POSSIBLE DIVISION OF MITOCHONDRIAL NUCLEOIDS
VISUALIZED BY BIPLANE FPALM / DSTORM

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Mitochondrial DNA (mtDNA) exists in complexes with accessory proteins and gene expression machinery proteins, termed nucleoids. It has been disputed whether a single nucleoid contains just a single or more mtDNA molecules, whether specific resting, transcriptional, or translational states exist, and whether these states are reflected by different apparent nucleoid sizes [1,2]. To elucidate the latter issue, 3D superresolution fluorescent photoactivable localization microscopy (FPALM) with Eos conjugated to the mitochondrial (mt) transcription factor-A (TFAM) [3], was employed to screen sizes of nucleoids in hepatocellular carcinoma HepG2 cells. Also, 3D-immunocytochemistry of TFAM, mt-single-stranded-DNA-binding protein (mtSSB), or DNA, was used with the direct stochastic optical reconstruction microscopy (dSTORM). The localized points of FPALM/dSTORM data were remodeled using Delauney tetrahedron modeling or by principal component analysis (PCA). Alternatively, PCA ellipsoid nucleoid images were normalized by Delauney volume to obtain symmetrical ellipsoid (DVSE) models. Their size and orientation distribution was analyzed.

For TFAM FPALM average DVSE of 80×80×159 nm dimensions were yielded, confirmed by TFAM dSTORM giving 78×78×170 nm; whereas mtDNA- and mtSSB-contoured ellipsoids were on average 65×65×152 nm and 44×44×133 nm, reflecting the nucleoid core and a space of unfolded mtDNA (or third-strand structures, such as D-loop), respectively. Due to the 2-fold lower axial vs. lateral resolution, only bulky DVSE models with a high aspect ratio and tilted towards the xy-plane were considered as two proximal nucleoids and suspicious to reflect a state immediately after the nucleoid division following mtDNA replication. Such nucleoid dividing pairs were visualized. The existence of twin nucleoids in mtDNA-dSTORM 3D images, representing snapshot of mtDNA “doubling”, i.e., predicted outcome of replication, supports our hypothetical assignments of similar nucleoids twins, when visualized via TFAM. We conclude that the visualized nucleoid pairs (bulky, exhibiting high aspect ratios along the mt tubular axis) represent direct observations of mt nucleoid division after mtDNA replication. Supported by grant 13-02033 (GACR).

Fig.1. Possible division of mitochondrial nucleoids imaged by antiDNA dSTORM - left: localized points; right: Delauney tetrahedron modeling (black), white points excluded.