ORIGIN OF THE RIBOSOME SPECIFIC FACTORS RESPONSIBLE FOR PEPTIDE CHAIN ELONGATION IN YEAST

Bruno PARISI and Rino CELLA

Laboratorio di Genetica Biochimica ed Evoluzionistica del C.N.R., Pavia and Istituto di Genetica, University of Pavia, 27100 Pavia, Italy

Received 10 March 1971

1. Introduction

The factors responsible for polypeptide chain elongation are specific for ribosomes of either the 70 S or the 80 S type [1–4]. Two different sets of elongation factors, one specific for 70 S ribosomes and one for 80 S ribosomes, are present in eukaryotic organisms: in *Neurospora crassa* [5], in yeast [6, 7] and in *Prototheca zopfii* [8]. Furthermore, evidence has been accumulated that mitochondria perform autonomous protein synthesis [9] and that the mitochondrial protein synthesizing system has different structural and functional properties from the cytoplasmic one [10–12], including the size of ribosomes which at least in *Neurospora* [11] and yeast [13] appear to be of the 70 S type. The elongation factors extracted from isolated *N. crassa* [5] and *Saccharomyces cerevisiae* [7] mitochondria are active only on 70 S ribosomes. In addition, bacterial initiation and elongation factors, specific for 70 S ribosomes, catalyze some of the reactions for protein synthesis on mitochondrial (70 S) but not on cytoplasmic (80 S) ribosomes [5, 14].

These results indicate that eukaryotic cells contain two specific and independent protein synthesizing systems, one of which is responsible for protein synthesis in mitochondria.

Since the mitochondrial apparatus of yeast is genetically controlled by nuclear and mitochondrial DNA [9, 15], we have attempted to answer the following questions: a) Is the genetic information for mitochondrial elongation factors located on the nuclear or on the mitochondrial DNA. b) Is the mRNA that carries the information for such factors translated on mitochondrial (70 S) or cytoplasmic (80 S) ribosomes.

The data reported in the present paper suggest that in yeast the genetic information for polypeptide chain elongation factors is encoded in the nuclear DNA and that such information is translated on the cytoplasmic ribosomes.

2. Materials and methods

The following diploid strains of *S. cerevisiae*, kindly supplied by Dr. G. Morpurgo, were used: parental strain DM (ρ^+) and derived cytoplasmic 'petites' strains 32 and DM_1 (ρ^-) [16]. The density of mitochondrial DNA of these strains was 1.686, 1.678, 1.671, respectively [16]. The C + G content of the mitochondrial DNA of strain DM_1 was 3.8% [17].

Cells were grown at 28° for at least seven generations in one liter flasks on a rotary bath in 300 ml of the medium described by Clark-Walker and Linnane [18], containing 1% glucose, and harvested during the exponential phase of growth. When present, chloramphenicol (CAP) was added at the beginning of the fermentation at a concentration of 4 mg/ml. Cells were collected by centrifugation, washed twice with distilled water and used immediately or stored frozen at - 70°.

Yeast ribosomes and polymerizing enzymes were prepared as already described [19] except that cells were disrupted by ultrasonic treatment (3 min at

0°, 1.5 A, in an M.S.E. sonicator). Chromatography on Sephadex G-200 was employed to separate elongation factors specific for 70 S and 80 S ribosomes from the preparations of polymerizing enzymes of strains DM and DM_1 [6]. 30 mg of protein were applied to a 2.8 × 38 cm column. 250 ml of the buffer described by Perani et al. [6] were passed through the column and protein synthesizing activity on *Escherichia coli* and yeast ribosomes was assayed on each 5 ml fraction. Two peaks of activity, one specific for *E. coli* ribosomes only and the other for yeast ribosomes were evident. The fractions corresponding to each peak were pooled and precipitated with ammonium sulfate (560 g/l) and the precipitate resuspended and used as source of elongation factors. *E. coli* ribosomes and polymerizing enzymes were prepared according to the procedure of Lucas-Lenard and Lipmann [20]. Poly U directed polyphenylalanine synthesis from *E. coli* ^{14}C-phenylalanyl-tRNA was measured as described elsewhere [3], except that the final volume of the reaction mixture was 0.1 ml. ^{14}C-Phenylalanyl-tRNA was prepared according to Kaji, Kaki and Novelli [21]. Protein and ribosome concentration was determined as already described [19]. Cytochrome oxidase activity was measured in the 2400 *g* supernatant of the cell-free extract according to the method of Wharton and Tzagoloff [22].

The effect of CAP on the incorporation of ^{14}C-amino acids into mitochondrial proteins was performed as follows: strain DM was grown at 28° for three generations up to 10^7 cells/ml in two flasks, one containing minimal medium [23], the other minimal medium plus CAP (4 mg/ml). The cells from the two cultures were collected by centrifugation and resuspended at the same concentration in fresh medium with or without CAP. 10 ml aliquots were transferred to 100 ml flasks and supplemented with cycloheximide (CHE) (0.1 mg/ml). A control experiment without CHE was performed on cells grown in the absence of CAP. After incubation for three minutes, 2 μCi of a mixture of ^{14}C-amino acids (Amersham, specific activity 52 mCi/maton carbon) were added and the flasks incubated for another 25 min. At this time, 0.5 ml of a mixture of twenty ^{12}C-amino acids (5×10^{-4} M each) were added to the three experiments. The cells were immediately chilled, collected by centrifugation and washed twice with a buffer containing sucrose [24]. The cells, resuspended in 10 ml of the same buffer were broken in an Aminco French Press at a pressure of 7000 psi. The mitochondrial pellet was prepared according to the method of Gregory, Palakis and Wartley [24] and the radioactivity insoluble in hot trichloroacetic acid determined by conventional techniques [25] (counting efficiency 50%).

3. Results and discussion

The presence in yeast polymerizing enzymes of elongation factors specific for 80 S and 70 S ribosomes was ascertained by assaying poly U directed polyphenylalanine synthesis on *E. coli* (70 S) or *S. cerevisiae* (80 S) ribosomes.

Yeast polymerizing enzymes, whether prepared from the parental strain DM or the two 'petite' strains 32 and DM_1, catalyze peptide chain elongation on both types of ribosome (table 1)*. Fig. 1A shows that the activity on *E. coli* ribosomes of increasing concentration of these enzymes is of the same order for the three strains. Therefore it must be concluded that polymerizing enzymes from these strains of yeast are endowed with activity on both types of ribosome. The demonstration that such activity is due to the presence of two sets of ribosome specific elongation factors was obtained by the fractionation on a Sephadex G-200 column of yeast polymerizing enzymes as described in materials and methods. Table 2 shows that from the wild type strain DM and the 'petite' strain DM_1 two enzyme fractions may be separated, one (A) active on 70 S ribosomes only and the other (B) on 80 S ribosomes only.

Assuming that a) the factors specific for 70 S ribosomes are those responsible for protein synthesis by mitochondria and b) the mitochondrial DNA of the 'petite' strain DM_1 does not carry the genetic information for active protein [15, 17], the most obvious explanation for the presence in these cells of factors specific for 70 S ribosomes is that the nuclear DNA carries the genetic information for such enzymes.

A further question that may be asked is whether these factors are translated by the cytoplasmic or

* The activity on 70 S ribosomes in these strains is at variance with the results obtained with another strain of yeast [19]. It now appears that in the latter strain the activity of the transfer factor T specific for 70 S ribosomes was possibly lost during preparation of the extracts.

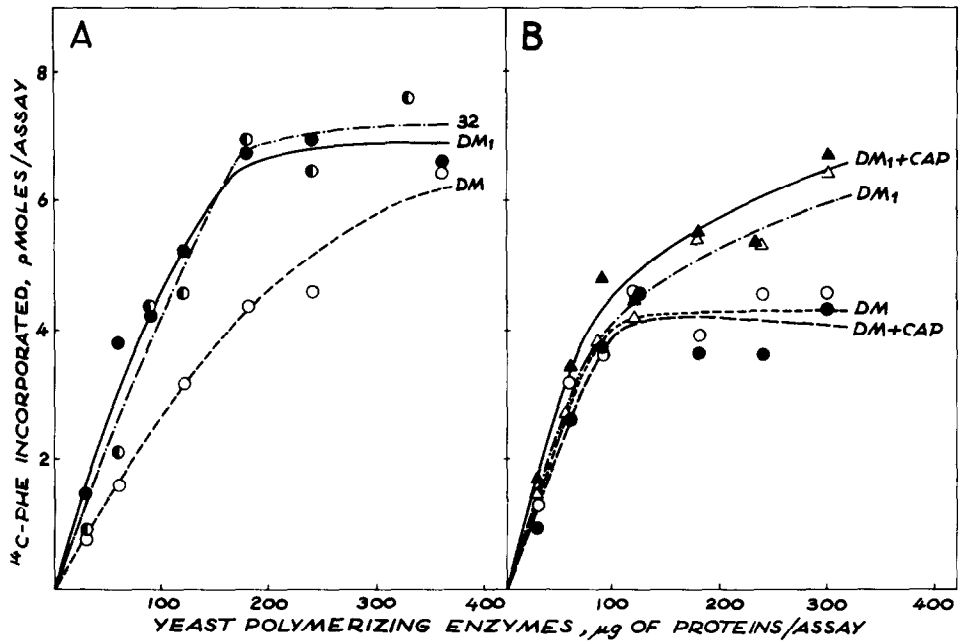

Fig. 1. Synthesis of polyphenylalanine by *E. coli* ribosomes in the presence of increasing concentrations of polymerizing enzymes prepared from yeast. Assay conditions as reported under materials and methods and table 1. The concentration of yeast polymerizing enzymes was varied as shown. A) Polymerizing enzymes prepared from the following strains: DM (wild type), ○——○; DM_1 (petite), ●——●; 32 (petite), ◐——◐. B) Polymerizing enzymes prepared from the following strains: DM, ○——○; DM_1, △——△; DM grown in the presence of CAP, ●——●; DM_1 grown in the presence of CAP, ▲——▲. In control experiments, in the presence of 100 μg of yeast ribosomes, the following amounts (in pmoles) of ^{14}C-phenylalanine were incorporated: 11.20 (DM), 8.97 (DM_1), 8.00 (DM + CAP) and 8.12 (DM_1 + CAP).

Table 1
Polyphenylalanine synthesis by polymerizing enzymes prepared from strains DM, DM_1 and 32 on 70 S and 80 S ribosomes.

Ribosomes	Polymerizing enzymes from strain	^{14}C-Phenylalanine incorporated (pmoles/assay)
E. coli (70 S)	DM	4.57
	DM_1	6.72
	32	6.47
S. cerevisiae (80 S)	DM	13.6
	DM_1	11.09
	32	12.20

Assay conditions as reported under materials and methods. Each assay contained 100 μg of *E. coli* or *S. cerevisiae* ribosomes, 240 μg of polymerizing enzymes and 40 pmoles of ^{14}C-phenylalanyl-tRNA.

Table 2
Ribosome specificity of separated yeast elongation factors.

Ribosomes	Polymerizing enzymes from strain	^{14}C-Phenylalanine incorporated (pmoles/assay)	
		Fraction A	Fraction B
E. coli	DM (wild type)	1.5	< 0.05
	DM_1 (petite)	2.8	< 0.05
S. cerevisiae	DM (wild type)	< 0.05	5.9
	DM_1 (petite)	< 0.05	4.1

Preparation of the fractions and experimental conditions are reported under materials and methods. Fraction A corresponds to the ammonium sulfate precipitate of column fractions from no. 29 to 36 and fraction B from no. 20 to 24. In each assay 30 μg of protein from fraction A or B were used.

Table 3
Cytochrome oxidase activity and polyphenylalanine synthesis on *E. coli* ribosomes by cell-free extracts prepared from yeast cells grown in the presence or the absence of CAP.

Strain	CAP	Activity (%)	
		Cytochrome oxidase	^{14}C-Phenylalanine incorporated
DM (wild type)	-	100	100
	+	0	96
DM_1 (petite)	-	0	146
	+	0	147

Cytochrome oxidase activity and polyphenylalanine synthesis were measured as reported under materials and methods. 100% of cytochrome oxidase activity corresponds to the oxidation of 11.3 nmoles of reduced cytochrome *c*/min/mg of protein. 100% of ^{14}C-phenylalanine incorporated (in the presence of 300 μg of polymerizing enzymes) corresponds to 7.23 pmoles of ^{14}C-phenylalanine incorporated per assay.

Table 4
In vivo incorporation of ^{14}C-amino acids into the mitochondrial fraction.

Exp. no.	Conditions	^{14}C-amino acids incorporated (cpm/mg of mit. prot.)	% of control
1	Control	171,965	100
2	+ CHE	8,519	4.96
3	+ CHE, CAP	184	0.11

Experimental conditions are reported under materials and methods. Wild type strain (DM) was grown in the presence (exp. no. 3) or in the absence (exps. 1 and 2) of CAP. CHE was added where indicated, followed by a pulse of ^{14}C-amino acids, and the radioactivity incorporated in the mitochondrial fraction determined.

the mitochondrial protein synthesizing system. Schatz and Saltzgraber [26] have found that defective mitochondria from yeast cytoplasmic 'petites' do not support protein synthesis. Such findings may indicate that the 70 S specific elongation factors present in cytoplasmic 'petites' (fig. 1A and tables 1 and 2) are translated on cytoplasmic ribosomes.

The results reported in fig. 1 B support such a contention. Polymerizing activity on *E. coli* ribosomes is present in experiments employing enzymes prepared from the wild type and a 'petite' strain grown in the presence of CAP, an antibiotic known to inhibit mitochondrial but not cytoplasmic protein synthesis [27–29]. Therefore, CAP does not seem to inhibit the synthesis of the elongation factors specific for 70 S ribosomes.

The activity of CAP in our experimental conditions was ascertained in the experiments shown in tables 3 and 4. Table 3 compares cytochrome oxidase activity and polymerizing activity on 70 S ribosomes in extracts prepared from the wild type and a 'petite' grown in the presence or in the absence of the antibiotic. While cytochrome oxidase activity is not detectable in the cell-free extracts prepared from the 'petite' strain or from the parental strain grown in the presence of CAP, both strains grown under either conditions display polymerizing activity on 70 S ribosomes.

In the experiment reported in table 4 mitochondria were isolated from cells of the wild type strain after a pulse of ^{14}C-amino acids in the presence of CHE, known to inhibit cytoplasmic protein synthesis [30, 31], or of CHE plus CAP. In the presence of CHE, the labeling of mitochondrial proteins by the cytoplasmic protein synthesizing system is completely inhibited and it is possible to measure the labeling dependent on the mitochondrial protein synthesizing system. The data show that the mitochondrial sediment is not labeled when the cells were exposed to both CAP and CHE.

In conclusion, the reported data indicate that in *S. cerevisiae* the genetic information for the mitochondrial elongation factors is coded by the nuclear DNA. Furthermore, such information appears to be translated by the cytoplasmic protein synthesizing system. In agreement with the latter results Davey, Yu and Linnane [32] have found that mitochondria prepared from yeast cells grown in the presence of CAP have an active protein synthesizing system. In addition Küntzel has demonstrated that also the ribosomal proteins of *Neurospora* mitochondria are synthesized on the cytoplasmic ribosomes [33].

Acknowledgements

The Authors are grateful to Dr. Orio Ciferri for critical discussions and to Mrs. Paola Alti for skilful

technical assistance. This work was supported by a grant from the Consiglio Nazionale delle Ricerche and carried out during the tenure by R.C. of a post-doctoral fellowship of the Ciclo biennale di ricerche per la specializzazione in Genetica e Biochimica applicate alla Microbiologia Industriale sponsored by C. Erba S.p.A., Farmitalia A.p.A., Italseber S.p.A., Lepetit S.p.A. and Pierrel S.p.A.

References

[1] D. Nathans and F. Lipmann, Proc. Natl. Acad. Sci. U.S. 47 (1961) 497.
[2] R. Rendi and S. Ochoa, J. Biol. Chem. 237 (1962) 3707.
[3] B. Parisi, G. Milanesi, J. Van Etten, A. Perani and O. Ciferri, J. Mol. Biol. 28 (1967) 295.
[4] O. Ciferri and B. Parisi, in: Progress in Nucleic Acid Research and Molecular Biology, eds. J.N. Davidson and W.E. Cohn, Vol. 10 (Academic Press, New York, 1970) p. 121.
[5] H. Küntzel, FEBS Letters 4 (1969) 140.
[6] A. Perani, O. Tiboni and O. Ciferri, J. Mol. Biol., in press.
[7] D. Richter and F. Lipmann, Biochemistry, in press.
[8] O. Tiboni, B. Parisi, A. Perani and O. Ciferri, J. Mol. Biol. 47 (1970) 467.
[9] D.B. Roodyn and D. Wilkie, The Biogenesis of Mitochondria (Methuen, London, 1968).
[10] R.P. Wagner, Science 163 (1969) 1026.
[11] H. Küntzel and H. Noll, Nature 215 (1967) 1340.
[12] H. Küntzel, J. Mol. Biol. 40 (1969) 315.
[13] H. Schmitt, European J. Biochem. 17 (1970) 278.
[14] F. Sala and H. Küntzel, European J. Biochem. 15 (1970) 280.
[15] P. Borst and A.M. Kroon, in: International Review of Cytology, eds. G.H. Bourne and J.F. Danielli, Vol. 26 (Academic Press, New York, 1969) p. 107.
[16] F. Carnevali, G. Morpurgo and G. Tecce, Science 163 (1969) 1331.
[17] G. Bernardi, F. Carnevali, A. Nicolajeff, G. Piperno and G. Tecce, J. Mol. Biol. 37 (1968) 493.
[18] G.D. Clark-Walker and A.W. Linnane, J. Cell Biol. 34 (1967) 1.
[19] O. Ciferri, B. Parisi, A. Perani and M. Grandi, J. Mol. Biol. 37 (1968) 529.
[20] J. Lucas-Lenard and F. Lipmann, Proc. Natl. Acad. Sci. U.S. 55 (1966) 1562.
[21] A. Kajii, H. Kaji and G.D. Novelli, J. Biol. Chem. 240 (1965) 1185.
[22] O.C. Wharton and A. Tzagoloff, in: Methods in Enzymology, eds. R.W. Estabrook and M.E. Pulmann, Vol. 10 (Academic Press, New York, 1967) p. 245.
[23] M. Polsinelli and M. Beretta, J. Bacteriol. 91 (1966) 63.
[24] D.W. Gregory, E.S. Palakis and W. Wartley, in: Methods in Enzymology, eds. L. Grossmann and K. Moldave, Vol. 12 A (Academic Press, New York, 1967) p. 472.
[25] M.W. Nirenberg, in: Methods in Enzymology, eds. S.P. Colowick and N.O. Kaplan, Vol. 6 (Academic Press, New York, 1963) p. 19.
[26] G. Schatz and J. Saltzgraber, Biochem. Biophys. Res. Commun. 37 (1969) 996.
[27] E. Wintersberger, Biochem. Z. 341 (1964) 409.
[28] A.G. So and E.W. Davie, Biochemistry 2 (1963) 132.
[29] G.D. Clark-Walker and A.W. Linnane, Biochem. Biophys. Res. Commun. 25 (1966) 8.
[30] H.L. Ennis and M. Lubin, Science 146 (1964) 1474.
[31] A.J. Lamb, G.D. Clark-Walker and A.W. Linnane, Biochim. Biophys. Acta 161 (1968) 415.
[32] P.J. Davey, R.Yu and A.W. Linnane, Biochem. Biophys. Res. Commun. 36 (1969) 30.
[33] H. Küntzel, Nature 222 (1969) 142.