-2–GDP complex, the only EF-2 dependent activity detected in these conditions, requires half the normal dotation of acidic proteins present in the untreated bacterial ribosome. On the other hand, about four copies of the acidic bacterial proteins seem to be needed to achieve the cyclic hydrolysis of the bound GTP since $P_{1.0}$ cores which support the uncoupled GTPase bind twice as much L7 and L12 as $P_{0.4}$ cores.

These results indicate that in our heterologous system it is possible to distinguish structurally between the binding of the substrate and its cyclic hydrolysis. The two dimers of L7 and L12 normally present in the bacterial ribosomes might, therefore, play a different role in this respect, perhaps one being directly involved in the binding of the substrates and the other in the GTP hydrolysis and recycling of the elongation factor. We have previously shown that the binding characteristics of the two dimers to the bacterial ribosome are different (Ballesta et al., 1976).

It is worth mentioning that our data bear some resemblance to the results reported by Schrier & Möller (1975) by using protein-deficient core particles from *E. coli* ribosomes. The particles reconstituted in the absence of L11 were able to form the EFG–GDP complex but unable to hydrolyze GTP. These reconstituted particles bind less L7 and L12 than the controls.

The stabilization of L7 and L12 binding to yeast cores promoted by the presence of the elongation factor EF-2 is also interesting and suggests a physical relationship between the factor and the acidic proteins in yeast, as has been shown by chemical cross-linking in bacterial ribosomes (Acharya et al., 1973).

As a final remark, we should like to point out that our results indicate that the heterologous reconstitutions are not a mere "oddity" of the biological systems but can be used to obtain interesting data about eukaryotic ribosomal structure.

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