MITOCHONDRIAL PEPTIDE CHAIN ELONGATION FACTORS FROM *NEUROSPORA CRASSA*

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Two complementary peptide chain elongation factors (G and T) have been isolated from a mitochondrial 100,000 *g* supernatant. Both factors are specific for 70 *S* ribosomes and can be crossed with T and G factors from *E. coli*.

1. Introduction

Mitochondrial 73 *S* ribosomes from *Neurospora* and bacterial 70 *S* ribosomes resemble each other in several functional aspects: they are insensitive to cycloheximide and sensitive to chloramphenicol [1,2], they contain peptide chain initiation factors recognizing and translocating *N*-formylmethionyl-tRNA [3]; and they require supernatant enzymes from bacteria or mitochondria for peptide chain elongation [4].

The latter observation suggests that mitochondria may contain peptide chain elongation factors similar to bacterial T and G factors. The bacterial G factor (translocase) is specific for 70 *S* ribosomes and cannot replace the corresponding cytoplasmic translocase in a eucaryotic cytoplasmic cell-free system [5,6]. A similar specificity for 70 *S* ribosomes of a mitochondrial G factor would explain why a mitochondrial supernatant fraction is much less active with 80 *S* than with 70 *S* ribosomes [4].

2. Methods

Growth of *Neurospora crassa* (wild type, Em 5256) and cytoplasmic ribosomes has been described previously [7].

Mitochondrial chain elongation factors (G and T) were isolated using a method similar as described for bacterial factors [8]. Crude mitochondria were washed once with 0.44 M sucrose containing 100 mM NH_4Cl, 10 mM $MgCl_2$, 10 mM tris (pH 7.5) mixed with a double weight of alumina, disrupted by grinding at 0° and extracted with two volumes of 20 mM tris (pH 7.8), 10 mM Mg acetate, 1 mM dithiothreitol. A high speed supernatant obtained after 90 min centrifugation at 240,000 *g* was treated with saturated ammonium sulfate (pH 7); the fraction precipitating between 40% and 70% saturation was dissolved in tris (pH 7.8), 1 mM dithiothreitol and passed through Sephadex G-25 ("crude polymerizing enzyme").

The solution was then passed through Sephadex G-150 and eluted with the same buffer. The active fractions were pooled, concentrated with aquacide I and dialyzed against 10 mM phosphate buffer (pH 7.0), 1 mM dithiothreitol. The separation of G and T factors on a hydroxylapatite column was performed according to Parmeggiani [8]. The peak fractions were concentrated with aquacide and precipitated with ammonium sulfate (peak I: 40% to 70%, peak II and III: 52% to 70%). The pellets were dissolved in 50 mM tris (pH 7.8), 1 mM dithiothreitol, and stored at 4°.

2.1. *Isolation of cytoplasmic polymerizing enzymes*

A mitochondria-free supernatant from *Neurospora* was centrifuged at 240,000 *g* for 90 min, the supernatant was treated with 0.17 mg/ml neutralized protamine sulfate, and the enzymes were precipitated

from the supernatant with ammonium sulfate (between 40 and 70% saturation). The precipitate was suspended in 20 mM tris, 5 mM $MgCl_2$, 1 mM dithiothreitol, 10 mM KCl, 40 μg/ml spermine, 0.1 mM glutathione.

Washed *E. coli* ribosomes were prepared according to Lucas-Lenard and Lipmann [9], ^{3}H-phenylalanyl-tRNA (spec. act. 1 Ci/mmole) was prepared according to Kaji, Kaji and Novelli [10].

The polymerizing activity of the enzyme fractions was tested in 100 μl assay mixtures containing 50 mM tris (pH 7.5), 13 mM Mg acetate, 50 mM NH_4Cl, 30 mM dithiothreitol, 5 mM PEP, 10 μg PK, 0.6 mM GTP, 5 mM ATP, 20 μg spermine, 10 mM glutathione, 2 mg *E. coli* ribosomes, 100 μg poly U and 6000 cpm ^{3}H-phenylalanyl-tRNA (58,000 cpm/mg). After incubation at 37° for 30 min, the hot TCA insoluble radioactivity was determined.

3. Results

Table 1 shows that the crude mitochondrial polymerizing enzyme contains an active G factor, because it is stimulated considerably by added T factor from *E. coli* which has little activity on washed ribosomes in the absence of G. The relative low activity of the mitochondrial enzyme in the absence of *E. coli* factors and the lack of stimulation by G (*Coli*) suggests that the T activity of this crude enzyme is much lower than the G activity.

Fig. 1 demonstrates the specificity of mitochondrial and cytoplasmic polymerizing enzymes in their interaction with 70 *S* and 80 *S* ribosomes, confirming earlier results [4]. Fig. 1 also shows the elution pattern of the crude mitochondrial enzyme from a Sephadex G-150 column. Two active fractions are eluted after the inactive exclusion peak, and both contain G activity because they polymerize phenylalanine in the absence of G (*Coli*). But the fact that T (*Coli*) stimulates much more the activity of the first than that of the second fraction indicates that most of the mitochondrial T activity elutes with the second peak. The much lower T activity of the enzyme before Sephadex filtration suggests that inhibiting proteins have been removed by this procedure.

The mitochondrial G and T activities have been completely separated by chromatography on a hydroxylapatite column. Fig. 2 shows the elution pattern of three peaks eluting with 40 mM, 70 mM and 100 mM phosphate buffer.

Table 2 indicates that the first peak contains G activity complementary to T (*Coli*), the third peak T activity complementary to G (*Coli*) and the middle peak both G and T activity. Virtually the same elution profile has been obtained with *E. coli* polymerizing enzymes [8]. Table 2 also shows the interchangeability of bacterial and mitochondrial G and T factors.

Table 1

Ribosomes	Polymerizing enzymes	*E. coli* elongation factors	^{3}H-Phenylalanine polymerized per assay (μμmoles)
E. coli	–	–	0.31
,,	–	G	0.31
,,	–	T	1.32
,,	–	G+T	6.60
,,	mitochondria	–	0.56
,,	,,	G	0.34
,,	,,	T	4.62
Neurospora cytoplasm	–	–	0.64
,,	mitochondria	–	0.30
,,	cytoplasm	–	6.62

Properties of crude polymerizing enzymes from mitochondria and cytoplasm. The preparation of the enzyme fractions and the assay conditions are described under Methods. The following amounts of enzymes were added per assay: 20 μg (mitochondrial or cytoplasmic crude enzymes), 0.75 μg (*E. coli* T factor), 6.3 μg (*E. coli* G factor).

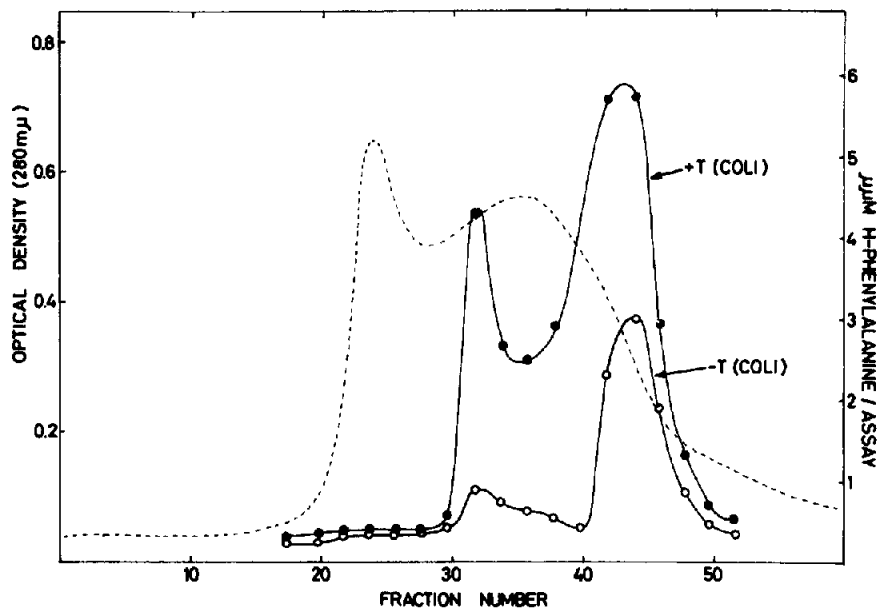

Fig. 1. Sephadex G-150 filtration of crude polymerizing enzymes from *Neurospora* mitochondria. 25 mg of crude mitochondrial enzyme (see Methods) were passed through a 80 ml G-150 column and eluted with 50 mM tris-HCl, pH 7.8, 1 mM dithiothreitol. 1 ml fractions were collected, and 60 μl of each were tested in a final volume of 100 μl. - - - - - - - - : optical density (280 mμ); o—o: polymerizing activity; •—•: polymerizing activity in the presence of 0.7 μg T factor from *E. coli*.

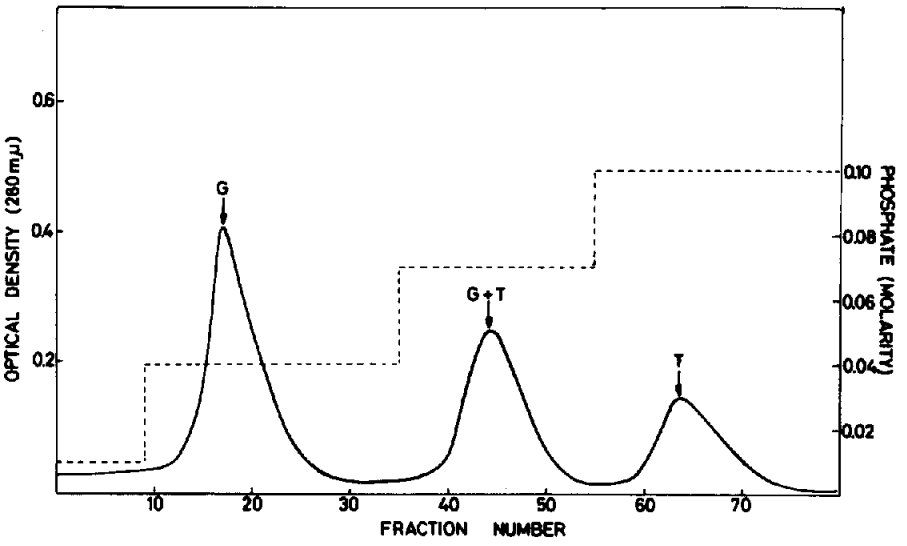

Fig. 2. Separation of mitochondrial G and T factors on a hydroxyapatite column. The peak fractions shown in fig. 1 were pooled, concentrated and dialyzed as described under Methods and absorbed onto a hydroxylapatite column (0.6 X 8 cm). After washing with 10 mM phosphate buffer pH 7 the peaks were eluted stepwise at the indicated phosphate concentrations and treated as described under Methods.

Table 2

Chain elongation factors	^{3}H-Phenylalanine polymerized per assay ($\mu\mu$moles)
none	0.34
G (mito)	0.36
G (mito) + T (*Coli*)	3.18
G (*Coli*)	0.38
G (*Coli*) + T (mito)	0.92
G (*Coli*) + T (*Coli*)	4.56
G+T (mito)	0.75
T (*Coli*)	0.85
T (mito)	0.42

Polymerizing activity of mitochondrial G and T factors complemented with T and G factors from *E. coli*. The factors were isolated from the peak fractions shown in fig. 2. The following amounts of enzymes were added per assay (in μg): 1.8 (G, mito), 1.8 (G, *Coli*), 4.1 (G+T, mito), 0.28 (T, mito) and 0.70 (T, *Coli*).

The G factors from mitochondria and *E. coli* have similar activity in combination with T (*Coli*), whereas the mitochondrial T factor is less active with G (*Coli*) than the *E. coli* T factor. The T activity of mitochondrial polymerizing enzymes varies with different preparations and is completely lost after freezing intact mitochondria prior to extraction, whereas the G activity seems to be stable.

4. Discussion

Our data show that mitochondria from *Neurospora crassa* contain chain elongation factors, which are specific for bacterial or mitochondrial ribosomes of the 70 *S* type, and which can be combined with complementary elongation factors from *E. coli*. Thus the bacterial and mitochondrial G and T factors seem to be strongly related, except that the mitochondrial T factor is more labil than the bacterial one.

A bacterial-like G factor specific for 70 *S* ribosomes and differing from the corresponding 80 *S*-specific T_2 factor has been found in the cytoplasm of *Prototheca zopfii*, yeast [6] and rat liver [11]. From our findings it appears possible that such factors, which cannot function in the cytoplasm because of the absence of 70 *S* ribosomes, have been released from mitochondria or, in the case of *Prototheca*, from chloroplasts during extraction. The lack of a 70 *S* specific T factor in yeast and rat liver cytoplasm could be explained by the lability of the mitochondrial T factor.

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References

[1] H. Küntzel, Nature 222 (1969) 142.
[2] W. Sebald, Th. Bücher, B. Olbrich and F. Kaudewitz, FEBS Letters 1 (1968) 235.
[3] F. Sala and H. Küntzel, European, J. Biochem., in press.
[4] H. Küntzel, FEBS Letters 4 (1969) 140.
[5] I. Krisko, J. Gordon and F. Lipmann, J. Biol. Chem. 244 (1969) 6117.
[6] O. Tiboni, B. Parisi, A. Perani and O. Ciferri, J. Mol. Biol. 47 (1970) 467.
[7] H. Küntzel and H. Noll, Nature 215 (1967) 1340.
[8] A. Parmeggiani, Biochem. Biophys. Res. Commun. 30 (1968) 613.
[9] J. Lucas-Lenard and F. Lipmann, Biochemistry 55 (1966) 1562.
[10] A. Kaji, H. Kaji and G.D. Novelli, J. Biol. Chem. 240 (1965) 1185.
[11] L. Felicetti and G.F. Di Matteo, J. Mol. Biol., in press.