Localization of riboproteins in a trypanosomatid mitochondrion

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Abstract There is growing evidence in support of mitochondrial translation in trypanosomes but mitoribosomes have never been characterized or localized in these parasites. On RNA–protein blots we identified several proteins from the trypanosomatid *Crithidia fasciculata* which bound the parasite's 12S and 9S mitochondrial ribosomal RNAs. Two of these proteins had significant amino acid sequence homology to riboproteins S8 and S21 across phyla. Immunoelectron microscopy revealed that antibodies raised against the two proteins react with matrix components in the *C. fasciculata* mitochondrion. Our data thus provide, we believe for the first time, evidence for the presence of riboproteins within a trypanosomatid mitochondrion, bound, possibly, to the 12S and 9S RNAs. The proteins were immunologically related to two cytosolic riboproteins which were also of identical size, suggesting the interesting possibility that the same set of riboproteins is shared between the cytosol and the mitochondrion in this parasite.

Key words: RNA–riboprotein complex; Mitochondrial localization; *Crithidia fasciculata*

1. Introduction

Trypanosomes are protozoan parasites of medical and veterinary importance. The major transcripts in their single mitochondrion are two mitochondrially encoded RNAs of 12S and 9S which have domains corresponding to bacterial ribosomal RNAs [1,2]. Mitochondrial translation in trypanosomes has been a controversial issue but there is growing evidence in support of it. For example, nuclear encoded transfer RNAs are targeted into the trypanosome mitochondrion [3,4]; and whole cells, crude mitochondrial extracts and purified mitochondria conduct protein synthesis in conditions known to reduce or eliminate cytosolic translation, such as in the presence of cycloheximide or ribonuclease [5–10]. Yet despite observations that the 12S and 9S RNAs can interact in vivo with several proteins [11], and that RNA–protein complexes containing the 12S and 9S RNAs and/or nascent peptides sediment at velocities different from those expected of cytosolic ribosomes [7,9], no mitoribosomal particle has so far been characterized in any trypanosome. This has prompted speculation as to whether the trypanosome mitoribosome is an unusual, even highly reduced, structure not responding to standard extraction procedures [12]. As a first step in investigating the nature of the trypanosome mitoribosome, we probed renatured protein blots of the insect trypanosome, *Crithidia fasciculata*, with radiolabelled transcripts derived from the parasite's 12S and 9S RNA genes. We identified several positive proteins which resemble riboproteins. In immunoelectron microscopy, antibodies raised against two of these proteins reacted strongly with components within the mitochondrion. Our data thus localize, for the first time, a class of riboproteins within the *C. fasciculata* mitochondrion, complexed, possibly, with the 12S and 9S RNAs.

2. Materials and methods

2.1. Protein purifications

C. fasciculata was grown at 27°C to late log phase ($\sim 8\times10^7$ cells/ml) in brain heart infusion medium (Difco) containing 20 μg/ml hemin and 100 μg/ml carbenicillin. All subsequent operations were at 0–4°C. Cells were harvested following two washes in phosphate buffered saline (PBS) and centrifugation at $3000\times g$ for 10 min. Cells were resuspended in buffer A (50 mM Tris–HCl pH 7.5, 1 mM EDTA, 50 mM ammonium sulfate, 1 mM dithiothreitol, 5% glycerol), broken as described previously [10], and the lysate clarified by two centrifugations at $1800\times g$ for 10 min. The supernatant over the loose pellet ('whole cell lysate') was centrifuged at $27\,000\times g$ for 15 min. The resulting pellet of enriched mitochondria was resuspended in buffer A and disrupted in a sonicator (Heat Systems) in volumes of 10 ml for 10 s using the 12.7 mm tip at setting 3. The lysate was treated with 400 U/ml RNase-free DNase I (Boehringer Mannheim) for 15 min on ice and clarified by centrifugation at $3000\times g$ for 10 min. The clarified lysate ('mitochondrial lysate') was fractionated by precipitation with ammonium sulfate, and the precipitates resuspended in and dialyzed extensively against buffer B (buffer A plus the following protease inhibitors: 0.1 mM phenylmethylsulfonyl fluoride, 0.05 mM TPCK, 0.005 mM TLCK, and 0.002 mg/ml pepstatin).

The 20–60% ammonium sulfate fraction from the mitochondrial lysate was applied to a column of heparin Sepharose CL-6B beads (Pharmacia Biotech) prepared according to the manufacturer's instructions. After washing extensively with buffer B, the bound proteins were eluted with a gradient of 0–2 M NaCl in buffer B. Fractions containing the 12S and 9S RNA binding proteins were monitored using RNA–protein blots (see Section 2.3). Relevant fractions were pooled and dialyzed extensively against buffer B. The pooled protein was applied to a 20 ml DNA Sepharose column prepared by coupling calf thymus DNA (Sigma) to CNBr-activated Sepharose 4B (Pharmacia Biotech) using standard procedures. After washing with buffer A, proteins were eluted with a gradient of 50–1000 mM ammonium sulfate in buffer B and monitored using RNA–protein blots.

Cytosolic ribosomes from *C. fasciculata* were purified and their subunits separated essentially as described by Gray [13].

2.2. Radiolabelled transcripts

Inserts a–f (Fig. 1A) were prepared by digesting the ~16 kb *Hin*dIII fragment (D5D1 fragment) of the *C. fasciculata* maxicircle [6,11] which contains the 12S and 9S RNA genes. The inserts were cloned into the Riboprobe Gemini System transcription vectors pGEM-3Z and 4Z (Promega) which had been restricted appropriately at their

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multiple cloning sites. The plasmids containing the inserts were linearized at restriction sites corresponding to the arrowheads on the inserts in Fig. 1A. They were then used as templates to produce radiolabelled run-off transcripts with either T7 or SP6 RNA polymerase (Promega) as appropriate, and [α-^{32}P]UTP (Amersham), according to Promega. Note that depending on the clone, a maximum of 30 extra bases from the pGEM plasmid, extending from the polymerase start site to the point of insertion of the maxicircle fragment on the cloning site, were included in the riboprobe. A sample of each radioactive probe was analyzed for size and counts prior to hybridization; the RNA transcripts had specific activities between $\sim$0.5 and 1.6×10^8 cpm/μg. Control radioactive riboprobes (Fig. 1A, probe f) were obtained from a region of the maxicircle (fragment R1D1 [6,11]) lying well outside the 12S/9S genes.

2.3. RNA–protein blots

Procedures were based on Cormack et al. [14]. Proteins were separated in 10–20% gradient SDS–PAGE gels and electroblotted onto a PVDF membrane (Millipore) using standard procedures. Efficient transfer was verified by checking passage of prestained protein markers from gel to membrane. After transfer the filter was washed and stored in TEN50 buffer (10 mM Tris–HCl, pH 8.0, 1 mM EDTA, 50 mM NaCl) at 4°C for about 20 h to renature bound protein. The filter was incubated with rolling for 90 min at 44°C in blocking buffer (TEN50 containing 0.02% Ficoll-400, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin (BSA, fraction V) and 250 μg/ml yeast RNA (Sigma)). Radioactive riboprobe in amounts specified in the figure legends, and yeast RNA to 1000 μg/ml final were added to the hybridization bottles and incubation continued for a further 90 min. Filters were washed at room temperature for 10 min in TEN50, 10 min in TEN50+200 mM NaCl, and 10 min in TEN50+500 mM NaCl, dried, and exposed to X-ray film.

2.4. Peptide sequencing

Protein in the gel band was reduced and alkylated with iodoacetamide, cleaved with trypsin, and the resulting peptides extracted. Part of the sample was desalted on Poros R2 beads in nano-scale using the nano-spray needles for the mass spectrometer. Analysis was performed with an electrospray tandem mass spectrometer (Q-tof, Micromass, Manchester, UK) using the manufacturer's software (Masslynx v3.2) for interpretation.

2.5. Antibody production

For production of polyclonal sera, protein bands m30 and m18, excised from gels (Fig. 2), were crushed, suspended in complete Freund's adjuvant and injected into rabbits. Polyclonal sera were affinity purified according to standard procedures [15] against corresponding protein bands which had been separated by SDS–PAGE and transferred to nitrocellulose membranes. Western blotting was done according to standard protocols on PVDF membranes using affinity purified antibodies. The blots were developed using alkaline phosphatase conjugated to goat anti-rabbit IgG (Bio-Rad).

2.6. Immunoelectron microscopy

Log phase *C. fasciculata*, washed in PBS, were fixed in 1.5% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3) for 20 min at room temperature. The fixed parasites were washed twice by centrifugation through PBS over a layer of fetal calf serum, and once through PBS supplemented with 50 mM glycine, to quench unreacted aldehyde groups. After blocking and permeabilizing with PBS containing 0.2% BSA, 0.1% fish gelatin and 0.1% saponin for 30 min, the parasites were incubated for 3 h at room temperature with affinity purified rabbit anti-m30 or anti-m18 antibodies, and washed in PBS for 30 min. The parasites were then incubated for 2 h at room temperature with biotinylated F(ab)′$_2$ fragments of donkey anti-rabbit IgG (Amersham) in PBS containing 0.2% BSA and 0.1% saponin. After washing in PBS, the parasites were incubated with peroxidase-conjugated streptavidin (Jackson Immunoresearch Laboratories) overnight at 4°C. After washing in PBS, the peroxidase activity was revealed with a substrate containing 0.05% 3,3′-diaminobenzidine tetrahydrochloride (Sigma) and 0.03% H_2O_2. The parasites were then fixed in 1.33% OsO_4 for 30 min, dehydrated in acetone, and embedded in a mixture of epon and Araldite (Fluka). Ultrathin sections were examined in a Zeiss EM 900 electron microscope without additional counterstaining. The specificity of the staining was confirmed by replacing primary antibodies with normal rabbit serum.

3. Results

3.1. Identification of 12S and 9S RNA binding proteins

Even on heavily loaded gels, riboprobes from the 12S and 9S genes (Fig. 1A) bound only to a limited set of proteins in the 20–60% ammonium sulfate fraction from a *C. fasciculata* mitochondrial lysate (Fig. 1B). Proteinase K treatment of the lysate (but not RNase or DNase treatment) abolished this binding (not shown). No signal was obtained with control radioactive riboprobes derived from a region of the maxicircle

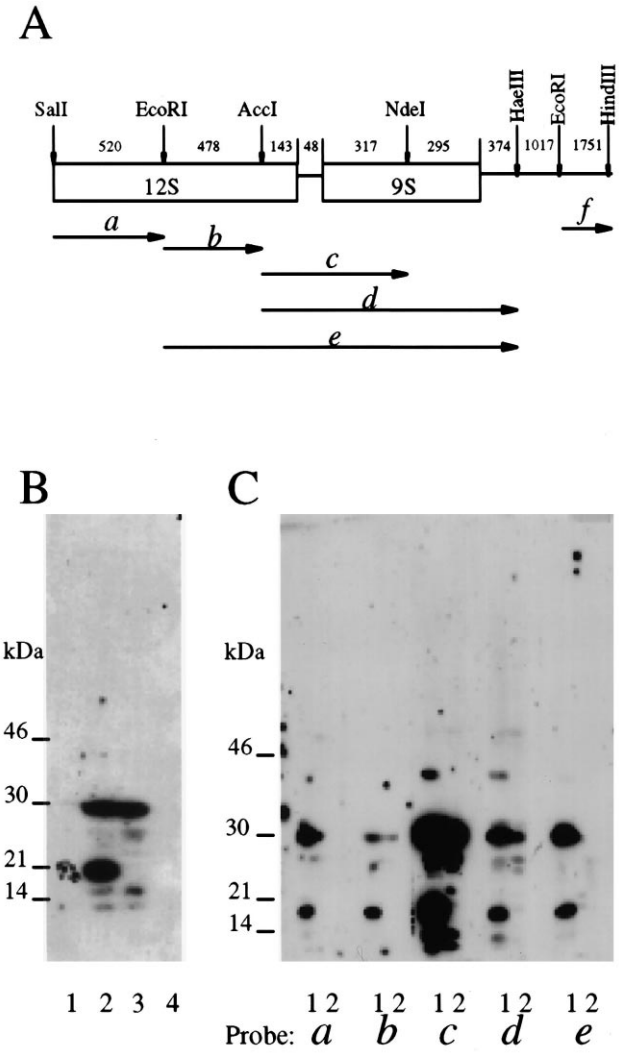

Fig. 1. Identification of 9S/12S RNA binding proteins. A: Part of *C. fasciculata* maxicircle DNA (not to scale) showing 12S and 9S RNA genes. Vertical arrows show relevant restriction sites; numbers give base pairs. Lettered horizontal arrows cover regions inserted into vectors for transcribing riboprobes, and point in the direction of transcription. B: Autoradiogram of protein blot of ammonium sulfate fractions from mitochondrial lysate probed with riboprobe c (8×10^5 cpm/ml) using standard protocol. Lane 1, 0–20% ammonium sulfate cut; lane 2, 20–40%; lane 3, 40–60%; lane 4, 60% saturation. Equivalent amounts of protein from each ammonium sulfate fraction were applied. C: Autoradiogram of protein blot of 20–40% (lane 1) and 40–60% (lane 2) ammonium sulfate cuts from whole cell lysate probed with riboprobes a, b, c, d and e, using standard protocol, but with only 250 μg/ml yeast RNA as competitor; equivalent amounts of protein from each ammonium sulfate fraction were applied. Probes a–e were each used at 5×10^5 cpm/ml, probe e at 2×10^5 cpm/ml.

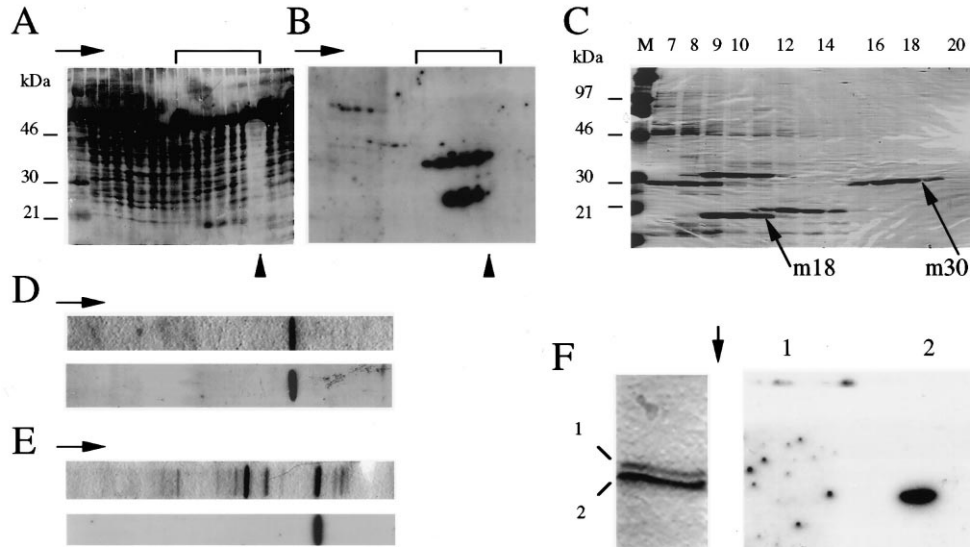

Fig. 2. Purification of 9S/12S RNA binding proteins. A, B: Analysis of protein eluting from heparin Sepharose column. Relevant column fractions were separated in a 10–20% SDS–PAGE gel, blotted onto a PVDF membrane and probed with probe c. B: Autoradiogram of membrane. A: Same membrane stained, post-exposure, with Coomassie blue. Arrows on top margin point to increasing salt in eluate; fractions under brackets eluted between ~1 and 1.4 M NaCl. Arrows on bottom margin show gel lane partially blocked by air bubble. C–E: Purification on calf thymus DNA–Sepharose of pooled positive fractions (bracketed in A and B) from the heparin-Sepharose column. C: Relevant fractions eluting from the calf thymus DNA–Sepharose column were separated in a 10–20% SDS–PAGE gel. Figure shows gel after silver staining and air drying. M: prestained standards. Bands containing m30 (fractions 15–19) and m18 (fractions 9–11) are arrowed. D, top panel: Silver stained gel lane in which material from fraction 17 (panel C) was separated; bottom panel: autoradiogram of an identical lane after blotting and probing with riboprobe c; arrow: direction of electrophoresis. E, top panel: Coomassie blue stained gel lane in which material from fraction 10 (panel C) was separated; bottom panel: autoradiogram of an identical lane after blotting and probing with riboprobe c; arrow: direction of electrophoresis. F: Further purification and identification of m18 by prolonged electrophoresis. Coomassie blue stained m18 band (E, top panel) was excised, crushed in sample buffer, loaded on to a 20–25% gradient gel and subjected to prolonged electrophoresis, when the band resolved into two (left panel). The two bands were carefully excised, electrophoresed in separate lanes of a softer gel, blotted, probed with probe c, and autoradiographed (right panel); arrow: direction of electrophoresis.

(Fig. 1A, probe f) lying outside the 12S/9S genes (not shown). We selected probe c for further work as it consistently gave the best signal (Fig. 1C). On the assumption that the positive proteins derive from mitochondria, we proceeded to purify the 20–60% ammonium sulfate cut from the mitochondrial lysate on a heparin Sepharose column. In stringent conditions, i.e. when hybridized in the presence of 1000 μg/ml competitor yeast RNA and washed in 500 mM NaCl, probe c bound to a set of proteins in the 15–30 kDa range eluting in a broad peak from this column (Fig. 2A,B). We pooled these fractions and purified them further on a calf thymus DNA–Sepharose column. A 30 kDa protein binding to probe c eluted from this column in fractions containing no other protein as judged by silver staining (Fig. 2C,D). An 18 kDa protein binding to probe c co-eluted with a few other proteins (Fig. 2C,E), but we were able to purify it further by prolonged electrophoresis

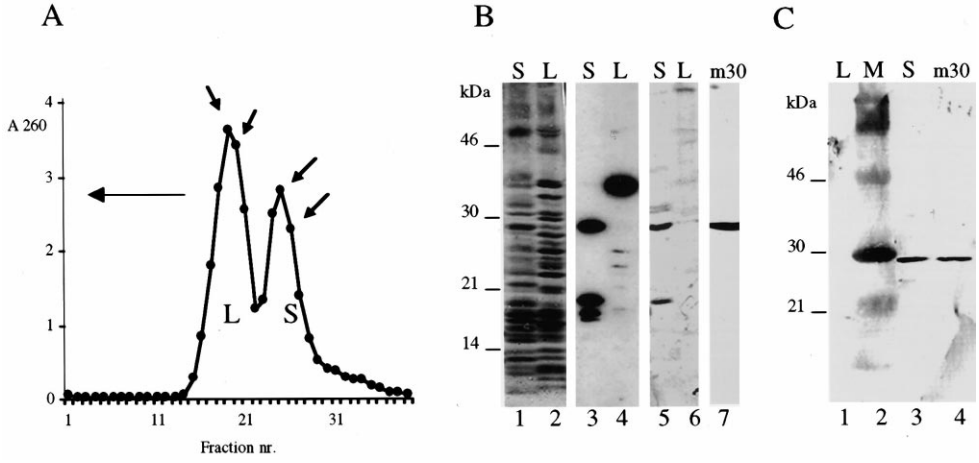

Fig. 3. 9S/12S RNA binding proteins resemble riboproteins. A: Sucrose gradient profile of separated large (L) and small (S) cytosolic ribosomal subunits. Long arrow shows direction of sedimentation; small arrows point to fractions used in the blots. B and C: Protein blots of single gels. Proteins analyzed are indicated at the top of each lane: S (small subunit); L (large subunit); m30; and M (prestained markers). B: Lanes 1 and 2, Coomassie blue stained filter. Lanes 3 and 4, autoradiogram after probing with riboprobe c. Lanes 5 and 6, Western blot probed with the anti-m18 antibody. Lane 7, Western blot probed with the anti-m30 antibody. C: Western blot, probed with anti-m30.

A

m30:	VLEGAELSFYQK
Lm:	lLEGAELqFYlK
Zm:	iLEGAELeFYmK
Hs:	VLEGAELeFYlr

B

m18:	NEEGENVDLYLPR
Sp:	NEaGqlVDLYvPR
Zm:	NEEGktVDLYvPR
Rn:	NdaGEfVDLYvPR

Fig. 4. Alignment of m30 and m18 peptides with matching ribosomal subunit proteins S8 (A) and S21 (B), respectively. Mismatches are in lower case letters. *Lm*: *Leishmania major*, SwissProt accession number P25204; *Zm*: *Zea mays*, SwissProt accession number Q08069 (panel A) and EMBL accession number Y09636.1 (panel B); *Hs*: *Homo sapiens* [25]; *Sp*: *Schizosaccharomyces pombe*, SwissProt accession number P05764; *Rn*: *Rattus norvegicus*, SwissProt accession number P05765.

in a 20–25% gradient gel (Fig. 2F). We shall refer to these two proteins, obtained from the mitochondrial lysate, as m30 and m18, respectively, to distinguish them from similar proteins obtained from a cytosolic ribosomal fraction (see below).

3.2. Proteins m30 and m18 resemble riboproteins

We asked if any cytosolic riboproteins have 12S and 9S RNA binding domains. We purified *C. fasciculata* cytosolic ribosomes, separated them into their large and small subunits in low magnesium sucrose gradients, and resolved their proteins by SDS–PAGE (Fig. 3A; B, lanes 1 and 2). On RNA–protein blots, probe c bound strongly to riboproteins of 30 and 18 kDa, and relatively weakly to riboproteins of ~16 and ~17 kDa from the small subunit (Fig. 3B, lane 3). Probe c bound strongly also to a riboprotein of ~35 kDa from the large subunit (Fig. 3B, lane 4). The control RNA probe (Fig. 1A, probe f) failed to bind to any of these riboproteins (not shown). We shall, for convenience, refer to these 30 and 18 kDa riboproteins as r30 and r18, respectively, to distinguish them from m30 and m18, which we purified from the mitochondrial lysate (see above).

Antibodies raised against m30 and m18 cross-reacted with these 12S and 9S RNA binding cytosolic riboproteins. Thus polyclonal antibodies raised against m30 reacted with a 30

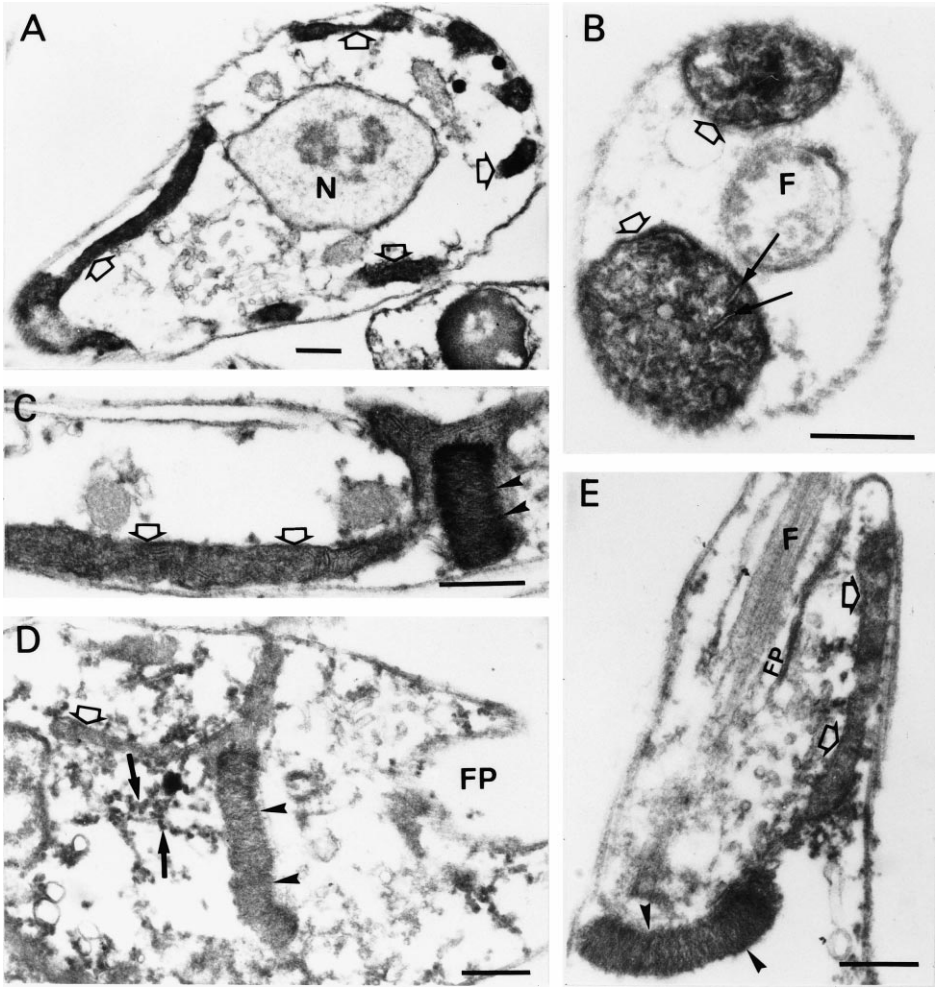

Fig. 5. Electron micrographs of parasites sectioned after staining with anti-m30 (A–C) or anti-m18 (D, E) antibodies. Open arrows: mitochondrial lobes; arrowheads: kinetoplasts; thin arrows: cristae; thick arrows: cytosolic polyribosomes; F: flagellum; FP: flagellar pocket; N: nucleus. Bar: 0.5 μm.

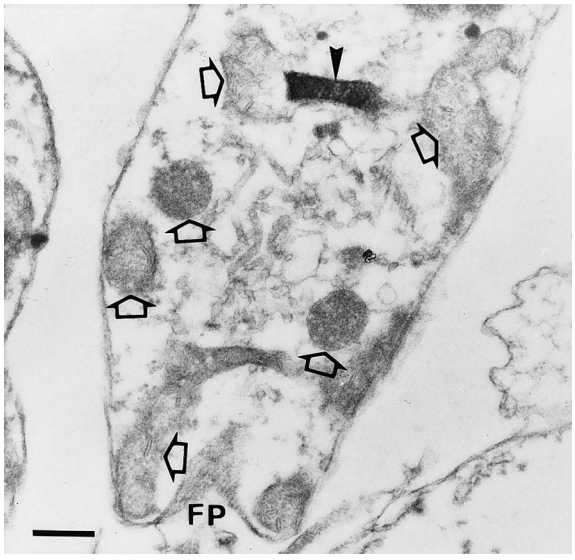

Fig. 6. Electron micrograph of a parasite sectioned after staining with normal rabbit serum. For labelling code see Fig. 5. Bar: 0.5 μm.

kDa protein from the cytosolic ribosomal small subunit (Fig. 3C, lane 3); this immunopositive band aligns with r30 in the RNA–protein blot and probably represents the same riboprotein (Fig. 3B, compare lanes 7 and 3). Antibodies raised against m18 also reacted only with proteins from the small subunit, but these antibodies were less specific. They bound to an 18 kDa protein which aligns with r18, suggesting similarity (Fig. 3B, compare lanes 5 and 3). The m18 antibody bound also to a 30 kDa riboprotein; alignment with the RNA–protein blot suggests that this 30 kDa protein is r30 (Fig. 3B, compare lanes 5 and 3).

We do not know if the binding of anti-m18 antibody to both the 18 kDa and 30 kDa riboproteins is due to the presence of common epitopes on these proteins, or to a peptide from the 30 kDa protein contaminating the m18 gel band (Fig. 2F) that we injected for antibody production. In any case, the blotting data clearly demonstrate that m30 and m18 are very similar, if not identical, to cytoriboproteins r30 and r18, respectively.

We confirmed the riboprotein nature of m30 and m18 by analyzing their amino acid sequence. Peptides derived from m30 and m18 had sequences highly homologous to riboproteins S8 and S21, respectively, across several phyla. This is illustrated in Fig. 4, where the sequences of two peptides, analyzed via ms fingerprinting and ms/ms sequencing, are aligned with representative homologous sequences from database searches.

3.3. Antibodies against m30 and m18 react with components within the mitochondrion

Binding of 12S and 9S RNA to m30 and m18 suggested that these proteins or their homologues might be localized in the mitochondrion. Preparations of mitochondria tend to retain cytosolic components such as polyribosomes attached tightly to the mitochondrial outer surface. Although the activity of these contaminants may be suppressed in in vitro assays [10] the contaminants themselves are virtually impossible to clear. This is a limitation if using subcellular fractionations alone to localize a particular component in a mitochondrial fraction. We therefore used immunoelectron microscopy to determine with greater certainty if mitochondria contain proteins similar to m30 and m18.

Fig. 5 shows fixed *C. fasciculata* parasites sectioned in various planes after staining with anti-m30 (panels A–C) or anti-m18 antibodies (panels D, E). We can readily identify positively stained mitochondria by their characteristic ramifications [16] and their disk-shaped genome (kinetoplast) lying perpendicular to the flagellar base [17]. The immunostaining is clearly intramitochondrial. In ultrathin sections of mitochondria the depositions of the electron-dense reaction product are uniformly distributed inside the mitochondrial matrix, contrasting with unstained intermembrane spaces of cristae (Fig. 5B,C). This is further illustrated by staining in sections cutting through the kinetoplast into adjoining mitochondrial lobes, and by the matrix staining in transverse sections of mitochondrial lobes lying alongside the flagellar base (Fig. 5B). There was no staining of mitochondria in control experiments in which primary antibody was replaced with normal rabbit serum (Fig. 6).

Note that the anti-m30 antibody, which bound to a cytosolic riboprotein in a Western blot (Fig. 3C, lane 3), does not stain cytosolic ribosomes to any significant degree in sectioned parasites (Fig. 5A–C); the anti-m18 antibody, by contrast, stains cytosolic components, possibly polyribosomes, as well as mitochondria (Fig. 5D,E; see Section 4).

4. Discussion

We have shown that two proteins, m30 and m18, from a *C. fasciculata* mitochondrial lysate bind to the parasite's 12S and 9S RNAs in stringent conditions in vitro; i.e. in competition with 1000 μg/ml yeast RNA, and at 500 mM NaCl. The proteins, furthermore, did not bind to RNAs derived from a part of the maxicircle lying outside the 12S and 9S RNA genes. Thus 12S and 9S RNA appears to have a specific affinity for m30 and m18. These proteins resemble riboproteins by two criteria. First, antibodies raised against the two proteins react with *C. fasciculata* cytoribosomal proteins of identical size which themselves bind 12S and 9S RNA. Second, amino acid sequences from m30 and m18 match riboproteins S8 and S21, respectively, from a variety of organisms. We also found that antibodies raised against m30 and m18 stain components in the mitochondrial matrix. Our observations suggest that riboprotein-like proteins are located within the *C. fasciculata* mitochondrion, possibly in complexes with 12S and 9S RNA.

Their close immunological, RNA binding and size relationships suggest that m30 and m18 are identical to r30 and r18, respectively; i.e. that they are the same set of riboproteins shared between the cytosol and the mitochondrion to perform related functions. We are investigating the m30/m18 complexes in greater detail to confirm this novel and evolutionarily interesting possibility.

In UV cross-linking studies of *C. fasciculata*, Leegwater et al. [18] have shown that several poly(U) binding proteins, including a 30 kDa protein, also bind to U-tailed 12S and 9S RNAs in vivo. These tails are added post-transcriptionally, probably via a terminal uridylyltransferase, an enzyme which has been detected in trypanosome extracts [19,20]. Our riboprobes, which were obtained by direct transcription of cloned

12S and 9S genes in vitro, do not have such poly(U) tails; and when probing blots with labelled synthetic poly(U) RNA, we failed to see any significant binding to m30 (not shown). Further, m30 is not immunologically related to the 30 kDa poly(U) binding protein reported by Leegwater et al. [18] (R. Benne, personal communication). It is therefore unlikely that m30 belongs to the class of poly(U) binding proteins reported by these authors.

In trypanosomes the 12S and 9S RNAs appear to be transcribed as polycistronic precursors containing large stretches (>1.2 kb) of RNA upstream of the 12S gene [21] and processed subsequently. Also, the steady-state levels of 12S and 9S RNAs may be regulated developmentally [21]. Are m30 and m18 enzymes involved in these processes? Although we cannot exclude this at present, it seems unlikely for several reasons. Firstly, the transcripts we used are devoid of large upstream stretches of RNA or poly(U) tails upon which such enzymes might act. Secondly, to the best of our knowledge, no riboproteins which also process rRNAs have been reported in either bacteria or eukaryotes. Lastly, m30 and m18 appear to bind to all parts of the 12S and 9S RNA, albeit with varying affinities (Fig. 1C), suggesting that these RNAs serve more as a scaffold for these proteins than as an enzymatic substrate.

The anti-m30 antibody cross-reacted with a 30 kDa cytoriboprotein (r30) in Western blots (Fig. 3C, lane 3) but failed to stain cytoribosomes in sectioned parasites (Fig. 5A–C); the anti-m18 antibody, on the other hand, did stain cytosolic components, possibly polyribosomes (Fig. 5D,E). Note that we sectioned the parasites only *after* permeabilization and treatment with primary antibody; r30 would escape staining in this procedure if it is a concealed cytoribosomal protein not accessible to primary antibody. By this reasoning, the mitochondrial protein which is stained by the anti-m30 antibody must be an exposed protein. If this m30 homologue is part of a mitoribosomal particle, such a surface location would suggest that the particle is a simplified structure, containing few, if any, outlying proteins.

The immunoelectron microscopy indicates that m30 and m18 are located throughout the mitochondrial matrix. The maxicircle of *C. fasciculata* encodes genes, some of whose products, such as the cytochrome *c* oxidase subunits, are established components of mitochondrial inner membrane electron carrier assemblies. There is evidence from baker's yeast, *Saccharomyces cerevisiae*, that such proteins are co-translationally inserted into these assemblies by a process in which nuclear encoded activator proteins tether the mitochondrial translational initiation complexes to the inner membrane [22]. If the staining in the *C. fasciculata* mitochondrial matrix does represent components of a translational machinery, the extensive staining would be in keeping with a distribution of mitoribosomes predicted by the *S. cerevisiae* model.

It is nonetheless important to ask if these mitochondrially localized riboprotein homologues have functions other than translation. There is indeed evidence for bifunctionality for several ribosomal proteins; classic examples being bacteriophages Qβ and λ which recruit host riboproteins for viral RNA replication (Qβ) and antitermination of transcription (λ) [23,24]. We are now attempting to understand in greater detail the complexes in which the m30/m18 homologues exist in the mitochondrion, and whether their role is truly translational.

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