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

Title of Project：Structure, regulation and physiology of ATP synthase

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Research Area：Biology, Biological science, Functional biochemistry
Keyword：bioenergetics, regulation of enzyme

【Purpose and Background of the Research】

Without any exception, all cells in all lives on the earth use ATP as an energy coin. ATP should be re-synthesized from ADP and Pi and ATP synthase carries out this task. It consists of proton-motor Fo and ATP-driven motor F1. Two motors are connected by a common rotary shaft that converts energy of down-hill proton flow into ATP synthesis or visa versa. Demand for ATP and energy supply for ATP synthesis are varying in living cells and ATP synthase should be regulated accordingly but the mechanism of regulation has been understood very little. Aims of this research project are clarification of molecular mechanism of regulation and physiological consequence of the fault of regulation in cultured cells and living animals. In addition we pursue crystallization of whole ATP synthase complex and determination of its atomic structure.

【Research Methods】

1. Molecular mechanism of regulation of ATP synthase

In general, core function of ATP synthase is common in all organisms but regulation mechanism can differ from one organism (cell) to another organism (cell). However, our recent studies reveal that there are at least four regulation mechanisms and cells combine them appropriately dependent on the situations. They are: ADP-inhibition (all organisms), inhibition by ε subunit (bacteria and plant chloroplasts), disulfide bond formation in γ subunit (plant chloroplasts), and specific inhibitor protein (animal and plant mitochondria). We recently developed two powerful experimental systems. One of them is expression of human F1 in E. coli cells, which enables us to introduce mutations for the first time into animal ATP synthase. Also single molecule observation of rotation is now possible for human F1.

2. Physiological consequence of failure of the regulatory system

Another powerful method we developed is high-throughput screening system of mitochondrial ATP synthesis. Combined with RNAi techniques, we already started the screening of hundred of unknown gene products in mitochondria. This screening also enable us to find chemical drugs that affect mitochondrial functions. Screening of chemical libraries of kinase inhibitors, for example, will reveal the protein kinase commitment of mitochondrial function.

3. X-ray crystallography of whole complex of ATP synthase

Even though partial structures of ATP synthase have been solved, whole structure is not known. We generate ATP synthase that is fixed at certain rotary angle. Also, mono-clonal antibodies that sometimes help crystallization of difficult membrane proteins.

【Expected Research Achievements and Scientific Significance】

For example, when starved, mitochondria is not fueled and ATP synthase starts the reverse reaction: ATP hydrolysis. This wasteful reaction should be prevented but we don't know how. Clarification of the regulation mechanisms of ATP synthase and their coordination will contribute to understanding cellular energy regulation system in living cells.

【Publications Relevant to the Project】

Saita E, et al. Activation and stiffness of the inhibited states of F1-ATPase probed by single-molecule manipulation. J Biol Chem. 2010: 285:11411-7

Masaike T, et al. Cooperative three-step motions in catalytic subunits of F_1-ATPase correlate with 80° and 40°substep rotations. Nat Struct Mol Biol. 2008; 15: 1326-33.

【Term of Project】 FY2011-2013

【Budget Allocation】 81,700 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.cc.kyoto-su.ac.jp/~fmotojim/index-j.html