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Crystal structure of yeast Sec2p, a guanine nucleotide exchange factor for yeast Rab GTPase, Sec4p.

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Exocytosis is a basic membrane traffic event including transport, docking, and fusion of secretory vesicles. The exocytic events are regulated by the interactions between Ras-related small GTPases, such as Rho, Rab, and Ral, and their respective effectors. In yeast, a Rab GTPase, Sec4p transports secretory vesicles in a GTP-dependent fashion, and interacts with a multiprotein complex, Exocyst, so as to tether the secretory vesicles to the proper sites of the plasma membrane. After the membrane fusion, Sec4p can reassociate with a new round of secretory vesicles, and get ready for the next fusion. This localization cycle of Sec4p is controlled by two types of regulators: GTPase-activating protein (GAP) stimulates the hydrolysis of GTP bound to Sec4p, whereas guanine nucleotide exchange factor (GEF) triggers GDP release, thus promoting GDP-GTP exchange and Sec4p activation. The GEF for Sec4p was identified in yeast as Sec2p. Yeast Sec2p is an essential protein for the polarized growth. The amino terminal region encompassing residues 1-160 interacts with Sec4p and exhibits the GEF activity, while it shows no sequence homology to any other GEFs with known structures. To elucidate the recognition mechanism of Sec4p, we have determined the 3.0-Å-resolution crystal structure of the Sec4p binding region of *S.cerevisiae* Sec2p. The structure of this region revealed a tropomyosin-like fold consisting of a coiled-coil dimer.

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Involvement of Rabs in the intracellular trafficking of secretory granules in mast cells

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Mast cells contains intracellular secretory granules and secrete their contents by exocytotic release. For this exocytotic release, secretory granules are transported to the plasma membrane where the membrane fusion between secretory granules and the plasma membrane occurs. Recently, the molecules responsible for the membrane fusion between granules and the plasma membrane has been elucidated, such as SNARE (soluble N-ethyl maleimide-sensitive factor attachment protein receptor) proteins. However, the molecular mechanism for the transport of secretory granules is poorly understood. In the present study, we investigated the mechanism of transport of secretory granules, especially the involvement of Rab small GTPase. We found the expression of Rabs in a mast cell line, RBL-2H3 cell. Analysis of intracellular distribution of fluorescent protein tagged Rabs revealed that Rab27a is localized on secretory granules, suggesting the involvement of Rab27a in granule transport. Furthermore, myosinV, a motor protein of actin dependent transport, was co-immunoprecipitated by antibody against Rab27a. In Rab27a overexpressing cell lines, degranulation induced by antigen stimulation was inhibited, while that induced by co-stimulation of Ca-ionophore and PMA, an activator of PKC was not affected. These results suggest that Rab27a is involved in antigen induced exocytosis, probably in the actin dependent manner. The effects of expression of dominant negative form and knockdown of Rab27a expression will be also discussed.

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Structural analysis of the Mammalian Exocyst Complex

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Exocytosis is an essential process for secretion, targeting membrane proteins onto cell surface, and lipid delivery to the plasma membrane. Fusion between the secretory vesicle and the plasma membrane completes the exocytic event. Two distinct stages have been postulated for the membrane fusion: (i) a loose, reversible, and small GTPase dependent tethering of the vesicle to the target membrane, and then, (ii) engagement of Q- and R-SNAREs, which leads to full SNARE complex formation and membrane fusion. The tethering step is defined as the initial contact of the vesicle via a protein bridge with its target compartment, and requires a multimeric protein complex. The tethering complex for the exocytosis is the Exocyst complex, which consists of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84. The mammalian Exocyst complex is an effector for the small GTPases, Ral, Rab11, and TC10. Loss of the interactions with these small GTPase causes mistargeting of several membrane proteins. To understand the tethering mechanism of the Exocyst complex, we purified the Exocyst complex from porcine brain, and observed its negatively stained images by using electron microscopy. The Exocyst complex was typically observed as a cluster of particles with a diameter of >400 Å. Addition of high concentration of salt increased the number of smaller single particles with a diameter of 60-150 Å. Further improvement of the sample preparation is in progress for 3D image reconstruction by a single particle analysis.

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Imaging of COPII vesicle formation and budding from artificial membrane.

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In eukaryotic cells, coat protein complex II (COPII) is responsible for the vesicle formation from endoplasmic reticulum membrane. The released vesicles from ER membrane diffuse or are moved on cytoskeleton to Golgi complex in order to transport particular cargo proteins. This vesicle formation was reconstituted in vitro from a v-SNARE protein (Bet1p), a small GTPase (Sar1p), and two COP II coat protein complexes (Sec23/24p and Sec13/31p)[Sato, K. and Nakano, A., Nat. Struct. Mol. Biol., 2005]. The in vitro assay offers ways to investigate the fundamental questions on the mechanism of the vesicle formation; how each complex form vesicle? What is the role of GTP hydrolysis? To access these tasks, we developed an imaging system to observe the vesicle formation in a planar bilayer at a single molecule level. A v-SNARE protein, Bet1p, was fluorescently labeled with Cy3, and reconstituted into a lipid bilayer formed on microscope stage. The lateral motion of the Cy3-labeled Bet1p in an artificial membrane was observed with an objective-type total internal reflection fluorescent microscope (TIRFM). When Sar1p and COPII coat proteins were added to the system, drastic change of the lateral diffusion was observed; cargo proteins quickly bind together to form large assemblies of few thousand molecules within 20 sec. In this poster, we will discuss about details of the vesicle formation; how many cargo proteins are incorporated in an assembly, how GTP hydrolysis does enhance this process.