Biochimica et Biophysica Acta, 1101 (1992) 206–209

BBABIO 43673

The mitochondrial $tRNA^{Leu(UUR)}$ mutation in MELAS: a model for pathogenesis

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(Received 14 April 1992)

Key words: Mitochondrion; Mitochondrial disease; mtDNA; Ribosome; Translation; rRNA

The A → G transition at nucleotide 3243 of the mitochondrial $tRNA^{Leu(UUR)}$ gene has been associated with MELAS, a maternally-inherited mitochondrial disorder. We recently transferred mitochondria harboring this mtDNA mutation into a human cell line devoid of endogenous mtDNA (ρ^0 cells), and showed: (1) decreased rate of synthesis and of steady-state levels of mitochondrial translational products, (2) reduced respiratory chain function and (3) increased amounts of a novel unprocessed RNA species (termed by us RNA 19) derived from transcription of the 16S rRNA + $tRNA^{Leu(UUR)}$ + ND 1 genes. Because RNA 19 contains rRNA sequences, we propose that this molecule is incorporated into mitochondrial ribosomes, and interferes disproportionately with mitochondrial translation, thereby causing the phenotypic changes associated with MELAS.

MELAS is a maternally-inherited mitochondrial disorder characterized by vomiting, seizures, lactic acidosis, migraine-like headaches, and recurrent cerebral insults resembling strokes and which cause hemiparesis, hemianopia or cortical blindness [1]. A point mutation in the human mitochondrial $tRNA^{Leu(UUR)}$ gene has been found in the mtDNA of many MELAS patients [2–4]. The mutation is an A → G transition at mtDNA map position 3243 [5] and is invariably heteroplasmic, with the proportion of mutated mtDNAs often exceeding 80% in muscle [6,7].

We recently described the use of a cell culture system to analyze the phenotypic consequences of the MELAS-3243 mutation [8]. This system is based on repopulation of a human cell line that is completely devoid of mtDNA (ρ^0 cell lines) with exogenous mitochondria [9]. We repopulated ρ^0 cells with mitochondria from two different patients with the MELAS-3243 mutation, and created clonal cytoplasmic hybrid (cybrid) cell lines harboring varying proportions of mutant mtDNAs, including lines with 100% normal mtDNAs ('wild-type' cybrids) and 100% mutant mtDNAs ('mutant' cybrids). Using morphological, biochemical, and genetic techniques, we found that cybrids harboring 100% mutated mtDNAs, but not those harboring 100% normal mtDNAs, displayed quantitative deficiencies in protein synthesis, respiratory chain activity, and cell growth [8]. These deficiencies are most likely not a direct result of a defect in leucine incorporation during translation as a result of a mutation in $tRNA^{Leu(UUR)}$, as there was no correlation between the efficiency of translation of the various mitochondrial mRNAs and the number of Leu(UUR) codons present in those mRNAs. Moreover, even in cybrids with 100% mutant mtDNAs, all the mtDNA-encoded polypeptides appeared qualitatively normal by polyacrylamide gel electrophoresis (except, perhaps, for a slight difference in the migration of ND1).

In Northern blot analysis, a novel RNA transcript corresponding to the 16S rRNA + $tRNA^{Leu(UUR)}$ + ND 1 genes (which are contiguous in the mtDNA) was identified [8]. This species was designated RNA 19, by extension of the nomenclature for the identified human mitochondrial transcripts [10]. A hybridizing band corresponding to RNA19 was identified in total RNA isolated from both the wild-type and mutant cybrids. However, there was a significant increase in the steady-state levels of RNA 19 (relative to the amount of either cytoplasmic β-actin mRNA or to other analyzed mitochondrial RNA species) in the mutant cybrids as compared to the wild-type cybrids (Table I).The

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Abbreviations: COX, cytochrome *c* oxidase; KSS, Kearns-Sayre syndrome; MERRF, myoclonus epilepsy with ragged-red fibers; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes; mtDNA, mitochondrial DNA; ND, NADH dehydrogenase; RRF, ragged-red fibers.

TABLE I

Levels of RNA 19 and oxygen consumption in ρ^o-MELAS cybrids

Cybrid [a]	Genotype [b]	RNA 19/β-actin [c]	O_2 consumption [d]
WS214	W	0.040	5.120
WS241	W	0.058	5.060
RN204	W	0.046	3.850
RN236	W	0.046	5.420
WS227	M	0.069	2.710
WS216	M	0.127	0.330
RN164	M	0.080	1.010
RN223	M	0.105	0.490

[a] Cell lines and methods described in Ref. 8.
[b] W, wild-type cybrid; M, mutant cybrid.
[c] Dimensionless value; see Fig. 6 in Ref. 8.
[d] Values in fmol/cell per min.

ratios of each of the other mitochondrial RNA species examined (ND1 mRNA, ND5 mRNA, 12S rRNA and 16S rRNA) to β-actin were not significantly different between the mutant and the wild-type cybrid cell lines. These results imply that, aside from RNA 19, the steady-state levels of each mitochondrial RNA species per cell was unchanged in the mutant as compared to the wild-type lines.

It is likely that RNA 19 is a partially-processed product derived from the normally-produced full-length polycistronic H-strand precursor RNA. This transcript is normally processed to produce the mRNAs and most of the tRNAs. The exact cause of the elevated levels of RNA 19 in mutant cybrids as compared to wild-type cybrids is currently unknown. Presumably, the MELAS-3243 mutation alters normal processing of the primary transcript, resulting in an elevated level of RNA 19, due to decreased levels of cleavage at the 5′ and 3′ ends of the $tRNA^{Leu(UUR)}$ portion of the primary transcript.

The average RNA 19 level in the mutant cybrids was approximately twice that in the wild-type cybrids. This level was approx. 28% of the amount of mature ND 1 mRNA in mutant cybrids. However, the levels of RNA 19 in all cybrids were extremely low (less than 1%) in comparison with the levels of mature 16S rRNA, which is an abundant species. In spite of the low amounts of RNA 19 present, we observed a very strong inverse correlation between the levels of RNA 19 per cell (i.e., RNA 19/β-actin) and the rates of oxygen consumption in the cybrid cell lines (Table I and Fig. 1). The correlation of increased levels of RNA 19 with decreases in mitochondrial respiratory function is also supported by our estimates of the decrease in the rates of protein synthesis in the mutant cybrids. The mutant cybrid with the lowest amount of RNA 19 (cybrid WS227) had the lowest impairment in the rate of protein synthesis (as measured by densitometry of labelled mtDNA-encoded polypeptides); the other mutant cybrids, which had higher levels of RNA 19, were more severely impaired.

Normally, a small increase in the already low level of a species like RNA 19 would not be expected to have phenotypic consequences. However, the inverse correlation of RNA 19 levels with the rates of oxygen consumption suggests that a cause-and-effect relationship may exist. If so, how can a molecule present at less than 1% of the level of mature rRNAs exert a deleterious effect on mitochondrial translation and respiratory chain function? We believe that the unique composition of the RNA 19 molecule may account for this phenomenon. Specifically, RNA 19 contains 16S rRNA as one of its 3 components. Therefore, it is possible that RNA 19 can be incorporated into ribosomes, rendering them functionally deficient (e.g., ribosomes might not be able to travel efficiently on the mRNA, or they might not be able to incorporate charged tRNAs as rapidly).

If mRNAs were translated only by monoribosomes, the incorporation of RNA 19 molecules into 1% of all ribosomes would impair the translation of only 1% of all mRNAs, resulting in a decrease in translation that would be considered insignificant, both practically and theoretically. However, since mitochondrial mRNAs are translated on polyribosomes [11], even a low level of RNA 19 (if incorporated into ribosomes) could have disproportionate effects on mitochondrial translation. If an RNA 19-containing ribosome paused during translation of an mRNA, it could prevent efficient translation of all the normal ribosomes following behind it on the mRNA (Fig. 2). This hypothesis is also consistent with our observation [8] that the synthesis of

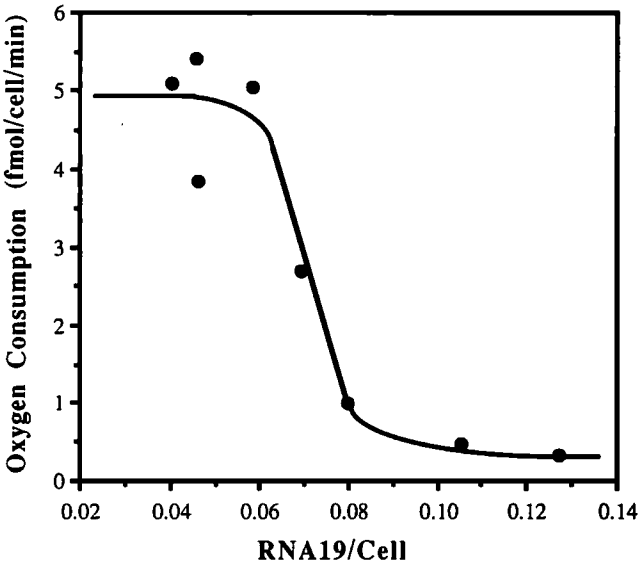

Fig. 1. Correlation of levels of RNA 19 per cell with oxygen consumption in ρ^o-MELAS cybrids. The ratios of RNA 19:β-actin mRNA (arbitrary units) was plotted against oxygen consumption for each of the MELAS cybrids so analyzed.

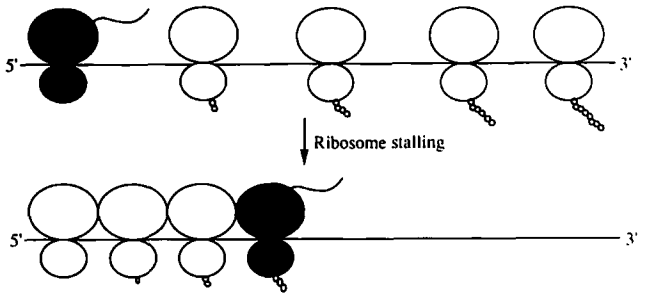

Fig. 2. 'Ribosome stalling' model for pathogenesis of MELAS. Upper figure: a nascent polypeptide (protruding chain of small circles) is produced at a rapid basal rate as normal ribosomes (open symbols) traverse the mitochondrial mRNA (line). An altered ribosome (solid symbol) containing RNA 19 (protruding line) enters at the 5′ end of the mRNA and travels more slowly on it. Lower figure: the RNA 19-containing ribosome becomes rate-limiting for translation, as all normal ribosomes entering subsequently stall behind the RNA 19-containing ribosome.

the larger mitochondrial polypeptides (such as ND 4, ND 5, COX I, and Cyt *b*) appeared to be inhibited disproportionately as compared to that of most of the smaller polypeptides (ND 6, ND 4L, and ATPase 8). Since the smaller polypeptides are encoded by shorter mRNAs than are the larger polypeptides, there would be fewer ribosomes per message on shorter mRNAs, and, therefore, there would be a proportionally lower probability that any single mRNA would contained a stalled RNA 19-containing ribosome. Conversely, the longer mRNAs would be more likely to contain a stalled ribosome. Thus, this hypothesis could explain the observed decrease in protein synthesis, which does not correlate with the number of Leu(UUR) codons present in each mRNA.

The MELAS phenotype is relatively unusual compared to other mitochondrial disorders associated with mitochondrial proliferation in muscle (characterized by RRF), such as MERRF (associated with a point mutation in the $tRNA^{Lys}$ gene [12,13]) and KSS (associated with deletions of mtDNA [14–16]). In MERRF and KSS, the RRF are predominantly COX-negative, but in MELAS they are predominantly COX-positive [7,17]. This observation is consistent with the finding that the translational defect in MELAS resulting from the mutation in $tRNA^{Leu(UUR)}$ is essentially quantitative (i.e., mitochondria containing only mutated genomes can probably still synthesize normal-sized, functional translation products, albeit at reduced rates), while the translational errors in MERRF and KSS may be more qualitative in nature (i.e., mitochondria containing only mutated genomes probably synthesize aberrantly-sized translational products, which are likely non-functional [18,19]). Perhaps only a mutation associated with a transcript that can be incorporated into ribosomes, such as a mutation in the $tRNA^{Leu(UUR)}$ gene, would lead to a strictly quantitative decrease in protein synthesis.

While the hypothesis for the pathogenesis of MELAS presented here is speculative, it is consistent with the data obtained in our analysis of the ρ^0-MELAS cybrids [8]. Investigations of the presence of RNA 19 in ribosomes isolated from wild-type and mutant cybrids, as well as analyses of changes in polysome composition and number, will be required to assess the validity of this model. Furthermore, the recent finding of a second point mutation in the $tRNA^{Leu(UUR)}$ gene that is also associated with MELAS, at nucleotide position 3271 [20], may provide another test of this hypothesis.

Acknowledgements

This work was supported by grants from the National Institutes of Health (NS28828 and AG08702), the Muscular Dystrophy Association, the Aaron Diamond Foundation, and by a grant from Libero and Graziella Danesi (Milano, Italy). M.P.K. is a recipient of the Harry Zimmerman Neuromuscular Disease Research Fellowship of the Muscular Dystrophy Association. Y.K. is the David Warfield Fellow in Ophthalmology of the New York Community Trust and the New York Academy of Medicine.

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