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Clathrin-mediated endocytosis: lessons from yeast

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Abstract

Clathrin-mediated endocytosis (CME) is the major pathway for internalization of membrane proteins from the cell surface. A half-century of studies have uncovered tremendous insights into how a clathrin-coated vesicle is formed. More recently, the advent of live-cell imaging has provided a dynamic view of this process. Since CME is highly conserved from yeast to man, budding yeast provides an evolutionary template for this process, and has been a valuable system for dissecting the underlying molecular mechanisms. In this review we trace the formation of a clathrin-coated vesicle from initiation to uncoating, focusing on key findings from the yeast system.

Introduction

Since electron micrographs first identified compelling dense regions of the curved plasma membrane, there has been continued interest in how endocytosis occurs¹. Following their initial visualization, careful purification led to the identification of clathrin as the source of the polygonal lattices surrounding these endocytic pits^{2,3}. The biochemical characterization of neurons, enriched for these pits, resulted in the purification of key constituents and began our now vast knowledge of the factors involved in clathrin-mediated endocytosis (CME). Additionally, several *in vitro* systems helped to determine the biochemical properties of clathrin including its self-assembly association with adaptors⁴.

However, at this time, biology in mammalian systems was hamstrung by a lack of tools for examining endocytosis in a dynamic way in live cells. To escape those pitfalls and difficulties, studies of endocytosis turned towards the yeast model, *Saccharomyces cerevisiae*. Yeast provided a facile genetic system, which when combined with technological advances, greatly accelerated discovery. Yeast screens for *endF* and *dim* mutants⁶ began illuminating what factors are involved and were the first to identify a role for actin in endocytosis^{7,8}. Breakthroughs in live-cell fluorescence microscopy permitted real-time visualization of cellular function – a powerful tool when combined with the capabilities to delete or manipulate genes in yeast. This allowed wholesale testing of endocytic factor recruitment in live yeast, yielding a more detailed understanding of the cellular roles of these components^{9–12}. Recently, quantitative immunoelectron microscopy has provided more

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detailed spatial resolution and fresh insight into the organization of the endocytic machinery¹³. Buoyed by additional findings in mammalian systems and new *in vitro* assays to investigate aspects such as actin assembly and membrane tubulation, our understanding of CME has now dramatically changed from a static picture of clathrin coated pits to a well-orchestrated chain of events.

In yeast, a multitude of modular proteins (Figure 1), with conserved homologues in metazoans, have been categorized into distinct temporal phases based on their recruitment and function at the endocytic site (Figure 2). During the immobile phase, endocytic coat factors appear in cortical patches at the plasma membrane and cargo collection commences. Then, additional endocytic factors are recruited and positioned for the activation of actin assembly. In the slow mobile phase, actin assembly drives invagination; meanwhile, membrane-binding proteins sculpt and hold the membrane in order to prepare the site for vesicle scission. After vesicle release there is a fast mobile phase characterized by immediate disassembly of the endocytic coat and the nascent vesicle, driven by actin assembly, moves deep into the cell. This review presents an overview of the endocytic process in yeast, focusing on newer discoveries, and highlights major similarities and differences between yeast and man.

Establishment of clathrin-coated pits

Understanding the precise events necessary to select a membrane site for initiation of a clathrin-coated pit (CCP) has remained outside of our range of detection. Since many endocytic factors bind to phosphatidylinositol (4,5)-bisphosphate (PtdIns(4,5)P₂), lipid phosphorylation by inositol kinases plays a role in the formation and size of CCPs^{14,15}. In yeast the earliest proteins seen at the incipient CCP are clathrin, the EH domain protein Ede1 (Eps15 homologue), and Syp1, the F-BAR/ μ 2 homology counterpart to FCHo1/2^{10,11,16–19}. After 1–2 minutes these factors are joined by the Hip1/R homologue, Sla2^{9,20}. Rapidly after Sla2, the final major constituents of the endocytic coat are recruited: Sla1, a SH3 domain protein; and End3 and Pan1, two EH domain factors^{9,10}. The clathrin adaptors Yap1801/2 (AP180/CALM) and Ent1/2 (epsins) arrive around the time of these middle/late coat factors (R.C. unpublished observation; reference²¹).

The roles of the three earliest arriving endocytic factors are still being dissected. Clathrin heavy chain (HC) or light chain (LC) mutants (*chc1* or *clc1*) exhibit reduced ability to uptake α -factor by the Ste2 receptor^{22,23}. Live-cell imaging has shown that clathrin mutants exhibit delays in cortical patch maturation as measured by increases in the cortical lifetime of Sla2, reduced numbers of Sla1-positive cortical patches, and abnormal actin structures, often seen as deep invaginations with Sla2 at their tip^{10,24}. Deletion of the Eps15 homologue, Ede1, also results in endocytic phenotypes similar to clathrin nulls^{10,25}. However, the early arriving Syp1 is dispensable for endocytic patch formation in yeast^{10,16–18}. In contrast, in mammalian cells, the Syp1-like FCHo1/2, as well as Eps15 and their interacting partner intersectin are reported to be essential for endocytic site initiation²⁶.

How clathrin is recruited to the cortex is an ongoing question. The HC terminal domain (TD) forms a β -propeller, which provides a platform for association with adaptors that

mediate clathrin recruitment. A major site on the TD interacts with adaptors containing short linear peptides with the consensus sequence L ϕ p ϕ p, also referred to as “clathrin box motifs”, where ϕ is hydrophobic and p represents a polar residue²⁷. Initial yeast studies suggested that clathrin assembly at cortical patches is dependent on the adaptors Ent1/2 and Yap1801/2¹¹, which contain C-terminal clathrin box motifs. However, AP180 proteins and epsins arrive at the cortex after clathrin (R.C. unpublished observation; reference²¹), likely discounting their role in clathrin recruitment. Also, mutation of the clathrin L ϕ p ϕ p binding site in the yeast HC-TD does not prevent cortical association of clathrin²⁸. Moreover, endocytosis is nearly normal in this TD mutant²⁸. Another clathrin box motif binding site on the TD was predicted in this study, which could explain the limited phenotypic consequences of the TD mutation. Consistent with this, studies in mammals have now identified as many as four distinct binding sites involved in clathrin-adaptor interaction in the clathrin TD and ablation of all four sites is required to significantly affect CME^{29,30}.

Identification of specific cargo sorting adaptors in yeast is ongoing. The only known cargo for AP-2 is the K28 killer toxin, but its receptor remains unknown³¹. Yeast AP-2 is not essential in yeast, so it does not play a central role in CME like its animal counterpart^{32,33}. Yap1801/2 are cargo-selective adaptors for recycling of the v-SNARE Snc1³⁴. Deletion of both AP180 homologues causes no general endocytic phenotype³², but these factors are partially redundant with yeast epsins³⁵. Syp1 is also likely a cargo adaptor, since overexpression improves internalization of the cell wall stress sensor, Mid2, and this is dependent on its C-terminal AP-2 μ 2 homology region¹⁷. Sla1 interacts directly with NPFxD sorting motifs (e.g. in Ste2, Wsc1) through its SHD1 domain^{36,37}. Sla1 also binds clathrin via a variant clathrin box motif (LLDLQ), an interaction that is subject to auto-regulation mediated through Sla1's SHD2 region³⁸. However, like the epsins and AP180 adaptors, Sla1 appears with later coat factors⁹, trailing clathrin recruitment. Thus clathrin at the patch seems to serve as a “basket” to capture adaptors and their cargo for internalization, and in turn adaptor association with clathrin stabilizes the endocytic site. Consistent with this, fluorescent α -factor bound by its receptor Ste2 is collected into pre-existing early endocytic patches¹⁹.

It remains unknown what adaptors collect ubiquitinated, endocytic cargo, such as aSte2 and many down-regulated permeases. The epsins (through ubiquitin interacting motifs (UIM)) and Ede1 (via its ubiquitin association domain (UBA)) were thought to be redundant adaptors for ubiquitinated cargo. This idea came from work demonstrating that removal of the epsin UIMs had little effect on internalization of Ste2, but in combination with *ede1* the defect became severe³⁹. However, recent studies showed that internalization of both ubiquitinated cargo and cargo depending on short peptide sorting motifs (eg. NPFxD) were equally defective in these cells, so these effects were not specific to ubiquitin modified cargos⁴⁰. Furthermore, ubiquitinated receptors were internalized efficiently even when the epsin UIMs and Ede1's UBA region were deleted. The epsin UIMs and Ede1 UBAs may still have cargo-binding functions, but these must be redundant with other ubiquitin-binding endocytic factors, such as SH3 domain proteins like Sla1, whose third SH3 binds ubiquitin⁴¹.

Controlling actin assembly

Yeast endocytosis requires the assembly of a dense branched actin network to promote plasma membrane invagination. This involves multiple layers of regulation that create a small timeframe of actin assembly. Branched actin filaments are formed through Arp2/3 complex, which appears only during the actin phase of endocytosis^{9,42}. Arp2/3 complex activity relies on activation by nucleation promoting factors (NPFs). There are five known endocytic NPFs (Pan1, Las17, Myo3, Myo5 and Abp1) that increase Arp2/3 complex potency by delivering actin monomers to the actin assembly site⁴³. Furthermore, additional endocytic factors, including Syp1, Sla2, Sla1, Vrp1, Bzz1 and Bbc1, control the ability of NPFs to activate the Arp2/3 complex (Figure 3).

The earliest arriving NPFs are Pan1 and the WASp homologue Las17, which together are proposed to play a role in priming actin assembly at the cortical patch. While ablation of the NPF activity of Pan1 (*pan1*-*WA*) or Las17 (*las17*-*WCA*) alone causes little phenotype, combining the two leads to dramatic endocytic progression defects⁴⁴. Both Pan1 and Las17 arrive 20–25 seconds prior to detectable levels of actin at the cortical patch, so both NPFs must be initially maintained in an inhibited state^{9,45}. Evidence suggests that Sla2 negatively regulates Pan1-NPF activity⁴⁶. However, both Sla2 and Pan1 are together in the coat and how their interaction is regulated is not known.

Syp1 and Sla1 are known negative regulators of Las17 and likely help thwart premature actin assembly prior to full endocytic coat maturation. Syp1 binds Las17 through its unstructured central domain to attenuate Las17 stimulation of Arp2/3 complex¹⁶. One hypothesis is that this inhibition is lifted as the Syp1 F-BAR domain senses changes in membrane curvature resulting in Syp1 departure from the patch just prior to the major burst of actin assembly¹⁶. The first two SH3 domains of Sla1 negatively regulate Las17 NPF activity⁴⁷. Unlike Syp1, Sla1 does not leave the endocytic site; instead it remains in close proximity to Las17 until actin assembly deepens the invagination¹³. Subsequently, Sla1 follows the clathrin coat to the tip of the invagination, whereas Las17 remains near the neck where constriction takes place¹³. This spatial separation may facilitate release of Sla1's hold on Las17. Just before the rapid actin assembly phase, another F-BAR domain protein, Bzz1 (related to syndapin) is recruited to the CCP along with the WIP homologue Vrp1⁴⁴. Bzz1 SH3 domains may interact with Las17, which could lift the negative regulation of Sla1 through competitive binding^{44,48}. Another possibility is that the prolines of Vrp1 act as a sink for the Sla1 SH3 domains to recruit it from Las17 and lift Sla1-mediated inhibition.

The type-1 myosins (Myo3/Myo5) are seen at cortical patches only during the actin assembly phase^{42,44}. Prior to that, they are bound by calmodulin in the cytosol in a conformational state that prevents their assembly and activity⁴⁹. Vrp1 is crucial for cortical recruitment and activation of Myo3/Myo5, which together with Las17 are the most potent Arp2/3 complex activators during internalization^{42,44,50}. Although defects in Pan1 and Las17 NPF activities cause elongated endocytic lifetimes, internalization is ultimately completed. In contrast, *vrp1* or *myo3 myo5* yeast are defective for inward movement, indicating myosin activity provides the push needed to drive invagination⁴⁴.

Immuno-electron microscopy has shown that as the membrane is deformed there is a spatial split between the major NPFs¹³. The type-1 myosins stay at the cortex while Las17 moves inward to the middle of the elongating endocytic tubule, and Pan1 is found at the tip of the invagination. During the actin phase another NPF inhibitor appears, the SH3 domain protein Bbc1, which inhibits both Las17 and myosin1 *in vitro*^{44, 47}. However, Bbc1 localizes near the neck of the invagination, suggesting that it chiefly restrains Las17 activity¹³. Overall, the locations of the Arp2/3 complex activators and negative regulators are also critical for productive invagination and scission.

The final NPF, Abp1, is a weak activator of the Arp2/3 complex, but curiously it also inhibits NPF activities of Las17 and the myosins *in vitro*⁴⁴. Furthermore, *abp1* does not cause major endocytic defects. Instead Abp1 seems to be more important for recruitment of coat disassembly factors following vesicle scission¹⁰.

In mammalian cells, the Arp2/3 complex, WASp and other actin regulators (*e.g.* WIP, syndapin, and cortactin) have been visualized at the endocytic site^{51–53}. N-WASp is autoinhibited, but syndapin binding (via its SH3 domain) relieves this inhibition and promotes actin assembly for endocytosis^{54–56}. Another BAR-domain protein, PICK1, is a negative regulator of Arp2/3 activity. Loss of PICK1 leads to actin aggregation and reduction in AMPA receptor internalization⁵⁷. However, relatively few studies dissecting the roles of Arp2/3 complex regulators in endocytosis have been reported in animal cells. This may be due to general morphological changes that result from perturbation of the actin cytoskeleton.

Translating actin assembly into membrane invagination

As substantial actin is assembled by Arp2/3 complex in yeast, the membrane is deformed creating an extended tubule with a clathrin coat at its tip¹³. New discoveries have begun to explain how actin assembly is leveraged into membrane curvature. The talin-like proteins, including Sla2 in yeast and Hip1/Hip1R in mammals, play a key role in translating actin assembly into membrane tension due their binding to the endocytic coat, the actin cytoskeleton and the membrane^{58–61}. This family of proteins is composed of an AP180 N-Terminal Homology domain (ANTH), a central coiled-coil region, and a Talin Hip1/R/Sla2 Actin-Tethering C-Terminal Homology domain (THATCH). Sla2 is initially recruited to a forming cortical patch through ANTH binding to PtdIns(4,5)P₂^{62, 63}. The Sla2 coiled-coil mediates dimerization and direct interaction of Sla2 with clathrin LC, Sla1, and Pan1,^{20,46,64} while the C-terminal THATCH domain mediates interaction with F-actin^{65–67}. In *sla2* yeast, early endocytic factors including clathrin are recruited, but these patches do not invaginate and instead waving F-actin assemblies emanate from the cortex, consistent with a membrane-actin anchoring role for Sla2^{9,11}. Similar F-actin structures are observed in mammalian cells depleted of HIP1R⁶¹.

When Hip1R is bound by the N-terminus of clathrin light chain (LC), this induces a conformational change in Hip1R to a closed state^{67,68}, which decreases Hip1R affinity for F-actin⁶⁷. This conformational change is consistent with yeast studies showing a self-interaction between the Sla2 THATCH and coiled-coil regions⁶⁰. Yeast LC also regulates Sla2, since overexpression of the Sla2-binding region of LC can suppress endocytic defects

of *chc1* yeast²⁴. Newer yeast studies support the conformational regulation model. A mutant LC lacking its Sla2-interacting residues can suppress endocytic defects caused by impaired Arp2/3 complex activation (*las17*, *vrp1*, or *myo3 myo5*)⁶⁹. Furthermore, this suppression is dependent upon the Sla2 actin binding region⁶⁹. Therefore preventing clathrin LC-mediated release of Sla2-actin attachments can bypass reduced actin assembly by increasing and prolonging anchoring to the membrane.

The confluence of Hip1R and Sla2 studies has led to a new model for the translation of actin assembly into membrane invagination (Figure 4)^{67,69}. As actin is assembled at the endocytic patch, it is bound by Sla2 at the periphery of the clathrin-coated pit. As the pit matures, clathrin LC binds Sla2 causing it to undergo a conformational change releasing actin. In this manner membrane invagination continues unidirectionally and is timed with clathrin coat formation. It is noteworthy that the THATCH domain of Sla2 is not required for general endocytosis^{59,60,70}; thus other unidentified membrane-actin tethering factors must exist to provide additional anchoring.

Actin-generated tension facilitates membrane tubule scission

Once the coat module factors move inward, the resultant elongated membrane tubules are targeted by the membrane scission apparatus, including the N-BAR-domain-containing amphiphysins (Rvs161/Rvs167) and the dynamin homologue Vps1. The amphiphysins form heterodimers that assemble at the narrowing neck of the tubular invagination just above the clathrin coat^{10,13}. Amphiphysin-null mutations lead to wildly inconsistent patch behaviors including tubules that retract to the cortex^{9,69,71,72}. In mammals the GTPase dynamin is required for scission to complete internalization⁷³. Earlier reports suggested that dynamins are not involved in yeast scission^{10,74}. However, recent studies have resurrected Vps1 as the yeast endocytic dynamin. Although it is not required for endocytosis, Vps1 is seen at endocytic patches and *vps1* causes a retraction phenotype, albeit one that is much weaker than amphiphysin nulls^{71,75}.

Yeast studies have led to a model of how scission may occur^{76,77}. In this model, membrane binding by the amphiphysins along with other PtdIns(4,5)P₂ binding proteins (e.g. Sla2, epsins, and Yap1801/2) functions to filter the charged lipids to a concentrated region of the membrane bilayer, as well as protect them from dephosphorylation by the synaptojanins (Inp51/Sjl1 and Inp52/Sjl2). Meanwhile, PtdIns(4,5)P₂ outside of this region continues to be dephosphorylated causing a charge differential that produces a lipid phase boundary. When the boundary force is combined with tension supplied by actin assembly and myosin motors, scission becomes energetically favorable⁷⁶.

The retraction phenotypes of amphiphysin-null mutants are consistent with the lipid boundary model, as are the stalled elongated invaginations present in *inp51 inp52* or *inp51 inp52-ts inp53 (inp-ts)* mutants^{78, 79}. In these mutants, a lipid boundary may never form. However, it remains counterintuitive that *SLA2* over-expression suppresses the *inp-ts* endocytic defects, since increased Sla2 should protect more PtdIns(4,5)P₂ and further retard lipid boundary formation⁷⁸. Likely, *SLA2* suppression is instead due to additional force caused by increasing actin attachments. Consistent with this, disruption of the Sla2 binding

region of LC, also suppressed the growth and endocytic defects of *rvs161 / rvs167*⁶⁹. The result was an increase in successful internalization events, even after cortical retraction, and suggests that increases in membrane tension can overcome deficits in phase separation. During deep invagination of the membrane, it is likely that the amphiphysins function as the equivalent of surgical retractors, holding the membrane tubule shape as the actin cytoskeleton is extended by Arp2/3 complex-mediated actin assembly creating more tension on the peripheral Sla2. In *S. pombe* two F-BAR domain proteins, Cdc15 and Bzz1, seem to be critical for scission during endocytosis⁸⁰. Interestingly Cdc15 is located near the cortex where it also activates myosin-1, while Bzz1 is located above the coat activating the WASp (Wsp1). Thus these actually coordinate constriction of the tubule neck with actin assembly at two zones. Bzz1 also seems to play a role to coordinate scission in budding yeast as well⁸¹. Similar BAR domain protein-actin relationships during endocytosis are also being uncovered in animal cells^{82–84}.

Disassembly of the endocytic coats and recycling of factors

Following scission, coat disassembly is initiated by the synaptojanins and Ark1/Prk1 kinases (Figure 2)^{21,62,85}. Arf3, its GAP Gts1, and Lsb5 (a GGA homologue) are also suspected to be involved in coat disassembly, but their roles are still not well defined²¹. Along with a role in vesicle scission, synaptojanin dephosphorylation of PtdIns(4,5)P₂ promotes uncoating of epsins, AP180s and Sla2 by releasing their PtdIns(4,5)P₂-binding domains²¹. After actin assembly peaks, Inp52 is recruited by Abp1's SH3 domain, consistent with a disassembly role late in endocytosis^{21,62}.

Arp2/3 complex activity and/or binding to the SH3 domain of Abp1 also mediates late recruitment the Ark1 and Prk1 kinases (related to AAK1 and GAK1 in animal cells)^{21,86–88}. These kinases phosphorylate a plethora of endocytic coat factors including Pan1, Sla1, Ent1/2, Yap1801/2, and Scd5 at Lxx(Q/T)xTG motifs^{87,89–94}, and interaction of some of these factors is diminished by this phosphorylation^{87,92}. Furthermore, *ark1 prk1* mutants have endocytic defects and accumulate large cytoplasmic aggregates of endocytic factors, F-actin and membrane, also supporting a disassembly role^{85,95–97}. Mutation of all Prk1 phosphorylation sites on Pan1 to alanine leads to similar phenotypes⁹⁷. Additionally, Prk1 inhibits Pan1 binding to F-actin and its Arp2/3 complex activation activity⁹⁷. Thus Prk1 phosphorylation may negatively regulate actin assembly by turning off Pan1 at the end of internalization.

Ark1/Prk1 modifications must be reversed by dephosphorylation to reactivate coat proteins for new rounds of internalization. In yeast this is achieved by Scd5 targeting of protein phosphatase 1 (PP1) to Ark1/Prk1-phosphorylated endocytic factors^{87,98,99}. Scd5 is recruited to the cortex during the late immobile phase of internalization^{87,100}, although cortical recruitment is not required for its endocytic function⁹⁶. Consistent with a role in recycling endocytic factors, *scd5* mutants are suppressed by deletion of *PRK1*^{87,91}, and mutation of the PP1 binding site in Scd5 impairs endocytosis^{89,98}. Scd5 itself is a target of Prk1, so its phosphorylation after vesicle scission may be another means to accelerate uncoating^{87,89,91}.

Although Ark1/Prk1 mediate disassembly of endocytic coats in yeast, the major mammalian homologue, AAK1, instead promotes endocytosis by phosphorylating the μ -subunit of AP-2 increasing the adaptor's affinity for membranes and cargo sorting motifs^{101–103}. The related kinase, GAK, also phosphorylates AP μ -chains, but GAK more likely plays a role in uncoating of clathrin coats during endocytosis via its auxilin domain^{104–107}. Yeast have an auxilin too, but it is not required for endocytosis²¹. The paradigm most similar to yeast phosphoregulation of endocytosis is found at the nerve synapse, where in resting neurons several endocytic factors are cytosolic and inactive due to phosphorylation by inhibitory kinases such as Cdk5^{108–111}. Synaptic transmission leads to calcium influx and activation of calcineurin, which dephosphorylates and reactivates the endocytic factors for synaptic vesicle recycling.

Finally, the actin network is quickly broken down by actin binding and severing molecules including Cof1 (ADF/cofilin), Aip1, Crn1 (coronin) and Srv2. Cof1, Aip1 and Crn1, like Ark1/Prk1 and Sjl2, are recruited with a delay after actin peaks, so their arrival is timed with disassembly of actin^{112,113}. ADP-actin is preferentially bound by Cof1, and many Cof1 molecules can bind cooperatively to the sides of the actin cable to produce biochemical twisting leading to actin severing between cofilin attachment sites^{114–116}. Aip1 interacts with the Cof1-ADP-actin complex and caps the newly exposed barbed end, thus preventing elongation^{117,118}. Srv2 binds to Cof1 to displace the ADP-actin monomers from Cof1^{117,119–121}, while Crn1 enhances the severing by Cof1 on older/ADP actin filaments¹²². The cooperation of these factors allows for efficient and rapid disassembly of the actin network, which may also help to propel the vesicle into the cell.

Conclusions and new frontiers

CME in yeast and higher eukaryotes requires nearly all the same factors and proceeds with similar timing^{53,123}. While actin's role in CME in animal cells had been controversial, overwhelming evidence has now validated its importance^{82,83,124–128}. Recent electron micrographs have allowed clear visualization of actin filaments, including their orientation, at the CCP¹²⁵. While yeast endocytosis is uniformly dependent on actin, in mammalian cells, actin becomes more important during specialized events including uptake of viral particles or endocytosis at focal adhesions or other regions of membrane tension^{124,126–129}. These events likely require the force of actin assembly to overcome resistance because of particle size or membrane attachment. These “specialized” internalizations may be more comparable to yeast CME, given that the yeast plasma membrane is constantly in contact with the cell wall and under high turgor pressure¹³⁰. In fact, actin is less essential for yeast endocytosis following hypertonic treatment of cells¹³¹. Additionally, cell shrinkage may also break connections between the plasma membrane and cell wall, thereby reducing the necessity for actin assembly-induced tension.

Emerging technologies have uncovered additional similarities between yeast and animal CME. A new cell free system utilizing GTP γ S to block membrane fission combined with electron microscopy and high-resolution fluorescence microscopy enabled determination of the three-dimensional localization of endocytic factors on arrested invaginations⁸³. These resultant long membrane tubules are reminiscent of yeast endocytic invaginations described

by Idrissi *et al*¹³. Live-cell imaging of endocytosis in animal cells has also seen major advances over the last 10 years. One recent study measured the cortical recruitment signatures of 34 mammalian endocytic factors in relation to scission, demonstrating conserved endocytic behaviors with analogous yeast components⁵³. Now, a new targeting technique may enable the tagging of single copies of mammalian genes at their endogenous loci for study of CME, much like what has been possible in yeast¹²³. Finally, new studies uncovered a non-clathrin-mediated, Arp2/3 complex-independent internalization pathway in yeast that is dependent on formin and Rho1, similar to the RhoA pathway in animal cells¹³². This opens the door to using molecular genetic approaches in yeast to study another conserved endocytic pathway.

Our expanding knowledge of clathrin-mediated endocytosis is validating that the yeast model is an evolutionary template for this process. Attention is now being focused on endocytic specialization in the mammalian system to understand the vast diversity of endocytic patches and clathrin structures. Yet yeast remains an ideal tool for uncovering the detailed molecular mechanisms of this essential process, because these fundamentals are transferable to all living organisms.

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Figure 1. Yeast endocytic factors are comprised of many conserved modular domains. Shown are the major endocytic factors with domain structures. Each factor is listed in the order of its recruitment during endocytosis. A red * indicates the EH ligand NPF motifs and ‡ indicates the acidic motif needed for actin nucleation promoting activity.

Figure 2.

Endocytic pathway in yeast. Early factors (Clathrin, Syp1, Ede1) are recruited during the immobile phase, which is followed by the ordered assembly of the mid/late coat (Sla2, Ap1801/, Ent1/2, Pan1, Sla1). Las17 is also recruited around this time. Shortly before the mobile phase Syp1 and Ede1 depart from the cortex and this is rapidly followed by the WASp/myosin/actin slow mobile invagination phase (Actin, Abp1, Arp2/3, Myo3/5, and Vrp1). Once the extended tubule forms, the vesicle scission apparatus (Rvs161/167 and Vps1) narrows the neck of the vesicle forming at the invagination tip to promote scission. Upon release, the nascent vesicle is immediately uncoated via synaptojanin and Prk1/Ark1 and then moves rapidly inward, shedding its actin shell via action of Cof1, Aip1, Srv2, and Crn1. Mid/late coat factors are reactivated via Scd5/PP1(Glc7) dephosphorylation and recruited back to the membrane for new rounds of CME.

Figure 3.

Arp2/3 complex-mediated actin assembly is controlled temporally and spatially by many regulatory mechanisms during endocytosis. In this figure, NPFs are indicated in green, positive regulators of NPFs in blue and negative regulators in red. The colors of the negative regulators are changed to pink and then white to indicate their inhibitory activity is partial or relieved. F-actin is shown as a gray cloud surrounding the endocytic site. Initially actin assembly is blocked by Syp1, Sla1 and Sla2, which prevent early activation of Las17 and Pan1 (left). Also myosin 1 is inhibited by calmodulin in the cytosol. Next, the activator Bzz1 arrives and Vrp1 is recruited by Las17 (middle). This may allow early F-actin seeding and the beginning of membrane invagination, as inhibitors start to lose their influence (pink). Release of Syp1 from the cortex and recruitment of the myosins (by Vrp1) and Arp2/3 complex lead to robust actin assembly and invagination (right). Another negative regulator, Bbc1, arrives in the late phase, preventing excessive endocytic actin accumulation. It is not known when the Pan1 inhibitory function of Sla2 is relieved since they move together into the coat. Sla2 is shown here as being increasingly less influential as the endocytic site invaginates.

Figure 4.

Translating actin assembly into membrane invagination is regulated by clathrin light chain. Sla2 is recruited to the cortex prior to actin assembly (I). As F-actin is assembled, Sla2 at the perimeter of the clathrin coat binds to the filaments through its C-terminal THATCH domain (II). This binding is used to tether the membrane to actin for invagination (III). As the clathrin coat continues to form at the tip, Sla2 is bound by clathrin LC causing a conformational shift (to closed) that releases Sla2's hold on F-actin (IV). This may direct the force of actin assembly towards formation of a tubule, as well as promote vesicle scission at the neck.