

The ins and outs of tRNA transport

Transfer RNAs (tRNAs) are involved in many cellular functions distributed throughout the cellular space. In addition to their role in protein translation, these molecules are engaged in tasks as diverse as the regulation of gene expression, amino-acid synthesis, protein degradation, cell-wall synthesis, porphyrin biosynthesis, priming of replication, RNA interference and the transport of macromolecules. The nucleus and the mitochondria of eukaryotic cells, as well as the chloroplasts in plants, contain their own genome that encodes a range of proteins and nucleic acids. Early evidence suggested that some of the RNA content of mitochondria could be transcribed from non-mitochondrial DNA (Suyama, 1967). It is now well established that a variable number of tRNA species present in the mitochondria are indeed nucleus-encoded (Fig 1). The phenomenon of mitochondrial tRNA import has been reported in plants, marsupials, the yeast *Saccharomyces cerevisiae*, and the protozoa *Leishmania*, *Trypanosoma* and *Tetrahymena*. When certain tRNA species are not encoded by the mitochondrial genome, there is a predictable requirement for the import of nucleus-encoded tRNAs into the mitochondria (Schneider & Marechal-Drouard, 2000). This is the case, for example, for the mitochondria of *Leishmania*, which are completely devoid of tRNA-encoding genes. However, there are a few examples of tRNA import into mitochondria that already have a complete set of mitochondria-encoded tRNAs. Other tRNA transport systems have also been identified (Fig 1), which include their retrograde transport from the cytoplasm to the nucleus (Takano *et al*, 2005; Shaheen & Hopper, 2005), the export of mitochondria-encoded tRNA to the cytoplasm (Maniataki & Mourelatos, 2005), and the packaging of tRNAs into retroviruses (Waters & Mullin, 1977). The roles of some of these transport systems have yet to be defined.

In yeast, the cytoplasmic, nucleus-encoded tRNA^(Lys, CUU) is imported into the mitochondria, although the mitochondrial genome encodes the full set of tRNAs, including an authentic tRNA^{Lys} (Tarassov & Entelis, 1992). Furthermore, the yeast mitochondrial lysyl-tRNA synthetase (mLysRS) cannot aminoacylate the nucleus-encoded tRNA^(Lys, CUU). This is because the mitochondrial, prokaryotic-type enzyme requires an adenine at position 73 to aminoacylate its cognate tRNA, whereas the eukaryotic enzyme accepts any of the four nucleotides at this position. Therefore, the physiological significance of this import is unclear. When the import of a tRNA species is not a prerequisite for mitochondrial protein synthesis, one interesting possibility is that import of that tRNA is a means to transport a protein into the mitochondrial compartment in a co-import mechanism. This suggests that the

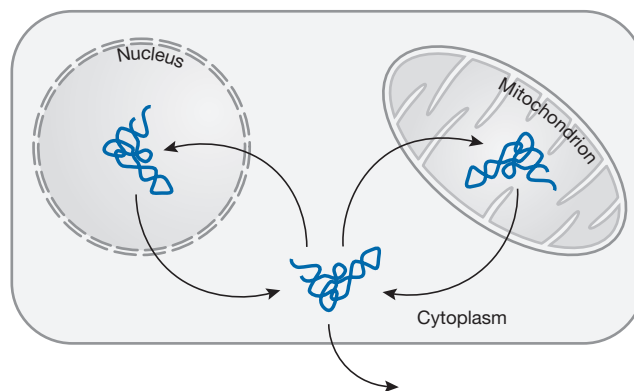


Fig 1 | The travels of transfer RNA. tRNAs are either nucleus- or mitochondria-encoded. Several tRNA transport systems have been described: from the nucleus or the mitochondria to the cytoplasm; from the cytoplasm to the nucleus or the mitochondria; and export out of the cell.

co-imported protein is not able to use the protein-import system. Such a mechanism has been described for the nuclear export of human elongation factor EF1A, which is efficiently excluded from the nucleus by a co-transport process involving exportin 5 and tRNA as a carrier molecule (Bohnsack *et al*, 2002). This explanation might hold true in some circumstances for mitochondrial import, but is not a universal answer to the problem. Indeed, the nucleus-encoded tRNA^(Lys, CUU) referred to above is actually co-imported through the protein-import pathway.

The essential requirement of tRNA import for protein synthesis to occur in the mitochondria of some organisms stimulated the initial research in this field (Marechal-Drouard *et al*, 1988; Hancock & Hajduk, 1990; Lye *et al*, 1993; Schneider *et al*, 1994). However, no unifying mechanism has emerged from the various *in vitro* and *in vivo* studies performed so far. In *S. cerevisiae*, cytosolic factors are involved in this process. Co-import of a tRNA^(Lys, CUU) with the mitochondrial precursor of LysRS has been established (Tarassov *et al*, 1995), but recruitment of this tRNA to the mitochondrial compartment also requires the glycolytic enzyme, enolase (Entelis *et al*, 2006). In this system, it is likely that the protein-import channels are used to translocate tRNA^(Lys, CUU). By contrast, import of tRNA in *Leishmania* mitochondria does not require cytosolic factors and the existence of a receptor-mediated import pathway has been uncovered (Mahapatra *et al*, 1994; Mahapatra & Adhya, 1996). A large multisubunit complex, isolated from the inner mitochondrial membrane of this organism, promotes tRNA import when incorporated into phospholipid vesicles (Bhattacharyya *et al*, 2003). Transport is ATP-dependent. Previous studies identified two components of this RNA import complex—RIC1 and RIC8A—as tRNA import receptors. RIC1 is homologous to the α -subunit of

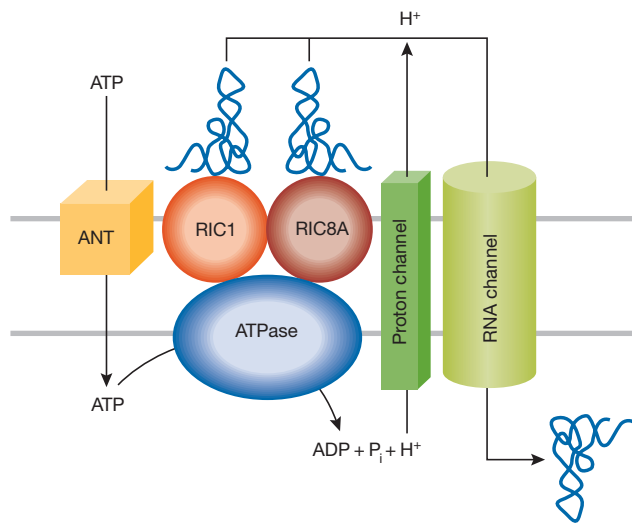


Fig 2 | The machinery of nucleus-encoded tRNA import through the inner mitochondrial membrane in *Leishmania*. Adapted with the permission of Landes Bioscience from Bhattacharyya & Adhya, 2004. ANT, a transporter similar to the adenine nucleotide translocator; RIC, RNA import complex.

F_1 ATP synthase (Goswami *et al*, 2006), and RIC8A is homologous to subunit 6b of the respiratory complex III (Chatterjee *et al*, 2006). Interestingly, RIC1 and RIC8A are responsible for the transport of type I and type II RNAs, respectively: RIC1 specifically recognizes import determinants localized in the DHU stem loop of tRNAs, whereas RIC8A recognizes determinants in the variable-TΨC stem-loop structure.

In this issue of *EMBO reports*, Mukherjee and colleagues provide a comprehensive description of the isolation and preliminary characterization of the RIC that mediates mitochondrial tRNA import in the kinetoplastid protozoan *Leishmania tropica* (Mukherjee *et al*, 2007). The authors affinity-purified the RIC through a column containing a tRNA-import signal oligonucleotide, and, by using mass spectrometry, the various components were identified. A total of 11 subunits were detected, eight of which are nucleus-encoded. Probably the most surprising feature of the RIC is that five of its components are shared with respiratory complexes, and therefore are likely to be bifunctional: RIC6 and RIC8A are the iron sulphur protein and subunit 6b of the respiratory complex III, respectively; RIC5 and RIC9 are components of complex IV; and RIC1 is also known as the F_1 ATP synthase subunit of complex V. Consequently, RIC provides a spectacular example of a complex that is a reservoir of multifunctional proteins and strengthens the view that the association of a polypeptide with a variable set of partners expands the functions of a given protein. Systematic knockdown of the RIC components by RNA interference was used to determine their role in mitochondrial tRNA import. The six subunits—RIC1, 4A, 6, 8A, 8B and 9—are essential *in vivo*. They are also necessary and sufficient to support tRNA transport *in vitro* in a reconstitution assay. Indeed, the various components were expressed in *Escherichia coli* and an active RIC could be reconstituted into liposomes. This complex displayed the regulatory properties of a native RIC, suggesting that

the identified components are necessary and sufficient for tRNA import. This is a significant advance towards the understanding of the import process, and these new data will certainly stimulate further investigations in the field. Now that the components of the molecular machinery responsible for mitochondrial tRNA import are known, further study will undoubtedly lead to the elucidation of the function of all the components of the RIC and provide a detailed description of the process.

Additional studies will be necessary to understand fully the mitochondrial tRNA-import mechanism. Intriguingly, only a fraction of a cytosolic tRNA is imported into the mitochondria, so what mechanism governs the balance between the mitochondrial and cytosolic fractions of a given tRNA? Another challenge is to understand the role of ATP hydrolysis and the proton gradient in tRNA translocation. A working model has been proposed (Bhattacharyya & Adhya, 2004), which suggests that binding of tRNAs to the RIC triggers a series of events that leads to tRNA translocation: activation of an ATPase, ATP hydrolysis in the mitochondrial matrix, export of protons and, finally, proton-dependent tRNA import through an RNA channel (Fig 2). How universal this mechanism is remains to be established. The extent of mitochondrial tRNA import differs between species, suggesting that import machineries have been independently selected as a result of the loss of organellar tRNA genes during evolution. It is noteworthy that in plant mitochondria, a distinct import machinery—the voltage-dependent anion channel—has been identified (Salinas *et al*, 2006). Finally, several human diseases are associated with mutations in mitochondrial tRNA genes. Human mitochondria do not naturally import tRNA, but the possibility of rescuing mutations through the import of nucleus-encoded tRNAs has been established (Kolesnikova *et al*, 2004; Mahata *et al*, 2006). Therefore, a fuller understanding of the tRNA-import process as provided by Mukherjee and colleagues might also have an impact on the rescue of mitochondrial dysfunctions.

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