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Review

Common principles in clathrin-mediated sorting at the Golgi and the plasma membrane

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Abstract

Clathrin-mediated vesicular trafficking events underpin the vectorial transfer of macromolecules between several eukaryotic membrane-bound compartments. Classical models for coat operation, focused principally on interactions between clathrin, the heterotetrameric adaptor complexes, and cargo molecules, fail to account for the full complexity of the coat assembly and sorting process. New data reveal that targeting of clathrin adaptor complexes is generally supported by phosphoinositides, that cargo recognition by heterotetrameric adaptors depends on phosphorylation-driven conformational alterations, and that dedicated clathrin-associated sorting proteins (CLASPs) exist to promote the selective trafficking of specific categories of cargo. A host of accessory factors also participate in coat polymerization events, and the independently folded appendage domains that project off the heterotetrameric adaptor core function as recruitment platforms that appear to oversee assembly operations. It is also now clear that focal polymerization of branched actin microfilaments contributes to clathrin-coated vesicle assembly and movement at both plasma membrane and Golgi sites. This improved appreciation of the complex mechanisms governing clathrin-dependent sorting events reveals several common principles of clathrin operation at the Golgi and the plasma membrane.

Keywords: Clathrin-coated vesicle; AP-1/AP-2 adaptors; Receptor sorting; Cargo selection; Phosphoinositide; Phosphorylation

1. Introduction

A striking characteristic of the delicate Golgi cisternae clearly visible in transmission electron microscope images of mammalian cells is the profusion of small vesicular elements amassed at both *cis*- and *trans*-faces of the stacked organelle [1]. Many of these structures correspond to small, membrane-encapsulated transport intermediates, which shuttle proteins and lipids to the *cis*-Golgi from the endoplasmic reticulum, and from the *trans*-Golgi to the cell surface or endosomes. High-voltage electron microscope tomography studies have further uncovered that in mammalian cells, the Golgi apparatus is typically composed of five to seven closely apposed cisternae. The most distal element of the *trans*-face, which displays a highly pleomorphic and fenestrated morphology, is dedicated exclusively to the formation of clathrin-coated vesicles [2,3]. These vesicle carriers deliver newly synthesized lysosomal hydrolases to the endosomal compartment predominantly via an intracellular route; the trajectory of these transport vesicles does not routinely cross the plasma membrane as this represents only a subset of the trafficking operations of the *trans*-Golgi network (TGN). One important implication of these morphological findings is that molecules destined for transport to the cell surface must apparently exit the TGN from penultimate cisternae [2], while components destined for clathrin-mediated export from the TGN must be segregated to avoid entering the exit sites for these alternate traffic streams. This review focuses on recent advances in our understanding of the mechanics of clathrin-coat formation and cargo selection, specifically in nonpolarized mammalian cells, and on the functional properties of some of the many proteins now known to participate in clathrin-coated vesicle formation. The process of tubulo-vesicular sorting of cell surface or secreted proteins into post-Golgi carriers and specific trafficking of apical and basolateral proteins from the TGN are reviewed elsewhere in this special issue.

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2. Clathrin-based sorting in mammalian cells

Clathrin-coated vesicles are assembled solely for the purpose of selective transport of designated cargo molecules between membrane-bound intracellular compartments. The process occurs rapidly, within a few minutes and, in the simplest sense, coordinates membrane deformation with preferential garnering and retention of cargo to generate a roughly spherical, transitory transport carrier. In classical models for clathrin-dependent sorting, a triad of protein components–transmembrane cargo receptors, adaptor heterotetramers, and clathrin triskelia–are positioned centrally. The clathrin triskelion is composed of three ~190-kDa heavy chains that trimerize through a carboxy-terminal helical tripod arrangement [4]; each fibrous heavy chain radiates from the central hub oriented at roughly 120° to one another (Fig. 1). The heavy chain also has a ~25-kDa clathrin light chain bound in an extended manner near to the central vertex [4]. The unusual geometry of the trimer imparts a strong propensity on the molecule to assemble into regular polyhedral structures, established primarily through interactions involving antiparallel packing of the distal and proximal leg regions of adjacent trimers. Yet, the regions important for polymeric lattice assembly do not promote membrane association and, alone, clathrin has no affinity for biological membranes. Instead, two major clathrin-associated heterotetrameric adaptors (AP-1 upon the Golgi and endosomes, and AP-2 at the plasma membrane) bind to clathrin by directly engaging the amino terminal domain of the clathrin heavy chain, which is folded into a 7-bladed β-propeller structure [5]. This common property is due largely to the β subunit; the AP-1 β1 chain is approximately 85% identical to the AP-2 β2 subunit, and both contain a clathrin-binding sequence termed the clathrin box (Table 1). AP-1 and AP-2 (as well as the related AP-3 and AP-4) complexes exhibit a conserved subunit composition and overall architecture (Fig. 1) [6]. Each contains two large subunits, γ and β1 in AP-1 and α and β2 in AP-2, a medium (μ1 or μ2) and a small (σ1 or σ2) subunit. Despite little primary sequence identity, the amino-terminal segment of the large chains (termed the trunk) has an analogous tertiary structure, being composed of an α-helical solenoid fold [7,8]. During biosynthesis, the α or γ trunk likely forms a hemicomplex with the σ2 or σ1 subunit, respectively. The β1/2 subunits probably form a structurally similar hemicomplex with the μ1/2 subunit as the amino terminal region of the μ subunit displays primary sequence homology to the σ subunits [9], and adopts the same tertiary structure [7,8]. The two hemicomplexes associate to form the tightly associated, roughly bilaterally symmetrical heterotetrameric core [7,8] (Fig. 1; for a comprehensive review of adaptor structure, see [10]).

To gain preferential entry into forming clathrin coats, transmembrane proteins require a dedicated sorting signal or tag (Table 2) [11]. The carboxy-terminal β-sandwich domain of the μ subunit of AP-1 and AP-2 (and AP-3 and AP-4) binds directly to YXXØ-type (where X represents any amino acid and Ø indicates a residue with a bulky hydrophobic side chain) internalization sequences [10,11], although each μ subunit does display individual preferences for particular residues at the X and Ø positions [11,12]. Another sorting signal, the [DE]XXXL[LI] motif, binds to an as yet unknown surface upon an AP-1 γ/σ1 or AP-3 δ/σ3 hemicomplex [13]. Because [DE]XXXL[LI] is also a potent endocytic sorting signal [14], it is likely that in vivo a similar hemicomplex of α/σ2 engages this motif as well, although, on the basis of chemical cross linking experiments, it has been argued that the [DE]XXXL[LI] sequence binds physically to the β subunit [15]. Irrespective, adaptors clearly exhibit the functional capability of binding to both clathrin and cargo molecules synchronously. AP-1 can also bind to another poorly understood acidic cluster sorting sequence found within the cytosolic domains of the cation-dependent (CD) and cation-independent (CI) mannose 6-phosphate receptors (MPRs) [16–18], but the location of the contact site on the adaptor complex is currently unknown.

For some time now, this general information has been synthesized into the widely accepted 'text book' model for clathrin-mediated sorting [19,20]. In this model, coat assembly is initiated by a constitutive interaction between cytosol-oriented sorting signals and adaptors at the surface of the membrane. Concerted translocation of clathrin

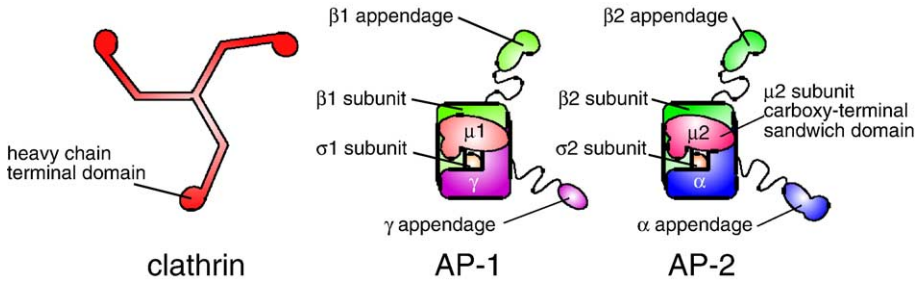

Fig. 1. Classical core clathrin-coat components. Schematic illustration of the general organization of the clathrin triskelion, AP-1, and AP-2. Images are modeled on the known molecular structures of the coat components, but are not drawn to scale. In both AP-1 and AP-2, the independently-folded globular appendages that project off the heterotetrameric adaptor core are flexible due to the unstructured properties of hinge region that connects each appendage to the core. The appendages serve as platforms to coordinate the protein–protein interaction networks established during coat assembly. Details of web connectivity are provided in Fig. 2, while the atomic structures, interaction surfaces, and differing modes of interaction motif engagement are presented in Fig. 3.