FURTHER ANALYSIS OF THE PROTEIN COMPOSITION OF YEAST RIBOSOMES

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1. Introduction

From several studies it has become clear that the ribosomal proteins of higher eukaryotes exceed those present in prokaryotic organisms both in number and in average molecular weight [1–17]. It was, therefore, of interest to examine a unicellular organism like yeast to see whether the same holds true for the proteins present in the ribosomes of primitive eukaryotes.

Previously we have reported the occurrence of 69 different proteins in the ribosomes of the yeast *Saccharomyces carlsbergensis* [18]. In this paper we extend our analysis of the protein composition of the yeast ribosome: two additional strongly acidic proteins (L44 and L45), which escape detection when the standard two-dimensional gel electrophoresis procedure is used, are described.

While the number of ribosomal proteins in yeast, therefore, is closely similar to that in higher eukaryotes [2,3,5,6,8,9,13,14], we found their average molecular weight to be intermediate between the values published for prokaryotic ribosomal proteins [15–17] on the one hand and ribosomal proteins from higher eukaryotes [2–4,7,9,11–14] on the other.

2. Experimental

2.1. *Preparation of ribosomal proteins*

Isolation of ribosomal subunits from *Saccharomyces carlsbergensis* and extraction of ribosomal proteins was performed as described before [18]. Dissociation of the ribosomes was carried out under conditions of high ionic strength.

* *Abbreviation:* SDS = sodium dodecylsulfate.

2.2. *Determination of protein and RNA content of the ribosomal subunits*

The protein content of the ribosomal subunits was estimated by the Lowry procedure [19] using lysozyme (Sigma) as a standard.

The RNA content of the subunits was estimated from the absorbancy of the solution at 260 nm (1 mg/ml of RNA corresponds to an E_{260} of 25). The absorption at 260 nm was corrected for the contribution of protein present in the solution.

2.3. *Determination of weight-average and number-average molecular weight of ribosomal proteins*

Ribosomal proteins isolated from the subunits were treated as described by Shapiro et al. [20]. A maximum of 100 μg of ribosomal protein was layered on top of a 10% polyacrylamide disc gel containing 0.1% SDS* and electrophoresis was carried out as described by Dunker and Rueckert [21]. Protein was visualised by staining with 0.1% Coomassie brilliant blue R250. The weight-average and number-average molecular weight of the ribosomal proteins were calculated from the densitometric tracings of the stained gels at 544 nm. Using several pure marker proteins, a perfectly linear relationship was found to exist between the logarithm of the molecular weight of a protein and its relative mobility under these electrophoresis conditions (data not shown).

2.4. *Two-dimensional polyacrylamide gel electrophoresis*

The first dimension of two-dimensional polyacrylamide gel electrophoresis in the presence of SDS and urea was performed as described previously ([18]; 24 h at 120 V). The first dimension gels were dialysed and electrophoresis in the second dimension was car-

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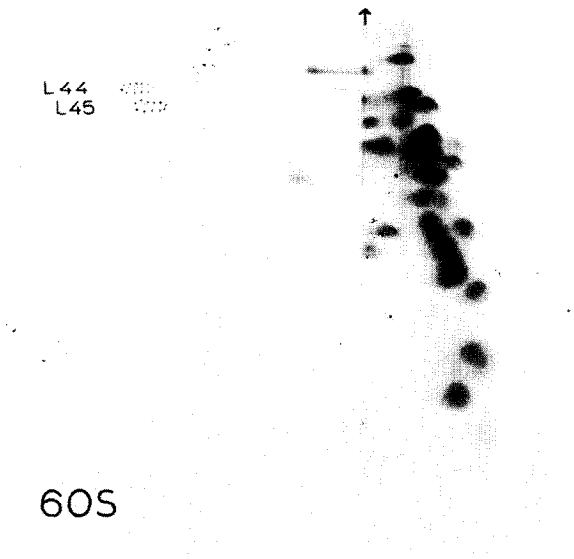

Fig.1. Two-dimensional separation of the proteins isolated from the 60 S ribosomal subunits. Electrophoresis in the first dimension was performed at 45 V during 24 h. The origin is indicated by an arrow. Electrophoresis in the second dimension was from top to bottom. The proteins L44 and L45 are indicated.

ried out according to Martini and Gould [2].

When only urea was used in the second dimension electrophoresis in the first as well as the second dimension was performed as described previously [18] with the modification that the first dimension electrophoresis was carried out at 45 V.

3. Results and discussion

The large subunit of yeast ribosomes, in addition to the 69 polypeptides described previously [18], contains two strongly acidic proteins that can only be detected by lowering the voltage during the first dimension run of a standard two-dimensional electrophoresis (fig.1).

These acidic proteins are similar to those found in rat liver 60 S subunits [6], which appear to be related immunologically to the *E. coli* proteins L7/L12 [22, 23]. The two eukaryotic acidic proteins might, thus, be homologous to L7/L12 which play an important role in prokaryotic protein biosynthesis. The detection of two additional proteins in yeast 60 S subunits brings the total number of polypeptide chains present on the yeast ribosome to 71, which is in close agreement with the number reported for other eukaryotes [1–3,5,6,8,9,13,14].

In order to determine the average molecular weight of yeast ribosomal protein two-dimensional polyacrylamide gel electrophoresis in the presence of SDS and urea was carried out. The ribosomal proteins of the large and small subunits were analysed separately and pure marker proteins were co-electrophoresed to provide a reference.

From fig.2 it is immediately clear that most of the yeast ribosomal proteins have mol. wts between 8000 and 32 000. The large subunit contains two proteins having a relatively high molecular weight (fig.2B). Similar high mol. wt proteins have been found in *Saccharomyces cerevisiae* 1 and in higher eukaryotic cells [2,8,22]. The average mol. wt of yeast ribosomal proteins as determined from experiments similar to the one shown in fig.2 is 19 200 for the 60 S subunit proteins and 18 500 for those from the 40S subunit. In higher eukaryotes the values reported for the average mol. wt of ribosomal proteins generally are around 25 000 [2–4,7,9,11–14]. The values for *E. coli* are 19 000 for the ribosomal proteins of the small subunit, including protein S1, which has a mol. wt of 65 000, and 16 300 for those from the large subunit [17]. Therefore, it can be concluded that, while the number of yeast ribosomal proteins is very similar to that found in other eukaryotes, their average mol. wt is intermediate between that of higher eukaryotes and that of prokaryotes.

In order to gain more insight into a possible structural heterogeneity of yeast ribosomal subunits, we determined the weight-average and number-average mol. wts of the ribosomal proteins by means of one-dimensional polyacrylamide gel electrophoresis in the presence of SDS. The values obtained can be combined with data on the total protein content of each subunit (see table 1) to calculate the number of protein molecules per subunit. In this way one arrives at a figure of 27–32 proteins for the 40 S subunit while on the 60 S subunit between 36 and 43 polypeptide chains must be located (table 1). These values are in excellent agreement with the data obtained previously from two-dimensional analysis of the protein composition of the yeast ribosomal subunits ([18]; table 1).

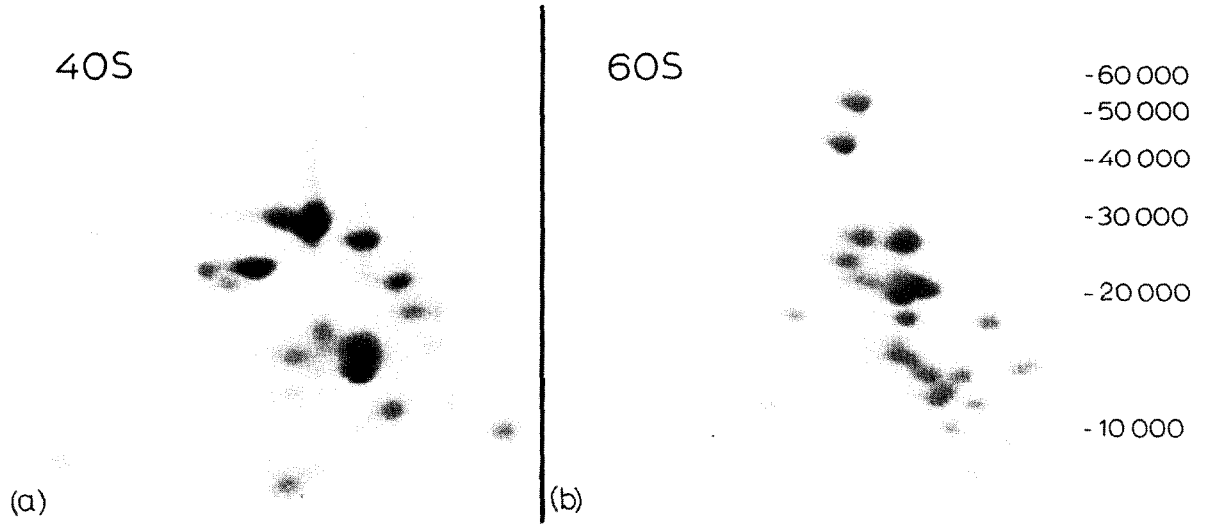

Fig.2. Two-dimensional polyacrylamide gel electrophoresis of yeast ribosomal proteins in the presence of SDS and urea. The scale to right of the figure indicating the mol. wt of the various polypeptides, was determined with the aid of pure marker proteins.

Table 1
Properties of the 40 S and 60 S ribosomal subunits of *Saccharomyces carlsbergensis*

Property	40 S	60 S
1. Mol. wt of rRNA-constituent	0.69×10^6 D.[a]	1.30×10^6 D.[b]
2. Protein content[c]	44.6%	38.1%
3. Total molecular mass of ribosomal proteins (calculated from 1 and 2)	0.55×10^6 D.	0.80×10^6 D.
4. Average mol. wt[c]	18 500 D.	19 200 D.
5. Number-average mol. wt[d]	17 500 D.	18 800 D.
6. Weight-average mol. wt[d]	20 500 D.	22 400 D.
7. Number of ribosomal proteins per subunit (calculated from 3, 5 and 6)	27–32	36–43
8. Number of ribosomal protein[e] species determined experimentally	30	41

[a] [24].
[b] [24,25].
[c] Determined as described in the text. The values are the mean of four independent determinations.
[d] Determined as described in Experimental. The values are the mean of six independent determinations.
[e] [18] and this paper.

Although these results do not point to a structrual heterogeneity of the yeast ribosomal subunits it can not be excluded that some proteins are present in multiple copies (repeated proteins) and are compensated for by protein species which occur in less than one copy per subunit (fractional proteins). In fact, we have observed reproducible differences in staining intensity between the spots on a two-dimensional electropherogram of yeast ribosomal proteins [18], which would tend to support the occurrence of a combination of repeated and fractional proteins on these ribosomes.

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