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Membrane Traffic: Editorial Overview

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The most outstanding feature of eukaryotic cells is compartmentalization by membranes, which enables them to achieve a broad spectrum of functions; some of them are common to all cell-types and others are specific to certain cell-types. Individual compartments, namely organelles, have unique sets of proteins that are specifically delivered from one compartment to another by membrane traffic. During the past several years, combinations of genomic, proteomic, structural and real-time imaging analyses with conventional genetical, biochemical and cell biological approaches have provided us with much new information not only about the intricate pathways and sophisticated regulatory mechanisms of membrane traffic but also about integration of membrane traffic with other cellular functions such as signaling and morphogenesis. This Minireview series composed of eight articles highlights the recent progress in this rapidly expanding research field.

Key words: endocytosis, Golgi apparatus, lysosome, membrane traffic, small GTPase.

Abbreviations: AP, adaptor protein; ER, endoplasmic reticulum; ERGIC, ER-Golgi intermediate compartment; fPEG-Chol, fluorescein ester of poly(ethyleneglycol)cholesteryl ester; GEF, guanine nucleotide exchange factor; NSF, *N*-ethylmaleimide-sensitive factor; SNAP, soluble NSF attachment protein; SM, sphingomyelin; SNARE, SNAP receptor; TGN, *trans*-Golgi network.

Unlike a prokaryotic cell that consists of a single compartment enclosed by the plasma membrane, a eukaryotic cell contains a variety of intracellular membrane-enclosed compartments. Referred to as organelles, these compartments appear to be static structures when fixed cells are observed under the microscope. For example, the endoplasmic reticulum (ER) has a typical network structure, whereas the Golgi apparatus appears as a collection of flattened cisternae that are piled like stacks of plates. However, recent real-time imaging analyses of living cells have shown that these organelles dynamically change their morphology and communicate with each other. Each compartment contains a unique set of proteins that have been transported there from other compartments where they were made or through which they were relayed. For example, proteins newly synthesized at the ER are transported through the Golgi apparatus to final destinations, such as endosomes, lysosomes and the plasma membrane. On the other hand, membrane proteins endocytosed from the cell surface are first delivered to endosomes, then transported further to the Golgi apparatus, delivered to lysosomes for degradation, or recycled back to the cell surface for another round of use.

Because in general individual compartments are not in contact with each other, the cargo proteins have to be delivered by membrane-bound carriers. In what was once referred to as "vesicular transport," the majority of the carriers are small membrane-enclosed vesicles that are covered with specific coat proteins. However, real-time imaging analyses have revealed that tubular carriers also mediate the protein transport. Therefore, the term "membrane traffic" has recently come to be used for the transport processes mediated by vesicles and tubules together. The protein transport processes are achieved with incredible accuracy and specificity and require interactions of the coat proteins with signals that are built in the cargo protein sequences. The accurate targeting of proteins to the proper organelles ensures the integrity and functions of organelles. Furthermore, integrated with other cellular functions, such as signaling, differentiation and morphogenesis, "membrane traffic" underlies normal functions of our bodies as systematically organized bands of cells. This Minireview series deals with several current topics in this research field of "membrane traffic."

Coated vesicle formation and small GTPases

Three classes of coated carrier vesicles that mediate transport between compartments along the exocytic and endocytic pathways have been well characterized. COPII-coated vesicles are involved in transport of proteins from the ER to the ER-Golgi intermediate compartment (ERGIC) or the Golgi apparatus. COPI-coated vesicles are responsible for retrograde transport from the Golgi to the ERGIC or ER and intra-Golgi transport. Clathrin-coated vesicles with specific adaptor protein (AP) complexes mediate various post-Golgi transport processes. For example, vesicles with the heterotetrameric AP-1 complex or with the monomeric adaptor GGA are involved in transport of certain transmembrane proteins between the *trans*-Golgi network (TGN) and endosomes, although it is currently a matter of debate in which direction, anterograde or retrograde, these vesicles deliver cargoes. Clathrin/AP-2–coated vesicles are responsible predominantly for receptor-mediated endocytosis from the cell surface. The formation of these coated vesicles,

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except for clathrin/AP-2, is triggered by the activation of small GTPases from the GDP- to GTP-bound form catalyzed by guanine nucleotide exchange factors (GEFs), and the activated GTPase then recruits the coat protein complexes onto membranes. Sar1 is involved in membrane recruitment of the COPII complex, and Arfs are involved in recruitment of COPI, AP-1, GGA and so on.

Sato (in this issue) reviews the mechanism of COPII-coated vesicle biogenesis. In particular, he discusses the possible roles of the Sar1 GTP hydrolysis in the coordination of coat protein assembly and cargo selection at the ER exit sites, on the basis of his recent data obtained using an *in vitro* reconstituted proteoliposome system for COPII vesicle formation. Shin and Nakayama (in this issue) review the structures and diverse functions of Arf-GEFs in membrane traffic. It had previously been believed that different Arf isoforms determined where distinct coated vesicles, such as COPI- and clathrin/AP-1–coated vesicles, are formed. On the basis of recent data of them and others, however, they discuss the possibility that different Arf-GEFs determine where Arfs are activated, and that activated Arfs subsequently determine where distinct coated vesicles are formed.

Targeting to lysosomes for degradation

When cell surface receptors undergo endocytosis, they are delivered first to endosomes, from where some are recycled back to the cell surface for another round of use (recycling pathway) and others are transported to lysosomes for degradation (degradation pathway or down-regulation). Recent studies have underscored the novel and crucial role of ubiquitin in this sorting event. Namely, receptors destined for degradation are ubiquitinated and sequentially recognized by sets of proteins, all of which belong to the so-called "Class E Vps" family. Komada and Kitamura (in the next issue) will overview the ubiquitin-dependent degradation pathway and discuss the critical roles of the Hrs/STAM complex, which first recognizes ubiquitinated proteins on the surface of endosomes and subsequently transfers them for lysosomal degradation to other Class E Vps proteins that constitute the ESCRT (endosomal sorting complex required for transport) complexes.

Roles of Rab27 in organelle transport and maturation

Rab/Ypt proteins constitute the largest family of small GTPases, are distributed to distinct intracellular compartments and are implicated in temporal and spatial regulation of a broad spectrum of membrane trafficking events. Of more than 60 family members, Rab27A is the first protein whose dysfunction has been found to cause a human hereditary disorder, the Griscelli syndrome. Patients with this syndrome and its mouse models manifest pigmentary dilution and immunodeficiency that are ascribed to defects in transport of melanosomes in melanocytes and in that of lytic granules in cytotoxic T lymphocytes, respectively. Rab27 is present on a wide range of lysosome-related and other secretory organelles. To date, three classes of effector proteins that bind to Rab27A and its close relative Rab27B have been identified and shown to function in different cell-types. Fukuda (in the next issue) will review the molecular mechanisms that underlie the Rab27-dependent membrane trafficking, in particular focusing on granule exocytosis in endocrine and exocrine cells and melanosome transport in melanocytes.

Membrane fusion in membrane traffic and organelle biogenesis

After their formation, coated vesicles shed their coat and become competent to fuse with membranes of target organelles. The fusion of uncoated vesicles with target membranes is thought to be a multi-step process. The initial step is the recognition of the target membrane by the vesicle, referred to as tethering or docking, and often involves Rab GTPases and multi-subunit complexes composed of coiled-coil proteins. Once the vesicle has docked on the target membrane, it fuses with the membrane to deliver its cargo. This step is mediated by complementary receptors on the vesicle and target membranes termed v- and t-SNAREs (SNAP receptors), respectively, with the aid of a AAA-ATPase, NSF (*N*-ethylmaleimide sensitive factor) and α-SNAP (soluble NSF attachment protein). Oka (in a future issue) will review the structures and functions of the multi-subunit complexes, including COG, GARP and TRAPP, and their interacting partners including SNAREs and Rab proteins in membrane traffic in the Golgi.

Like the vesicular transport process, assembly and disassembly of organelles require membrane fusion and fission, respectively. For example, the Golgi apparatus is fragmented into a number of vesicles and short tubules at the onset of mitosis and reassembled by fusion of the fragments at telophase. The Golgi reassembly depends on two distinct fusion pathways. One is the NSF pathway, which proceeds like membrane fusion of vesicles as described above and therefore requires NSF, SNAREs and α-SNAP. A second pathway requires another AAA-ATPase, p97, and its cofactor p47 as well as SNAREs. Uchiyama *et al.* (in a future issue) will review the regulation of the p97/p47 pathway during the cell cycle, in particular focusing on the changes in phosphorylation and subcellular localization of p47 and on its implication in the ubiquitin system.

Membrane traffic and lipids

Changes in lipid composition not only underlie membrane deformation that leads to formation of vesicles and tubules but also are important for regulation of membrane traffic, signaling and cytoskeletal remodeling. Many cytosolic proteins are recruited onto membranes by interacting with specific phospholipids as well as proteins. Especially, it has become increasingly clear that spatially and temporarily regulated production and degradation of phosphoinositides play a pivotal role in regulation of clathrin-mediated endocytosis. Takei *et al.* (in a future issue) will first overview major molecules that participate in clathrin-mediated endocytosis. They will then discuss the regulation of endocytosis by proteins interacting with phosphoinositides, in particular focusing on amphiphysin and the dynamin GTPase.

Some lipids, together with specific proteins, regulate membrane dynamics and are known to form specialized membrane microdomains transiently or constitutively in a variety of compartments, especially on the plasma

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membrane. They can serve as signaling platforms and mediate clathrin-independent endocytosis. Sphingomyelin (SM) and cholesterol are major constituents of a lipid raft, one of such microdomains. However, little is known about distribution and dynamics of these lipids. Ishitsuka *et al.* (in a future issue) will describe their analyses using two probes: lysenin, a sphingomyelin-specific protein obtained from the coelomic fluid of the earthworm *Eisenia foetida*, and fluorescein ester of poly(ethyleneglycol) cholesteryl ether (fPEG-Chol), which partitions into cholesterol-rich membranes. Lysenin reveals that the organization of SM differs between different cell types and between different membrane domains within the same cell. When added to live cells, fPEG-Chol is distributed exclusively on the outer leaflet of the plasma membrane and dynamically clustered upon activation of receptor signaling. By contrast, microinjected fPEG-Chol is found exclusively on the Golgi apparatus.

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