PROTEIN SYNTHESIS IN AN *E. COLI* SYSTEM DIRECTED BY YEAST MITOCHONDRIAL RNA

M. J. EGGITT

Division of Biochemistry, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA UK

Received 12 November 1975

1. Introduction

It is established that a few mitochondrial proteins are synthesized on mitoribosomes but that some 90% of the total mitochondrial protein is made on cytoribosomes and coded by nuclear DNA [1,2]. Most of the work attempting to identify the proteins made within the mitochondria has been done using inhibitors [3–5]. By blocking cytoplasmic protein synthesis with cycloheximide or emetine, it has been possible to show that a small number of polypeptides are made within the mitochondria, and these include some subunits of large enzyme complexes [6]. Recently, it has been shown that 3 of the enzyme subunits of cytochrome oxidase can be made within isolated mitochondria and then assembled together with 4 cytoplasmic synthesized subunits into a holoenzyme [7,8].

In this communication, evidence is presented that mitochondrial RNA from *Saccharomyces cerevisiae* is capable of stimulating protein synthesis in an *E. coli* S-30 protein synthesizing system. The polypeptides synthesized in this system were specifically precipitated by anti-mitochondrial antisera and were found to have molecular weights similar to those polypeptides which are labelled in vivo in the presence of cycloheximide.

2. Materials and methods

Radiochemicals were obtained from Amersham, Bucks., England. *S. cerevisiae* strain αDV 147 (original source, R. K. Mortimer) and *E. coli* strain MRE 600 (supplied frozen by MRE, Porton, Wilts, England) were used.

Isolation of mitochondrial RNA, preparation of anti-mitochondrial antisera, and preparation of mitochondrial proteins labelled in vivo, was as described by Eggitt and Scragg [9].

Polyacrylamide-SDS gel electrophoresis of proteins was as described by Weber and Osborn [10], except that ethylene diacrylate was used as cross linker instead of *NN'*-methylenebisacrylamide.

The *E. coli* S-30 was prepared from *E. coli* MRE 600 basically as described by Matthaei and Nirenberg [11]. The 30 000 *g* supernatant was dialysed overnight against TMA (0.01 M Tris–HCl pH 7.7, 0.01 M magnesium acetate, 0.06 M ammonium chloride, 6 mM mercaptoethanol) at 4°C. The S-30 preparation was then divided into 0.5 ml samples, and stored at −70°C until required.

The *E. coli* S-30 cell free protein synthesising system was based on the method described by Matthaei and Nirenberg [11] and Nirenberg and Matthaei [12] but without CTP and UTP, and in addition tRNA was used in the procedure described here. The standard reaction mixture contained in a final volume of 185 μl, 0.7 mM ATP, 0.7 mM GTP, 40 μg tRNA, 0.4 mM of each of 19 amino acids minus methionine, 50 μg pyruvate kinase, 2.7 mM phosphoenolpyruvate, 11 mM magnesium acetate, 50 μg tetrahydrofolic acid, 5–10 μCi of [^{35}S]methionine (200 Ci/mmol), 40–80 μg RNA and 40–90 μg protein of *E. coli* S-30 preparation. Incubation was at 37°C for 30 min then anti-mitochondrial antisera (prepared against yeast mitochondrial proteins), were added to each cell free preparation to precipitate any specific mitochondrial proteins synthesised. After adding antisera (20 μl) the tubes were incubated for 30 min at 37°C, then incubated for a further 45 min with 200 μl goat anti-rabbit serum, and left overnight at 4°C. The

 North-Holland Publishing Company – Amsterdam

mixture was centrifuged at 9000 *g* for 10 min and the resultant pellet washed with 3 X 3 ml of phosphate-buffered saline, before analysis on polyacrylamide-SDS gels.

3. Results

Total mitochondrial RNA from yeast has already been shown to contain messenger RNA which is capable of stimulating protein synthesis when injected into oocytes of the frog *X. laevis* [9]. Evidence presented below indicates that yeast mitochondrial RNA can also direct protein synthesis in an *E. coli* S-30 system and that this protein is immunologically recognizable as mitochondrial protein.

Stimulation of incorporation of [^{35}S]methionine is enhanced by the addition of total mitochondrial RNA, and is saturated at about 200 μg RNA per ml (fig.1a). The time course of the reaction is shown in fig.1b; incorporation of [^{35}S]methionine reaches a plateau at 30–60 min.

A consistent problem with yeast mitochondrial proteins has been their insolubility and their tendency to aggregate and precipitate out of solution. Rubin and Tzagoloff [13] have shown that KCl and Triton X-100 are required to solubilize certain mitochondrial proteins. To illustrate this problem of insolubility and aggregation, yeast mitochondrial and cytoplasmic RNA were added to *E. coli* S-30 systems (endogeneous controls were also included), and incubated at 37°C for 30 min. To each tube anti-mitochondrial antisera were added (no KCl or Triton X-100 added), and the precipitation procedure carried out as described in the methods section. The precipitates were analyzed by polyacrylamide-SDS gel electrophoresis (only the results in which mitochondrial RNA was added to the cell free system are illustrated, fig.2).

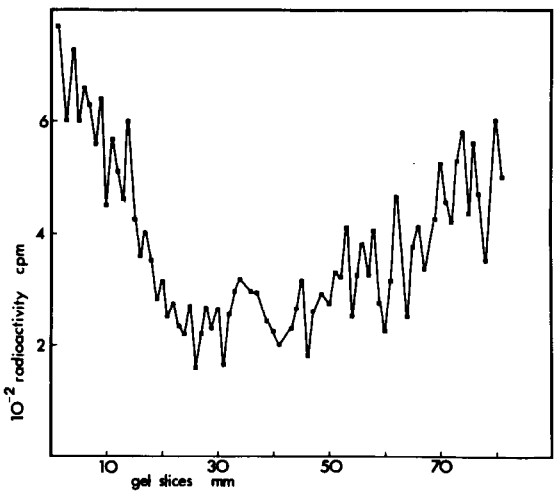
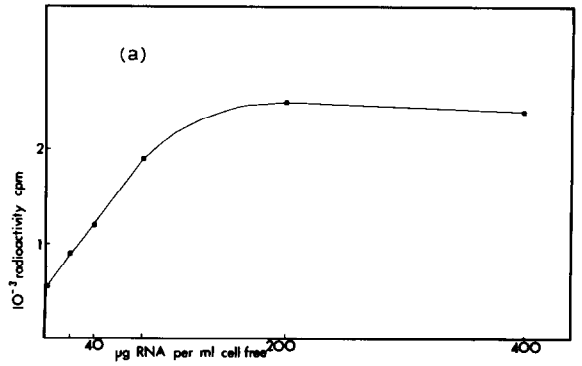


Fig.2. The *E. coli* S-30 system was set up using mitochondrial, cytoplasmic and no RNA (endogeneous control); each assay being 185 μl total volume. After incubation anti-mitochondrial antisera were added to each tube (see Materials and methods), and the precipitate analysed on SDS-polyacrylamide gels. Only the results using mitochondrial RNA are shown here.



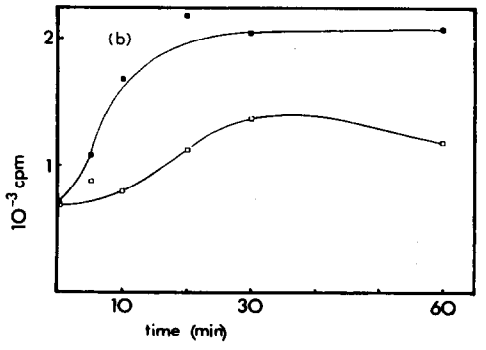


Fig.1(a). *E. coli* S-30 cell free systems (each assay 185 μl total) were prepared as described in Materials and methods, except that the mitochondrial RNA concentration was varied between 0–400 μg/ml. Incubation was at 37°C for 30 min. (b) *E. coli* S-30 cell free systems (total vol of each 350 μl) were prepared as described in Materials and methods section. 50 μl samples were removed from the assay mix for TCA precipitation at different time intervals up to 60 min. Incubation was at 37°C. (■——■) Mitochondrial RNA. (□——□) No RNA (endogeneous control) (For this experiment the RNA concentration was 250 μg/ml).

The *E. coli* S-30 system was set up again, exactly as described above, but each tube was made up to 1 M KCl and 1% Triton X-100 before addition of the anti-mitochondrial antisera. In order to compare the polypeptides synthesised in the *E. coli* S-30 system with those made by mitochondria of whole cells, yeast cells were labelled in the presence of cycloheximide, and the mitochondria separated as described in Materials and methods. A comparison of in vivo and in vitro preparations of mitochondrial proteins when analysed by polyacrylamide-SDS gel electrophoresis is shown in fig.3. The apparent molecular weights of the polypeptides from the two systems are compared in table 1.

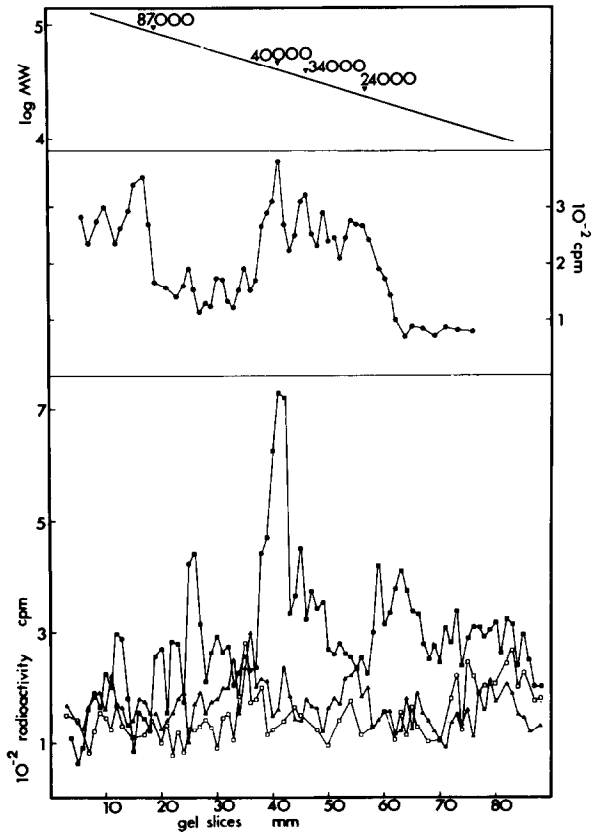


Fig.3. Mitochondrial proteins labelled in vivo were prepared and analysed as described by Eggitt and Scragg [9]. The *E. coli* cell free assay was set up as described in the legend to fig.2. After incubation of the cell free, each assay tube was made up to 1 M KCl and 1% Triton X-100 before addition of anti-mitochondrial antisera. The precipitates were analysed by SDS-polyacrylamide gel electrophoresis. (a) Mitochondrial proteins labelled with intact yeast for 2 h with [^{35}S]methionine (2 μCi/ml) and [^{3}H]leucine (10 μCi/ml), in the presence of cycloheximide (100 μg/ml) (●——●) (b) Antisera precipitates from *E. coli* S-30 system using mitochondrial RNA (■——■), cytoplasmic RNA (▲——▲), and no RNA (endogeneous control) (□——□).

Table 1
Comparison of the apparent molecular weights of mitochondrial proteins produced in vivo with antiserum-precipitated products from an *E. coli* S-30 system directed by mitochondrial RNA

In vivo products labelled in the presence of cycloheximide	Antiserum precipitate from an *E. coli* S-30 system directed by mitochondrial RNA
90 000 +	80–100 000
70 000	70 000
60 000	60 000
50 000	
39–44 600	39–44 600
34 000	34 000
28–31 500	33–31 500
22–25 000	
	22–18 000
Occasionally smaller proteins present in in vivo preparations	Some smaller proteins but not well defined

The results in the table above have been determined from fig.3.

4. Discussion

These results show that total mitochondria RNA from yeast is capable of stimulating the synthesis of discrete polypeptides in an *E. coli* S-30 system. Although the range of polypeptides produced in vivo and in vitro is similar, the relative proportions of the different polypeptides is variable between the two systems. This could be due to variable antigenicity of the different mitochondrial proteins, or it could reflect some sort of translational control. The *E. coli* ribosomes may preferentially select different messenger RNA molecules, or translate them at different speeds. Although the relative proportions of the different polypeptides is variable between the systems, the same range of polypeptides is made in both systems.

It is interesting to note that of the 8 enzyme subunits which have been shown to be made in the mitochondrial (4 of the ATPase complex [14], 3 of the cytochrome oxidase complex [15], and 1 of the cytochrome *b* complex [16]) 6 have molecular weights between 25 000 and 42 000 which coincides with the range of polypeptides always found in the in vitro systems (the high and low mol. wt material is somewhat variable between different experiments), while the two remaining polypeptides (of the ATPase complex) are 12 000 and 7800 which could correspond to the low molecular weight material found occasionally in both in vivo and in vitro preparation.

These results from the *E. coli* S-30 cell free also compare well with results obtained when total yeast mitochondrial RNA was injected into *X. laevis* oocytes [9] and also to the results obtained by Scragg and Thomas [17] using an *E. coli* system to transcribe mitochondrial DNA, and translate the resultant RNA. Three of the proteins made within a system similar to the latter represent 3 subunits of cytochrome oxidase in size and antigenicity (personal communication A. H. Scragg and D. Y. Thomas).

Recently Halbreich et al. [18] have demonstrated that RNA isolated from yeast petite mitochondria is capable of stimulating an *E. coli* cell free system. Analysis by SDS-polyacrylamide gel electrophoresis of the protein products revealed three proteins with apparent mol. wts. of 11 000, 13 500 and 17 000. It could prove useful and instructive to compare the proteins synthesised when mitochondrial RNA from wild type and from a variety of petite mutants are used in an *E. coli* protein synthesising system.

Acknowledgements

I would like to thank Dr T. S. Work and Dr A. H. Scragg for help and guidance in this work, and to thank the Medical Research Council for the scholarship enabling this work to be carried out.

References

[1] Ashwell, M. and Work, T. S. (1970) Ann. Rev. Biochem. 39, 251–290.
[2] Linnane, A. W., Haslam, J. M., Lukins, H. B. and Nagley, P. (1972) Ann. Rev. Microbiol. 26, 163–198.
[3] Coote, J. L. and Work, T. S. (1971) Eur. J. Biochem. 23, 564–574.
[4] Thomas, D. Y. and Williamson, D. H. (1971) Nature New Biol. 233, 196–199.
[5] Tzagoloff, A. and Akai, A. (1972) J. Biol. Chem. 247, 6517–6523.
[6] Weiss, H., Sebald, W., Schwab, A. J., Kleinow, W. and Lorenz, B. (1973) Biochimie 55, 815–821.
[7] Poyton, R. O. and Groot, G. S. P. (1975) Proc. Nat. Acad. Sci. USA 72, 172–176.
[8] Groot, G. S. P. and Poyton, R. O. (1975) Nature 255, 238–240.
[9] Eggitt, M. J. and Scragg, A. H. (1975) Biochem. J. 149, 507–512.
[10] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[11] Matthaei, J. and Nirenberg, M. (1961) Proc. Nat. Acad. Sci. USA 47, 1580–1587.
[12] Nirenberg, M. and Matthaei, J. (1961) Proc. Nat. Acad. Sci. USA 47, 1588–1602.
[13] Rubin, M. S. and Tzagoloff, A. (1973) J. Biol. Chem. 248, 4275–4279.
[14] Tzagoloff, A., Rubin, M. and Sierra, M. F. (1973) Biochim. Biophys. Acta 301, 71–104.
[15] Mason, T. L. and Schutz, G. (1973) J. Biol. Chem. 248, 1355–1360.
[16] Weiss, H. (1972) Eur. J. Biochem. 30, 469–478.
[17] Scragg, A. H. and Thomas, D. Y. (1975) Eur. J. Biochem. 56, 183–192.
[18] Halbreich, A., Di Franco, A., Groudinsky, O., Cosson, J. and Slonimski, P. P. (1975) Biochem. Res. Commun. 64, 1286–1292.