SPATIAL REDISTRIBUTION OF MITOCHONDRIAL ATP SYNTHASE IN PANCREATIC β-CELLS UNDER GLUCOSE STIMULATED INSULIN SECRETION AS VISUALIZED BY 3D dSTORM AND STED MICROSCOPY

Andrea Dlasková, Anežka Kahancová, Tomáš Špaček and Petr Ježek

Institute of Physiology, Dept.75, Czech Academy of Sciences, Czech Republic

dlaskova@biomed.cas.cz, jezek@biomed.cas.cz

In pancreatic β-cells the mitochondrial ATP synthase is an inherent part of a glucose sensor for insulin secretion and studies dealing with its regulation are thus crucial. Previous reports have shown a link between the spatial distribution and regulation of energy metabolism [1]. We addressed a question how spatial (topological) organization of mitochondrial ATP synthase in model pancreatic β-cells (INS1E) depends on glucose availability. ATP-synthase was labeled by means of primary antibody against the subunit alpha directly conjugated to Alexa Fluor 647. By both 3D dSTORM and 3D STED microscopy we have shown that the ATP synthase is organized into clusters which distribution pattern is dependent on glucose concentration. While cells incubated with low glucose exhibited more diffuse pattern of ATP synthase clusters with more localized particles, cells incubated with standard or high glucose had more punctual localization of clusters and less localized particles (Fig.1). By western blots we have found that the total amount of ATP synthase is constant under all conditions indicating that observed changes are solely due to ATP synthase spatial reorganization. This view is also supported by our biochemical studies showing that oligomerization status of ATP synthase varies between the examined conditions.

Next, we studied regulation of mitochondrial ATP synthase by the inhibitor factor IF1, which is supposed to bind ATP synthase only under conditions of nutrient starvation or hypoxia. Nevertheless, using blue native electrophoresis (BN-PAGE) we have observed that in INS1E cells, IF1 binds to the ATP synthase also under standard physiological conditions. We set to examine this paradox finding by use of two color 3D STED microscopy. By colocalization analysis we have confirmed that IF1 binds to the ATP synthase not only under conditions of glucose deprivation but also in the presence of standard and high glucose concentrations, *i.e.* concentrations stimulating insulin secretion. We conclude that the regulatory role of IF1 is broader than thought and requires further examination.

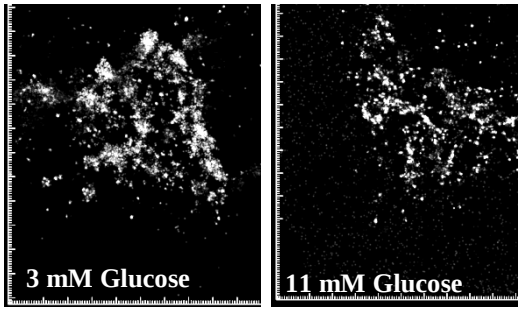

Fig. 1. ATP synthase subunit alpha as observed by 3D dSTORM microscopy in model pancreatic β-cells (positions in z-axis are gray scale coded, one division of the scale is 1 μm)

[1] L. Jimenez, D. Laporte, S. Duvezin-Caubet, F. Courtout, I. Sagot, Mitochondrial ATP synthases cluster as discrete domains that reorganize with the cellular demand for oxidative phosphorylation., J. Cell Sci. 127 (2014) 719–26. doi:10.1242/jcs.137141.