

Decreased mitochondrial tRNA^{Lys} steady-state levels and aminoacylation are associated with the pathogenic G8313A mitochondrial DNA mutation

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Mutations in human mitochondrial tRNA genes cause a number of multisystemic disorders. A G-to-A transition at position 8313 (G8313A) in the mitochondrial tRNA^{Lys} gene has been associated with a childhood syndrome characterized by gastrointestinal-system involvement and encephaloneuropathy. We have used transmitochondrial cybrid clones harbouring patient-derived mitochondrial DNA with the G8313A mutation for the study of the molecular pathogenesis. Our results showed that mutant mitochondrial cybrids respired poorly, and had severely defective

mitochondrial protein synthesis and respiratory-chain-enzyme activity. Mutant cybrids also showed a marked decrease in tRNA^{Lys} steady-state levels and aminoacylation, suggesting that these molecular abnormalities may underlie the pathogenesis of the mitochondrial G8313A mutation.

Key words: mitochondrial DNA (mtDNA), mitochondrial translation, myoclonus epilepsy with ragged red fibres (MERRF).

INTRODUCTION

A large number of mutations in the mitochondrial DNA (mtDNA) have been associated with clinical syndromes [1]. Pathogenic mtDNA mutations have been described in protein-coding genes, as well as in rRNA- and tRNA-coding genes [1]. The mitochondrial G-to-A transition at position 8313 (G8313A) has been associated with a childhood disorder involving the gastrointestinal system, accompanied by seizures, peripheral neuropathy, retinitis pigmentosa and neural deafness [2]. The mutation maps to the tRNA^{Lys} gene, which is also mutated in patients with myoclonus epilepsy with ragged-red fibres (MERRF) [3], as well as in patients with other multisystem disorders [1].

The molecular pathogenesis of mitochondrial tRNA^{Lys} mutations is not completely understood, but experiments using ρ^0 -mediated transmitochondrial cybrids [4,5] have unveiled important biochemical and genetic clues. Transmitochondrial cybrids with the A8344G and T8356C mutations showed a defect in mitochondrial protein synthesis [6]. The A8344G mutation in mitochondrial tRNA^{Lys} has also been associated with defects in aminoacylation capacity, as well as with a decrease in steady-state levels of tRNA^{Lys} [7]. Yasukawa et al. [8] have reported that the A8344G mutation is associated with a defect in a 2-thiouridine modification in the tRNA^{Lys} 'wobble' position, which affects anticodon-codon pairing. Post-transcriptional modifications are important not only for anticodon-codon pairing, but also for tRNA folding, stability, aminoacylation and function in ribosomes [9].

We have constructed transmitochondrial cell lines harbouring the G8313A-mutated mtDNA. Our findings show that the G8313A transition causes a substantial decrease in the mitochondrial tRNA^{Lys} aminoacylation and steady-state levels, and that these decreases might account for the translational problems.

MATERIALS AND METHODS

Cell lines and culture conditions

The human cell line 143B and its mitochondrial-less derivative 143B- ρ^0 cell line [10] were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum. A fibroblast line from the patient harbouring a heteroplasmic G → A transition at position 8313 (56% mutated mtDNA) within the mitochondrial tRNA^{Lys} gene has been obtained and characterized previously [2]. Fibroblasts were enucleated and fused to 143B ρ^0 cells, as described previously [4]. Transmitochondrial cybrid clones were selected in high-glucose DMEM supplemented with 10% dialysed fetal bovine serum, bromodeoxyuridine (100 μ g/ml) and no uridine supplementation.

Genetic characterization of transmitochondrial cybrids

Individual cybrid clones were isolated and analysed for the presence of the G8313A transition by *DdeI* restriction fragment length polymorphism (RFLP) of PCR-amplified fragments [2]. Four heteroplasmic clones (C2, D1, G2 and G6) were treated with 100 ng/ml ethidium bromide for 2 weeks to deplete their mtDNA, followed by growth in complete medium for 24 days. Several clones containing apparently homoplasmic levels of the G8313A mutation were isolated, including the ones used in subsequent experiments (i.e. clones D1-30, D1-34 and D1-36). The percentage of mutated and wild-type molecules was determined by 'last-cycle hot' PCR, as described previously [11], followed by quantification of the electrophoretically separated restriction products with a Cyclone Storage Phosphor System (Packard, Meriden, CT, U.S.A.).

Abbreviations used: COXII, cytochrome c oxidase subunit II; DMEM, Dulbecco's modified Eagle's medium; MERRF, myoclonus epilepsy with ragged-red fibres; mtDNA, mitochondrial DNA; ND1, NADH dehydrogenase subunit 1; RFLP, restriction fragment length polymorphism; SDH(Fp), the Fp subunit of succinate dehydrogenase; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

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To determine the sensitivity of the RFLP assay, we PCR-amplified a DNA fragment encompassing the mtDNA 8313 position (oligonucleotide primers from mtDNA positions 7955–7979 and 8573–8549; numbers are as designated in [12]) from a wild-type and a mutant transmitochondrial cybrid line. These PCR fragments were subcloned into a plasmid vector (TA cloning/pCR II kit obtained from Invitrogen). Individual bacterial clones were isolated, and their plasmids were tested for the presence or absence of the 8313 mutation. We mixed known amounts of purified wild-type and mutated DNA-containing plasmids corresponding to 10, 5, 2, 0.5 and 0.1 % wild-type sequence. These mixtures were PCR-amplified in parallel with DNA from transmitochondrial cybrid cell lines used in our studies. By overexposing the gel for 4 days with an intensifying screen, we were able to visualize as little as 1 % wild-type mtDNA in the test mixture (results not shown).

Respiratory function assay

Oxygen-consumption studies in intact cells were performed as described previously [13,14] in 0.6 ml of standard medium [0.3 M mannitol/10 mM KCl/5 mM MgCl₂/1 mg/ml BSA/10 mM KH₂PO₄ (pH 7.4)] with a Clark oxygen electrode in a micro water-jacketed cell, magnetically stirred, at 37 °C (Hansatech Instruments Limited, Norfolk, U.K.). In addition, cells (approx. 3 × 10⁶ cells were used for each experiment) were permeabilized with digitonin and analysed for the oxidation of glutamate/malate, followed by the addition of rotenone. Succinate was then added, and respiration recorded. After inhibition of complex III with antimycin A, the oxidation of ascorbate plus *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) through the complex IV was measured, followed by KCN inhibition. Final concentrations of substrates and inhibitors were as follows: glutamate, 5 mM; malate, 5 mM; succinate, 5 mM; ascorbate, 10 mM; TMPD, 0.2 mM; rotenone, 100 nM; antimycin A, 20 nM; and KCN, 1 mM.

The measurement of the specific activity of the individual complexes of the electron-transport chain was performed spectrophotometrically, essentially as described in [13]. Whole-cell fractions were assayed for complex II + III (succinate cytochrome *c* oxidoreductase), complex II (succinate decylubiquinone oxidoreductase), complex IV (cytochrome *c* oxidase) and citrate synthase. Lactate released in the cultured medium was measured with a YSI Stat Plus system (YSI Inc., Yellow Springs, OH, U.S.A.), and the values were normalized to the number of cells, counted in a Coulter Particle Counter (Beckman Coulter, Miami, FL, U.S.A.). Briefly, 0.2 × 10⁶ cells were plated in a 12-well dish with 2 ml of high-glucose DMEM and 10 % fetal calf serum. After 3, 6 or 24 h, an aliquot of the medium was taken for lactic acid measurement, and the cells were treated with trypsin and counted.

Western blot analysis and immunodetection

Cultured transmitochondrial cybrids were treated with 1 % (w/v) SDS and a cocktail of protease inhibitors (Roche Biochemicals, Indianapolis, IN, U.S.A.) and the lysates (30 μg) were separated on an SDS/15 % polyacrylamide gel and electroblotted on to PVDF membranes. Blotted membranes were incubated overnight at 4 °C with 5 % milk in PBS with 0.05 % (v/v) Tween 20 as a blocking agent. Membranes were then incubated with cytochrome *c* oxidase subunit II (COXII) polyclonal antibody, NADH dehydrogenase subunit 1 (ND1) polyclonal antibody (gifts from Dr A. Lombes, Groupe Hospitalier Pitié-Salpêtrière, Paris, France) or SDH(Fp) [where SDH(Fp) represents the Fp subunit

of succinate dehydrogenase] monoclonal antibody (Molecular Probes, Eugene, OR, U.S.A.) for 2 h at room temperature, and subsequently incubated with anti-(mouse IgG) or anti-(rabbit IgG) peroxidase conjugates (Sigma, St Louis, MO, U.S.A.). Bands were developed with SuperSignal Chemiluminescent Substrate (Pierce, Rockford, IL, U.S.A.) for 5 min, and exposed to X-ray films.

Analysis of mitochondrial translation products

Exponentially growing transmitochondrial cybrids, a 143B cell line and 143B-ρ⁰ cells (2 × 10⁶ cells) were labelled with [³⁵S]methionine (1.000/mmol; 250 μCi/ml) for 30 min in the presence of emetine (100 μg/ml), as described previously [15]. After labelling, cells were washed with DMEM and cold PBS, and then treated with 1 % (w/v) SDS. Equal amounts of total protein [16] (120 μg) were analysed on 15 % polyacrylamide gels, subjected to fluorography after electrophoresis, and exposed to X-ray film for 48 h at –80 °C.

Analysis of tRNA aminoacylation

Isolation of RNA was performed with cell cultures with a lysis buffer containing 4 M guanidinium thiocyanate and 1 % 2-mercaptoethanol, and the pH was adjusted with 3 M sodium acetate, pH 4.3. Total RNA (10–15 μg) from each cell line was obtained under acid conditions (pH 4.1) to preserve aminoacylated tRNAs [7]. RNA (5 μg) was separated on a 1-mm-thick 6.5 % polyacrylamide gel (29:1)/7 M urea in 0.1 M sodium acetate, pH 5.0. Electrophoresis was performed at 80 V for 18 h and 200 V for 5 h in a 20 cm gel, with the buffer being recirculated continuously at 4 °C. The gel was then transferred to a Zeta-probe membrane in Tris/EDTA buffer for 30 min at 15 V (0.5 A), then for a further 2.5 h at 25 V (0.8 A), and the membranes were then hybridized and detected with the following [³²P]P_i 5'-end-labelled probes: a 42-mer oligonucleotide corresponding to mtDNA position 8365–8326 in the *tRNA^{Lys}* gene, which excludes the mutated site (8313); a 36-mer oligonucleotide corresponding to mtDNA positions 10441–10406 complementary to *tRNA^{Arg}*; and a 39-mer oligonucleotide (AACTTGAGTCTGGCGCCTTAGACCCTCGGCCATCTGA) complementary to the human cytosolic *tRNA^{Lys}*.

RESULTS

Isolation and characterization of transmitochondrial cybrids

Forty-five individual transmitochondrial cybrid clones were isolated from a fusion of enucleated fibroblasts from the patient (containing 54 % G8313A mtDNA) with mtDNA-less (143B-ρ⁰ cells). PCR-amplified fragments encompassing mtDNA nt 7955–8573 were obtained and analysed by 'last-cycle hot' PCR-RFLP, as described in the Materials and methods section. Using this procedure, we obtained several homoplasmic wild-type mtDNA clones (e.g. clone G4), as well as mtDNA G8313A heteroplasmic clones. A heteroplasmic cell line was treated with 100 ng/ml ethidium bromide in an attempt to obtain additional cell lines with increased levels of the mutated mtDNA. After 15 days of ethidium bromide treatment, cells were grown in complete medium for 24 days, and clones were isolated. Of the clones, two became ρ⁰ cell lines, three clones were the homoplasmic mutant (D1-30, D1-34, D1-37), one clone was 97 % mutant (D1-31),

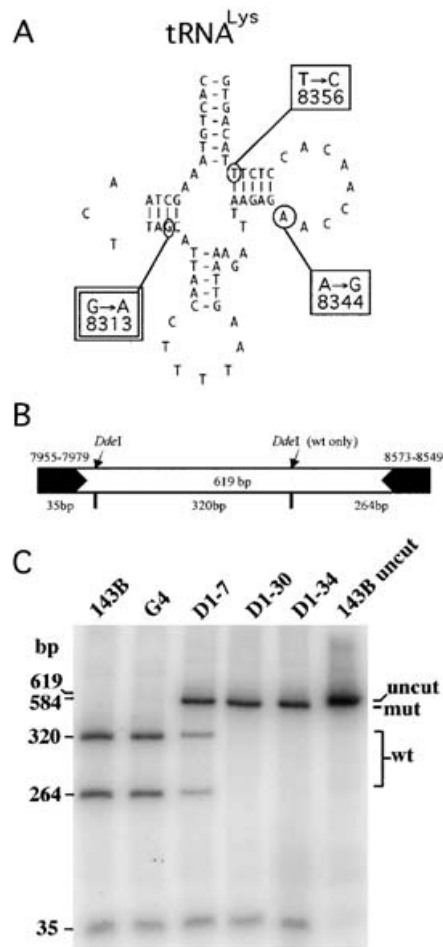


Figure 1 Characterization of transmitochondrial cybrid cell lines

(A) The secondary structure of the mitochondrial tRNA^{Lys} is shown, with the position of the most common pathogenic mutations as well as the G8313A transition. (B) illustrates the PCR-RFLP analysis of the G8313A transition. PCR products (619 bp) that are originated from wild-type (wt) genomes have an additional *DdeI* site (when compared with mutant cybrids), creating a diagnostic size difference in a non-denaturing polyacrylamide gel after digestion of PCR products (C). mtDNA from different clones were characterized. The following clones are shown in (C): a cell line containing essentially 100% wt mtDNA (G4), a heteroplasmic mutant cell line containing 82% mutated mtDNA (D1-7), two homoplasmic mutant cell lines (D1-30 and D1-34) and a control cell line (143B).

and one clone was 82% mutant (D1-7). Our RFLP assay for the detection of wild-type sequences was sensitive to 1% wild-type mtDNA (see the Materials and methods section), indicating that the homoplasmic mutant clones had >99% mutated mtDNA (Figure 1).

Mutant cybrids exhibit a severe respiratory-chain defect

Lactate release into the medium was increased in mutant clones (Figure 2A), suggesting a defective oxidative phosphorylation. The respiratory capacity of the transmitochondrial cybrid cell lines was determined by measuring the rates of ascorbate/TMPD-driven oxygen consumption of intact cells (Figure 2B). A severe defect was observed in cells containing high levels of mutated mtDNA. Using digitonin-permeabilized cells (Figure 2C), respiration driven by site I (glutamate/malate) and site IV substrates (ascorbate/TMPD) was severely decreased

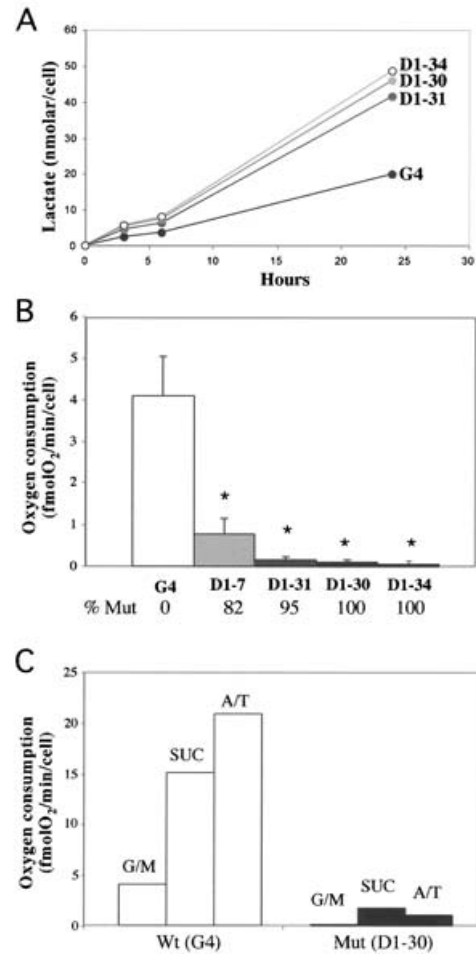


Figure 2 Cell respiration in transmitochondrial cybrid cell lines

(A) Release of lactate into the cultured medium was measured as described in the Materials and methods section, and normalized to the number of cells. (B) KCN-inhibited oxygen consumption driven by ascorbate/TMPD was measured for the different mutant and wild-type intact cells ($n \geq 3$). % Mut, % mutant. The asterisks (*) denote values statistically different from the control cybrid cell lines ($P < 0.001$ according to the Student's *t* test for equality of means for independent samples). Error bars represent S.D. (C) Polarographic determinations were performed in digitonin-permeabilized cells using the respective inhibitors/substrates glutamate and malate (G/M), rotenone and succinate (SUC) and antimycin A-ascorbate and TMPD (A/T). Wt, wild-type.

(97, 95 and 97% respectively) when compared with the homoplasmic wild-type clone G4.

Because of the respiratory defect, we analysed the respiratory-chain-enzyme activities of transmitochondrial cybrid cell lines. Figure 3 illustrates the activity of different respiratory enzyme complexes normalized to the activity of citrate synthase (a mitochondrial-matrix enzyme). The activity of the cybrids with 82, 97 and 100% mutated mtDNA showed a severe defect in complex II + III (Figure 3A) and complex IV (Figure 3C). Complex II activity was not significantly different between the tested clones.

Synthesis and steady-state levels of mitochondrial polypeptides

Steady-state levels of COXII, a subunit of cytochrome *c* oxidase, and ND1, a subunit of complex I (both mtDNA-encoded subunits),

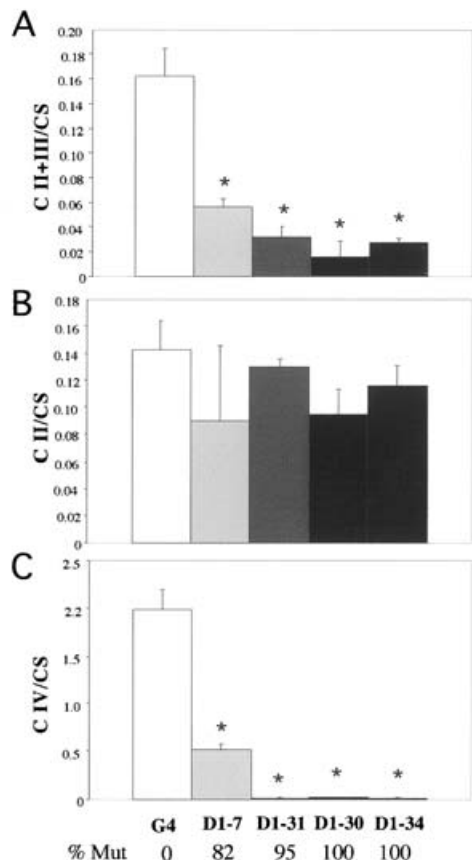


Figure 3 Spectrophotometric assay of the respiratory-chain-enzyme activities of human transmitochondrial hybrid cell lines

Specific activity of the respiratory-chain-enzyme complexes normalized to citrate synthase (CS) activity. Assays include complex II + III [C II + III (succinate cytochrome *c* oxidoreductase); **A**], complex II [C II (succinate decylubiquinone oxidoreductase); **B**] and complex IV [C IV (cytochrome *c* oxidase); **C**]. The asterisks (*) denote values statistically different from the control hybrid cell lines ($P < 0.001$, according to the Student's *t* test for equality of means for independent samples). Error bars represent S.D. ($n \geq 3$). % Mut, % mutant.

were markedly reduced in the mutant hybrid cell lines, as shown by Western blotting, whereas the steady-state levels of a nuclear-coded mitochondrial protein (SDH[Fp]) was not (Figure 4A).

The effect of the mtDNA G8313A mutation on mitochondrial protein synthesis was investigated by labelling cells with [35 S]methionine in the presence of emetine, a cytoplasmic ribosome inhibitor. Cells that were labelled with [35 S]methionine for 50 min were solubilized and analysed by SDS/15%-PAGE (Figure 4B). A mutant hybrid clone showed many bands with an altered migration, and normally sized bands were virtually absent. This observation suggests that a dysfunctional tRNA^{Lys} leads to premature termination of translation products. The absence of COXII and ND1 bands on Western blots (Figure 4A) suggests that such abnormal products are unstable.

Aminoacylation of mutated tRNA^{Lys}

To look for a potential deficiency in the aminoacylation of the mutated tRNA^{Lys}, we isolated total RNA from cell lysates under acidic conditions and electrophoresed them through a

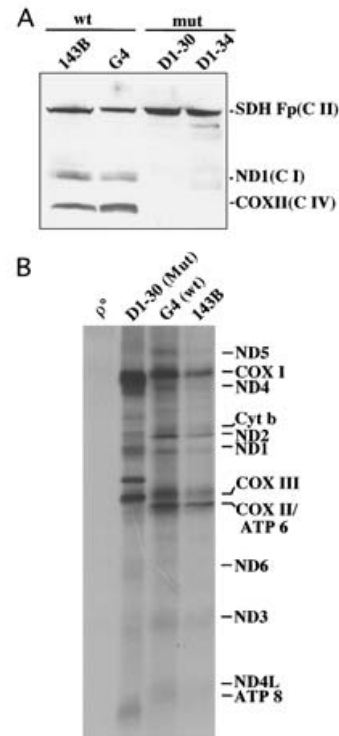


Figure 4 Mitochondrial translation of hybrid clones

(**A**) Steady-state levels of respiratory-chain subunits. Western blots of SDS lysates from different transmitochondrial cell lines were incubated with specific antibodies against complex IV subunit II (C IV; COXII), complex I subunit 1 (C I; ND1) and the flavoprotein subunit of complex II [C II; SDH(Fp)]. Colour development was stopped before previously determined half-maximum band intensities. (**B**) Fluorogram of mitochondrial protein synthesis products labelled with [35 S]methionine after electrophoresis through an SDS/15% polyacrylamide gel. Wild-type (wt) and mutant (Mut) hybrid lines were labelled for 50 min in the presence of emetine. A mtDNA-less ρ^0 cell line and the parental 143B cell line were included as a negative control. Equal amounts of SDS lysates of total cellular protein (120 μ g) were loaded in each lane. Bands were assigned as described by Chomyn [23].

6.5% polyacrylamide/7 M urea gel in acid buffer to preserve the amino acid moiety. After electroblotting, the membranes were hybridized to specific mitochondrial and cytosolic tRNA oligonucleotide probes. Figure 5(A) shows the acid Northern blots for the mitochondrial and cytosolic tRNA^{Lys}, and mitochondrial tRNA^{Arg}. The bands were quantified in a phosphorimager, and the percentage of aminoacylated tRNA is shown in Figure 4(B). We found that homoplasmic mutant clones showed a mean decrease in tRNA^{Lys} aminoacylation of 55% when compared with homoplasmic wild-type lines. We found no differences between homoplasmic mutant and homoplasmic wild-type RNA samples when cytosolic tRNA^{Lys} or mitochondrial tRNA^{Arg} were used (Figures 5A and 5B).

The steady-state levels of the mitochondrial tRNA^{Lys} were also significantly decreased. Although the blots shown in Figure 5(A) were adjusted to a similar intensity, the inset in Figure 5(C) exemplifies the decreased signal in mutant samples obtained with the mitochondrial tRNA^{Lys} probe. Figure 5(C) shows the ratio of mitochondrial tRNA^{Lys} to the mitochondrial tRNA^{Arg}, a ratio that was decreased by almost one order of magnitude. It is important to emphasize that the oligonucleotide probe used to detect the mitochondrial tRNA^{Lys} did not encompass the mutated site (see the Materials and methods section).

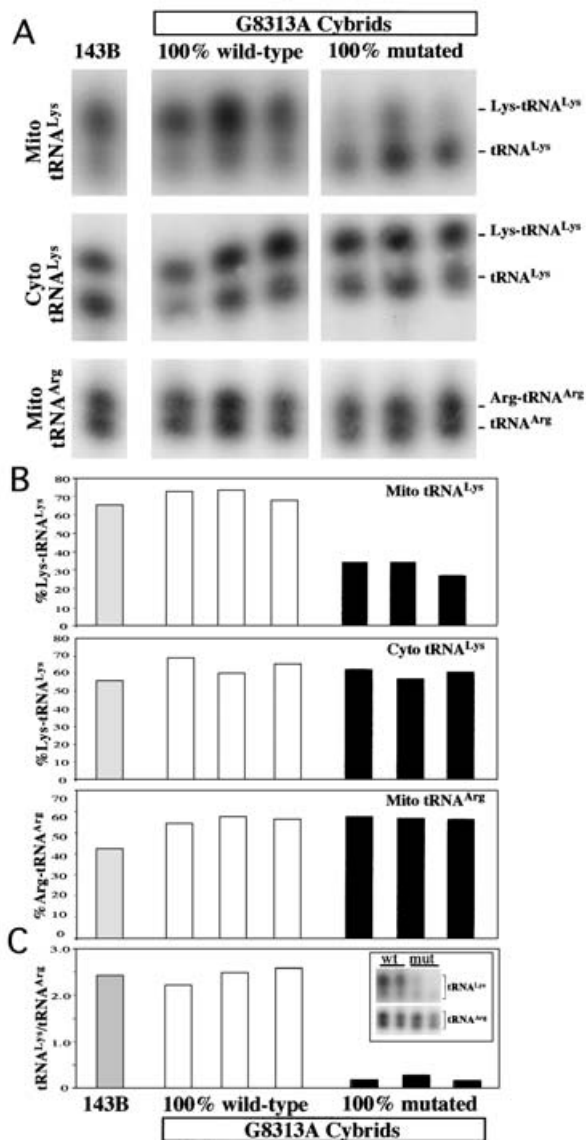


Figure 5 tRNA aminoacylation and steady-state levels

(A) Acidic Northern blots for the determination of tRNA aminoacylation in 143B, homoplasmic wild-type and G8313A mutant cell lines. Northern blots were performed for the mitochondrial tRNA^{Lys} (Mito tRNA^{Lys}), cytoplasmic tRNA^{Lys} (Cyto tRNA^{Lys}) and mitochondrial tRNA^{Arg} (Mito tRNA^{Arg}) species. The presence of the amino acid retards the migration of the tRNA. (B) The percentage of aminoacylated tRNA above that of total tRNA was calculated from phosphorimager readings of the acid Northern blots shown in (A). (C) The steady-state levels of Mito tRNA^{Lys} normalized against Mito tRNA^{Arg} were also determined from the acid Northern blots. The inset shows a 'same exposure' image to compare the mitochondrial tRNA levels in wild-type (wt) and mutant (Mut) lines.

DISCUSSION

Mutations in the mitochondrial tRNA^{Lys} have been associated with MERRF [3,17,18], encephalomyopathy [19], hypertrophic cardiomyopathy [20], diabetes/deafness [21] and Leigh syndrome [22]: therefore a better understanding of the molecular pathogenesis of these mutations is critical to the diagnosis and treatment of these diseases.

The repopulation of mtDNA-less cells (ρ^0 cells) with mtDNA harbouring point mutations is a valuable tool for investigating the molecular mechanisms of mtDNA alterations [4,5], and it

has been extensively used in the study of mitochondrial tRNA^{Lys} gene mutations [6,7,23,24]. We used this approach to investigate the molecular pathogenesis of the G8313A mutation in the mitochondrial tRNA^{Lys} gene, previously identified in a patient with gastrointestinal symptoms and progressive encephaloneuropathy [2]. The mtDNA G8313A mutation was heteroplasmic in the muscle biopsy (74%), and muscle extracts showed marked reduction in the activity of enzymes containing mtDNA-coded subunits. In agreement, transmitochondrial cybrids harbouring the mtDNA G8313A transition with a high load of the mutation showed a severe deficiency in respiratory-chain function, as demonstrated by increased lactate production, decreased oxygen consumption and impaired respiratory-complex enzyme activities. These biochemical defects seem to be caused by a drastic impairment in mitochondrial protein synthesis and in steady-state levels of mtDNA-coded polypeptides. Therefore a defect in tRNA^{Lys} function is likely to be the cause of the biochemical collapse.

Among several reactions in which tRNA molecules participate during protein synthesis, aminoacylation is the most specific, requiring a different synthase for each amino acid, and is presumably very sensitive to tRNA chemical modifications [25]. The G8313A mutation affects both steady-state levels and the aminoacylation efficiency of tRNA^{Lys}. The decrease in steady-state levels was particularly striking, which, in combination with the aminoacylation defect, resulted in a fraction of lysyl-tRNA^{Lys} that was approx. 15 times lower than that observed in controls. These observations are similar to (although more striking than) those made with cybrids harbouring the tRNA^{Lys} A8344G mutation [7]. We also detected a significant reduction in aminoacylation in the A8344G tRNA^{Lys} (D.P. Atencio and C.T. Moraes, unpublished work). Chomyn et al. [26] also found a defect in aminoacylation associated with the A3243G mutation in the mitochondrial tRNA^{Leu(UUR)} gene, and suggested that such defect would lead to a reduced association of mRNAs with ribosomes [26]. In contrast, Borner et al. [27], using an RT-PCR-based technique, did not find aminoacylation defects in tissue samples (heteroplasmic) from patients harbouring the A8344G mutation, but did find these in some patients with the A3243G mutation.

The tRNA^{Lys} aminoacylation defect was specific, because there was no change in aminoacylation levels of the mitochondrial tRNA^{Arg} in the homoplasmic mutant clones. This observation demonstrates that the decrease in aminoacylation is not a simple consequence of a reduced supply of ATP or any other biochemical 'collateral damage'.

Recently, the role of decreased aminoacylation associated with the A8344G mtDNA mutation has been downplayed by the observation that the mutated tRNA^{Lys} lacks a uridine modification in the anticodon 'wobble' position, which seems to impair codon-anticodon pairing [8]. However, other authors could not detect a difference in post-transcriptional modifications between A8344G and wild-type tRNA^{Lys} [28]. At this point, we cannot exclude the possibility that the defects associated with the G8313A mutation are caused by a codon-anticodon-pairing-problem interaction with elongation factors or ribosomes.

Although the G8313A mutation may lead to a defect in post-transcriptional modifications which could affect the functions described above, such an alteration is likely to affect the instability of tRNA^{Lys}, since its steady-state levels were dramatically lowered. This scenario would be similar to a report of a mutation in tRNA^{Ile} associated with a defect in post-transcriptional modification [29]. In that report, there was also a dramatic reduction in steady-state levels of tRNA^{Ile}. A decrease in steady-state levels of mutated mitochondrial tRNAs has been also observed in many other cell lines harbouring different tRNA gene

mutations [7,24,26,29,30]. Such a decrease, possibly as a consequence of changes in post-transcriptional modifications, might be a common pathogenetic mechanism for many mitochondrial tRNA gene mutations.

In addition to the extremely low tRNA^{Lys} levels, there are some additional differences between the molecular abnormalities observed in association with the G8313A when compared with the A8344G or T8356C mutations. In contrast with other studies [6,7,23], we observed a larger number of what appear to be truncated polypeptides in the mitochondrial protein-synthesis experiment. Moreover, some of the normally sized bands were absent. Therefore the translational defect in the G8313A mutant cells appears to be stronger than those observed for the other tRNA^{Lys} gene mutations [6,7,23].

In conclusion, we have provided evidence that steady-state levels of tRNA^{Lys}, tRNA^{Lys} aminoacylation and mitochondrial protein synthesis are severely altered in transmitochondrial cybrids harbouring high percentages of the mitochondrial G8313A tRNA^{Lys} mutation. As with other mtDNA mutations, it is not clear if (and how) this specific molecular pathogenesis dictates the clinical presentation of the disease.

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