STRAIN-DEPENDENT VARIATION OF THE PROTEIN COMPOSITION OF *TETRAHYMENA* RIBOSOMES

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1. Introduction

We have described the preparation and preliminary characterization of active ribosomal subunits from the amicronucleate strain CGL of the protozoa *Tetrahymena pyriformis* [1]. Further development of these studies and their extension to micronucleate and to other amicronucleate strains of *Tetrahymena* has led to revised (higher) estimates of the number of proteins in the subunits of ribosomes of this microorganism and to the observation of large differences between the ribosomal protein complements of different strains. Here we compare the ribosomal proteins of the previously studied amicronucleate strain *T. pyriformis* CGL and of the micronucleate strain *T. thermophila* B IV.

The revised estimates of the number of proteins in the subunits of ribosomes of *T. pyriformis* CGL with, in parenthesis, the number reported [1] are:

40 S subunit; 5(2) acidic and 35(31) basic proteins

60 S subunit; 6(2/3) acidic and 40(35) basic proteins

Ribosomal subunits of *T. thermophila* B IV have the following protein contents:

40 S subunit; 4 acidic and 34 basic proteins

60 S subunit; 5 acidic and 38 basic proteins

Comparison of the electrophoretic properties of the basic ribosomal proteins of *T. pyriformis* CGL and *T. thermophila* B IV in Wittmann 2D system shows that only 40 out of 75 in the former and 72 in the latter strain are identical as judged by this criterion. A quantitatively similar result has been obtained (in preparation) by comparison of the electrophoretic properties of the ribosomal subunit proteins of these two *Tetrahymena* strains (acidic/basic proteins) in the presence of SDS using the two dimensional system [2]. However since the positions of ribosomal proteins in these two separation systems have not yet been correlated it cannot be concluded that proteins from different strains which comigrate in one system also comigrate in the other. Comparison of the electrophoretic mobilities of the acidic ribosomal proteins of *T. pyriformis* CGL and *T. thermophila* B IV in the presence of 6 M urea at pH 8.6 (1st dimension, Wittman 2D system) shows that only 7 out of 11 (CGL) and 9 (B IV) behave identically in this respect. In spite of the large difference between protein complements of the ribosomal subunits of *T. pyriformis* CGL and *T. thermophila* B IV revealed by these experiments these particles do not appear to differ significantly in conformation since active 80 S ribosomes can be formed in vitro in high yield by reassociation of subunits from the two strains.

2. Materials and methods

2.1. Tetrahymena *strains and cell culture conditions*

The amicronucleate strain *T. pyriformis* CGL*, referred to in [1,3–5] as *T. pyriformis* GL was obtained from Dr R. Charret (Laboratoire de Biologie Cellulaire, Université de Paris Sud) and the micronucleate strain *T. thermophila* B IV, previously known

* The phenoset as defined [11] to which this amicronucleate strain belongs has not been established and the appelation 'pyriformis' is therefore not legitimate. However we propose to continue its use until the phenoset affiliation of the strain is determined

as *T. pyriformis* B 1868 IV was provided by Dr Engberg (Department of Biochemistry B, Panum Institute, University of Copenhagen). Both strains were grown as described [1,3] for *T. pyriformis* CGL.

2.2. *Preparation of ribosomal subunits and ribosomal proteins*

Ribosomal subunits were isolated as in [1] and their protein complements were extracted as in [6].

2.3. *Electrophoretic analysis of ribosomal proteins*

The following one- and two-dimensional electrophoresis systems were used:

1. Basic proteins were separated in the two dimensional system described [7]. First dimension: cylindrical gels, 3.5 × 120 mm, 4% acrylamide, 0.13% bisacrylamide, 6 M urea (pH 8.6). Migration conditions: 16 h at 120 V or 16 h at 100 V followed by 2.5 h at 200 V and 2.5 h at 300 V ('short' and 'long' gels, respectively). Second dimension: slab gels 130 × 100 × 3 mm, 18% acrylamide, 0.5% bisacrylamide, 6 M urea (pH 4.5). Migration conditions: 16 h at 80 V, or 16 h at 160 V ('short' and 'long' gels, respectively, see fig.1).
2. Acidic proteins were compared by one dimensional electrophoresis in 130 × 100 × 3 mm gel slabs, 4% acrylamide, 0.13% bisacrylamide, 6 M urea (pH 8.6). Migration conditions: 16 h at 100 V.

2.4. *Reassociation of ribosomal subunits in vitro*

Equimolar amounts of separated 40 S (1 A_{260}) and 60 S (2 A_{260}) subunits were combined in 0.1 ml 20 mM Tris–HCl (pH 7.6); 120 mM KCl, 10 mM Mg acetate, 6 mM β-mercaptoethanol and the mixtures were layered on 4 ml 5–20% (w/v) linear sucrose gradients prepared in the same buffer. Gradients were centrifuged for 18.5 h at 14 000 rev./min at 4°C in three place adapters [8] in an SW 25 rotor and distributions of A_{260} absorbing material were then recorded.

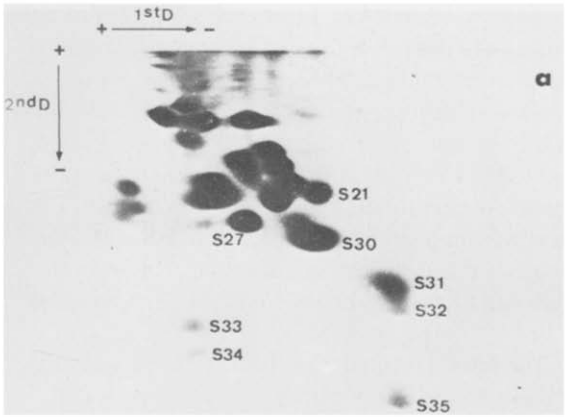
2.5. *Polyphenylalanine synthesis in vitro*

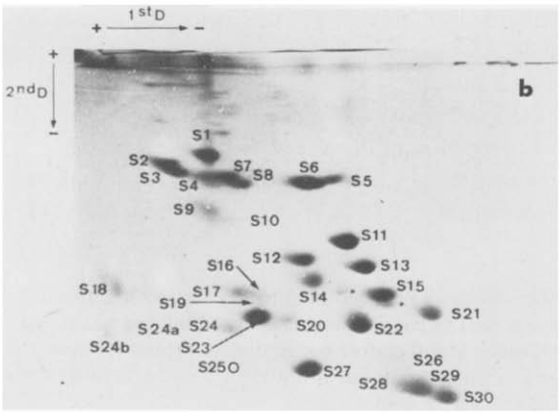
Separated subunits were recombined in equimolar amounts in 20 mM Tris–HCl (pH 7.6), 200 mM KCl; 10 mM Mg acetate; 6 mM β-mercaptoethanol. Polyphenylalanine synthesis was carried out as in [1] except that reaction mixtures were incubated at 28°C instead of 37°C. Undissociated 80 S ribosomes used in control reactions were isolated as in [1].

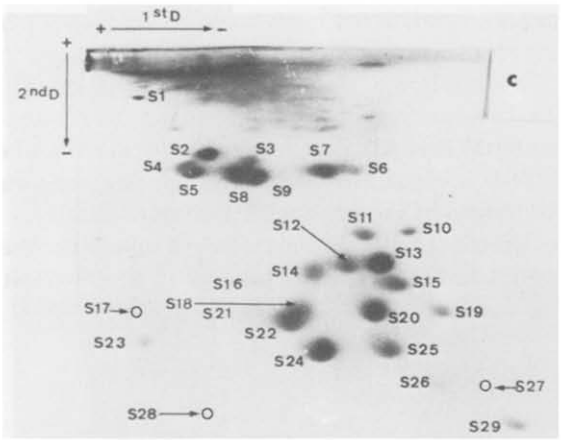
3. Results and discussion

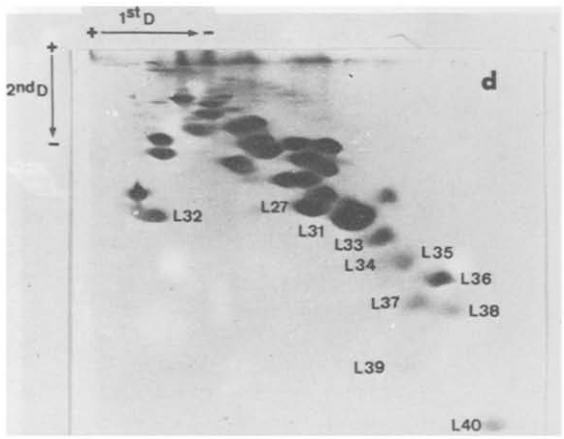
3.1. *Ribosomal proteins of* T. pyriformis *CGL*

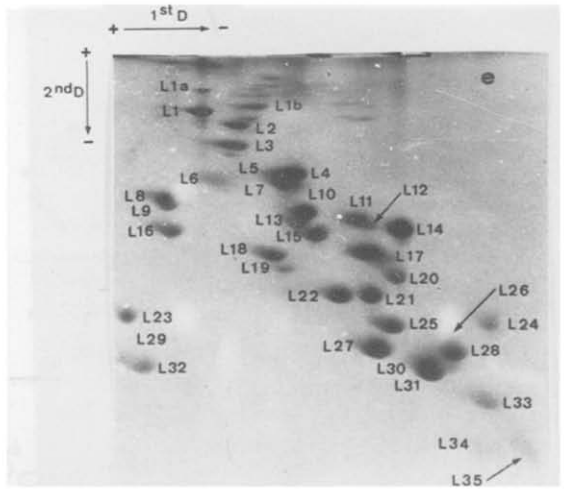
By use of longer and shorter migration times and in the case of acidic proteins larger samples, additional basic and acidic proteins have been detected in both subunits of the ribosomes of *T. pyriformis* CGL. The results of these experiments are summarized in fig.1a,b,d,e and 3. Since acidic and basic proteins were analysed independently, a unified revised nomenclature has not yet been established. In fig.1–3 basic and acidic proteins of the small and large subunits of ribosomes of *T. pyriformis* CGL and *T. thermophila*











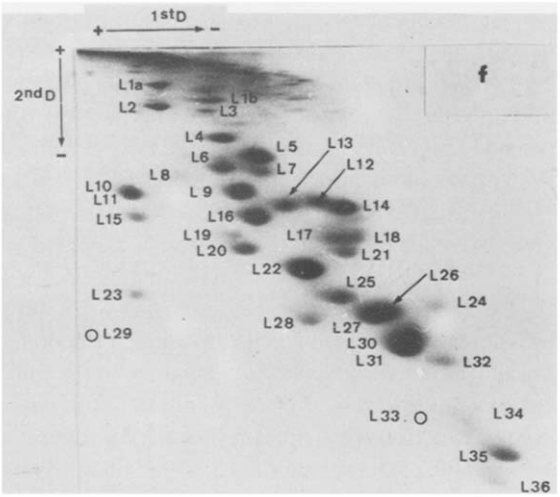


Fig.1. Two dimensional electrophoresis of basic ribosomal proteins of *Tetrahymena*. (a, b) *T. pyriformis* CGL 40 S subunit proteins: (a) 300 μg, short migration; (b) 200 μg, long migration. (c) *T. thermophila* B IV 40 S subunit proteins, 200 μg, long migration. (d, e) *T. pyriformis* CGL 60 S subunit proteins: (d) 400 μg, short migration; (e) 400 μg, long migration. (f) *T. thermophila* B IV 60 S subunit proteins, 400 μg long migration. Protein S1 of *T. thermophila* B IV (c) is observed in all preparations of 40 S proteins of this strain and does not disappear if proteins are treated with high concentrations of β-mercaptoethanol before analysis. Protein L1 of the same strain is not visible in (f) where it is hidden in the smear of material with low second dimension mobility but is seen in many other analyses of the 60 S subunit proteins of this strain. After treatment of 60 S subunit proteins of *T. thermophila* B IV with a high concentration of β-mercaptoethanol spots L1a and L1B (f) disappear and spot L3 becomes more intense. Spots indicated in (b) were not observed in preparations of proteins of *Tetrahymena* strains other than *T. pyriformis* CGL and only in a single sample of 40 S proteins of that strain. They are considered to be degradation products derived probably from S15. Spots indicated (○) in (b, c, f) are clearly but faintly visible in stained gel slabs but are not evident in photographs of the gels.

B IV are numbered independently, e.g., for the 40 S subunits GS1–GS35 (basic), GSα–GSε (acidic) and BS1–BS38 (basic), BSα–BSδ (acidic).

Some of the additional proteins detected in ribosomal subunits of *T. pyriformis* CGL (G-S34, G-S35, fig.1a; G-L39, G-L40, fig.1d; G-Lδ, G-Lε, G-Lζ in fig.3) have high electrophoretic mobilities due to their low molecular weight (basic proteins) or high charge (acidic proteins) and were lost in earlier anal-

ysis. Others have been detected as a result of improvement of the resolution of electrophoretic separations (e.g., 40 S protein pairs G-S2 + G-S3, and G-S16 + G-S19 in fig.1b were not resolved in earlier experiments [1] and were numbered respectively S3 and S17; 60 S protein groups, G-L4 + G-L5 + G-L7, G-L8 + G-L9, G-L10 + G-L13 and G-L11 + G-L12 in fig.1d were also reported [1] as single species designated L5, L6, L7 and L8, respectively). Protein G-S25 (fig.1b) which was not observed in earlier work has been detected in small variable amounts in the 40 S subunit proteins of five *Tetrahymena* strains in addition to *T. pyriformis* CGL and is considered to correspond to a ribosomal protein. Insufficient data is as yet available to determine the nature of the proteins present in spots numbered G-S24a, and G-S24b in fig.1b. These spots which are present in significant amounts in two dimensional analyses of proteins of preparations of *T. pyriformis* CGL 40 S subunits were not observed in our earlier experiments.

The results of these analyses increase the number of proteins detected in ribosomes of *T. pyriformis* CGL from 66 to 86 and the number of acidic proteins from 4/5 to 11, totals which are close to those reported for a *T. pyriformis* GL strain of different origin [9], for rat liver ribosomes [10] and for ribosomes of *Saccharomyces carlsbergensis* [11].

3.2. *Comparison of the ribosomal proteins of* T. pyriformis *CGL and* T. thermophila *B IV*

Comparison of the electrophoretic behaviour of the basic proteins of ribosomal subunits of *T. pyriformis* CGL (fig.1b,c) and *T. thermophila* IV (fig.1d,e) reveals marked differences. This surprising result was confirmed by electrophoretic analysis of mixtures of proteins prepared from ribosomal subunits of the two strains. As shown in fig.2 only 19 small subunit

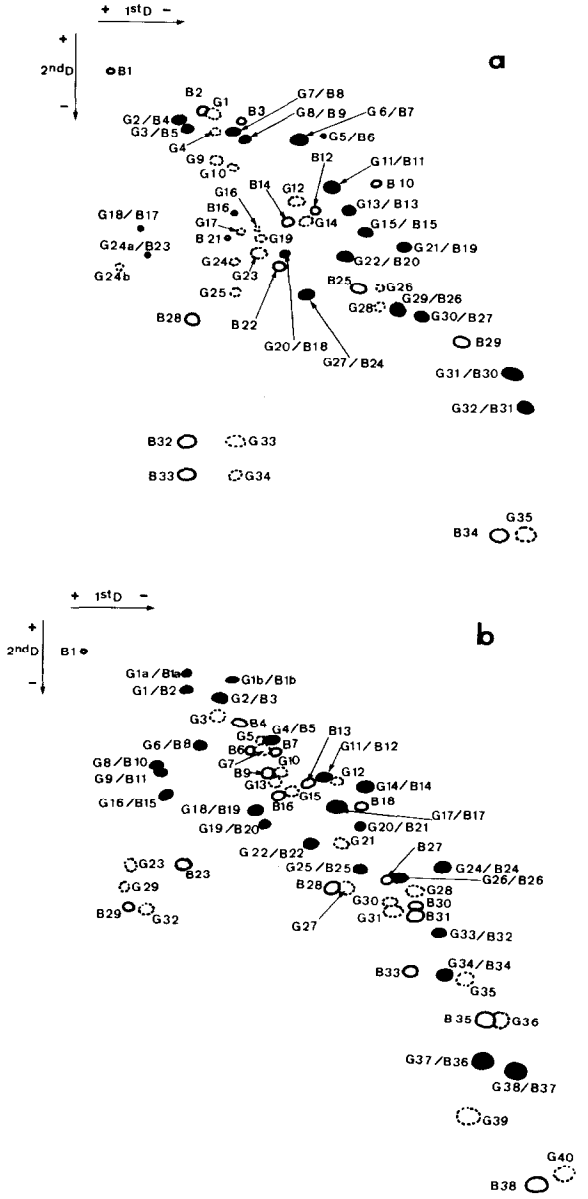


Fig.2. Results of coelectrophoresis in two dimensions of ribosomal subunit proteins of *T. pyriformis* CGL and *T. thermophila* B IV. Mixtures containing equal amounts of the proteins of the 40 S (150 μg + 150 μg) and 60 S (200 μg + 200 μg) subunit proteins of the two strains were analysed by two dimensional electrophoresis using short and long migrations. To identify the components present in the spots observed in the stained gel slabs the patterns observed were compared with those obtained when subunit proteins from the two strains were analysed separately (e.g. fig.1). In fig.2 in which the results of these experiments are combined in schematic form, the prefixes G and B added to protein designations indicate proteins of *T. pyriformis* CGL, and *T. thermophila* B IV, respectively. Spots corresponding to *T. pyriformis* CGL and *T. thermophila* B IV proteins which migrate independently of each other are indicated (⊘) and (○) and spots corresponding to proteins of the two strains which display identical electrophoretic mobilities are shown (●). (a) Small subunit proteins. Nineteen proteins comigrate. (b) Large subunit proteins. Twenty one proteins comigrate (spots corresponding to comigrating β-mercaptoethanol sensitive complexes G-1a/B 1a, and G-1b/B 1b are not included in this total).

and 21 large subunit proteins from these strains display identical electrophoretic properties and comigrate in the two dimensional system. The differences in the electrophoretic properties of the remaining proteins appear to be small in several cases (e.g., G-S14/B-S14, G-S25/B-S25, G-S35/B-S34, G-S33/B-S32, G-S34/B-S33 and G-L27/B-L28, G-L32/B-L29) but until further information is obtained (e.g., trypsin fingerprints) the degree of relatedness of closely migrating proteins remains problematic. Comparison of the acidic ribosomal proteins of *T. pyriformis* CGL and *T. thermophila* B IV by one dimensional electrophoresis gave an analogous result (fig.3). Of the 11 acidic ribosomal proteins of the former strain and the 9 acidic proteins of the latter only 7 display the same electrophoretic behaviour. In experiments whose results are not shown the basic and acidic ribosomal proteins of four additional *Tetrahymena* strains have been analysed as described here and their electrophoretic distributions compared to those shown in fig.1 and 2. All six strains were found to possess distinctive and different 40 S and 60 S ribosomal protein complements. The differences between the ribosomal proteins of *T. pyriformis* CGL and *T. thermophila* B IV described here therefore appear to be a general characteristic of *Tetrahymena* strains.

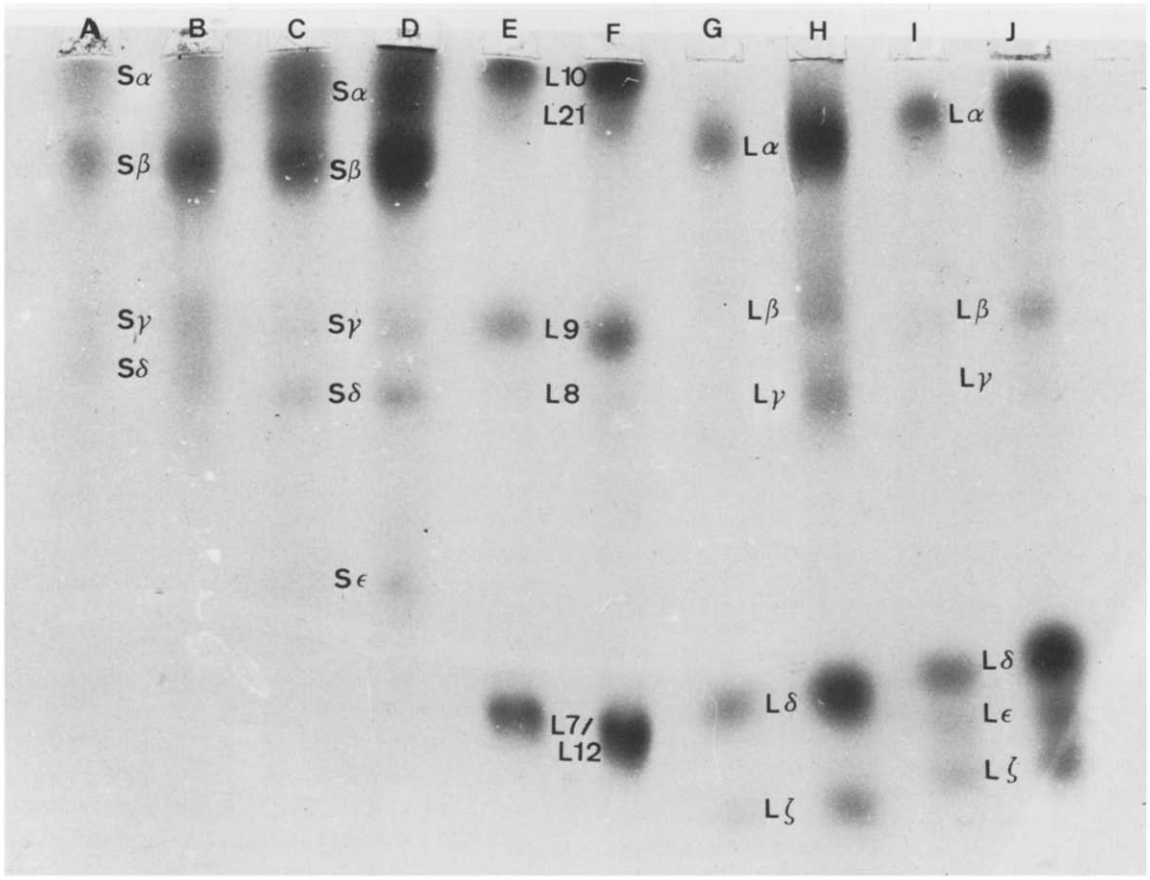


Fig.3. One dimensional electrophoresis of acidic ribosomal proteins of *T. pyriformis* CGL and *T. thermophila* B IV. Sample slots in the gel slab were loaded with the following amounts of total subunit proteins: (a) 500 μg, (b) 700 μg, *T. thermophila* B IV, 40 S; (c) 500 μg, (d) 700 μg, *T. pyriformis* CGL, 40 S; (e) 400 μg, (f) 600 μg, *E. coli* D10 (K12 strain), 50 S; (g) 300 μg, (h) 600 μg, *T. thermophila* B IV, 60 S; (i) 300 μg, (j) 600 μg, *T. pyriformis* CGL, 60 S. Three small subunit proteins (Sβ, Sγ, Sδ) and four large subunit proteins (Lα, Lγ, Lδ, Lζ) from each strain possess similar or identical electrophoretic mobilities. The large subunit protein Lδ of both strains has the same electrophoretic mobility as *E. coli* 50 S protein L7/L12.

3.3. *Reassociation of ribosomal subunits of* Tetrahymena *strains in vitro and polyphenylalanine synthesizing activity of reassociated ribosomes*

Figure 4 shows the results of sucrose gradient sedimentation of products of in vitro reassociation of homologous and heterologous mixtures of 40 S and 60 S ribosomal subunits of *T. pyriformis* CGL and *T. thermophila* B IV. Efficient formation of 80 S ribosomes was observed in all cases showing that the different protein compositions of the ribosomal subunits of the two strains have no influence on their reassociation specificity. A more rigorous test of the nature of the interaction between heterologous 40 S and 60 S subunits was made by measuring the polyphenylalanine synthesizing activity of 80 S ribosomes formed in vitro. Table 1 shows that all four combinations of 40 S and 60 S subunits gave products with similar activities which were 45–55% of that of control (undissociated) 80 S ribosomes.

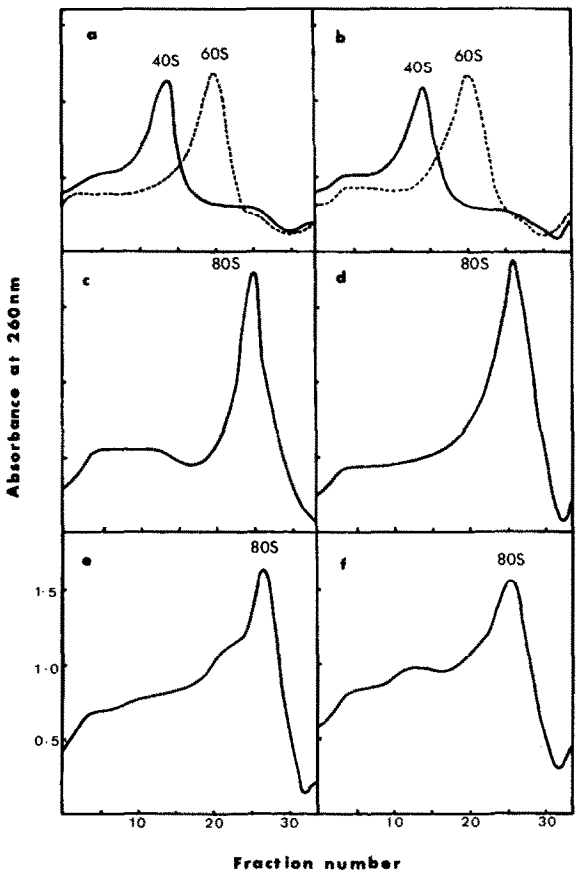


Fig.4. Homologous and heterologous reassociation of separated subunits of *Tetrahymena* ribosomes. Sedimentation analyses were carried out in reassociation buffer (20 mM Tris–HCl (pH 7.6); 120 mM KCl; 10 mM Mg acetate, 6 mM β-mercaptoethanol) as in section 2. (a, b) The results obtained by sedimentation of separated ribosomal subunits on different sucrose gradients have been combined; (a) *T. thermophila* B IV, (——) 40 S subunit; (b) *T. pyriformis* CGL, (– – –) 60 S subunit; (c, d) Results obtained by sedimentation of homologous mixtures of subunits. (c) *T. thermophila* B IV, 40 S + 60 S, (d) *T. pyriformis* CGL, 40 S + 60 S; (e, f) Results obtained by sedimentation of heterologous mixtures of subunits. (e) 40 S - B IV + 60 S - CGL, (f) 40 S - CGL + 60 S - B IV.

4. Conclusions

Until recently classification of *Tetrahymena* strains was based on morphological and mating characteristics. The use of biochemical criteria for classification became possible following studies in [12–14] which demonstrated the existence of significant strain-related differences in the electrophoretic mobility patterns of a series of isoenzyme families and the demonstration [15] of strain-dependent differences in the primary sequence of *Tetrahymena* DNA. The differences in the electrophoretic distribution patterns of ribosomal proteins of *T. pyriformis* CGL and *T. thermophila* B IV shown in fig.1,2 have been reproduced in numerous analyses of protein samples prepared from different batches of cells. In addition analyses of ribosomal proteins of four other *Tetrahymena* strains (results to be presented elsewhere) has shown that all six can be clearly distinguished by comparison of the two-dimensional electrophoretic distributions of the basic proteins of either ribosomal subunit. These results therefore reveal a degree of strain specificity of ribosomal proteins which is fully consistent with the results of isoenzyme mobility pattern analysis and provides a potentially useful additional method for strain identification and classification.

Our estimates [1] of the number of proteins in ribosomal subunits of *T. pyriformis* CGL differed from those in [9] for a *T. pyriformis* GL strain of different origin and were closer to the values reported for rat liver [10] than for yeast ribosomes [11]. The protein compositions of ribosomal subunits of *T. pyriformis* CGL and *T. thermophila* B IV reported here as well as

Table 1
Polyphenylalanine synthesis in vitro

Ribosomes / ribosomal subunits		[^{14}C]Phenylalanine incorporation	
Species	A_{260} units	cpm/A_{260} units of ribosomes	% control
80 S CGL (control)	1	1370	–
40 S CGL	0.5		
+		760	55
60 S CGL	1		
40 S B IV	0.5		
+		690	50
60 S B IV	1		
40 S B IV	0.5		
+		680	50
60 S CGL	1		
40 S CGL	0.5		
+		630	46
60 S B IV	1		

Polyphenylalanine synthesis was carried out as in section 2 using homologous and heterologous mixtures of the separated ribosomal subunits of *T. pyriformis* CGL and *T. thermophila* B IV, and, as control, undissociated 80 S ribosomes

those of ribosomal subunits of four other *Tetrahymena* strains (in preparation) are however closer to the compositions found in *T. pyriformis* GL [9], in ribosomes of *Saccharomyces carlsbergensis* [11] and in ribosomes of plants (pea, maize) [16]. Hence the protein composition of their ribosomes suggests that *Tetrahymena* species are more closely related to lower than to higher eucaryotic cells.

The extensive variation in the protein composition of *Tetrahymena* ribosomes revealed by the present study poses problems of several kinds:

(a) Its genetic origin;
(b) The structural relationships between the variable and non-variable proteins of different strains.

Do the electrophoretically identical proteins of two strains possess identical primary structures? Are the primary structures of variable proteins of different strains related or unrelated?

(c) The respective roles of variable and non-variable proteins in the structure and function of *Tetrahymena* ribosomes.

Work on these problems is being undertaken.

Acknowledgements

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