Membrane Trafficking


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Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

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Literature references


Reactome database release: 82

This document contains 9 pathways (see Table of Contents)
Membrane Trafficking

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The secretory membrane system allows a cell to regulate delivery of newly synthesized proteins, carbohydrates, and lipids to the cell surface, a necessity for growth and homeostasis. The system is made up of distinct organelles, including the endoplasmic reticulum (ER), Golgi complex, plasma membrane, and tubulovesicular transport intermediates. These organelles mediate intracellular membrane transport between themselves and the cell surface. Membrane traffic within this system flows along highly organized directional routes. Secretory cargo is synthesized and assembled in the ER and then transported to the Golgi complex for further processing and maturation. Upon arrival at the trans Golgi network (TGN), the cargo is sorted and packaged into post-Golgi carriers that move through the cytoplasm to fuse with the cell surface. This directional membrane flow is balanced by retrieval pathways that bring membrane and selected proteins back to the compartment of origin.

Literature references

Secretory cargo destined to be secreted or to arrive at the plasma membrane (PM) leaves the ER via distinct exit sites. This cargo is destined for the Golgi apparatus for further processing.

About 25% of the proteome may be exported from the ER in human cells. This cargo is recognized and concentrated into COPII vesicles, which range in size from 60-90 nm, and move cargo from the ER to the ERGIC. Soluble cargo in the ER lumen is concentrated into COPII vesicles through interaction with a receptor with the receptor subsequently recycled to the ER in COPI vesicles through retrograde traffic.

The ERGIC (ER-to-Golgi intermediate compartment, also known as vesicular-tubular clusters, VTCs) is a stable, biochemically distinct compartment located adjacent to ER exit sites.

Retrograde traffic makes use of microtubule-directed COPI-coated vesicles, carrying ER proteins and membrane back to the ER.

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The mammalian Golgi complex, a central hub of both anterograde and retrograde trafficking, is a ribbon of stacked cisterna with biochemically distinct compartments (reviewed in Glick and Nakano, 2009; Szul and Sztul, 2011). Anterograde cargo from the ERGIC and ER is received at the cis-Golgi, trafficked through the medial- and trans-Golgi and released through the trans-Golgi network (TGN) to the endolysosomal system and the plasma membrane. Although still under debate, current models of Golgi trafficking favour the cisternal maturation model, where anterograde cargo remain associated with their original lipid membrane during transit through the Golgi and are exposed to sequential waves of processing enzymes by the retrograde movement of Golgi resident proteins. In this way, cis-cisterna mature to medial- and trans-cisterna as the early acting Golgi enzymes are replaced by later acting ones (reviewed in Pelham, 2001; Storrie, 2005; Glick and Nakano, 2009; Szul and Sztul, 2011). More recently, a kiss-and-run (KAR) model for intra-Golgi trafficking has been proposed, which marries aspects of the cisternal maturation model with a diffusion model of transport (reviewed in Mironov et al, 2013).

Like the anterograde ERGIC-to Golgi transport step, intra-Golgi trafficking between the cisterna appears to be COPI-dependent (Storrie and Nilsson, 2002; Szul and Sztul, 2011). Numerous snares and tethering complexes contribute to the targeting and fusion events that are required to maintain the specificity and directionality of these trafficking events (reviewed in Chia and Gleeson, 2014). Golgi tethers include long coiled coiled proteins like the Golgins, as well as multisubunit tethers like the COG complex. These tethers make numerous interactions with other components of the secretory system including RABs, SNAREs, motor and coat proteins as well as components of the cytoskeleton (reviewed in Munro, 2011; Willet et al, 2013).

Retrograde traffic from the cis-Golgi back to the ERGIC and ER depends on both the COPI-dependent
pathway, which appears to be important for recycling of KDEL receptors, and a more recently described COPI-independent pathway that relies on RAB6 (reviewed in Szul and Sztul, 2011; Heffernan and Simpson, 2014). RAB6 and RAB9 also play roles at the TGN side of the Golgi, where they are implicated in the docking of vesicles derived from the endolysosomal system and the plasma membrane (reviewed in Pfeffer, 2011)

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After passing through the Golgi complex, secretory cargo is packaged into post-Golgi transport intermediates (post-Golgi), which translocate plus-end directed along microtubules to the plasma membrane.

There at least two classes of clathrin coated vesicles in cells, one predominantly Golgi-associated, involved in budding from the trans-Golgi network and the other at the plasma membrane. Here the clathrin-coated vesicles emerging from the Golgi apparatus are triggered by the heterotetrameric adaptor protein complex, AP-1 at the trans-Golgi network membrane. The cargo can be transmembrane, membrane associated or golgi luminal proteins. Each step in the vesicle sculpting pathway, gathers cargo and clathrin triskelions, until a complete vesicular sphere is formed. With the scission of the membrane the vesicle is released and eventually losses its clathrin coat.

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Gap junctions are clusters of intercellular channels connecting adjacent cells and permitting the direct exchange of ions and small molecules between cells. These channels are composed of two hemichannels, or connexons, one located on each of the two neighboring cells. Each connexon is composed of 6 trans-membrane protein subunits of the connexin (Cx) family. A gap of approximately 3 nm remains between the adjacent cell membranes, but two connexons interact and dock head-to-head in the extracellular space forming a tightly sealed, double-membrane intercellular channel (see Segretain and Falk, 2004). The activity of these intercellular channels is regulated, particularly by intramolecular modifications such as phosphorylation which appears to regulate connexin turnover, gap junction assembly and the opening and closure (gating) of gap junction channels.

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Clathrin-mediated endocytosis (CME) is one of a number of processes that control the uptake of material from the plasma membrane, and leads to the formation of clathrin-coated vesicles (Pearse et al., 1975; reviewed in Robinson, 2015; McMahon and Boucrot, 2011; Kirchhausen et al., 2014). CME contributes to signal transduction by regulating the cell surface expression and signaling of receptor tyrosine kinases (RTKs) and G-protein coupled receptors (GPCRs). Most RTKs exhibit a robust increase in internalization rate after binding specific ligands; however, some RTKs may also exhibit significant ligand-independent internalization (reviewed in Goh and Sorkin, 2013). CME controls RTK and GPCR signaling by organizing signaling both within the plasma membrane and on endosomes (reviewed in Eichel et al., 2016; Garay et al., 1996; Sorkin and von Zastrow, 2014; Di Fiori and von Zastrow, 2014; Barbieri et al., 2016). CME also contributes to the uptake of material such as metabolites, hormones and other proteins from the extracellular space, and regulates membrane composition by recycling membrane components and/or targeting them for degradation.

Clathrin-mediated endocytosis involves initiation of clathrin-coated pit (CCP) formation, cargo selection, coat assembly and stabilization, membrane scission and vesicle uncoating. Although for simplicity in this pathway, the steps leading to a mature CCP are represented in a linear and temporally distinct fashion, the formation of a clathrin-coated vesicle is a highly heterogeneous process and clear temporal boundaries between these processes may not exist (see for instance Taylor et al., 2011; Antonescu et al., 2011; reviewed in Kirchhausen et al., 2014). Cargo selection in particular is a critical aspect of the formation of a mature and stable CCP, and many of the proteins involved in the initiation and maturation of a CCP contribute to cargo selection and are themselves stabilized upon incorporation of cargo into the nascent vesicle (reviewed in Kirchhausen et al., 2014; McMahon and Boucrot, 2011).

Although the clathrin triskelion was identified early as a major component of the coated vesicles, clathrin does not bind directly to membranes or to the endocytosed cargo. Vesicle formation instead relies on many proteins and adaptors that can bind the plasma membrane and interact with cargo molecules. Cargo selection depends on the recognition of endocytic signals in cytoplasmic tails of the cargo proteins by adaptors that interact with components of the vesicle's inner coat. The classic adaptor for clathrin-coated vesicles is the tetrameric AP-2 complex, which along with clathrin was identified early as a major component of the coat. Some cargo indeed bind directly to AP-2, but subsequent work has revealed a large family of proteins collectively known as CLASPs (clathrin-associated sorting proteins) that mediate the recruitment of diverse cargo into the emerging clathrin-coated vesicles (reviewed in Traub and Bon-
Many of these CLASP proteins themselves interact with AP-2 and clathrin, coordinating cargo recruitment with coat formation (Schmid et al, 2006; Edeling et al, 2006; reviewed in Traub and Bonifacino, 2013; Kirchhausen et al, 2014).

Initiation of CCP formation is also influenced by lipid composition, regulated by clathrin-associated phosphatases and kinases (reviewed in Picas et al, 2016). The plasma membrane is enriched in PI(4,5)P2. Many of the proteins involved in initiating clathrin-coated pit formation bind to PI(4,5)P2 and induce membrane curvature through their BAR domains (reviewed in McMahon and Boucrot, 2011; Daumke et al, 2014). Epsin also contributes to early membrane curvature through its Epsin N-terminal homology (ENTH) domain, which promotes membrane curvature by inserting into the lipid bilayer (Ford et al, 2002).

Following initiation, some CCPs progress to formation of vesicles, while others undergo disassembly at the cell surface without producing vesicles (Ehrlich et al, 2004; Loerke et al, 2009; Loerke et al, 2011; Aguet et al, 2013; Taylor et al, 2011). The assembly and stabilization of nascent CCPs is regulated by several proteins and lipids (Mettlen et al, 2009; Antonescu et al, 2011).

Maturation of the emerging clathrin-coated vesicle is accompanied by further changes in the lipid composition of the membrane and increased membrane curvature, promoted by the recruitment of N-BAR domain containing proteins (reviewed in Daumke et al, 2014; Ferguson and De Camilli, 2012; Picas et al, 2016). Some N-BAR domain containing proteins also contribute to the recruitment of the large GTPase dynamin, which is responsible for scission of the mature vesicle from the plasma membrane (Koh et al, 2007; Lundmark and Carlsson, 2003; Soulet et al, 2005; David et al, 1996; Owen et al, 1998; Shupliakov et al, 1997; Taylor et al, 2011; Ferguson et al, 2009; Aguet et al, 2013; Posor et al, 2013; Chappie et al, 2010; Shnyrova et al, 2013; reviewed in Mettlen et al, 2009; Daumke et al, 2014). After vesicle scission, the clathrin coat is dissociated from the new vesicle by the ATPase HSPA8 (also known as HSC70) and its DNAJ cofactor auxilin, priming the vesicle for fusion with a subsequent endocytic compartment and releasing clathrin for reuse (reviewed in McMahon and Boucrot, 2011; Sousa and Laufer, 2015).

Literature references


Many plasma membrane proteins are in a constant flux throughout the internal trafficking pathways of the cell. Some receptors are continuously internalized into recycling endosomes and returned to the cell surface. Others are sorted into intraluminal vesicles of morphologically distinctive endosomes that are known as multivesicular bodies (MVBs). These MVBs fuse with lysosomes, resulting in degradation of their cargo by lysosomal acid hydrolases.

Endosomes can be operationally defined as being either early or late, referring to the relative time it takes for endocytosed material to reach either stage. Ultrastructural studies indicate that early endosomes are predominantly tubulovesicular structures, which constitute a major sorting platform in the cell, whereas late endosomes show the characteristics of typical MVBs and are capable of fusing with lysosomes.

A well characterized signal for shunting membrane proteins into the degradative MVB pathway is the ubiquitylation of these cargoes. At the center of a vast protein:protein and protein:lipid interaction network that underpins ubiquitin mediated sorting to the lysosome are the endosomal sorting complexes required for transport (ESCRTs), which are conserved throughout all major eukaryotic taxa.

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Translocation of SLC2A4 (GLUT4) to the plasma membrane

**Location:** Membrane Trafficking

**Stable identifier:** R-HSA-1445148

**Compartments:** cytoplasmic vesicle membrane, cytosol

In adipocytes and myocytes insulin signaling causes intracellular vesicles carrying the GLUT4 (SLC2A4) glucose transporter to translocate to the plasma membrane, allowing the cells to take up glucose from the bloodstream (reviewed in Zaid et al. 2008, Leney and Tavare 2009, Bogan and Kandror 2010, Foley et al. 2011, Hoffman and Elmendorf 2011, Kandror and Pilch 2011, Jaldin-Fincati et al. 2017). In myocytes muscle contraction alone can also cause translocation of GLUT4.

Though the entire pathway leading to GLUT4 translocation has not been elucidated, several steps are known. Insulin activates the kinases AKT1 and AKT2. Muscle contraction activates the kinase AMPK-alpha2 and possibly also AKT. AKT2 and, to a lesser extent, AKT1 phosphorylate the RAB GTPase activators TBC1D1 and TBC1D4, causing them to bind 14-3-3 proteins and lose GTPase activation activity. As a result RAB proteins (probably RAB8A, RAB10, RAB14 and possibly RAB13) accumulate GTP. The connection between RAB:GTP and vesicle translocation is unknown but may involve recruitment and activation of myosins.

Myosins 1C, 2A, 2B, 5A, 5B have all been shown to play a role in translocating GLUT4 vesicles near the periphery of the cell. Following docking at the plasma membrane the vesicles fuse with the plasma membrane in a process that depends on interaction between VAMP2 on the vesicle and SNAP23 and SYNTAXIN-4 at the plasma membrane.

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Human cells have more than 60 RAB proteins that are key regulators of intracellular membrane trafficking. These small GTPases contribute to trafficking specificity by localizing to the membranes of different organelles and interacting with effectors such as sorting adaptors, tethering factors, kinases, phosphatases and tubular-vesicular cargo (reviewed in Stenmark et al, 2009; Wandinger-Ness and Zerial, 2014; Zhen and Stenmark, 2015).

RAB localization depends on a number of factors including C-terminal prenylation, the sequence of upstream hypervariable regions and what nucleotide is bound, as well as interaction with RAB-interacting proteins (Chavrier et al, 1991; Ullrich et al, 1993; Soldati et al, 1994; Farnsworth et al, 1994; Seabra, 1996; Wu et al, 2010; reviewed in Stenmark, 2009; Wandinger-Ness and Zerial, 2014). More recently, the activity of RAB GEFs has also been implicated in regulating the localization of RAB proteins (Blumer et al, 2103; Schoebel et al, 2009; Cabrera and Ungermann, 2013; reviewed in Barr, 2013; Zhen and Stenmark, 2015).

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