Review

Vesicle coats: structure, function, and general principles of assembly

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The transport of proteins and lipids between distinct cellular compartments is conducted by coated vesicles. These vesicles are formed by the self-assembly of coat proteins on a membrane, leading to collection of the vesicle cargo and membrane bending to form a bud. Scission at the bud neck releases the vesicle. X-ray crystallography and electron microscopy (EM) have recently generated models of isolated coat components and assembled coats. Here, we review these data to present a structural overview of the three main coats: clathrin, COPII, and COPI. The three coats have similar function, common ancestry, and structural similarities, but exhibit fundamental differences in structure and assembly. We describe the implications of structural similarities and differences for understanding the function, assembly principles, and evolution of vesicle coats.

Transport vesicle formation

Eukaryotic cells segregate functions in membrane-delimited compartments. These intracellular compartments are not static: they exchange proteins and lipids continuously in a directional and regulated manner [1]. The exchange of material (cargoes) between compartments is mostly conducted by coated transport vesicles that bud from one membrane and fuse with another. Transport vesicles are hence essential for maintaining organelle identity and lipid homeostasis and for the secretion of proteins.

The formation of transport vesicles is mediated by cytosolic coat proteins. These proteins can bind each other as well as the membrane of a compartment and can interact with cargoes. To form a transport vesicle, the coat proteins must collect cargo, must induce membrane bending to form a coated bud, must coordinate membrane scission to release a vesicle, and must then disassemble to allow fusion of the vesicle with the target membrane.

The molecular mechanisms underlying these processes are, despite extensive research, still not fully understood. Recent advances using structural biology approaches including X-ray crystallography, cryo-EM, and cryoelectron tomography (cryo-ET) have given new structural insights into the protein complexes involved (Box 1). Combining structural and biochemical approaches is advancing our understanding of the dynamic and complex mode of assembly and disassembly of coated transport carriers.

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The three best-characterized types of vesicular carrier involved in intracellular trafficking are distinguished by their different coat proteins and their different trafficking routes. Clathrin-coated vesicles (CCVs) act in the late secretory pathway and in the endocytic pathway, COPII-coated vesicles export proteins from the endoplasmic reticulum (ER), and COPI-coated vesicles shuttle within the Golgi organelle and from the Golgi back to the ER. Despite having different compartment specificities and different structural components, the mechanisms of their formation follow similar rules. The time and place at which vesicle formation occurs are most often regulated by small GTP-binding proteins. In these cases, vesicle formation is initiated by activation of a small GTPase, stimulated by specific guanine exchange factors. The small GTPase exposes an N-terminal amphipathic helix that anchors the protein to the outer leaflet of the membrane, then recruits coat protein complexes that further interact with cytosolic cargo-recognition sequences [2–4]. In endocytosis, a small GTPase is not required for initiation; instead, the AP2 adaptor complex is recruited to the membrane by phosphatidylinositol phosphates (PIPs) [5]. Coat protein complexes have a common organization: they can be functionally divided into adaptor and cage complexes. In the case of clathrin or COPII, the adaptor complexes (including AP1–5, AP180, and the Golgi-localizing, γ-adaptin ear containing, ARF-binding (GGA) proteins for clathrin, and Sec23-24, for COPII) are first recruited to the membrane, followed by the cage complexes that polymerize to form the protein lattice or meshwork that constitutes the 'cage' of a coated vesicle. In the case of the COPI coat, the adaptor and cage complexes are associated as a single heptameric complex, which is recruited to the membrane *en bloc* [6]. Assembly of the protein coat, in some cases with the assistance of other cellular machineries such as the actin cytoskeleton, leads to concentration of the vesicle cargo and membrane curvature to form a bud. Additional activities, either present within the coat proteins or mediated through the GTPase [7,8], then induce scission at the neck of the bud, releasing the vesicle from the donor membrane (Box 2). Lastly, the coat depolymerizes under the effect of GTP hydrolysis mediated by GTPase-activating proteins (GAPs) [9] or by GAP activity within the coat protein complex. Alternatively, clathrin coats are destabilized by ATP hydrolysis of HSC70 [10]. Depolymerization uncoats the vesicle, making it competent for fusion with its target membrane.

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Box 1. Methods in structural biology of coats

Coat proteins are complex machineries that represent a challenge for structural biology. They have a broad range of sizes (from 50 to 600 kDa) and they are often flexible, because they have to interact with multiple cargo proteins and bend membranes. To understand them, it is necessary to use a combination of diverse structural techniques that span different sizes and resolutions.

X-ray crystallography is a structural technique whereby a crystal, formed from purified protein, is irradiated with X-rays and the resulting diffraction pattern is interpreted to obtain an atomic model. Proteins can be cocrystallized with binding peptides or other proteins to identify sites of interaction. The main limitation of the technique is the requirement for the formation of a protein crystal. This involves selection of suitable protein constructs that will usually only include stable, less flexible parts of a complex.

In single particle electron microscopy, thousands of noisy images of copies of the same biological object are combined in a 3D electron-density model. The sample is either stained with a heavy metal salt or frozen in vitreous ice, preserving all of its physiological conformations. The technique is applicable to purified complexes, with an effective minimum size limitation of about 150 kDa. The resolution attainable is limited by the conformational flexibility and size of the sample, but is typically between 4 Å and 30 Å. Where the sample contains more than one conformation of the protein complex, these can often be sorted from one another and analyzed separately. The resulting electron density reveals the shape of the protein and may reveal structural differences between conformations. When combined with atomic models of individual subunits, pseudoatomic models can be built to identify protein-interaction interfaces.

Cryo-electron tomography is a related technique whereby a unique object is imaged from several directions by rotating it within the electron microscope. These views are reconstructed as a 3D density map. It has been used to show the structure of unique assembled coated vesicles and to assess their heterogeneity. The resolution is limited to about 40 Å and is not the same in all directions (anisotropic resolution).

Subtomogram averaging is an emerging structural technique based on local averaging of volumes extracted from electron tomograms. For example, where cryo-ET has been applied to coated vesicles, subtomogram averaging can subsequently be used to identify and average the many copies of the basic building block of the coat contained within the tomogram. In this way, higher-resolution structural data can be obtained, typically at a resolution of between 20 Å and 40 Å, and the resolution is the same in all directions. The positions at which the many copies of the structure were identified can be mapped in 3D in the original position in the tomogram to observe, for example, the arrangement of the building blocks within the assembled coat.

Here, we will describe and compare the structural biology of the coats at multiple levels: their component proteins and protein domains, their cytosolic complexes and subcomplexes, and their assembled cage-like forms. In all cases, the structural biology has profound implications for understanding function and mechanism. Structural biology can help us to answer questions such as: 'how is cargo identified and distinguished?'; 'how does coat polymerization form a curved protein shell?'; 'how can the same proteins form different-sized vesicles?'; and 'how is formation of a coated vesicle initiated and regulated?'. For other recent reviews of related topics, the reader is

Box 2. A function of coats in membrane scission involving GTPases?

Various models have been advanced to explain the function of dynamin in endocytosis [60–63]. Repeated cycles of GTP loading and hydrolysis trigger conformational changes in a helical dynamin lattice on the bud neck. This leads to narrowing of the helix and constriction of the bud neck [64]. Recent work suggests that the change in curvature at the boundary of the dynamin lattice causes an increase in the local elastic energy of the membrane, reducing the scission energy barrier [65]. Scission therefore occurs at the edge of the dynamin helix. Another recent report on scission in clathrin-mediated endocytosis implicates epsin as a major contributor to scission [66]; shallow insertion of the amphipathic helix of epsin into the membrane induced scission. Downregulation of epsin isoforms led to fission arrest of clathrin-coated structures in mammalian cells, whereas slight overexpression of epsin could palliate a CCV scission defect on downregulation of dynamin. When dynamin disassembly was blocked, epsin did not support formation of CCVs [66]. These findings hint that dynamin may play a regulatory role where its disassembly is required to allow shallow insertion of the epsin amphipathic helix contributing to scission.

Like epsin, small GTPases tubulate liposomes *in vitro* using amphipathic helices that are inserted shallowly into the outer leaflet of membranes, inducing bilayer curvature. *In vivo*, shallow insertion into a membrane does not necessarily cause curvature in the absence of additional proteins, such as vesicular coat proteins, but can be a prerequisite for curvature. We define the contribution of shallow insertion of amphipathic helices into membranes as 'potentiating membrane curvature'. In the COPII system, Sar1p was shown *in vitro* to potentiate membrane curvature [8], depending on its N-terminal amphipathic helix. In the COPI system, Arf1 with its myristoylated N-terminal amphipathic helix potentiates membrane curvature, strictly depending on dimerization of the small GTPase [67].

COPI- and COPII-coated vesicles were originally accumulated and purified using non-hydrolyzable analogs of GTP [33,68,69]. This independence of GTP hydrolysis was questioned with the suggestion that the vesicles were not released by a biochemical mechanism but rather by mechanical shearing during the experimental preparation, [64,70,71]. Nevertheless, we are convinced by the multiple reports showing that small GTPase-dependent COPI and COPII vesicles form and are released without hydrolysis of GTP from native membranes [33,68,72–74] or chemically defined liposomes [56,57,75]. Free vesicles are observed by cryo-EM of incubations of liposomes with coat proteins and GTPγS under conditions where a 'fission-arrest' point mutant of Arf1 yields no free vesicles but a series of buds that are continuous with the donor membrane [7]. A similar fission arrest was observed *in vitro* for COPII when a Sar1p variant was used, lacking its N-terminal amphipathic helix [76]. This suggests it is unlikely that GTP hydrolysis-independent scission is an experimental artifact. When directly compared in semi-intact cells, COPI and COPII vesicles are formed (and released without any further manipulation) with GTP, GTPγS or GMPPNP or using constitutively activated small GTPases (our observation).

A role in regulating vesicle formation has recently been discussed for arfaptin-1. Depending on arfaptin-1's phosphorylation state, it can sequester Arf1-GTP and antagonize the formation of insulin granules *in vivo* or COPI vesicles *in vitro* [77].

These findings suggest to us that, in all three systems – clathrin, COPI, and COPII – scission may be mediated by the insertion of amphipathic helices into the membrane by epsin, Arf1, or Sar1. This mechanism would be independent of GTP hydrolysis (e.g., epsin is not a GTPase). There are many possible models of how such insertion could drive scission. We have suggested [7] that insertion of the amphipathic helices creates a high-energy state at the neck of forming buds in zones of growing negative curvature. The helices would be prevented from moving out of this zone by their interactions with the coat proteins, so the high-energy state would be relaxed by separation of the vesicular membrane from the donor membrane. Regulation of this process would then be mediated by preventing access of the scissase to the neck of the bud (by dynamin) or by sequestering the activated scissase (by arfaptin).