論文の内容の要旨

論文題目

Regulation of macropinocytosis by PIPs metabolism

(PIPs 代謝によるマクロピノサイトーシスの制御機構)

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Endocytosis is defined as the uptake of membrane proteins, lipids, extracellular ligands and soluble molecules from cell surface/extracellular space into the cell interior by endocytic vesicles. This process is a vital process required for many cellular activities, including extracellular nutrient uptake, antigen uptake, membrane recycling and signal transduction. Eukaryotic cells are capable of different types of endocytosis ranging from receptor-mediated uptake of soluble ligands by clathrin-coated vesicles to ingestion of large particles by phagocytosis. Endocytosis occurs by multiple mechanisms that fall into two broad categories, “phagocytosis” (uptake of large particles) and “pinocytosis” (uptake of fluid and solutes). Phagocytosis is restricted to specialized mammalian cells, whereas pinocytosis occurs in all cells by at least four basic mechanisms: macropinocytosis, clathrin-dependent endocytosis, caveolin-dependent endocytosis and clathrin/caveolin-independent endocytosis.

Macropinocytosis is unique because, unlike other pinocytic processes, it is preceded by vigorous plasma membrane activity in the form of ruffling. Macropinocytosis can be classically defined as a transient, growth factor induced,

actin-dependent endocytic process that leads to internalization of fluid and membrane into large vacuoles, called macropinosome, by the closure of lamellipodia generated at ruffling membrane domains. By its nature, macropinocytosis is thought to be used by cells for the non-selective internalization of fluid. However, recent studies have shown that, under special circumstances, macropinocytosis can facilitate the uptake of "particles" including bacteria and viruses for their infections. In the immune system, macropinocytosis is means by which antigen-presenting cells sample their immediate environment antigens. In the case of dendritic cells, immature dendritic cells utilize macropinocytosis to sample large quantities of exogenous solute from their immediate environment for circulating antigens as part of their sentinel function. Furthermore, in the aspect of therapeutics, macropinocytosis gains growing interests as a potential tool for delivery of nucleic acids, peptides and therapeutics into cells.

Considerable efforts have recently been made to elucidate the molecular mechanism underlying macropinocytosis. One of key differences between clathrin-dependent endocytosis and macropinocytosis is that the latter requires actin cytoskeleton reorganization. The remodeling of the cytoskeleton that leads to macropinocytosis requires phosphatidylinositol-3-kinase (PI3K) activity at the plasma membrane. Although the picture of macropinocytosis at molecular level is elusive, several molecules essential for macropinocytosis have been identified. The GTPase Rac1 and Cdc42, as well as p21-activated kinase-1 (PAK-1), are involved in the actin polymerization, and CtBP1/BARS is required for macropinosome closure. The activation of PI3K and the engagement of Rho family GTPase are common to a variety of actin-dependent processes such as phagocytosis and chemotaxis. Thus, treatment with inhibitors like wortmannin effectively blocks these processes, as well as macropinocytosis. In contrast, macropinosome formation appears to be uniquely susceptible to inhibition by amiloride and its analogues, and this property has been extensively used as an identifying feature of macropinocytosis. Amiloride, a guaniginium-containing pyrazine derivative, has been used as an inhibitor of Na^+/H^+ exchangers, and inhibits macropinocytosis by lowering submembranous pH and preventing Rac1 and Cdc42 signaling. Also, recent studies have shown that SHIP2, $PI(3,4,5)P_3$ 5-phosphatase, and SNX5 were required for macropinocytosis.

Several studies utilized the worm *C. elegans* to examine endocytosis in a multicellular organism. A number of endocytosis assays have been developed to study the mechanisms of endocytosis and endocytic trafficking in *C. elegans*. Greenwald and Fares have established fluid phase endocytosis assays in *C. elegans*. Six scavenger cells, called coelomocytes, reside in the *C. elegans* body cavity and actively and continuously endocytose fluid and macromolecules from the body cavity. These cells of 10 ± 15 μm diameter have relatively fixed positions, and are not essential for the survival of the animal. Most foreign substances that are microinjected into the body cavity of *C. elegans* are rapidly taken up by coelomocytes. Though their biological role in *C. elegans* is currently unknown, the coelomocytes constitute an excellent tissue to study fluid-phase endocytosis. An *in vivo* assay for endocytosis by coelomocytes has been developed. The assay utilizes transgenic worms in which a secretory signal sequence-GFP chimera (*myo-3p::ssGFP*) is secreted into the pseudocoelom from body-wall muscle cells. The GFP is endocytosed primarily by the coelomocytes, and is subsequently degraded. Several groups revealed that some genes were required for fluid phase uptake in coelomocytes.

In the present study, we first hypothesized that macropinocytosis contributes, in part, to this fluid-phase endocytosis in *C. elegans* coelomocytes. We focused on *mtm-6* and *mtm-9*, phosphoinositides 3-phophatases, previously reported that mutations of these genes caused defects in fluid phase endocytosis in coelomocytes and showed that coelomocytes of *mtm-6* and *mtm-9* mutants did not endocytose fluid-phase marker. We then showed that MTMR6 and MTMR9, mammalian homologues of *mtm-6* and *mtm-9*, were essential for macropinocytosis, not for clathrin-dependent endocytosis in A431 cells. Furthermore, we showed that phosphoinositides 3-phosphatase activity of MTMR6 was required for macropinocytosis. We revealed that MTMR6 was involved not in membrane ruffle formation but in macropinosome formation in the process of macropinocytosis. These data indicate that MTMR6 and MTMR9 are novel regulators of macropinocytosis. Next, we focused on PIPs metabolism during macropinocytosis. We found that sequential conversion of $PI(3,4,5)P_3$ to PI is essential for membrane closure of macopinocytosis and identified INPP4B, 4-phosphatase of $PI(3,4)P_2$, as a novel molecule required for macropinocytosis. Finally, we found that pretreatment of

TRAM-34, a specific inhibitor of Ca dependent potassium channel KCa3.1, caused defects in membrane closure of macropinocytosis. This channel is activated by PI3P and inactivated by MTMR6 through PI3P degradation. These data indicate that proper activation of KCa3.1 by PI3P and inactivation of KCa3.1 through PI3P degradation by MTMR6 is important for membrane closure of macropinocytosis.