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Correction of Translational Defects in Patient-derived Mutant Mitochondria by Complex-mediated Import of a Cytoplasmic tRNA*

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A variety of clinical disorders result from mutations in mitochondrial tRNA genes, leading to translational defects. We show here that a protein complex from the kinetoplastid protozoon *Leishmania* induces specific, ATP-dependent import of human cytoplasmic $tRNA_1^{Lys}$ into human mitochondria *in vitro*. The imported tRNA undergoes efficient aminoacylation within the organelle and supports organellar protein synthesis. Moreover, translation in mitochondria from patients with myclonic epilepsy with ragged red fibers (MERRF) and Kearns-Sayre syndrome (KSS), containing mutant $tRNA^{Lys}$ genes, is stimulated to near-wild-type levels and the formation of aberrant polypeptides suppressed by complex-mediated import. These results suggest a novel way to introduce RNAs for the modulation of mitochondrial gene expression.

The mitochondrial genomes of a wide variety of protist, plant, and animal species contain an insufficient number of functional tRNA genes, and translation of mitochondrial mRNAs is sustained by import of nucleus-encoded tRNAs (reviewed in Ref. 1). Although human mitochondria do not normally import tRNA, a number of neuromuscular degenerative diseases are caused by mutations in mitochondrial tRNA genes (2). For example, the genetic disease known as myoclonic epilepsy with ragged red fibers (MERRF)[1] is caused by the A8344G mutation in the mitochondrial $tRNA^{Lys}$ gene (3). Other diseases such as the Kearns-Sayre syndrome (KSS) are characterized by mitochondrial genomic deletions (4). Correction of the tRNA defect may be possible by engineering the import machinery derived from other species into human cells. Crude extracts from yeast support the import of a yeast tRNA into human mitochondria (5), and transfection of modified yeast tRNA genes into cybrid cells rescues translation in, and activity of, MERRF-derived mitochondria (6), but the effect is only partial, and there is considerable clonal heterogeneity.

An alternative approach is suggested by the observation that certain yeast and human tRNAs, including human $tRNA_1^{Lys}$ are imported into the mitochondria of the kinetoplastid protozoa *Trypanosoma* (7) and *Leishmania* (8), indicating a broad specificity of the endogenous tRNA import machinery in these organisms, which import a wide variety of tRNAs to support mitochondrial translation in the complete absence of organellar tRNA genes (9, 10). In *Leishmania*, short sequence motifs in various tRNA domains are recognized as import signals (11). A previously characterized motif YGGUAGAGC is present in a number of kinetoplastid tRNAs, including *Leishmania* $tRNA^{Tyr}$(GUA), where it acts as an import signal (12). An identical sequence is present in the D domain of human $tRNA_1^{Lys}$ (Fig.1), explaining its recognition by the *Leishmania* import apparatus.

Recently, we have isolated a large protein complex (the RNA import complex or RIC) with an apparent molecular mass of ~640 kDa from the inner membrane of *Leishmania* mitochondria that induces the import of *Leishmania* $tRNA^{Tyr}$ into phospholipid vesicles (13). This complex resolves into multiple protein species, including two, of apparent mass 45 and 21 kDa, respectively, that bind to different tRNAs ($tRNA^{Tyr}$ and $tRNA^{Ile}$) in an allosterically regulated manner (13). Thus, it was of interest to determine whether RIC is capable of delivering tRNAs into the matrix of human mitochondria.

MATERIALS AND METHODS

Cell Culture and Preparation of Mitochondria—The hepatocellular carcinoma cell line HepG2 was grown in RPMI 1640 medium supplemented with 10% fetal bovine serum. The following homoplasmic transmitochondrial cybrids were derived by fusing mitochondria-less 143B206 cells with enucleated patient fibroblasts containing wild-type or mutant mitochondria and cultivated in Dulbecco's modified Eagle's medium (containing 4.5 g/liter glucose and 110 mg/liter sodium pyruvate) supplemented with 10% fetal bovine serum and 50 mg/liter uridine: LB58, containing wild-type mitochondria (14); LB64, containing MERRF mitochondria with the A8344G mutation in the mitochondrial $tRNA^{Lys}$ gene (14); and FLP32.39, derived from a patient with the Kearns-Sayre syndrome, harboring a 1902-bp mitochondrial DNA deletion between nucleotides 7846 and 9748 (15). Mitochondria were isolated from cell lysates by differential centrifugation as described (16). To prepare mitoplasts, the outer membrane was selectively removed with 320 μM digitonin (17).

Purification of RIC—Sodium deoxycholate extracts of *Leishmania tropica* mitochondrial inner membranes were subjected to RNA affinity chromatography and the 1 M Na^+ eluate concentrated to yield RIC, as described (13).

Import Substrates—Leishmania $tRNA^{Tyr}$(GUA) and $tRNA^{Gln}$(CUG) and 3′-CCA terminated human cytoplasmic $tRNA_1^{Lys}$(UUU) were prepared by T7 RNA polymerase transcription of cloned or PCR-amplified templates as described (8, 18). Lysylation of ^{32}P-labeled $tRNA^{Lys}$ transcript was carried out as described (16), using a 0–80% ammonium sulfate concentrated HepG2 cytosolic extract as the enzyme source.

Import Assay—Import into intact mitochondria or mitoplasts was assayed by RNase protection as described previously (17). Briefly, mitoplasts (100 μg of protein) were incubated with ^{32}P-labeled RNA (2.5 nM) in the presence of 4 mM ATP and ~0.2 μg of affinity-purified RIC at 37 °C for 15 min, then treated with RNases A plus T1, washed, and the

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[1] The abbreviations used are: MERRF, myoclonic epilepsy with ragged red fibers; KSS, Kearns-Sayre syndrome; RIC, RNA import complex; CCCP, *m*-chlorocarbonylcyanide phenylhydrazine; ND, NADH dehydrogenase, CO, cytochrome oxidase; A, F_1F_0 ATPase.

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RNase-resistant mitochondrial RNA recovered for gel electrophoretic analysis. Intact mitochondria were preincubated for 1 h on ice before the import incubation. Aminoacylated tRNAs were analyzed on 7 M urea, 8% PAGE in 0.1 M sodium acetate, pH 5.0, at 4 °C and 7 V/cm, with buffer recirculation (19).

In Organello Translation—Unless otherwise stated, mitoplasts or mitochondria (100 μg) were incubated for 1 h at 30 °C in 20 μl of translation mixture (0.25 M sucrose, 20 mM Tris-HCl, pH 7.5, 30 mM KH_2PO_4, 5 mM sodium succinate, 50 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 12 mM creatine phosphate, 0.16 mg/ml creatine phosphokinase, 4 mM ATP, 0.5 mM GTP, 5 mM NADH, 2.5 mg/ml bovine serum albumin, 100 μg/ml cycloheximide, 0.125 mM concentration each unlabeled amino acid except methionine, 20 μCi of [^{35}S]methionine (1000 Ci/mmol), 100 fmol of lysyl-tRNA^{Lys}, and 280 ng of RIC. After washing the mitoplasts with sucrose-Tris-EDTA buffer, [^{35}S]methionine incorporation into protein was measured as hot trichloroacetic acid-precipitable counts/min (20). Translation products were analyzed by 15–20% exponential gradient SDS-PAGE (21) followed by fluorography.

RESULTS

Mitoplasts prepared from the human cell line HepG2 were incubated with *Leishmania* tRNA^{Tyr} and tested for import by RNase protection. ATP- and RIC-dependent import was observed (Fig. 1*A*). Import was tRNA-specific, since *Leishmania* tRNA^{Gln}(CUG), which is not recognized by RIC (13), was not imported (Fig. 1*A*). Moreover, import was sensitive to a range of respiratory inhibitors (Fig. 1*A*), including *m*-chlorocarbonylcyanide phenylhydrazine (CCCP, a protonophore), valinomycin (a K^+ ionophore), nigericin (a K^+/H^+ exchanger), and oligomycin (an inhibitor of F_1F_0 ATPase). CCCP disrupts transmembrane proton gradients, thus reducing the protonmotive force that drives synthesis of ATP as well as various transport processes across the inner mitochondrial membrane; valinomycin neutralizes the electrical component of this gradient, while nigericin equalizes the proton concentration across the membrane without affecting the membrane potential; and oligomycin blocks proton flow-through the F_1F_0 ATPase. Similar sensitivities have been observed in the reconstituted phospholipid vesicle system and reflect the intrinsic property of RIC as a proton pump capable of generating a membrane potential at the expense of ATP hydrolysis, as observed recently (22). Apparently, hydrolysis of ATP generates a pH gradient that actually drives import (22). Significantly, human cytoplasmic tRNA^{Lys} was transferred to the matrix of HepG2 mitoplasts in an RIC- and ATP-dependent manner (Fig. 1*C*), thus opening up the possibility of modulating mitochondrial translation.

The RNA import complex was isolated from the inner membrane and appears to work quite well when simply added to phospholipid vesicles (13) or to human mitoplasts (Fig. 1) or to RIC-depleted *Leishmania* mitoplasts (data not shown). For successful tRNA delivery, however, it is essential to reconstitute intact mitochondria, *i.e.* organelles with an intact outer membrane. When RIC was simply added to intact HepG2 mitochondria and import incubations carried out without a preincubation step, little or no uptake of human cytoplasmic tRNA^{Lys} was observed (Fig. 1*D*). However, upon preincubation of intact mitochondria with RIC for 60 min on ice, uptake was greatly stimulated (Fig. 1*D*). A HepG2 cytosolic extract could not substitute for the preincubation (data not shown), and addition of the extract to preincubated mitochondria did not have any effect (Fig. 1*D*), indicating the lack of a requirement for cytosolic factors. The requirement of a preincubation step could be due to some intrinsic feature of the composition or structure of the outer membrane, such as the presence of sterols, resulting in a slower rate of RIC insertion into the membrane.

To determine the intramitochondrial location of the tRNA^{Lys} after transfer through the outer membrane of preincubated HepG2 mitochondria, postimport, RNase-treated mitochondria were fractionated into outer membrane plus intermembrane space and mitoplasts by treatment with digitonin, which selectively solubilizes the outer membrane (17), and labeled tRNA recovered from each fraction. At the concentration of digitonin

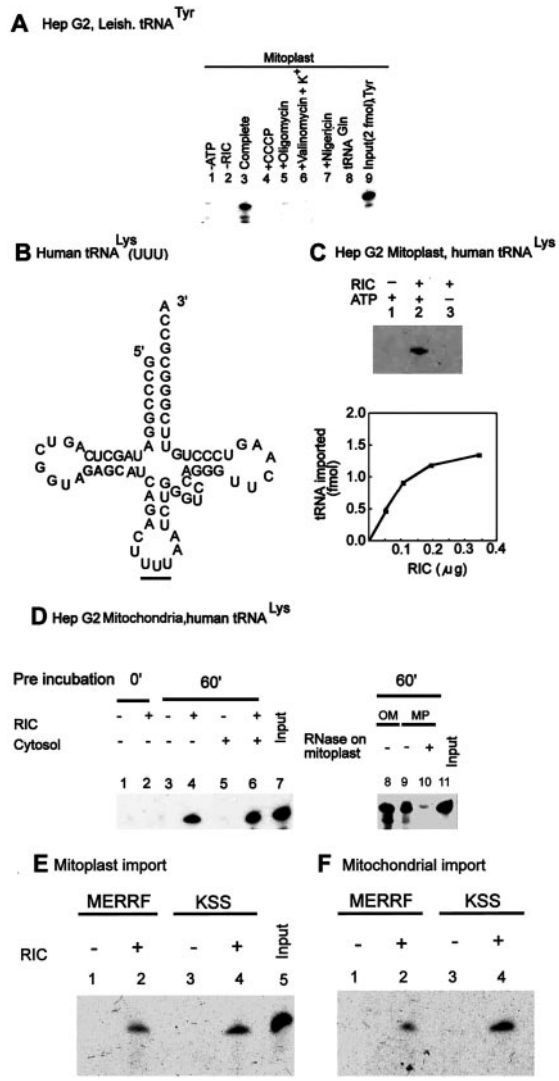

FIG. 1. **RIC-induced import of tRNAs into human mitochondria.** *A*, import of *Leishmania* tRNA^{Tyr} into HepG2 mitoplasts. The complete system (*lane 3*) contained 100 fmol of ^{32}P-labeled tRNA^{Tyr}, 4 mM ATP, 0.2 μg of RIC, and 100 μg (as protein) of digitonin-resistant mitoplasts. *Lanes 1* and *2*, ATP or RIC omitted, respectively. *Lanes 4–7*, import assayed in the presence of CCCP, oligomycin, valinomycin plus 50 mM KCl, and nigericin, respectively (all inhibitors at 50 μM). *Lane 8*, *Leishmania* tRNA^{Gln}(CUG) replaced tRNA^{Tyr}. *Lane 9*, input tRNA^{Tyr} (2 fmol). *B*, cloverleaf structure of human cytoplasmic tRNA^{Lys}(UUU). The anticodon is *underlined*. *C*, *upper*, import of human cytoplasmic tRNA^{Lys}(UUU) into HepG2 mitoplasts in the presence of ATP and 0.2 μg RIC (*lane 2*) or with RIC (*lane 1*) or ATP (*lane 3*) omitted; *lower*, titration of RIC. *D*, import of human tRNA^{Lys} into intact HepG2 mitochondria. Mitochondria were incubated with tRNA and ATP for 15 min at 37 °C without (*lanes 1* and *2*) or after a preincubation for 60 min on ice (*lanes 3–6* and *8–10*), before RNase treatment and recovery of imported RNA. In *lane 2*, RIC (0.2 μg) was added after addition of import substrate, whereas in *lanes 4*, *6*, and *8–10*, RIC was present during the preincubation. In *lane 6*, a concentrated HepG2 cytosolic extract (10 μg of protein) was present. Postimport, RNase-treated mitochondria were treated with digitonin and the digitonin-soluble fraction, representing the outer membrane plus intermembrane space (*OM*; *lane 8*) was separated from the mitoplasts (*MP*; *lanes 9* and *10*), half of which was treated with RNase (*lane 10*). *Lanes 7* and *11*, input tRNA^{Lys} (2 fmol). *E* and *F*, import of cytoplasmic tRNA^{Lys} into mutant mitoplasts (*E*) or mitochondria (*F*) derived from transmitochondrial cybrids LB64 (MERRF; *lanes 1* and *2*) or FLP32.39 (KSS; *lanes 3* and *4*) in the absence (*lanes 1* and *3*) or presence (*lanes 2* and *4*) of RIC. In *F*, mitochondria were preincubated with RIC as above. *Lane 5*, input tRNA^{Lys}, 2 fmol.

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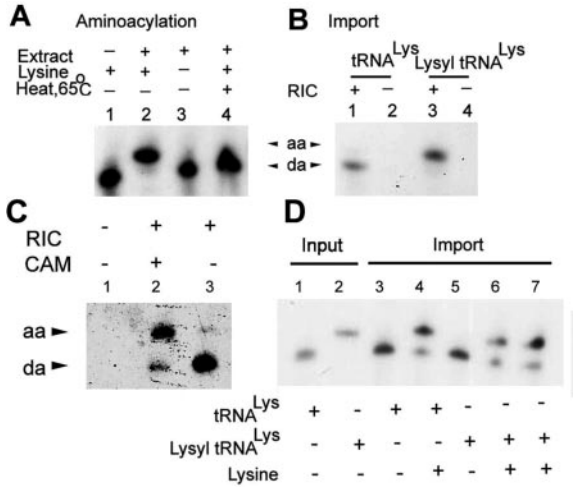

FIG. 2. **RIC-induced import of charged and uncharged tRNA into human mitochondria.** *A*, lysylation of cytosolic $tRNA^{Lys}$(UUU) *in vitro*. ^{32}P-Labeled $tRNA^{Lys}$ was incubated with crude human aminoacyl-tRNA synthetase, unlabeled lysine, and ATP and the product resolved by acid gel electrophoresis. *lane 1*, enzyme omitted; *lane 2*, complete system; *lane 3*, lysine omitted; *lane 4*, the product was deacylated by heating at 65 °C for 10 min at pH 9. *B*, import into HepG2 mitoplasts of uncharged (*lanes 1* and *2*) or lysyl-$tRNA^{Lys}$ (*lanes 3* and *4*) in the presence (*lanes 1* and *3*) or absence (*lanes 2* and *4*) of RIC. *aa*, lysylated form; *da*, deacylated or uncharged form of $tRNA^{Lys}$. *C*, aminoacylation status of imported $tRNA^{Lys}$ during translation *in organello*. ^{32}P-Labeled lysyl-$tRNA^{Lys}$ was incubated with HepG2 mitoplasts in the presence of a lysine-deficient amino acid mixture and RIC in the presence (*lane 2*) or absence (*lane 3*) of 100 μg/ml chloramphenicol (*CAM*) and the imported RNA analyzed by acid gel electrophoresis. *D*, aminoacylation of cysolic $tRNA^{Lys}$ within human mitochondria. HepG2 mitoplasts were incubated with RIC, lysine-deficient amino acid mixture, and ^{32}P-labeled $tRNA^{Lys}$ (*lanes 3* and *4*) or lysyl-$tRNA^{Lys}$ (*lanes 5–7*) and the imported RNA analyzed as above. Lysine (0.125 mM) was added before import incubation (*lanes 4* and *6*) or after 1 h of import incubation (*lane 7*) followed by 1 more h of incubation at 37 °C. *Lanes 1* and *2*, input deacylated and acylated forms, respectively, of $tRNA^{Lys}$.

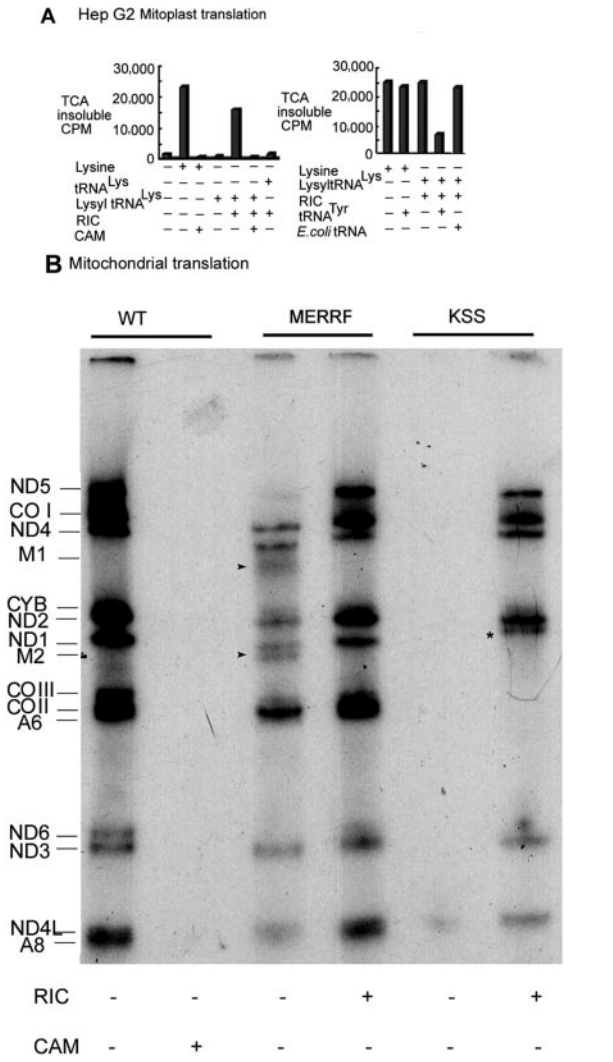

FIG. 3. **Rescue of mitochondrial translation by RIC-mediated import.** *A*, RIC- and lysyl-$tRNA^{Lys}$-dependent translation in human mitoplasts. HepG2 mitoplasts were incubated in translation mixture containing cycloheximide, all amino acids (including [^{35}S]methionine) except lysine, and other components as indicated. Incorporation was measured as hot trichloroacetic acid-precipitable counts/min. *B*, mitochondria from wild-type (LB58; *lanes 1* and *2*), MERRF (LB64, *lanes 3* and *4*), and KSS (FLP32.39; *lanes 5* and *6*) were incubated in the presence of a complete amino acid mixture (including lysine) and cycloheximide and translation products analyzed by SDS-PAGE. In *lane 2*, chloramphenicol was added. *Lanes 3–6* contained lysyl-$tRNA^{Lys}$. RIC was present in *lanes 4* and *6*. Radiolabeled mitochondria-encoded proteins are indicated at the *left*. *CYB*, apocytochrome b; *M1* and *M2*, aberrant translation products.

used (320 μM), about 95% of the outer membrane marker kynurenine hydroxylase, but less than 1% of the inner membrane marker succinate deydrogenase was solubilized, as observed previously (17), attesting to the specificity of the solubilization procedure. About 40% of the internalized tRNA (*i.e.* resistant to RNase treatment of the intact mitochondrion) was recovered from the mitoplasts (Fig. 1*D*, *lane 9*) and the remainder from the outer membrane-intermembrane space fraction (Fig. 1*D*, *lane 8*). About 15% of the internalized tRNA was resistant to RNase treatment of the recovered mitoplasts (Fig. 1*D*, *lane 10*); this represents the tRNA in the matrix; the remainder is presumably stuck to the outer surface of the inner membrane and therefore RNase-sensitive.

Next, we checked the ability of RIC to introduce cytoplasmic $tRNA^{Lys}$ into mitochondria containing mutations in the organellar $tRNA^{Lys}$ gene and thus complement the translational defects arising from such mutations. For this purpose, mitochondria were prepared from cybrid cell lines derived from human patients with mitochondrially inherited genetic disorders. Cybrid LB58 contains wild-type mitochondria (14). Cybrid LB64 is nearly homoplasmic for mitochondria derived from a MERRF patient, which contain a single A-to-G transition in the mitochondrial $tRNA^{Lys}$ gene (14). Cybrid FLP32.39 is similarly derived from a patient with the Kearns-Sayre syndrome and is characterized by a 1.9-kb mitochondrial deletion covering the $tRNA^{Lys}$ gene, as well as the COII, COIII, A6, and A8 genes (15). RIC-induced import of cytoplasmic $tRNA^{Lys}$ into LB64 and FLP32.39 mitoplasts (Fig. 1*E*) as well as preincubated intact mitochondria (Fig. 1*F*) was observed.

We next determined the effect of aminoacylation of the cytoplasmic $tRNA^{Lys}$ on RIC-mediated import into human mitochondria. Indirect evidence indicates that aminoacylation is not a requirement for import in kinetoplastid protozoa (23) but appears to be necessary in yeast (24) and plants (25). The CCA-terminated $tRNA_1^{Lys}$ transcript (Fig. 1) was lysylated *in vitro* with an enzyme preparation from HepG2 cytosol, and the charged and uncharged forms were resolved by electrophoresis under acidic conditions (19). Nearly complete charging of the transcript with lysine occurred, with the amino acid-RNA linkage characteristically labile to heat at alkaline pH (Fig. 2*A*). RIC-dependent import of both the charged and uncharged species into HepG2 mitoplasts was observed, with about the same efficiency (Fig. 2*B*). This experiment simultaneously shows that import itself does not cause appreciable deacylation of the tRNA (but see later).

Isoaccepting mitochondrial and cytosolic tRNAs may have

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considerable structural differences, and it is by no means certain that an imported cytosolic species would be recognized by the prokaryotic-type organellar ribosome or by the mitochondrial aminoacyl-tRNA synthetase. To test the ability of imported cytosolic $tRNA^{Lys}$ to support mitochondrial translation, HepG2 mitoplasts were incubated in the presence of cycloheximide (to block residual cytoplasmic translation) under lysine-limiting conditions with RIC, ATP, and lysyl-$tRNA^{Lys}$; RIC-mediated delivery of the charged tRNA should then rescue organellar protein synthesis. The mitoplasts used were of greater than 95% purity, as shown by marker assays (see above), and by the lack of significant cycloheximide-sensitive amino acid incorporation due to the near-complete solubilization of the outer membrane and any associated cytoplasmic polysomes by digitonin treatment. As shown in Fig. 3*A*, *left panel*, in the presence of RIC, lysyl-$tRNA^{Lys}$, but not the uncharged species, could partially replace lysine for chloramphenicol-sensitive protein synthesis in lysine-deficient mitoplasts. Lysyl-tRNA-dependent translation was competed out by excess *Leishmania* $tRNA^{Tyr}$, indicating the involvement of the RIC tRNA import pathway, whereas lysine-dependent translation, presumably involving an inner membrane amino acid carrier, was not (Fig. 3*A*, *right panel*). There was no detectable deacylation of lysyl-$tRNA^{Lys}$ imported alone in the presence of RIC (Fig. 2*B*). However, in the presence of a lysine-deficient amino acid mixture, the imported $tRNA^{Lys}$ underwent deacylation, and deacylation was inhibited by chloramphenicol (Fig. 2*C*), indicating the direct transfer of lysine from the imported tRNA to the elongating polypeptide chains. These results argue against the possibility that free lysine, generated by deacylation of the cytoplasmic tRNA before or during import, was responsible for the observed effects. Additionally, about 75% of $tRNA^{Lys}$ underwent acylation within the mitochondrion in the presence of lysine (Fig. 2*D*, *lanes 3* and *4*), and imported lysyl-$tRNA^{Lys}$, which underwent deacylation in the presence of a lysine-deficient translation mixture (Fig. 2*D*, *lane 5*), became reacylated on addition of lysine added simultaneously (Fig. 2*D*, *lane 6*) or subsequently (Fig. 2*D*, *lane 7*). Thus, the cytoplasmic $tRNA^{Lys}$ is recognized by the mitochondrial lysyl-tRNA synthetase, as observed previously (26), and undergoes multiple rounds of decylation and reacylation during translation within the organelle.

The mutant mitochondria exhibited qualitative as well as quantitative defects of translation. In *in organello* translation assays, a variable degree of inhibition of individual translation products was observed with LB64 mitochondria, ranging from 4.5% of the wild-type level for ND5 to 56.7% for ND3 (Fig. 3*B*, *lane 3*); the overall decline in translation rate was about 75%. Moreover, aberrant translation products of 47 and 34 kDa were present. These results are consistent with those obtained with intact cells (14), except that the two smallest aberrant products observed in that study were not clearly apparent. FLP32.39 mitochondria were completely defective for synthesis of all polypeptides except for ND4L, which was reduced to 22% of the wild-type level (Fig. 3*B*, *lane 5*). In the presence of RIC and cytoplasmic lysyl-$tRNA^{Lys}$, the translation of individual polypeptides was significantly stimulated to 35–100% of wild-type levels in both of the mutants (Fig. 3*B*, *lanes 4* and *6*). Under these conditions, the aberrant products of LB64 mitochondria disappeared. In RIC-treated FLP32.39 mitochondria, COII, COIII, A6, ΔA6, and A8 were missing due to deletion of the corresponding genes, but unexpectedly, ND1 was also absent, being replaced by a slightly larger polypeptide (Fig. 3*B*, *lane 6*). The reason for this is unknown; it may be due to translational read-through caused by a previously undetected frameshifting mutation in the ND1 gene.

DISCUSSION

The A8344G mutation in the mitochondrial $tRNA^{Lys}$ gene of MERRF patients causes severe translational defects through inefficient aminoacylation of the mutant tRNA (27) and/or ineffective codon recognition (28). The system of translational correction described above exploits the broad specificity of the protozoal RNA import complex to introduce a human cytosolic tRNA into human mitochondria. The imported tRNA is highly efficient in translational rescue; the small fraction of the tRNA transferred to the matrix (Fig. 1) suffices to restore translation to nearly wild-type levels, as well as to suppress aberrant polypeptide formation (Fig. 3). This may be due to the high level of aminoacylation of the imported tRNA within the organelle and direct transfer of the lysine to the translating ribosome (Fig. 2). In contrast, yeast tRNA derivatives are aminoacylated with variable efficiency within human mitochondria, and this, together with variable expression levels, may account for the observed clonal heterogeneity (6).

Traditionally, it has been difficult to introduce exogenous nucleic acids into mitochondria. The RIC is a molecular machine that easily inserts into membranes in a defined orientation (22) and is active through double membranes, enabling transfer into the matrix of intact mitochondria (Fig. 1). The issue of membrane permeability of this large complex is interesting and needs further mechanistic studies.

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REFERENCES

1. Schneider, A., and Marechal-Drouard, L. (2000) *Trends Cell Biol.* **10,** 509–513
2. Wallace, D. C. (1999) *Science* **283,** 1482–1487
3. Shoffner, J. M., Lott, M. T., Lezza, A. M., Seibel, P., Ballinger, S. W., and Wallace, D. C. (1990) *Cell* **61,** 931–937
4. Mita, S., Rizzuto, R., Moraes, C. T., Shanske, S., Arnaudo, E., Fabrizi, G. M., Koga, Y., DiMauro, S., and Schon, E. A. (1990) *Nucleic Acids Res.* **18,** 561–567
5. Kolesnikova, O. A., Entelis, N. S., Mireau, H., Fox, T. D., Martin, R. P., and Tarassov, I. A. (2000) *Science* **289,** 1931–1933
6. Kolesnikova, O. A., Entelis, N. S., Jaquin-Becker, C., Goltzene, F., Chrzanowska-Lightowlers, Z. M., Lightowlers, R. N., Martin, R. P., and Tarassov, I. A. (2004) *Hum. Mol. Genet.* **13,** 2519–2534
7. Hauser, R., and Schneider, A. (1995) *EMBO J.* **14,** 4212–4220
8. Goswami, S., Chatterjee, S., Bhattacharyya, S. N., Basu, S., and Adhya, S. (2003) *Nucleic Acids Res.* **31,** 5552–5559
9. Simpson, A. M., Suyama, Y., Dewes, H., Campbell, D., and Simpson, L. (1989) *Nucleic Acids Res.* **17,** 5427–5445
10. Hancock, K., and Hajduk, S. L. (1990) *J. Biol. Chem.* **265,** 19203–19215
11. Bhattacharyya, S. N., Chatterjee, S., and Adhya, S. (2002) *Mol. Cell. Biol.* **22,** 4372–4382
12. Bhattacharyya, S. N., Mukherjee, S., and Adhya, S. (2000) *Mol. Cell. Biol.* **20,** 7410–7417
13. Bhattacharyya, S. N., Chatterjee, S., Goswami, S., Tripathi, G., Dey, S. N., and Adhya, S. (2003) *Mol. Cell. Biol.* **23,** 5217–5224
14. Masucci, J. P., Davidson, M., Koga, Y., Schon, E., and King, M. P. (1995) *Mol. Cell. Biol.* **15,** 2872–2881
15. Sobreira, C., King, K. P., Davidson, M. M., Park, H., Koga, Y., and Miranda, A. F. (1999) *Biochem. Biophys. Res. Comm.* **266,** 179–186
16. Enriquez, J. A., and Attardi, G. (1996) *Methods Enzymol.* **264,** 183–196
17. Mukherjee, S., Bhattacharyya, S. N., and Adhya, S. (1999) *J. Biol. Chem.* **274,** 31249–31255
18. Adhya, S., Ghosh, T., Das, A., Bera, S. K., and Mahapatra, S. (1997) *J. Biol. Chem.* **272,** 21396–21402
19. Varshney, U., Lee, C.-P., and RajBhandary, U. L. (1991) *J. Biol. Chem.* **266,** 24712–24718
20. Merrick, W. C. (1983) *Methods Enzymol.* **101,** 606–615
21. Chomyn, A. (1996) *Methods Enzymol.* **264,** 197–211
22. Bhattacharyya, S., and Adhya, S. (2004) *J. Biol. Chem.* **279,** 11259–11263
23. Schneider, A., Martin, J., and Agabian, N. (1994) *Mol. Cell. Biol.* **14,** 2317–2322
24. Tarassov, I., Entelis, N., and Martin, R. P. (1995) *EMBO J.* **14,** 3461–3471
25. Delage, L., Duchene, A. M., Zaepfel, M., and Marechal-Drouard, L. (2003) *Plant J.* **34,** 623–633
26. Tolkunova, E., Park, H., Xia, J., King, M. P., and Davidson, E. (2000) *J. Biol. Chem.* **275,** 35063–35069
27. Enriquez, J. A., Chomyn, A., and Attardi, G. (1995) *Nat. Genet.* **10,** 47–55
28. Kirino, Y., Yasukawa, T., Ohta, S., Akira, S., Ishihara, K., Watanabe, K., and Suzuki, T. (2004) *Proc. Natl. Acad. Sci. U. S. A.* **101,** 15070–15075

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