SPECIFICITY OF MITOCHONDRIAL AND CYTOPLASMIC RIBOSOMES FROM *NEUROSPORA CRASSA* IN POLY-U DEPENDENT CELL FREE SYSTEMS

Hans KÜNTZEL

Max-Planck-Institut für experimentelle Medizin, Abteilung Chemie, 3400 Göttingen, Germany

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1. Introduction

Two different types of ribosomes have been isolated from *Neurospora crassa:* cytoplasmic 77 *s* and mitochondrial 73 *s* ribosomes [1]. The cytoplasmic ribosomes are structurally related to 80 *s* ribosomes from eucaryotic organisms whereas mitochondrial ribosomes resemble procaryotic 70 *s* particles [2–4].

A functional relationship between mitochondrial and bacterial ribosomes is suggested by the observation that both ribosomes are sensitive to chloramphenicol and resistent to cycloheximide, in contrast to the cytoplasmic ribosomes from *Neurospora* [5,6].

Another functional difference between ribosomes from procaryotic and eucaryotic organisms is their specific interaction with peptide chain elongation factors. It has been demonstrated that heterologous Poly-U dependent systems containing ribosomes and supernatant factors from different organisms are active only when systems within the procaryotic class or within the eucaryotic class are interchanged; systems combining ribosomes from bacteria with supernatant enzymes from higher cells, and vice versa, are inactive [7].

To find out whether mitochondrial ribosomes belong to the procaryotic class when tested for their specificity in chain elongation, poly-U dependent cell-free systems from *Neurospora* mitochondria and cytoplasm were combined with each other and with systems from *E. coli* and rat liver.

Part of these results has been presented at the 6th FEBS meeting in Madrid (1969) [8].

2. Methods

Mitochondrial and cytoplasmic ribosomes were isolated from *Neurospora crassa* (wild type, Em 5256) as described previously [6]. Preparation of mitochondrial supernatant enzymes: *Neurospora* mitochondria were suspended in 10 mM Tris, pH 7.5, 10 mM magnesium acetate, 10 mM KCl and 5 mM β-mercaptoethanol, and disrupted by sonification (4 × 15 sec). The extract was centrifuged for 2 hr at 100,000 × *g*, the supernatant was dialyzed against the same buffer and stored at −20°C. Preparation of cytoplasmic enzymes: *Neurospora* hyphae were homogenized with sea sand in the same buffer as above; the extract was centrifuged for 2 hr at 100,000 × *g* and the supernatant was dialyzed against the same buffer. Ribosomes and supernatant enzymes from *E. coli* and rat liver cytoplasm were prepared according to Parisi et al. [7]. The homologous and heterologous systems from *Neurospora* mitochondria and cytoplasm (table 1, fig. 1) had the following composition (μmoles per ml): Tris pH 7.5 (100), KCl (50), magnesium acetate (20), spermidine (2), β-mercaptoethanol (16), glutathione (2), GTP (0.5), ATP (5), PEP (5), PK (20 μg), 19 amino acids minus phenylalanine (0.04 each), Poly-U (120 μg), cytoplasmic tRNA from *Neurospora* (0.6 mg), L-^{14}C-phenylalanine (0.5 μC; sp. act. 325 mC/mM), ribosomes and supernatant enzymes as indicated in the legends of table 1 and fig. 1.

The homologous and heterologous systems from *Neurospora*, *E. coli* and rat liver were composed as follows (μmoles per ml): Tris pH 7.5 (100), KCl (50),

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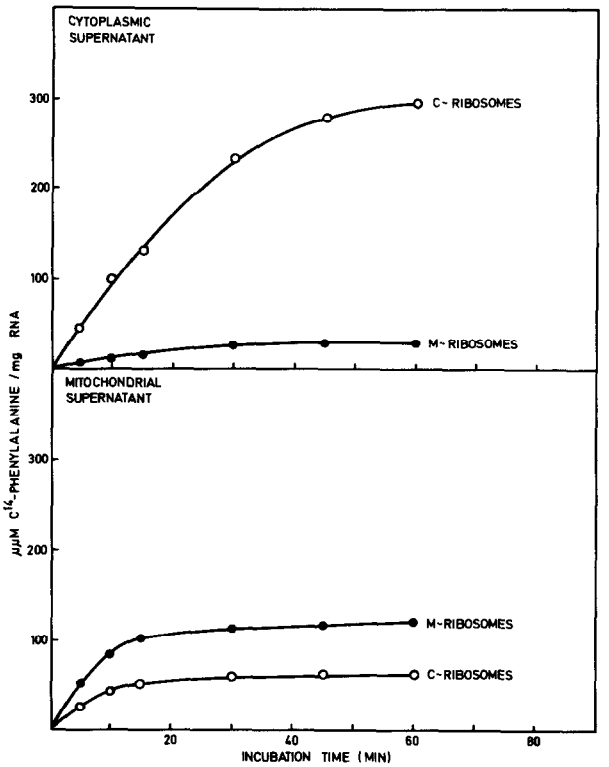

Fig. 1. Polymerization of ^{14}C-phenylalanine in homologous and heterologous systems from *Neurospora* mitochondria and cytoplasm. The composition of the reaction mixture is given under Methods. The mixtures contained per ml: cytoplasmic ribosomes (12.5 O.D.) or mitochondrial ribosomes (13.2 O.D.); cytoplasmic supernatant enzymes (1.1 mg protein) or mitochondrial supernatant enzymes (1.3 mg). After incubation at 37°C 50 μl aliquots were removed, added to the same volume of 10% TCA containing an excess of ^{12}C-phenylalanine and assayed as described [6].

magnesium acetate (14), spermidine (2), β-mercaptoethanol (6), glutathione (2), GTP (0.6), ATP (2). PEP (5), PK (10 μg), Poly-U (120 μg), ^{14}C-phenylalanine in the form of phenylalanyl-tRNA from *E. coli* (71 μμmoles), ribosomes and supernatant enzymes as indicated in the legend of table 2.

3. Results

Table 1 shows the requirements for the poly-U dependent polyphenylalanine synthesis in a cell-free system from *Neurospora* cytoplasm. Spermidine

Table 1

	Incorporation of ^{14}C-phenylalanine	
	μ μmoles/mg RNA	%
Complete	95.1	100
−ribosomes	1.9	2
−supernatant	4.7	5
−poly-U	20.0	21
−spermidine	46.6	49
tRNA from *Neurospora* cytoplasm replaced by		
yeast tRNA	90.3	95
E. coli tRNA	103.1	108
mitochondrial tRNA	78.0	82

Properties of a cell free system from *Neurospora* cytoplasm. The composition of the reaction mixture is given under Methods. 0.1 ml mixtures were indubated for 10 min at 37°C and assayed as described [6].

stimulates two-fold the incorporation activity. Cytoplasmic tRNA from *Neurospora* can be replaced by mitochondrial tRNA from the same cell, by yeast, or by *E. coli* tRNA without loss of activity.

Fig. 1 shows the time course of ^{14}C-phenylalanine incorporation in homologous and heterologous systems from *Neurospora* cytoplasm and mitochondria. Cytoplasmic ribosomes are much more active than mitochondrial ribosomes when combined with cytoplasmic supernatant enzymes. With mitochondrial supernatant enzymes the mitochondrial ribosomes are more active than the cytoplasmic ones. The initial incorporation rate is similar in both homologous systems, but in the system containing mitochondrial supernatant enzymes the plateau is reached earlier; this could be explained with the presence of endonucleases in mitochondrial extracts from *Neurospora* [10]. Table 2 summarizes the results obtained with homologous and heterologous systems from different species. It is obvious that the mitochondrial and the *E. coli* system can be interchanged with each other, but not with systems from the cytoplasm of *Neurospora* or rat liver; the two cytoplasmic systems can again be interchanged with each other. The incompatibility between the *E. coli* and the rat liver system is more strict than the incompatibility between the two *Neurospora* systems; it is, however, difficult to ex-

Table 2
Incorporation of ^{14}C-phenylalanine from *E. coli* Phe-tRNA into polyphenylalanine in various cell free systems.

Supernatant enzymes	Ribosomes	Incorporation of ^{14}C-phenylalanine	
		μ μmoles/mg RNA	%
E. coli	*E. coli*	9.8	100
	N.C. mitochondria	9.2	94
	N.C. cytoplasm	0.2	2
	rat liver cytoplasm	< 0.1	< 1
N.C. mitochondria	*E. coli*	4.4	108
	N.C. mitochondria	4.1	100
	N.C. cytoplasm	0.9	23
	rat liver cytoplasm	0.8	19
N.C. cytoplasm	*E. coli*	0.1	1
	N.C. mitochondria	2.8	38
	N.C. cytoplasm	7.4	100
	rat liver cytoplasm	6.8	92
Rat liver cytoplasm	*E. coli*	< 0.1	< 1
	N.C. mitochondria	0.8	7
	N.C. cytoplasm	9.8	90
	rat liver cytoplasm	11.0	100

The composition of the reaction mixtures is given under Methods. The mixtures contained 12 O.D.$_{260}$ ribosomes per ml and the following amounts of supernatant enzymes (mg protein/ml): *E. coli* (1.2), *Neurospora* mitochondria (0.9), *Neurospora* cytoplasm (1.1) and rat liver cytoplasm (1.1). The mixtures (0.5 ml) were incubated at 37°C for 10 min and assayed as described [6].

clude a certain degree of cross-contamination of the mitochondrial and cytoplasmic ribosome and supernatant preparations.

A contamination of mitochondrial ribosomes with bacterial ones can be avoided by working under sterile conditions and by the fact bacteria are not lysed with Triton-X.

4. Conclusion

The two protein-synthesizing systems in *Neurospora* exhibit a distinct incompatibility, which may reflect a different specificity of mitochondrial and cytoplasmic ribosomes in their interaction with chain elongation factors.

The observation that the mitochondrial system can be freely interchanged with the *E. coli* system, but not with cytoplasmic systems from the same cell or from rat liver, suggests a functional relationship between mitochondrial and bacterial ribosomes and chain elongation factors.

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