

# Import of small RNAs into *Leishmania* mitochondria *in vitro*

Sridam Mahapatra, Trina Ghosh and Samit Adhya\*

Genetic Engineering Laboratory, Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Calcutta 700032, India

Received May 6, 1994; Revised and Accepted July 15, 1994

EMBL accession no. X51821

## ABSTRACT

Using an *in vitro* ribonuclease protection assay, it was shown that synthetic antisense transcripts from the 5'-upstream region of the  $\beta$ -tubulin gene are efficiently imported into isolated *Leishmania* mitochondria. Import occurred after a lag of about 30 min at 25°C and was dependent on ATP. Preincubation experiments suggested that import consists of a slow interaction of mitochondria with RNA, followed by rapid ATP-dependent uptake. Import was saturable with antisense RNA at about 1 nM concentration, and sequence-specific, as shown by lack of import of other labelled transcripts. Deletion analysis demonstrated a correlation between efficiency of import and the number of oligopurine motifs on the antisense RNA. Several small ribosomal RNAs (srRNAs) and *Leishmania* tRNA competed with antisense RNA for import. Incubation of mitochondria with srRNAs and tRNA in the presence of radiolabelled UTP resulted in the ribonuclease-resistant labelling of these RNAs by the mitochondrial terminal uridylyl transferase. Extracts of isolated mitochondria contain a factor binding to antisense RNA, as shown by gel retardation assay. These observations indicate the presence of a receptor-mediated import pathway for srRNAs and tRNA in *Leishmania* mitochondria.

## INTRODUCTION

The majority of proteins of mitochondria and chloroplasts are not coded for by the organellar genome but synthesized in the cytoplasm and imported post-translationally into specific compartments by mechanisms involving import signals on the protein, receptors, import channels and soluble protein factors (reviewed in ref. 1). Additionally, recent studies indicate import of specific cytoplasmic tRNAs into yeast (2) and plant (3) mitochondria. The mechanism of RNA import is not understood, but the system suggests novel methods for mitochondrial gene targeting.

The mitochondrial genome of kinetoplastid protozoa such as *Leishmania* codes for a number of components of the respiratory chain and oxidative phosphorylation (4). The primary transcripts

of many of these genes are modified by RNA editing to yield mature mRNAs (5). However, there are serious deficiencies in the mitochondrially-coded translation machinery. Kinetoplast-mitochondrial ribosomal RNAs (9S and 12S) are among the smallest known, lacking many structural domains universally present in their eukaryotic and prokaryotic counterparts (6–8). Furthermore, mitochondrial DNA does not appear to code for any tRNA genes (9,10), but mitochondria contain a large number of functional tRNAs, most of which are also present in the cytoplasm and are nuclear-coded (11–14). Therefore the case exists for large-scale import of tRNAs into kinetoplast-mitochondria. Recently, *in vivo* import of tRNA<sup>Tyr</sup> into *Trypanosoma brucei* mitochondria has been demonstrated (15).

Previous experiments in this laboratory led to the identification of a sequence-specific RNA binding factor (TAS factor) in *Leishmania* which interacts with synthetic antisense transcripts from the 5'-untranslated region of the  $\beta$ -tubulin gene (16). In the present report we show that *Leishmania* mitochondria contain TAS factor and that antisense transcripts, srRNAs and tRNA are imported into mitochondria *in vitro*, apparently utilizing the same receptor-mediated import pathway.

## MATERIALS AND METHODS

### Cell culture

Promastigotes of *Leishmania* strain UR6 were cultured on blood-agar media as previously described (17).

### DNA clones and RNA probes

Clones pSG3B, pSG3Q, pSG3P and pSG3S contain 25 bp of the *Leishmania*  $\beta$ -tubulin coding sequence and 53, 31, 27 and 20 bp, respectively, of the 5'-untranslated region, inserted in the dual transcription vector pGEM3Zf(+) or pSPT19 (16). <sup>32</sup>P-Labelled antisense RNAs of high or low specific activity were prepared from the linearized templates using phage T7 or SP6 RNA polymerase and [ $\alpha$ -<sup>32</sup>P]UTP, as described (16).

### Preparation of small ribosomal and transfer RNAs

Total promastigote RNA was passed through an oligo dT-cellulose column, and the poly A<sup>-</sup> fraction collected (18). Poly A<sup>-</sup> RNA was electrophoresed on a denaturing 5% acryl-

\*To whom correspondence should be addressed

amide gel and the srRNA and tRNA bands were excised, eluted and ethanol precipitated.

### Isolation of mitochondria

Mitochondrial vesicles were isolated from promastigotes by hypotonic lysis and Percoll centrifugation (19). All operations were carried out at 0–4°C. Promastigotes were harvested, washed with phosphate-buffered saline containing 20 mM glucose, and suspended in hypotonic lysis buffer (1 mM Tris-HCl, pH 8.0, 1 mM EDTA) at  $1.2 \times 10^9$  cells/ml. The suspension was homogenized with 5 strokes of a type B pestle in an all-glass Kontes homogenizer. Cells were lysed by forceful passage of the homogenate through a 26-gauge hypodermic needle. Lysis was monitored microscopically. Sucrose (2 M stock) was immediately added to the lysate to 0.25 M, and the lysate centrifuged at 11,500 rpm in a Sorvall SS34 rotor. The pellet was suspended in one-fifth the original lysate volume of STM buffer (0.25 M sucrose, 20 mM Tris-HCl, pH 8.0, 2 mM MgCl<sub>2</sub>, 0.1 mM PMSF). RNase-free DNase I was added to 40–50 units/ml and the suspension incubated on ice for 30–45 min. The lysate was diluted 5-fold with STE-A buffer (0.25 M sucrose, 20 mM Tris-HCl, pH 8.0, 2 mM EDTA, 0.1 mM PMSF) and centrifuged. The pellet of crude mitochondria was resuspended in one-fifth volume of STE-A, layered on a 20–35% Percoll gradient, and centrifuged at 24,000 rpm in a Beckman SW28 rotor for 45 min. The turbid band of purified mitochondria near the bottom of the tube was withdrawn, washed 4 times with STE-B (0.25 M sucrose, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1 mM PMSF), and suspended in storage buffer (50% glycerol, 0.25 M sucrose, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1 mM PMSF) at a protein concentration of 7–8 mg/ml. Mitochondria were stored at –20°; at this temperature they retained import activity for about 2 weeks. The preparations consisted of mitochondrial vesicles of uniform size, essentially free of flagella and other cell debris, and staining positively for the inner mitochondrial membrane marker succinate dehydrogenase (using succinate as substrate, phenazine methosulphate as electron acceptor and nitroblue tetrazolium as chromogenic indicator; our unpublished data).

### RNA import assay

Unless otherwise stated, import assays (20  $\mu$ l) contained 10 mM Tris-HCl, pH 7.5, 5 mM MgCl<sub>2</sub>, 2 mM DTT, 1 mM ATP, 6.7 mM creatine phosphate, 15 mg/ml creatine phosphokinase, 100 fmol <sup>32</sup>P-labelled antisense RNA and 10  $\mu$ l mitochondrial suspension (about 80  $\mu$ g protein). After incubation for 45 min at 25°C, 1  $\mu$ l RNase mix (2 mg/ml RNase A plus 40,000 units/ml RNase T1) was added and incubation continued for 15 min at 25°C. This was followed by addition of 1  $\mu$ l of 0.1 M EDTA and 25  $\mu$ g proteinase K, incubation for 5 min, then 0.5 ml of 10% SDS, and incubation for a further 10 min at the same temperature. RNA was extracted with phenol-chloroform, ethanol precipitated and electrophoresed on a 5% acrylamide–8 M urea gel before autoradiography. In some experiments, mitochondria were re-isolated by centrifugation after the RNase treatment, resuspended in STE-B (see above) and then proteinase K digested. This was done to minimize degradation of imported RNA in lysing mitochondria by partially inactivated RNases.

### UTP labelling of imported RNA

A mixture of unlabelled *Leishmania* srRNAs and tRNA (0.6 pmol each) was incubated with mitochondria under import conditions

(see above) in presence of 8 mCi of [ $\alpha$ -<sup>32</sup>P]UTP (3000 Ci/mmol). After RNase treatment of the mitochondria followed by protease digestion, RNA was purified and analyzed as above.

### Preparation of mitochondrial extracts

Purified, washed mitochondria were collected by centrifugation and lysed with one-tenth the original volume of 10 mM Tris-HCl, pH 7.5, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, 20% glycerol and 0.5% Triton X-100. After incubation for 30 min on ice, the extract was clarified by centrifugation and stored at –70°.

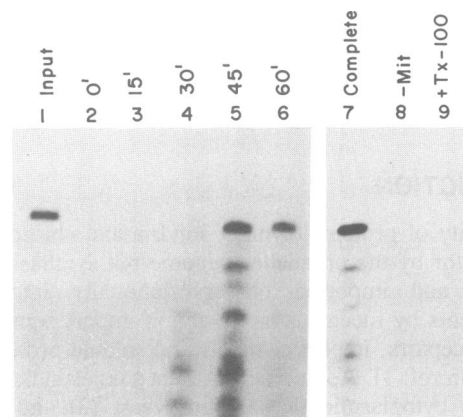
### Gel retardation assay

<sup>32</sup>P-Labelled pSG3B antisense RNA (2 fmol) was incubated with mitochondrial extract in the presence of 10 mM Tris-HCl, pH 7.5, 5 mM MgCl<sub>2</sub>, 2 mM DTT in a total volume of 10  $\mu$ l, for 30 min at 0°C. Heparin was added to 5 mg/ml and the reaction incubated for a further 10 min, before electrophoresis on a native 5% acrylamide gel, as described (16).

## RESULTS

### Kinetics of import of antisense RNA into mitochondria

Import of RNA into isolated mitochondria *in vitro* was assayed by incubating purified mitochondrial vesicles with <sup>32</sup>P-labelled antisense RNA under appropriate conditions, digesting excess RNA with ribonucleases, followed by inactivation of nuclease, disruption of the vesicles, deproteinization and analysis of the protected material. Figure 1 shows a time course of the reaction at 25°C. RNase-resistant RNA appeared after a lag of about 30 min and reached a maximum at 45 min (Fig. 1, lanes 2–6). No protection was observed in the absence of mitochondria (Fig. 1, lane 8), or upon disruption of the vesicles with Triton X-100 after a 45-min incubation (Fig. 1, lane 9), indicating the requirement for intact vesicles for import.

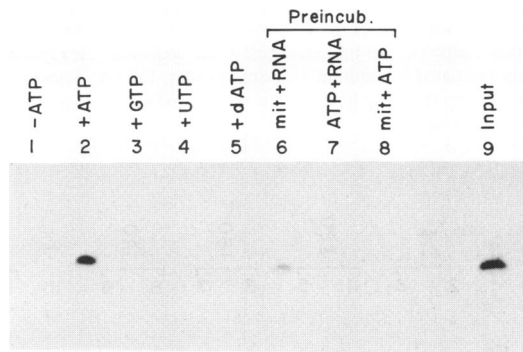


**Figure 1.** Time course of import of antisense RNA into mitochondria. Mitochondria were incubated with <sup>32</sup>P-labelled pSG3B antisense RNA (19 fmol) under import conditions for the following times at 25°C: 0, 15, 30, 45 and 60 min, respectively (lanes 2–6). Then RNase was added and the resistant material analyzed by gel electrophoresis. In lanes 7–9, incubations were for 45 min. Lane 7, complete system. Lane 8, mitochondria omitted. Lane 9, Triton X-100 (0.5%) was added after 45 min, followed by RNase. The size of pSG3B RNA is 105 nt (based on sequence, ref. 16).

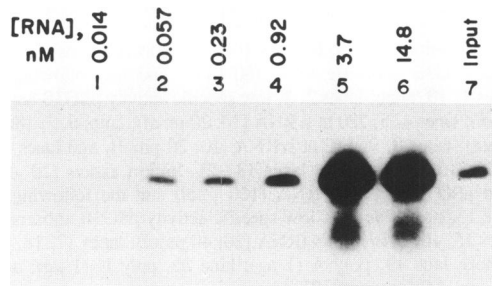
**ATP-dependence of RNA import**

Import of antisense RNA was dependent on addition of ATP (Fig. 2, lanes 1,2). ATP could not be substituted by GTP, UTP or dATP (Fig. 2, lanes 3-5).

The time course of import (Fig. 1) suggested a slow and a fast step involving three components: mitochondria, RNA and ATP. To define the components participating in each step, pairwise preincubations for 30 min were performed, followed by addition of the third component and incubation for a further 15 min prior to RNase treatment. When mitochondria were incubated with RNA, then ATP was added, import was observed at about 30% of the control level (Fig. 2, lane 6). However, no import occurred when ATP and RNA were preincubated before addition of mitochondria (Fig. 2, lane 7). When mitochondria were incubated with ATP, then RNA was added, the amount imported was reduced by more than 90% (Fig. 2, lane 8). These observations suggest that import consists of a slow interaction of mitochondria with RNA, followed by rapid ATP-dependent uptake.



**Figure 2.** Dependence of RNA import on ATP. Mitochondria were incubated with <sup>32</sup>P-labelled pSG3B antisense RNA (19 fmol) under import conditions in the absence (lane 1) or presence (lane 2) of 1 mM ATP. In lanes 3-5, ATP was replaced with GTP, UTP or dATP, respectively (each at 1 mM). In lanes 6-8, mitochondria, RNA and ATP were incubated pairwise for 30 min, then the third component was added and incubation continued for a further 15 min. Lane 6, mitochondria + RNA, then ATP. Lane 7, RNA + ATP, then mitochondria. Lane 8, mitochondria + ATP, then RNA. Lane 9, input RNA (0.4 fmol). Overexposure of the gel (not shown) indicated a faint band in lane 8.



**Figure 3.** Dependence of import on RNA concentration. Import assays were carried out at the following concentrations of <sup>32</sup>P-labelled pSG3B antisense RNA: 0.014, 0.057, 0.23, 0.92, 3.7 and 13.6 nM, respectively (lanes 1-6). Lane 7, input RNA (0.4 fmol). Densitometric analysis of the autoradiogram showed that the relative amounts imported in lanes 1-6 were in the ratio 1.0:1.9:2.2:3.6:29:24.

**Dependence of import on RNA concentration**

In titration experiments, import could be detected at very low concentrations of antisense RNA (about 0.01 nM), increased between 0.01 and 0.04 nM, then remained relatively unchanged until 1 nM, registering a second sharp increase (by about 10-fold) between 1 and 4 nM. Above this concentration, saturation was observed (Fig. 3).

**Specificity of import**

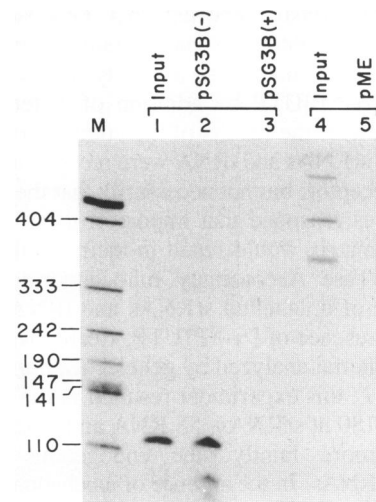
<sup>32</sup>P-Labelled  $\beta$ -tubulin antisense RNA was imported, but the corresponding sense strand was not (Fig. 4, lanes 2,3). Transcripts from the cloned mini-exon-derived RNA gene (20) were not imported (Fig. 4, lane 5). Thus the system is sequence-specific.

**Deletion analysis of antisense RNA**

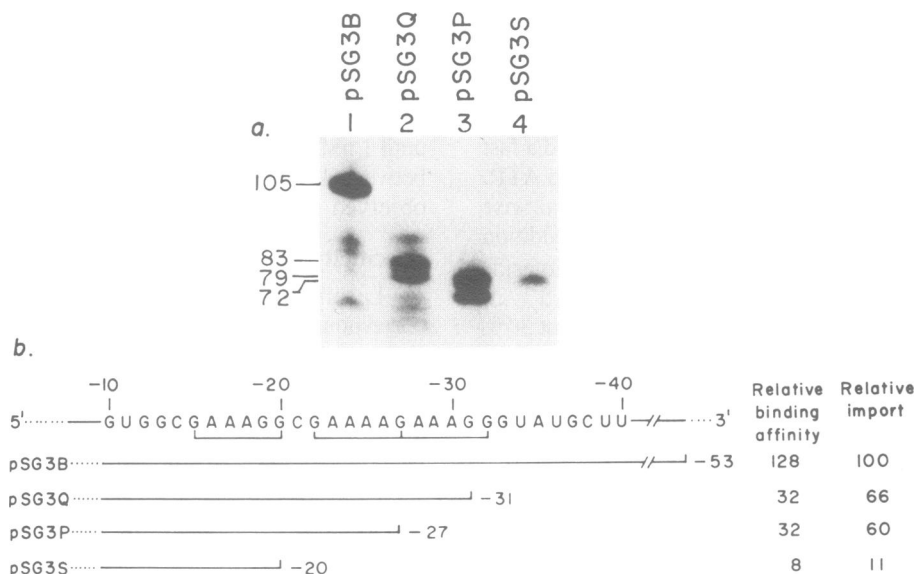
To delineate the sequences on the antisense RNA required for import, deletion clones were assayed. As shown in Fig. 5a, antisense RNAs from all 4 clones were imported, but with different efficiencies. By counting the radioactivity in each band it was determined that the relative molar amounts imported were in the ratio pSG3B (deletion endpoint -53): pSG3Q (-31): pSG3P (-27): pSG3S (-20) = 100:66:60:11. These results indicate the requirement of sequences between -53 and -20 for optimum levels of import.

**Competition of antisense RNA import by small ribosomal and transfer RNA**

The specificity of the RNA import system was further examined by competition assays. Import of <sup>32</sup>P-labelled antisense RNA was competed out by excess of the same RNA labelled to low specific activity (Fig. 6, lanes 1-3). The 220 nt, 180 nt, 140 nt and 120 nt small ribosomal RNAs were effective competitors



**Figure 4.** Specificity of import. The following <sup>32</sup>P-labelled RNAs (50 fmol each) were incubated with mitochondria under import conditions: lane 2, pSG3B antisense; lane 3, pSG3B sense; lane 5, pME1 RNA, from the *Leishmania* med RNA clone (20). Lanes 1 and 4, input pSG3B and pME1 RNA, respectively. Multiple bands in lane 4 are prematurely terminated RNAs synthesized at limiting UTP concentrations. M, molecular weight markers [pGEM3Zf(+)] digested with *Hpa*II].

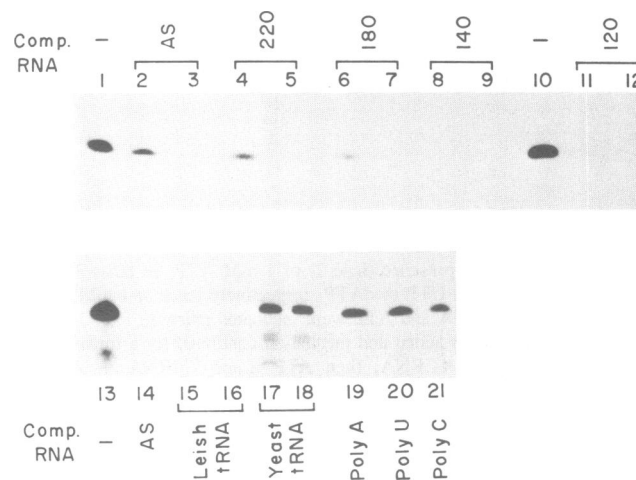


**Figure 5.** Deletion analysis of antisense RNA. (a) Import assays were performed with  $^{32}\text{P}$ -labelled antisense RNA (50 fmol each) from *EcoRI*-linearized clones pSG3B, pSG3Q, pSG3P and pSG3S, respectively (lanes 1–4). (b) Sequence of deletion clones. The sequence of the antisense strand of the *Leishmania*  $\beta$ -tubulin 5'-untranslated region (16) between  $-10$  and  $-40$  with respect to the AUG, is shown. Deletion endpoints are indicated below the sequence. Hexapurine motifs are bracketed. Relative affinities of the clones for binding TAS factor in solution were previously estimated by titration experiments (16). Relative import efficiency was determined by excising each band from the dried gel, liquid scintillation counting, dividing the cpm by the chain length of the corresponding RNA, and normalizing with respect to pSG3B (= 100). Mean values from several experiments (standard deviation 5%) are shown.

(Fig. 6, lanes 4–12), as was *Leishmania* tRNA (Fig. 6, lanes 15–16). However, neither yeast tRNA, nor poly A, poly U nor poly C (Fig. 6, lanes 17–21) competed to any significant extent. Thus the import system appears to have broad specificity for a range of srRNAs and the homologous tRNA.

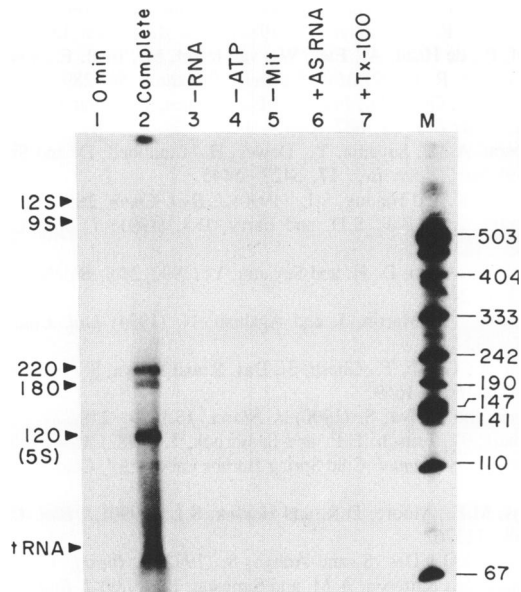
#### UTP labelling of imported srRNAs and tRNA

It has been previously reported that *Leishmania* (21) and *Trypanosoma* (19) mitochondria contain a terminal uridylyl transferase (TUTase) that labels a variety of cytoplasmic RNAs *in vitro* with  $[\alpha\text{-}^{32}\text{P}]\text{UTP}$  by addition of 3'-terminal uridylyl residues. In view of the results of the competition assay above, it appeared that srRNAs and tRNA were recognizing the antisense RNA import receptor, but not necessarily that they were actually imported. It was reasoned that import of these RNAs into the mitochondrial matrix would result in their labelling by UTP in presence of TUTase. Accordingly, mitochondria were incubated with a mixture of unlabelled srRNAs and tRNA under import conditions in presence of  $[\alpha\text{-}^{32}\text{P}]\text{UTP}$ , treated with RNase, and the resistant material analyzed by gel electrophoresis. As shown in Fig. 7, lane 2, this experiment resulted in the labelling of all 4 RNAs (220, 180 nt srRNAs, 5S RNA and *Leishmania* tRNA) as well as, more faintly, the endogenous 9S and 12S mitochondrial rRNAs. In the absence of incubation at  $25^\circ\text{C}$ , there was no labelling (Fig. 7, lane 1). Labelling was dependent on the presence of added RNA, ATP and mitochondria (Fig. 7, lanes 3–5). Disruption of mitochondria with Triton X-100 after incubation resulted in digestion of the imported material (Fig. 7, lane 7). Furthermore, labelling was effectively competed out by excess antisense RNA (Fig. 7, lane 6). Labelling of the individual srRNAs and *Leishmania* tRNA was also observed by this protocol; however, yeast tRNA was not labelled (data not



**Figure 6.** Competition assays. In lanes 1–12, import reactions (10  $\mu\text{l}$ ) contained  $^{32}\text{P}$ -labelled pSG3B antisense RNA (50 fmol) and the following competitor RNAs: lanes 1, 10, none; lanes 2, 3, low specific activity pSG3B antisense RNA (10, 20 pmol); lanes 4, 5, 220 nt srRNA (10, 20 pmol); lanes 6, 7, 180 nt srRNAs (10, 20 pmol); lanes 8, 9, 140 nt srRNA (10, 20 pmol); and lanes 11, 12, 120 nt (5S) RNA (10, 20 pmol). In lanes 13–21, import assays (20  $\mu\text{l}$ ) contained  $^{32}\text{P}$ -labelled pSG3B antisense RNA (100 fmol) and the following competitor RNAs: lane 13, none; lane 14, low specific activity pSG3B antisense RNA (40 pmol); lanes 15, 16, *Leishmania* tRNA (20, 40 pmol); lanes 17, 18, yeast tRNA (20, 40 pmol); lane 19, poly A (1  $\mu\text{g}$ ); lane 20, poly U (1  $\mu\text{g}$ ); and lane 21, poly C (1  $\mu\text{g}$ ). AS, antisense RNA.

shown). These observations indicate that srRNAs and tRNA are imported into the mitochondrial matrix using the antisense RNA import receptor, to become labelled by the mitochondrial TUTase.



**Figure 7.** UTP labelling of imported srRNAs and tRNA. An equimolar mixture of unlabelled 220 nt srRNA, 180 nt srRNA, 5S RNA and *Leishmania* tRNA was incubated under import conditions in the presence of [ $\alpha$ - $^{32}$ P]UTP. After RNase digestion the products were analyzed by gel electrophoresis. Lane 1, 0 min incubation control. Lane 2, complete system, 45 min at 25°C. Lane 3, RNA omitted. Lane 4, ATP omitted. Lane 5, mitochondria omitted. Lane 6, unlabelled pSG3B antisense RNA added. Lane 7, Triton X-100 (0.5%) added after incubation. Lane M, molecular weight markers [*Hpa*II fragments of pGEM3Zf(+)].

### Association of TAS factor with mitochondria

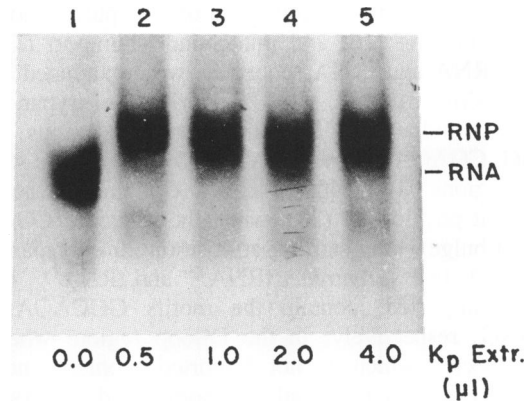
Extracts of purified mitochondria were incubated with  $^{32}$ P-labelled antisense RNA. A heparin-resistant RNA:protein complex was detected by the gel retardation assay (Fig. 8), showing the presence of TAS factor in mitochondria.

### DISCUSSION

In this report we demonstrate the existence of an RNA import system in *Leishmania* mitochondria that has the hallmarks of a receptor-mediated process but differs substantially from the tRNA import system recently described in yeast (2).

In common with numerous other receptor-mediated phenomena such as hormone signalling or solute transport across plasma membranes, import of  $\beta$ -tubulin antisense RNA into *Leishmania* mitochondria is saturable with ligand (Fig. 3) and specific, as shown by the lack of import of other labelled RNAs (Fig. 4) and by competition experiments (Fig. 5). The nature of the titration curve further suggests that more than one import receptor, operating at about 0.01 and 1 nM RNA respectively, may be present.

Import of RNA occurs with a pronounced lag of about 30 min and is dependent on ATP (Figs 1, 2). In these respects it resembles the yeast tRNA import (2) and mitochondrial protein import (22) systems. However, in contrast to the yeast tRNA import system, the *Leishmania* system does not apparently require soluble cytoplasmic proteins. Preincubation experiments indicate that the rate-limiting step involves interaction of RNA with the mitochondrion (Fig. 2); in yeast, the corresponding step involves RNA and soluble factor (2). The apparent differences in the two



**Figure 8.** Presence of antisense RNA binding activity in isolated mitochondria.  $^{32}$ P-labelled pSG3B antisense RNA (2 fmol) was incubated with 0, 0.5, 1, 2 and 4  $\mu$ l (lanes 1–5) of mitochondrial extract (7 mg/ml protein). Heparin-resistant RNA:protein complex was detected by native gel electrophoresis.

systems may be explained by the presence of a soluble vs. membrane-bound RNA binding protein acting as import receptor. A slow, ATP-independent binding of RNA to receptor is followed by rapid uptake in presence of ATP.

Although additional cytoplasmic proteins are not absolutely required in this *in vitro* system, *in vivo* the RNAs are probably imported as ribonucleoprotein particles. This possibility is currently under investigation.

The import signal on antisense RNA was analyzed with deletion clones. Progressive deletion of the region between -53 and -20 led to decrease in the efficiency of import (Fig. 5). This region contains hexapurine repeats of the sequence GAAA A/G G (Fig. 5). The number of intact repeats correlates with import efficiency, and also, as shown previously (16), with the relative affinity of binding to TAS factor in solution. This observation, together with the presence of antisense RNA binding activity in mitochondria (Fig. 8), implies that TAS factor acts as an RNA import receptor, but this should be confirmed by further experiments.

Whereas the import system in yeast mitochondria is apparently specific for cytoplasmic tRNA<sub>1<sup>Lys</sup></sub> (2), the *Leishmania* system appears to recognize a wide variety of cellular RNAs, including srRNAs, 5S RNA and tRNA. This was shown by their ability to effectively compete with antisense RNA for import (Fig. 6). The imported RNAs also became labelled with UTP by mitochondrial TUTase, and this reaction was inhibited by antisense RNA (Fig. 7). We have previously shown (16) that srRNAs and tRNA bind to TAS factor in solution. It seems reasonable to conclude that the TAS system mediates mitochondrial import of these RNAs. However, one discrepancy should be noted. Whereas both *Leishmania* and yeast tRNAs bind with low affinity to TAS factor in solution and on Northwestern blots (16), the homologous tRNA is a much more effective competitor of antisense RNA import than yeast tRNA (Fig. 6), and while the former is post-import UTP-labelled (Fig. 7), the latter is not (data not shown). One explanation of these differences is the presence of distinct forms of TAS factor with different affinities for *Leishmania* and yeast tRNAs; alternatively, tRNA import may require sequences additional to those necessary for binding to TAS factor, and which are absent from the yeast species.

In view of the apparent requirement of oligopurine motifs for TAS factor binding (16) and mitochondrial import (Fig. 5), available srRNA and tRNA sequences were examined for the presence of similar motifs. The 180 nt srRNA of trypanosomes (23) and crithidia (24) contains the homologous motifs GAAGGG, CGAGAGG and CGAAGGG within single-stranded (hairpin) regions. The 140 nt srRNA contains the sequence CGAGAG at position 18 (23), while the sequence CGAAUG occurs in a bulge-loop starting at position 20 in trypanosome 5S RNA (25). In *L. tarentolae*, tRNA<sup>Thr</sup> and tRNA<sup>Tyr</sup>, both of which are imported, contain the motifs GGCAGAG and GGUAGAG, respectively, in the D-loop region, whereas a tRNA<sup>Gln</sup> species which is not imported, contains no such sequence (14). Thus the small ribosomal and tRNAs share sequence motifs recognized by the mitochondrial import receptor.

Previously, Bakalara *et al.* observed that incubation of intact *L. tarentolae* mitochondria with exogenously added cytoplasmic RNAs led to their labelling by mitochondrial TUTase (21). Our experiments reproduce this result (Fig. 7), and show, additionally, that the labelled species is RNase resistant, i.e. imported. Small amounts of 5S RNA have also been detected by hybridization in *L. tarentolae* mitochondrial RNA (14).

The demonstration of tRNA import in *Leishmania* mitochondria confirms previous studies based on comparative analysis of mitochondrial and cytoplasmic tRNAs, and hybridization (11–15). Furthermore, our experiments for the first time show the import of nuclear-coded srRNAs *in vitro*. The significance of this latter phenomenon is unclear at present. Kinetoplastid srRNAs are derived by post-transcriptional processing of the cytoplasmic 28S rRNA precursor (23,24). Chloroplasts of *Chlamydomonas* (26) and higher plants (27), and mitochondria of *Paramecium* (28) and *Tetrahymena* (29) also contain similarly processed srRNAs, but these are encoded in the organellar genomes. Given the association of trypanosomal srRNAs with cytoplasmic polysomes (30), a role of these RNAs in mitochondrial translation is a possibility deserving further experimentation.

Regardless of functional significance, the TAS RNA import system may provide a novel means of targeting genes to *Leishmania* mitochondria for studies of organellar gene expression and regulation.

## ACKNOWLEDGEMENTS

We are grateful to Ms Subhagata Ghosh for technical assistance, to Mr Swadesh Sahu for the artwork, and to Dr A.Nandi for help with the computer work. This work was partly supported by the UNDP Project no. IND/87/018/A/01/99. S.M. was supported by a research associateship of CSIR, and T.G. by a senior research fellowship of UGC.

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