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.

The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

Literature references


Reactome database release: 77

This document contains 2 pathways and 20 reactions (see Table of Contents)
Clathrin-mediated endocytosis 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.

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 Bonifacino, 2013). 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...
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**


**Editions**

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https://reactome.org
Cargo recognition for clathrin-mediated endocytosis

**Location:** Clathrin-mediated endocytosis

**Stable identifier:** R-HSA-8856825

Recruitment of plasma membrane-localized cargo into clathrin-coated endocytic vesicles is mediated by interaction with a variety of clathrin-interacting proteins collectively called CLASPs (clathrin-associated sorting proteins). CLASP proteins, which may be monomeric or tetrameric, are recruited to the plasma membrane through interaction with phosphoinositides and recognize linear or conformational sequences or post-translational modifications in the cytoplasmic tails of the cargo protein. Through bivalent interactions with clathrin and/or other CLASP proteins, they bridge the recruitment of the cargo to the emerging clathrin coated pit (reviewed in Traub and Bonifacino, 2013). The tetrameric AP-2 complex, first identified in early studies of clathrin-mediated endocytosis, was at one time thought to be the primary CLASP protein involved in cargo recognition at the plasma membrane, and indeed plays a key role in the endocytosis of cargo carrying dileucine- or tyrosine-based motifs.

A number of studies have been performed to test whether AP-2 is essential for all forms of clathrin-mediated endocytosis (Keyel et al, 2006; Motely et al, 2003; Huang et al, 2004; Boucrot et al, 2010; Henne et al, 2010; Johannessen et al, 2006; Gu et al, 2013; reviewed in Traub, 2009; McMahon and Boucrot, 2011). Although depletion of AP-2 differentially affects the endocytosis of different cargo, extensive depletion of AP-2 through RNAi reduces clathrin-coated pit formation by 80-90%, and the CCPs that do form still contain AP-2, highlighting the critical role of this complex in CME (Johannessen et al, 2006; Boucrot et al, 2010; Henne et al, 2010).

In addition to AP-2, a wide range of other CLASPs including proteins of the beta-arrestin, stonin and epsin families, engage sorting motifs in other cargo and interact either with clathrin, AP-2 or each other to facilitate assembly of a clathrin-coated pit (reviewed in Traub and Bonifacino, 2013).

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## Editions

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Initial binding of AP-2 and clathrin to PI(4,5)P2

**Location:** Clathrin-mediated endocytosis

**Stable identifier:** R-HSA-8871196

**Type:** binding

**Compartments:** plasma membrane

Assembly of an endocytic clathrin-coated pit (CCP) at the plasma membrane depends on the recruitment of the AP-2 adaptor protein complex and clathrin triskelions to the lipid bilayer (reviewed in McMahon and Boucrot, 2011; Robinson, 2015). Transient interactions between the plasma membrane-enriched lipid phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) and AP-2 initiate coated pit formation (Beck et al, 1991; Honing et al, 2005; Loerke et al, 2009; Cocucci et al, 2012). A proportion of the transient complexes between AP-2, clathrin and the plasma membrane are rapidly stabilized by the recruitment of a number of proteins, including FCHo proteins, intersectins (ITSNs), EPS15 and SGIP1 among others (Henne et al, 2010; Stimpson et al, 2009; Reider et al, 2009; Cocucci et al, 2012; reviewed in McMahon and Boucrot, 2011). Many of these early players in CCP formation bind both to the plasma membrane and to the AP-2 complex and/or clathrin.

CCP formation is a highly heterogeneous and dynamic process and includes abortive initiation of nearly half of nascent CCPs (Loerke et al, 2009; Aguet et al, 2013). Heterogeneity is in part the result of the widely varied cargo proteins, which compete for a limited number of interaction hubs on AP-2 and clathrin and influence the other protein components of the CCPs. Heterogeneity may also be partly stochastic, or be influenced by the presence of CCP 'hot spots' in the plasma membrane (Taylor et al, 2011; Antonescu et al, 2011; Gaidarov et al, 1999; Ehrlich et al, 2004; Saffarian et al, 2009; Nunez et al, 2011). It is important to note that although events in this pathway are depicted as occurring sequentially in a defined order, in reality the assembly of a clathrin-coated vesicle may be highly variable and the temporal boundaries are likely less clearly defined. Moreover, not every CCP will have all of the proteins indicated in this pathway.

**Followed by:** FCHo proteins bind nascent clathrin-coated pit

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FCHo proteins bind nascent clathrin-coated pit

**Location:** Clathrin-mediated endocytosis

**Stable identifier:** R-HSA-8862280

**Type:** binding

**Compartments:** plasma membrane

Stabilization of the transient binding of AP-2 and clathrin at the plasma membrane is effected by the recruitment of a number of early acting proteins, including FCHo (F-BAR domain-containing Fer/Cip4 homology domain-only) proteins 1 and 2, intersectins (ITSNs), EPS15, EPS15L1, REPS1 and SGIP1 among others (Henne et al, 2010; Stimpson et al, 2009; Reider et al, 2009; Dergai et al, 2010; Antonescu et al, 2011; reviewed in McMahon and Boucrot, 2011).

FCHo proteins interact with the plasma membrane-enriched PI(4,5)P2 through the F-BAR domain, which recognizes curvature in the membrane (Henne et al, 2010; Henne et al, 2007; Shimada et al, 2007; Umasankar et al, 2012). Other F-BAR proteins, such as FNBP1 and FNBP1L may join the nascent clathrin-coated pit at a slightly later stage (Shimada et al, 2007). Recruitment of EPS15 and ITSN1 and 2 appears coincident with binding of FCHo2 and depends on direct interaction with the AP2 mu homology domain of FCHo2 (Henne et al, 2010).

SGIP1 (Src homology 3-domain growth factor receptor-bound 2-like (endophilin) interacting protein 1) interacts with numerous endocytic proteins including AP-2, ITSN1, REPS1, EPS15, endophilin and amphiphsyin1 and is thought to play a role in clathrin-mediated endocytosis (Trevaskis et al, 2005; Dergai et al, 2010; Uezu et al, 2007). SGIP1 is related to the FCHo proteins and is co-immunoprecipitated in a tripartite complex containing ITSN1 and REPS1 (Dergai et al, 2010). The exact function of SGIP1 in clathrin-mediated endocytosis remains to be elucidated, however recent work suggests SGIP1 and FCHo proteins may contribute to allosteric changes in AP-2 that promote membrane binding and cargo recognition (Hollopeter et al, 2014).

The recruitment of this group of early CCP proteins is rapidly followed by the incorporation of many AP-2 and clathrin molecules, stimulated in part by the FCHo- and SGIP-dependent stabilization of the open, membrane binding conformation of AP-2 (Hollopeter et al, 2014). Alternately, a proportion of the nascent CCPs may undergo abortive initiation (Loerke et al, 2009; Aguet et al, 2013; Antonescu et al, 2011).
This is prompted in part through the early recruitment of the 170 kDa isoform of synaptojanin 1 (SYNJ1-170, not shown in this reaction). SYNJ1 catalyzes the hydrolysis of PI(4,5)P2 to PI(4)P and destabilizes the interaction of many early CCP components with the plasma membrane (Perera et al, 2006).

**Preceded by:** Initial binding of AP-2 and clathrin to PI(4,5)P2

**Followed by:** Recruitment of AP-2 complex and clathrin

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Recruitment of AP-2 complex and clathrin

**Location:** Clathrin-mediated endocytosis

**Stable identifier:** R-HSA-8856808

**Type:** binding

**Compartments:** plasma membrane

Recruitment of early acting proteins such as the FCHO and ITSN proteins stabilizes the transient AP-2:clathrin complex at the plasma membrane and is rapidly followed by incorporation of many more molecules of AP-2 and clathrin. AP-2 binding to the plasma-membrane enriched PI(4,5)P2 is reinforced early in the formation of a CCP by the interaction of AP-2 with PIP5K1C, which synthesizes PI(4)P to PI(4,5)P2 (Krauss et al, 2006; Bairstow et al, 2006; Thieman et al, 2009).

AP-2 recruitment is also promoted by conformational changes upon lipid and protein binding. AP-2 is a heterotetramer consisting of two large subunits (alpha and beta1 adaptin), a medium mu2 subunit and a small sigma2 subunit, and exists in a closed conformation when not part of a clathrin-coated pit (Jackson et al, 2010).

Interactions between the AP-2 mu2 subunit and PIP2 within the lipid bilayer stabilize the 'open' conformation of AP-2, exposing binding sites for cargo proteins. The open conformation is also promoted by interaction of AP-2 with early CCP proteins such as SGIP and FCHO2 (Hollopeter et al, 2014). Recruitment of clathrin stimulates the activity of AAK1, an AP-2 kinase that phosphorylates the mu2 subunit of the adaptor complex at Thr156, further stabilizing the open conformation and promoting cargo recruitment (Olusanya et al, 2001; Ricotta et al, 2002; Conner et al, 2002; Conner et al, 2003).

NECAP1 and 2 may also aid in the assembly of an emergent clathrin-coated pit. NECAP proteins have a WxF motif at the C-terminus that binds with high affinity to the alpha-ear sandwich domain of AP-2 and an N-terminal PH ear domain that interacts both with AP-2 and a wide range of endocytic accessory proteins containing FxDxF motifs (Ritter et al, 2003; Wasiak et al, 2002; Ritter et al, 2013). Clathrin and the NECAP PH ear domain appear to compete for an AP-2 binding site. Clathrin-mediated displacement of the NECAP PH ear domain from its lower affinity AP-2 site may allow release this domain, allowing it to transition to a role in recruiting endocytic accessory proteins and cargo (Ritter et al, 2007; Ritter et al, 2013; reviewed in McMahon and Boucrot, 2011).
Finally, studies have highlighted a role for ARF6 and its GTPase activating protein ARFGAP1 in CCP formation, although the details remain to be established.

ARFGAP1 and ARF6 appear to contribute to the recruitment of some cargo, but may also play a more generalized role in CCP formation (Moravec et al, 2012; Bai et al, 2011). ARFGAP1 binds directly to AP-2 and its GAP activity is required for CME. Consistent with this, silencing of ARFGAP1 impairs CME (Schmid et al, 2006; Rawet et al 2010; Bai et al 2011). ARFGAP1 has activity towards several ARFs, including ARF6 which is found in some CCPs and is known to regulate CME under some circumstances (Moravec et al, 2003; Palacios et al, 2002; Paleotti et al, 2005; Kraus et al, 2003). ARF6 is thought to contribute to the recruitment of AP-2 and clathrin to the plasma membrane, possibly in part by affecting the lipid composition (Paleotti et al, 2002; Krauss et al, 2003).

Preceded by: FCHo proteins bind nascent clathrin-coated pit

Followed by: AAK1 phosphorylates AP-2 mu subunit at T156, PIP5K1C phosphorylates PI(4)P to PI(4,5)P2

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**PIP5K1C phosphorylates PI(4)P to PI(4,5)P2**

**Location:** Clathrin-mediated endocytosis

**Stable identifier:** R-HSA-8868066

**Type:** transition

**Compartments:** plasma membrane

Plasma membrane enrichment of PI(4,5)P2 is maintained in part through the action of PI 4-phosphatase 5 kinases (PIPKIs) such as PIP5K1C (Di Paolo and De Camilli, 2006). PIP5K1C interacts directly with AP-2 and the interaction activates the kinase, generating a positive feedback loop for the recruitment of AP-2 to the plasma membrane (Krauss et al, 2006; Bairstow et al, 2006; Thieman et al, 2009; reviewed in Daumke et al, 2014).

**Preceded by:** Recruitment of AP-2 complex and clathrin

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[https://reactome.org](https://reactome.org)
AAK1 phosphorylates AP-2 mu subunit at T156

**Location:** Clathrin-mediated endocytosis

**Stable identifier:** R-HSA-8856813

**Type:** transition

**Compartments:** plasma membrane

AAK1 is a serine-threonine kinase that phosphorylates T156 of the AP2 mu2 subunit (Olusanya et al, 2001; Conner et al, 2002; Conner et al, 2003). This phosphorylation is thought to stabilize the open conformation of the AP-2 complex, exposing the cargo-binding sites and promoting cargo capture (Ricotta et al, 2002). AAK1 kinase activity is stimulated by interaction with clathrin (Conner et al, 2003; Henderson et al, 2007).

**Preceded by:** Recruitment of AP-2 complex and clathrin

**Followed by:** CLASP proteins and cargo are recruited to the nascent clathrin-coated pit

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https://reactome.org
CLASP proteins and cargo are recruited to the nascent clathrin-coated pit

**Location:** Clathrin-mediated endocytosis

**Stable identifier:** R-HSA-8867756

**Type:** binding

**Compartments:** plasma membrane

CLASP proteins are recruited to nascent clathrin-coated pits (CCPs) through interactions with AP-2 and clathrin. Although in this pathway cargo recruitment is depicted as occurring after the recruitment of bulk AP-2 and clathrin, a number of studies suggest that they are largely recruited concomitantly (Liu et al, 2010; reviewed in McMahon and Boucrot, 2011). Concurrent interactions with sorting signals in cargo cytoplasmic tails and with clathrin and/or AP-2 ensure that CLASPs and cargo are incorporated into the emerging CCP (Schmid et al, 2006; Edeling et al, 2006; reviewed in Traub, 2009; Traub and Bonifacino, 2013; Kirchausen et al, 2014). In addition, incorporation of CLASPs and cargo may play a role in regulating the timing and dynamics of endocytosis (Loerke et al, 2009; Mettlen et al, 2009; Soohoo et al, 2013; Mettlen et al, 2010; Puthenveedu et al, 2005).

**Preceded by:** AAK1 phosphorylates AP-2 mu subunit at T156

**Followed by:** Clathrin recruits PIK3C2A

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PIK3C2A is a member of the class II PI 3 kinases, and phosphorylates PI(4)P to PI(3,4)P2 at the plasma membrane. PIK3C2A interacts with clathrin through a clathrin-binding domain in its unique N-terminal tail and localizes to late-stage clathrin-coated pits (Domin et al, 2000; Gaidarov et al, 2001; Gaidarov et al, 2005). Binding to clathrin stimulates the kinase activity of PIK3C2A and promotes the production of PI(3,4)P2 at the plasma membrane (Gaidarov et al, 2001). PI(3,4)P2 formation by PIK3C2A contributes to maturation of clathrin-coated pits by promoting the recruitment of BAR-domain containing proteins such as SNX9, which stimulate membrane curvature required for vesicle formation and eventual fission (Posor et al, 2013; reviewed in Daumke et al, 2014).

**Preceded by:** CLASP proteins and cargo are recruited to the nascent clathrin-coated pit

**Followed by:** Clathrin-associated PIK3C2A phosphorylates PI(4)P to PI(3,4)P2, F- and N- BAR domain proteins bind the clathrin-coated pit

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Clathrin-associated PIK3C2A phosphorylates PI(4)P to PI(3,4)P2

Location: Clathrin-mediated endocytosis

Stable identifier: R-HSA-8868072

Type: transition

Compartments: plasma membrane

Clathrin-associated PIK3C2A catalyzes the conversion of PI(4)P to PI(3,4)P2, which contributes to the recruitment of BAR domain proteins such as SNX9 to the clathrin-coated pit (Domin et al, 2000; Gaidarov et al, 2001; Gaidarov et al, 2005; Posor et al, 2013; reviewed in Daumke et al, 2014).

Preceded by: Clathrin recruits PIK3C2A

Literature references


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2016-05-10 Reviewed Antonescu, CN.
2016-05-11 Authored, Edited Rothfels, K.
**F- and N- BAR domain proteins bind the clathrin-coated pit**

**Location:** Clathrin-mediated endocytosis

**Stable identifier:** R-HSA-8867754

**Type:** binding

**Compartments:** plasma membrane

BAR (BIN/amphiphysin/Rvs) domain proteins sense and contribute to membrane curvature. BAR domain proteins generally form long, coiled-coil homo- or hetero-dimers with a concave inner surface that interacts with membranes (reviewed in Gallop and McMahon, 2005; Daumke et al, 2014). F-BAR domain proteins such as FCHo1 and 2 recognize shallow membrane curvature and are generally recruited early in the formation of clathrin-coated pit (Itoh et al, 2005; Kamioka et al, 2004; Henne et al, 2007; Shimada et al, 2007; Henne et al, 2010). FNBP proteins and N-BAR containing endocytic proteins such as SNX9 and 18, amphiphysin (AMPH) and endophilins recognize regions of membrane with greater curvature, interact with dynamin and likely play a later role in CCP formation with spatiotemporal coupling to vesicle scission (Kamioka et al, 2004; Itoh et al, 2005; Soulet et al, 2005; Shimada et al, 2007; Shin et al, 2008; Taylor et al, 2011; reveiwed in McMahon and Boucrot, 2011). These proteins are recruited to the complex through interactions with core components of the clathrin-coated pit, and in the case of SNX9, also through interaction with PI(3,4)P2, which is generated at late stages by clathrin-associated PIK3C2A (Lundmark and Carlson, 2003; Schmid et al, 2006; Dergai et al, 2010; Brett et al, 2002; Posor et al, 2013; reviwed in Daumke et al, 2014). Early BAR domain containing proteins such as FCHO1 and 2 are not present in either late stage clathrin-coated pits or in free clathrin-coated vesicles. Although the precise timing of their dissociation is not known, in this pathway, they are shown leaving the clathrin-coated pit upon recruitment of the more highly curved N-BAR proteins (Taylor et al, 2011).

**Preceded by:** Clathrin recruits PIK3C2A

**Followed by:** SNX9 recruits components of the actin polymerizing machinery

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SNX9 recruits components of the actin polymerizing machinery

**Location:** Clathrin-mediated endocytosis

**Stable identifier:** R-HSA-8868230

**Type:** binding

**Compartments:** plasma membrane

Actin polymerization is not absolutely required for clathrin-mediated endocytosis, and disruption of actin does not interfere with the early stages of clathrin-coated pit formation. Actin is required to complete vesicle formation under conditions of high membrane tension, such as on the apical side of polarized epithelial cell, while actin is dispensable for this process in the absence of membrane tension (Boulant et al, 2011). In cases where actin is required, it appears to be recruited late to the emerging clathrin-coated pit, just prior to or coincident with the recruitment of dynamin and vesicle scission (Taylor et al, 2011; Taylor et al, 2012; reviewed in McMahon and Boucrot, 2011). Recruitment of actin depends on the ARP2/3 complex, and cortactin or the neural Wiscott-Aldrich syndrome proteins WASL. These proteins, in turn, are recruited through interactions with N-BAR domain containing proteins such as SNX9 (Yarar et al, 2007; Shin et al, 2007; Shin et al, 2008; Ferguson et al, 2009; reviewed in Lundmark and Carlsson, 2009; McMahon and Boucrot, 2011).

HIP1 and HIP1R are additional components of the late clathrin-coated pit that interact with clathrin and AP-2 and may contribute to actin nucleation (Waelter et al, 2001; Mishra et al, 2001; Metzler et al, 2001; Legendre-Guillemin et al, 2002; Wilbur et al, 2008; Taylor et al, 2011).

**Preceded by:** F- and N-BAR domain proteins bind the clathrin-coated pit

**Followed by:** BAR domain proteins recruit dynamin

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BAR domain proteins recruit dynamin

Location: Clathrin-mediated endocytosis

Stable identifier: R-HSA-8868236

Type: binding

Compartments: plasma membrane

Dynamin is a large GTPase whose GTP hydrolysis activity is required for the scission of clathrin-coated vesicles from the plasma membrane (reviewed in Ferguson and De Camilli, 2012). Dynamin is recruited to the plasma membrane through protein-protein interactions with many components of the clathrin-coated pit including ITSNs, SNX9 and 18 and amphiphysin (Lundmark and Carlsson, 2003; Soulet et al, 2005; David et al, 1996; Owen et al, 1998; Shupliakov et al, 1997). Although dynamin is recruited at lower levels throughout formation of the clathrin-coated pit, the bulk of dynamin is recruited at late stages, after the incorporation of BAR domain-containing proteins and actin-polymerizing factors (Ferguson et al, 2009; Taylor et al, 2011; Taylor et al, 2012; Posor et al, 2013; Meineke et al, 2013; Aguet et al, 2013; reviewed in Daumke et al, 2014). Several BAR domain proteins have SH3 domains that bind the proline rich domain (PRD) of dynamin. These interactions regulate dynamin GTPase activity and vesicle formation (Neuman and Schmid, 2013). To facilitate scission of a clathrin-coated pit from the plasma membrane, dynamin self assembles into helical oligomers, stimulating its GTPase activity and contributing to the membrane remodeling required to form the neck of the emerging vesicle (Sweitzer and Hinchaw 1998; Yoshida et al, 2004; Chappie et al, 2010; Faelber et al, 2011; Ford et al, 2011; reviewed in McMahon and Boucrot, 2011; Daumke et al, 2014).

Preceded by: SNX9 recruits components of the actin polymerizing machinery

Followed by: Endophilins recruit synaptojanins to the clathrin-coated pit

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Endophilins recruit synaptojanins to the clathrin-coated pit

Location: Clathrin-mediated endocytosis

Stable identifier: R-HSA-8868651

Type: binding

Compartments: plasma membrane

Synaptojanin (SYNJ) 1 and 2 are inositol-5-phosphatases that sequentially convert PI(4,5)P2 to PI(4)P and PI (Cremona et al 1999; reviewed in Billcliff and Lowe, 2014). Conversion of PI(4,5)P2 to PI(4)P and PI accompanies maturation of the clathrin-coated pit, and consistent with this, SYNJ proteins are recruited to the clathrin-coated pit through interactions with a number of endocytic proteins including ITSNs, EPS15, PACSIN proteins and endophilins, as well as with clathrin and AP-2 (Haffner et al, 1997; Cestra et al, 1999; Maire et al, 2004; Schuske et al, 2003; Verstreken et al, 2003; Modregger et al, 2000; Perera et al, 2006; Milosevic et al, 2011; reviewed in Dittman and Ryan, 2009). SYNJ1 exists in two isoforms, a longer 170 kDa isoform and a shorter 145 kDa isoform, with slightly different roles. The recruitment and activity of SYNJ1-145 appears to largely coincide with that of dynamin at later stages of vesicle formation, while the SYNJ1-170 isoform also plays earlier roles in stabilizing the growing clathrin-coated vesicle (Perera et al, 2006; Taylor et al, 2011; Antonescu et al, 2011). SYNJ-mediated hydrolysis of PI(4,5)P2 to PI(4)P is most efficient on highly curved, endophilin-coated tubules of the vesicle neck and contributes to dynamin-mediated membrane scission (Chang-Ileto et al, 2011; reviewed in Daumke et al, 2014; McMahon and Boucrot, 2011).

In addition to SYNJ1 and 2, other inositol-5-phosphatases are also recruited to the CCP at the time of scission. These include OCRL, which is recruited through interaction with clathrin as well as the RAB5 interactors APPL1 (Erdmann et al, 2007; Mao et al, 2009; Taylor et al, 2011; Nandez et al, 2014).

Preceded by: BAR domain proteins recruit dynamin

Followed by: SYNJ hydrolyze PI(4,5)P2 to PI(4)P

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**SYNJ hydrolyze PI(4,5)P2 to PI(4)P**

**Location:** Clathrin-mediated endocytosis

**Stable identifier:** R-HSA-8868648

**Type:** transition

**Compartments:** plasma membrane

Inositol-5-phosphatases like SYNJs and OCRL hydrolyze PI(4,5)P2 to PI(4)P. In the context of CME, this promotes the abortive turnover (disassembly) of some CCPs, contributes to the dynamin-mediated scission of the clathrin-coated vesicle neck, and promotes clathrin uncoating following scission (Guan et al, 2010; Cremona et al, 1999; Mani et al, 2007; Chang-Ileto et al, 2011; Antonescu et al, 2011; reviewed in McMahon and Boucrot, 2011; Daumke et al, 2014).

**Preceded by:** Endophilins recruit synaptojanins to the clathrin-coated pit

**Followed by:** Dynamin-mediated GTP hydrolysis promotes vesicle scission

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Dynamin-mediated GTP hydrolysis promotes vesicle scission

**Location:** Clathrin-mediated endocytosis

**Stable identifier:** R-HSA-8868661

**Type:** transition

**Compartments:** plasma membrane, clathrin-coated endocytic vesicle membrane

Self-assembly of dynamin around the neck of the emerging clathrin-coated vesicle stimulates its GTPase activity. This in turn promotes a conformational change in dynamin organization that is required for membrane fission (Hinshaw and Schmid, 1995; Sweitzer and Hinshaw, 1998; Takei et al, 1999; Yoshida et al, 2004; Chappie et al, 2010; Chappie et al, 2011; Ford et al, 2011; Faelber et al, 2011; reviewed in Daumke et al, 2014).

**Preceded by:** SYNJ hydrolyze PI(4,5)P2 to PI(4)P

**Followed by:** Clathrin recruits auxilins to the clathrin-coated vesicle

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[https://reactome.org](https://reactome.org)
Clathrin recruits auxilins to the clathrin-coated vesicle

**Location:** Clathrin-mediated endocytosis

**Stable identifier:** R-HSA-8868659

**Type:** binding

**Compartments:** clathrin-coated endocytic vesicle membrane

After fission from the plasma membrane, auxilin proteins DNAJC6 and GAK are recruited to the vesicle through interaction with clathrin and phosphoinositides, in particular PI4P (Greener et al, 2000; Lee et al, 2006; Massol et al, 2006; Taylor et al, 2011; Scheele et al, 2001; Fotin et al, 2004a; Fotin et al, 2004b; Guan et al, 2010; reviewed in McMahon and Boucrot, 2011; Sousa and Lafer, 2015). Auxilin in turn recruits the ATPase HSPA8 (also known as HSC70), which uses the energy from ATP hydrolysis to remove the clathrin-coat from the vesicle, priming it for fusion with a subsequent endosomal compartment (Schlossman et al, 1984; Ungewickell et al, 1995; Rappoport et al, 2008; Xing et al, 2010; Bocking et al, 2011; Rothnie et al, 2011; reviewed in McMahon and Boucrot, 2011; Sousa and Lafer, 2015).

**Preceded by:** Dynamin-mediated GTP hydrolysis promotes vesicle scission

**Followed by:** Auxilin recruits HSPA8:ATP to the clathrin-coated vesicle

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Auxilin recruits HSPA8:ATP to the clathrin-coated vesicle

**Location:** Clathrin-mediated endocytosis

**Stable identifier:** R-HSA-8868660

**Type:** binding

**Compartments:** clathrin-coated endocytic vesicle membrane

HSPA8 (also known as HSC70) is recruited to the clathrin-coated vesicle through interaction with DNA J proteins GAK and DNAJC6 (Rapoport et al, 2008; Xing et al, 2010; reviewed in Sousa and Lafer, 2015). Recent studies examining the stoichiometry of uncoating predict between one and three HSPA8 molecules are required per clathrin triskelion for maximal uncoating in vitro (Bocking et al, 2011; Rothnie et al, 2011). After ATP hydrolysis, HSPA8 remains associated with the liberated clathrin, which prevents aberrant repolymerization and association of clathrin (Schlossman et al, 1984; reviewed in Sousa and Lafer, 2015).

**Preceded by:** Clathrin recruits auxilins to the clathrin-coated vesicle

**Followed by:** HSPA8-mediated ATP hydrolysis promotes vesicle uncoating

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HSPA8-mediated ATP hydrolysis promotes vesicle uncoating

**Location:** Clathrin-mediated endocytosis

**Stable identifier:** R-HSA-8868658

**Type:** transition

**Compartments:** clathrin-coated endocytic vesicle membrane

HSPA8 hydrolyzes ATP to promote dissociation of the clathrin coat from the vesicle (reviewed in Sousa and Lafer, 2015). Interaction of HSPA8 with the C-terminal tail of clathrin may sterically block re-stabilization of the clathrin coat, which is thought to undergo transient cycles of 'breathing', or loosening of the interactions between the triskelions (Barouch et al, 1997; Rapoport et al, 2008; Xing et al, 2010). Alternatively, HSPA8 may destabilize the clathrin coat through intermolecular collisions with the coat (reviewed in Sousa and Lafer, 2015). The HSPA8-clathrin interaction persists once clathrin has been removed from the vesicle. This is thought to preclude aberrant repolymerization of clathrin by sequestering free clathrin (Schlossman et al, 1984; reviewed in Sousa and Lafer, 2015).

**Preceded by:** Auxilin recruits HSPA8:ATP to the clathrin-coated vesicle

**Followed by:** RAB5 and GAPVD1 bind AP-2

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RAB5 and GAPVD1 bind AP-2

Location: Clathrin-mediated endocytosis

Stable identifier: R-HSA-8871194

Type: binding

Compartments: clathrin-coated endocytic vesicle membrane

RAB5 is a small GTPase that is implicated in clathrin-mediated endocytosis (Chavrier et al, 1990; McLauchlan et al, 1998; Shin et al, 2002; Taylor et al, 2011; reviewed in Stenmark, 2009; Wandiger-Ness and Zerial, 2014). Recent studies have shown that RAB5 and its associated GEF GAPVD1 may contribute to AP-2 uncoating by displacing AAK1 and promoting the net dephosphorylation of the AP-2 mu2 subunit. This is predicted to destabilize interactions with the plasma membrane and promote uncoating (Sato et al, 2005; Hunker et al, 2006; Smerdjieva et al, 2008). RAB5 and GAPVD1 also increase PI(4,5)P2 turnover, likely through recruitment of a class I PI3K or a PI phosphatase (Christoforidis et al, 1999; Shin et al, 2005).

Preceded by: HSPA8-mediated ATP hydrolysis promotes vesicle uncoating

Followed by: Dissociation of AAK1 and dephosphorylation of AP-2 mu2

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Dissociation of AAK1 and dephosphorylation of AP-2 mu2

Location: Clathrin-mediated endocytosis

Stable identifier: R-HSA-8871193

Type: omitted

Compartments: clathrin-coated endocytic vesicle membrane

GAPVD1 binds the alpha adaptin ear domain of AP-2 mu2, activating its RAB5-directed GEF activity and displacing AAK1. AAK1 displacement results in a net dephosphorylation of the AP-2 mu2 subunit, destabilizing the interaction of AP-2 with the vesicle membrane (Sato et al, 2005; Smerdjieva et al, 2008). In addition, RAB5 contributes to PI(4,5)P2 turnover through recruitment of a PI3K or PI phosphatase, and this also destabilizes the interaction of AP-2 with the membrane (Smerdjieva et al, 2008; Christoforidis et al, 1999; Shin et al, 2005).

Preceded by: RAB5 and GAPVD1 bind AP-2

Followed by: Dissociation of clathrin-associated proteins

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https://reactome.org
**Dissociation of clathrin-associated proteins**

**Location:** Clathrin-mediated endocytosis

**Stable identifier:** R-HSA-8869438

**Type:** omitted

**Compartments:** clathrin-coated endocytic vesicle membrane

After the removal of the clathrin coat, it is likely that many of the proteins that contributed to vesicle formation are lost, although the timing and mechanism of this step are poorly understood (reviewed in McMahon and Boucrot, 2011; Lemmon, 2001).

**Preceded by:** Dissociation of AAK1 and dephosphorylation of AP-2 mu2

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