*Clipboard*

# Leishmanial proteins can correct human mitochondrial mutations

The kinetoplastid parasites are a class of flagellate protozoan microorganisms. They are characterized by the possesion, within their single mitochondrion, of a DNA-containing granule known as the kinetoplast. Trypanosomatids, which constitute an Order among kinetoplastids, contain parasitic genera that are major agents of human disease – for example, sleeping sickness and Chagas disease (caused by species of *Trypanosoma*) and leishmaniasis (caused by species of *Leishmania*). A recent publication by Mahata *et al* (2006) advances the surprising possibility that proteins from *Leishmania* may in fact be useful for treating human disease – specifically, for curing disease originating from certain mitochondrial defects.

Mitochondria are the energy-producing symbiotic organelles that are present in the cells of most eukaryotes. In general, they are essential for the survival of the cell. Testifying to their independent ancestry, they contain their own DNA. The human mitochondrial genome is a small (16,569 bp) circular DNA molecule encoding 13 proteins required for oxidative phosphorylation, 22 tRNAs and 2 rRNAs. When mutated, it can lead to various neurological and muscular disorders that can be identified by their maternal inheritance pattern. Deletions and missense mutations in mitochondrial genes have been mapped for diseases such as Leber's hereditary optic neuropathy (LHON), myoclonic epilepsy associated with ragged red muscle fibres (MERRF, indicative of mitochondrial proliferation in muscle), Kearns-Sayre syndrome (KSS) and a neurological syndrome leading to retinitis pigmentosa, ataxia, seizures and dementia. In most of these diseases, the affected individual's cells are heteroplasmic, meaning that they contain a mixture of normal and mutant mitochondria. The severity of the disease is affected by the ratio of mutant to normal mitochondria (Lander and Lodish 1990).

It turns out that mitochondria encode only a small subset of the proteins and RNAs that they require for their functioning. This means that they need to import many nuclear genome-encoded proteins and RNAs. However, in the case of human mitochondria, all the tRNAs required for making mitochondrial proteins are encoded by the mitochondrial genome itself. Therefore human mitochondria do not normally import tRNAs. In contrast, trypanosomatid genomes do not encode any mitochondrial tRNA genes at all. Their evolution has resulted in a condition in which they import (from the host) all the tRNAs that are required for translation by the kinetoplastid. In trypanosomatids, this typically parasitic property of a loss of function is accompanied by the co-evolution of an RNA import complex (RIC), a large multi-subunit aggregate containing several tRNA binding proteins.

Myoclonic epilepsy is associated with point mutations in human mitochondrial DNA at positions 8344 and 8356. These alter the $tRNA^{Lys}$ T$\psi$C loop and stem respectively (Shoffner *et al* 1990; Masucci *et al* 1995). By making use of cytoplasmic hybrids or 'cybrids', Masucci *et al* (1995) demonstrated that both mutations result in aberrant or inefficient mitochondrial translation and lead to the same biochemical and phenotypic alterations. Subsequent work suggested that one might try to rescue human mitochondrial defects in two ways. Goswami *et al* (2003) discovered that human $tRNA^{Lys}$ was imported by the *Leishmania* mitochondrion. Working with cell cultures, Kolesnikova *et al* (2004) were able to functionally complement the human mitochondrial A8344G $tRNA^{Lys}$ mutation by inducing defective mitochondria to import yeast $tRNA^{Lys}$ . These experiments showed that tRNAs from different species could be efficiently interchanged and loss of mitochondrial function restored. However, the methods suffered from too many difficulties – for example, low efficiency and toxicity caused by the transfection vehicle – to make it possible to think of modifying them for therapeutic purposes.

The latest report by Mahata *et al* (2006) makes use of an ingenious method to overcome the low efficiency and toxicity of transfection vehicles: they introduce the RNA import complex (RIC) of

**Keywords.** Human mitochondrial mutations; *Leishmania* RNA import complex; maternally inherited disorders

*Leishmania* into human cells. The exogenously added RIC was taken up by a caveolin-1 dependant pathway and subsequently facilitated the import of cytosolic tRNAs in cybrids that harboured mutants of human mt-tRNA$^{Lys}$ . Using siRNA targeted to caveolin and clathrin, they show that *Leishmania* RIC is internalized specifically by the caveolin-1 pathway and not by the clathrin-mediated endocytotic pathway. After having demonstrated the uptake of RIC in cybrids, they tested the hypothesis that the RIC could cause tRNA to be imported in whole cells. Untreated MERRF mitochondria show a general reduction in mitochondrial protein synthesis and accumulation of aberrant translational products. Treatment with RIC completely restored the protein synthesis profile to wild-type levels and suppressed the formation of aberrant products. Additionally, as a consequence of its tRNA import by the RIC, the respiratory activity of mitochondria was restored. The recovery of wild-type functions was stable for at least 4 generations in these cells; the authors argue that this could be due to dilution of the RIC over time.

The observations of Mahata *et al* (2006) have opened up interesting possibilities for the therapy of inherited mitochondrial diseases in humans. Many years ago Lander and Lodish (1990) drew attention to the inherent difficulties to be overcome in thinking of gene therapy for mitochondrial diseases. The application of protozoan proteins to correct human disorders was surely unforeseen both by human geneticists and protozoologists.

## References

Goswami S, Chatterjee S, Bhattacharyya S N, Basu S and Adhya S 2003 Allosteric regulation of tRNA import: interactions between tRNA domains at the inner membrane of Leishmania mitochondria; *Nucleic Acids Res.* **31** 5552–5559

Kolesnikova O A, Entelis N S, Jacquin-Becker C, Goltzene F, Chrzanowska-Lightowlers Z M, Lightowlers R N, Martin R P and Tarassov I 2004 Nuclear DNA-encoded tRNAs targeted into mitochondria can rescue a mitochondrial DNA mutation associated with the MERRF syndrome in cultured human cells; *Hum. Mol. Genet.***13** 2519–2534

Lander E S and Lodish H 1990 Gene mapping and gene therapy; *Cell* **61** 925–926

Masucci J P, Davidson M, Koga Y, Schon E A and King M P 1995 In Vitro Analysis of Mutations Causing Myoclonus Epilepsy with Ragged-Red Fibers in the Mitochondrial tRNALys Gene: Two Genotypes Produce Similar Phenotypes; *Mol. Cell Biol.* **15** 2872–2881

Mahata B, Mukherjee S, Mishra S, Bandyopadhyay A and Adhya S 2006 Functional Delivery of a Cytosolic tRNA into Mutant Mitochondria of Human Cells; *Science* **314** 471–474

Shoffner J M, Lott M T, Lezza A M S, Seibel P, Ballinger S W and Wallace D C 1990 Myoclonic Epilepsy and Ragged-Red Fiber Disease (MERRF) Is Associated with a Mitochondrial DNA tRNALys Mutation; *Cell* **61** 931–93

Anuradha Lohia
*Department of Biochemistry, Bose Institute,*
*Kolkata 700 054, India*
*(Email, anuradha@bic.boseinst.ernet.in)*

*e*Publication: 13 November 2006