OPINION

Coat proteins: shaping membrane transport

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Coat proteins allow the selective transfer of macromolecules from one membrane-enclosed compartment to another by concentrating macromolecules into specialized membrane patches and then deforming these patches into small coated vesicles. Recent findings indicate that coat proteins might also participate in the differentiation of membrane domains within organelles and large transport carriers, as well as in the association of the carriers with the cytosketelon and with acceptor organelles.

A hallmark of eukaryotic cells is the presence of an elaborate endomembrane system that is responsible for the exchange of macromolecules between cells and their environment. In this system, the secretory pathway delivers newly synthesized proteins, carbohydrates and lipids to the outside of the cell, whereas the endocytic pathway takes up macromolecules into the cell. Transport along these pathways occurs by the transfer of secretory or endocytic cargo between different membrane-enclosed organelles. Despite this transfer, each organelle maintains its characteristic set of resident macromolecules.

How selective transport between membrane-enclosed organelles occurs and how, in the face of this transport, organellar identity can be maintained are questions that have fascinated biologists for decades. In this article, we discuss how these problems have been addressed in the framework of the coated-vesicle model, and consider the recent discoveries that are leading to a broader view of coat-protein function.

Clathrin-coated vesicles

Early electron microscopy (EM) studies of protein trafficking led to the formulation of the vesicle-transport hypothesis[1]. This hypothesis proposed that transport intermediates, which are enclosed by a membrane ('vesicles'), pinch off a donor organelle and fuse with an acceptor organelle, and that these vesicles carry a specific set of macromolecules ('cargo') as part of the process. For this process to achieve selectivity, the sorting of cargo away from resident macromolecules would have to occur at sites of vesicle formation on the donor organelle. The resulting vesicles would then need to be targeted accurately for fusion with the correct acceptor organelle. In this way, the directed transport of macromolecules through secretory and endocytic pathways could be achieved and compartmental identity retained.

Given this conceptual framework, the challenge was to identify the vesicular transport carriers and to explain how they are formed and consumed. The initial clues to the nature of the carriers came from EM studies that identified a population of small vesicles (60–100 nm in diameter) that were encircled by a thick, electron-dense 'coat'[2,3] (FIG. 1a). These 'coated vesicles' were generally observed in close proximity to the plasma membrane or the *trans*-Golgi network (TGN), and membranes that were covered with the same type of coat could be seen budding from these organelles. Both the coated vesicles and buds were found to contain cargo macromolecules that traffic between different intracellular compartments — hinting that these vesicles were the hypothetical carriers.

Coated vesicles were later purified to homogeneity and found to consist of spherical membrane vesicles that are encased in a lattice-like shell[4]. Biochemical analyses subsequently showed that the main constituent of the coats was a structural protein — clathrin[5] — that is composed of three heavy chains and three lights chains[6] (FIG. 2). The coats also contained either of two heterotetrameric adaptor protein (AP) complexes, AP1 or AP2, which are present in clathrin-coated vesicles (CCVs) that are derived from the TGN or the plasma membrane, respectively[7] (FIG. 2; TABLE 1). These AP complexes are known to have many roles in the coats, such as the recruitment of clathrin to specific organellar membranes[8], the selection of specific transmembrane proteins for incorporation into CCVs[9], and the binding of accessory factors that regulate coat assembly and disassembly, vesicle formation or targeting and interactions with the cytoskeleton[10]. Recent studies have identified a family of monomeric adaptors that are known as the GGAs (for Golgi-localized, γ-ear-containing, ADP-ribosylation factor-binding proteins), which are components of TGN clathrin coats and fulfil many of the same functions as the AP complexes[11] (FIG. 2; TABLE 1). In addition, other clathrin-associated proteins — such as epsins, epidermal growth factor receptor substrate 15 (Eps15), Eps15 related sequence (Eps15R) and hepatocycte growth factor-regulated tyrosine kinase substrate (Hrs) — participate in the recruitment of ubiquitylated transmembrane proteins to clathrin-coated areas of the plasma membrane and endosomes[12,13].

The discovery and biochemical characterization of CCVs provided the foundation for general models of intracellular transport that is mediated by coated vesicles[14–16]. In the most common model, AP1 or AP2 are first recruited to the membrane of a donor

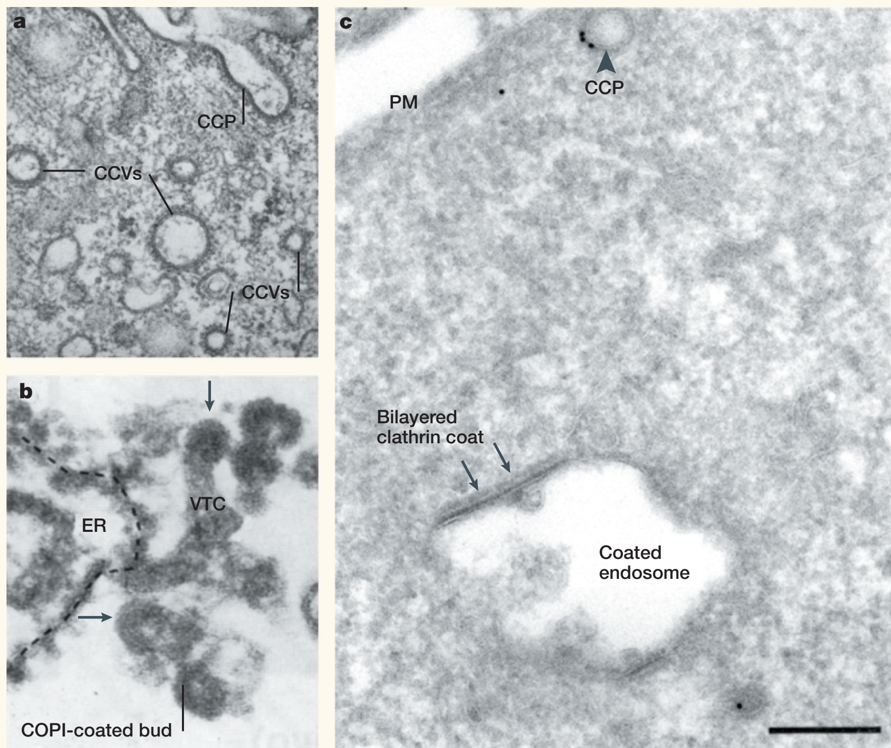

Figure 1 | **Morphological diversity of coated structures. a** | Electron microscopy (EM) of conventional clathrin-coated vesicles (CCVs) in an epithelial cell. The micrograph shows the different sizes of CCVs (60–100 nm in diameter). A clathrin-coated pit (CCP) is also evident in the picture. Reproduced with permission from REF. 3 © the Rockefeller University Press (1967). **b** | EM of a vesicular–tubular carrier (VTC) that was formed by the incubation of permeabilized cells with activated Sar1 (a COPII-associated GTP-binding protein) and ADP-ribosylation factor 1. VTCs consist of clusters of ~60 nm vesicles or buds that are linked by membrane tubules. The dashed line indicates the endoplasmic reticulum (ER) membrane. Reproduced with permission from REF. 40 © the Rockefeller University Press (1995). **c** | ImmunoEM of an endosome covered with a bilayered clathrin coat. Cells were immunolabelled with an antibody to the β1 and β2 subunits of adaptor protein (AP)1 and AP2, respectively. Notice the presence of labelling on a plasma membrane (PM) CCP, but not on the bilayered clathrin coat. The scale bar represents 200 nm. Reproduced with permission from REF. 47 © the American Society for Cell Biology (2002) and kindly provided by M. Sachse and J. Klumperman (Utrecht University, the Netherlands). COP, coatomer protein.

organelle by binding to a putative docking factor or factors. Members of the ADP-ribosylation factor (Arf) family of small GTP-binding proteins — in particular, Arf1 and Arf3 — regulate the recruitment of AP1 to the TGN and endosomes. The Arfs function as binary switches for coat formation. In their GTP-bound state, the Arfs are active and coats are assembled, whereas in their GDP-bound state they are inactive and coats are disassembled. The factors that allow the Arfs to be converted to their active state (guanine nucleotide exchange factors or GEFs) or inactive state (GTPase-activating proteins or GAPs) therefore have a key role in regulating the assembly and disassembly of AP1-containing coats[17]. The binding of AP2 to membranes, on the other hand, is apparently not regulated by Arfs but by phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2)[16]. Clathrin subsequently polymerizes onto the membrane-bound AP1 or AP2 complexes, which leads to the assembly of the coat scaffold.

Specific transmembrane proteins and their lumenal cargo molecules become concentrated at the coated membrane domains by virtue of interactions between sorting signals in the cytoplasmic domains of the transmembrane proteins and the AP complexes, the GGAs or other clathrin-binding proteins (TABLE 1). The initially flat, coated membrane domains become curved, probably as a result of the mechanical deformation that is induced by the remodelling of the clathrin lattices. Recently, the accessory factor epsin 1 has been found to contribute to membrane bending and budding by inserting part of its epsin amino-terminal homology (ENTH) domain into the inner leaflet of the plasma membrane[18]. Other accessory factors might have a similar role at the TGN. The resulting coated buds pinch off as small CCVs that subsequently uncoat and fuse with an acceptor organelle (FIG. 3a).

Non-clathrin coats

The applicability of the CCV model expanded markedly with the discovery of other coat-protein complexes (FIG. 2; TABLE 1). These included: coatomer protein (COP)I (REF. 19), which associates with pre-Golgi and Golgi membranes and is involved in membrane trafficking between the endoplasmic reticulum (ER) and the Golgi complex; COPII (REF. 20), which binds to ER exit sites and mediates export from the ER; and AP3 (REFS 21,22) and AP4 (REFS 23,24), which function in protein sorting at endosomes and/or the TGN.

COPI, COPII and AP4 do not interact with clathrin and are therefore referred to collectively as 'non-clathrin coats'. Mammalian AP3 can interact with clathrin[25], but this interaction is not thought to be essential for function[26]. Four of the COPI subunits, as well as the four subunits of AP3 and AP4, are homologous to the subunits of AP1 and AP2 (REF. 27; FIG. 2; TABLE 1). The subunits of COPII, on the other hand, are structurally unrelated to those of the other coats[28,29]. EM analyses have shown that all of these coat proteins form electron-dense deposits on 60–100 nm buds or vesicles[24,25,30,31]. Small vesicles that are coated with COPI and COPII have been produced *in vitro* and functionally characterized[31,32]. As is the case for AP1, the recruitment of COPI, AP3 and AP4 to membranes is regulated by Arf1 and Arf3, whereas the recruitment of COPII is regulated by the Arf-related protein Sar1. In addition, COPI, COPII, AP3 and AP4 recognize sorting signals that are present in the cytoplasmic domains of transmembrane proteins (TABLE 1). So, by many criteria, these new coats resemble clathrin coats and are likely to function in similar ways. It remains to be determined, however, whether all forms of cargo transport occur as described by the CCV model.

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Pleiomorphic transport carriers

Until recently, the manner in which proteins are transported between compartments of the secretory and endocytic pathways was inferred largely from EM studies of fixed cells and from the biochemical characterization of isolated organelles. Recently, imaging of the transport of green fluorescent protein (GFP)–cargo protein constructs — between the ER and the Golgi complex[33], from the TGN to the plasma membrane[34] or the endosomal system[35–37], and along the axon[38,39] — in mammalian cells has shown that there are carriers that are larger and more pleiomorphic than conventional CCVs. These carriers can appear as vesicles up to 1 µm in diameter, tubules up to 10 µm in length, or vesicular–tubular structures of various sizes and shapes. These carriers show considerable plasticity, often changing shapes or dividing during transport. They move at speeds of ~1 µm s^{-1} predominantly in one direction. The depolymerization of microtubules or interference with microtubule motors causes immobilization of the carriers, which indicates that they are too big to diffuse freely through the cytoplasm and must therefore move along microtubule tracks.

The morphology of these transport carriers is therefore quite distinct from that of conventional CCVs. Some of these carriers — such as post-Golgi carriers that transport the G protein of the vesicular stomatitis virus (VSV-G) from the TGN to the plasma membrane — do not seem to have any known associated coat[34]. Other carriers, however, have classic coats. The large intermediates that carry the VSV-G protein from ER exit sites to the Golgi complex, for example, contain COPI (REF 33; FIG. 4a) — a coat that, paradoxically, mediates the retrograde transport of some proteins from the Golgi complex to the ER (TABLE 1). These intermediates probably correspond to the vesicular–tubular carriers that have been seen previously by EM and that consist of a proliferation of tubules with 60–100-nm coated buds[40] (FIG. 1b). Other examples of such coated carriers are the vesicular–tubular structures that bud from the TGN and contain associated clathrin[37], AP1 (REFS 35–37) or GGA1 (REF. 37). Coats containing these proteins often appear as swellings on tubules, which is indicative of an association with specific domains. The existence of these large coated intermediates therefore signals a departure from models that are based on small quantum packets of cargo as the only mediators of vesicular transport.

Conventional CCVs that are labelled with GFP-tagged clathrin have been visualized budding from 'hot-spots' at the plasma membrane[41]. However, CCVs have been more difficult to pinpoint in other cellular locations, probably because of their small size, faint labelling, transient nature or proximity to larger organelles. The role of CCVs and other small coated vesicles might be limited to the short-range transfer of cargo between neighbouring membrane-enclosed organelles (for example, CCV-mediated transfer between the plasma membrane and nearby endosomes, COPII-coated-vesicle transport from the ER to vesicular–tubular carriers, and the shuttling of COPI-coated vesicles between adjacent Golgi cisternae), whereas the large transport carriers could allow long-range distribution of cargo through the cytoplasm. The large transport carriers could form either by the fusion of small vesicles (FIG. 3a,b) or by direct budding from the donor organelles (FIG. 3b). So far, the presence of these large transport carriers has not been shown in lower eukaryotes such as yeast, and they could therefore be an adaptation that is needed to cope with the longer distances that have to be travelled in the cytoplasm of metazoan cells.

Cargo transport that is mediated by large carriers could offer other advantages to cells. First, these carriers might accommodate a wider range of cargoes, from small molecules to large supramolecular complexes (for example, procollagen) that do not fit inside conventional coated vesicles. Second, the presence of coats on the carriers could facilitate their maturation either by creating partitioned domains or by allowing the removal of constituents through coated-vesicle budding. Third, the persistence of the

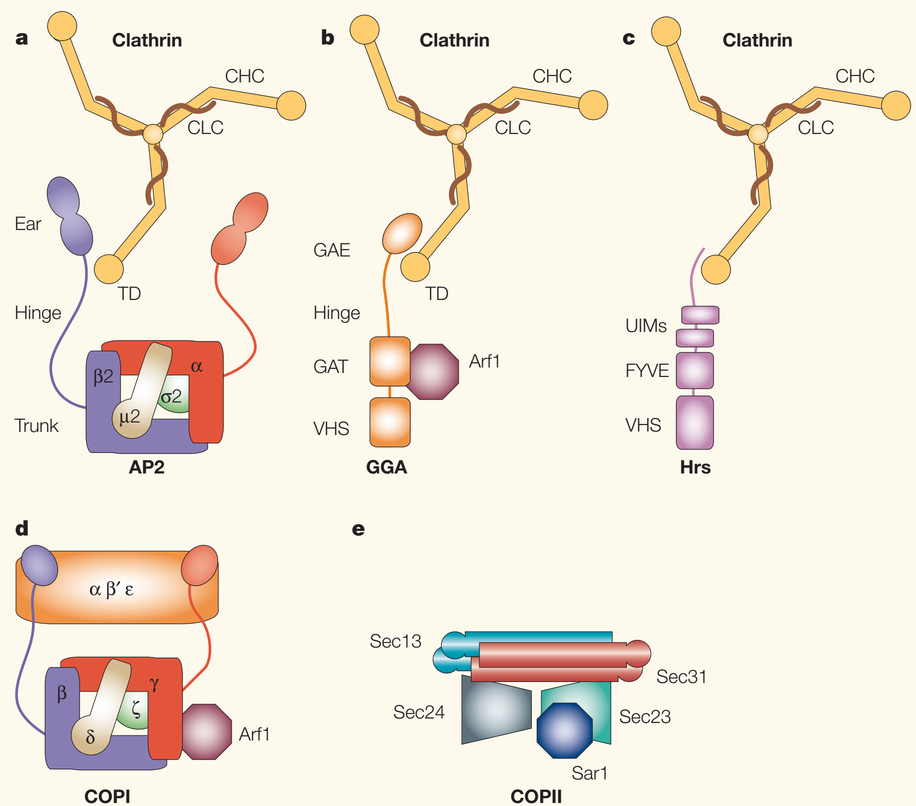

Figure 2 | **A schematic representation of the components of various protein coats. a** | A clathrin triskelion (CHC, clathrin heavy chain; CLC, clathrin light chain) is shown interacting through its terminal domain (TD) with the appendage domains of the β2 subunit of adaptor protein (AP)2. The AP2 scheme incorporates features that were revealed by the recent high-resolution crystal structure of the AP2 core[51]. AP1, AP3 and AP4 are expected to display a similar subunit architecture. **b** | Clathrin is shown interacting with the hinge and GAE (γ-adaptin ear) domains of a generic GGA (for Golgi-localized, γ-ear-containing, ADP-ribosylation factor-binding protein). **c** | Clathrin is shown interacting with Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate). This interaction is mediated by a clathrin-box motif in Hrs. **d** | The structure of the coatomer protein (COP)I βγδζ subcomplex is based on the model of AP2 because of the homology of their subunits[27]. The arrangement of the other COPI subunits has not been elucidated. **e** | The COPII scheme has been modelled on the basis of recent electron-microscopy and crystallographic analyses[29,52]. The small G proteins ADP-ribosylation factor (Arf)1 and Sar1 are shown next to the coat proteins that they regulate. FYVE, for Fab1, YOTB, Vac1, EEA1; GAT, for GGA and TOM1; UIM, ubiquitin-interacting motif; VHS, for Vps27, Hrs, STAM.

Table 1 | **The properties of protein coats**

Coat	Subunit composition	Regulators of recruitment to membranes	Sorting signals recognized*	Localization	Known or presumed functions
Clathrin–AP1	CHC, CLCa or CLCb; γ1 or γ2, β1, μ1A or μ1B, σ1A, σ1B or σ1C	Arf1, Arf3	YXXØ, [DE]XXXL[LI]	TGN, endosomes	Sorting between TGN and endosomes, basolateral sorting (μ1B)
Clathrin–AP2	CHC, CLCa or CLCb; αA or αC, β2, μ2, σ2	$PtdIns(4,5)P_2$	YXXØ, [DE]XXXL[LI], FXNPXY‡	Plasma membrane	Endocytosis
Clathrin–GGAs§	CHC, CLCa or CLCb; GGA1, GGA2 and/or GGA3	Arf1, Arf3	DXXLL	TGN, endosomes	Sorting from TGN to endosomes
Clathrin–Hrs	CHC, CLCa or CLCb; Hrs	PtdIns(3)P	Ubiquitin	Endosomes	Sorting from early to late endosomes
COPI	α-COP, β′-COP, ε-COP, γ1- or γ2-COP, β-COP, δ-COP, ζ1- or ζ2-COP	Arf1, Arf3	KKXX, KXKXX, FFXXRRXX	Golgi, ER-to-Golgi intermediates	Retrograde transport from the Golgi to the ER, maintenance of Golgi integrity
COPII	Sec13, Sec31, Sec23 Sec24	Sar1	DXE	ER exit sites	Protein export from the ER
AP3‖	δ, β3A or β3B, μ3A or μ3B, σ3A or σ3B	Arf1, Arf3	YXXØ, [DE]XXXL[LI]	Endosomes, TGN	Biogenesis of melanosomes and platelet dense bodies
AP4	ε, β4, μ4, σ4	Arf1, Arf3	YXXØ	TGN	Sorting from the TGN to endosomes, basolateral sorting

*Ø represents leucine, isoleucine, phenylalanine, methionine or valine, and X represents any amino acid. ‡FXNPXY signals have been shown to interact with the clathrin terminal domain, the μ2 subunit of AP2, disabled-2 and the autosomal recessive hypercholesterolemia (ARH) protein. §GGAs and AP1 might be part of the same clathrin coats. ‖AP3 binds clathrin but does not require it for function. AP, adaptor protein; Arf, ADP-ribosylation factor; CHC, clathrin heavy chain; CLC clathrin light chain; COP, coatomer protein; ER, endoplasmic reticulum; GGA, Golgi-localized, γ-ear-containing, ADP-ribosylation factor-binding protein; Hrs, hepatocyte growth factor-regulated tyrosine kinase substrate; PtdIns(3)P, phosphatidylinositol-3-phosphate; $PtdIns(4,5)P_2$, phosphatidylinositol-4,5-bisphosphate; TGN, *trans*-Golgi network.

coats could allow the recruitment of microtubule motor proteins that are necessary for long-range directional movement through the cytoplasm. In this regard, the plus-end-directed microtubule-dependent motor protein KIF13A has been shown to interact with the β1-adaptin subunit of AP1 and to mediate centrifugal transport of AP1-coated carriers that are derived from the TGN[42]. Finally, the coats could also participate in the recruitment of tethering factors that are necessary for the fusion of the carriers with acceptor organelles. In this regard, the Rab4/Rab5 effector Rabaptin-5, which functions in endosomal fusion events, has recently been shown to interact with the γ-adaptin subunit of AP1 (REF. 43).

Bilayered clathrin coats on endosomes

In another departure from the 'one-size-fits-all' models of transport, EM studies have uncovered more varied coated structures than were recognized originally. From COPI-coated 'megavesicles' that transport protein aggregates between Golgi cisternae[44], to flat clathrin lattices that function as reservoirs or precursors of coated vesicles[45], coats can adopt various shapes.

One new type of clathrin-containing coat has recently been observed in association with pre-melanosomes[46] and early endosomes[13,47]. This coat consists of two electron-dense layers — an outer layer that is composed of clathrin and an inner layer that is composed of other peripheral membrane proteins (FIG. 1c). Strikingly, these 'bilayered' coats do not seem to contain any of the conventional clathrin adaptors (that is, the AP complexes). Instead, the coats that are attached to these early endosomes contain the protein Hrs[13,47], which binds to ubiquitylated membrane proteins through two ubiquitin-interacting motifs (UIMs) and to clathrin using a 'clathrin-box' motif[13] (FIG. 2; TABLE 1). The growth hormone receptor and epidermal growth factor receptor, which are ubiquitylated and targeted to lysosomes on ligand binding, are concentrated in these bilayered, coated endosomal domains[47]. By contrast, receptors that recycle to the plasma membrane, such as the transferrin receptor, are distributed uniformly throughout the endosomal membranes[47].

It has been proposed that these bilayered coats do not to lead to the formation of coated vesicles or other coated intermediates[47]. Rather, the coats might function to retain lysosomally targeted cargo proteins when recycling proteins are being removed by vesicles or tubules that bud from the early

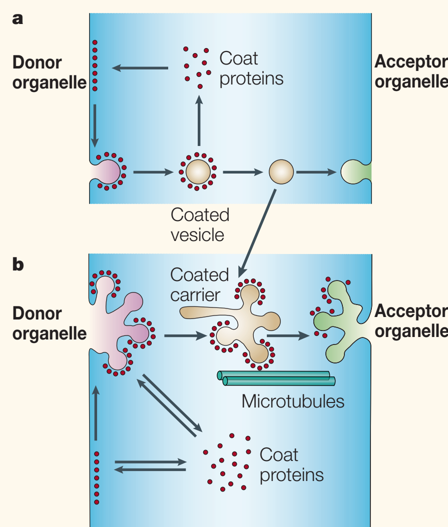

Figure 3 | **Models for the generation of coated carriers. a** | The formation of a small coated vesicle. Coat proteins are recruited from the cytosol to membranes to form a flat lattice. The coated membrane domain of a donor organelle bends to form a coated bud. A spherical coated vesicle pinches off, after which the coat proteins dissociate back into the cytosol. The uncoated vesicle fuses with an acceptor organelle. **b** | The formation of a large, pleiomorphic carrier. Coat proteins cycle constantly between the cytosol and the membrane of the donor organelle. The pleiomorphic carrier forms and detaches from the coated domain, after which it translocates along microtubules. The carrier eventually fuses with an acceptor organelle. The formation of these carriers from the donor organelle probably requires a mechanism of scission that is distinct from that of small coated vesicles. The large carriers could also form by the fusion of small coated vesicles, which is indicated by the arrow from part **a** to part **b**.

endosomes. These coats could therefore mediate the retention of cargo in a 'stationary' phase (that is, in the organelle from which other transport vesicles bud), rather than incorporation into a 'mobile' phase (that is, into a newly-formed transport vesicle). The possibility that these bilayered coats eventually give rise to coated transport intermediates, however, cannot be excluded at this time.

The dynamics of coat exchange

Inherent to the classical model of transport mediated by small coated vesicles is the concept that coats are put on and taken off only once in a single cycle of coated-vesicle formation and consumption (FIG. 3a). In this model, the coats are thought of as rigid assemblies that are similar to viral capsid structures. Indeed, the exchange of free clathrin for clathrin on isolated coated vesicles *in vitro* is extremely slow and inefficient[48]. However, experiments involving fluorescence recovery after photobleaching (FRAP) of GFP-tagged clathrin, AP1, AP2, GGA1 and COPI have shown that there is a rapid ($t_{1/2}$ = 10–32 s) exchange of coat proteins between membrane-bound and cytosolic pools[33,37,48,49] (FIG. 4b). Moreover, the exchange proceeds even when the detachment of coated carriers from the membranes is inhibited[33,37,48,49]. So, the binding and release of coat components are not necessarily one-time events that are coupled to vesicle budding and preparation for fusion, respectively, but are, instead, processes that can occur even in the absence of vesicle formation (FIG. 3b).

This exchange could be a manifestation of 'proof-reading' mechanisms that ensure that only cargo-associated coated structures become stable and give rise to coated vesicles[50]. In this case, the extensive exchange that is observed *in vivo* would indicate that much of the coat-protein recruitment that occurs in the cell is unproductive. An alternative explanation could be that the continuous exchange generates kinetically stable membrane domains that allow the membranes to differentiate through the progressive alteration of their protein and lipid composition[33].

Concluding remarks

Coat proteins are known to carry out two principal tasks — the concentration of specific membrane proteins into a specialized patch and the mechanical deformation of the patch into a small coated vesicle. This concept, which came from the study of clathrin-coated vesicles, has been extended over the past 12 years to various non-clathrin coats. However, as research into the structure and dynamics of these coats has progressed, our understanding of coat function has broadened.

Recent findings indicate that the job of coat proteins might go beyond their widely accepted roles in protein concentration and membrane deformation. Coat proteins probably also carry out post-budding functions through the recruitment of accessory factors that mediate interactions with the cytoskeleton and tethering to acceptor organelles. These functions might be made possible by the persistence of the coats on the carriers as these carriers migrate, often for long distances, in the cytoplasm. Strikingly, the long-range transportation of cargo in mammalian cells seems to be mediated by large membrane-enclosed carriers, some of which contain associated coats. The coats on these carriers, as well as the endosomal, bilayered clathrin coats, might participate in the differentiation of membrane domains. Finally, protein coats seem to be much more dynamic than originally envisioned, because they undergo continuous exchange between membranes and the cytosol by a process that can be uncoupled from vesicle formation. The new challenge that is posed by these observations will be to elucidate how protein coats contribute to the generation, maturation and targeting of large transport carriers, and to the differentiation of organellar domains.

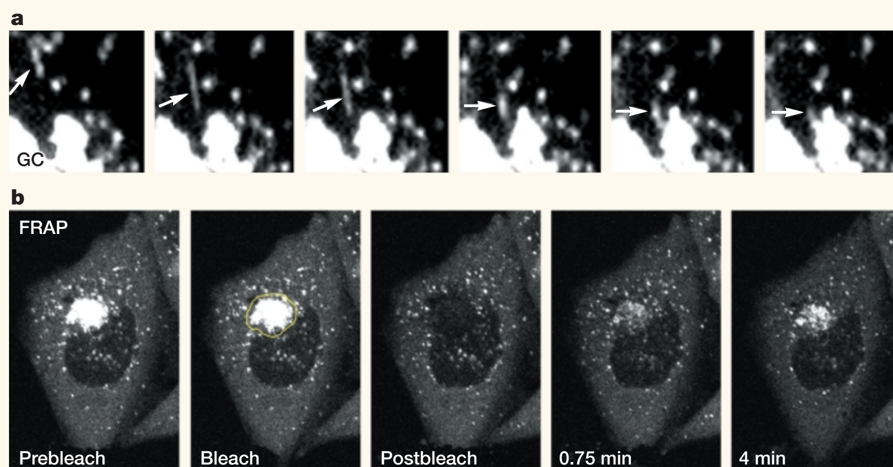

Figure 4 | **The dynamic properties of coat proteins in living cells. a** | The translocation of a coatomer protein (COP)I-coated carrier (see arrow) from the cell periphery to the Golgi complex (GC) over a time-period of 57 s. It should be noted that εCOP–green fluorescent protein (GFP) remains associated with the carriers as they move through the cytosol. **b** | The binding and release kinetics of COPI to and from Golgi membranes are shown by fluorescence recovery after photobleaching (FRAP) in εCOP–GFP-expressing cells. The rapid recovery after photobleaching occurs as a result of the rapid exchange of COPI between Golgi membranes and the cytosol. Part **a** is modified and part **b** is reproduced with permission from REF. 33 *Nature* © (2002) Macmillan Magazines Ltd.

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TIMELINE

Lipids on the frontier: a century of cell-membrane bilayers

Michael Edidin

Our present picture of cell membranes as lipid bilayers is the legacy of a century's study that concentrated on the lipids and proteins of cell-surface membranes. Recent work is changing the picture and is turning the snapshot into a video.

All of the membranes of eukaryotic cells separate functional compartments, but the cell-surface membrane — the plasma membrane — is an extreme. It is the frontier between the cell and its environment. Exploration of this frontier has revealed its physical and functional properties. The plasma membrane is a lipid bilayer, the composition of which regulates frontier crossings by molecules between a cell's surroundings and its interior, and the properties of the bilayer are different from those of any of its components alone.

Explorers of the cell frontier draw their resources from the physical chemistry of pure lipid ensembles, that is, model membranes made *in vitro* from just one or two kinds of lipid. The data from these simplified membranes allow the exploration of more complicated cell membranes that are rich in proteins and that contain a bewildering array of lipids. The approach of physical chemistry provides information on how lipids associate with one another and on their dynamic interplay. However, it is hard to capture the dynamic interplay between the components of cell membranes. We have information on the interactions of membrane lipids with one another and with membrane proteins, but, until recently, it has not been easy to apply this information to the membranes of living cells. Often, spatial resolution has been sacrificed for the sake of temporal resolution and vice versa. However, in recent years, new techniques have allowed us to visualize cell-membrane structure and dynamics on scales that match those of studies of model membranes. The next step to take is one towards a new integrated model of membrane structure and dynamics, that is, towards a model that spans many timescales and spatial scales. Here, I look back and discuss the way in which the lipid-bilayer model developed over the past one-hundred years (TIMELINE). Then, I look forward and suggest some elements for a dynamic model of the plasma membrane.

Membrane history: cells and models

Cell boundaries and cell permeability. To use the style of Rudyard Kipling, "In the high and far off times cells, O best beloved, had no plasma membranes". They had only an 'end layer' — an outer layer of protoplasm of unknown composition and properties,