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**LS-2-P-5958 Three-dimensional distribution of the mitochondrial DNA in the mammalian cell revealed by FIB/SEM tomography.**

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[Introduction]
Mitochondrial fission and fusion events are fundamental mechanisms for quality control of the mitochondrial functions. It has been known that the mitochondrial DNA (mtDNA) frequently divide into offspring mitochondria after the fission that has been observed as nucleoid by fluorescent microscopy and the mtDNA dynamics is considered to be coordinated with the mitochondrial turnover. Candidates of molecular mechanisms of the relationships between mtDNA division and the mitochondrial fission have been suggested recently, but their ultrastructural aspect are still unclear. Visualization of the mtDNA at electron microscopic level is a quite important step to understand how they involved in this mechanism, but it is quit difficult to observe by a conventional electron microscopic method. In the present study, we tried to visualize the localization of the mtDNA / nucleoid at EM level by immuno-electron microscopy. We also attempted to visualize 3D distribution of the nucleoid within the mitochondria using focused ion beam scanning electron microscopy (FIB/SEM).

[Materials & Methods]
After chemical fixation in mixture of 4% paraformaldehyde and 0.05% glutaraldehyde in 0.1M phosphate buffer, HeLa cells were immunohistochemically labeled with anti-TFAM IgG antibody(Abnova, USA)and anti-DNA IgM antibody(Progen, Germany). For pre-embedding method, HRP conjugated secondary antibody and DAB reaction was preformed. For immunogold method, nanogold conjugated secondary antibody was used. Immunologically labeled specimens were then strained by OTO method, embedded in resin and applied for the FIB/SEM tomography(Quanta 3D FEG, FEI) and 3D reconstruction was done on computer software(Amira, USA).

[Results & Discussion]
We could not identify any nucleoid-like structure within the mitochondria even in a complete 3D reconstruction by FIB/SEM tomography of the HeLa cells prepared by conventional specimen preparation. In pre-embedding immuno-electron microscopy, DAB immunoreaction products (IR)were observed in the matrix of some mitochondria. Interestingly, DAB-IR depicted in the globular region of the mitochondrial matrix(0.4µm diameter)frequently localizing in the peripheral end of the mitochondrial matrix just adjacent to the inner membrane. In post-embedding immunogold method, gold labels were also observed in a part of the matrix adjacent to the mitochondrial inner membrane. These immunocytochemical results were well coincide with the fluorescent microscopic observation.