COP and clathrin-coated vesicle budding: different pathways, common approaches

Harvey T McMahon[1] and Ian G Mills[2]

Vesicle and tubule transport containers move proteins and lipids from one membrane system to another. Newly forming transport containers frequently have electron-dense coats. Coats coordinate the accumulation of cargo and sculpt the membrane. Recent advances have shown that components of both COP1 and clathrin-adaptor coats share the same structure and the same motif-based cargo recognition and accessory factor recruitment mechanisms, which leads to insights on conserved aspects of coat recruitment, polymerisation and membrane deformation. These themes point to the way in which evolutionarily conserved features underpin these diverse pathways.

Addresses
[1]MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK
e-mail: hmm@mrc-lmb.cam.ac.uk
[2]Department of Oncology, University of Cambridge, Hutchison MRC Cancer Research Centre, Hills Road, Cambridge, CB2 2XZ, UK
e-mail: igm23@cam.ac.uk

Current Opinion in Cell Biology 2004, **16**:379–391

This review comes from a themed issue on
Membranes and organelles
Edited by Judith Klumperman and Gillian Griffiths

Available online 20th June 2004

0955-0674/$ – see front matter

DOI 10.1016/j.ceb.2004.06.009

Abbreviations

AP	adaptor protein
Arf	ADP ribosylation factor substrate
ARH	autosomal recessive hypercholesterolemia protein
COP	coat protein complex
ER	endoplasmic reticulum
GGA	Golgi-localised, γ-ear containing, ADP-ribosylation factor-binding protein
Hrs	hepatocyte growth factor receptor tyrosine kinase substrate
TGN	trans-Golgi network

Introduction: The coated vesicle budding hypothesis

Early electron microscopy studies of cells led to a vesicle-transport hypothesis for protein trafficking. Vesicular transport intermediates bud from a donor organelle and then fuse with an acceptor organelle. Budding intermediates were initially identified by their electron-dense 'coats' and were found on the plasma membrane and intracellular organelles [1] (Figure 1). Three major classes of these coated vesicles have now been purified: COPI-(coatomer) and COPII-coated vesicles (where COP stands for coat protein complex) and clathrin-coated vesicles [2–6] (Figure 1). Coat components are needed for generation of highly curved membrane areas, recruitment of cargo (and exclusion of non-cargo proteins/lipids), vesicle scission and uncoating factor recruitment. Not all budding pathways lead to small vesicles, but the high membrane curvature of these transporters makes them intrinsically more fusogenic than larger vesicle structures like macro-phagosomes. There may be many ways to bud a membrane but we would argue that producing a small vesicle that concentrates cargo molecules requires a coat.

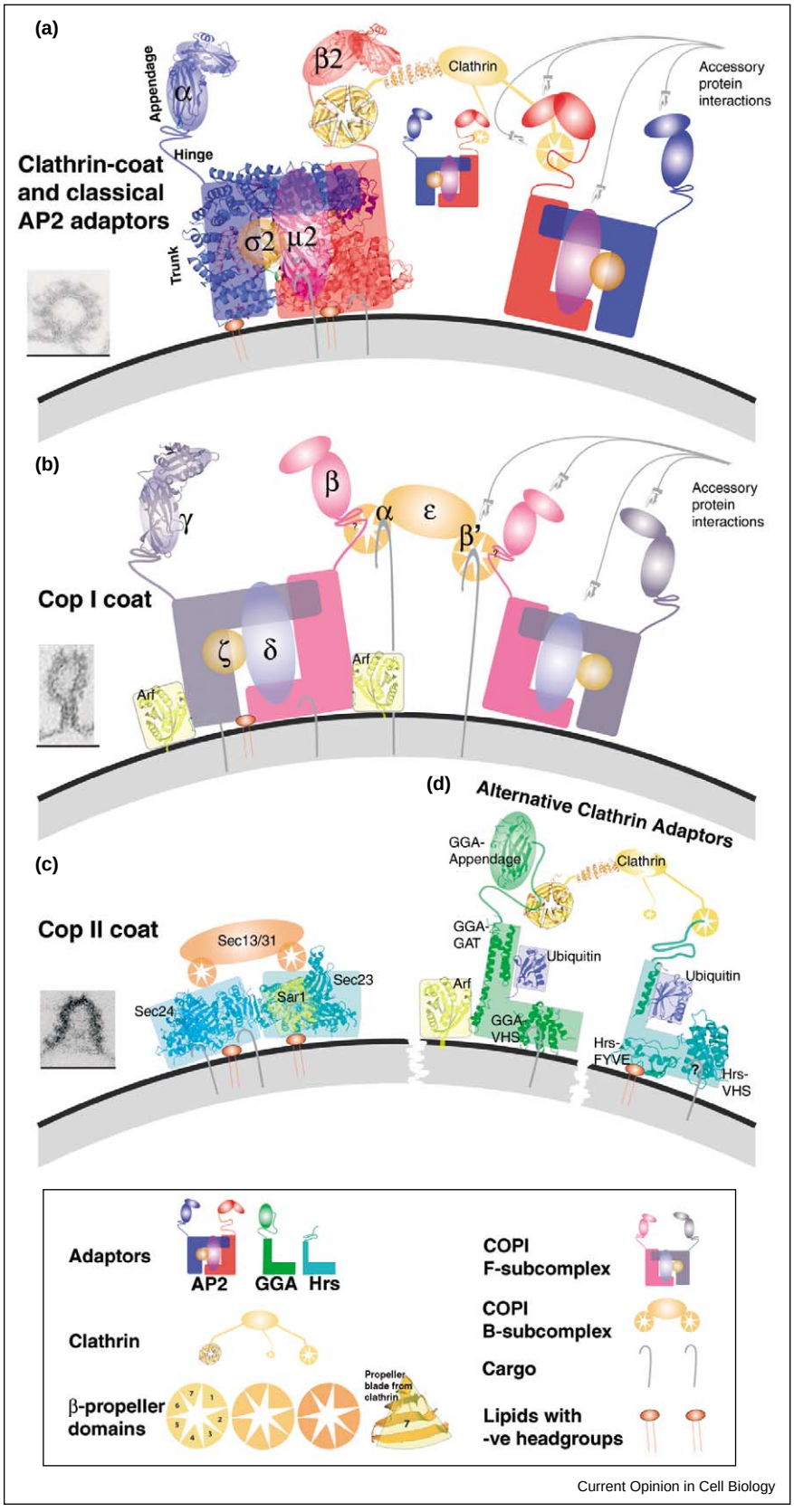
Just as a SNARE-based fusion underpins the hypothesis of a common mechanism for all membrane fusion, so a coat-based budding hypothesis has emerged as a common theme in vesicle budding. This review highlights conserved mechanisms underpinning diverse budding pathways and the differences that often reflect specificity and regulation.

Background to coated vesicle budding

Clathrin-coated vesicles are named after the protein that self-polymerises into a lattice around these vesicles as they bud from the plasma membrane, trans-Golgi network (TGN) and endosomes [7,8]. For all clathrin-coated vesicles, clathrin is the central organiser (Box 1). It concentrates cargo adaptors, leading to a diverse protein and lipid load in the forming vesicle, and its polymerisation into a curved lattice stabilises the nascent membrane bud as it forms. Each clathrin molecule contains an N-terminal domain that has a β-propeller structure and binds to peptide motifs between its blades [9]. This multi-bladed propeller allows for multiple protein interactions with various specificities [10], recruiting a versatile array of different cargo adaptors and membrane attachment proteins [11,12,13••,14].

The main cargo adaptor proteins characterised to date are the classical adaptor protein (AP) complexes, AP1, AP2, AP3 and AP4 [15–19] (Table 1). Most AP complexes adapt and/or link clathrin to selected membrane cargo and lipids, and they also bind accessory proteins that regulate coat assembly and disassembly (such as AP180, epsins and auxilin; see Table 2). 'Alternative adaptors' can also function in clathrin-mediated vesicle budding. These adaptors are sometimes called 'monomeric adaptors' but this does not accurately describe some of the members of this group, which are dimeric. GGA adaptors

Figure 1


(a)
Appendage
α
β2
Clathrin
Accessory protein interactions
Hinge
Clathrin-coat and classical AP2 adaptors
σ2
μ2
Trunk
(b)
γ
β
α
ε
β'
Accessory protein interactions
Cop I coat
ζ
δ
Arf
Arf
(c)
Cop II coat
Sec13/31
Sec23
Sar1
Sec24
(d)
Alternative Clathrin Adaptors
GGA-Appendage
Clathrin
GGA-GAT
Ubiquitin
Arf
GGA-VHS
Ubiquitin
Hrs-FYVE
?
Hrs-VHS
Adaptors
AP2
GGA
Hrs
Clathrin
β-propeller domains
Propeller blade from clathrin
COPI F-subcomplex
COPI B-subcomplex
Cargo
Lipids with -ve headgroups
Current Opinion in Cell Biology

Box 1 Definitions

Clathrin
Clathrin is a trimer with a central hub domain from which extends three legs ending in terminal (β-propeller/WD40) domains. This building block of a cage structure is known as a triskelion. During polymerisation the legs on neighbouring triskelia twist around each other and make a lattice. Recruitment proteins bind to these terminal domains and help to concentrate clathrin on membranes. Concentrated clathrin self-assembles [7].

Cargo adaptors
Proteins that link cargo into the clathrin-coated pit

Classical clathrin adaptors:
AP1, AP2, AP3 and AP4 complexes each have four subunits: two large, one medium and one small, which are believed to be stably associated in the cell. AP1 is involved in protein sorting from the TGN and endosomes; AP2 traffics from the plasma membrane; AP3 traffics to lysosomes and AP4 is found on vesicles in the vicinity of the TGN [19].

Alternative clathrin adaptors
Monomeric and dimeric cargo/clathrin adaptors like GGAs and Hrs

Accessory proteins
Clathrin recruitment, membrane bending and scission molecules. This group can cover all proteins involved in clathrin-mediated endocytosis except clathrin. But the definition is more usefully applied when it neither covers clathrin nor cargo adaptors.

COP coat subcomplexes
There are seven COPI subunits (α, β, β', δ, γ, ε and ζ) divided into two subcomplexes: the F subcomplex composed of β, δ, γ and ζ-COPs, of which the β and γ subunits display homology to AP complex appendage domains, and the B subcomplex, within which the α and β' subunits contain WD40 repeats akin to clathrin terminal domains [28].

COPII coat proteins can also be considered as subcomplexes consisting of Sec13/31p and Sec23/Sec24p. Both Sec13/31p have WD40 repeats akin to clathrin whereas Sec23/24p functionally resemble the AP complexes in a cargo recruitment capacity.

Motif domains
Generally regions lacking tertiary structure in proteins containing short sequence motifs that serve as binding sites for ligand. This is a generic term that can be applied to all proteins with similar domains across the proteome and stands in contrast to 'structured domains'. It is likely that many of these domains have a limited structure.

(Golgi-localized, γ-ear containing, ADP-ribosylation-factor-binding proteins) are associated with TGN clathrin coats and bind cargo, membranes, clathrin and accessory factors (Figure 1 and Table 1) [20,21]. GGAs and Hrs (hepatocyte growth factor receptor tyrosine kinase substrate) have similar but not identical domain compositions (Figure 1). By analogy with cargo binding to the GGA VHS domain, Hrs may also bind cargo, but this protein is targeted via its FYVE domain to endosomes. Arrestins, epsins, disabled-2 and ARH (autosomal recessive hypercholesterolemia protein) can also be classified as alternative adaptors as they also link cargo and membranes to the clathrin lattice (Table 2) [11,13••,14,22]. These may work in some cases alongside or independently of classical adaptors to recruit their cargo into budding vesicles [23•]. Since multiple adaptors are found in individual coated pits, it is not necessary for all adaptors to have a direct clathrin interaction.

COP coats come in two very different flavours, belying the homologous nomenclature (Figure 1 and Table 1). COPI coat components have sequence homology to clathrin AP complex proteins [24–28]. Just as clathrin and adaptors come together to form clathrin-coated vesicles, so the two subcomplexes of COPI coats come together to recruit cargo into buds [28]. COPI coat components traffic primarily from the Golgi to the endoplasmic reticulum (ER) and between Golgi cisternae.

COPII-coated vesicles traffic from the ER to the Golgi. There are again two subcomplexes (Figure 1 and Table 1) that come together to recruit cargo into buds. In the clathrin-like subcomplex of COPI coats (the B-subcomplex) and in the Sec13/31p subcomplex of COPII coats there are β-propeller domains (made from WD40 repeats) [29] that form a protein interaction face just like the β-propeller clathrin terminal domains. COP coats are therefore also multisubunit protein complexes, whose components select cargo, drive membrane curvature and vesicle budding (Table 1). We will now look at some commonalities in these diverse vesicle budding pathways.

Conservation of coat recruitment and membrane curving mechanisms

A long-recognised commonality between COPI, COPII and many clathrin budding pathways is that small

(Figure 1 Legend) Structural and functional homology between COP1 coats and clathrin-adaptor coats. **(a)** The 'classical' AP2 clathrin adaptor with its four subunits forms a complex that links cargo recruitment to clathrin. Clathrin is a trimer and each terminal domain is represented as being bound to a β2 adaptin appendage and hinge domain. The other heterotetrameric AP complexes, AP1, AP3 and AP4, have the same overall structure. **(b)** The COPI F-subcomplex probably has a similar topology to the AP complex and the B-subcomplex is likely to be the functional equivalent of clathrin. **(c)** Likewise, COPII has two subcomplexes, Sec23/24 and Sec13/31. **(d)** Alternative clathrin adaptors GGAs and Hrs are like the large subunits of AP complexes in that they bind to cargo, clathrin and membranes. Hrs is specifically recruited to endosomes via its FYVE domain, while GGAs are membrane recruited via Arf binding just like the COPI F-subcomplex and AP1, AP3 and AP4 clathrin adaptor complexes. GGA has been shown to bind cargo via its VHS domains and both GGA and Hrs also have ubiquitin binding regions. Also, both bind to clathrin, but only GGAs have the appendage domain necessary for accessory protein recruitment. PDB codes: AP2 core complex 1GW5; Clathrin terminal domain 1BP0; β2-appendage 1E42; α-appendage 1B9K; COP1 γ-appendage 1R4X; sec23/24 1M2V; Hrs VHS-FYVE 1VDP; VPS27 UIM-ubiquitin 1Q0W; GGA1 appendage 1NA8; GGA3 VHS 1JPL; GGA GAT-Arf 1J2J. We acknowledge the kind permission of Pietro DeCamilli, Randy Schekman and Lelio Orci to use the following electron micrographs of budding vesicles from liposomes: clathrin-coated vesicle [58] (reproduced with permission from Elsevier); COPI vesicle [59] (copyright 1998 National Academy of Sciences, USA); COPII vesicle [60] (reproduced with permission from Elsevier). Scale bar for EM images of vesicle budding is 100 nm.

Table 1

Coat subunit functions and domains Colour coding: blue text, AP1/GGA-associated proteins; red text, AP2-associated proteins; purple text, COPI-associated proteins; green text, COPII-associated proteins.

Adaptor/coat	Salient features	Peptide motifs	Functions
Clathrin			**Endocytosis, sorting from TGN to endosomes, sorting from early to late endosomes**
Heavy chain	Subunits polymerise into a triskelion; atomic structures of several fragments reveal α-zigzag repeats and a β-propeller terminal domain.	Binds LLDLD type 1 clathrin motif and PWxxW type 2 clathrin motif.	Recognition of peptide motifs through the β-propeller formed by WD40 repeats (terminal domain). Binding partners include auxilin, amphiphysin, epsin and AP180. The triskelial arms define skeleton of the clathrin coat.
Light chain: LCa/b	Interacts with Hsc70, calmodulin and the central helical/coiled coil domain of HIP1/HIP12.		Regulates clathrin self-assembly into polyhedral lattices.
AP1 adaptors			**TGN-endosome sorting**
γ	Large AP complex subunit with truncated appendage domain.	Binds DFGxØ and DFxDF motifs.	Membrane binding via Arf1. Recruitment of accessory factors to AP1 complexes e.g. EpsinR.
β1		Clathrin box motif in hinge: LLNLD. Binds dileucine cargo motifs: [DE]xxxL[LI].	Membrane binding via Arf1. Binds to clathrin via hinge domain. Binds accessory proteins via appendage domain. Cargo recognition.
μ1 (A/B)		Binds YxxØ cargo motifs.	Cargo recognition. Membrane interaction.
σ1	σ1 sequence is weakly related to the N-terminal portion of μ.	Binds [D/E]xxx[L/I] cargo motifs in complex with the μ subunit	Stabilises the AP core complex by mediating interactions between subunits.
AP2 adaptors			**Plasma membrane endocytosis**
αA/C	Atomic structure of the appendage and trunk domain are known.	Binds DxF, FxDxF and WVxF motifs	Membrane binding. Recruitment of accessory proteins e.g. Epsin1
β2	Atomic structure of the appendage and trunk domain are known.	Clathrin box motif in hinge: LLNLD. Binds cargo motif: [DE]xxxL[LI].	Binds to cargo dilucine motifs. Binds clathrin via hinge domain. Binds accessory proteins via appendage domain.
μ2	Atomic structure of μ2 and of interactions with YxxØ sorting motifs is known.	Binds YxxØ in cargo and WVxF in stonin 2.	Membrane interaction. Cargo recognition. Also interacts with accessory proteins such as stonin 2.
σ2	σ2 sequence is weakly related to N-terminal portion of μ.		Stabilises the AP core complex by mediating interactions between subunits.
AP3 adaptors			**Melanosome biogenesis**
δ	Appendage domain and trunk domains conserved.		Like binds to accessory proteins via the appendage domain, but these have not been identified as yet.
β3 (A/B)	Appendage domain and trunk domains conserved.	Clathrin box motif in hinge: LLDLD. Binds cargo motif: [DE]xxxL[LI].	Binds clathrin. Appendage domain with conserved ligand binding pocket likely to bind to accessory proteins. Cargo recognition.
μ3 (A/B)		Binds cargo motif YxxØ.	Cargo recognition.
σ3 (A/B)		Binds [D/E]xxx[L/I] cargo motifs in complex with the μ subunit	Stabilises the AP3 complex.
AP4 adaptors			**Basolateral sorting/ TGN-endosome sorting**

Table 1 Continued

Adaptor/coat	Salient features	Peptide motifs	Functions
ε			Membrane binding via Arf1. Conserved appendage domain so likely to recruit accessory proteins.
β4		No obvious clathrin box	Binds the neuronal protein tyrosine phosphatases PTP-SL and PTPBR7. Has conserved appendage domain.
μ4		Binds YxxØ weakly and also interacts with other non-classical cargo motifs like DLYYDPM	Cargo recognition.
σ4			Stabilises the AP4 complex.
GGAs			**TGN-endosome/lysosome sorting**
GGA-1	Multidomain alternative adaptors with VHS, GAT, hinge and appendage domains.	Clathrin box motif in hinge: LLDDE. Appendage domain binds DFGxØ motifs. VHS domain binds DxxLL motifs. WNSF sequence in the hinge is bound by the AP1 γ appendage.	Clathrin binding. Recruitment of accessory factors such as rabaptin-5 and p56 by appendage domain. Cargo recognition (e.g. Ci-M6PR) via VHS domain. Membrane recruitment via Arf binding to GAT domain. Ubiquitin binding via GAT domain and thus potentially binds ubiquitinated cargo.
GGA-2		Clathrin box motifs in hinge: LIDLE and LLDLL. Other interactions are same as for GGA-1.	Same as for GGA-1
GGA-3		Unidentified clathrin-binding motif and other interactions are same as for GGA-1.	Same as for GGA-1
COPI F-subcomplex: βδγζ B-subcomplex:αβ′ε			**Retrograde transport from the Golgi to the ER, maintenance of Golgi integrity**
Arf1	Small GTPase; Ras family.		Recruitment of COPI coatomer to membranes in a GTP dependent manner.
αCOP / Ret1p	WD40 repeats (β-propeller domain).	Binds KKxx, KxKxx motifs.	Recruitment of cargo and accessory factors (eg Dsl1p).
βCOP	Binds Arf1, and has weak sequence identity to β-adaptin. Has appendage domain like large AP subunits.		Binds to diacidic cargo motifs. Recruitment of cargo and accessory factors via appendage domain but these have not been identified.
β′COP / Sec26p	WD40 repeats (β-propeller).	Binds KxKxx motif.	Recruitment of cargo.
γCOP / Sec21p	Binds members of the p24 family. Has appendage domain like large AP subunits.		Recruitment of cargo. Recruitment of cargo and accessory factors (ArfGAP) via appendage domain.
δCOP / Ret2p	Weak sequence identity to μ-adaptin.	Binds WxxxW motif in the acidic domain of Dsl1p.	Binds accessory protein via acidic tryptophan motif.
εCOP / Sec28p			May stabilise the complex (point mutations are often lethal in yeast).
ζCOP / Ret3p	Weak sequence identity to σ-adaptin.		Stabilises the interaction between β-COP and γ-COP.
ARFGAPs Glo3p	GTPase activating protein for ARF. Binds β′ WD40 domain and γ-COP appendage domain.		Activates Arf GTP hydrolysis to promote coat disassembly.
ARFGEFs Gea1p/Gea2p	GEF for ARF		

Table 1 Continued

Adaptor/coat	Salient features	Peptide motifs	Functions
COPII			**Protein export from the ER**
hSar1p	Small GTPase of the Ras family.		Recruits coat components to membranes in a GTP-dependent manner.
hSec13p/hSec31p	Both subunits have WD40 repeats (β-propeller domains).	Not identified as yet.	Induces coat polymerisation.
hSec23p/hsec24p	Sec23 is a Sar1 GTPase activating protein and Sec24 binds cargo.	Sec24 binds DxE, YNNSNPF and Lxx[L/M]E motifs.	Has GAP activity for Sar1. Cargo recognition and membrane curvature selection.
hSec12p	Has a GEF domain.		ER localized GEF and thus leads to the recruitment of Sar1 and COPII coat components to the ER.
hSec16p	Forms a ternary complex with Sec23/24p *in vitro*.		Stabilises Sec23/24 complex and stimulates vesicle budding.

This table is referenced in full and updated online at: http://www2.mrc-lmb.cam.ac.uk/groups/hmm/adaptors/Table1.htm.

G-proteins are required for membrane recruitment [18,20,30,31]. COPI components and clathrin adaptors (AP1, AP3, AP4 and GGAs) are recruited by Arfs (ADP ribosylation factor substrates), whereas COPII depends on the Arf-related protein Sar1. An amphipathic helix is extended from the N terminus of these G-proteins in the GTP-bound forms [32]. It is likely that this amphipathic helix lies on the surface of the membrane between the lipid head groups and, when concentrated at the bud site, either drives or facilitates membrane curvature like epsin [13••]. The exception to Arf recruitment is clathrin-AP2 budding from the plasma membrane, where PtdIns(4,5)P_2 binds directly to AP2 and other accessory proteins and epsins help to generate membrane curvature. This difference may be due to different topological constraints applying to the generation of curvature from a relatively flat membrane as opposed to a positively curved membrane, and also to the rigidity of this bilayer.

Coat polymerisation leads to cargo concentration

COPI and COPII coats all have the equivalent of a clathrin and an adaptor subcomplex (Figure 1). These subcomplexes can be purified and in each case one subcomplex binds to membranes and the other associates with the first to promote polymerisation of the coat. The outer sheath, or clathrin-equivalent, can in all cases bind either directly or indirectly (via adaptors) to cargo using conserved β-propeller domains [33,34] (Table 1). The self-polymerisation of clathrin has been studied in great detail using cryo-electron microscopy [7] and it is this oligomerisation that leads to the presentation of a concentrated field of adaptor/cargo binding domains (the β-propeller domains). Self-oligomerisation of clathrin is also promoted by β-propeller binding proteins [13••,14,35•]. Thus coat assembly and cargo recruitment are dependent on each other.

Coat polymerisation leads to membrane curvature

Coat assembly may also lead to membrane budding. Clathrin, in the absence of membranes and other proteins, self-assembles into a coat with a similar curvature to coated vesicles. The COPII membrane-associated sec23/24 complex has an intrinsic curvature like that found in COPII vesicles and thus by polymerisation this curvature will be favoured [36•]. The distinction between the structural curvature of a clathrin lattice or Sec23/24p and the forces required to impose curvature on a membrane are well illustrated by the recent study of BAR domain proteins. The crystal structures of the *S. cerevisiae* Sec23/24–Sar1 complex and the BAR domain of *Drosophila* amphihysin are both concave or 'banana-like' in appearance with positively charged amino acids providing an electrostatic interface with the membrane. A protein will curve membranes if the difference in the energy of binding to curved versus flat membranes is greater than the energy required for membrane deformation. The BAR domain of *Drosophila* amphiphysin induces membrane deformation, and mammalian amphiphysin 2 binds clathrin promoting polymerisation *in vitro*. Give the association of Sec23/24–Sar1 complex with a clathrin-like COPII subcomplex (Sec13/31p-containing WD40 terminal-like domains) perhaps it would be timely to investigate whether Sec23/24–Sar1 is both a sensor and inducer of membrane curvature.

Just because the coat preferentially adopts a given curvature this does not mean that coat polymerisation provides enough energy for curving membranes and that additional factors will not also promote this curvature. We have already proposed that small G-proteins will aid membrane bending, and for clathrin-AP2 vesicles, epsin, amphiphysin and endophilin may also help to drive the membrane curvature in conjunction with clathrin polymerisation (Table 2) [13••,35•,37]. Thus coat assembly may facilitate membrane curvature in two ways: first,

Table 2

Accessory proteins involved in clathrin and COP coat formation. Colour coding: blue text, AP1/GGA-associated proteins; red text, AP2-associated proteins; purple text, COPI-associated proteins; green text, COPII-associated proteins.

Accessory factor	Salient features	Peptide motifs	Functions
Amphiphysin	All forms have an N-terminal BAR domain (a dimerisation, lipid-binding and curvature-sensing module). All mammalian forms have a C-terminal SH3 domain that binds dynamin. The non-muscle splice forms have an insert that binds clathrin and the AP2 complex.	AP2 α-adaptin appendage binding motifs: FxDxF and DxF. Clathrin β-propeller binding motifs: LLDLD and PWxxW. SH3 domain binds to PSRPNR motif in dynamin.	Induces/senses membrane curvature. Recruits dynamin to membranes. Promotes clathrin polymerisation on membranes.
AP180/CALM	N-terminal ANTH domain binds PtdIns(4,5)P_2. C-terminal region binds clathrin and the AP2 complex.	AP2 α-adaptin appendage binding motifs: FxDxF and DxF motifs. CALM has DxF and NPF motifs (bind to EH domains). Clathrin binding motifs are not well defined.	Recruits clathrin and promotes polymerisation on membranes containing PtdIns(4,5)P_2. Regulates vesicle size.
ARFGAP	GTPase activating protein for ARF. Binds βWD40 and γ-COP appendage.	The peptide motif in ARFGAP has not been described but is likely to resemble that found α and γ appendage binding partners.	Activates ArfGTP hydrolysis to promote coat disassemby
ARFGEF	GEF for Arf.		Converts ArfGDP to ArfGTP.
ARF1 ARF1	Small G-protein.	GTP binding motifs G1 to G4: G1(GKT); G2(T); G3(VGG); G4(NKQD). Adaptor complexes and COPI subunits are effector molecules.	Helps to recruit AP1, AP3, AP4, GGA and COPI F-subcomplex to membranes in a GTP-dependent manner.
ARH1/2	N-terminal PTB domain binds to inositol lipids and receptor tails. Binds to NPXY motifs and AP2.	PTB domain recognizes NPVY motif in LDLR. Clathrin β-propeller binding motif: LLDLE. Also binds β2-adaptin (motif not defined).	An alternative cargo adaptor. May also be found in combination with other adaptors.
β-arrestin	Similar to visual arrestin whose structure has been solved. This domain binds inositol lipids and G-protein coupled receptors. A C-terminal extension binds clathrin and the AP2 complex.	Clathrin β-propeller binding motif: LIEFE. AP2 β2-adaptin appendage binding motif: IVFEDAFR with the arginine being essential and corresponding to R-394 in β2-arrestin.	An alternative cargo adaptor that helps recruit clathrin and AP2 in G-protein coupled receptor endocytosis.
Auxilin	Binds clathrin and contains a DnaJ domain and is a cofactor for Hsc70.	AP2 α-adaptin appendage binding motifs: DPW and WDW. Clathrin β-propeller binding motifs: DxF and DLL. DnaJ domain has a HPDK signature motif conserved across all auxilins and is required for Hsc70 binding and stimulation of its ATPase activity.	Involved in uncoating of clathrin-coated vesicles.
Dab2/Doc2	N-terminal PTB domain binds to PtdIns(4,5)P_2 and receptor tails. NPXY motifs and AP2.	PTB domain recognises FxNPxY motif in the LDL receptor. AP2 appendage binding motifs: FxDxF and DxF.	An independent cargo-specific adaptor that also binds to the AP2 complex.
Dsl1p	Interacts with α- and δ-COP and ER resident protein Tip20p.	Contains WxW and WxxxW motifs in an acidic central domain	Integrates cargo with the 'clathrin-' and 'AP-like' subcomplexes of COPI
Dynamins	Large GTPase that self-oligomerises into a helix on negatively charged membranes. Changes conformation on GTP hydrolysis.	GTP binding motifs G1 to G4: G1(GKS); G2(T); G3(DLPG); G4(TKLD).	Vesicle scission on GTP hydrolysis.
Endophilins	N-terminal BAR domain (a dimerisation, lipid-binding and curvature-sensing module). C-terminal SH3 domain that binds synaptojanin and dynamin.	SH3 domain binds to PPxRP motif in dynamin and PxRPP motif in synaptojanin.	Induces/senses membrane curvature. Recruits the lipid phosphatase, synaptojanin, to membranes, and thus is involved in uncoating.

Table 2 Continued

Accessory factor	Salient features	Peptide motifs	Functions
Epsin1 EpsinR	N-terminal ENTH domain of epsin1/ epsinR binds PtdIns(4,5)P_2 and PtdInsP respectively and promotes initial membrane curvature. Epsin1 has three ubiquitin binding motifs (UIMs). Both epsin1 and epsinR have a clathrin/adaptor binding domain.	AP2 α-adaptin appendage binding motifs in epsin1: DPW. AP1 γ-adaptin appendage binding motifs in epsinR: DFxDF. Clathrin β-propeller binding motif in epsin1 is LMDLADV and LVDLD, and is DLFDLM in epsinR. Eps15 EH domain binding motifs: three copies of NPF motif in epsin1. The ubiquitin binding signature is ExxxLxLAxAxS[K/R/Q].	Induces membrane curvature in conjunction with promoting clathrin polymerisation. Epsin1 may bind ubquitinated cargo via its UIMs.
Eps15 Eps15	N terminus has three EH domains that bind the NPF motifs in epsin1. Also has a clathrin/adaptor binding domain.	AP2 α-adaptin and β-adaptin appendage binding motifs in epsin1: multiple DxFs	Located at the edges of clathrin-coated pits. Thought to organise epsins and other molecules at the leading edge of coated pit formation.
HIP1/HIP1R	N-terminal ANTH domain binds PtdIns(4,5)P_2. C-terminal region binds clathrin, the AP2 complex and actin.	AP2 α-adaptin appendage binding motifs in HIP1: FxDxF and DxF. Clathrin β-propeller binding motif in HIP1: LMDMD. Clathrin β-propeller binding motif in HIP1R: LIEIS.	Thought to provide a link between actin and clathrin nucleation.
Intersectin/ DAP160	Has multiple EH and SH3 domains and a RhoGEF domain. Binds N-WASP, cdc42, dynamin and SNAP-25 among other proteins.	Binds proline-rich motifs in ligands through its SH3 domains and NPF motifs in ligands via its EH domains.	Links actin polymerisation to proteins involved in clathrin-coated vesicle formation.
P56	Binds to γ-adaptin and GGA appendage domains *in vitro* and co-localises with GGAs *in vivo*.	GGA/AP1 γ-adaptin appendage binding motifs: DFxxF.	Unknown function
PACS1	Binds to AP1 and AP3 complexes and acidic cluster motifs in cargo such as M6PR, Nef and furin.	AP1 complex interaction via ETELQLTF sequence (with mu and sigma subunits).	Recruits cargo containing diacidic motifs.
Sar1p	Small G-protein.	GTP binding motifs G1 to G4: G1(GKT); G2(T); G3(DLGG); G4(NKID).	Binds to membranes when occupied by GTP and recruits COPII coat components. GTPase activity implicated in coat disassembly upon vesicle budding.
Stonins (1/2)	Binds Eps15, AP2 and synaptotagmin.	AP2 α-adaptin appendage binding motif: WVxF. Eps15 EH domain binding motifs: two copies of NPF in stonin2.	Assists AP2/synaptotagmin recruitment
Synaptojanin	Phosphoinostide 5′-phosphatase and SacI phosphatase domain. Binds to the AP2 complex and has a proline-rich domain that binds to endophilin and amphiphysin.	AP2 α-adaptin appendage binding motifs: FxDxF and WxxF are found in the 170kDa splice form and may be involved in the recruitment of synaptojanin to coated pits.	A lipid phosphatase that is recruited to coated vesicle via endophilin. By dephosphorylation of inositol lipids this will weaken the attachment of coat proteins and thus this protein is likely involved in uncoating.
Synaptotagmin1	Calcium sensor for exocytosis. Gets endocytosed by binding to the AP2 complex and to stonin2.	WHxL motif is essential for plasma membrane association.	May integrate exo-and endocytosis in nerve terminals
Syndapin/Pacsin	C-terminal SH3 domain. Binds N-WASP and dynamin.		Regulates clathrin-coated vesicle motility
Hsc70	ATPase that binds to auxilin.		Clathrin coat release. Hsc70 is also called the ‘uncoating ATPase’.
γ-synergin	Has EH domain that binds to NPF motifs in SCAMP. Also binds to the AP1 complex.	AP1 γ-adaptin appendage binding motif: DFxDF.	Unknown function in clathrin-AP1 trafficking.

Some of the accessory proteins are alternative adaptors while others are involved in organising cargo adaptors, sculpting the vesicle and in vesicle scission and uncoating. This table is referenced in full and updated online at: http://www2.mrc-lmb.cam.ac.uk/groups/hmm/adaptors/Table2.htm.

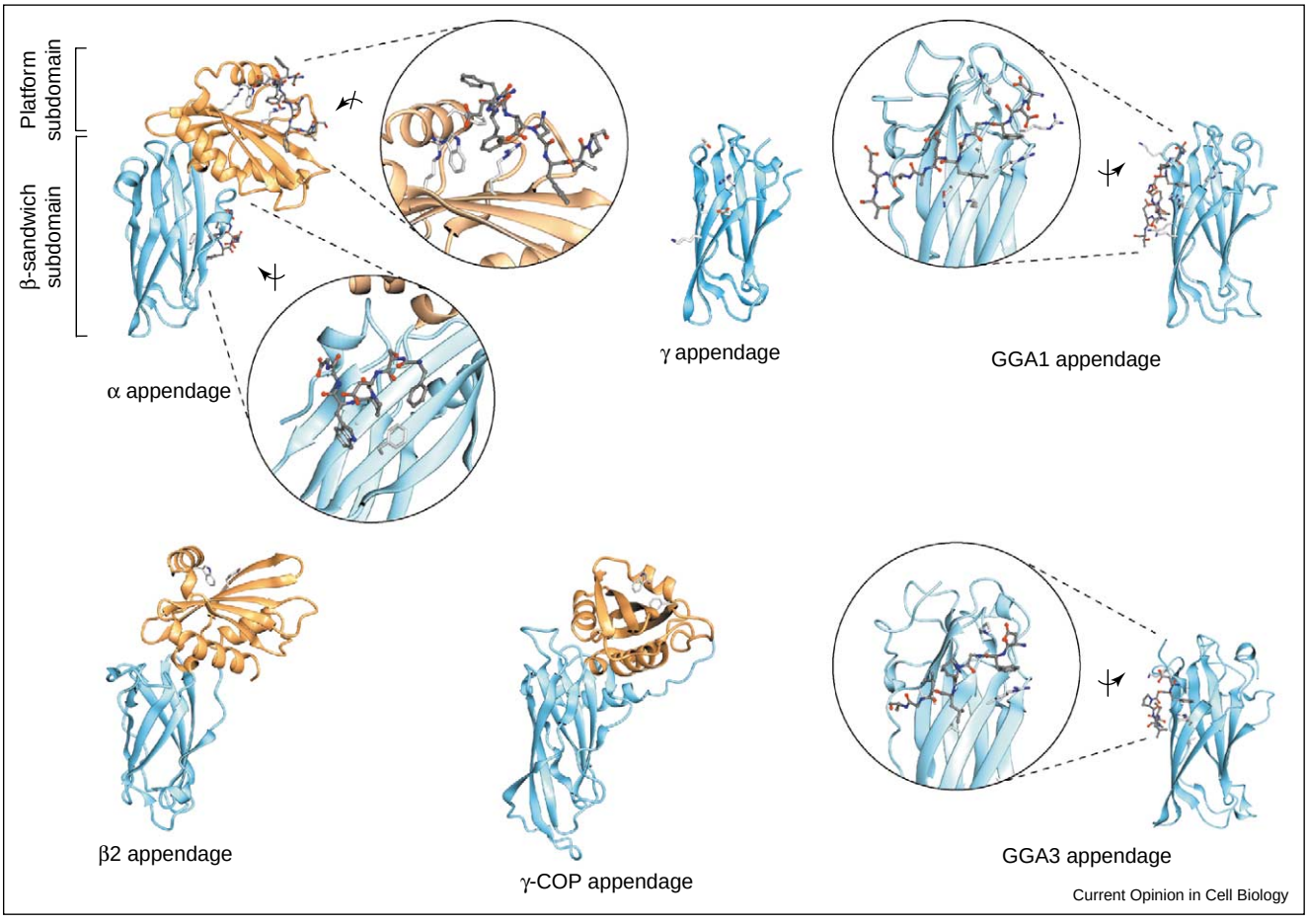
directly through the inherent curvature of the coat complex, and second, indirectly through the recruitment of accessory factors capable of promoting curvature.

Structural and functional similarity between COPI and adaptor protein complex appendage domains

The aforementioned similarities are largely mechanistic but there is also structural similarity between COPI and clathrin-AP vesicle budding [38••,39••]. The large subunits of adaptor complexes have domains called ears or appendages that, as shown by electron microscopy, are attached to the main trunk of the AP complex by a flexible linker known as a hinge (Figure 1). The first appendage structure to be solved was from the AP2 α subunit [40,41,42•]. It contained two sub-domains (Figure 2), a platform with α-helical and β-sheet content and a support β-sandwich subdomain. These two subdomains are present in most appendage domains, whereas γ-adaptin and the structurally-related GGA adaptors have only the β-sandwich subdomain [43–47]. At first it was thought that the platform subdomain contained the only ligand-binding site, but ligand binding to both the β sandwich subdomains of γ and GGA appendages has prompted a re-evaluation of the possibility that each subdomain has a distinct binding site for ligands. These studies are still ongoing in various laboratories. In an AP complex there are always two appendage domains and thus there may be up to four independent protein interaction sites per AP complex in addition to clathrin binding sites on the flexible hinge region (Figure 1).

There is strong structural homology between AP complex appendages and the γ-COP appendage (Figure 2). We

Figure 2



The amazing structural similarity of appendage domains from COPI (γ-COP), clathrin-AP complex (α-, β- and γ-appendages) and GGAs (GGA1 and GGA3). Binding sites are highlighted with important residues and where available structures of bound peptides are shown in greater detail. In GGA and γ-adaptin appendages the upper platform domain is absent. It is likely that this lower β-sandwich binding site is conserved in other appendages, alongside the platform binding site. Movies of individual appendage domain highlighting their binding sites are available at http://www2.mrc-lmb.cam.ac.uk/groups/hmm/adaptors/Appendages.html. PDB codes: α-appendage 1KY7, 1KYD; β2-appendage 1E42; COP1 γ-appendage 1R4X; γ-adaptin appendage 1GYU; GGA1 appendage 10M9; GGA3 appendage 1P4U. Drawings were made with AESOP (Martin Noble, unpublished work).

note by sequence homology that β-COP also has a conserved appendage. The presence of two subdomains again points to the possibility of two ligand-binding sites on COP appendages. Indeed the tryptophan on the platform subdomain that forms the central binding pocket in α-adaptin is conserved in both β- and γ-COP, and mutagenesis of γ-COP abolished the interaction with ArfGAP [39••]. This shows a structural conservation in coat components and points to a common ancestral origin for coated vesicle budding mechanisms. It also shows a common function for appendage domains in recruiting accessory proteins needed for the budding process.

Accessory proteins promote vesicle uncoating in COPI and clathrin-coated vesicles

In cases where we know the functions of the appendage-domain binding partners we can say that they are involved in congregating the proteins necessary to polymerise, invaginate and promote fission of the nascent vesicle (Table 2). For example, AP2 complexes bind to epsin1, which is involved in driving membrane bending in addition to promoting the polymerisation of clathrin [13••]. The AP1 appendage domains bind a homologue of this protein, epsinR, which also binds clathrin and membranes but has a different lipid specificity, consistent with the different membrane localisation. AP appendage domains also recruit synaptojanin1 [48], a lipid phosphatase that will deposphorylate the lipids in the coated vesicle to which many of the coat attachment proteins bind. This should make way for coat disassembly. In a similar manner γ-COP appendage domains bind ArfGAP, which stimulates the hydrolysis of Arf-GTP. Arf-GDP will become detached from the membrane and with it the coat will be released from areas of metastability. ArfGAP activity in COPI vesicles is sensitive to membrane curvature and thus vesicle uncoating is precisely controlled to occur after budding [49••]. Thus there is also commonality between different budding pathways at the level of the accessory protein recruitment and function. Further commonalities can now be explored, including the possibility that clathrin-coated vesicle uncoating is also sensitive to membrane curvature.

'Motif domains' in accessory proteins

Proteins that interact with the appendage domains do so by short peptide motifs found in regions lacking tertiary structure, which we call 'motif domains'. Appendage binding motifs in these domains are often found in multiple copies, especially where they have a low appendage affinity. Thus there are eight DPW motifs (required for AP2 appendage interaction) in the motif domain of epsin1. The unstructured nature and thus greater fluidity of this domain increases the chance of an individual motif finding its interaction partner. The multiple copies of motifs and their low affinity means that these binding partners have higher avidity for appendage domains when concentrated by clathrin polymerisation. When the clathrin cage is disassembled and the adaptors dispersed, these low affinity interactions will readily fall apart. Thus, this mode of interaction by COPs and clathrin coats is well adapted for proteins that only need to interact during coat assembly.

Given the similarity of the appendage platform binding site of COP to the binding site on α-adaptin and the conservation of surrounding basic residues, it is likely that the COP accessory protein binding motifs will be similar and may be of the form [D/E]x[F/W]. Looking through the ArfGAP2 sequence we find multiple copies of these motifs. When a more precise idea of the motif identity is achieved, one will be able to search the protein database for other potential COP ligands and accessory proteins for COP vesicle budding, as has previously been done for AP-dependent pathways.

Clathrin versus COPI coats

COPI and COPII coats are used in the biosynthetic and recycling pathways between the Golgi and the ER, whereas clathrin coats are found on endocytic and recycling pathways between the plasma membrane and the lysosomes or the TGN. COPII coats are likely to be the most ancestral, as they are used on the biosynthetic pathway that will have been essential for all organisms. The recycling back to the ER and the endocytic pathways are likely to be later specialisations. The strong homology between COP1 and clathrin-AP coats implies a common ancestral origin, but there is clearly a wide divergence between these pathways. Why? One possibility is that there would be major advantages to being able to regulate the clathrin endocytic and recycling pathways at the transcriptional level or by post-translational modifications (principally phosphorylation and ubiquitination) independently of effects on COPI/II ER–Golgi trafficking. The clathrin pathway may also be more adaptable to making larger vesicles, as illustrated by the vesicular studies of Roth and Porter on mosquito oocytes [1]. It may also be that the cholesterol- and sphingomyelin-rich membrane composition of the early endocytic pathways means that specialist molecules are needed.

Clathrin coats may be more versatile in their cargo selection and recruitment. Classical adaptors in clathrin-coated vesicles have many ways to recognise cargo. The μ-subunit is specialised to recognise YxxΦ motifs (see Table 1) and each AP complex has a different μ-subunit [50]. β subunits of AP complexes can also bind to dileucine motifs in cargo. Clathrin adaptors can also use other alternative adaptors (e.g. arrestins, Dab2, GGAs and Hrs) to bind to other types of cargo. By contrast, COPI coats do not use a range of alternative adaptors (although cargo can be bound directly to the WD40 domains of the B-subcomplex); they use the equivalent of the μ-subunit

(δ-COP) in the F-subcomplex to bind the accessory protein Dsl1p, which in turn binds to cargo [51]. Similarly, stonin 2 interacts with the μ-subunit of the AP complex [52]. Dsl1p and stonin 2 alter the cargo repertoire of COPI and AP complexes through these interactions. This implies that adaptations of cargo recruitment by accessory proteins may be more ancestral, with the versatility of the AP μ-subunit and other alternative clathrin adaptors being subsequent evolutionary events.

Another feature of the versatility of the clathrin coat is its ability to make flat lattices. These flat lattices are seen on endosomes where they colocalise with Hrs and on the plasma membrane adjacent to clathrin-coated buds [53,54]. (As a side point, this shows that clathrin polymerisation itself does not drive membrane curvature, a point discussed from a different angle above.) What is the function of these flat lattices? They probably function to sequester molecules, either keeping them from entering budding pathways or serving as a store or initial recruitment site. It is easy to envisage their formation in the absence of curvature-driving molecules. But how are these excluded? On the endosome the clathrin adaptor is Hrs [55•]; significantly, this molecule has a similar domain organisation to that of GGA but is missing the appendage domain (Figure 1), and thus will not recruit the epsins, amphiphysins and dynamins necessary for the budding reaction.

Given the use of structurally similar appendage domains in clathrin-AP complexes and in COPI β and γ subunits, what can we learn from the differences? There are at least 10 binding partners of the AP2 complex, six for the AP1 γ-appendage, one for the AP3 complex and one for COPI (Table 2). We propose that the diversity of binding partners for AP2 mainly reflects the additional levels of regulation in this pathway. Most of the AP2 ligands were identified in brain extracts where clathrin-mediated endocytosis is important for synaptic vesicle recycling. In the synapse, vesicle retrieval is stimulated by calcium activation of calcineurin, which results in the dephosphorylation of many of these AP2 accessory components [56]. This calcium regulation means that AP2-dependent endocytosis only occurs in response to exocytosis at the synapse. It also means that there may be partial preassembly and recruitment of endocytic proteins to endocytic zones and thus the process is primed to occur immediately following exocytosis. This diversity of accessory proteins is absent in other coated vesicle pathways. This could reflect difficulties in detecting low-affinity and low-abundance proteins. However, for many budding pathways there may not be the necessity for these additional layers of regulation.

Final remarks

While much evolutionary conservation is apparent in vesicle budding, the study of tubular transport intermediates is at a much more preliminary stage. However, even now it is clear that they are important and that the mechanistic basis for their formation may also be highly conserved in many trafficking pathways. The recent structure of a BAR domain and the long list of predicted homologues provides a pointer [35•]. Tubular transport intermediates have high membrane curvatures at their ends and thus will have the same advantages as small vesicles in fusion reactions. Certain lipid compositions may stabilise this shape [57]. Similarly, caveoli represent putative trafficking invaginations about which much remains to be discovered, but we can infer by analogy with other coats that caveolin polymerisation leads to cargo recruitment and membrane curvature. Caveolin may even use a predicted amphipathic helix in curvature formation in a manner akin to epsins and Arfs.

Given that evolution militates against obsolescence, establishing the reasons for divergence will become the final challenge in completing our view of why distinct coat complexes exist and how they work in the cell.

Update

Accessory proteins

We have used stonin2 and Dsl1p as examples of accessory proteins interacting with components of the AP complex (μ subunit) and COPI (δ) to alter cargo selection. It is now becoming clear that monomeric adaptors like the GGAs have their own array of accessory factors that bind to the GAT (GGAs and TOM1) domain, including Rabaptin-5 and TSG101. This domain also contains an Arf-binding site and a binding site for ubiquitin. A recent paper by Mattera *et al.* has defined the binding site for Rabaptin-5 in the GAT domain and the relationship between this and the binding of ubiquitin and TSG101 [61]. This paper provides a springboard to look at the relationship between accessory proteins and the trafficking of ubiquitinated cargo by describing the competitive and cooperative interactions between these factors and a trihelical bundle in the GAT domain. It is likely that phosphorylation and ubiquitination will be a key to regulating these interactions *in vivo* just as appears to be the case for multimeric adaptor complexes.

'Motif domains' in adaptor proteins

Bai *et al.* have recently reported that a sequence, WNSF, in the hinge region of GGA1 is an interaction site for the γ ear of AP-1 [62]. This suggests that the long-reported colocalisation of GGAs and AP-1 in cells may reflect a physical interaction and raises further combinatorial questions about cargo selection at the trans-Golgi network. Multi-hybrid yeast assays to investigate sorting signal recognition may in future need to incorporate combinations of GGAs and AP-1 subunits to account for the possibility of AP-GGA complexes. It also becomes intriguing to consider why, given this interaction, GGAs are often reported to be de-enriched in clathrin-coated

vesicles in contrast to AP-1 enrichment. Are GGAs both accessory proteins for AP complexes and functional adaptors in their own right?

Acknowledgements

We thank Marijn Ford for transforming pdb files into structures.
We also thank members of the lab for discussion and helpful comments.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Roth TF, Porter KR: **Yolk protein uptake in the oocyte of the mosquito *Aedes aegypti. L.*** *J Cell Biol* 1964, **20**:313-332.

2. Pearse BM: **Coated vesicles from pig brain: purification and biochemical characterization**. *J Mol Biol* 1975, **97**:93-98.

3. Malhotra V, Serafini T, Orci L, Shepherd JC, Rothman JE: **Purification of a novel class of coated vesicles mediating biosynthetic protein transport through the Golgi stack**. *Cell* 1989, **58**:329-336.

4. Hicke L, Yoshihisa T, Schekman R: **Sec23p and a novel 105-kDa protein function as a multimeric complex to promote vesicle budding and protein transport from the endoplasmic reticulum**. *Mol Biol Cell* 1992, **3**:667-676.

5. Salama NR, Yeung T, Schekman RW: **The Sec13p complex and reconstitution of vesicle budding from the ER with purified cytosolic proteins**. *EMBO J* 1993, **12**:4073-4082.

6. Barlowe C, Orci L, Yeung T, Hosobuchi M, Hamamoto S, Salama N, Rexach MF, Ravazzola M, Amherdt M, Schekman R: **COPII: a membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum**. *Cell* 1994, **77**:895-907.

7. Crowther RA, Pearse BM: **Assembly and packing of clathrin into coats**. *J Cell Biol* 1981, **91**:790-797.

8. Hanover JA, Willingham MC, Pastan I: **Kinetics of transit of transferrin and epidermal growth factor through clathrin-coated membranes**. *Cell* 1984, **39**:283-293.

9. ter Haar E, Harrison SC, Kirchhausen T: **Peptide-in-groove interactions link target proteins to the β-propeller of clathrin**. *Proc Natl Acad Sci USA* 2000, **97**:1096-1100.

10. Miele AE, Watson PJ, Evans PR, Traub LM, Owen DJ: **Two distinct interaction motifs in amphiphysin bind two independent sites on the clathrin terminal domain β-propeller**. *Nat struct mol biol* 2004, **11**:242-248.
There are two distinct clathrin terminal-domain binding motifs in amphiphysin, and these are now shown to bind to separate regions of the propeller. It is proposed that other distinct motifs may also bind the terminal domain and thus there may be multiple simultaneous protein interactions.

11. Goodman OB Jr, Krupnick JG, Santini F, Gurevich VV, Penn RB, Gagnon AW, Keen JH, Benovic JL: **β-arrestin actsas a clathrin adaptor in endocytosis of the β2-adrenergic receptor**. *Nature* 1996, **383**:447-450.

12. Ford MG, Pearse BM, Higgins MK, Vallis Y, Owen DJ, Gibson A, Hopkins CR, Evans PR, McMahon HT: **Simultaneous binding of PtdIns(4,5)P_2 and clathrin by AP180 in the nucleation of clathrin lattices on membranes**. *Science* 2001, **291**:1051-1055.

13. •• Ford MG, Mills IG, Peter BJ, Vallis Y, Praefcke GJ, Evans PR, McMahon HT: **Curvature of clathrin-coated pits driven by epsin**. *Nature* 2002, **419**:361-366.
Epsin1 directly modifies membrane curvature on binding to PtdIns(4,5)P_2 by the folding and membrane insertion of an N-terminal helix amphipathic helix. In conjunction with clathrin polymerisation epsin can drive the formation of clathrin-coated buds.

14. Mishra SK, Keyel PA, Hawryluk MJ, Agostinelli NR, Watkins SC, Traub LM: **Disabled-2 exhibits the properties of a cargo-selective endocytic clathrin adaptor**. *EMBO J* 2002, **21**:4915-4926.

15. Pearse BM, Robinson MS: **Purification and properties of 100-kd proteins from coated vesicles and their reconstitution with clathrin**. *EMBO J* 1984, **3**:1951-1957.

16. Dell'Angelica EC, Ohno H, Ooi CE, Rabinovich E, Roche KW, Bonifacino JS: **AP-3: an adaptor-like protein complex with ubiquitous expression**. *EMBO J* 1997, **16**:917-928.

17. Dell'Angelica EC, Mullins C, Bonifacino JS: **AP-4, a novel protein complex related to clathrin adaptors**. *J Biol Chem* 1999, **274**:7278-7285.

18. Hirst J, Bright NA, Rous B, Robinson MS: **Characterization of a fourth adaptor-related protein complex**. *Mol Biol Cell* 1999, **10**:2787-2802.

19. Robinson MS, Bonifacino JS: **Adaptor-related proteins**. *Curr Opin Cell Biol* 2001, **13**:444-453.

20. Dell'Angelica EC, Puertollano R, Mullins C, Aguilar RC, Vargas JD, Hartnell LM, Bonifacino JS: **GGAs: a family of ADP ribosylation factor-binding proteins related to adaptors and associated with the Golgi complex**. *J Cell Biol* 2000, **149**:81-94.

21. Hirst J, Lui WW, Bright NA, Totty N, Seaman MN, Robinson MS: **A family of proteins with γ-adaptin and VHS domains that facilitate trafficking between the trans-Golgi network and the vacuole/lysosome**. *J Cell Biol* 2000, **149**:67-80.

22. He G, Gupta S, Yi M, Michaely P, Hobbs HH, Cohen JC: **ARH is a modular adaptor protein that interacts with the LDL receptor, clathrin, and AP-2**. *J Biol Chem* 2002, **277**:44044-44049.

23. • Motley A, Bright NA, Seaman MN, Robinson MS: **Clathrin-mediated endocytosis in AP-2-depleted cells**. *J Cell Biol* 2003, **162**:909-918.
RNAi was used to ablate the function of AP2 in binding cargo. Clathrin-coated pits were still formed at the plasma membrane and EGF endocytosis continued but transferrin endocytosis was inhibited. This paper demonstrates that AP2 adaptors are not the only plasma membrane adaptors associated with clathrin-mediated endocytosis.

24. Serafini T, Stenbeck G, Brecht A, Lottspeich F, Orci L, Rothman JE, Wieland FT: **A coat subunit of Golgi-derived non-clathrin-coated vesicles with homology to the clathrin-coated vesicle coat protein β-adaptin**. *Nature* 1991, **349**:215-220.

25. Fiedler K, Veit M, Stamnes MA, Rothman JE: **Bimodal interaction of coatomer with the p24 family of putative cargo receptors**. *Science* 1996, **273**:1396-1399.

26. Schledzewski K, Brinkmann H, Mendel RR: **Phylogenetic analysis of components of the eukaryotic vesicle transport system reveals a common origin of adaptor protein complexes 1, 2, and 3 and the F subcomplex of the coatomer COPI**. *J Mol Evol* 1999, **48**:770-778.

27. Eugster A, Frigerio G, Dale M, Duden R: **COP I domains required for coatomer integrity, and novel interactions with ARF and ARF-GAP**. *EMBO J* 2000, **19**:3905-3917.

28. Takatsu H, Futatsumori M, Yoshino K, Yoshida Y, Shin HW, Nakayama K: **Similar subunit interactions contribute to assembly of clathrin adaptor complexes and COPI complex: analysis using yeast three-hybrid system**. *Biochem Biophys Res Commun* 2001, **284**:1083-1089.

29. Garcia-Higuera I, Gaitatzes C, Smith TF, Neer EJ: **Folding a WD repeat propeller. Role of highly conserved aspartic acid residues in the G protein β subunit and Sec13**. *J Biol Chem* 1998, **273**:9041-9049.

30. Seaman MN, Sowerby PJ, Robinson MS: **Cytosolic and membrane-associated proteins involved in the recruitment of AP-1 adaptors onto the trans-Golgi network**. *J Biol Chem* 1996, **271**:25446-25451.

31. Ooi CE, Dell'Angelica EC, Bonifacino JS: **ADP-Ribosylation factor 1 (ARF1) regulates recruitment of the AP-3 adaptor complex to membranes**. *J Cell Biol* 1998, **142**:391-402.

32. Antonny B, Beraud-Dufour S, Chardin P, Chabre M: **N-terminal hydrophobic residues of the G-protein ADP-ribosylation**

factor-1 insert into membrane phospholipids upon GDP-to-GTP exchange. *Biochemistry* 1997, **36**:4675-4684.

33. Stenbeck G, Harter C, Brecht A, Herrmann D, Lottspeich F, Orci L, Wieland FT: **β-COP, a novel subunit of coatomer**. *EMBO J* 1993, **12**:2841-2845.

34. Eugster A, Frigerio G, Dale M, Duden R: **The α- and β-COP WD40 domains mediate cargo-selective interactions with distinct di-lysine motifs**. *Mol Biol Cell* 2003.

35. • Peter BJ, Kent HM, Mills IG, Vallis Y, Butler PJ, Evans PR, McMahon HT: **BAR domains as sensors of membrane curvature: the amphiphysin BAR structure**. *Science* 2004, **303**:495-499.
It is interesting that clathrin polymerisation on membranes is promoted by β-adaptin, epsin, amphiphysin, AP180 and disabled-2. It is likely that other proteins will soon be added to this list.

36. • Bi X, Corpina RA, Goldberg J: **Structure of the Sec23/24-Sar1 pre-budding complex of the COPII vesicle coat**. *Nature* 2002, **419**:271-277.
The sec23/24 structure shows an intrinsic curvature on the lipid binding face that matches that of COPII coated vesicles.

37. Farsad K, Ringstad N, Takei K, Floyd SR, Rose K, De Camilli P: **Generation of high curvature membranes mediated by direct endophilin bilayer interactions**. *J Cell Biol* 2001, **155**:193-200.

38. •• Hoffman GR, Rahl PB, Collins RN, Cerione RA: **Conserved structural motifs in intracellular trafficking pathways: structure of the γCOP appendage domain**. *Mol Cell* 2003, **12**:615-625.
This structure of the γCOP appendage domain confirms the suspected homology between coatomer subunits and clathrin adaptors. This initial structure will provide impetus for further parallels to be drawn between these vesicle budding pathways.

39. •• Watson PJ, Frigerio G, Collins BM, Duden R, Owen DJ: **γCOP appendage domain — structure and function**. *Traffic* 2004, **5**:79-88.
This paper identifies ArfGAP2 as a ligand of the γCOP appendage domain. Mutagenesis of W776 in γCOP abolishes the interaction and is equivalent to the ligand interaction pocket centred around W840 in the α-adaptin appendage.

40. Owen DJ, Vallis Y, Noble ME, Hunter JB, Dafforn TR, Evans PR, McMahon HT: **A structural explanation for the binding of multiple ligands by the α-adaptin appendage domain**. *Cell* 1999, **97**:805-815.

41. Traub LM, Downs MA, Westrich JL, Fremont DH: **Crystal structure of the α appendage of AP-2 reveals a recruitment platform for clathrin-coat assembly**. *Proc Natl Acad Sci USA* 1999, **96**:8907-8912.

42. • Brett TJ, Traub LM, Fremont DH: **Accessory protein recruitment motifs in clathrin-mediated endocytosis**. *Structure* 2002, **10**:797-809.
This paper reveals a novel AP2 α-appendage binding motif, FxDxF, which is found in several binding partners.

43. Kent HM, McMahon HT, Evans PR, Benmerah A, Owen DJ: **γ-adaptin appendage domain: structure and binding site for Eps15 and γ- synergin**. *Structure* 2002, **10**:1139-1148.

44. Nogi T, Shiba Y, Kawasaki M, Shiba T, Matsugaki N, Igarashi N, Suzuki M, Kato R, Takatsu H, Nakayama K *et al.*: **Structural basis for the accessory protein recruitment by the γ -adaptin ear domain**. *Nat Struct Biol* 2002, **9**:527-531.

45. Shiba T, Takatsu H, Nogi T, Matsugaki N, Kawasaki M, Igarashi N, Suzuki M, Kato R, Earnest T, Nakayama K *et al.*: **Structural basis for recognition of acidic-cluster dileucine sequence by GGA1**. *Nature* 2002, **415**:937-941.

46. Collins BM, Praefcke GJ, Robinson MS, Owen DJ: **Structural basis for binding of accessory proteins by the appendage domain of GGAs**. *Nat Struct Biol* 2003, **10**:607-613.

47. Miller GJ, Mattera R, Bonifacino JS, Hurley JH: **Recognition of accessory protein motifs by the gamma-adaptin ear domain of GGA3**. *Nat Struct Biol* 2003, **10**:599-606.

48. Jha A, Agostinelli NR, Mishra SK, Keyel PA, Hawryluk MJ, Traub LM: **A novel AP-2 adaptor interaction motif initially identified in the long-splice isoform of synaptojanin 1, SJ170**. *J Biol Chem* 2004, **279**:2281-2290.
This paper extends the range of α-adaptin binding motifs from DxF and FXDXF to include a WxxF motif found in synaptojanin, AAK, GAK and stonin-2.

49. •• Bigay J, Gounon P, Robineau S, Antonny B: **Lipid packing sensed by ArfGAP1 couples COPI coat disassembly to membrane bilayer curvature**. *Nature* 2003, **426**:563-566.
It has long been a puzzle how vesicle uncoating is regulated. This paper gives the first clues and is worth a read.

50. Ohno H, Aguilar RC, Yeh D, Taura D, Saito T, Bonifacino JS: **The medium subunits of adaptor complexes recognize distinct but overlapping sets of tyrosine-based sorting signals**. *J Biol Chem* 1998, **273**:25915-25921.

51. Andag U, Schmitt HD: **Dsl1p, an essential component of the Golgi-endoplasmic reticulum retrieval system in yeast, uses the same sequence motif to interact with different subunits of the COPI vesicle coat**. *J Biol Chem* 2003, **278**:51722-51734.

52. Walther K, Diril MK, Jung N, Haucke V: **Functional dissection of the interactions of stonin 2 with the adaptor complex AP-2 and synaptotagmin**. *Proc Natl Acad Sci USA* 2004, **101**:964-969.

53. Sachse M, Urbe S, Oorschot V, Strous GJ, Klumperman J: **Bilayered clathrin coats on endosomal vacuoles are involved in protein sorting toward lysosomes**. *Mol Biol Cell* 2002, **13**:1313-1328.

54. Heuser J: **Effects of cytoplasmic acidification on clathrin lattice morphology**. *J Cell Biol* 1989, **108**:401-411.

55. • Raiborg C, Bache KG, Gillooly DJ, Madshus IH, Stang E, Stenmark H: **Hrs sorts ubiquitinated proteins into clathrin-coated microdomains of early endosomes**. *Nat Cell Biol* 2002, **4**:394-398.
An interesting approach using a transferrin-receptor–ubiquitin conjugate to highlight the importance of the Hrs–ubiquitin interaction in cargo sorting to multivesicular bodies at the early endosome.

56. Marks B, McMahon HT: **Calcium triggers calcineurin-dependent synaptic vesicle recycling in mammalian nerve terminals**. *Curr Biol* 1998, **8**:740-749.

57. Stowell MH, Marks B, Wigge P, McMahon HT: **Nucleotide-dependent conformational changes in dynamin: evidence for a mechanochemical molecular spring**. *Nat Cell Biol* 1999, **1**:27-32.

58. Takei K, Haucke V, Slepnev V, Farsad K, Salazar M, Chen H, De Camilli P: **Generation of coated intermediates of clathrin-mediated endocytosis on protein-free liposomes**. *Cell* 1998, **94**:131-141.

59. Spang A, Matsuoka K, Hamamoto S, Schekman R, Orci L: **Coatomer, Arf1p, and nucleotide are required to bud coat-protein-complex-I-coated vesicles from large synthetic liposomes**. *Proc Natl Acad Sci USA* 1998, **95**:11199-11204.

60. Matsuoka K, Orci L, Amherdt M, Bednarek SY, Hamamoto S, Schekman R, Yeung T: **COPII-coated vesicle formation reconstituted with purified coat proteins and chemically defined liposomes**. *Cell* 1998, **93**:263-275.

61. Mattera R, Puertollano R, Smith WJ, Bonifacino JS: **The tri-helical bundle subdomain of the GGA proteins interacts with multiple partners through overlapping but distinct sites**. *J Biol Chem* 2004, in press.

62. Bai H, Doray B, Kornfeld S: **GGA1 interacts with the adaptor protein AP-1 through a WNSF sequence in its hinge region**. *J Biol Chem* 2004, **279**:17411-17417.