【Grant-in-Aid for Scientific Research (S)】

Broad Section G

Title of Project： Molecular basis of the protein trafficking system for mitochondrial biogenesis and functional maintenance

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Keyword：mitochondria, translocator, cryo-electron microscopy, protein trafficking

【Purpose and Background of the Research】

Mitochondria have central roles in cellular energy production, metabolic pathways, signaling, and programmed cell death in eukaryotic cells. Biogenesis and maintenance of mitochondria require transport of over 1,000 different mitochondrial proteins from the cytosol to pre-existing mitochondria, and detection and removal of defective mitochondrial proteins. We found that the mitochondrial protein transport system is controlled by dynamic re-organization of the components of the translocator machineries, and protein quality control (QC) operates not only as degradation but also a proofreading to re-deliver mistargeted proteins to the ER. On the basis of these findings, we will perform cryo-electron microscopy (EM) based structural analyses and biochemical and cell biology analyses of the yeast mitochondrial protein trafficking and related QC systems. We will also search for factors facilitating expansion of mitochondria. By doing so, we aim to understand the molecular mechanisms of the fundamental question of how mitochondria are made and maintained in the cell.

【Research Methods】

The questions to be answered in this project are as follows: (1) What is the structural basis of the mechanism for the TOM complex, the outer membrane (OM) translocator, to import over 1,000 different proteins by its subunit re-organization? (2) How does the SAM complex, another OM translocator, facilitate β-barrel formation and OM integration of substrate proteins by dynamic subunit exchange? (3) How does Msp1, an AAA-ATPase of the OM, recognize mistargeted proteins, extract them from the outer membrane, and re-deliver them to the ER, and how

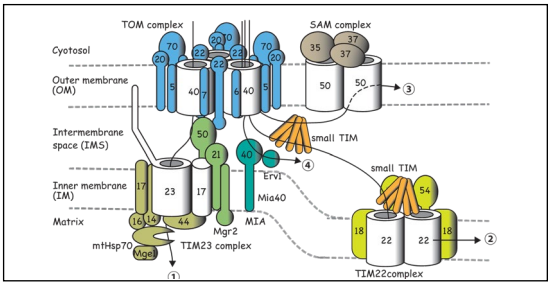

Figure 1 Mitochondrial protein trafficking; pathways and translocators

can we generalize the concept of the “proofreading of the protein trafficking” in terms of substrates and organelles? (4) How do the TOM-Ubx2 complex and Msp1 handle mitochondrial precursor proteins accumulated at the OM due to functional failure of the mitochondrial protein trafficking? (5) What are the factors and pathways that control expansion of pre-existing mitochondria? To answer these questions, we will perform structure-biology analyses of the translocators and related factors, together with *in vivo* and *in vitro* biochemical, cell biology analyses, and yeast molecular genetic analyses.

【Expected Research Achievements and Scientific Significance】

This project will reveal dynamic and high-resolution structures of the proteins involved in mitochondrial protein trafficking, which will allow us to understand the basic principle of the operation and regulation of the mitochondrial protein trafficking systems as a whole. In addition, the entire picture will be revealed for the new concept of re-trial or proofreading of the protein trafficking. Identification of the factors promoting expansion of mitochondria could be linked to new concept of mitochondrial biogenesis. The expected outcome of this project will lead to development of a novel strategy for human health maintenance and preventative as well as therapeutic treatments of aging and aging-related diseases. Furthermore, the obtained results are not limited to mitochondria, but will have a broad impact on protein trafficking to other organelles and on the construction of the intracellular membrane structures in general.

【Publications Relevant to the Project】

- Araiso, Y. *et al.* Structure of the mitochondrial import gate reveals distinct preprotein paths. ***Nature*** 575, 395-401 (2019).
- Matsumoto, S. *et al*. Msp1 clears mistargeted proteins by facilitating their transfer from mitochondria to the ER. ***Mol. Cell*** 76, 191-205 (2019).

【Term of Project】

FY2020- 2024

【Budget Allocation】

151,300 Thousand Yen

【Homepage Address and Other Contact Information】

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