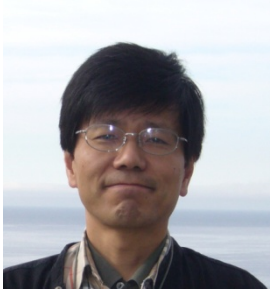
【Grant-in-Aid for Scientific Research(S)】
Biological Sciences (Medicine, dentistry, and pharmacy Ⅰ)



Title of Project : Molecular mechanisms and biological roles of electro-chemical coupling mediated by voltage-sensor domain proteins

Yasushi Okamura
(Osaka University,Graduate School of Medicine, Professor)

Research Area : Physiology, Basic Medical Sciences

Keyword : membrane, channel, transporter, active transport

【Purpose and Background of the Research】

Voltage-sensor has been studied as the key module for electrical signals such as action potentials in neuron and muscle. We have recently identified new proteins that contain voltage sensor but not pore domain. Voltage-sensing phosphatase (VSP) has a voltage-sensor linked to PTEN, and operates as voltage-regulated PIP2/PIP3 phosphsatase. Voltage-sensor only protein (VSOP) operates as the voltage-gated proton channel. These proteins indicate that voltage sensor is widely used for electro-chemical coupling in multiple biological events beyond a module of voltage-gated ion channels. This project aims at elucidation of molecular mechanisms and physiological roles of voltage-sensor domain proteins by characterizing protein complexes containing voltage sensor and measuring membrane potentials from cells that express voltage-sensor domain proteins such as blood cells.

【Research Methods】

This research utilizes the following three approaches.

(1) Voltage sensor domain proteins, including VSP and VSOP1, will be studied by electrophysiology, biochemistry and flurescence imaging. Role of VSOP1 in phagocytes such as neutrophils and macrophages will be studied with reference to interaction to Nox2.

(2) Mermaid protein is a protein-based voltage-probe. It consists of the voltage-sensor domain from VSP and the two fluorescent proteins with distinct excitation wavelength derived from coral that act as a FRET pair. Mermaid will be modified for imaging of membrane potentials in cells that express voltage-sensor domain proteins.

(3) VSOP1 knockout mice will be studied with special reference to roles of VSOP1 in phagocytosis and immune functions. Cell-specific knockout mice will also be established. Knockout mice will also be established for other proteins. Membrane potential changes will be measured using voltage-probe from cells that express those voltage-sensor domain proteins. Results will be compared between wild-type mice and knockout mice.

【Expected Research Achievements and Scientific Significance】

Understanding mechanisms of membrane potential change mediated by voltage-sensor domain proteins will lead to deep understanding of mechanisms of electrical signals, including EC coupling in skeletal muscle and polyspermy block. Results of this research will also help understand basic mechanisms of phagocytosis and other physiological events of immune cells and testis. Findings of VSP will potentially lead to understanding mechanisms of tumor suppression.

【Publications Relevant to the Project】

・A voltage sensor-domain protein is a voltage-gated proton channel. Sasaki M, Takagi M & Okamura Y. *Science*, 312(5773), 589-92. (2006).
・Phosphoinositide phosphatase activity coupled to an intrinsic voltage sensor. Murata Y, Iwasaki H, Sasaki M, Inaba K & Okamura Y. *Nature*, 435:1239-1243.(2005).

【Term of Project】 FY2009-2013

【Budget Allocation】 130,700 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.med.osaka-u.ac.jp/pub/phys2/tougouseiri/menu.htm