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# Molecular dynamics at the endocytic portal and regulations of endocytic and recycling traffics

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ABSTRACT

Endocytic and recycling pathways involve the transportation of soluble and transmembrane cargos to destinations within the cell or back to the plasma membrane for reuse. Common mechanistic themes for the traffic pathways in eukaryotic cells from yeast to mammalian cells are well-conserved, manifested by the molecular choreography of cargo segregation, membrane budding and coating, pinching off of the invaginated vesicle, cytoskeleton-mediated vesicle motility and fusion with target compartments. Here, we discuss recent insights into the spatiotemporal dynamics of endocytic machinery at the plasma membrane and the molecular details of bifurcating traffics at the endosome either to the lysosome or to the *trans*-Golgi network (TGN).



## Introduction

Membrane trafficking is a highly conserved pivotal process by which essential membrane materials, such as proteins and lipids, are transported between membrane-bound compartments, including the plasma membrane. The trafficking consists of various types of traffic pathways and starts with the entry of materials into the cell, termed endocytosis. The targeting and exchange of the internalized materials between compartments are subsequently achieved through a diverse array of intracellular pathways, and the transportation of materials out into the extracellular space is accomplished through exocytic traffic. Emerging lines of evidence clearly exhibits that dysfunction in the traffic pathways is strongly associated with many human disorders, such as Alzheimer's disease and Danon syndrome (Aridor and Hannan, 2000; Li and Cohen, 1996; Neefjes and van der Kant, 2014). Understanding the molecular mechanisms underlying membrane trafficking, therefore, is crucial to the treatment and cure of human aliments. The focus of this review includes spatiotemporal dynamics of endocytic factors at the endocytic portal, cargo delivery traffic toward the *trans*-Golgi Network (TGN) and cargo degradation traffic to the lysosome.

## The entry: Endocytosis

Endocytosis is an imperative process through which cells internalize extracellular materials in company with parts of the plasma membrane, such as proteins and lipids. It is becoming more evident that the process plays a central role in the regulation of communication with extracellular environment, orchestration of the plasma membrane organization, and many intracellular signaling cascades (Hoeller et al., 2005; Kotowski et al., 2011). Considering the complexity of the process, it is not surprising that there exists many different uptake portals, and each has distinct characteristics. In mammalian cells, at least ten putative endocytic mechanisms have been identified in appreciation of transmission electron and fluorescence microscopy techniques; Clathrin mediated, Caveolae dependent, CLIC/GEEC portal, IL2Rβ pathway, Arf6 dependent, Flotillin dependent, Phagocytosis, Macropinocytosis, Circular dorsal ruffles and entosis (Doherty and McMahon, 2009). However, these endocytic portals can be classified into two general categories based on the existence of the clathrin coats around endocytic buds and vesicles: clathrin-mediated and clathrin-independent endocytosis. Clathrin-mediated endocytosis (CME), also called receptor-mediated endocytosis (RME), requires specific receptors that recognize their cargo at the plasma membrane. A malfunction of the low-density lipoprotein receptor (LDLR) involved in RME has been found to negatively affect the hepatic uptake and degradation of LDL, which leads to familial hypercholesterolemia (FH) that is characterized by raised serum LDL cholesterol levels (Goldstein and Brown, 1973; Soutar and Naoumova, 2007). Dysregulation of CME by losing some of the key players has

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also been reported to be associated with many human diseases. Breast cancer and renal cancer showed associations with the functional loss of the clathrin heavy chain (Kan et al., 2010), and increased expression level of dynamin was observed in patients with Schizophrenia (Prabakaran et al., 2004). In addition, defects in adaptor protein (AP)-2 and Eps1/Eps2 functions were reported to be linked with embryonic lethality (Chen et al., 2009; Ferguson et al., 2009; Mitsunari et al., 2005). Years of investigations in this field improved our fundamental understanding of the mechanism of CME. Therefore, the first part of this review will focus on discussing the mechanism of clathrin-mediated endocytosis.

*Clathrin-mediated endocytosis in mammalian systems*

Among a diverse array of endocytic pathways, the mechanism that has received most attention is clathrin-mediated endocytosis (CME). CME can easily be distinguished from other endocytic mechanisms by electron microscopy (EM) due to its distinct morphology; clathrin coats are observable around the external surface of endocytic buds or vesicles (Kirchhausen, 2009). A various range of transmembrane receptors and their ligands are transported to internal organelles via CME, and the general steps of the process include (1) formation of clathrin-coated pit (CCP), (2) membrane scission mediated by dynamin, (3) movement of clathrin-coated vesicle (CCV) away from the plasma membrane, and (4) uncoating event of clathrin from the vesicle.

To form a functional CCP, an orchestrated selection of adaptor and accessory proteins at the endocytic site is essential (Doherty and McMahon, 2009). Recruitment of different adaptors and accessory factors is most likely responsible for the formation of different subtypes of CCP, containing distinct cargo materials (Benmerah and Lamaze, 2007; Puthenveedu and von Zastrow, 2006). A recent study of G-protein-coupled receptors (GPCRs) internalization indeed showed that two purinergic GPCRs, $P2Y_1$ and $P2Y_{12}$, enter two populations of CCPs in a mutually exclusive manner (Mundell et al., 2006). The study also revealed that the internalization of $P2Y_{12}$ is mediated by GPCR kinases (GRKs) and arrestin, whereas $P2Y_1$ internalization requires protein kinase C, in a GRK- and arrestin-independent manner. It may be that the physiological relevance of the existing subtype CCPs is to secure that these subtype CCPs have the capacity to control their own pathway, thus increasing control options for clathrin-mediated endocytosis.

In mammalian system, CME begins with the arrival of early endocytic adaptor/scaffold proteins including Eps15 and FCHO1/SGIP1 to a nascent endocytic site, followed by the clustering of cargo and the early coat protein clathrin. More adaptor proteins, including Epsin (Koshiba et al., 2001), AP-2 (Gaidarov and Keen, 1999; Keen et al., 1979), and BAR (Bin-Amphiphysin-Rvs) domain proteins (Lundmark and Carlsson, 2003), are reported to be recruited to the endocytic site via interactions with phosphatidylinositol 4,5-bisphosphate ($PIP_2$) enriched in the plasma membrane (Fig. 1). Among these, the most abundant adaptor AP-2 of RME process has been considered as a pivotal player, serving as a linker between membrane cargoes and clathrin due to its interaction with both of them (McMahon and Boucrot, 2011) (Fig. 1). Loss of AP-2 in mammalian cells causes a severe defect in the uptake of transferrin and a clathrin recruitment defect, pointing the significance of AP-2 for endocytic process (Boucrot et al., 2010). The recruited adaptor proteins promote membrane distortion by generating membrane curvature (Peter et al., 2004) and stabilize the deformed membrane by recruiting and linking clathrin coat layer to cargo (Aridor and Traub, 2002; Wilbur et al., 2005). In addition, BAR domain proteins have been reported to recruit dynamin to initiate the subsequent membrane fission event (Lundmark and Carlsson, 2004) (Fig. 1). The continuous polymerization of clathrin in concert with recruitment of dynamin by BAR domain proteins facilitates the construction of vesicle neck (Peter et al., 2004). Dynamin is an essential player that mediates the scission of fully invaginated CCP from the membrane (Ferguson and De Camilli, 2012). Its molecular properties and mechanistic details in CME will be further discussed below. Upon the fission, the yielded CCV, whose size is approximately 100 nm in diameter, is internalized into the interior of the cell, and the clathrin coat is disassembled by auxilin and heat shock cognate 70 (Hsc70) for reuse (Krantz et al., 2013; Stoorvogel et al., 1996; Young et al., 2013).

Here, the fascinating molecular features of dynamin will be described in more detail. Dynamin is a mammalian cytosolic GTPase, mostly recognized for its key role in membrane fission event during endocytosis. It was discovered as a nucleotide-sensitive, microtubule-binding protein during the purification process of the calf brain microtubules (Shpetner and Vallee, 1989). The significance of dynamin in the endocytic process was first investigated in presynaptic plasma membrane of *Drosophila melanogaster*; cells expressing dynamin mutants failed to yield properly formed CCVs, rather they displayed an arrest of deeply invaginated endocytic pits, indicating the impairment in the fission process (Chen et al., 1991; Kosaka and Ikeda, 1983; Poodry and Edgar, 1979; van der Bliek and Meyerowitz, 1991). In subsequent studies, the distinctly structured pits surrounded by clathrin coat were observed in various mammalian cell lines with dynamin mutations, strongly implying the conserved function of dynamin in eukaryotic systems and its imperative participation in clathrin-coated endocytosis (Damke et al., 1994; Ferguson et al., 2009; Herskovits et al., 1993; van der Bliek et al., 1993). Recently, the crystal structure of the full length of dynamin except its C-terminal PRD (Proline-Rich Domain) (Faelber et al., 2011; Ford et al., 2011) has modified the classic structural view of dynamin domains, allowing a better prediction of its three-dimensional structure and putative mechanism. The structure includes the N-terminal GTPase domain forming a head-like structure, the middle domain as a component comprising a stalk, the PH (Pleckstrin Homology) domain a foot, and the GED (GTPase Effector Domain) as another stalk component (Faelber et al., 2011; Ferguson et al., 1994; Ford et al., 2011; Gao et al., 2010). The hydrolytic activity of the GTPase domain is highly coupled with its dimerization to achieve its basic unit and conformational change to mediate vesicular scission (Faelber et al., 2011; Ford et al., 2011; Marks et al., 2001). The dimerization is mediated by the cross-linkage of two stalks resulting in the two GTPase heads pointing in opposite direction (Faelber et al., 2011; Ford et al., 2011; Gao et al., 2010). The GED portion that is comprising half of the stalk is considered to be the intrinsic GAP (guanine nucleotide-activating proteins) for dynamin (Narayanan et al., 2005). The PH domain is responsible for binding to $PIP_2$ in the plasma membrane with low-affinity, allowing the dynamic interaction (Bethoney et al., 2009; Ferguson et al., 1994; Zheng et al., 1996). Lastly, the PRD contains many SH3-binding motifs which synergistically link dynamin to many important SH3 domain proteins at endocytic patches to aid in proper endocytic processes (Grabs et al., 1997; Shpetner et al., 1996). Extensive studies have revealed that the functions carried out by each domain of dynamin are conserved in the dynamin-like protein (DLP) family member (Low and Lowe, 2010), except the fact that DLPs lack PH and PRD domains. Nevertheless, DLPs have capacity to bind lipid because they contain variable lipid binding motifs. Like dynamins, the activity of DLPs does not dependent on the conventional regulation of GEFs (guanine nucleotide-exchange factors) or GAPs (Narayanan et al., 2005). Instead, they load GTP spontaneously and have built-in mechanisms for stabilizing the GTP hydrolysis-induced transition state (Gasper et al., 2009). The detailed mechanism of dynamin in membrane fission has been controversial and has been intensely investigated by many research groups. In order to achieve the proper GTPase activity for the fission, dimerization of dynamin is reported to be critical (Chappie