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The Evolution of Organellar Coat Complexes and Organization of the Eukaryotic Cell

Michael P. Rout¹ and Mark C. Field²

¹The Rockefeller University, New York, NY 10021; email: rout@rockefeller.edu

²Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, Dundee DD1 5EH, United Kingdom; email: mfield@mac.com

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Abstract

Eukaryotic cells possess a remarkably diverse range of organelles that provide compartmentalization for distinct cellular functions and are likely responsible for the remarkable success of these organisms. The origins and subsequent elaboration of these compartments represent a key aspect in the transition between prokaryotic and eukaryotic cellular forms. The protein machinery required to build, maintain, and define many membrane-bound compartments is encoded by several paralog families, including small GTPases, coiled-bundle proteins, and proteins with β -propeller and α -solenoid secondary structures. Together these proteins provide the membrane coats and control systems to structure and coordinate the endomembrane system. Mechanistically and evolutionarily, they unite not only secretory and endocytic organelles but also the flagellum and nucleus. The ancient origins for these families have been revealed by recent findings, providing new perspectives on the deep evolutionary processes and relationships that underlie eukaryotic cell structure.

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INTRODUCTION

The transition from prokaryotic to eukaryotic cells occurred over a billion and a half years ago and represents one of the most important and spectacular changes to cellular structure in all of evolution (1, 2). Although many prokaryotes possess some internal organization that may even include membranous structures (3–5), in the eukaryotic cell this has become elevated to a far greater level of sophistication and includes the multiple organelles of the endomembrane system, the nucleus, and the flagellum. The elaborate internal membrane compartments of modern eukaryotes are a testament to functional flexibility, which we presume evolved to respond to changing environmental conditions and new requirements by a cell in a multitude of ways (i.e., controlling nuclear egress and ingress, entry and exit of bulk materials across the plasma membrane, and sorting of proteins into specific compartments).

Intracellular organelles originated either from the acquisition of some preexisting external biological structure, as is the case for the endosymbiotically derived mitochondrion and plastid/chloroplast, or via adaptation and elaboration of preexisting intrinsic cellular structures and molecules, which require duplication and neofunctionalization of existing genes and their products (6–10). The origins of these endogenous organelles, the order in which they arose, and their subsequent adaptation within modern lineages are central to understanding eukaryotic evolutionary cell biology and also their impact on mechanisms of development, disease and pathogenesis. Over the last two decades or so, many of the paralog protein families that constitute the specificity machinery and facilitate the evolution of new compartments have been identified through

biochemical, comparative genomic, and structural analyses; as we will detail below, these families include GTPases, longins, SNAREs (soluble NSF attachment protein receptors), tethering complexes, and a group of proteins that includes the vesicle coating complexes central to intracellular transport (11–14).

GENERAL PRINCIPLES OF COMPARTMENT CONSTRUCTION

The material from which internal compartments are constructed includes a membrane—a lipid bilayer, comprised chiefly of various phospholipids and sterols, in which peripheral and transmembrane proteins are embedded. Depending on lipid composition, membranes are usually planar and thus require stretching and/or deformation to mold them into tubules, sacs, cisternae, or more complex structures (15). As a result, the eukaryotic endomembrane system is assembled and dynamically maintained by protein complexes, many of which induce membrane deformation to generate compartments and the vesicles that ferry membrane and luminal cargoes among these compartments. Other complexes mediate interactions between endomembrane compartments and vesicles or with the cytoskeleton, regulating fission, budding movement, docking, as well as the subcellular positioning of the endomembrane compartments themselves. As a consequence of specific transport, these complexes define the identity of each membrane compartment and trafficking vesicle. Membrane manipulations require several factors. First, local compartmentalization in the plane of the membrane is required to recruit specific factors to the site of bending, such as where a vesicle is to form. Second, energy is needed, both to increase the local concentration of recruited factors and to overcome the membrane's own resistance to deformation. Third, an asymmetry across the lipid bilayer is also essential to define the direction of bending (15).

Because the mechanisms cells employ for membrane bending are constrained by these architectural, biophysical, and functional requirements, at a fundamental level all bending machineries follow similar operating parameters (16). To initiate the process of generating a compartment, curvature must be induced at a site on the target membrane. Local compositions, including asymmetric conical lipids, are likely contributors (17). However, recent work has underscored the importance of amphipathic protein helices to this process; the insertion of these helices into one leaflet of the lipid bilayer likely assists in initiating curvature, and because they also preferentially interact with the already-curved membrane, they also create a positive feedback cascade (15, 16). There is synergy between lipid and protein recruitment in this initial step—for example, PI(4,5)P₂ (phosphatidylinositol 4,5-bisphosphate) is required to recruit epsin, CALM, and amphiphysin, all of which have N-terminal amphipathic helices for membrane insertion, to initiate clathrin-coated vesicle formation (18–20). However, this initial induced curvature appears to be insufficient for the degree of membrane deformation needed for compartmentalization and vesiculation, and so a crucial next step involves recruitment of extrinsic coat-forming proteins. Even as monomers, the shape alone of these coat proteins can help further drive membrane bending (21, 22), but polymerization into larger curved scaffolds, with the considerable energy expenditure involved and a precurved structure being assembled, truly drives membrane curvature and creates a scaffold to support and stabilize continued membrane deformation; most coat proteins oligomerize to form a roughly spherical lattice, whose tight interaction with underlying proteins ensures the membrane remains in its deformed state and ultimately likely contributes to the thermodynamic energy required to achieve fission (23). Thus, many examples of eukaryotic membrane manipulation ultimately involve a coating event. If the eventual goal is vesiculation, this coating step is followed by a scission event, once again mediated by recruitment of specific proteins, and possibly lipids, to create a transient scission complex and a membrane environment conducive to fission. However, the dynamics of these different events can vary greatly. For example, if the goal of the

process is trafficking between compartments, then, as with clathrin-mediated endocytosis (CME), the coating complexes are maintained for milliseconds to seconds (24, 25); conversely, if the goal of the process is to assemble a stable endomembrane compartment, then, as with the nuclear pore complex (NPC), the assembled coat complex persists for days or even years (26).

EUKARYOGENESIS AND HOW COMPARTMENTALIZATION WAS ACHIEVED

The revolution in genomics has allowed an unprecedented level of detailed information on the components of any eukaryotic cell to be obtained and has allowed for comparisons among essentially all the major eukaryotic lineages to be made. Moreover, advances in structural biology have also propelled comparisons among the detailed architectures of many of the protein complexes associated with endomembranes. Together, these insights allow exploration of the different ways that endomembrane systems are potentially modified in cells and the evolutionary origins of these membrane-manipulating machineries. Such studies have likely identified the major families of protein players, and it has emerged that the overall configuration of the endomembrane system was established very early in the evolutionary history of eukaryotes (27–29). By the time of the last eukaryotic common ancestor (LECA), approximately one and one half billion years ago, a final consensus endomembrane arrangement and associated complement of components had become established. Remarkably, reconstructions from comparative genomic studies established that the LECA's endomembrane system was actually extraordinarily complex (**Figure 1**). As well as the core exocytic, endocytic, and phagocytic apparatuses, these systems were, according to the complement of genes predicted as present, already differentiated into multiple endosomal and recycling pathways, such that elaborate transport and tethering systems were functioning. The majority of modern eukaryotes, including the plants, protists, amoeba, and opisthokonts (animals and fungi), have retained much of this complexity along with the components that established them.

Peering beyond the LECA to events surrounding the first eukaryotic common ancestor (FECA) and events leading up to eukaryogenesis has been significantly more challenging (**Figure 1**). However, connections between mechanisms of intracellular vesicle and intraflagellar transport (IFT) and their dependence on Rab and Rab-like proteins and further similarities with Ran, the GTPase principally involved in nucleocytoplasmic transport, have been obvious for a considerable time, and all of these GTPases are comparatively closely related within the greater Ras superfamily (30–34).

Eukaryotic forms emerged most likely within the Archaea bacteria and close to the Thaumarchaeota, Aigarchaeota, Crenarchaeota, and Korarchaeota (TACK) clade, based on extensive whole genome sequencing and greater similarity among many genes shared between TACK archaea and eukaryotes (35–37). Recently, the predicted gene complement of a metagenome from a Lokiarchaeum, which itself is closely related to the TACK clade and was isolated from hydrothermal vent sediments, has strengthened this paradigm further, as the reconstructed genome contains close relatives to hallmark eukaryotic genes (i.e., genes so far considered restricted to eukaryotic genomes). Even more recently, additional relatives to Lokiarchaea have been identified and termed the Asgard Archaea (38, 39). Significantly, these are derived from geographically widely dispersed locations, suggesting this is a major branch of previously uncharacterized Archaea. Taken together, the Asgard archaea host homologs to many trafficking genes within their predicted metagenomes.

The reconstructed genomes of these organisms notably contain predicted protein domains that in eukaryotes are associated with intracellular trafficking and secretion. These include ESCRT (endosomal sorting complexes required for transport), TRAPP (transport protein particle), and homologs of the Sec23/24 COPII (coat protein complex II) vesicle coatomer protein complex,

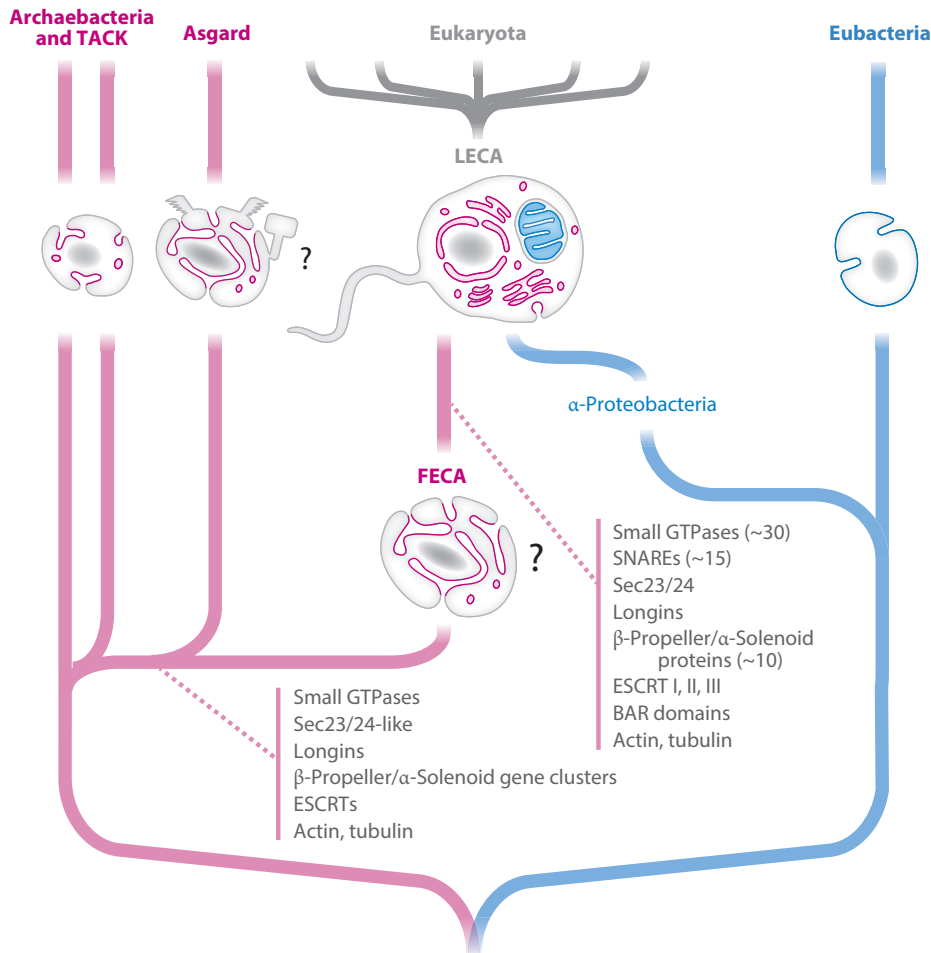


Figure 1

The pathway to eukaryotic structure. According to the now well-accepted two domains of life model, eukaryotes arose from within the Archaea, with the closest known lineage being the TACK/Asgard archaea. These organisms share a number of clear similarities to the eukaryotic state, for example, a role for ESCRT in cytokinesis, together with small GTPases, longins, homologs of the COPII transport system (resembling Sec23 and Sec24), and other potential distant homologs to trafficking genes. This lineage gave rise to the organism that was the FECA, which is assumed to have already acquired a nucleus and possibly some internal structure. However, uncertainty in the order of events of evolution of the internal membrane (e.g., if phagocytosis or secretory structures arose first) has made a definition of FECA elusive. By contrast, the LECA is well defined and contained a clear, complex cell architecture with fully differentiated internal systems. At some point in the transition from FECA to LECA the mitochondrion was acquired, and despite the clear importance of the mitochondrion for bioenergetics, the genetic contribution of this endosymbiont to cellular complexity appears minor. LECA was in fact more complex than many well-studied model eukaryotes, such as *Saccharomyces*, *Chlamydomonas*, and *Trypanosoma*. Abbreviations: BAR, Bin/amphiphysin/Rvs domains; COPII, coat protein complex II; ESCRT, endosomal sorting complexes required for transport; FECA, first eukaryotic common ancestor; LECA, last eukaryotic common ancestor; SNAREs, soluble NSF attachment protein receptors; TACK, Thaumarchaeota, Aigarchaeota, Crenarchaeota, and Korarchaeota. The question mark indicates cellular forms for which the precise internal configuration is uncertain. Archaeal lineages are shown in magenta, eubacteria in blue, and eukaryotes in gray. Thorarchaeal image courtesy of Jack Kirby.

together with many small GTPases that are closely related to Rabs. Perhaps most intriguing are the presence of gene clusters in the Thorarchaea that encode proteins with predicted β -propeller or α -solenoid secondary structures (38–41). As we discuss below, these architectures are signatures for the huge diversity of vesicle coat and tethering complexes found throughout the eukaryotes. Moreover, Asgard archaea possess the prenylation system important for Rab posttranslational modification and function, together with longin domain proteins, indicating that the potential precursors to at least some small GTPases, SNAREs, and other systems are present within this phylum. It is a comparatively short step from this configuration to a simple eukaryotic cellular system, envisaged as being on board in the FECA (**Figure 1**). However, given the absence of any information concerning the internal organization of these fascinating organisms, the precise functions of these proteins remain unclear. Nevertheless, in terms of establishing the link between prokaryotes and eukaryotes, these findings are extremely provocative. Indeed, it is an exciting possibility that, despite a shared ancestor, the modern Asgard archaea and the eukaryotes have derived distinct solutions and configurations for intracellular compartmentalization.

Among the proteins that are central components for compartmentalization and defining organelle identity are Rab and ARF/ARL (ADP-ribosylation factor/ARF-like) family GTPases, coiled-bundle SNARE proteins, and a subset of these and related proteins possessing a longin domain. There is also a large cohort of regulatory factors—many of which are also paralog families, including the Sec7 domain guanosine triphosphate (GTP) guanosine exchange factors (GEFs) and the Tre-2/Bub2/Cdc16 (TBC) domain GTPase-activating proteins (GAPs). These protein families mediate vesicular transport and, equally significantly, control specificity. As a consequence, and as the result of the flux of vesicular delivery and removal, the transport system dictates the protein and lipid composition and hence the function of donor and target organelles.

The evolution of Rabs, ARFs, SNAREs, GEFs, and GAPs has likely been the result of stepwise growth in sophistication from a rather simple system and is consistent with gene duplication and neofunctionalization (27). For the Rab proteins a putative endocytic and exocytic clade can be resolved, suggesting a deep division and very early acquisition of these distinct arms of the endomembrane system (13), as proposed by the organelle paralogy hypothesis for the origins of new compartments. It is also possible, albeit not formally proven, that a similar situation holds for the SNAREs and, as discussed below, the coat protein complexes. This paradigm is also fully consistent with the concept of stepwise increments to pathway complexity and the idea of a basic exocytic and endocytic system that has become elaborated (42); a large number of modern eukaryotes have a simpler cellular plan than predicted for the LECA, indicating that secondary loss is also a frequent contributor to cellular evolution (43).

COATS AND TETHERS: THE CHIEF PLAYERS?

The physical constraints of membrane deformation, mechanistic commonalities at the heart of many transport steps, and constraints arising from the limits of evolution together may explain why modern eukaryotes actually possess a limited repertoire of membrane manipulating machines, with the major players comprising a handful of paralogous families. The organelle paralogy hypothesis suggests that paralogous duplication is a far more flexible mechanism for the diversification of new organelles than defining organelles with disparate groups of unrelated proteins or requiring coevolution of a large cohort of proteins to achieve new specificity (27).

Having settled upon a simple set of principles and protein complexes and membranes to enact them, these systems duplicated and diverged to meet the needs of the diverse functions of endomembrane systems. Thus, all the currently known membrane-deforming complexes fall into three major structural classes. The first of these is the ESCRT complex, including the

membrane-deforming Snf7 domain subunits. ESCRT chiefly functions in late endosomal sorting and cytokinesis, although numerous additional roles have now been described (44). The second is a group of Bin/amphiphysin/Rvs (BAR) domain-containing proteins, with roles mainly at the Golgi and endosomal interface, in phagocytosis, and once more in cytokinesis (20, 22, 45).

However, the third group, the protocoatomer-derived complexes, has come to dominate, in terms of both the number of processes that they facilitate and the range of architectures that these complexes are able to accommodate (11). Protocoatomer complexes all consist of four classes of components: coat-forming proteins, small GTPases, coiled-coil-containing proteins, and longin domain-containing proteins (**Figure 2**). These complexes are mostly thought to act in exocytosis, endocytosis, and intracellular vesicular transport, such as the COPI, COPII, and clathrin complexes. However, membership of this family extends to proteins that do not form transport vesicles but instead build stable membrane-associated coats, such as the NPC; that form tethering complexes, such as the HOPS (homotypic fusion and vacuole protein sorting)/CORVET (class C core vacuole-endosome tethering) and SEA (Seh1-associated) tethering complexes; or that, similarly to the NPC, associate with membranes to direct protein transport, such as the IFT complexes that mediate trafficking inside flagella (46–50).

COMMON ARCHITECTURES WITHIN MEMBRANE COAT AND TETHER COMPLEXES

Several domains are key features of membrane deforming systems.

β - α Protocoatomer Architecture

The architectural similarities among many membrane coat components, specifically COPI and clathrin/adaptin complexes, have been recognized since their structures were elucidated and have suggested a possible common evolutionary origin (51–53). COPI and clathrin/adaptin complexes consist of one or two β -proPELLERS, an α -solenoid-like domain, or both, exclusively in the order β - α (54). More recently, the same architecture was recognized in subunits of the NPC, COPII, and elsewhere. On the basis of these observations, the hypothesis that NPCs and clathrin, COPI, and COPII vesicle coats share a common origin in an ancestral protocoatomer was proposed, with their modern descendants retaining key elements of this ancestral β - α protocoatomer architecture (54–57) (**Figure 2**). X-ray crystallography, electron microscopy, and structural modeling all support this hypothesis and have extended the number of protocoatomer architecture-containing complexes to include IFT and the SEA and HOPS/CORVET complexes (**Figure 2**) (46, 50, 58–66). Furthermore, the same α -solenoid architecture found in adaptins and NPC proteins is also present in NPC-interacting karyopherins (55, 67) and possibly also the retromer, involved in Golgi/late endosomal transport (68–70), further extending these relationships.

Coiled-Bundle Complexes

This family is largely comprised of SNAREs, low-molecular-weight proteins, most of which are tail-anchored and inserted into membranes via a C-terminal transmembrane domain and/or a lipid modification. SNAREs assemble into heterocomplexes, with each complex targeted to a unique membrane or subdomain, providing both specificity as well as fusogenic activity. All share a SNARE motif in their cytosolic domain consisting of ~ 60 amino acids containing heptad repeats with the ability to form coiled-coil bundles, and formation of a core parallel four-helix coiled-coil bundle brings membranes into close apposition to trigger fusion (71–73). Notably, coiled-coil

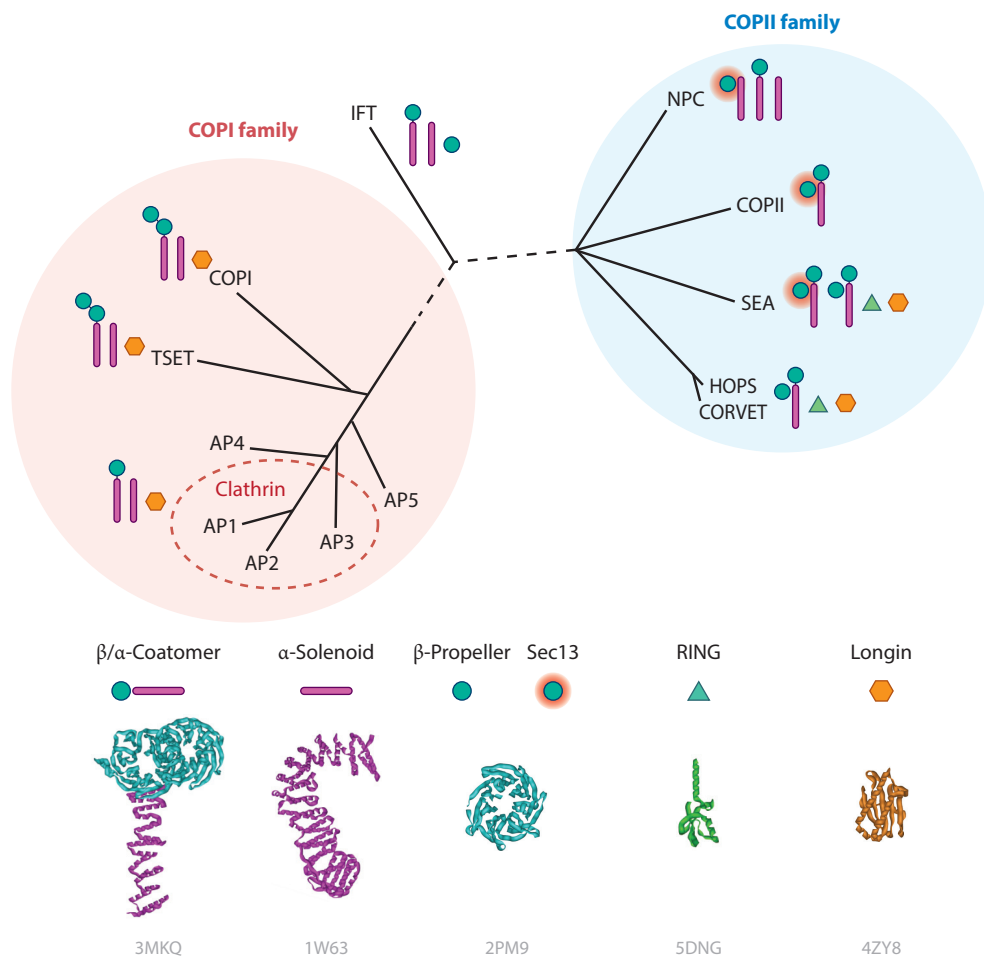


Figure 2

Major relationships among protocoatomeer architecture-containing complexes. At least twelve complexes possess protocoatomeer-related subunits, but the precise relationships among these systems remain difficult to characterize. This difficulty is likely in part due to the presence of multiple complexes within some of the structures (e.g., IFT and the NPC), but subunit sharing and coevolutionary constraints are also important factors. Overall data suggest the presence of two groups in the protocoatomeer evolutionary history, a major adaptin/COPI/TSET cluster (*red*) and a COPII cluster (*blue*), with IFT remaining refractory to accurate placement owing to the lack of discriminatory architectural information. The adaptin/COPI cluster shares the common architecture of a heteromeric complex containing α -solenoid and longin domain subunits that interact with a β -propeller/ α -solenoid-containing membrane coat system. The second COPII cluster either shares Sec13 or, in the case of HOPS/CORVET, is a clear relative to SEA. Several of these complexes also integrate a RING domain within their protocoatomeer subunits and are perhaps notable by the absence of longin domain-containing subunits. Significantly, the expansion of the adaptins could be explained as a recent event, as these complexes retain high degrees of sequence identity and several share a coating complex, whereas all other systems have distinct protocoatomeer subunits. Abbreviations: AP, adaptor protein; COPI, coat protein complex I; COPII, coat protein complex II; CORVET, class C core vacuole-endosome tethering; HOPS, homotypic fusion and vacuole protein sorting; IFT, intraflagellar transport; NPC, nuclear pore complex; RING, really interesting new gene; SEA, SEH1 associated; TSET, TPLATE complex. The Protein Data Bank numbers for the various domains are provided below each structure.

complexes are also found at the heart of the NPC (74, 75), but though suggestive, their relationship to SNAREs is currently unclear.

Amphipathic Lipid Packing Sensor Motifs

Amphipathic lipid packing sensor (ALPS) motif sequences are 20–40 amino acids in length and contain regular bulky hydrophobic residues spaced by 3–4 small polar uncharged residues, such that all the hydrophobic residues align at one face of an α -helix (76). ALPS motifs are intrinsically soluble but bind efficiently to positively curved membranes; however, rather than recognize the curved surface geometry of membranes per se, ALPS motifs recognize defects in lipid packing that arise from curvature. ALPS motifs are present in coat-forming proteins, membrane tethers, nucleoporins, and lipid transporters in which they essentially act as detectors of membrane curvature. This is certainly the case for the ALPS domain of ArfGAP1, which detects packing defects in the curved bilayers of COPI vesicles, specifically inserting into membranes that are under curvature stress. As a result, GTPase stimulation activity is restricted to curved membrane regions (77). ArfGAP1 ALPS motifs help to organize two reactions: the assembly/disassembly cycle of COPI and the attachment of vesicles to long coiled-coil tether proteins (78, 79).

GTPases

As discussed above, GTPases and their GEFs and GAPs are central to membrane-associated transport processes. GTPases of the ARF family control cargo selection by coat complexes, whereas GTPases of the Rab family are required for the fusion of vesicles with the appropriate target membrane. Coat assembly is initiated by the activation of an Arf protein, and Arf1 regulates formation of COPI vesicles and of clathrin-coated vesicles that contain the adaptor protein (AP) complexes AP1, AP3, and AP4 (80). Likewise, COPII vesicle budding involves Sar1, an Arf relative, for initiation (81). When activated by a cognate GEF, localized to the target membrane, the ARF/Sar-GDP (guanosine diphosphate) becomes ARF/Sar-GTP and then recruits the coatomer through direct interactions.

Longins

The longin or uDENN domain is a regulatory element in many SNAREs but is also present as a component of several coat systems, including Npr1/2 of SEA, δ -COP (delta-subunit of COPI) of COPI, and adaptin subunits, and also many GTPase interacting proteins, including the prokaryotic MlgA (12, 71). The importance of the vast contributions of longin domains to cell structure and their potentially ancient origin has come to the fore only recently. The ~15 kDa longin domain is a conserved α - β - α sandwich fold (a very common architecture) that in trafficking contributes to regulation of assembly and fusion, including budding, tethering, and regulation of Rab GTPases. The longin domain regulates the fusogenic activity of SNAREs by mediating intramolecular interactions with their coiled bundle domain. Longin domain-containing proteins are classified into seven superfamilies: longin SNAREs, adaptins, sedlins, SANDs (Sp100, AIRE-1, NucP41/75, DEAF-1), targetins, DENNs (differentially expressed in normal and neoplastic cells), and APL2 VPS1 synthetic lethal proteins (AVLs) (12, 71). A subset of SNAREs, the VAMPs (vesicle-associated membrane proteins), have a highly conserved longin domain, and these SNAREs act to mediate intracellular membrane fusion and define localization through association with coat proteins, as in longin Sec22b with the Sec23/24 subunits of COPII. Longin proteins also bring membranes together without initiating their fusion—for example, Sec22b brings the

endoplasmic reticulum (ER) and plasma membranes into close proximity, stabilizing close contacts between these two membranes. The small σ and μ adaptin subunits and their homologs σ and ζ in COPI are all longin-like domain proteins and important mediators of function through signaling and protein–protein interaction pathways (12, 82). Furthermore, longin domains are involved in regulating Rab GEFs and tethering complexes, including DENN, SAND, and TRAPP complexes, and also in the TOR (target of rapamycin) pathway through nutrient sensing and autophagy via their incorporation in the SEA complex. Longin domains appear to be very ancient, being present in Archaea and possibly also related specific prokaryotic GTPase-activating proteins, with some indication that prokaryotic GTPase circuits have persisted into eukaryotic forms (12, 40).

ARCHITECTURE AND MORPHOLOGY

Most coated vesicles are formed by a mechanism that bears some clear similarities. In most cases, formation is initiated by exchange of GDP for GTP in a small GTPase that induces a conformational change allowing recruitment or insertion into the donor organelle membrane. The GTPase recruits APs, which in turn recruit coat proteins that polymerize to form the outer coat or cage. There are also often two layers to the coat scaffold, a membrane proximal layer (adaptin or Sec23/24 for CME or COPII, respectively) and a membrane distal layer (clathrin, Sec13/31), suggesting functional and structural subdivisions between adaptor and coat functions (63) (**Figure 3**). Significantly, the distinction between these layers in COPI is less strict, with the subunits more intertwined; nevertheless, similar to the other examples, α -solenoids serve to distribute cargo and membrane-binding domains on the vesicle surface (82). The NPC also contains an intertwining of coatomer-like and adapter-like proteins (57, 74).

THE COAT AND TETHER COMPLEXES

The packing of the coat proteins into a lattice, which can be defined as edges and vertices, demonstrates significant structural variation (**Figure 3**). Whereas the edges invariably involve α -solenoid-like domains, vertices can be comprised of β -propellers or α -solenoids. Most significantly, a single lattice architecture is able to accommodate differing curvatures and hence vesicles of different sizes. Evolutionarily, this coat architecture benefits from the ability of α -solenoid-like domains to relatively easily extend or contract in length or to change their packing (54, 55, 83–85). Crucially, the β -propeller and α -solenoid configuration therefore has a high degree of evolvability, consistent with these folds being involved in many additional cellular processes (84, 86). As shown in **Figure 3**, this simple combination of motifs has been adapted into an extremely diverse form of architectures, producing complexes with very distinct forms and functions.

Adaptin/Coat Protein Complex I (COPI)/Clathrin

The adaptin/COPI/clathrin group is important for transport of proteins through recognition of cytosolic tails of transmembrane cargoes or receptors that act as a bridge to the cargo—selecting, sorting, and concentrating cargo into vesicles for transport through the secretory and endocytic pathways (80, 82, 87, 88). The structural repeat unit of clathrin is a trimeric triskelion, in which three clathrin heavy chain edges meet at the vertex. Different curvatures are achieved by varying packing angles between the edges of neighboring triskelions (62) (**Figure 3**). The outer triskelion coat interacts with the inner layer of adaptins through flexible linkers on the latter. There are

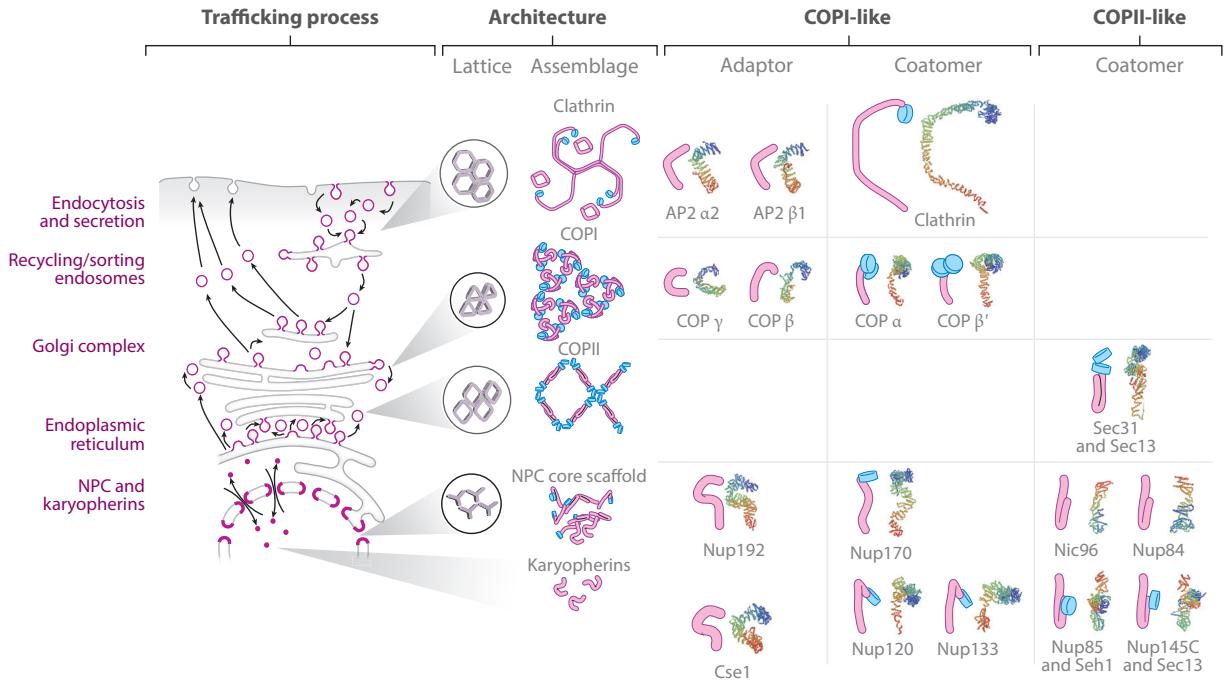


Figure 3

Molecular anatomy of coat complexes. Extreme left, idealized endomembrane system, emphasizing transport events and the compartments with which they are associated. Locations of protocoatamer architecture–contacting complexes are shown in purple. Middle left, architectures of coats showing the lattice (*purple*) and its association with the membrane (*gray*). The assemblage details at higher resolution and the position of subunits forming the lattices with approximate structures and β -propeller (*cyan*) and α -solenoid (*pink*) are indicated. Middle right, detailed architectures of monomers constituting COPI and COPI-related complexes with atomic resolution models compared with the schematic structures. Extreme right, detailed architectures of monomers constituting COPII and COPII-related complexes with atomic resolution models compared with the schematic structures. Protein Data Bank accession numbers for the shown structures are 2JKR, 3IYV, 5A1X, 4BZJ, 1WA5, 4XMN, 2RFO, and 4IFQ. Abbreviations: AP, adaptor protein; COPI, coat protein complex I; COPII, coat protein complex II; NPC, nuclear pore complex.

now known to be five adaptin complexes, all of which share a common heterotetrameric structure, with two large subunits (γ 1, α 2, δ 3, ϵ 4, ζ 5, and β 1–5) and two smaller subunits (μ 1–5 and σ 1–5). Both the μ and σ subunits contain a longin domain, which also suggests an ancient duplication from a simpler heterodimer—a theme one can also clearly see in the NPC (see below) (57, 74). These are a clear example of paralogous expansion generating new complexes, with evidence that, for AP1 and AP2, this expansion is still ongoing (89). A recently discovered divergent member of this group, TSET (TPLATE complex), despite a patchy distribution across eukaryotes, is likely a LECA component and contains all the adaptin-like protein equivalents as well as coat-like proteins. TSET is likely more closely related to COPI than the other adaptins (**Figure 2**) (90).

COPI comprises an F-COPI adaptor and a B-COPI coat. F-COPI comprises two large subunits (γ -COP and β -COP), a medium subunit (δ -COP), and a small subunit (ζ -COP) (82), which are likely distant paralogs to the equivalent adaptin subunits. The B-COPI coat complex consists of α , β' , and ϵ subunits (91). The F-COPI adaptor and B-COPI coat combine in a soluble heteroheptameric complex that is recruited in its entirety to the membrane. Though the subunit structures are broadly similar to clathrin, F-COPI does not assemble into triskelions. Instead, the

COPI coat is built of a repetition of building blocks, termed triads, that contain all the important functional elements organized in a precise three-dimensional structure such that F+B COPI proteins all intertwine together into one large layer (**Figure 3**) (82). These “triads” are connected by flexibly attached domains, to propagate curvature over larger areas while allowing a malleable architecture to eventually form buds, and may have arisen to facilitate accommodation of a diverse range of transport vesicle geometries.

A further candidate member of this group is the exomer, a divergent adaptin-like complex that interestingly does not recruit a coat but bends the membrane directly (80, 88). Finally, the retromer is a heteropentameric complex involved in recycling transmembrane receptors from endosomes to the *trans*-Golgi network. The largest subunit, Vps35, is an α -solenoid protein and resembles AP complexes—however, membrane curvature and vesiculation are induced by BAR domain-containing proteins within the complex, and currently no clear evidence indicates the retromer is part of the protocoatmer class of membrane-deforming systems (68–70, 88).

Together these data suggest a great deal of similarity between COPI, TSET, and clathrin/adaptin complexes. Although the combined coat and adaptor of TSET have been suggested as a missing link in the evolution of this family, recent data indicates instead a common ancestor for TSET and COPI (47). Significantly, this may also reflect mechanistic difference in the assembly processes of the adaptin and TSET/COPI subfamilies, whereby adaptin and clathrin recruitment occurs as two distinct steps that are well documented (92, 93), but the entire COPI coatmer, and possibly TSET (47), is recruited to the membrane en bloc. Significantly, there are separate coats for AP5 (SPG11) (94) and TSET (TTRAY) (90), underscoring the surprising complexity within the adaptin/COPI family. Presumably stepwise assembly facilitates flexibility in cargo recruitment and may be a distinct solution to the roles of different coat proteins.

Coat Protein Complex II (COPII)

This complex is primarily involved in anterograde transport from the ER to the Golgi apparatus. COPII appears at first glance to be rather different from the adaptin/COPI/clathrin group, but closer inspection demonstrates both mechanistic and structural similarities. The Sec23/24 subunits form a bow tie-shaped structure, with a concave positively charged membrane proximal surface to interact with the acidic phospholipid composition of the COPII vesicle (60, 66). Sec23 and Sec24 are clearly ancient duplicates of each other, following the theme of generation of complexity through paralogous duplications and subsequent divergence within a complex (see section above). Sec23/24 possess a smorgasbord of features including a β -barrel, a zinc finger, an α/β vWA (von Willebrand A domain) or so-called trunk domain, an all-helical region, and a carboxy-terminal gelsolin module (60, 66, 95)—another ancient domain found in the Asgard archaeal superphylum (38, 39). The inner Sec23/24 coat can also form a regular lattice independent of the outer Sec31/13 coat, suggesting it may not only function to link cargo and membrane to the outer coat but also play a structural role in determining vesicle morphology (66, 95). There is a considerable degree of flexibility in the geometry of the COPII coat. At least three properties contribute to outer coat adaptability: variability of intersection angles at the vertices, flexibility within the central rod hinge, and the absence of any inherent asymmetry in the Sec13/31 rods, allowing them to make head-to-head, head-to-tail, and tail-to-tail contacts. Together this versatility allows coating of not only spherical, but also tubular membranes and therefore accommodation of large elongated cargoes such as procollagen. This configuration also suggests that the unstructured C-terminal region of Sec31, which connects the inner and outer coats, constrains the coat layers but does not fix their absolute positions relative to one another (66, 95).

The Nuclear Pore Complex

This structure is the largest structurally characterized member of the protocoatome complexes. It appears to be in essence a supercomplex of COPI-like and COPII-like systems and indicates a rather complex relationship with the other protocoatome members (**Figure 3**) (11, 54, 55), although for now we have placed it for convenience with other COPII-like systems due to the defining presence of Sec13 (**Figure 2**). The structural diversity within the NPC suggests that there must have been a progenitor NPC with duplications that gave rise to a complicated core scaffold, itself made up from a pair of outer rings flanking a pair of inner rings. It seems possible that the COPI + COPII architecture of the core scaffold arose through a merging of different coat complexes when the endomembrane was less differentiated (11, 57, 67, 96).

The architecture of the NPC itself does not currently provide clear answers—the core scaffold carries both COPII-like and COPI-like protein architectures; additionally, the inner ring also includes the adaptin-like proteins Nup188 and Nup192, which share similar architectures with the major karyopherin family of soluble cargo-carrying transport factors (57, 74). These transport factors interact with the Rab-like GTPase, Ran, to drive bidirectional macromolecular transport across the NPC (58). Flexible connectors, analogous to the unstructured C-terminal region of Sec31 and flexible connectors of the adaptins in COPI and clathrin/adaptin complexes, connect between the outer and inner rings (97). Whereas longin domains seem absent from the NPC, coiled-bundle domains are present in numerous subassemblies throughout the NPC (74, 75).

Valuable evidence in support of a closer relationship between COPII and the NPC can be inferred from the presence of a shared component, Sec13 (56). However, this shared component is evidence only for a relationship between the outer ring Nup84 NPC subcomplex and COPII and not additional NPC elements, and it would perhaps imply that the split between COPII and the NPC was at the stage of a very primitive proto-NPC. Complicating this is the late endosomal SEA complex, which shares both Sec13 and Seh1 with the NPC (at least in the yeast *Saccharomyces*) in addition to possessing several β - α protocoatome subunits. One of these, SEA4, is similar to Sec31, which suggests an additional connection with COPII, and is possibly also shared with Vps39, a component of the HOPS/CORVET complex (46, 50, 98). Although this remains rather tentative, these pieces of evidence suggest a close relationship among HOPS/CORVET, COPII, SEA, and the NPC (**Figure 2**).

The Intraflagellar Transport System

The flagellum (cilium) is also an ancient eukaryotic structure predicted to be present in the LECA. Flagellum assembly requires a specialized IFT system, which comprises three complexes: IFT-A, IFT-B, and the BBsome. These complexes are loosely associated biochemically and assemble in cells into IFT trains, structures several hundred nanometers in length that are closely engaged with the axoneme, and bind directly to tubulin (99, 100). IFT is powered by kinesin 2 and cytoplasmic dynein, to facilitate bidirectional transport. IFT-A contains 7 subunits and IFT-B together with the BBsome 17 subunits, with the BBsome contributing 10 more, to achieve a compositional complexity rivaling the NPC. IFT complexes A and B are invariably present to some degree in organisms that possess conventional flagella and/or cilia, whereas the association is less strong for the BBsome, as some flagellates lack this complex. Several subunits of each complex (WDR19, WDR35, IFT140, IFT122, IFT172, and IFT80) are clearly members of the protocoatome family, containing the hallmark β - α architecture. IFT subunits are also remarkably well conserved at the sequence level across eukaryotes, substantially more than for the NPC. In evolutionary terms, it is likely that IFT-B evolved first from a simpler system pre-LECA and then duplicated to form the BBsome and finally IFT-A. Significantly, the modern IFT system possesses several GTPases

that are closely related to Rab8 (IFT22, IFT27), whereas the BBSome contains an ARL protein (ARL6/BBS3) (101, 102).

Clearly the presence of all these elements indicates a fundamental connection to the proto-coatomer, with the presence of GTPases controlling both vesicle fusion (Rabs) and coat assembly (ARLs). A short region of ~150 residues that sits between the β -propeller- and α -solenoid-like segments in all β - α IFT subunits possesses some similarity toward the α and β' subunits of the COPI complex, whereas TTC21, IFT88, TTC26, TTC30A/B, BBS4, and BBS8 also exhibit architectural similarity to the COP- ϵ subunit, which together suggests an evolutionary relationship between IFT and COPI (49). The presence of coat proteins and GTPases is potentially a further example of a coat system, together with fusion and control element equivalents, remaining in biochemical association, but the sheer number of potential coating proteins in the IFT system indicates considerable complexity. Significantly, a novel means of association between proto-coatomer subunits not so far described elsewhere was revealed from the structure of a complex of *Chlamydomonas reinhardtii* IFT70/IFT52, in which the IFT70 solenoids wrap tightly around a proline-rich and mainly hydrophobic fragment of IFT52 that runs through the center of the solenoid tube (103). Interaction maps and partial structures are available for much of the IFT system but currently remain insufficient to fully understand how the quaternary structure of IFT resembles other members of the proto-coatomer family, if at all.

The IFT complexes thus remain difficult to place evolutionarily, at least in part owing to the absence of full structural information, which raises the possibility that IFT is, similarly to the NPC, a chimera of COPI-like and COPII-like components, such that placing the entire complex is in truth impossible and that subcomplexes should be the unit of consideration (**Figure 2**). Although some evidence links several IFT components to COPI (49), the presence of some NPC proteins at the base of the cilium may indicate more complex level of interactions and evolutionary relationships (104).

Multi-Subunit Tethering Complexes

Although SNAREs are sufficient for fusion in *in vitro* reconstitutions, it is a rather slow reaction with poor specificity. Additional components are often recruited to assist with the docking and fusion events and are referred to as tethering complexes. Mechanistically these complexes all interact with SNAREs and/or GTPases to control the specificity of vesicle fusion and, in many instances, nucleotide exchange reactions. Multi-subunit tethering complexes (MTCs) can also interact with coat complexes: for example, COPI with the Dsl1 (CATCHR family) tether, COPII with the TRAPP I tether, and AP3 with the HOPS tether. Significantly, the HOPS complex contains proto-coatomer-like subunits, strongly suggesting that HOPS is derived from primordial coat complexes. In ancestral eukaryotic cells, both donor and acceptor membranes may have been covered by different bona fide coats, and fusion may have been initiated by the direct contact between them. During evolution, one of these coats acquired and improved its capability to induce membrane curvature, whereas the other, with a preference for flat membranes, developed into a tethering factor and lost some coat-forming abilities (47, 105).

MTCs are categorized into three groups that also share some domains with the proto-coatomer complexes. Recent findings have blurred the distinction between true coat complexes and tethering complexes, traditionally considered to be involved in vesicle docking. HOPS/CORVET is classed as a tethering complex but possesses β/α domain subunits, recognizes curved membranes, acts in late endocytic targeting (106, 107), and is a near-universal eukaryotic feature (29). It is unknown if HOPS/CORVET forms a lattice contributing to formation of transport intermediate complexes or HOPS is important for homotypic fusion and vacuole protein sorting and later recruits CORVET

for endosomal transport. Similar to the adaptins, a degree of subunit expansion to create at least three variant complexes, but retaining the core Vps11, 16, 18, and 33 protocoatmer subunits, has extended the functionality of this system and facilitates a switch between being controlled by Rab5 or Rab7; most clearly, these complexes were derived from a single ancestral complex (47, 105). At some level HOPS/CORVET appears as an intermediate in structural sophistication between the simple adaptins and NPC/IFT supercomplexes.

Further, the SEA complex, which interacts with the TOR pathway at vacuolar/late endosomal membranes, is very clearly HOPS/CORVET-related and significantly shares two subunits, Seh1 and Sec13, with the NPC as well as a structural analog of Sec31 (as discussed above). This indicates that not all members of the protocoatmer system likely are true coats and suggests a degree of subunit promiscuity that may have facilitated the evolution of additional complexes by a mixing of preexisting components. Hence, the protocoatmer family has functions that have diverged from the core process of membrane deformation. Whether membrane deformation or tethering represents the ancestral role is unclear, as all of these complexes are sufficiently retained across eukaryotes to support a presence for them in the LECA. It is unclear if the presence of these complexes within the endosomal system, rather than other transport routes, has any significance, but it could be the case that all of these tethers are derived from a single ancestral complex that has come to populate the multiple steps between endocytosis and vacuole delivery. It has yet to be determined if the newly characterized SEA complex, with some structural similarities to the HOPS/CORVET complex, is involved in tethering, coat formation, or another function (98).

HOW TO BUILD THE EUKARYOTIC CELL

Several distinct groups of protocoatmer-containing complexes can be discerned, based on common architectural principles, and with differing levels of complexity (**Figure 2**). When considered individually, distinct evolutionary mechanisms are clearly responsible for this variability.

For the adaptin/COPI group, the possibility that these complexes arose by sequential paralogous expansion is high. This is well established for the adaptins (51, 89, 108) and, given the level of architectural similarity, likely includes the COPI and TSET complexes as well. Significantly, TSET and COPI each have a unique coat, whereas most of the adaptins (AP1, AP2, and AP3) share the clathrin coat and AP5 has a distinct coat. This suggests a possible scenario in which COPI/TSET arose as a Golgi complex trafficking system that was later duplicated to TSET for post-Golgi transport and COPI for intra-Golgi transport. Similarly, for the AP complexes, paralogous expansion from a progenitor adaptin complex became associated with endosomal/recycling systems, and presumably the progenitor fulfilled a similar but less differentiated role. This model suggests that a basic Golgi trafficking system existed that subsequently gave rise to post-Golgi and sorting systems for additional routes but that the differentiation between COPI/TSET and adaptins took place quite early in establishing this configuration. It may be significant in this regard that adaptin complexes and TSET are not that infrequently lost (108–110), suggesting continual lineage-specific sculpting.

Complexes within the second major group are united by their shared Sec13 subunit (i.e., NPC, COPII, and SEA). Clear structural similarities among additional subunits indicate that COPII and SEA are related, and although the functions of these two complexes are distinct, they are both potentially involved in ER-related functionality. The NPC also shares elements of the COPII-type architecture, and its association with the NE (nuclear envelope) indicates that potential ER functions connect all three of these complexes, once more suggesting a route of paralogous expansion.

The final group includes the HOPS/CORVET complexes. In this case, the mechanism of evolution is very clear for the complexes within the cluster, as all share a heterotetrameric core but bolt on additional subunits to differentiate the individual complexes. A connection to the SEA complex is evidenced by the presence of a RING (really interesting new gene) domain and additional features (46, 98). Hence the HOPS group could be viewed as equivalent to the adaptin expansion, with the exception that the majority of subunits in HOPS are shared, rather than fully differentiated into distinct genes as in the adaptins.

In summary, current data suggest Golgi- and ER-associated complexes are possible ancestral forms, existing in the transition between the FECA and LECA. Expansions and differentiation of these complexes generated over a dozen distinct complexes in the LECA, which have come to differentiate the endosomal and other transport systems.

CONCLUSIONS: WHY ONE SYSTEM DOMINATES THE EUKARYOTIC CELL

To allow cells to grow, membranous cellular structures must be dynamic and moldable—for example, in both postmitotic separation of daughter nuclei and cytokinesis. Cell division represents a fundamental need for the ability to bend membranes, as even the most primitive cell must replicate and hence complete fission. Significantly, the ESCRT complex is required for cytokinesis in both archaeal and eukaryotic cells, and emerging evidence suggests a role in NE maintenance, perhaps hinting at a unified role in cell division (111).

However, overall the eukaryotic membrane coat repertoire is dominated by the protocoatmer signature β -propeller/ α -solenoid proteins. As the core role of the protocoatmer is forming scaffolds, we propose that some aspect of their molecular architecture is exceptionally flexible, allowing scaffolding of many membranes and complexes ranging in sophistication from simple adaptins to the NPC and IFT (11). This implies a level of evolvability and architectural flexibility apparently absent from the BAR domain and ESCRT systems in which, essentially, the same complex or subsections of that complex may operate in more than one context, but paralog expansion and neofunctionalization do not occur. For ESCRT, essentially only a single set of coat proteins is present (112), and hence, only one architecture is possible.

In conclusion, reconstructing the order and relationships among membrane coating systems currently represents the best path to unravel the order of the majority of events in eukaryogenesis. In viewing the proteins that make up the coating complexes (**Figure 3**), one is struck not only by their basic similarities but also by the remarkable diversity in form, size, and architecture of the protein networks that they form. This provides a spectacular example of how biological complexity can arise from simplicity.

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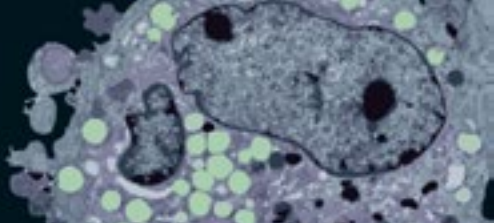
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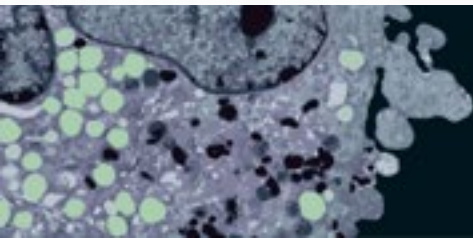
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