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COMMENTARY

Endocytic internalization in yeast and animal cells: similar and different

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SUMMARY

The internalization step of endocytosis has been the focus of several laboratories during the last forty years. Unlike some other budding events in the cell, many fundamental questions regarding the molecular machinery involved in the mechanism of budding itself still remain unsolved. Over the last few years the general picture of the field has quickly evolved from the originally simplistic view which postulated that clathrin polymerization is the major force driving budding at the plasma membrane. Refinement of the assays and molecular markers to measure endocytosis in animal cells has shown that other factors in addition to the clathrin coat are required and that endocytosis can also take place through clathrin-independent mechanisms. At the same time, recent introduction of genetic approaches to study endocytosis has accelerated the identification of molecules required for this process. The isolation of endocytosis mutants in budding yeast has been especially fruitful in this respect. Preliminary comparison of the results obtained in yeast and animal cells did not seem to coincide, but further progress in both systems now suggests that part of the divergence originally seen may be due to the particular experimental approaches used rather than fundamental differences in endocytic mechanisms. In this review we present a short historical overview on the advances made in yeast and animal cells regarding the study of endocytosis, underlining both emerging similarities and still interesting differences.

Key words: Endocytosis, Uptake, Yeast, Animal cell

INTRODUCTION

The endocytic pathway defines membrane traffic from the plasma membrane to the degradative compartment: the lysosome in animal cells and the vacuole in fungi and plants. All eukaryotic cells studied thus far are able to internalize extracellular components as well as components of their plasma membrane. A portion of the lipid bilayer engulfs the surrounding extracellular macromolecules and sequesters them through formation of an endocytic vesicle that detaches from the plasma membrane. Some of the internalized substances are then delivered to the lysosome/vacuole after passage through two endocytic compartments termed early and late endosomes.

The original description of this route relied mainly on extensive morphological studies of animal cells. Later development of assays to quantitatively measure endocytic traffic (in vivo and in vitro) allowed more fundamental questions about the molecular mechanism to be addressed. Only recently, genetic approaches have been applied to its study using *Drosophila melanogaster, Dictyostelium discoideum* and *Saccharomyces cerevisiae.* Isolation and characterization of *Saccharomyces cerevesiae* mutants blocked in the endocytic pathway has been especially productive during the last few years regarding the identification of molecules involved in the internalization step at the plasma membrane.

An earlier, cursory examination of the requirements for endocytosis in yeast and animal cells did not point to a common endocytic mechanism. Therefore, use of yeast as a model to study higher eukaryotic endocytosis was brought into doubt (Trowbridge et al., 1993). However, recent advances in the understanding of both systems reveals much stronger connections than originally thought. The fundamental differences between the approaches used in the different systems might be of great importance for further development of the field, as it has been for study of the secretory pathway.

The general organization of endocytic traffic inside yeast cells resembles that of mammalian cells. Both early and late endosomes have been morphologically and biochemically identified (Singer-Krüger et al., 1993; Hicke et al., 1997; Prescianotto-Baschong and Riezman, 1998), and the molecular machinery required for transport to proceed does not seem to differ fundamentally (Riezman et al., 1996). However, lack of exhaustive ultrastructural studies and quantitative assays that measure post-internalization steps in yeast make comparison between the two systems difficult. In contrast, the internalization step in yeast can be monitored quantitatively by measuring uptake of radiolabeled α-factor pheromone after binding to its receptor, Ste2p (Dulic et al., 1991), facilitating the identification and characterization of several genetic requirements of this event.

The aim of this review is to depict briefly what is known about the internalization step of endocytosis in mammalian and yeast cells following their historically different approaches, to stress common features, and to discuss possible differences in light of recent results.

INTERNALIZATION IN ANIMAL CELLS

Several morphologically and mechanistically different types of endocytic internalization occur in mammalian cells: phagocytosis is responsible for the uptake of solid particles, pinocytosis for the endocytosis of extracellular fluid, macromolecules and solutes. Pinocytosis can further be subdivided into macropinocytosis, which is associated with the formation of relatively large vacuoles that are heterogeneous in size and shape (0.5-200 μm), and micropinocytosis which occurs through the generation of small vesicles (50 to 150 nm). Phagocytosis and macropinocytosis are triggered in specialized cells in response to specific stimuli and they will not therefore be the main subject of this review. Pinocytosis of bulk solutes is generally referred to as fluid-phase endocytosis. In contrast, receptor-mediated endocytosis defines the adsorptive uptake of specific macromolecules bound to plasma membrane receptors.

The first insight into the mechanism of endocytosis came from electron microscopy studies. Electron-dense coated invaginations were discovered at the plasma membrane in insect oocytes that accumulated yolk proteins and appeared to give rise to endocytic coated vesicles (CV) (Roth and Porter, 1964). CVs were isolated from brain tissue and shown to be coated with a polygonal lattice whose main component is clathrin (Pearse, 1975; Crowther et al., 1976). Later, coated pits and vesicles of mammalian cells were shown to be enriched in membrane receptors carrying endocytosis signals (Anderson et al., 1977a,b).

The clathrin coat has been the subject of extensive biochemical characterization. A triskelion of three clathrin heavy chains tightly associated with three clathrin light chains constitutes the assembly unit of the polygonal lattice. A heterotetramer (AP-2) consisting of two large (α and β) and two small (σ2 and μ2) subunits mediates assembly of clathrin cages on the plasma membrane (Pearse and Robinson, 1990; Schmid, 1992). The β subunit interacts with clathrin (Ahle and Ungewickell, 1989), whereas the μ2 subunit is capable of binding the tyrosine-based endocytic signals that mediate clathrin-dependent internalization of a number of membrane proteins (Ohno et al., 1995; Marks et al., 1997; Robinson, 1997).

The fact that purified clathrin spontaneously forms coat-like structures that resemble those found at the plasma membrane suggests that clathrin oligomerization may be the major driving force generating endocytic vesicles. Inhibition of transferrin (Tfn) and liposome uptake by microinjection of antibodies against clathrin or α adaptin in living cells further supported their direct involvement in endocytosis (Chin et al., 1989). However, development of assays that differentially measured coat assembly, invagination and vesicle fission in vivo and in vitro (Schmid, 1992) demonstrated that additional energy requirements exist and most likely other proteins are involved. Growth and invagination of planar coats already assembled at the plasma membrane can take place in the absence of ATP. However, ATP depletion blocks the fission event, causing accumulation of constricted coated pits connected to the cell surface through thin necks (Smythe et al., 1989; Schmid and Carter, 1990; Schmid and Smythe, 1991). On the other hand, growth and invagination of clathrin lattices is inhibited in the presence of GDPβS whereas the fission event is blocked by GTPγS indicating that invagination requires GTP binding whereas fission requires hydrolysis (Carter et al., 1993).

These results suggested that at least one GTPase might be required. Shortly afterwards, the involvement of the GTPase, dynamin, was demonstrated. Dynamin was identified as the product of the *Shibire* gene of *Drosophila* (van der Bliek and Meyerowitz, 1991; Chen et al., 1991). Flies bearing a temperature sensitive (ts) mutation in this gene are paralyzed after short incubation at the restrictive temperature (Grigliatti et al., 1973), their synapses are depleted of vesicles and accumulate constricted pits (Kosaka and Ikeda, 1983). In cultured cells, overexpression of a mutant dynamin, that is unable to bind GTP, inhibits Tfn and epidermal growth factor (EGF) internalization blocking coated vesicle budding in an intermediate stage before constriction of the neck (van der Bliek et al., 1993; Damke et al., 1994). Wild-type dynamin is localized homogeneously on planar and shallowly invaginated clathrin coated pits, but it concentrates at the neck of deeply invaginated constricted pits. In contrast, mutant dynamin fails to relocate (Damke et al., 1994; Warnock and Schmid, 1996). Additionally, nerve terminals incubated with GTPγS accumulate long tubular invaginations bearing transverse electron dense rings that can be decorated with dynamin antibodies and are often capped with coated pits (Takei et al., 1995).

Purified dynamin can self assemble into rings and stacks of interconnected rings under certain conditions (Hinshaw and Schmid, 1995). Additionally, the high GTP affinity and hydrolysis rate of dynamin make this molecule much more similar to the mechanochemical enzymes than to the classical GTPases that work as molecular switches of different cellular process (Warnock and Schmid, 1996). This growing body of data has been used to suggest that after GDP/GTP exchange, dynamin might constrict the invaginated coated pits while polymerizing at their neck. GTP hydrolysis might finally trigger a conformational switch that could promote vesicle fission (Warnock and Schmid, 1996). Whereas there is evidence for the involvement of dynamin in coated pit constriction, no direct experiments address its role in the fission event itself. GTP promotes depolymerization of the in vitro oligomerized dynamin rings. It is therefore possible that GTPγS inhibits release of dynamin from the constricted neck preventing the binding of other proteins that are required for the fission event. The additional ATP requirement in this late event may suggest the involvement of other key players.

Even though the involvement of clathrin in micropinocytosis is convincing, growing evidence indicates that both fluid-phase and receptor-mediated endocytosis can take place under conditions that prevent clathrin polymerization (Lamaze and Schmid, 1995). In fact, the discovery of non-coated pits and vesicles preceded the discovery of CVs by about one decade (Palade, 1953). Still, however, very little is known about the cargo and the molecular machinery involved in clathrin-independent pinocytic pathways. The best characterized of these alternative endocytic routes is internalization through caveolae. Caveolae are flask-shaped invaginations of 50-80 nm in diameter and are highly abundant in epithelial cells that have

been proposed to participate in transcytosis (Schnitzer et al., 1994). Planar freeze-fracture views reveal a spiral-shaped coat surrounding caveolae which is very different from clathrin lattices (Rothberg et al., 1992). Caveolae can be identified by the presence of a 21 kDa integral membrane protein called caveolin (Rothberg et al., 1992) and are enriched in sphingolipids and cholesterol (Parton, 1996). Whether endocytic uptake really occurs through caveolae has been a matter of debate for a long time. However, in vivo data showing caveolae-dependent internalization of cholera toxin B (Tran et al., 1987), GPI-anchored horseradish peroxidase (Parton et al., 1994) or simian virus 40 (SV40) (Stang et al., 1997), as well as recent development of an in vitro assay to measure caveolae budding (Schnitzer et al., 1996) leave little doubt about their capacity to detach from the cell surface. It is not clear how different the molecular machinery implicated in caveolae-dependent, clathrin-independent pinocytosis is from that described above. Caveolae budding in vitro also requires cytosol and ATP, and budding in vitro or in vivo is inhibited by GTPγS (Schnitzer et al., 1996). Recent data suggest that dynamin might also be required for caveolae fission (Oh and Schnitzer, 1996). Furthermore, it is worth noting that overexpression of caveolin in lymphoblastoid cells that do not normally express it causes accumulation of these structures (Fra et al., 1995), indicating that any additional molecular machinery involved in their formation and possibly their budding is already present in these cells.

INTERNALIZATION IN YEAST

The study of yeast endocytosis is relatively young compared with studies in mammalian cells and the original approach is fundamentally different: genes that are not essential for viability can easily be deleted from the yeast genome to directly address their requirement in a given cellular process. However, the question often remains of whether an effect observed is a direct or indirect consequence of the gene deletion. This problem, as well as addressing the function of essential genes can be overcome by using thermosensitive (ts) or other conditional alleles that are only inactivated at a given temperature or under certain conditions. Rapid installation of a defect after shift to the restrictive condition is normally considered to be good evidence supporting a direct role for the corresponding protein in that process.

Development of an assay that specifically measures the uptake step of receptor mediated endocytosis in yeast (see above) permitted the identification of potential genes that are required for this event. A candidate protein to be tested was the main component of the clathrin coat: the clathrin heavy chain. Surprisingly, deletion of the only gene encoding this protein (*CHC1)* or its inactivation using a ts allele, only caused a 50% reduction in the α-factor internalization rate (Tan et al., 1993). Even though the installation of the internalization defect in the *chc1* ts mutant is quite rapid, the data depicted above brought into doubt whether receptor-mediated internalization in yeast was analogous to clathrin-dependent endocytosis in mammalian cells. In addition, identification of a lysine-dependent endocytosis signal in the α-factor receptor that did not resemble the classical tyrosine-based mammalian signals further supported this view (Rohrer et al., 1993).

In order to search for other requirements, several available mutants were tested and different screening procedures to isolate endocytosis mutants (*end, ren, dim* mutants) were set up in the next years (Chvatchko et al., 1986; Davis et al., 1993; Raths et al., 1993; Munn and Riezman, 1994; Wendland et al., 1996; Luo and Chang, 1997). Identification of proteins specifically required in the uptake step has been especially fruitful (Table 1). The initial analysis of these mutants revealed two fundamental aspects of the internalization process in yeast largely unnoticed in animal cells: the requirement for actin and a subset of actin-associated proteins, and the involvement of certain lipids (Table 1). The requirement of actin in the internalization of Ste2p seems to be direct for a number of reasons: (1) different actin ts alleles are completely blocked for receptor-mediated and fluid-phase endocytosis immediately after shift to the restrictive temperature before other obvious defects in actin-dependent functions are installed (Kübler and Riezman, 1993). (2) A subset of proteins associated with the actin cytoskeleton such as the actin bundling protein Sac6p, calmodulin or the type I myosins are also required in the process (Table 1), however, this machinery seems to be very specific since mutations in many other genes with gross defects in the actin cytoskeleton do not affect endocytosis (tropomyosin, myosins V, profilin...) (Riezman et al., 1996). (3) Analysis of a collection of ts actin mutants (Wertman et al., 1992) reveals that different actin-dependent functions are

Table 1. Yeast genes required for the uptake step of receptor-mediated endocytosis

Yeast gene	Mammalian homologies and comments	References
I.		
ACT1/END7	Actin	Kübler and Riezman, 1993 Munn et al., 1995
ARP2	Actin related protein	Moreau et al., 1997
CMD1	Calmodulin	Kübler et al., 1994
MYO5	Type I myosin	Geli and Riezman, 1996
SAC6	Fimbrin	Kübler and Riezman, 1993
END3	Eps15 (EH domain)	Raths et al., 1993 Bénédeti et al., 1994
PAN1/DIM2	Eps15 (EH domains)	Wendland et al., 1996 Tang et al., 1997
SLA2/END4/MOP2	Talin (C terminus)	Raths et al., 1993 Wesp et al., 1997
RVS161/END6	Amphiphysin	Munn et al., 1995
RVS167	Amphiphysin	Munn et al., 1995
VRP1/END5	-	Munn et al., 1995
AKR1	Ankyrin repeat	Givan and Sprague, 1997
II.		
LCB1/END8	Ceramide biosynthesis enzyme	Munn and Riezman, 1994
ERG2/END11	Ergosterol biosynthesis enzyme	Munn and Riezman, 1994
III.		
CHC1	Clathrin heavy chain	Tan et al., 1993
CLC1	Clathrin light chain	Huang et al., 1997
END9	-	Munn and Riezman, 1994

I. Genes that when mutated can cause disorganization of the actin cytoskeleton.

II. Genes involved in lipid biosynthesis.

III. Genes with no obvious connection to the actin cytoskeleton or lipid biosynthesis. The internalization of certain proteins also requires their ubiquitination.

differentially affected by distinct alleles suggesting that a distinct machinery is involved in endocytosis (Hill et al., 1996; Yang et al., 1997; M. I. Geli, unpublished data).

The second exciting and as yet unexplored mechanistic aspect of the endocytic internalization has been revealed by the cloning and sequencing of two *END* genes blocked in the uptake step of endocytosis: *END8* encodes an enzyme involved in ceramide biosynthesis (*LCB1*) and *END11* encodes a gene involved in the synthesis of ergosterol (*ERG2*), the major yeast sterol (Riezman et al., 1996) (Table 1).

IS INTERNALIZATION IN YEAST AND ANIMAL CELLS SO DIFFERENT?

Up to this point endocytosis in animals and yeast seemed to appear rather different, however, further insight into both systems in the last few years has highlighted some striking similarities and some fundamental differences.

One difference between clathrin-dependent endocytosis in animal cells and endocytosis in yeast is that no dynamin-like proteins seem to be involved in the uptake step in yeast. Three different genes have been identified in the yeast genome that share some homology to animal dynamins: Mgm1p, Vps1p and Dnm1p. Mgm1p is a mitochondrial protein involved in mitochondrial DNA maintenance (Jones and Fangman, 1992). Vps1p and Dnm1p are both involved in membrane traffic, Golgi to vacuole and early to late endosome, respectively (Vater et al., 1992; Gammie et al., 1995). Strains deleted for either *DNM1* or *VPS1* do not show any internalization defect (Gammie et al., 1995; Alan Munn, personal communication) suggesting that they are not involved in the process. The possibility still exists that these proteins are redundant for their endocytic internalization function, in which case a defect would only be noticed by introducing mutations in both genes. Whether they are true functional homologs of dynamin is not clear. Their homology lies mainly in the GTPase domain and they lack an important portion of the C-terminal tail that seems to be important for self assembly, binding to membranes and stimulated GTPase activity (Warnock and Schmid, 1996, and references therein). If dynamin works as a mechanochemical enzyme in endocytosis, a possible replacement has recently been identified in yeast: the unconventional myosins I (Geli and Riezman, 1996). Myosins I are ubiquitous actin/ATP-dependent molecular motors that bind cellular membranes through their short, positively-charged C-terminal tail (Pollard et al., 1991; Cheney and Mooseker, 1992). Extensive biochemical analysis has been performed on mammalian and protozoa myosins I, and some of their features strikingly resemble those of dynamin. They both hydrolyze a similar number of nucleotide molecules per minute and their nucleotidase activity is modulated in vitro through binding to acidic phospholipids, Ca^{2+} and phosphorylation events (Pollard et al., 1991; Cheney and Mooseker, 1992; Warnock and Schmid, 1996). Type I myosins are additionally assembled with light chains. In yeast, Myo5p requires calmodulin as a light chain for its function in the endocytic internalization step (Geli et al., 1998).

A second striking feature of the endocytic internalization in yeast when compared to animal cells is the mild requirement for clathrin in that organism. Clathrin coated pits and vesicles have not yet been identified ultrastructurally in yeast and therefore the possibility still exists that the effect observed in *chc1* mutants is indirect and is caused by primary defects in other membrane trafficking events. However, several pieces of evidence indicate that this is not the case: (1) some of the isolated *end* mutants affect genes sharing homology with recently identified mammalian proteins associated with clathrin/dynamin function (eps15 and amphiphysin). (2) Further insights in the analysis of the endocytic signals in yeast and mammals demonstrate some important common features.

The *END3* and *PAN1*/*DIM2* (Table 1) N-terminal portions share homology to the recently identified EGF receptor kinase substrate, eps15 (Fazioli et al., 1993; Bénédetti et al., 1994; Wendland et al., 1996; Tang et al., 1997). They contain an imperfect repeat of about 70 amino acids with an overall 60% homology: the EH (Eps15 homology) domain (Wong et al., 1994, 1995). The role of Eps15 in clathrin-mediated endocytosis is strongly suggested by its interaction with α adaptin (Benmerah et al., 1995, 1996) and its recent ultrastructural localization to the rim of coated pits (Tebar et al., 1996). The function of the EH domain is unknown; this domain does not seem to be required for binding to α adaptin (Benmerah et al., 1996), but has recently been shown to bind proteins with an NPF motif (Salcini et al., 1997).

Other *END* genes with significant homology to mammalian clathrin/dynamin-associated counterparts are *END6/RVS161* and *RVS167* (Table 1). They share homology to the neuronal amphiphysin identified as the major autoantigen in the stiff-Man syndrome (David et al., 1994). Amphiphysin interacts both with dynamin and clathrin (David et al., 1996; McMahon et al., 1997). The functional significance of the amphiphysin-dynamin interaction has recently been elegantly demonstrated. Microinjection of peptides corresponding to the amphiphysin and dynamin interacting domains into neurons inhibited synaptic vesicle endocytosis at the stage of invaginated clathrin-coated pits (Shupliakov et al., 1997).

Additionally, an important insight into the mechanism involved in the lysine-mediated internalization of the α-factor receptor revealed that functionally analogous signals may also play a role in clathrin-dependent endocytosis in animal cells. The lysine residue undergoes ubiquitination in response to ligand binding. Preventing ubiquitination either by a point mutation in the receptor or by mutations in the ubiquitination machinery abolished receptor uptake, therefore demonstrating its role as an endocytic signal (Hicke and Riezman, 1996). Ubiquitination of plasma membrane receptors is also a common phenomenon in mammalian cells. Many growth factor receptors receive ubiquitin upon binding to their ligands (Riezman et al., 1996, and references therein). In particular, the ubiquitination machinery has been proposed to be required for internalization of the growth hormone receptor which occurs through clathrin-coated pits (Strous et al., 1996).

Two new yeast endocytosis signals that resemble those that mediate clathrin-dependent internalization of mammalian proteins have recently been identified: The NPFSD and NPF peptides (similar to FDNPVY of the LDL receptor) present in the yeast Kex2p and Ste3p, respectively, are sufficient to trigger internalization. Uptake of these two proteins is dependent on clathrin and other *END* genes to a similar extent as Ste2p which depends upon ubiquitination, suggesting that a similar endocytic machinery is involved in their uptake (Tan et al., 1996). On the other hand, the general amino acid permease bears a dileucine

sequence similar to the mammalian endocytic dileucine signals (Aiken et al., 1994) and which is required for its downregulation. In addition, ubiquitination of Gap1p is also apparently required for its internalization (Hein and André, 1997).

How can one explain the partial effect of clathrin mutants in endocytosis in yeast? Two hypothesis are consistent with the accumulated data. The first one postulates that clathrin is not essential for the budding process itself but functions to recruit receptors. In this context, deleting clathrin heavy chain would diminish the efficiency of, but not abolish, receptor-mediated endocytosis. An alternative possibility would postulate that at least two endocytic pathways exist in yeast, one that is clathrin- and actin-dependent and another that is clathrin-independent, but still actin-dependent. As mentioned above, clathrin-independent endocytic pathways have been described in mammalian cells. Some interesting mechanistic parallels can be drawn between caveolar uptake and endocytosis in yeast. Caveolae structure and function seem to be dependent on actin and cholesterol in analogy to the endocytic internalization in yeast. Actin depolymerizing and cholesterol sequestering drugs both disturb caveolae structure and prevent internalization through this pathway (Schnitzer et al., 1994; Parton et al., 1994). It is worth noting that no caveolin homolog has been identified in the yeast genome, however, there are to date no data demonstrating that caveolin itself is a fundamental component of the machinery required for internalization though caveolae. It is also possible that this protein causes the accumulation of non-clathrin coated endocytic vesicles at the plasma membrane by preventing their fission. Many GPI anchored proteins seem to be internalized through caveolae upon antibody crosslinking in an actin and cholesterol dependent manner (Parton et al., 1994; Schnitzer et al., 1994). Interestingly, the GPI-anchored protein CD59 is internalized with the same requirements in lymphoblastoids cells (Deckert et al., 1996), which as previously mentioned do not express endogenous caveolin but accumulate caveolae structures when this protein is overexpressed (Fra et al., 1995).

In contrast to the important role of clathrin in mammalian internalization versus its controversial role in yeast, actin is clearly required to support endocytosis in yeast whereas its involvement in mammalian cells has been a matter of debate for a long time. The actin requirement for clathrin-dependent and -independent internalization in mammalian cells has been addressed by analyzing the effect of actin depolymerizing drugs such as cytochalasin derivates. The results obtained seem to be conflicting and to depend on the cell type and the assay utilized to assess the endocytic defect. Fluid-phase endocytosis was inhibited by these drugs in different cell types (Wagner et al., 1971; Pratten and Lloyd, 1979) but not in others (Piasek and Thyberg, 1980; Kyle et al., 1988). Cytochalasin also showed selective effects within the same cell. It inhibited uptake of ricin and Lucifer Yellow CH, while it had no effect on clathrin-dependent internalization of transferrin in A431 cells (Sandvig and van Deurs, 1990). It also completely blocked clathrin-dependent and independent endocytosis at the apical side of polarized MDCK cells but not at the basolateral surface (Gottlieb et al., 1993). Are these results indicative of a selective requirement for actin in endocytosis or is it possible that the contribution of actin to mammalian endocytosis has been underestimated?

The cortical actin cytoskeleton can be stabilized by specialized proteins in different cell types and even in different regions of the same cell (Luna, 1991). Different actin depolymerizing drugs could therefore disrupt the cortical actin cytoskeleton to different extents depending on the cell type or the cell domain. Recent results from S. Schmid's laboratory point to this direction. Latrunculin A, a drug that prevents actin polymerization (Coué et al., 1987), but not cytochalasin D, completely blocked clathrin-dependent endocytosis (Lamaze et al., 1997). Interestingly, latrunculin A, but not cytochalasin D, is efficient as an actin depolymerizating drug in yeast (Ayscough and Drubin, 1996).

Ultrastructural and biochemical experiments suggesting a functional interaction between clathrin and the actin cytoskeleton have previously been reported in mammalian cells (Salisbury et al., 1980; Kohtz et al., 1990). Additionally, recent data demonstrate that clathrin associated proteins might be involved in the organization of the actin cytoskeleton. Strikingly, overexpression of dominant dynamin mutants in HeLa cells (Damke et al., 1994) or depletion of neuronal amphiphysin using antisense RNA (Mundigl et al., 1997) have a major effect on the structure of the cortical actin cytoskeleton. Additionally, calmodulin, which is essential for endocytosis and actin localization in yeast (Kübler et al., 1994; Ohya and Botstein, 1994) binds to clathrin light chains in animal cells (Pley et al., 1995).

In summary, the studies of endocytosis in animal and yeast cells are beginning to converge as the knowledge in both systems progresses. Even though, it is still early to decide exactly what is common and what is different, a few general guidelines can be drafted: the data indicate that clathrin-dependent and -independent endocytosis can take place in both cell types. An increasing number of proteins, with animal and yeast counterparts, seem to be involved in the process but it is not yet clear which machinery if at all is shared by clathrin-dependent and -independent pathways. Finally, the requirement of actin for the endocytic internalization which might have been underestimated in animal cells might turn out to be of key importance for the mechanism of formation of the endocytic vesicles.

What is it in particular about the formation of endocytic vesicles that requires actin, whereas the formation of most vesicles at the ER or Golgi membranes does not? Vesicle formation from internal membranes may occur at the rims of these structures, whereas endocytic internalization takes place from the mainly flat or convex surface. Two additional forces may come into play here that work against vesicle formation, surface tension (Dai and Sheetz, 1995) and osmotic pressure. We postulate that the force generated through actin polymerization and or actin-dependent molecular motors is used to deform the plasma membrane against these counterforces.

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REFERENCES

Aiken, C., Konner, J., Landau, N. R., Lenburg, M. E. and Trono, D. (1994). Nef induces CD4 endocytosis: requirement for a critical dileucine motif in the membrane proximal CD4 cytoplasmatic domain. *Cell* **76**, 853-864.

Anderson, R. G. W., Brown, M. S. and Goldstein, J. L. (1977a). Role of coated endocytic vesicles in the uptake of receptor bound L.D.L. lipoprotein in human fibroblast. *Cell* **10**, 351-364.

Anderson, R. G. W., Goldstein, J. L. and Brown, M. S. (1977b). A mutation that impairs the ability of lipoprotein receptors to localise in coated pits on the cell surface of human fibroblast. *Nature* **270**, 695-699.

Ahle, S. and Ungewickell, E. (1989). Identification of the clathrin binding subunit in the HA2 adaptor protein complex. *J. Biol. Chem.* **264**, 20089-20093.

Ayscough, K. R. and Drubin, D. G. (1996). Actin: general principles from studies in yeast. *Annu. Rev. Cell. Dev. Biol.* **12**, 129-160.

Bénédetti, H., Raths, S., Crausaz, F. and Riezman, H. (1994). The *END3* gene encodes a protein that is required for the internalization step of endocytosis and for actin cytoskeleton organization in yeast. *Mol. Biol. Cell* **5**, 1023-1037.

Benmerah, A., Gagnon, J., Begue, B., Megarbane, B., Dautry-Varsat, A. and Cerf-Bensussan, N. (1995). The tyrosine kinase substrate eps15 is constitutively associated with the plasma membrane adaptor AP-2. *J. Cell Biol.* **131**, 1831-1838.

Benmerah, A., Begue, B., Dautry-Varsat, A. and Cerf-Bensussan, N. (1996). The ear of α-adaptin interacts with the COOH-terminal domain of the eps15 protein. *J. Biol. Chem.* **271**, 12111-12116.

Carter, L. L., Redelmeier, T. E., Woollenweber, L. A. and Schmid S. L. (1993). Multiple GTP-binding proteins participate in clathrin-coated vesicle-mediated endocytosis. *J. Cell Biol.* **120**, 37-45.

Chen, M. S., Obar, R. A., Schroeder, C. C., Austin, T. W., Poodry, C. A., Wadsworth, S. C. and Vallee, R. B. (1991). Multiple forms of dynamin are encoded by Shibire, a *Drosophila* gene involved in endocytosis. *Nature* **351**, 583-586.

Cheney, R. E. and Mooseker, M. S. (1992). Unconventional myosins. *Curr. Opin. Cell Biol.* **4**, 27-35.

Chin, D. J., Straubinger, R. M., Acton, S., Nathke, I. and Brodsky, F. M. (1989). 100-kDa polypeptides in peripheral clathrin-coated vesicles are required for receptor-mediated endocytosis. *Proc. Nat. Acad. Sci. USA* **86**, 9289-9293.

Chvatchko, Y., Howald, I. and Riezman, H. (1986). Two yeast mutants defective in endocytosis are defective in pheromone response. *Cell* **46**, 355-364.

Coué, M., Brenner, S., Spector, I. and Korn E. (1987). Inhibition of actin polymerization by latrunculin A. *FEBS Lett.* **213**, 316-318.

Crowther, R. A., Finch, J. T. and Pearse, B. M. F. (1976). On the structure of coated vesicles. *J. Mol. Biol.* **103**, 785-798.

Dai, J. and Sheetz, M. P. (1995). Regulation of exocytosis, endocytosis and membrane shape by membrane tension. *Cold Spring Harb. Symp. Quant. Biol.* **60**, 567-571.

Damke, H., Baba, T., Warnock, D. E. and Schmid, S. L. (1994). Induction of human dynamin specifically blocks endocytic coated vesicle formation. *J. Cell Biol.* **127**, 915-934.

David, C., Solimena, M. and de Camilli, P. (1994). Autoimmunity in stiff-Man syndrome with breast cancer is targeted to the C-terminal region of human amphiphysin, a protein similar to the yeast proteins, Rvs167 and Rvs161. *FEBS Lett.* **351**, 73-79.

David, C., McPherson, P. S., Mundigl, O. and de Camilli, P. (1996). A role of amphiphysin in synaptic vesicle endocytosis suggested by its binding to dynamin in nerve terminals. *Proc. Nat. Acad. Sci. USA* **93**, 331-335.

Davis, N. G., Horecka, J. L. and Sprague, G. F. (1993). Cis- and trans-functions required for endocytosis of the yeast pheromone receptors. *J. Cell Biol.* **122**, 53-65.

Deckert, M., Ticchioni, M. and Bernard, A. (1996). Endocytosis of GPI-anchored proteins in human lymphocytes: role of glycolipid-based domains, actin cytoskeleton, and protein kinases. *J. Cell Biol.* **133**, 791-799.

Dulic, V., Egerton, M., Elguindi, I., Raths, S., Singer, B. and Riezman, H. (1991). Yeast endocytosis assays. *Meth. Enzymol.* **194**, 697-710.

Fazioli. F., Minichiello, L., Matoskova, B., Wong, W. T. and Di Fiore, P. P. (1993). Eps15, a novel tyrosine kinase substrate, exhibits transforming activity. *Mol. Cell Biol.* **13**, 5814-5828.

Fra, A. M., Williamson, E., Simsons, K. and Parton, R. G. (1995). *De novo* formation of caveolae in lymphocytes by expression of VIP21-caveolin. *Proc. Nat. Acad. Sci. USA* **92**, 8655-8659.

Gammie, A. E., Kurihara, L. J., Vallee, R. B. and Rose, M. D. (1995). *DNM1*, a dynamin related gene, participates in endosomal trafficking in yeast. *J. Cell Biol.* **130**, 533-566.

Geli, M. I. and Riezman, H. (1996). Role of type I myosins in receptor-mediated endocytosis in yeast. *Science* **272**, 533-535.

Geli, M. I., Wesp, A. and Riezman, H. (1998). Distinct functions of calmodulin are required for the uptake step of receptor-mediated endocytosis in yeast: the type I myosin Myo5p is one of the calmodulin targets. *EMBO J.* **17**, 635-647.

Givan, S. A. and Sprague, G. F. (1997). The ankyrin repeat-containing protein Akr1p is required for the endocytosis of yeast pheromone receptors. *Mol. Biol. Cell* **8**, 1317-1327.

Grigliatti, T. A., Hall, L., Rosenbluth, R. and Suzuki, D. T. (1973). Temperature sensitive mutants in *Drosophila melanogaster*. XIV. Selection of immobile adults. *Mol. Gen. Genet.* **120**, 107-114.

Gottlieb, T. A., Ivanov, I. E., Adesnik, M. and Sabatini, D. D. (1993). Actin microfilaments play a critical role in endocytosis at the apical but not the basolateral surface of polarized epithelial cells. *J. Cell Biol.* **120**, 695-710.

Hein, C. and André, B. (1997). A C-terminal di-leucine motif and nearby sequences are required for NH4(+)-induced inactivation and degradation of the general amino acid permease, Gap1p, of *Saccharomyces cerevisiae*. *Mol. Microbiol.* **24,** 607-616.

Hicke, L. and Riezman, H. (1996). Ubiquitination of a yeast plasma membrane receptor signals its ligand-stimulated endocytosis. *Cell* **84**, 277-287.

Hicke, L., Zanolari, B., Pypaert, M., Rohrer, J. and Riezman, H. (1997). Transport throught the yeast endocytic pathway occurs through morphologically distinct compartment and requires an active secretory pathway and Sec18p/N-ethylmaleimide-sensitive fusion protein. *Mol. Biol. Cell* **8**,13-31.

Hill, K. L., Catlett, N. L. and Weisman, L. S. (1996). Actin and myosin function in directed vacuole movement during cell division in Saccharomyces cerevisiae. *J. Cell Biol.* **135**, 1535-1549.

Hinshaw, J. E. and Schmid, S. L. (1995). Dynamin self-assembles into rings suggesting a mechanism for vesicle coated budding. *Nature* **374**, 190-192.

Huang, K. M., Gullberg, L., Nelson, K. K., Stefan C. J., Blumer, K. and Lemmon, S. K. (1997). Novel functions of clathrin light chains: clathrin heavy chain trimerization is defective in light chain deficient yeast. *J. Cell Sci.* **110**, 899-910.

Jones, B. A. and Fangman, W. L. (1992). Mitochondrial DNA maintenance in yeast requires a protein containing a region related to the GTP-binding domain of dynamin. *Genes Dev.* **6**, 380-389.

Kohtz, D. S., Hanson, V. and Puszkin, S. (1990). Novel proteins mediate an interaction between clathrin coated vesicles and polymerizing actin filaments. *Eur. J. Biochem.* **192**, 291-298.

Kosaka, T. and Ikeda, K. (1983). Possible temperature-dependent blockage of synaptic vesicle recycling induced by a single gene mutation in *Drosophila*. *J. Neurobiol.* **14**, 207-225.

Kübler, E. and Riezman, H. (1993). Actin and fimbrin are required for the internalization step of endocytosis in yeast. *EMBO J.* **12**, 2855-2862.

Kübler, E., Schimmöller, F. and Riezman, H. (1994). Calcium-independent calmodulin requirement for endocytosis in yeast. *EMBO J.* **13**, 5539-5546.

Kyle, M. E., Nakae, D., Sakaida, I., Miccadei, S. and Farber, J. L. (1988). Endocytosis of superoxide dismutase is required in order for the enzyme to protect hepathocytes from the cytotoxicity of hydrogen peroxide. *J. Biol. Chem.* **263**, 3784-3789.

Lamaze, C. and Schmid, S. L. (1995). The emergence of clathrin-independent pinocytic pathways. *Curr. Opin. Cell Biol.* **7**, 573-580.

Lamaze, C., Fujimoto, L. M., Yin, H. L. and Schmid, S. L. (1997). The actin cytoskeleton is required for receptor-mediated endocytosis in mammalian cells. *J. Biol. Chem.* **272**, 20332-20335.

Luna, E. J. (1991). Molecular links between the cytoskeleton and membranes. *Curr. Opin. Cell Biol.* **3**, 120-126.

Luo, W. J. and Chang, A. (1997). Novel genes involved in endosomal traffic in yeast revealed by suppression of a targeting-defective plasma membrane ATPase mutant. *J. Cell Biol.* **138**, 731-746.

McMahon, H. T., Wigge, P. and Smith, C. (1997). Clathrin interacts specifically with amphiphysin and is displaced by dynamin. *FEBS Lett.* **413**, 319-322.

Marks, M. S., Ohno, H., Kirchhausen, T. and Bonifacino, J. S. (1997). Protein sorting by tyrosine-based signals: adapting to the Ys and wherefores. *Trends Cell Biol.* **7**, 124-128.

Moreau, V., Galan, J. M., Devilliers, G., Haguenauer-Tsapis, R. and Winsor, B. (1997). The yeast actin-related protein Arp2p is required for the internalization step of endocytosis. *Mol. Biol. Cell.* **8**, 1361-1375.

Mundigl, O., Ochoa, G. C., David, C., Kabanov, A. V. and de Camilli, P. (1997). Amphiphysin, a dynamin binding protein is implicated in the function of the actin based cytoskeleton. ASCB abstract number 486.

Munn, A. L. and Riezman, H. (1994). Endocytosis is required for the growth of vacuolar H(+)-ATPase-defective yeast: identification of six new *END* genes. *J. Cell Biol.* **127**, 373-386.

Munn, A. L., Stevenson, B. J., Geli, M. I. and Riezman, H. (1995). *end5*, *end6* and *end7* mutations that cause actin delocalization and block the internalization step of endocytosis in *Saccharomyces cerevesiae*. *Mol. Biol. Cell.* **6**, 1721-1742.

Oh, P. and Schnitzer, J. E. (1996). Dynamin-mediated fission of caveolae from plasma membranes. *ASCB.* Abstract number 483.

Ohno, H., Steward, J., Fournier, M. C., Bosshart, H., Rhee, I., Miyatake, S., Saito, T., Gallusser, A., Kirchhausen, T. and Bonifacino, J. S. (1995). Interaction of tyrosine-based sorting signals with clathrin-associated proteins. *Science* **269**, 1872-1875.

Ohya, Y. and Botstein, D. (1994). Diverse essential functions revealed by complementing yeast calmodulin mutants. *Science* **263**, 963-966.

Palade, G. E. (1953). The fine structure of blood capillaries. *J. Appl. Phys.* **24**, 1424.

Parton, R. G., Joggerst, B. and Simons, K. (1994). Regulated internalization of caveolae. *J. Cell Biol.* **127**, 1199-1215.

Parton, R. G. (1996). Caveolae and caveolin. *Curr. Opin. Cell Biol.* **8**, 542-548.

Pearse, B. M. F. (1975). Coated vesicles from pig brain: purification and biochemical characterization. *J. Mol. Biol.* **97**, 93-98.

Pearse, B. M. F. and Robinson, M. S. (1990). Clathrin, adaptors and sorting. *Annu. Rev. Cell Biol.* **6**, 151-171.

Piasek, A. and Thyberg, J. (1980). Effects of colchicine on endocytosis of horseradish peroxidase by rat peritoneal macrophages. *J. Cell Sci.* **45**, 59-71.

Pley, U. M., Hill, B. L., Alibert, C., Brodsky, F. M. and Parham, P. (1995). The interaction of calmodulin with clathrin coated vesicles, triskelions and light chains. Localization of a binding site. *J. Biol. Chem.* **270**, 2395-2402.

Pollard, T. D., Doberstein, S. K. and Zot, H. G. (1991). Myosin-I. *Annu. Rev. Physiol.* **53**, 653-681.

Pratten, M. K. and Lloyd, J. B. (1979). Effect of temperature, metabolic inhibitors and some other factors in fluid-phase adsorptive pinocytosis by rat peritoneal macrophages. *Biochem. J.* **180**, 567-571.

Prescianotto-Baschong, C. and Riezman, H. (1998). Morphology of the yeast endocytic pathway. *Mol. Biol. Cell.* **9**, 173-189.

Raths, S., Rohrer J., Crausaz, F. and Riezman, H. (1993). *end3* and *end4*: two mutants defective in receptor-mediated and fluid-phase endocytosis in *Saccharomyces cerevesiae. J. Cell Biol.* **120**, 55-65.

Riezman, H., Munn, A., Geli, M. I. and Hicke, L. (1996). Actin-, myosin- and ubiquitin-depedent endocytosis. *Experientia* **52**, 1033-1041.

Robinson, M. S. (1997). Coats and vesicle budding. *Trends Cell Biol.* **7**, 99-102.

Rohrer, J., Benedetti, H., Zanolari, B. and Riezman, H. (1993). Identification of a novel sequence mediating regulated endocytosis of the G-protein couple alpha-pheromone receptor in yeast. *Mol. Biol. Cell.* **4**, 511-521.

Roth, T. F. and Porter, K. R. (1964). Yolk protein uptake in the oocyte of the mosquito *Aedes aegypti*. *J. Cell Biol.* **20**, 313-330.

Rothberg, K. G., Heuse, J. E., Donzell, W. C., Wing, Y. S., Glenney, J. R. and Anderson, R. G. W. (1992). Caveolin, a protein component of caveolae membrane coats. *Cell* **68**, 673-682.

Salcini, A. E., Confalonieri, S., Doria, M., Santolini, E., Tassi, E., Minenkova, O., Cesareni, G., Pelicci, P. G. and Di Fiore, P. P. (1997). Binding specificity and in vivo targets of the EH domain, a novel protein-protein interaction module. *Genes. Dev.* **11**, 2239-2249.

Salisbury, J. L., Condeelis, J. S. and Satir, P. (1980). Role of coated vesicles, microfilaments and calmodulin in receptor-mediated endocytosis by cultured B lymphoblastoid cells. *J. Cell Biol.* **87**, 132-141.

Sandvig, K. and van Deurs, B. (1990). Selective modulation of the endocytic uptake of ricin and fluid phase markers without alteration in transferrin endocytosis. *J. Biol. Chem.* **265**, 6382-6388.

Schmid, S. L. and Carter, L. L. (1990). ATP is required for receptor-mediated endocytosis in intact cells. *J. Cell Biol.* **111**, 2307-2318.

Schmid, S. L. and Smythe, E. (1991). Stage-specific assay for coated pit formation and coated vesicle budding in vitro. *J. Cell Biol.* **114**, 869-880.

Schmid, S. L. (1992). The mechanism of receptor-mediated endocytosis: more questions than answers. *BioEssays* **14**, 589-596.

Schnitzer, J. E., Oh, P., Pinney, E. and Allard, J. (1994). Filipin sensitive caveolae-mediated transport in endothelium: reduced transcytosis, scavenger endocytosis and capillary permeability of select macromolecules. *J. Cell Biol.* **127**, 1217-1232.

Schnitzer, J. E., Oh, P. and McIntosh, D. P. (1996). Role of GTP hydrolysis in fission of caveolae directly from plasma membranes. *Science* **274**, 239-242.

Singer-Krüger, B., Frank, R., Crausaz, F. and Riezman, H. (1993). Partial purification and yeast characterization of early and late endosomes in yeast. *J. Biol. Chem.* **268**, 14376-14386.

Shupliakov, O., Low, P., Grabs, D., Gad, H., Chen, H., David, D., Takei, K., de Camilli, P. and Brodin, L. (1997). Synaptic vesicle endocytosis impaired by disruption of dynamin-SH3 domain interactions. *Science* **276**, 259-263.

Smythe, E., Pypaert, M., Lucocq, J. and Warren, G. (1989). Formation of coated vesicles from coated pits in broken A431 cells. *J. Cell Biol.* **108**, 843-853.

Stang, E., Kartenbeck, J. and Parton, R. G. (1997). Major histocompatibility complex class I molecules mediate association of SV40 with caveolae. *Mol. Biol. Cell* **8**, 47-57.

Strous, G. J., Van Kerkhof, P., Govers, R., Ciechanover, A. and Schwartz, A. L. (1996). The ubiquitin conjugating system is required for ligand-induced endocytosis and degradation of the growth hormone receptor. *EMBO J.* **15**, 3806-3812.

Takei, K., McPherson, P. S., Schmid, S. L. and de Camilli, P. (1995). Tubular membrane invaginations coated by dynamin rings are induced by GTP-γS in nerve terminal. *Nature* **374**, 186-190.

Tan, P. K., Davis. N. G., Sprague, G. F. and Payne, G. S. (1993). Clathrin facilitates the internalization of seven transmembrane segment receptors for mating pheromones in yeast. *J. Cell Biol.* **123**, 1707-1716.

Tan, P. K., Howard, J. P. and Payne, G. S. (1996). The sequence NPFXD defines a new class of endocytosis signal in *Saccharomyces cerevisiae. J. Cell Biol.* **135**, 1789-1800.

Tang, H. Y., Munn, A. and Cai, M. (1997). EH domain proteins Pan1p and End3p are components of a complex that plays a dual role in organization of the cortical actin cytoskeleton and endocytosis in Saccharomyces cerevisiae. *Mol. Cell. Biol.* **17**, 4294-4304.

Tebar, F., Sorkina, T., Sorkin, A., Ericsson, M. and Kirchhausen T. (1996). Eps15 is a component of clathrin-coated pits and vesicles and it is located at the rim of coated pits. *J. Biol. Chem.* **271**, 28727-28730.

Tran, D. J., Carpentier, J. L., Sawano, F., Gorden, P. and Orci, L. (1987). Ligands internalized through coated and non-coated invaginations follow a common intracellular pathway. *Proc. Nat. Acad. Sci. USA* **84**, 7957-7961.

Trowbridge, I. S., Collawn, J. F. and Hopkins, C. R. (1993). Signal-dependent membrane protein trafficking in the endocytic pathway. *Annu. Rev. Cell Biol.* **9**, 129-161.

van der Bliek, A. M. and Meyerowitz, E. M. (1991). Dynamin-like protein encoded by the Drosophila *Shibire* gene associated with vesicular traffic. *Nature* **351**, 411-414.

van der Bliek, A. M., Redelmeier, T. E., Damke, H., Tisdale, E. J., Meyerowitz, E. M. and Schmid S. L. (1993). Mutations in human dynamin block an intermediate stage in coated vesicle formation. *J. Cell Biol.* **122**, 553-563.

Vater, C. A., Raymond, C. K., Kena, K., Howald-Stevenson, I. and Stevens, T. H. (1992). The VPS1 protein, a homolog of dynamin required for vacuolar protein sorting in *Saccharomyces cerevesiae*, is a GTPase with two functional separable domains. *J. Cell Biol.* **119**, 773-786.

Wagner, R., Rosenberg, M. and Estensen, R. (1971). Endocytosis in chang liver cells. Quantitation by sucrose-^{3}H uptake and inhibition by cytochalasin B. *J. Cell Biol.* **50**, 804-817.

Warnock, D. E. and Schmid, S. L. (1996). Dynamin GTPase, a force-generating molecular switch. *BioEssay*s **18**, 885-893.

Wendland, B., McCaffrey, J. M., Xiao, Q. and Emr, S. D. (1996). A novel-fluorescence-activated cell sorter-based screen for yeast endocytosis mutants identifies a yeast homologue of mammalian eps15. *J. Cell Biol.* **135**, 1485-1500.

Wertman, K. F., Drubin, D. G. and Botstein, D. (1992). Systematic mutational analysis of the yeast *ACT1* gene. *Genetics* **132**, 337-350.

Wesp, A., Hicke, L., Palecek, J., Lombardi, R., Aust, T., Munn, A. L., and Riezman, H. (1997). End4/Sla2 interacts with actin-associated proteins for endocytosis in yeast. *Mol. Biol. Cell.* **8**, 2291-2306.

Wong, W. T., Kraus, M. H., Carlomagno, F., Zelano, A., Druck, T., Croce, C. M., Huebner, K. and Di Fiore, P. P. (1994). The human eps15 gene, encoding a tyrosine substrate, is conserved in evolution and maps to 1p31-32. *Oncogene* **9**, 1591-1597.

Wong, W. T., Schumacher, C., Salcini, A. E., Romano, A., Castagnino, P., Pelicci, P. G. and di Fiore, P. P. (1995). A protein-binding domain, EH, identified in the receptor tyrosine kinase substrate eps15 and conserved in evolution. *Proc. Nat. Acad. Sci. USA* **92**, 9530-9534.

Yang, S., Ayscough, K. R. and Drubin, D. G. (1997). A role of the actin cytoskeleton of *Saccharomyces cerevisiae* in bipolar bud-site selection. *J. Cell Biol.* **136**, 111-123.