RESOLVING OF DISTRIBUTION OF ENDOPLASMIC RETICULUM PROTEIN WOLFRAMINE1 AND TETHERING OF OUTER MITOCHONDRIAL MEMBRANE BY 3D dSTORM/PALM MICROSCOPY

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Wolfram syndrome (WS) is a rare hereditary disorder that causes diabetes insipidus, diabetes mellitus, optical and brain atrophy, and deafness. Mutations in Wolframin1 (*Wfs1*) gene, which encodes endoplasmic reticulum (ER) protein, are responsible for WS. Symptomatically is WS similar to mitochondrial disorders. Indeed, Wfs1 deficiency and mutations affect mitochondrial dynamics and neuronal morphology. Silencing of Wfs1 in neurons using specific siRNA had strong negative impact on mitochondrial fusion frequency and caused mitochondrial fragmentation, the same effect was observed in neurons isolated from the Wfs1 deficient mice [1]. It could be mediated by ER stress, calcium signalling and/or by direct tethering of mitochondrial and ER membrane. The latter assumption is supported by the fact, that Mitofusin2, the main protein facilitating mitochondrial fusion, is located both in the membrane of ER and outer mitochondrial membrane as well.

We employed the 3D-PALM/STORM superresolution microscopy to visualize a spatial distribution of Wfs1 protein in respect to outer mitochondrial membrane in axons of rat cortical neurons. We observed that Wfs1 formed clusters of different distinct sizes probably corresponding to different oligomers homogeneously distributed throughout the ER. Moreover, some clusters of Wfs1 were in close contact with mitochondria. Therefore, we hypothesize that clusters of Wfs1 might, at least in part, contribute to tethering of ER and mitochondria.

Future perspective: in the nearest future we plan to exploit the advantage of the PALM/STORM microscopy for two-color visualization of co-localization between Wfs1 and Mitofusin2 as well as for visualization of Wfs1 in the ER membrane.

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[1] Cagalinec et al., 2016, PLoS Biol. Jul 19;14(7)