主論文の要旨

Regulation of cargo-selective endocytosis by dynamin 2 GTPase-activating protein girdin

（ダイナミン 2 活性化分子であるガーディンによる
積み荷選択的エンドサイトーシスの制御）

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Introduction

Eukaryotic cells utilize clathrin-mediated endocytosis (CME) to internalize various cargoes (e.g., receptors, nutrients) in a well-organized manner. CME determines various cellular behaviours and biological properties, such as cell signaling pathways, migration, and polarity. Due to the vital role of CME in cells and the diversity of cargoes that are internalized via CME, there is no doubt that the specificity and selectivity for cargoes and their timing and spacing must be precisely controlled to maintain cell homeostasis. Previous studies have demonstrated that cargo specificity is determined by the recognition of different cargoes by cargo-specific adaptors at the cargo selection stage. However, given the limited number of known adaptor proteins and the variety of cargoes that are selectively internalized, additional mechanism(s) that govern selective CME remain to be identified.

During the process of endocytosis, the large GTPase dynamin plays a critical role in endocytic membrane fission events through assembling into helical polymers at the necks of budding vesicles. As a GTPase, the function of dynamin is largely dependent on binding to nucleotide and its transition from the GTP-bound form to the GDP-bound form. This process requires the involvement of GTPase activating protein (GAP).

We and others previously characterized the actin-binding protein girdin (girders of actin filament) as a critical regulator of migration of endothelial cells, cancer cells, and neuroblasts, all of which depend on extracellular cues including growth factor stimulation. Although girdin was reported to be a component of a protein complex that included dynamin, the role of girdin in CME has not been investigated. Also, our previous finding that girdin colocalizes with submembranous actin network, which possess critical roles in CME, led to the idea of girdin's involvement in CME.

Materials and Methods

Cell culture, transfection, and RNAi. HeLa, COS7 and MDCK cells were purchased from American Tissue Type Culture (Rockville, MD) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). BS-C-1 cells stably expressing AP-2 σ2-EGFP or LCa (clathrin light chain A)-EGFP were kindly provided by Tomas Kirchhausen (Harvard Medical School) and cultured in DMEM supplemented with 10% FBS and 1 mg/mL of Geneticin (Invitrogen). siRNA or plasmids were transfected into cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Lipofectamine LTX (Invitrogen) or FuGene HD (Roche Diagnostics, Indianapolis, IN) was used to obtain a high expression level of plasmid in HeLa or BS-C-1 cells, respectively. The targeted sequences of siRNA used in this study, the specificity of which has been previously demonstrated, were as follows: girdin (5'-AAGAAGGCTTAGGCAGGAATT-3'), clathrin

heavy chain (5'-AATCCAATTCGAAGACCAATT-3'), dynamin 2 (5'-CTGCAGCTCATCTTCTCAAAA-3'). For short hairpin RNA (shRNA)-mediated knockdown of girdin, the targeted sequence 5'-GGAACAAACAAGATTAGAA-3' was inserted into pSIREN-RetroQ retroviral shRNA expression vector (Clontech, Palo Alto, CA) as previously described.

Dynamin sedimentation assays. Purified recombinant dynamin 2 (2 μM) was incubated in sedimentation buffer (10 mM HEPES, 2 mM MgCl2, 150 mM KCl, pH 7.2) at room temperature for 30 min with increasing amounts of tested GST fusion proteins (GST, GST-NT, or GST-Grb2). The reaction mixture (50 μL) was centrifuged at 4°C for 10 min at 14,000 rpm. Equal volumes of the pellets and supernatant fractions were separated by SDS-polyacrylamide gels (SDS-PAGE), followed by Coomassie blue staining.

Data Analysis. The data are presented as means ± SE. Statistical significance was evaluated using the Student's t-test. All of the experiments were repeated at least 3 times.

Results

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Discussion

Dynamin is an atypical multidomain GTPase containing a large GTPase domain, which is well known for its critical role in budding or scission of transport vesicles. Distinct from other GTPases, previous biochemical studies and recent structural analyses showed that its GTPase catalytic activity is stimulated by oligomerization, which requires the involvement of the middle domain and the GED domain, where the GED domain servers as an intramolecular GAP to regulate its GTPase activity.

In this study, we revealed that a new dynamin GAP protein girdin regulated cargo-selective CME via a novel mechanism. Girdin preferentially regulated CME that occurred in central cell membranes by controlling CCP formation, although its mechanisms remain unknown at present. In addition, girdin also selectively controlled the scission of CCPs in this area via competition between cargoes, dynamin, and itself. However, the generality of our hypothesis in other cargo-selective endocytosis remains to be proven, which should await further studies.

Conclusion

1. The actin-binding protein girdin interacts with dynamin 2 and functions as a GTPase-activating protein for dynamin 2.

2. Girdin selectively inhibits the endocytosis of some cargoes (E-cadherin and transferrin) through the center of cells, whereas it has no apparent effects on the endocytosis at cell periphery.

3. The competitive interaction between cargoes and dynamin 2 for binding with girdin is involved in the selective endocytosis.

4. Girdin regulates clathrin-coated pits formation in the center of the cells, which is also contribute to girdin's role in selective endocytosis.