Biochimica et Biophysica Acta 1817 (2012) S72–S76

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

S8 Mitochondrial Dynamics

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Mitochondrial ultrastructure and complex formation are determining factors for the mobility of OXPHOS complexes in the IMM

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Mitochondrial fusion and fission dynamics are indispensably linked to mitochondrial quality control and thus cell health and performance. Their distortion is a characteristic element during neuronal cell death in neuro-degenerative diseases. In this context, our group is interested in the impact of mitochondrial fusion and fission on the re-mixing of mitochondrial compounds. Outer membrane proteins, proteins in the inner membrane space and matrix proteins mix fast and homogeneously. This is not the case for proteins located in the inner mitochondrial membrane: in recently fused mitochondria, complexes of the oxidative phosphorylation (OXPHOS) display a patchy distribution for hours [2]. To understand this heterogeneous distribution of OXPHOS complexes, the inner membrane architecture as well as the localization and mobility of OXPHOS complexes has to be taken into consideration. In our work, we addressed two principal subjects: (i) the dynamics of the inner mitochondrial membrane during organelle fusion, and (ii) the spatio-temporal organization of OXPHOS and IMM protein complexes in this situation.

By means of superresolution techniques, we determined the distribution of OXPHOS complexes on cryo-slides of mitochondria from mammalian cells by immuno-electron microscopy and revealed the spatio-temporal dynamics of OXPHOS complexes by single molecule tracking and localization microscopy (TALM). TALM, we recently employed, is a method based on the analysis of fluorescent signals from single membrane proteins in mitochondria [1]. Subunits of OXPHOS complexes were fused to the HaloTag® and posttranslational labeled with TMR-HTL in sub-stoichiometric amounts to obtain single molecule signals. TMR is a bright and photostable organic dye that is superior to fluorescent proteins in single particle tracking. TALM allowed the dissection of mitochondrial membrane microcompartments through successive exploration of their accessibilities by mobile membrane proteins. We show that alteration of mitochondrial ultrastructure as well as variable supercomplex-formation are pivotal factors that influence the localization and mobility and thus distribution of IMM proteins.

References

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doi:10.1016/j.bbabio.2012.06.201

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Role of mitochondrial dynamics on the relationship of amoeba and bacteria, as host–parasite and/or symbiont

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From various hot water habitats in our environment including fresh water, hot spring, sewage and other sources of water supply in Japan, many kinds of free-living amoeba, such as genera Acanthamoeba, Naegleria, Hartmannella and others, have been isolated. Including the host candidates of Legionella, we investigated isolates taken from thermal waters and identified species by PCR and/or IP-PAGE. Amoebae were observed under various conditions with bacteria. Not only Acanthamoeba but also Naegleria permitted bacteria to harbor in their cytosolic area. Our experimental system of amoeba and bacteria is shown as one of the models to study on parasite–host and/or symbiotic system as a natural circumstance and medical lung model. In the case of Legionella and amoeba, and amoeba and human host, it shows a kind of three-layered parasite–host relationship. On the other hand, mitochondrial dynamics has a very important role in a live cell, as the mitochondrion itself is indispensable for cell life by producing ATP, regulating ions, such as Ca, apoptosis and so on. Mitochondria always change their own shape and take various forms when they repeat mitosis and/or fusion. The investigation of mitochondrial dynamics was started but its physiological role is yet unclear.

An observation of this system of live cells by each half day which is also able to harbor pathogenic bacteria indicates the public health importance of amoebae. Here preliminary experiments were tried using *Acanthaomeba castellanii*/ATCC30010 strain and *Legionella pneumophila*/80-045 strain, and other unknown bacteria: cultured Acanthamoeba, 15 strains and 30 strains, with Legionella, observed as a membranous structure using mitoTracker and LysoTracker. Two strains showed different sensitivity against Legionella infection and obtained two fluorescence staining patterns, Mito and Lyso. Results of an amoeba with free-living bacteria in its

0005-2728/$ – see front matter.