Protein synthesis in mitochondria isolated from the trypanosomatid protozoan *Crithidia fasciculata*

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Abstract Evidence presented over the years in support of mitochondrial translation in trypanosomes, based largely on studies using differential inhibitors such as cycloheximide and chloramphenicol, remains controversial. I have studied endogenous mRNA-dependent translation in a mitochondrial fraction isolated from the trypanosomatid protozoan *Crithidia fasciculata*. By using pancreatic ribonuclease to inactivate contaminating cytosolic activity, I show that these mitochondria can conduct protein synthesis in their own right. The mitochondrial translational products differed from cytosolic products as judged by SDS-PAGE, and had sizes expected of some proteins encoded in the mitochondrial genome of *C. fasciculata* and other trypanosomatids. Some evidence is provided suggesting that the seat of translation might be the kinetoplast.

Key words: Protein synthesis; Isolated mitochondrion; *Crithidia fasciculata*

1. Introduction

Trypanosomatid protozoa include parasites of medical and veterinary importance. They have a single large mitochondrion per cell, containing a complex mitochondrial genome, the kinetoplast. They have features suggestive of mitochondrial translation. For example, the kinetoplast has a pair of strongly conserved genes encoding two small but abundant RNA species, the 9S and 12S RNAs, which have sequence and structural similarities with ribosomal RNA (rRNA) [1–5]. Cytosolic transfer RNAs (tRNAs) are imported into the *Trypanosoma brucei* mitochondrion [6–9]. And in *Crithidia fasciculata*, some proteins associating with the 9S and 12S RNA are immunologically related to proteins co-purifying with the parasite's cytosolic ribosomes, and to riboprotein S11 of the bacterium *Escherichia coli*, suggesting a mito-ribosomal complex [10]. Yet the case for mitochondrial translation in trypanosomes has remained controversial for several important reasons. Despite much effort, no ribosomes have been isolated from trypanosome mitochondria [11,12]. No sequences encoding obvious tRNA-like structures or riboproteins have been found in the trypanosome kinetoplast, including that of *C. fasciculata* where there is little RNA editing [13]. And some important early investigations, based on differential effects of the antibiotics chloramphenicol and cycloheximide, have given contradictory results [14–19]. This may be due to variations in permeability of trypanosome membranes to antibiotics depending on conditions which varied in these studies, such as the metabolic state of cells at harvest and the intactness and age of mitochondria following isolation. I have here studied endogenous mRNA-dependent translation by a mitochondrial fraction from the trypanosomatid protozoan *C. fasciculata*. By using pancreatic ribonuclease to differentially inactivate any contaminating cytosolic activity, I show that mitochondria from this protozoan can conduct protein synthesis in their own right.

2. Materials and methods

2.1. Preparation of cytosolic and mitochondrial fractions

This procedure is summarized in Fig. 1. *C. fasciculata* was grown at 27°C to mid-log phase ($\sim 6\times10^7$ cells/ml) in brain heart infusion medium (Difco) containing 20 μg/ml hemin and 100 μg/ml carbenicillin. All fractionation procedures were at 0–4°C, using sterile buffers and equipment. Cells were harvested after two washes in buffer A (10 mM Tris-HCl pH 7.5, 10 mM magnesium acetate, 100 mM ammonium chloride, 400 mM sorbitol) and centrifugation at $3000\times g$ for 10 min. Cells were suspended in 30 ml buffer A at about 8×10^9 cells/ml. 8 g washed glass beads (425–600 μm; Sigma) were added and the cells were broken in an Omnimixer (model 17106; OCI Instruments), using 10×30 s cycles at full speed, with 30 s intervals between cycles for cooling. The homogenate was clarified three times by centrifugation at $1800\times g$ for 10 min. The supernatant, over the loose pellet, was centrifuged at $27\,000\times g$ for 15 min. The resulting supernatant was clarified again ($27\,000\times g$/15 min), and stored at −80°C in small aliquots (cytosolic fraction). The pellet from the first $27\,000\times g$ centrifugation was resuspended gently (using a Kontes glass homogenizer) in a small volume of buffer A containing 400 units/ml RNase-free DNase I (Boehringer Mannheim) and incubated on ice for 15 min. The suspension was diluted in buffer A and centrifuged at $27\,000\times g$ for 15 min. The pellet was washed twice more in buffer A by this procedure and resuspended in 0.5 ml buffer A using the homogenizer. The crude mitochondrial suspension was made up to about 40 ml with 50% Percoll (Pharmacia and Upjohn) in buffer A, and centrifuged in a Beckman Ti-70 rotor at $165\,000\times g$ for 30 min. A turbid band about two thirds the way down the gradient indicated the approximate location of the mitochondria (Fig. 1b). 1.3 ml fractions were collected from the bottom of the gradient using a peristaltic pump, and equal aliquots were assayed for translation in the presence and absence of RNase, as given in Section 2.2. The three fractions showing most translational activity were pooled, diluted to 40 ml in buffer A and centrifuged at $27\,000\times g$ for 20 min to remove Percoll. The pellet was resuspended in buffer A and washed once more as above. The final pellet was resuspended in a small volume of buffer A and stored in aliquots at −80°C (mitochondrial fraction). The fractions retained activity for several weeks, but the cytosolic fraction tended to be more sensitive to thawing than the mitochondrial fraction.

Both the cytosolic and mitochondrial fractions were checked for residual whole *Crithidia* cells and bacterial contaminants. No *Crithidia* cells were detected by phase contrast microscopy. Aliquots were used to inoculate brain heart infusion liquid cultures and agar plates. No growth of *Crithidia* was detected after several days of incubation at 27°C. Bacterial contamination was checked by spreading aliquots on L-broth plates and incubating for 36 h at 37°C. Not more than 10 contaminant bacterial colonies were found per ml of either fraction. (Note that only 0.04–0.15 ml of the fractions were used per translation assay.)

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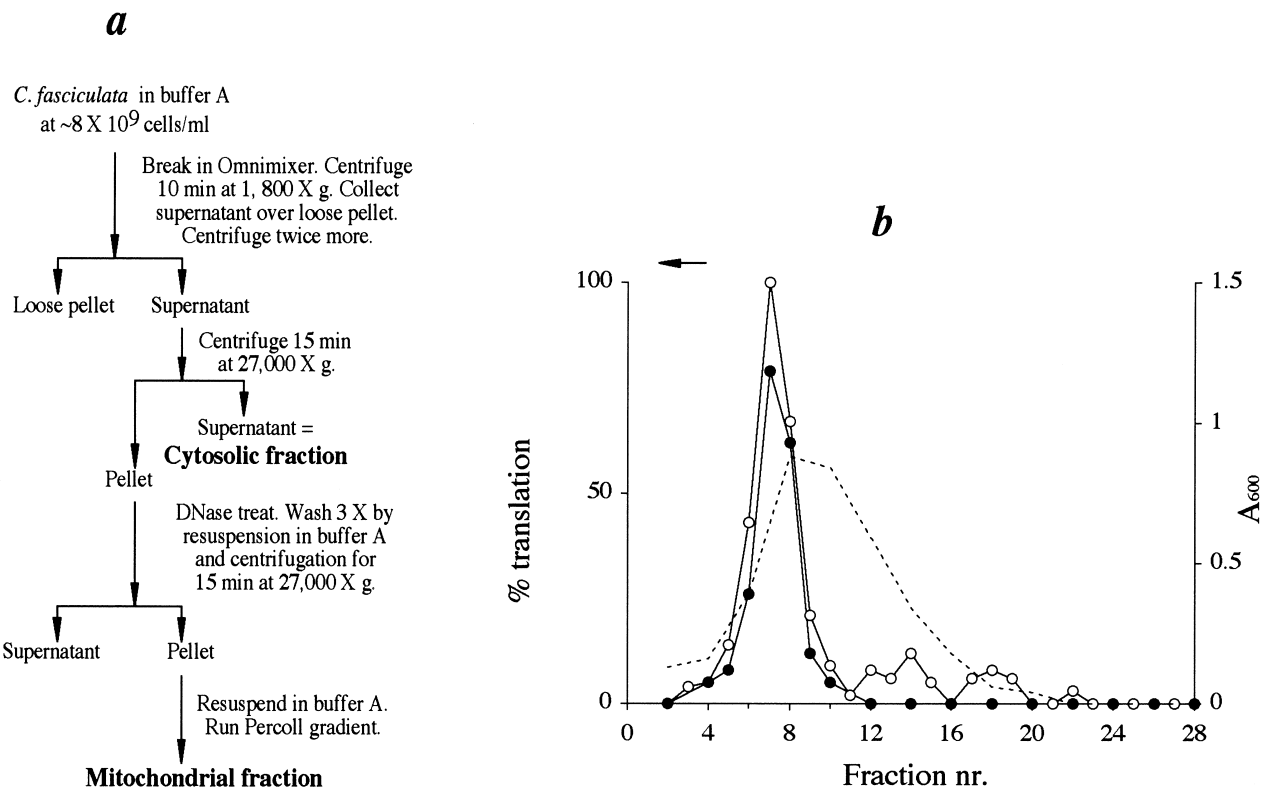

Fig. 1. Separation of cytosolic and mitochondrial fractions (see Section 2). a: Summary of procedure. b: Percoll purification of crude mitochondrial fraction. 40 μl of indicated fractions was assayed for translation directly, i.e. without removing Percoll. Translation was as given in Section 2, except that the TCA washes were all done in a beaker, i.e. without suction. Open circles, no RNase in assay; filled circles, +80 μg/ml RNase. Arrow points to bottom of gradient.

2.2. Translation assays

Translation assays were based on a standard procedure, with variations as given in figure legends. Only endogenous mRNA was used as template throughout. The standard assay had the following components in a final volume of 200 μl. 0.4 mM ATP, 0.035 mM GTP, 15 μg/ml creatine phosphokinase, 3.75 mM creatine phosphate, 6.5 mM magnesium acetate, 50 mM potassium acetate, 10 mM HEPES, 5 mM Tris-HCl (pH 7.5), 50 mM ammonium chloride, 220 mM sorbitol, and all amino acids except methionine, at 50 μM each. All ingredients except radioactive precursor were mixed on ice. L-[^{35}S]Methionine (Amersham; 1000 Ci/mmol; 25 μCi/ml final) was added and the assay tubes were shifted immediately to a 30°C water bath for 30 min. After incubation, the reaction mixture was deposited on a 2.5 mm GF/F filter (Whatman), and the filter placed in a beaker of ice-cold 5% trichloroacetic acid (TCA); separate beakers were used for each filter. After >30 min, the filter was rinsed once with 5% ice cold TCA and transferred to 5% TCA at 75°C for 15 min to hydrolyze [^{35}S]methionyl tRNA. The filter was washed under suction with 4×10 ml batches of ice cold 5% TCA and 10 ml acetone, dried, and counted for acid-insoluble radioactivity by liquid scintillation spectrometry. All values were corrected for appropriate zero time controls.

2.3. Electron microscopy

Sample fixation and staining were at 4°C. Aliquots from the cytosolic and mitochondrial fractions were prefixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer for 1 h, and fixed in 1.33% OsO_4 for 1 h. The samples were then washed in 0.1 M phosphate buffer for 1 h and stained with 10% uranyl acetate in 70% methanol overnight. The samples were dehydrated with a graded series of acetone and embedded in a mixture of Epon and Araldite. Ultrathin sections were stained with lead citrate and examined in a transmission electron microscope (EM 900; Carl Zeiss).

3. Results and discussion

3.1. Separation of cytosolic and mitochondrial fractions

Cytosolic and mitochondrial fractions were prepared ensuring that they were free of unbroken *Crithidia* cells and contaminating bacteria (Section 2.1; Fig. 1). Only the mitochondrial fraction yielded minicircle DNA (~2.5 kbp), the major component of the kinetoplast, both in a crude extract and in a procedure designed to enrich for kinetoplast DNA networks (Fig. 2a,b). Both fractions also contained much RNA. Northern blots were prepared from total RNA extracted from equivalent amounts of material from the two fractions. Probes covering the cytosolic small and large rRNA genes hybridized mainly to RNA derived from the cytosolic fraction (Fig. 2c); and a probe covering the mitochondrial 9S rRNA gene and part of the 12S rRNA gene hybridized mainly to RNA extracted from the mitochondrial fraction (Fig. 2d). In electron microscopy, the cytosolic fraction showed arrays of polyribosomes, with occasional membrane vesicles (Fig. 2e). The mitochondrial fraction showed a marked enrichment in intact mitochondria, with their characteristic double membranes and cristae (Fig. 2f,g). However, as the isolation procedure used here was controlled to minimize disruption of mitochondria, the inclusion of a small amount of cytoribosomes in the mitochondrial fraction was unavoidable.

3.2. Response of cytosolic and mitochondrial fractions to pancreatic ribonuclease

In extensively washed mitochondria such as those used in this study the contaminating cytoribosomes would have very

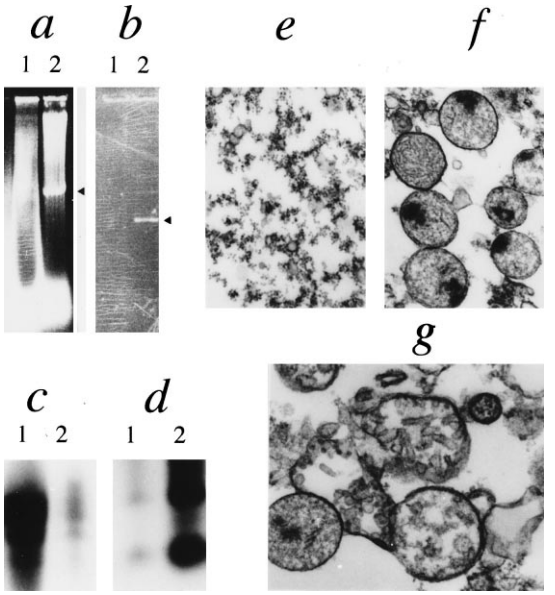

Fig. 2. Characterization of cytosolic and mitochondrial fractions. a–d: Nucleic acid analyses. Nucleic acids were extracted from equivalent amounts of each fraction as judged by the Bradford protein assay (Bio-Rad). a: Ethidium bromide stained agarose gel in which crude nucleic acids were electrophoresed. Nucleic acids were prepared by incubating fractions in 1% SDS and 200 μg/ml proteinase K (Boehringer Mannheim) for 1 h at 37°C, followed by organic extraction and ethanol precipitation. The nucleic acids were restricted with *Xba*I (which cuts most of the minicircles once) prior to electrophoresis. Lane 1, cytosolic fraction; lane 2, mitochondrial fraction. Arrow: minicircle DNA. b: Extracts enriched for kinetoplast DNA according to the procedure of Perez-Morga and Englund [24], and digested with *Xba*I. Lane 1, cytosolic fraction; lane 2, mitochondrial fraction. Arrow: minicircle DNA. c: Northern blot of total RNA extracted from equivalent amounts of material (lane 1, cytosolic fraction; lane 2, mitochondrial fraction) according to standard procedures [25], and hybridized to a mixture of plasmids carrying the *C. fasciculata* small and large subunit cytosolic rRNA genes (plasmids pCf1 and pCf2, respectively [26,27]). d: Northern blot of equivalent amounts of RNA (lane 1, cytosolic; lane 2, mitochondrial) hybridized to plasmid pIT101 [10] carrying a DNA insert covering the mitochondrial 9S rRNA gene and part of the 12S rRNA gene. e–g: Electron micrographs of material from the two fractions. e: Cytosolic fraction, original magnification 32 500×. f: Mitochondrial fraction, original magnification 20 700×. g: Mitochondrial fraction, original magnification 33 000×.

little, if any, access to soluble factors required for polypeptide synthesis. It was nonetheless essential to suppress any cytosolic activity which might contribute to translation in the mitochondrial fraction. Pancreatic ribonuclease is an enzyme which rapidly degrades mRNA, tRNA and exposed rRNA domains on ribosomes. This enzyme has, furthermore, been shown not to penetrate mitochondria isolated from the amphibian *Xenopus laevis* [20]. I therefore used pancreatic ribonuclease to differentiate between cytosolic and mitochondrial translation. As shown in Fig. 3a, 3 μg/ml of the nuclease reduced translation by the cytosolic fraction to 5%; and 25 μg/ml of the nuclease inhibited it completely. In contrast, 80% of translation by the mitochondrial fraction remained resistant to 140 μg/ml of the nuclease. (This varied from 80–95% in different experiments.) The slight sensitivity of the mitochondrial fraction to nuclease need not be due to inactivation of cytoribosomal activity alone; it could also be due to some damaged mitochondria allowing entry of the nuclease. These results demonstrate that the bulk of translational activity in the mitochondrial fraction is not of cytoribosomal origin.

Pancreatic ribonuclease (80 μg/ml) was routinely included in assays of the mitochondrial fraction to suppress sensitive translation. Fig. 3b shows the time course of protein synthesis

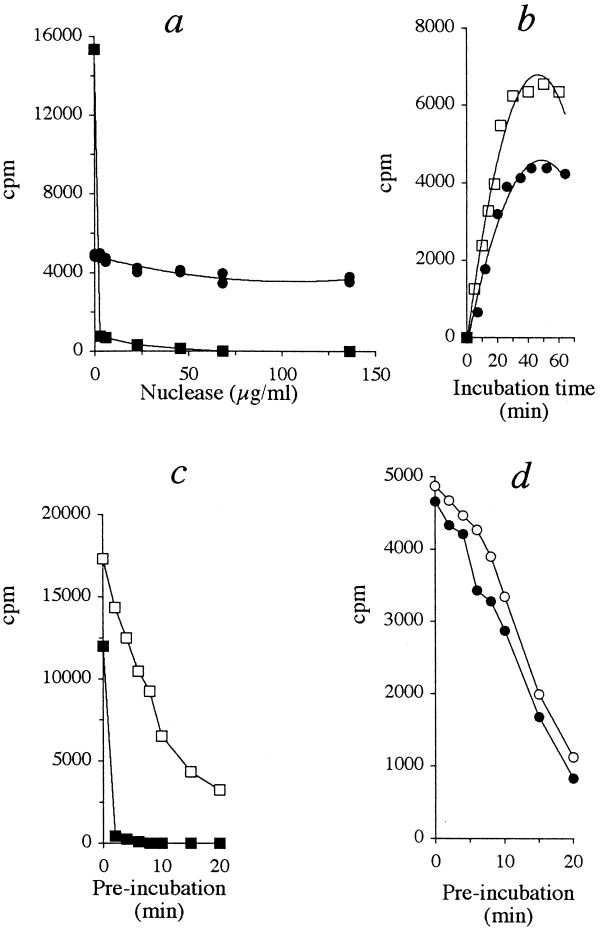

Fig. 3. Translation by cytosolic and mitochondrial fractions. a: Effect of bovine pancreatic ribonuclease (Boehringer Mannheim) on cytosolic (squares) and mitochondrial (circles) fractions. The standard (200 μl) assay was performed, except that the assay mixture was incubated with protein and nuclease for 10 min in ice before adding label and shifting to 30°C. The assay mixtures had about 22 mg/ml cytosolic protein and 50 mg/ml mitochondrial protein, respectively. b: Time-course of label incorporation by cytosolic (open squares) and mitochondrial (filled circles) fractions. Two standard assay mixtures, but in larger volumes, containing either ~10 mg/ml cytosolic protein or ~40 mg/ml mitochondrial protein, were incubated in ice for 10 min with (for mitochondrial extract) or without (for cytosolic extract) 80 μg/ml nuclease, and shifted to 30°C. At the times indicated 200 μl aliquots were withdrawn and assayed for acid-insoluble radioactivity. c,d: Effect of nuclease on incorporation of [^{35}S]methionine by cytosolic and mitochondrial fractions following periods of pre-incubation at 30°C. Values give average of duplicate estimates. The standard translation assay was performed with the following modifications. All ingredients except label, in a larger volume, were incubated in ice for 10 min and transfered to 30°C. At the indicated times after transfer (pre-incubation period), 200 μl aliquots were taken into tubes containing [^{35}S]methionine and incubation continued for a further 30 min at 30°C. Hot TCA-insoluble incorporation of label was measured as given for the standard assay. c: Cytosolic fraction. ~25 mg/ml protein was used per assay. Open squares, no nuclease; filled squares, +10 μg/ml nuclease. d: Mitochondrial fraction. ~40 mg/ml of protein was used per assay. Open circles, no nuclease; filled circles, +80 μg/ml nuclease.

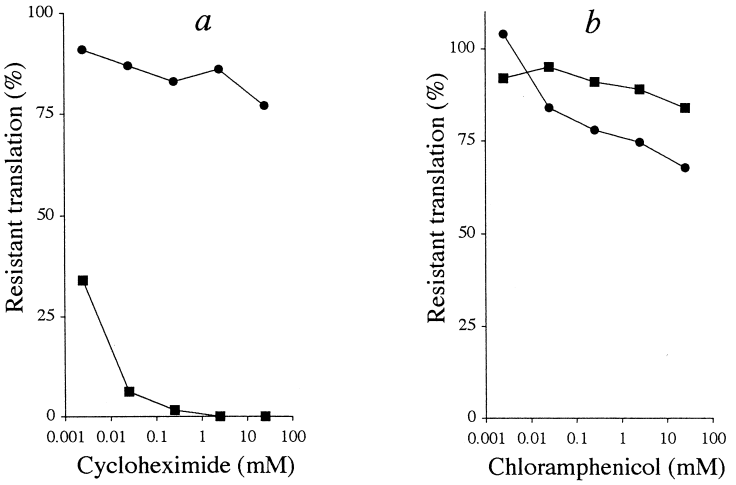

Fig. 4. Effect of antibiotics on translation by cytosolic (squares) and mitochondrial (circles) fractions. Cytosolic assay tubes had ~25 mg/ml protein; mitochondrial assay tubes had ~40 mg/ml protein. Note that the mitochondrial fraction was assayed in the presence of 80 μg/ml pancreatic ribonuclease. All ingredients, except [^{35}S]methionine, were incubated on ice for 10 min. Label was then added and the tubes shifted to 30°C. The standard translation assay was performed. The antibiotics were dissolved in DMSO. Corrections were made for reduction in translation caused by the DMSO by assaying control tubes without drug. (Tubes having the highest drug level had 2.5% DMSO, which reduced translation by ~15%.) Corrected no-drug cpm/assay were ~15 000 and ~4000 for cytosolic and mitochondrial fractions, respectively.

by the mitochondrial fraction (assayed in the presence of nuclease) and by the cytosolic fraction. Both fractions have comparable kinetics, with label being incorporated at a linear rate for about 20 min, and reaching a maximum around 40 min.

3.3. Evidence against co-translational insertion of cytosolic proteins into the mitochondria

I considered the possibility that amino-terminal mitochondrial targeting sequences of cytosolic proteins might already be inserted at 'contact sites' in the mitochondrial membranes, forming complexes with their cognate polyribosomes and mRNA [21]. If these complexes were resistant to the nuclease, the targeting sequences would be extended into the mitochondria during assay, with incorporation of label. In order to chase such polyribosomes, and to expose the mRNA to prolonged nuclease attack, I allowed the reaction mixture to translate for a period in the presence of the nuclease before adding label. Note that pre-incubation even without nuclease inhibits subsequent label incorporation in this assay (Fig. 3c,d); this may be due to depletion of translational factors, to reduced recycling of ribosomes and to messenger decay. However, whereas a 2-min pre-incubation of the cytosolic extract with 10 μg/ml nuclease was sufficient to inhibit subsequent translation completely (Fig. 3c), a 20-min pre-incubation of the mitochondrial fraction with 80 μg/ml nuclease still failed to reduce subsequent translation much below the no-nuclease control (Fig. 3d). This indicates that there is no significant extension of cytosolic targeting sequences into the mitochondrion during the assay.

Table 1
Recovery of acid-insoluble radioactivity after Triton X-100 treatment of mitochondrial fraction

Sample	cpm
Assay mixture	7659 (100%)
Pellet 1	4702 (61%)
Pellet 2	4103 (53%)
Pellet 3A	3583 (47%)
Pellet 3B	163 (2%)

Translation was performed according to the standard procedure, using about 100 mg/ml mitochondrial protein per assay, and 80 μg/ml ribonuclease. After the 30 min incubation at 30°C, the assay tube was transferred to ice and Triton X-100 was added to a final concentration of 2%. After 10 min in ice, the tube was centrifuged at 30 000×*g* for 15 min at 4°C. The supernatant was carefully removed, and the pellet (pellet 1) was gently but completely resuspended in 500 μl buffer A at 4°C. The tube was centrifuged again as above to give pellet 2. This pellet was resuspended in 500 μl buffer A and divided into two equal aliquots. To one, DNase (200 units/ml; Boehringer Mannheim) was added, and both aliquots were incubated at room temperature for 15 min. The two tubes were centrifuged again as above to give pellets 3A (no DNase) and 3B (+DNase). After removing the supernatant, the pellets were resuspended in buffer A. Acid-insoluble cpm at each stage was estimated by withdrawing small aliquots, counting as given for the standard assay, and correcting for the volumes; note that cpm obtained for pellets 3A and 3B were further corrected by a doubling to account for the halving of pellet 2. Values give the mean of triplicate estimates.

3.4. Effect of cycloheximide and chloramphenicol on translation

The antibiotic cycloheximide inhibits the peptidyl transferase activity of the 60S ribosomal subunit of eukaryotes and is therefore a potent inhibitor of cytosolic translation. Bacterial and organellar translation is resistant to this antibiotic, and cycloheximide is therefore commonly used to distinguish between cytosolic and mitochondrial translation in eukaryotes. As shown in Fig. 4a, the *C. fasciculata* cytosolic and mitochondrial fractions differed significantly in their response to this antibiotic. Cytosolic translation was completely inhibited by 0.25 mM (0.07 mg/ml) cycloheximide, whereas 80% of mitochondrial translation remained resistant to this concentration of the drug; 75% of mitochondrial translation was still resistant to 25 mM (7 mg/ml) of this drug. (Interestingly, the *C. fasciculata* cytosolic extract appears to be more sensitive to cycloheximide than a *T. brucei* extract, which retained as much as 40% activity at a drug concentration of 7 mM [22]).

Both cytosolic and mitochondrial fractions were, however, quite resistant to chloramphenicol (Fig. 4b), an antibiotic which inhibits peptidyl transferase activity of the 50S riboso-

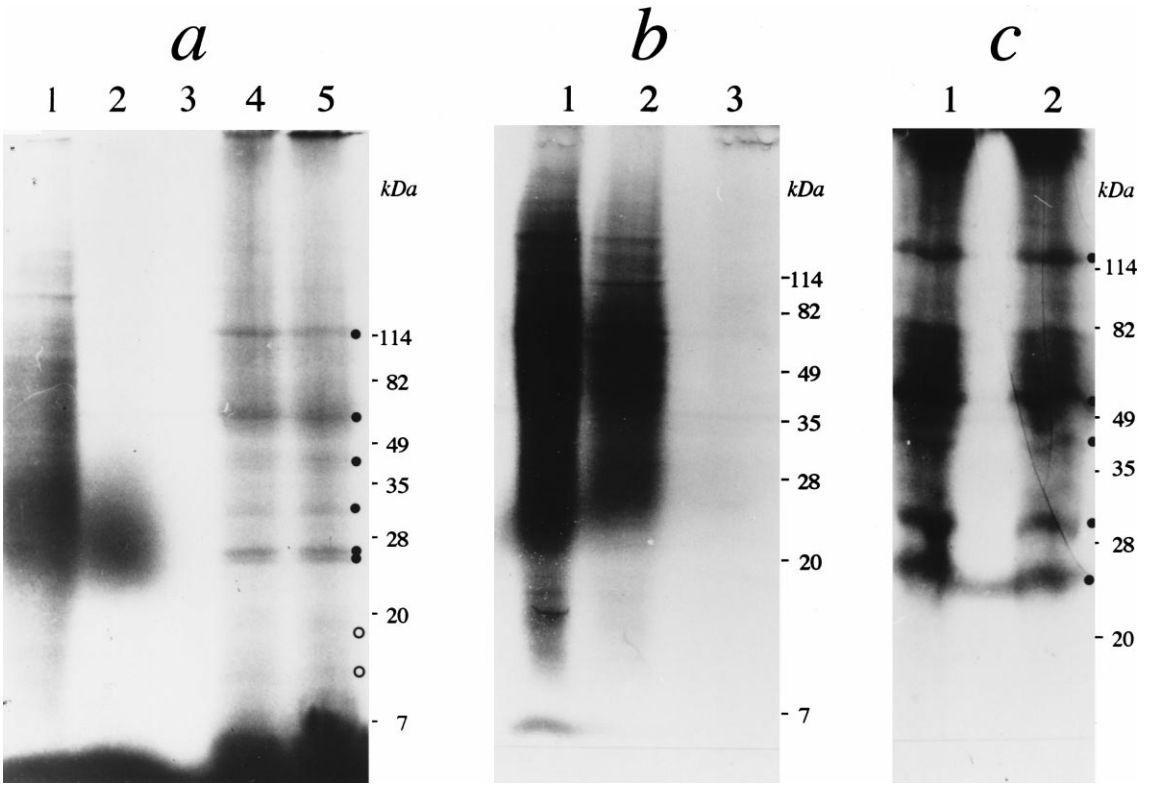

Fig. 5. SDS-PAGE on cytosolic and mitochondrial translational products. Standard translation reactions were performed with or without pancreatic ribonuclease and cycloheximide, as indicated below. The reaction mixtures contained all the unlabelled amino acids except cysteine and methionine. The assay tubes had either ~50 mg/ml cytosolic protein or ~90 mg/ml mitochondrial protein. All ingredients except label were incubated in ice for 10 min and the assay tubes were shifted to 30°C. After 2 min at 30°C, a mixture of L-[^{35}S]methionine and L-[^{35}S]cysteine (Pro-Mix, Amersham; 100 Ci/mmol) was added to each assay tube (90 μCi/ml final for cytosolic fraction; 360 μCi/ml final for mitochondrial fraction) and incubation continued for a further 35 min. The tubes were returned to ice and the contents precipitated with 50% acetone at −20°C. After evaporating off the acetone, the pellet was dissolved in Laemmli sample buffer+10% β-mercaptoethanol, sonicated briefly, and heated at 95°C for 5 min. The proteins were electrophoresed in gradient SDS polyacrylamide slab gels (10–20% for a and b; 12–17.5% for c) together with pre-stained markers (Bio-Rad), using standard procedures. The gels were fixed in a mixture of isopropanol:water:acetic acid (25:65:10) for 30 min, soaked in Amplify solution (Amersham), dried, and fluorographed. a: Effect of pancreatic ribonuclease; lane 1, cytosolic fraction; lane 2, cytosolic fraction+3 μg/ml ribonuclease; lane 3, cytosolic fraction+80 μg/ml ribonuclease; lane 4, mitochondrial fraction; lane 5, mitochondrial fraction+80 μg/ml ribonuclease. Dots mark bands probably corresponding to those dotted in c. Open circles mark two faint bands often seen on the film. b: Effect of cycloheximide on cytosolic translation; lane 1, no cycloheximide; lane 2, +0.7 μg/ml cycloheximide; lane 3, +70 μg/ml cycloheximide. c: Effect of cycloheximide on mitochondrial translation; lane 1, +80 μg/ml ribonuclease; lane 2, +70 μg/ml cycloheximide.

mal subunit in prokaryotes. This unexpected resistance of trypanosome mitochondria to chloramphenicol has been observed and commented on before; it was suggested that a nucleotide sequence on the 12S mito-rRNA of trypanosomatids, analogous to the chloramphenicol binding site on bacterial 23S rRNA, has been altered in such a way as to prevent the drug from binding [16].

Trypanosomatids have unusual properties, and the resistance of mitochondrial translation to these two antibiotics may be the result simply of reduced permeability of the mitochondrial membranes to drugs. In any case the resistance to cycloheximide at least demonstrates that the mitochondria contain a translational capability physically separated from the cytosolic system.

3.5. *Analysis of cytosolic and mitochondrial translational products by SDS-PAGE*

The translational products of the cytosolic and mitochondrial fractions gave different protein profiles in SDS-PAGE (Fig. 5). In these experiments I used [^{35}S]cysteine in addition to [^{35}S]methionine, as mitochondrial proteins in trypanosomes appear to be unusually rich in cysteine residues. The distribution of cytosolic proteins was relatively even in the gel and, as expected, translation was highly sensitive to both pancreatic ribonuclease and cycloheximide (Fig. 5a,b). In contrast, translation by the mitochondrial fraction produced preferentially a limited set of proteins, of approximately 15, 20, 25, 30, 40, 55 and 120 kDa (Fig. 5a). Their synthesis was resistant to both ribonuclease and cycloheximide (Fig. 5a,c). This greatly reduces the likelihood that these proteins are, say, translated off a limited set of nuclear encoded mRNAs by cytosolic ribosomes sequestered by mitochondria during isolation. It is more likely that these proteins represent translation products of mRNAs normally available only to a mitochondrial protein synthesising system. The sizes of some proteins encoded by the maxicircle component of the kinetoplast of *C. fasciculata* (deduced using the mean value of 135 Da/amino acid) do indeed fall within the size range of proteins synthesized by the mitochondrial fraction. These include cytochrome *c* oxidase subunit II, i.e. COII (~29 kDa), COIII (~41 kDa), maxicircle unidentified reading frame 2, i.e. MURF2 (~50 kDa) and NADH dehydrogenase subunit 7, i.e. ND7

(~54 kDa) [23]. Other proteins encoded by trypanosome mitochondrial genes not yet fully characterized in *C. fasciculata* also fall within this size range; for example, in *T. brucei* one of the G versus C strand biased genes, i.e. CR1, is ~25 kDa [23]. Other CR genes may account for faint bands of ~15–20 kDa often seen in these experiments (e.g. Fig. 5a). The ~120 kDa protein (Fig. 5a,c) may be an unprocessed precursor or a protein unique to *C. fasciculata*, whose maxicircle is larger than those of *Leishmania* or *Trypanosoma*.

3.6. Translation probably occurs in the kinetoplast

When the mitochondrial fraction, after translation, is washed in Triton X-100 and centrifuged, a significant proportion of acid precipitable counts sediments in the pellet (Table 1). However, much of this label cannot be re-sedimented if the pellet is treated with DNase, indicating that labelled polypeptides are trapped in complexes within the kinetoplast network. The kinetoplast may therefore be a major site of translational activity within the mitochondrion.

4. Conclusion

The cell fraction used in this study was highly enriched in mitochondria as judged by nucleic acid analyses and by electron microscopy. Translation by this fraction was virtually unaffected by pancreatic ribonuclease at a concentration >3-fold that needed to completely inhibit translation by a fraction enriched in cytosolic ribosomes. Prolonged pre-incubation of the mitochondria in nuclease prior to label addition did not significantly reduce subsequent acid-insoluble label incorporation below the no-nuclease controls, arguing against co-translational activity by cyto-ribosomes. In the presence of ribonuclease and cycloheximide the mitochondrial fraction synthesised preferentially a class of proteins similar in size to those encoded by some maxicircle genes. And about half the acid-insoluble translation products in the mitochondrial fraction appeared to sediment with the kinetoplast. Taken together, these results provide compelling evidence that the ribonuclease resistant protein synthesis observed in the mitochondrial fraction from *C. fasciculata* is due not to contaminating cytosolic activity, but to a translational capability residing within the mitochondrion itself, possibly in the kinetoplast.

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