Living with Clathrin: Its Role in Intracellular Membrane Traffic

FRANCES M. BRODSKY

Clathrin polymerization at the cytoplasmic side of the plasma membrane forms coated pits and vesicles that mediate uptake of cell surface receptors. Clathrin-coated vesicles have also been implicated in protein export but definition of their precise role has been controversial. Recent advances in characterization of the clathrin subunits and additional coated vesicle components have identified molecular interactions involved in clathrin polymerization and coated vesicle formation, and have provided new approaches to investigating its function. These studies suggest that clathrin's role, in both inward and outward membrane traffic, is to facilitate receptor transport by a concentration and sorting process that initiates targeting to specific intracellular compartments.

MOVEMENT OF MEMBRANE-BOUND AND SECRETED PROteins to and from the plasma membrane and between intracellular membrane compartments requires budding and fusion of membrane vesicles (*1–3*). For more than 10 years, cell biologists have been investigating molecular interactions that regulate this process of intracellular membrane traffic. The protein clathrin was first identified as a participant when Pearse purified it from coated vesicles isolated from pig brain (*4*). Recent advances in the characterization of clathrin include the cloning and sequencing of DNA coding for the clathrin subunits (*5–9*) and the production of monoclonal antibodies (MAbs) to clathrin (*10–14*). This progress made it possible to examine clathrin's role in intracellular traffic by production of yeast mutants lacking clathrin (*5, 8*) and by cytoplasmic delivery of MAbs to clathrin (*15*). Although these studies initially generated controversy about clathrin function (*16*), they have helped formulate a more precise conception of how clathrin participates in membrane transport. This review will summarize the recent insights into the structure of clathrin and the formation of coated vesicles as well as the experiments that have led to current paradigms about its function.

Experimental Analysis of Clathrin Function

Initially, clathrin was assigned a role in intracellular membrane traffic based on microscopy studies (*17*). These showed clathrin forming a dense protein coat on the cytoplasmic side of invaginating membranes, during receptor-mediated endocytosis. Clathrin-coated pits and vesicles were associated with internalization of a variety of ligands including nutrients, hormones, proteins, and viruses (*18*). Morphological analysis of coated pits showed that clathrin forms a regular polyhedral network on the membrane (*19*). Purified clathrin was found to assemble into polyhedral protein baskets (*20*) with a similar morphology (Fig. 1A), and it was assumed that clathrin assembly at the plasma membrane promoted endocytosis of receptors (*18*).

Initial observation of clathrin in the region of the Golgi apparatus (Fig. 2) led to the generalization that clathrin mediates exocytic as well as endocytic transport (*21*). Subsequent detailed electron microscopy has specifically implicated Golgi clathrin in the transport of lysosomal enzymes (*22*) and of proteins destined for secretory granules (*23, 24*). However, in cells undergoing viral infection, the viral glycoproteins are not transported to the cell surface in clathrin-coated vesicles. For example, influenza hemagglutinin transport is mediated by noncoated vesicles (*24*), and vesicles transporting the surface glycoprotein (G protein) of vesicular stomatitis virus appear to be coated, but not with clathrin (*3*). These morphological results collectively suggest that clathrin is not involved in constitutive export but participates in the transport of proteins to specialized compartments (*2, 21*).

Direct evidence for clathrin function in the exocytic or endocytic pathway has been sought with three different experimental approaches. Several laboratories have purified clathrin-coated vesicles and analyzed their contents. In these experiments, newly synthesized viral glycoproteins (*25*), histocompatibility molecules, immunoglobulin, and cholinesterase (*26*), as well as lysosomal enzyme precursors (*27*), have been found in clathrin-coated vesicles. There is some discrepancy between these biochemical results and the data obtained by immunoelectron microscopy, which may be a function of the purity of the coated vesicle preparations examined in the different experiments. Contamination with other types of vesicles has been suggested as an explanation for finding newly synthesized viral glycoproteins in clathrin-coated vesicles (*16*). However, in the other studies cited above (*26, 27*), care was taken to examine the purity of the preparation as well as to isolate clathrin-coated vesicles in several ways. These better characterized results support the morphological evidence that clathrin-coated vesicles are involved in transport of lysosomal enzymes (*22, 27*) and suggest these vesicles may export some cell surface and constitutively secreted proteins (*26*), for which no morphological analysis is yet available.

Production of yeast strains lacking a functional clathrin heavy chain gene has provided a second approach to studying the intracellular role of clathrin. Two classes of mutants lacking clathrin have been obtained: those that live and those that die. The viable mutants isolated by Payne and Schekman (*5, 28*) are able to secrete several proteins, so clathrin is apparently not essential for these export functions. These mutants are also capable of pheromone uptake, but at 36 to 50% of normal levels. This demonstration of "life without clathrin" led to a debate about the importance of clathrin's role in

The author is at the Department of Pharmacy, School of Pharmacy, University of California, San Francisco, Box 0446, 513 Parnassus Avenue, San Francisco, CA 94143.

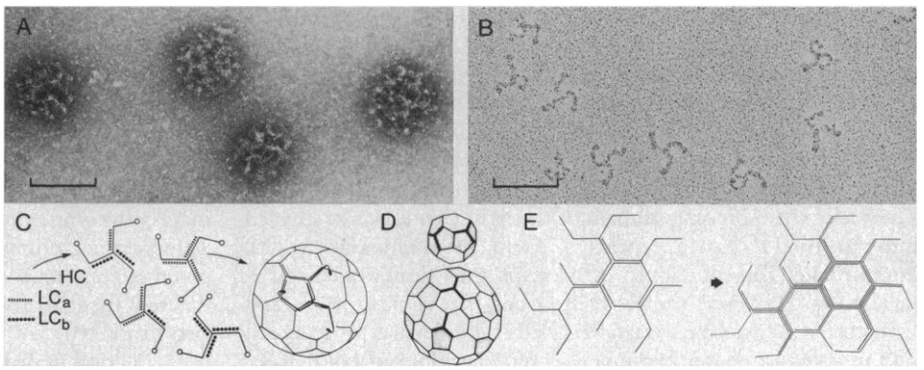

Fig. 1. Structure and assembly of clathrin triskelions. (**A**) Clathrin baskets produced by assembly of purified triskelions at *p*H 6.2 (*11, 20*) (bar, 100 nm). [Reprinted from (*11*) with permission, copyright 1986 IRL Press] (**B**) Clathrin triskelions visualized by rotary shadowing with platinum (*34, 76*) (bar, 120 nm). (**C**) Triskelions are composed of three identical heavy chains (HC). A random distribution of two types of light chain, LC_a and LC_b, has been observed for purified triskelions (*35*). Baskets are formed with one triskelion at every vertex, spanning two polyhedron edges, with the terminal domain projecting inward (*33, 77*). [Adapted from (*78*) with permission, copyright 1984 Elsevier] (**D**) All polymerized clathrin structures contain 12 pentagons (shaded) and variable numbers of hexagons (*43*). Consequently, there are four possible vertices (in bold face) and coincidentally four types of triskelion. The ratios of LC_a to LC_b expressed in different mammalian tissues vary from 2:1 (*34*) to 10:1 (*49*), which presumably influences the forms of triskelion predominant in different tissues. The size of clathrin-coated vesicles varies among tissues (*43*) and could be a function of the different triskelions present. [Reprinted courtesy of Heuser (*43*), copyright 1985 Academic Press] (**E**) Complete assembly of clathrin involves two types of triskelion-triskelion contacts (*11*). Triskelions at alternate vertices make contacts between the distal portion of one arm and the proximal portion of another. At the remaining vertices, contacts form between proximal arms from two triskelions. This diagram does not reflect the actual intertwining of the arms (*33*) or the mechanism for forming pentagons to introduce curvature (*19*). Terminal domains are left out for simplicity (*11*). [Reprinted from (*11*) with permission, copyright 1986 IRL Press]

intracellular traffic and its necessity for yeast viability (*16*). Lemmon and Jones (*8*) found that introduction of clathrin heavy chain mutations in some yeast strains was lethal, but other strains survived because of the presence of a suppressor gene. Identification of the function supplied by this suppressor will provide insight into a critical role clathrin normally plays in these cells. From independent experiments, the role of clathrin in yeast has been difficult to assess. For example, there is controversy about whether yeast carry out receptor-mediated endocytosis across the cell wall (*29*). Study of the yeast mutants should resolve these questions as well as provide clues about the function of clathrin in other organisms.

Clathrin function in mammalian intracellular transport was examined after bulk delivery of high concentrations of antibody to clathrin into the cytoplasm of CV-1 monkey kidney cells (*15*). This procedure reduced endocytic activity by 50%. Results indicated that clathrin participates actively in receptor-mediated internalization as well as fluid-phase pinocytosis. An earlier experiment (*30*) in which microinjection of immunoglobulin from clathrin antiserum had no effect on receptor uptake is not necessarily contradictory, since inhibition of clathrin assembly with antibodies is quite sensitive to the concentration and specificity of the antibodies (*11*). In fact, incomplete inhibition of endocytosis in the CV-1 cells might be explained by variations in the antibody concentrations delivered to individual cells (*15*). Alternatively, some endocytosis may occur through a pathway in which clathrin is not involved (*31, 32*). For cells receiving MAbs to clathrin in concentrations that inhibited endocytosis, the export of endogenously synthesized hemagglutinin to the cell surface was not affected (*15*).

These experiments in which clathrin function was examined demonstrate that clathrin plays an active role in endocytosis and is not essential for secretion of yeast enzymes or transport of viral glycoproteins in mammalian cells. The function of clathrin in the Golgi apparatus is not yet well defined, although biochemical and morphological data strongly suggest a role in sequestration of proteins in organelles and possibly in the export of some endogenous proteins to the plasma membrane. Further analysis of the role of clathrin in intracellular membrane traffic will be facilitated by our understanding the molecular structure of clathrin and the components of clathrin-coated vesicles.

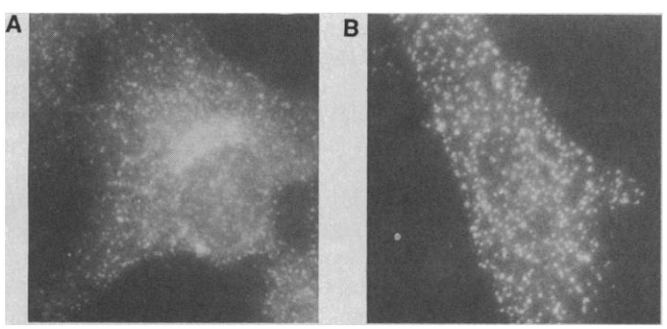

Fig. 2. Intracellular distribution of clathrin and assembly polypeptides. Immunofluorescent staining (*15*) of human fibroblasts with MAbs to coated vesicle components. (**A**) Staining with antibody X22 to clathrin heavy chain (*11*). (**B**) Staining with antibody AP.6 to 100-kD assembly polypeptides (*79*) specifically found in peripheral coated vesicles (*54*). Note the prominent Golgi staining with antibody to clathrin and its absence from staining with AP.6 antibody to a subset of 100-kD assembly polypeptides. [Cell staining and photography by S. Acton]

Molecular Properties of Clathrin

Clathrin molecules have a triskelion shape (*33, 34*) (Fig. 1B) and their self-assembly into polyhedral protein baskets can occur in the absence of membranes at *p*H 6 to 7 (Fig. 1A) (*20, 33*). Although triskelion means "three legged," the projections are often described as arms, which are formed from three noncovalently linked heavy chains and three light chains (*34*) that extend from the vertex to the "elbow" (Fig. 3) (*35, 36*). Proteolysis studies have indicated that the clathrin heavy chain is oriented with the carboxyl terminus near the center of the triskelion and the amino terminus at the globular tips of the arms, forming the terminal domain (*9*) (Fig. 3).

DNA clones coding for rat, bovine (*9*), and yeast (*5, 8*) clathrin heavy chain have been isolated and DNA blotting indicates there is only one heavy chain gene in these species. The complete deduced rat heavy chain sequence is 1675 amino acids, giving a calculated molecular mass of 191 kD, although it appears to be 180 kD by electrophoretic analysis. The yeast and plant clathrin heavy chains have a slower electrophoretic mobility (*5, 37*). The molecular basis for clathrin heavy chain trimerization is not known but Kirchhausen *et al.* (*9*) have proposed that a proline-rich region near the carboxyl terminus of the heavy chain sequence might be involved.

A light chain binding region of the clathrin heavy chain has been

localized to a segment between 56 and 62 kD from the carboxyl terminus (*11*). Between residues 1107 and 1184 of the heavy chain sequence [54 to 62.5 kD from the carboxyl terminus (*38*)] there is a weak pattern of heptad repeats (*9*) with nonpolar residues at positions 1 and 4, characteristic of coiled-coil α helices (*39*). Identification of a similar region in the clathrin light chains suggests a structural motif for molecular interactions with the heavy chain. The heavy chain binding domain of the light chains can be divided into 10 to 11 heptad repeats (*7*) and has homology to the intermediate filament family of proteins that form coiled-coil α helices (*6*). However, the interaction between clathrin heavy and light chains may not be a classical coiled-coil α helix as prolines and skip residues are found in the heptad repeat regions of both chains (*7, 9*). Data in favor of some kind of extended helical interaction between the light and heavy chains include the observations that (i) the light chains span the entire proximal portion of the triskelion arm (*36*) and that (ii) the residues involved in the possible interaction face between the two helices are highly conserved between different forms of the light chains (*40*). The putative coiled-coil region in clathrin has been implicated in the process of self-assembly by inhibition with the X35 MAb that binds to heavy chain in the light chain binding region (*11*). Other filamentous molecules composed of coiled-coil α helices also self-assemble into larger structures (*41*).

Every edge of the assembled clathrin polyhedron is composed of heavy chains from four triskelions as well as two light chains (Fig. 1, C and E) (*33*). Isolated clathrin heavy chains can assemble into polyhedral structures (*42*), which suggests that the light chains are not strictly required for clathrin assembly. These heavy chain baskets have not been extensively analyzed with respect to the efficiency of their assembly or the geometry of the polyhedra formed. Furthermore, when light chains are removed from clathrin heavy chains by proteolysis, the heavy chains are no longer assembly competent (*34*). When one considers that the light chain binding region of the heavy chain is involved in interactions that are critical for correct assembly, it would be surprising if the light chains had no influence on the assembly process. The possible role of light chains in improving the efficiency of assembly, stabilizing the basket structure, or influencing basket geometry (*43*) (Fig. 1D) has yet to be investigated.

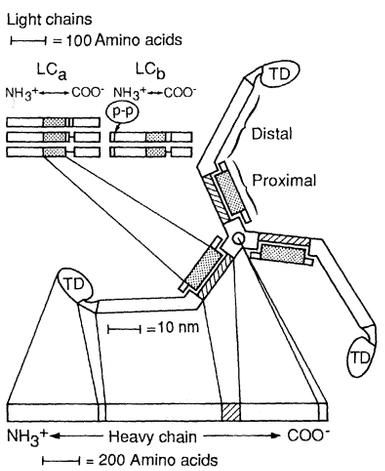

Fig. 3. Primary structural features of clathrin subunits and their disposition in the triskelion. Bar diagrams of the primary sequences of the light chains (upper left) and heavy chain (bottom) are to scale as indicated (*6, 7, 9*). Features of the subunit sequences are projected onto the triskelion, which is also drawn to scale (*43*). For the light chains, the heavy chain binding sequence is stippled. The inserted sequences, characteristic of the brain forms of the light chains, are included in the top sequences and a horizontal line indicates their excision in the peripheral forms. The LC_b light chains can be phosphorylated by the casein-kinase activity in coated vesicles at serines 11 and 13 (*50*). The orientation of the light chains on the triskelion is based on immunolocalization of the CVC.6 antigenic site, near the carboxyl terminus of LC_a, to the triskelion vertex (*35*). For the heavy chain, the light chain binding region is shaded and is projected onto the triskelion, illustrating a potential contact region of 115 Å, if the heptad repeat regions were strictly α helical. The terminal domain is composed of residues 1 to 478, adjacent to a protease-sensitive linker region, composed of residues 479 to 522. The three heavy chains are noncovalently attached. The carboxyl-terminus sequence from residues 1628 to 1675 may participate in trimerization. The heavy chain sequence analysis and orientation is based on the work of Kirchhausen *et al.* (*9*).

The light chains of mammalian and avian clathrin are heterogeneous (*34, 44*), whereas yeast clathrin has a single light chain (*5*). Perhaps the diversification of light chain structure correlates with increased complexity of clathrin function in higher vertebrates. Transport in mammalian and avian cells is not limited by a cell wall and these cells have specialized secretory and endocytic functions in different tissues. In all mammalian and avian cells analyzed there are two major types of light chain, LC_a and LC_b (*34, 44*), which are 60% identical in protein sequence and coded for by separate genes, present in single copies (*6, 7*). All forms of the mammalian light chains appear to be 30 to 40 kD by electrophoretic analysis, whereas the protein sequences deduced from complementary DNA (cDNA) clones predict actual molecular masses of 23 to 27 kD (*6*). Three subtypes of LC_a and two subtypes of LC_b have been identified (*6, 7*) (Fig. 3). The subtypes vary in molecular weight because of the presence of inserted sequences near the carboxyl terminus that are the result of alternative RNA splicing.

Comparison of clathrin from different tissues shows that the higher molecular weight forms of LC_a (with 18– and 30–amino acid inserts) and LC_b (with an 18–amino acid insert) are expressed in brain tissue (bovine, human, rat, and chicken) and that LC_a and LC_b without insertion sequences are expressed in peripheral (nonbrain) tissue (*44*). The insertion sequences are highly exposed on the clathrin triskelion and in assembled coated vesicles as determined by MAb binding (*10*). These sequences introduce a region of hydrophobicity into the brain LC_a and LC_b (*6*) that may constitute a binding site for a cytoplasmic protein involved in some brain-specific function of clathrin. Our preliminary results indicate that the brain-specific forms of the clathrin light chains are detectable in neurons, which suggests these light chains may participate in the burst of endocytosis that mediates membrane recycling after synaptic vesicle release (*45*). One possibility is that specialized forms of light chains may be required for synchronized recruitment of clathrin for rapid assembly. Alternatively, the location of the insertion sequences near the polyhedral vertex may enable the formation of a particular vesicle geometry that is used in membrane recycling.

The biochemical and serological properties of LC_a and LC_b indicate they have a number of distinct functional sites. Some are shared by LC_a and LC_b and others represent specialization (*10, 12*). Both light chains bind to clathrin heavy chain and compete with each other for heavy chain association (*10, 36, 42*). They also bind to calmodulin and can bind Ca^{+2} [dissociation constant (K_d) of 25 to 55 μM] (*46, 47*). Both LC_a and LC_b are heat-resistant (*46, 48*) and acidic [isoelectric point (pI) 4 to 5]. Serological differences between LC_a and LC_b are conserved in human, bovine, rat, and chicken clathrin (*44*), which correlates with a 90% similarity between known LC_a and LC_b sequences (*40*). A major difference between the light chains is that LC_b is phosphorylated by a casein kinase present in isolated coated vesicles (*49, 50*). The target sequence for this phosphorylation (Fig. 3), which is missing from LC_a, has homology to other casein kinase phosphorylation sites. A second difference between LC_a and LC_b is the disposition and reactivity of cysteine residues near the carboxyl terminus (*51*). Mapping these shared and different characteristics with respect to the protein sequences divides the light chains into domains (*40, 51*). From the amino to the carboxyl terminus, these domains include (i) a phosphorylation domain, (ii) a conserved domain, (iii) a variable domain, (iv) a heavy chain binding domain, (v) an exposed domain where tissue-specific sequences can be inserted, (vi) and a domain containing cysteine residues. This domain structure suggests that the single yeast light

chain may regulate several different aspects of clathrin function. The multiple forms of the light chains that are found in the cells of higher vertebrates presumably serve to diversify this regulatory capacity.

The role of the light chain, or chains, in clathrin function remains unclear. Although not essential for clathrin assembly, light chains influence the activity of two extrinsic proteins that affect assembly. The light chains are the binding sites for an adenosine triphosphatase (ATPase), which depolymerizes clathrin from coated vesicles (*52*), and they enhance phosphorylation of the 50-kD assembly polypeptide that copolymerizes with clathrin (*53*). These results indicate that the light chains interact with both cytoplasmic proteins and internal vesicle proteins, providing a bridge between the regulatory elements that influence coated vesicle formation.

Clathrin-Coated Pit and Vesicle Formation

Two areas of investigation are advancing our understanding of how clathrin self-assembly nucleates on a membrane and how assembly is controlled in vivo: (i) the characterization of additional coated vesicle proteins that influence clathrin assembly (*33, 54*) and (ii) the development of an assay to study coated pit formation in vitro (*55*).

In addition to clathrin, there is a second major protein component associated with clathrin-coated vesicles. These large complexes (250 to 350 kD) are composed of peripheral membrane proteins, more tightly bound to the coated vesicle membrane than clathrin. These complexes have been called assembly polypeptide complexes because they coassemble with clathrin in vitro and, under physiological conditions, promote formation of clathrin baskets with homogeneous size and shape (*33, 54*). Image analysis of clathrin coassembled with assembly polypeptides indicates that they form a shell within the assembled clathrin coat and appear to be contacted by the terminal domain of the triskelion (*33*). However, the central portion of the triskelion has also been implicated in assembly polypeptide binding (*56*). Available experimental evidence suggests that the assembly polypeptide complexes serve two recognition functions that are critical for coated pit and vesicle formation. These complexes can interact with receptors as well as bind to clathrin and perhaps facilitate its assembly. Clathrin cannot reassociate with coated vesicle membranes stripped of assembly polypeptides by proteolysis (*56*). Furthermore, coassembly of clathrin with purified mannose-6-phosphate receptor requires the presence of assembly complexes (*33*).

Two subsets of assembly polypeptide complexes have been identified by fractionation on hydroxylapatite (*33, 54*). One subset (designated HA-II or AP-2) is associated with clathrin-coated vesicles in peripheral regions of the cell, away from the nucleus (Fig. 2); the other subset (HA-I or AP-1) is associated with clathrin-coated vesicles in the Golgi region. Each complex is composed of three sets of proteins of about 100, 50, and 17 kD. There are four types (α, β, β′, and γ) of 100-kD polypeptides (98 to 115 kD), each of which exhibits additional heterogeneity. The peripheral assembly complexes are composed of one α and one β 100-kD protein associated with a 50- to 52-kD and a 16- to 17-kD subunit. The Golgi complexes have one γ and one β′ 100-kD protein associated with 40- to 47-kD and 19- to 20-kD subunits. Measurements of stoichiometry have not been accurate enough to determine whether the pair of 100-kD subunits in each assembly complex is associated with one or two copies of the other subunits. Other coated vesicle–associated proteins (115 to 185 kD) that coassemble with clathrin in vitro have also been identified (*54*). Their intracellular distribution is unknown and it is therefore unclear if they represent additional subsets of assembly complexes.

The multiple subunit configuration of the assembly polypeptide complexes may contribute to their proposed role as a link between diverse receptor tails and the process of clathrin assembly (*33*). These complexes may differ in their receptor-binding domains but share a "constant" clathrin-binding domain that cross-links clathrin in some way to promote assembly (*54*). Some features of the assembly complex may also determine where clathrin assembly nucleates. However, the diversity of receptor tails that associate with clathrin still exceeds the diversity of assembly polypeptides, so additional transducing proteins could be involved in receptor recognition. Alternatively, the shared features of receptor tails are not yet appreciated.

Formation of a clathrin-coated pit involves recognition among cytoplasmic receptor tails, assembly polypeptides, and clathrin, as well as their recruitment from intracellular stores of dissociated components. A method for analyzing the dynamics of this process was recently developed by Moore *et al.* (*55*). Cultured human fibroblasts are sonicated to rupture cells, exposing coated pits on the membrane that remains attached to the cell culture substrate. When clathrin is depolymerized from these membranes, addition of fresh cytosol allows coated pits to reform in a saturable fashion, demonstrating a fixed number of nucleation sites. Proteins of 180, 36, and 110 kD, corresponding in size to clathrin heavy and light chains and an assembly polypeptide, are bound to the membranes from radioactively labeled cytosol. This suggests the presence of cytosolic pools of clathrin and assembly polypeptides. In previous studies, the presence of cytosolic pools and membrane-bound stores of clathrin have been demonstrated (*19, 57*). The amount of cytosolic clathrin seems to vary with cell type (*13*). The large sheets of assembled clathrin observed in macrophages and fibroblasts may represent frustrated phagocytosis or readily accessible pools of clathrin available for rapid recruitment (*19*). In other cell types both free triskelions and empty baskets have been observed as cytoplasmic forms of clathrin (*13, 57*). Whether clathrin is generally stored assembled or disassembled is not clear. Soluble assembly polypeptides can be detected in the cytosol but their cycling behavior is relatively uncharacterized (*49, 55*). If both assembly polypeptides and clathrin triskelions assemble on the membrane from a cystolic pool, there must be nucleating signals at the membrane that recruit these proteins.

Self-assembly of clathrin in vitro can be regulated by changes in *p*H, Ca^{2+} concentration, and ionic strength (*20*). Membrane-associated assembly and disassembly in vivo may be influenced by phosphorylation of clathrin and assembly polypeptides (*49, 58*). In reticulocytes, LC_b and 50-kD assembly polypeptide phosphorylation is increased in disassembled clathrin. The 50-kD assembly polypeptide is phosphorylated by a Ca^{2+}-independent kinase, which is associated with coated vesicles but distinct from the casein kinase that phosphorylates LC_b (*49, 53*). Phosphorylation of the 100-kD proteins is increased in assembled clathrin in reticulocytes (*49*) and observed in cultured neurons (*58*).

The dramatic effects induced by nonphysiological ion fluxes in cells suggest that more subtle ionic changes may contribute to regulation of clathrin assembly in vivo. Depletion of K^+ from extracellular medium disrupts assembled clathrin structures in some cell types, causing them to disappear (*59*). Lowering the *p*H of extracellular medium also effects clathrin-mediated processes, apparently preventing budding of clathrin-coated pits (*31, 60*). Both of these procedures cause clathrin to precipitate in small empty baskets in the cytoplasm or at the edge of coated pits (*60*). Identification of a proton pump associated with coated vesicles has suggested that the interior of a coated vesicle can acidify (*61*). Whether this is a property of both endocytic and exocytic coated vesicles is unclear, but regulation of enclosed *p*H might also affect the state of the

surrounding clathrin.

Two cytosolic proteins, a 70-kD heat-shock cognate protein (*52*) and tubulin (*62*), have been implicated in regulation of coated-vesicle formation and function. The heat-shock protein depolymerizes clathrin from coated vesicles in an adenosine triphosphate (ATP)–dependent manner, a requirement for such a protein having been predicted from a theoretical analysis of clathrin assembly and disassembly (*63*). The uncoating ATPase requires clathrin light chains for binding to triskelions (*52*), suggesting its binding could affect the light chain binding region of the triskelion involved in clathrin assembly. The MAbs binding to the clathrin heavy chain in this region induce partial clathrin depolymerization (*11*). The more complete disassembly induced by uncoating ATPase may result from its similarity to other heat-shock proteins that bind to and disrupt protein aggregates (*52*). Although uncoating by the ATPase has only been demonstrated in vitro, its physiological relevance is circumstantially supported by the observation that ATP depletion of cells causes arrest of clathrin recycling (*64*). Clathrin polymerization seems to provide the energy for the accompanying membrane vesiculation. However, tubulin is a component of coated vesicle preparations, which suggests that subsequent to budding, these vesicles are transported by microtubules (*65*). Since clathrin disassembles rapidly after vesicle formation, another coated vesicle–associated protein may mediate association of the uncoated vesicle with microtubules, thus accounting for the tubulin "contamination" of coated-vesicle preparations and providing a possible mechanism for vesicle targeting.

Role in Endocytosis

Expression of cell surface receptors is controlled by endocytosis, followed by recycling, degradation, or transcytosis (*1*). Clathrin-mediated internalization facilitates this process by concentrating receptors and increasing the efficiency of their delivery to endosomes where they are sorted into different intracellular pathways. Endocytosis caused by noncoated membrane invagination delivers internalized ligands and receptors to endosomes in a less efficient manner than clathrin-mediated uptake (*32*) and may suffice as a default pathway of internalization with no specificity for concentrating receptors. This pathway is involved in the internalization of ligands [toxins (*31, 32*) and virus (*15*)] for which no facilitated uptake would be expected and which may actually stimulate noncoated membrane invagination. Although fluid phase markers are internalized by all endocytic pathways (*15, 31*), most receptors are internalized by the facilitated route provided by clathrin. There are five documented exceptions (*66*), in contrast to the more than 20 different receptors that have been observed in clathrin-coated pits (*18*). Antibody to the "excluded" receptor Thyl causes capping, which suggests that these receptors are actively retained on the cell surface or that nonselective membrane invagination does not contribute substantially to endocytosis in the particular cells studied. Excluded Class I histocompatibility molecules have been observed in noncoated membrane invaginations after treatment with antibody, but the efficiency of their internalization is poor (*66*).

Attempts to define the molecular signals responsible for receptor uptake in clathrin-coated pits have implicated positive signals that can be either constitutive or regulated (Table 1). For receptors that serve an import function or bind to ligands on the surface of other cells, the signal for association with clathrin-coated pits is independent of ligand binding (*67*). Receptors that transduce signals when soluble ligands are bound are more concentrated in clathrin-coated pits when occupied by ligand (*68*). Ligand binding has differential effects on the postendosomal fate of receptors (*1, 18*), indicating that internalization and sorting are mediated by separate signals.

The effect of cytoplasmic tail mutations on receptor uptake by clathrin-coated pits has been studied for the low-density lipoprotein (LDL) receptor (*67*) and for hemagglutinin (*66*). For both molecules, the presence of a tyrosine residue in the cytoplasmic tail is critical for association with clathrin-coated pits. For other receptors that are internalized by coated pits, the effects of cytoplasmic tail mutations on internalization have been studied but their presence in clathrin-coated pits has not been examined (Table 1). Removal of the cytoplasmic tail from all receptors studied prevents internalization. In addition, phosphorylation has been studied as a possible signal for internalization. Removal of phosphorylation sites has no effect on transferrin and LDL receptor uptake (*67*), but phosphorylation events correlate with internalization of epidermal growth factor (EGF), insulin (*68*), and T cell receptors (*67*). These results suggest that the cytoplasmic tails of receptors carry positive but variable signals for association with clathrin-coated pits. However, the mutations studied might also prevent coated pit association through secondary effects on receptor aggregation or on the conformation of transmembrane or extracellular domains. Of molecules

Table 1. Characteristics of receptors internalized by clathrin-coated pits. The symbols indicate the following: +, required; −, not required; ±, event occurs with or without signal; NA, not applicable for this receptor; NE, not examined for this receptor.

Receptor	Trafficking (ligand requirement)				Cytoplasmic tail (internalization requirement)			
	Clathrin association	Recycling	Degradation	Transcytosis	Terminal	Presence	Phosphorylation	Tyrosine
Low-density lipoprotein receptor (*67*)	±	±	NA	NA	COOH	+	−	+
Asialogylcoprotein receptor (*67*)	±	±	NA	NA	NH_3^+	NE	NE	NE
Transferrin receptor (*67*)	±	±	NA	NA	NH_3^+	+	−	NE
Mannose-6-phosphate receptor (215 kD) (*67*)	±	±*	NA	NA	COOH	NE	NE	NE
Polymeric immunoglobulin receptor (*67*)	±	NA	NA	±	COOH	+	NE	+
F_c receptor (*67*)	±	−	+	NA	COOH	NE	NE	NE
Epidermal growth factor receptor (*68*)	±†	−	+	NA	COOH	+	+‡	NE
Insulin receptor (*68*)	+	+	NA	NA	COOH	+	+‡	NE
Class I histocompatibility antigen (*66*)§	±	−	+	NA	COOH	NE	NE	NE
T cell receptor (*67*)‖	−	−	NA	NA	COOH	NE	+	NE
Vesicular stomatitis G protein (*67*)	−	−	NA	NA	COOH	+	NE	NE
Hemagglutinin (*66*)¶	−	−	NA	NA	COOH	+	−	+

*Recycles to trans-Golgi rather than plasma membrane. †Ligand-independent internalization and recycling requires phosphorylation by protein kinase C. ‡Ligand-dependent internalization requires tyrosine kinase activity mediated by the receptor. §Clathrin association is observed only on activated T cells but not on B cells or fibroblasts. Antibodies induce uptake and degradation, and recycling occurs without antibodies, on activated T cells. ‖Internalization and recycling is assumed to be clathrin-mediated, but this has not been demonstrated directly. ¶Wild-type hemagglutinin is not internalized by clathrin even when cross-linked by antibody, but mutant hemagglutinin containing a tyrosine in the cytoplasmic tail associates with clathrin in a ligand-independent manner.

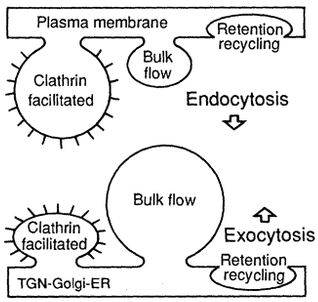

Fig. 4. Endocytosis compared with exocytosis. The diagram indicates relative contributions of membrane transport mechanisms. This scheme proposes that the import and export pathways differ in their relative use of bulk flow and clathrin-facilitated transport to move membrane-bound and -secreted proteins. Retention signals or recycling can exclude proteins from transport in either direction. Clathrin plays a facilitative role in both directions by concentrating the receptors involved. ER, endoplasmic reticulum; TGN, trans Golgi network (*21*).

that are excluded from clathrin-coated pits (*66*), glycophorin may be retained on the cell surface by association with the erythrocyte cytoskeleton and Thyl may have unusual properties because of its glycosyl-phosphatidylinositol membrane anchor. Whether receptors accumulate in clathrin-coated pits or whether assembly of coated pit components takes place at receptor tails has not been resolved. The end result is that before internalization many receptors are preferentially concentrated in clathrin-coated regions, which results in the facilitation of receptor delivery to endosomes.

Role in Exocytosis

In exocytic membrane traffic, clathrin-mediated transport is less general than the default or bulk flow pathway (*3*). However, protein export can be prevented by either retention signals or recycling in ways that are analogous to those found in receptor-mediated endocytosis (*69*).

Clathrin-coated vesicles facilitate the mannose-6-phosphate receptor-mediated transport of lysosomal enzymes from the Golgi apparatus to a prelysosomal compartment (*22, 27, 70*). Here they are sorted into lysosomes and the dissociated receptors are recycled back to the Golgi apparatus. In the formation of hormone-containing secretory granules (*2, 23, 24*), it appears that clathrin-coated pits and vesicles remove membrane from the site of a condensing secretory bud that will eventually pinch off from the trans-Golgi network to form a secretory granule. Although there is no direct evidence for receptors that bind prohormone, the presence of clathrin suggests that prohormone may be initially concentrated by a receptor-mediated process. After granule acidification (*2*) and prohormone processing, such receptors could be removed by clathrin, resulting in granule condensation. This scheme again postulates that the role of clathrin is to concentrate and sort receptors.

An outstanding question is whether mammalian clathrin is involved in the export of constitutively secreted proteins and normal membrane proteins. Export of constitutively secreted proteins does not seem to be receptor-mediated, nor to require concentration (*2*). However, both newly synthesized secretory immunoglobulin and cholinesterase have been found in preparations of clathrin-coated vesicles (*26*). Perhaps exported proteins, synthesized in large amounts, are trapped in clathrin-coated vesicles in the Golgi, just as a fluid phase marker undergoes endocytosis in clathrin-coated vesicles. Without morphological data examining the primary route of constitutive protein secretion, it is hard to assess the contribution of clathrin to this pathway in mammalian cells.

Export of viral glycoproteins, which are synthesized in vast quantities in infected cells, is not mediated by clathrin-coated vesicles (*21, 24*). However, the export of endogenous membrane proteins, expressed at lower levels on the cell surface, might be clathrin-facilitated, particularly in cases where targeting to a specialized compartment occurs en route. Transport of histocompatibility antigens may provide an example of this situation (*26*). These cell-surface glycoproteins bind antigen fragments and thus form the ligand for the T cell receptor (*71*). Class I histocompatibility proteins generally bind fragments of endogenously synthesized proteins, a process that involves protein synthesis and presumably occurs during the export pathway (*72*). Peptides derived from incorrectly folded and subsequently degraded proteins could bind Class I glycoproteins throughout the biosynthetic pathway. However, clathrin-facilitated transport to an acidic prelysosomal sorting compartment might enhance binding or exchange of peptides. Class II histocompatibility glycoproteins are generally involved in T cell responses to fragments of exogenous antigens (*73*). In this case peptide binding is independent of protein synthesis and is probably a function of endocytosis and recycling (*72*). However, the exocytic pathway may provide an additional route for peptide binding by Class II molecules. Cell-surface expression of Class II glycoproteins requires a *p*H-dependent dissociation of the invariant chain subunit (*74*). This may involve clathrin-mediated transport to an acidic compartment, a potential site for encountering antigenic peptides. Thus, for histocompatibility antigens, clathrin-facilitated export could enhance their biological function.

Universal Function for Clathrin

The experimental evidence for clathrin's involvement in the endocytic and exocytic pathways suggests a resolution to the controversy surrounding its role in intracellular membrane traffic and, indeed, its biological necessity. Clathrin's contribution to intracellular traffic is quantitative rather than absolute; clathrin assembly concentrates and sorts receptors, facilitating their transport from one membrane to another. It performs this function in both the import and export pathway, enhancing transport provided by bulk flow in either direction (Fig. 4). Because of differential requirements for facilitated transport in the endocytic as opposed to exocytic pathway, clathrin is used to different degrees in the two pathways. Localized control of clathrin assembly through diverse molecular signals from receptors and coated pit components allows its universal function to be applied to specific intracellular targeting (*75*).

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