Sequence-specific visualization of mitochondrial RNA in living cells

Jaroslav Zelenka, Lukáš Alán, Petr Ježek

Institute of Physiology, Czech Academy of Sciences, Dept.75
Vídeňská 1083, Prague 4, Czech Republic
E-mail: zelenka@biomed.cas.cz

KEY WORDS: fluorescence microscopy, mitochondrial import, RNA labeling

Mitochondria are metabolic powerplants of eukaryotic cells and important contributors to basic physiological processes such as oxygen sensing and hypoxic response of cells, insulin secretion of pancreatic β-cells, proliferation, apoptosis, signaling and responses to various stresses. Mitochondria contain autonomous DNA which encodes for 13 proteins, 22 tRNAs and 2 rRNAs. Mitochondrial transcription and further RNA processing are key steps of mitochondrial biogenesis and function, while their decline is implicated in ageing and pathogenesis of numerous diseases. Surprisingly, although studies of nuclear-encoded RNAs represent one of the most exposed fields of biology, little is known about mitochondrial RNA. We applied the previously reported innate ability of nuclear-encoded 5S rRNA [1] to enter mitochondria and constructed fluorescence RNA hybridization probes which could be useful for further development of fluorescence in vivo hybridization enabling detailed studies on mitochondrial RNAs [2]. We synthesized ssRNA probe composed of mitochondrial (mt) address, antisense sequence against 5´end of mt ND5 mRNA and fluorescent label at the 3´end. Constructs were transfected into HepG2 cells expressing mitochondria-addressed GFP. We observed colocalization of the probe with mitochondria-addressed GFP in living cells, proving the probe import into the mitochondrial matrix. In addition, the import of the probe was verified by RT-PCR of the purified mitochondria treated with Rnase A. Further use of these probes for fluorescent in vivo hybridization of mitochondrial RNAs will be discussed.

Supported by grants no. P305/12/P388 and P305/12/1247 given by the Grant Agency of the Czech Republic.
