CHAIN ELONGATION FACTORS OF YEAST MITOCHONDRIA

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1. Introduction

It is now clear that yeast mitochondria possess an autonomous protein synthesizing system similar to those found in prokaryotic organisms [1–3]. Mitochondrial ribosomes from yeast and *Neurospora crassa* exhibit activity in a Poly U-directed cell-free system when combined with a bacterial supernatant [1, 3, 4] in contrast with cytoplasmic ribosomes. This suggests that mitochondria contain chain elongation factors similar to those of bacteria [3, 4]. The results presented further demonstrate the similarities, and difference between yeast mitochondrial and bacterial chain elongation factors, and contrast them with those from the cytoplasm. Data are also presented on the possible reasons for the comparative inactivity of yeast mitochondrial supernatant when compared with that of *E. coli*, and on the origin of these factors.

2. Methods

Growth of *Saccharomyces cerevisiae* (strains 239; N.C.Y.C. OX 2 45 EB 2 XEB p^+ and p^-; αDV EB p^+ and p^-) and preparation of mitochondria has been described previously [5]. The method was slightly modified by the use of a Braun shaker to rupture the cells.

Mitochondrial, cytoplasmic, and *E. coli* chain elongation factors were prepared as 80% saturation ammonium sulphate preparations of the 100,000 *g* supernatants. Washed mitochondrial ribosomes were prepared as described previously [5], cytoplasmic ribosomes according to Richter et al. [6], and *E. coli* ribosomes according to Lucas-Lenard and Lipmann [7]. Krebs ascites tumor cell ribosomes were kindly provided by Dr. I. Kerr. The separation of T and G factors on hydroxylapatite columns was performed according to Parmeggiani [8]. The peak fractions were pooled, and stored at −20° in the presence of 40% glycerol.

^{3}H-GDP binding activity was assayed according to Ertel et al. [10]. ^{14}C-phenylalanyl-tRNA was prepared, and polymerizing activity of the enzyme fractions tested as described previously [5].

3. Results

The compatability of various ribosomes and supernatants is shown in table 1. Clearly the mitochondrial, and bacterial system are compatible. The partial activity obtained with yeast cytoplasmic ribosomes when combined with both mitochondrial and *E. coli* supernatants is probably due to the difficulty in removing G from these ribosomes as activity was greatly reduced with the use of Krebs ascites ribosomes. This possibility and, or the presence of mitochondrial factors in a cytoplasmic preparation can explain in the partial compatability obtained previously [11] when yeast cytoplasmic and *E. coli* system were compared.

Mitochondrial T and G factors can be separated using hydroxylapatite chromatography as shown in fig. 1. Here T_{mit} elutes at 10 mM and G_{mit} at 30 mM phosphate buffer, whereas T_{cyto}, and G_{cyto} elute at 10 mM and 70 mM respectively. *E. coli* T and G elute at 70 mM and 30 mM respectively under similar conditions. These results differ from those of Richter and Lipmann [3] using mitochondrial factors from *S. fragilis*, and *S. carlsbergensis* where T_{mit} elutes at 70 mM, and G_{mit} at 30 mM. *N. crassa* T and G factors elute at 100 mM and 40 mM respectively [4].

Table 1
Ribosome specificity of various supernatant preparations.

Ribosomes	Supernatant	^{14}C-phenylalanine Incorporated pmoles/mg prot.	%
E. coli	*E. coli*	178	100
	cytoplasm	9.5	5.3
	mitochondria	25	14
mitochondria	*E. coli*	57	380
	cytoplasm	4	26.5
	mitochondria	15	100
cytoplasm	*E. coli*	2.7	39
	cytoplasm	6.9	100
	mitochondria	3.3	48
Krebs ascites	*E. coli*	9.3	8.3
	cytoplasm	112.2	100
	mitochondria	9.6	8.6

Assay conditions as reported under Methods. Each assay contained 295 μg of *E. coli* ribosomes, 600 μg of mitochondrial ribosomes, 800 μg cytoplasmic ribosomes, and 300 μg of Krebs ascites tumor cell ribosomes. The supernatant added was 150 μg *E. coli*, 405 μg mitochondrial, 600 μg cytoplasmic.

The separated factors when combined show the complete compatability of mitochondrial and *E. coli* systems (table 2), in contrast with those from the cytoplasm (table 3). It has been reported [3] that *S. fragilis* and *S. carlsbergensis* T_{mit} can function with G_{cyto} and cytoplasmic ribosomes, as has been shown for *E. coli* T [12]. However, this does not appear to be the case as the activity of T_{mit} is low when compared with T_{cyto} on cytoplasmic ribosomes, and could represent some cross contamination. This would appear to be more likely as Perani et al. [13] have shown as absolute specificity for the 70 S type factors from *S. fragilis*, and T_{mit} is unable to bind ^{14}C-phenylalanyl-tRNA to cytoplasmic ribosomes (unpublished results).

It is clear from table 1 that although the mitochondrial supernatant is capable of cross reacting with *E. coli* ribosomes, the *E. coli* supernatant is far more active with either *E. coli* or mitochondrial ribosomes. This has also been shown for mitochondrial ribosomes from *S. carlsbergensis* [14]. This difference in activity is true even at saturating levels of supernatant enzymes (results not shown). Possible reasons for this apparent lack of activity are: (1) that the mitochondrial T and G are more labile; (2) that their function is different

Table 2
Cross reactivity of mitochondrial and *E. coli* T and G factors.

Ribosome	T Factor	G Factor	pmoles ^{14}C-phe. incorporated/ assay
E. coli	*E. coli*	*E. coli*	5.20
		Mito	2.70
	Mito	*E. coli*	2.85
		Mito	1.06
Mito	Mito	*E. coli*	4.85
		Mito	2.55
	E. coli	*E. coli*	12.34
		Mito	2.40

Assay conditions as reported in Methods. Where indicated 295 μg *E. coli* ribosomes, 800 μg mitochondrial ribosomes, were added. The following amounts of enzymes were added per assay; 1.9 μg of *E. coli* T and G, 1.5 μg of mitochondrial T, and 1.0 μg of mitochondrial G.

Table 3
Cross reactivity of mitochondrial and cytoplasmic T and G factors.

Ribosome	T Factor	G Factor	pmoles ^{14}C-phe. incorporated/ assay
Cyto	Cyto	Cyto	146.0
		Mito	17.8
	Mito	Cyto	7.7
		Mito	0.3
Mito	Mito	Cyto	1.5
		Mito	2.6
	Cyto	Cyto	0.1
		Mito	0.1

Assay condition as reported in Methods. Where indicated 600 μg of mitochondrial ribosomes and 1 mg of cytoplasmic ribosomes were added. The following amounts of enzymes were added per assay: 4.4 μg of cytoplasmic T and 2.0 μg of cytoplasmic G, 1.5 μg of mitochondrial T and 1.0 μg of mitochondrial G.

and hence less efficient or limiting; (3) the presence of inhibiting proteins [4].

The first possibility has been examined, and the relative heat stabilities of *E. coli*, and mitochondrial T are shown in fig. 2. T_{mit} is about twice as unstable as *E. coli* T. In addition, it has been shown that T_{mit}

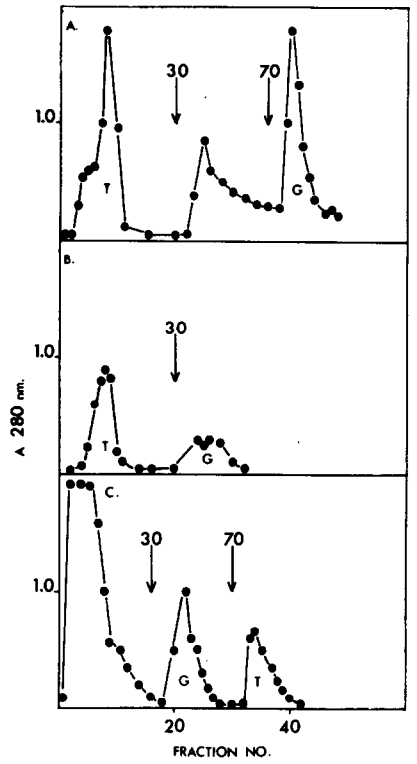

Fig. 1. Separation of *E. coli*, mitochondrial, and cytoplasmic T and G factors by hydroxylapatite chromatography. 80% saturation ammonium sulphate preparations were applied to a hydroxylapatite column (2.5 × 10 cm) and eluted stepwise at the indicated phosphate concentrations as described under Methods. The T factor was identified by its ability to bind ^{3}H-GDP, and G by its stimulation of a cell-free system containing washed-ribosomes and excess T. (A) cytoplasmic factors; (B) mitochondrial factors; and (C) *E. coli* factors.

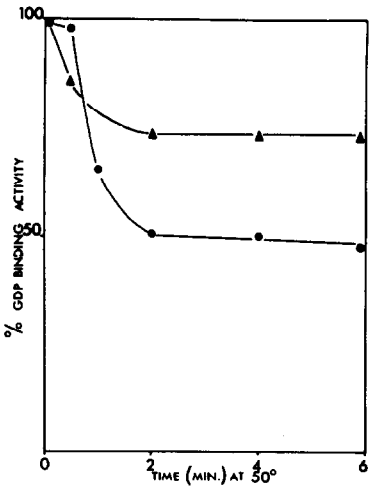

Fig. 2. Heat stability of mitochondrial and *E. coli* T factors. 100,000 *g* supernatant preparations of yeast mitochondria and *E. coli* were incubated at 50°. 20 µl aliquots were removed at intervals, cooled rapidly in ice, and assayed as described in Methods for ^{3}H-GDP binding activity. Mitochondrial activity ○—○; *E. coli* activity ▲—▲.

Table 4
Polyphenylalanine synthesis by polymerizing enzymes from *E. coli*, wild type and "peptide" yeast mitochondria.

Strain	pmoles ^{14}C-phenylalanine incorporated/mg prot.
Expt. I	
αDV EB ρ^+	5.3
αDV EB ρ^-	4.2
239	5.3
Expt. II	
E. coli	120
OX2 45EB XEB ρ^+	5.9
OX2 45EB XEB ρ^-	7.6

Assay conditions as reported under Methods. Each assay contained: Expt. I; 40 µg *E. coli* ribosomes, 158 µg of ρ^+ mitochondrial, and 135 µg of ρ^- mitochondrial polymerizing enzymes where indicated. Expt. II; 110 µg *E. coli* ribosomes, and 50 µg *E. coli*, 12 µg of ρ^+ mitochondrial, 14 mg of ρ^- mitochondrial polymerizing enzymes where indicated.

can be separated into Tu and Ts activities [5], and that mitochondrial Ts functions only in the stimulation of ^{3}H-GDP exchange with Tu or Tu-GDP. This is in contrast with *E. coli* Ts which stimulates both ^{3}H-GTP and ^{3}H-GDP exchanges.

The ability of mitochondrial enzyme to function with *E. coli* ribosomes, enables their presence and absence in various mitochondrial preparations to be determined. Two mutants have been investigated here which are "petites" lacking mitochondrial DNA completely (these are neutral, and mDNA cannot be detected by normal means, i.e. analytical ultracentrifugation [15]). As can be seen from table 4 both the mutants retain their ability to function with *E. coli* ribosomes, when compared with the "grandes" indicating that both T and G are present and therefore are coded for by the nucleus. Parisi and Cella [16]

have reported similar findings with "petite" yeast strain with a very low G+C content in their mitochondrial DNA.

4. Discussion

The data presented demonstrates that *S. cerevisiae* mitochondrial chain elongation factors are readily interchangeable with those from *E. coli*, in contrast with those from the cytoplasm, and exhibit an absolute specificity for 70 S type ribosomes. Some differences do exist in the chromatographic properties of T_{mit} when compared with *E. coli* T or T from *S. fragilis* or *S. carlsbergensis*. The significance of these differences are difficult to assess as they might be due to differences in source of hydroxylapatite.

The lower activity of the mitochondrial supernatant when compared with *E. coli* supernatant can in part be explained by the greater lability of T_{mit} (fig. 2). Another reason could be the slight difference in function of mitochondrial Ts [5]. Both of these results indicate that although *E. coli* T and T_{mit} are compatible there exists differences, the exact significance of which is not known, and which also may occur between mitochondria from different yeast species. It remains to be seen whether the G_{mit} also has slight differences capable of reducing its activity.

Although yeast mitochondria contains specific and separate chain elongation factors it is clear that these factors are coded for by the nucleus (table 4), and it also appears that they are also produced on 80 S ribosomes in the cytoplasm [16]. Work is in progress to determine whether the initiation factors are coded for by the nucleus or mitochondrion.

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References

[1] H. Küntzel, FEBS Letters 4 (1969) 140.
[2] A.H. Scragg, H. Morimoto, V. Villa, J. Nekhorocheff and H.O. Halvorson, Science 171 (1971) 908.
[3] D. Richter and F. Lipmann, Biochemistry 9 (1970) 5065.
[4] M. Grandi and H. Küntzel, FEBS Letters 10 (1970) 25.
[5] A.H. Scragg, H. Morimoto, V. Villa and H.O. Halvorson, submitted to Arch. Biochem. Biophys.
[6] D. Richter, H. Hameister, K.G. Petersen and F. Klink, Biochemistry 7 (1968) 3753.
[7] J. Lucas-Lenard and F. Lipmann, Biochemistry 5 (1966) 1562.
[8] A. Parmeggiani, Biochem. Biophys. Res. Commun. 30 (1968) 613.
[9] F.J. Bollum, J. Biol. Chem. 234 (1959) 2733.
[10] R. Ertel, B. Redfield, N. Brot and H. Weissbach, Arch. Biochem. Biophys. 128 (1968) 331.
[11] A.G. So and E.W. Davie, Biochemistry 2 (1963) 132.
[12] I. Krisko, J. Gordon and F. Lipmann, J. Biol. Chem. 244 (1969) 6117.
[13] A. Perani, O. Tiboni and O. Ciferri, J. Mol. Biol. 55 (1971) 107.
[14] P. Borst and L.A. Grivell, FEBS Letters 13 (1971) 73.
[15] D.H. Williamson, Heredity 25 (1970) 491.
[16] B. Parisi and R. Cella, FEBS Letters 14 (1971) 209.