CHANGES IN MITOCHONDRIAL NETWORK MORPHOLOGY OF MAMMALIAN CELLS OBSERVED BY 4Pi MICROSCOPY

Lydie Plecitá¹, Mark Lessard ², Jitka Šantorová¹, Joerg Bewersdorf ², Petr Ježek¹

¹Institute of Physiology, Czech Academy of Sciences
Videnska 1083, Prague 4, Czech Republic
²Institute of Molecular Biophysics, The Jackson Laboratory, Bar Harbor, Maine, USA

E-mail: hlavata@biomed.cas.cz; jezek@biomed.cas.cz

Key words: 3D mitochondrial morphology, 4Pi microscopy, oxidative phosphorylation

A wide number of mammalian cells possess mitochondrial reticular networks which are continuously remodeled by cycles of fusion and fission events. The observed various mitochondrial shapes show characteristic features at the 100 – 500 nm size scale in three dimensions which cannot be investigated properly by traditional confocal microscopy due to resolution limitations. We have used 4Pi microscopy [1], which provides ~250 nm lateral and ~100-150 nm axial resolution to follow changes in mitochondrial network morphology responding to variations in mitochondrial oxidative phosphorylation and energetic status in hepatocellular carcinoma HepG2 and insulinoma INS-1E cells.

Mitochondria of HepG2 cells showed bulkier (mitochondrial diameter increase from ~270 nm to ~390 nm) and less dense perinuclear mitochondrial networks in the presence of high (25mM) glucose. This corresponds to switching of energy metabolism towards cytoplasmic glycolysis at low intensity of mitochondrial oxidative phosphorylation. On the other hand, mitochondria of INS-1E cells, which play a central role in energy generation and insulin secretion, showed partly branched, dense mitochondrial networks comprising of thin ~260 nm tubules, hardly distinguishable in classical confocal microscope. Furthermore, inhibition of the mitochondrial respiration on Complex I together with induction of mild oxidative stress showed fragmentation of reticular network in both cell types. The de-energetization of mitochondria by uncoupling (setting zero proton-motive force), supressing oxidative phosphorylation and decreasing the oxidative stress, gave rise to solitary or integrated rings of mitochondrial reticulum, being short segments fused to such units. Inhibition of respiration together with uncoupling led in INS-1E cells to closing of the rings, forming shell-like objects. Thus despite of the reported cleavage of a subset of pro-fusion OPA1 isoforms into short forms by de-energization [2], other OPA1 isoforms or other pro-fusion mitodynamins help to fuse the segments into the rings or cisternae. In conclusion, using 4Pi microscopy for visualisation of mitochondria enables us to uncover the influence of mitochondrial activity, i.e. energy production on mitochondrial morphology in mammalian cells. Supported by grants NR/7917-6, NR/9183 - 3 (Czech Ministry of Health); and IAA500110701 (Academy of Sciences).