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Recycling endosomes

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The endosomal membrane recycling system represents a dynamic conduit for sorting and re-exporting internalized membrane constituents. The recycling system is composed of multiple tubulovesicular recycling pathways that likely confer distinct trafficking pathways for individual cargoes. In addition, elements of the recycling system are responsible for assembly and maintenance of apical membrane specializations including primary cilia and apical microvilli. The existence of multiple intersecting and diverging recycling tracks likely accounts for specificity in plasma membrane recycling trafficking.

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Plasma membrane recycling: a general requirement for surface homeostasis

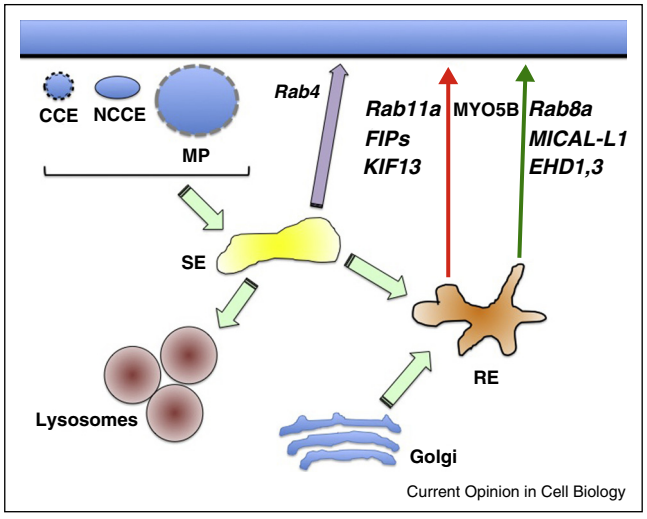
The presentation of proteins, including receptors, ion channels, ion pumps and adhesion molecules, at the membrane surface determines many of the aspects of differentiated cell physiology. It is increasingly clear that many of these proteins are internalized using a number of different endocytic pathways. Once internalized, membrane proteins must undergo a number of sorting steps that determine their fate including targeting trafficking to degradation pathways or processing for return to the membrane surface through membrane recycling (Figure 1). The list of proteins that are recognized to recycle to the membrane surface has expanded markedly over the past decade. It now appears that membrane recycling can regulate not only surface receptors and ion channels, but also mediates aspects of junctional protein maintenance as well as the maintenance of apical and basolateral specializations in polarized cells. Together these processes place the endosomal recycling system in the center of coordinating decisions in membrane trafficking that can radically affect the cell surface.

The recycling endosome is a dynamic structure

Maxfield's original studies on transferrin trafficking established that the membrane recycling system is a highly dynamic and morphologically diverse complex of membranous elements [1]. Recent studies have now focused on how regulators of the recycling system can alter the morphology as well as the function of the recycling system. The most prominent morphological dynamic in the recycling system involves dynamic tubulation of the recycling system membranes. This tubulation is less apparent in fixed cells where the endosomal morphologies are often fragmented by formaldehyde fixation. However, in live cells, the extensive tubulation is readily apparent and highly dynamic [2,3•]. Some of the characteristics of tubulation likely reflect the regulation of the passage of membrane and cargoes through the recycling system. Overexpression of Rab11-Family Interacting Proteins (Rab11-FIPs) can result in dynamic expansion of tubules, while dual overexpression with Rab11a can shift the dynamics towards a more vesicular phenotype, indicating that multiple effectors may be competing for a limiting pool of Rab11a [3•]. KIF13A can associate directly with Rab11a and promotes the formation of tubules in the recycling endosome system [4••]. Recent studies have implicated the microtubule-severing protein, spastin, and ESCRT in scission of recycling system tubules [5••]. Thus, regulation of the tubulation of the Rab11a-containing recycling system utilizes multiple mechanisms for the growth of tubules and their subsequent vesiculation.

Rab8a distributes into tubular and vesicular endosomes that also recruit EHD proteins [6]. Tubulation of the Rab8a-dependent recycling system, which is responsible for recycling of non-clathrin-dependent endocytosed cargoes, such as MHC Class I, is regulated by MICAL-L1 [7,8]. MICAL-L1 recruits both Arf6 and EHD1 to recycling membranes and promotes tubulation [7,9,10•,11]. The association of MICAL-L1 with tubular endosomes is regulated by Rab35 [8,12]. These studies suggest that regulation of the morphology of the endosomal recycling membranes may reflect alterations in trafficking patterns. It appears that recycling endosomes demarcated by Rab11a or Rab8a utilize distinguishable mechanisms for their dynamic regulation. It remains to be determined if the morphological alterations in the recycling system

Figure 1



A complex scheme for endosomal pathways leading to membrane recycling. Membrane proteins are internalized by multiple mechanisms including clathrin-dependent endocytosis (CDE), non-clathrin-dependent endocytosis (NCDE) and macropinocytosis (MP). The early endosomes from these pathways are thought to traffic through a common sorting endosome (SE). Proteins can then be shunted for degradation to the lysosome, recycled to the plasma membrane using a rapid recycling pathway (thought to be mediated by Rab4) or processed into slower recycling endosome (RE) pathways (thought to be regulated by Rab11a and Rab8a). While both Rab11a and Rab8a can interact with MYO5B, specific proteins interacting with membranes containing either Rab11a (Rab11-FIPs and KIF13) or Rab8a (MICAL-L1, EHD1 and EHD3) may regulate specific trafficking pathways out of the recycling system.

are reflected in either the kinetics or the specificity of recycling trafficking. Nevertheless, it seems likely that different sets of proteins associated with Rab11a or Rab8a-containing membranes will govern the formation tubular elements radiating from more central recycling system cisternae.

How many recycling systems are there?

When addressing the question of membrane recycling from endosomes, it is now clear that a number of pathways can lead to recycling of proteins following internalization. It is therefore of importance to ask how many pathways exist for recycling and are they acting separately or in concert? A number of regulatory proteins have been associated with recycling pathways. The greatest amount of literature addresses the role of Rab11a as a regulator of 'slow' recycling pathways that are present in all cells. These Rab11a-dependent slow recycling pathways are involved in a vast array of cell surface proteins from transferrin receptor to ion channels to junctional proteins and integrins. While there appears to be considerable overlap in the Rab11a-mediated recycling membranes, it is increasingly clear that there may be multiple pathways for both entry and exit from these recycling membranes. Recent studies in live cells have noted a considerable amount of subcompartmentalization of regions within the Rab11a-containing recycling system [2,3•]. What is even less clear is whether other Rab protein-dependent recycling pathways are truly distinct or are integrated into general recycling endosome systems. Previous investigations had suggested that Rab8a and Rab11a-containing recycling pathways may be distinct, with distinct Rab11a-containing vesicles separate from Rab8a-containing membranes [6]. However, Rab8a and Rab11a both interact with the actin motors Myosin VA and Myosin Vb [6,13•] as well as with the Rab8 GEF, Rabin8 [14]. A coordination of Rab8a and Rab11a with Rabin8 is required for initial lumen formation [15], but lumen formation is also regulated by Rab8a and Rab11a interacting with Myosin Vb [16]. Although their binding sites in Myosin Vb are distinct, there is no definitive evidence that Rab11a and Rab8a can bind simultaneously to Myosin Vb. It therefore remains unclear whether these Rab proteins may occupy parts of the same membrane cisternae or are present on separate membranes that either juxtapose each other during transport along microtubules or gather in 'crowded' regions in the pericentriolar space or in cell extensions. Rab10 appears to occupy domains at the ends of Rab8a-containing tubules [17]. Furthermore, the concept of recycling pathways may be more diverse. Thus, trafficking from the recycling endosome to the plasma membrane may also occur through shunting into autophagy pathways that lead to degradation or recycling through lysosomal exocytosis [18,19]. Finally, it should be noted that roles of Rab8a and Rab11a may likely extend past the recycling system. Thus, lumen formation and primary cilium formation, as will be discussed below, my involve specialized functions of Rab11a and Rab8a that do not necessarily have to be mediated by recycling membranes *per se*.

Defects in apical recycling system function influence polarized apical trafficking

Polarized cells require distinct regulation of trafficking to segregated apical and basolateral domains. Previous studies have established that polarized cells have a distinct Rab11a-containing apical recycling system that is separate from the basolateral recycling system which is not dependent on Rab11a [20,21]. Over the years, there has been much discussion of whether the Rab11a-containing recycling system functions as an intermediate for trafficking of *de novo* synthesized proteins to the apical membrane. Recently, substantial evidence has been reported analyzing the passage of newly synthesized Rhodopsin through the recycling endosome on its way to the plasma membrane [22•]. These studies indicate that the recycling endosome does function as an intermediate trafficking compartment, at least for proteins that normally undergo recycling. Other recent studies have now focused interest in the recycling system as a distinct coordinator of the integrity of apical specializations and polarity. Bryant,