Identification of a Luminal Sequence Specifying the Assembly of Emp24p into p24 Complexes in the Yeast Secretory Pathway*

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Leonora F. Ciufo‡ and Alan Boyd§
From the Department of Biomedical Sciences, University of Edinburgh, Hugh Robson Building, George Square, Edinburgh EH8 9XD, United Kingdom

The p24 proteins are transmembrane proteins of the endomembrane system that play a poorly defined role in vesicle traffic between the endoplasmic reticulum and the Golgi apparatus. Various lines of evidence indicate that p24 proteins fall into four subfamilies (α, β, γ, and δ) and that tetramers are assembled containing one representative from each subfamily; however, the nature of the protein-protein interactions within these hetero-oligomers is unknown. We have identified a luminal segment of yeast p24β (Emp24p) that is necessary for its assembly into p24 complexes. Replacement of 52 C-terminal residues of Emp24p with the corresponding sequence from Erv25p (p24δ) generates a chimeric protein able to replace Emp24p in p24 complexes that retain partial function in vivo, ruling out a role for the transmembrane and cytosolic domains in specifying p24 interactions. Substitution of a further 50 residues, encompassing a heptad repeat region, abolishes the ability of the chimera to replace Emp24p but instead creates a protein that resembles its Erv25p parent in its requirement for stabilization by Emp24p. These data point to a role for coiled-coil interactions in directing subfamily-specific assembly of p24 oligomers that project into the lumen of transport vesicles, where they may act to exclude secretory cargo from coat protein complex type I-coated retrograde transport vesicles.

The p24/gp25/Lemp24/Erp (p24) family of proteins has been implicated in membrane and protein trafficking between the endoplasmic reticulum (ER) and the Golgi apparatus, although the precise role of p24 proteins remains undefined. Members of the p24 family have been discovered in a wide range of eukaryotes, including mammals, other vertebrates, Caenorhabditis elegans, Schizosaccharomyces pombe, and Saccharomyces cerevisiae (1–3). With the completion of the sequence of the S. cerevisiae genome, it has emerged that in yeast there is a total of eight genes that encode p24s (4).

The p24 proteins are type 1 transmembrane proteins of about 25 kDa that have a C-terminal transmembrane domain and a short cytosolic tail, typically 12–15 residues, containing sequence motifs known to specify interactions with vesicle coat proteins. The N-terminal luminal domain of the proteins includes two conserved cysteine residues and, in the membrane proximal part of the domain, a region that includes a set of heptad repeats suggestive of a capability to participate in coiled-coil interactions.

Some of the first studies of p24 function (2, 5) were taken to point to a role in selecting cargo molecules for packaging into COPII-coated vesicles leaving the ER (6). Although recent evidence indicates that the COPII proteins can form vesicles from liposomes without any involvement of integral membrane proteins (7), the formation of vesicles in vivo appears to be linked to a cargo selection process (8–11). In yeast cells, the finding that p24 defects cause slowed kinetics of secretion of a subset of cargo proteins (2, 5) led to the suggestion that p24 proteins might act as cargo receptors, the function of which is to ensure the concentration of cargo into COPII-coated vesicles. Consistent with this idea are the findings that p24 proteins are packaged into COPII vesicles (5) and that p24 defects allow the bypass of the requirement for one component of the COPII coat, Sec13p (12), which could reflect the existence of a regulatory mechanism linking cargo recruitment with the completion of COPII coat assembly. Although associations involving COPII components, p24 molecules, and cargo have been reported (13), direct evidence for a cargo selection role involving interactions between p24 molecules and specific cargo molecules has not yet emerged.

Other evidence points to a role for the p24s in COPII-mediated retrograde vesicle traffic from the Golgi to the ER. The proteins contain COPII binding motifs in their cytosolic tails that have been shown to function in vivo and in vitro (14–16). Studies of the localization of the proteins in mammalian cells indicate that they cycle between the ER and Golgi but are to be found predominantly in the Golgi (15, 17). A role for the proteins in retrograde traffic would explain the finding that, in yeast, p24 defects cause elevated secretion of the ER protein Kar2p (12), normally retrieved from the Golgi via retrograde trafficking of the Erd2 receptor (18). However, this phenotype could also stem from a loss of cargo control at the ER: previous studies indicate that the Erd2p-mediated retrieval mechanism is easily saturated in yeast (19), so any increased flow of proteins, such as Kar2p, out of the ER would be expected to lead to a significant overspill into the secretory pathway.

On the basis of sequence comparisons, p24s can be divided into four subfamilies, p24α, p24β, p24γ, and p24δ (4, 15). Several lines of evidence support the view that p24s from each of these subfamilies are assembled into hetero-oligomeric complexes. The observation that two yeast p24s, Emp24p and Erv25p, are interdependent for stability (5) raised the possibility that the proteins might interact in a complex, and this work has been extended by Marzioch et al. (4), who have concluded from the results of immunoprecipitation experiments that four yeast p24s (Erp1p (p24α), Emp24p (p24β), Erp2p (p24γ), and Erv25p (p24δ)) function together in a heteromeric complex.
Furthermore, mammalian cells seem to contain heteromeric p24 complexes of similar composition: immunoprecipitation and coexpression studies (20) have shown that human gp27 (hp24y3) forms a specific stoichiometric complex with GMP25 (hp24o2), p24 (hp24b1), and p23 (hp24d1).

We set out to investigate the association between Emp24p and other p24s. Our data point to the involvement of a region in the lumenal domain of the proteins that includes a heptad repeat region, suggesting that coiled-coil interactions between p24 proteins are at least in part responsible for the assembly of p24 complexes.

**EXPERIMENTAL PROCEDURES**

**Strains, Media, and Growth Conditions**—Yeast strains used were ASY927 (MATa/MATα ade2-1 ade2-1 his3-11 his3-11 leu2-3, 112 leu2-3, 112 trp1-1 trp1-1 ura3-1 can1-100 can1-100 sdi1-d2/sdi1-d2 Gal"/Gal") and YLC200 (MATα ura3-52 trp1 leu231 his3Δ200 pep4 HLY1b1.6 R can1 GAL) (this latter strain is a renamed version of BJ2454 obtained from the Yeast Genetic Stock Center, University of California, Berkeley, CA). YLC200 was used for the construction of strains bearing deletions of either EVR25 or EMP24. In both cases, a flanking PCR method was used: primers amplified a 441-bp DNA fragment downstream of the gene disruptions (this latter strain is a Dhis3 strain) and then tested for the presence of the gene disruptions using PCR. The emp24Δ33 allele removes codons 16–189 of the EMP24 gene; the emp24Δ44 allele removes codons 16–197 of the EVR25 gene.

**DNA Manipulations**—The synthesis of protein A fusion proteins was induced in mid-log phase cultures of yeast transformants, using IgG-Sepharose (Amersham Pharmacia Biotech). The fusion proteins were purified by affinity chromatography using guest on July 23, 2018http://www.jbc.org/Downloaded from

**Fusion Protein and Antibody Production**—Polyclonal antisera directed against the mammalian forms of both p24 proteins were generated at the Scottish Antibody Production Unit. For Emp24p, the primers (GAATTCCATAATGTTACATTTCGACATTGGATC, CAGCTGCTGCAGTATTTCTAT); the flanking primers used were the RI site in the primer was lost during cloning. The resulting PCR product was ligated directly into the vector YCPp24 as template. The PCR product was ligated directly into Smal-digested pK19 (23) and sequenced; this revealed that parts of the flanking regions were lost during cloning. The PCR product was ligated directly into Smal-digested pK19 and sequenced; this revealed that parts of the flanking regions were lost during cloning. The PCR product was ligated directly into Smal-digested pK19 and sequenced; this revealed that parts of the flanking regions were lost during cloning. The PCR product was ligated directly into Smal-digested pK19 and sequenced; this revealed that parts of the flanking regions were lost during cloning. The PCR product was ligated directly into Smal-digested pK19 and sequenced; this revealed that parts of the flanking regions were lost during cloning.

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**Strain Construction**—Except where indicated, all plasmid constructs were created using DNA fragments generated by *Pfu* polymerase (Stratagene) and were verified by DNA sequencing of the coding region.

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directly comparable amounts of secreted material were analyzed. Kar2p was detected by immunoblotting using rabbit anti-Kar2p antiserum provided by Dr Jeremy Brown, University of Edinburgh.

**Sucrose Gradient Fractionation of Yeast Membranes**—The procedure used was essentially that described (26). Cells were grown in selective medium to an A600 of approximately 0.4, whereupon 400 A600 units of cells were harvested and converted to spheroplasts. Sucrose gradients consisted of (w/v) 18, 22, 26, 30, 34, 38, 42, 46, 50, and 54% sucrose. Following centrifugation for 2.5 h in an SW41 rotor at 38,000 rpm (174,000 × g), 330-μl fractions were collected and analyzed. GDPase was assayed using published methods (27). For immunoblot analysis, samples were pooled in pairs and analyzed directly by SDS-PAGE.

**Purification of Protein Complexes Using Ni-NTA Agarose**—Cultures were grown in selective medium to mid-logarithmic phase (A600 = 0.4). Cells were converted to spheroplasts and lysed by homogenization. Following a low speed centrifugation to remove unbroken spheroplasts, membranes were recovered by centrifugation for 30 min at 158,000 × g. Membranes (equivalent to 200 A600 units of cells) were resuspended in Buffer A (150 mM sodium phosphate, pH 8.0, 2% Triton X-100) containing 10 mM imidazole and incubated on ice for 30 min. Solubilized membrane proteins were recovered in the supernatant following centrifugation at 158,000 × g for 15 min. This supernatant was incubated with 20 μl of Ni-NTA agarose (Qiagen) for 1 h at 4 °C. Unbound material was recovered, and the agarose was then washed with 400 μl of Buffer B, containing 20 mM imidazole. Bound proteins were then eluted with Buffer C containing 250 mM imidazole. Equivalent samples of total solubilized material, unbound material, and eluate were analyzed by SDS-PAGE and immunoblotting.

**RESULTS**

We set out to define the region of Emp24p that is responsible for its assembly into p24 hetero-oligomeric complexes, using the stabilization of Erv25p as a convenient indicator of assembly. The approach taken was to create Emp24p-Erv25p chimeras. Although Emp24p and Erv25p are both members of the p24 family of proteins, they are only distantly related to one another: as shown in Fig. 1A, apart from two conserved cysteine residues in their N-terminal portions, the major region of close identity of these proteins lies in their C-terminal regions. We constructed three gene fusions encoding Emv proteins (Emp-Erv chimeras), in which C-terminal portions of Emp24p were replaced by the corresponding sequence from Erv25p (Fig. 1B). In Emv146p, the substituted sequence of 34 residues encompasses the C-terminal transmembrane domain and cytosolic tail, together with a short conserved region from the luminal domain. In Emv146p, a further 18 residues of the luminal sequence of Emp24p was replaced with the corresponding Erv25p sequence. Beyond this point in the two sequences, the alignment breaks down; the position of the junction in Emv98p corresponds to the second conserved cysteine residue, lying ~50 residues further upstream. There was no obvious basis upon which to construct Emv chimeras intermediate to Emv146p and Emv98p.

Cells expressing one of the three Emv chimeras in place of Emp24p were analyzed for the presence both of the chimeric proteins and of Erv25p (Fig. 1C). As expected, deletion of the EMP24 gene caused a marked reduction in the level of Erv25p, and the reintroduction of EMP24 on a centromeric plasmid restored Emp24p and Erv25p to normal levels. The chimeric proteins Emv164p and Emv146p were detected at levels similar to Emp24p, and their presence was accompanied by restored levels of Erv25p. However, Emv98p was only weakly detected, and in this strain, Erv25p remained at the same low level seen in the absence of an Emp24p-related protein. Because Emv chimeras are detected by both antisera direct comparisons of the levels of the different Emv proteins are not reliable.

Because Emv146p is able to stabilize Erv25p, we conclude that Emv146p can replace Emp24p in p24 complexes, indicating that a portion of Emp24p sequence lying to the N-terminal side of Lys-147 is sufficient to specify the assembly of Emp24p into heteromeric complexes; Emp24p sequences downstream of this point may participate in the inferred interaction with other p24 proteins, but these are not essential for Emp24p-specific interactions. The failure of Emv98p to stabilize Erv25p suggests that the region of Emp24 between residues Leu-101 and Lys-147 is, however, essential for this property of Emp24p.

The absence of Emp24p results in the secretion of luminal ER proteins, such as Kar2p (12). We tested the Emv chimeras
for their ability to reverse this effect (Fig. 1C): Emv164p was able to reduce secretion of Kar2p as effectively as Emp24p, Emv146p gave a partial restoration of Kar2p localization, and Emv98p had no effect. This result suggests that the p24 complex formed by Emv146p may be partially defective, even though Erv25p levels are fully restored, pointing to an important functional role for the portion of Emp24p that lies near the luminal surface of the ER membrane. Further support for this idea comes from the analysis of an Emp24p mutant in which residues 165–168 (ESTN) have been replaced with alanines. This form of Emp24p is also fully able to restore Erv25p but does not restore normal localization of Kar2p (Fig. 1C).

Reports that p24 proteins are found in both the ER and the Golgi (15, 17) are consistent with the suggestion that the proteins are involved in the formation of COPII vesicles at the ER membrane and of COPI vesicles at the Golgi membrane. To further characterize cells containing Emv164p, we compared the subcellular distribution of Erv25p in this strain with that seen in a wild-type strain in which Emp24p and Erv25p were found broadly distributed across a sucrose density gradient with peaks co-localizing with the Golgi marker enzyme GDPase and with the ER membrane protein Sec61p (Fig. 2). In cells containing Emv164p in place of Emp24p, the chimera and Erv25p showed a relative distribution similar to that seen in wild-type cells, indicating that the Erp complexes incorporating the chimera behave normally. We do not believe the apparent shift in distribution of Sec61p in the experiment shown to be significant because measurements of sucrose concentration in the gradient fractions indicate that the peaks of Sec61p occurred at similar densities on the two gradients.

If residues Leu-101 to Lys-147 of Emp24p are responsible for the assembly of the protein into a hetero-oligomeric complex with other p24 proteins, then perhaps Emv98p, containing the corresponding region from Erv25p, might show Erv25p-like behavior and be stabilized by Emp24p. To test this idea, we compared the levels of Emv98p in cells lacking either Emp24p or Erv25p. As shown in Fig. 3A, the instability of Erv25p in a strain lacking Emp24p is accentuated in stationary phase cells, with Erv25p being undetectable in this strain but fully stabilized by the presence of Emv146p. To assess the influence of Emp24p upon the stability of Emv98p, we therefore compared levels of the latter protein in cells containing either Emp24p or Erv25p, in both growing and stationary cultures, with Emv146p as a control (Fig. 3B). In the presence of Erv25p, Emv146p persisted into stationary phase (Fig. 3B, left lane). In contrast, in this same background, Emv98p disappeared from stationary phase cells along with Erv25p, consistent with the conclusion that Emv98p cannot replace Emp24p in p24 complexes and thus cannot stabilize Erv25p. In cells containing Emp24p but lacking Erv25p, Emv146p persisted into stationary phase in the same way that Emp24p itself does (Fig. 3B, second lane from right; also compare the right and second from right lanes of Fig. 3A), and, in marked contrast to the result seen in cells lacking Emp24p, so does Emv98p (Fig. 3B, right lane). Thus, Emv98p is stabilized in the presence of Emp24p, just like its Erv25p parent, suggesting that this chimera can mimic Erv25p by assembling into p24 complexes: in the transition from Emv146p to Emv98p, the replacement of ~50 residues downstream of the second conserved cysteine converts the protein from one that is able to act like Emp24p to one that, like Erv25p, depends upon Emp24p for stability.

We also tested the possibility that the region of Emp24p defined by this analysis as necessary for the stabilization of Erv25p could, if transplanted into Erv25p, convert that protein to one that was able to replace Emp24p in p24 complexes. Gene fusions were constructed to encode chimeras Rmr146p and Rmr164p in which an internal segment of Erv25p was replaced with the corresponding portion of Emp24p. The ability of these proteins to stabilize Erv25p in cells lacking Emp24p was then assessed (Fig. 4). In growing cells, both proteins had a small but significant stabilizing effect (Fig. 4A), and in stationary phase cells, a trace of Erv25p was still detected under condi-
tions in which it is normally undetectable. Thus, the Rmr chimeras have a weak ability to stabilize Erv25p, although it is clear that in the context of the Erv25p sequence, this region cannot confer full stabilizing activity.

The interpretation of the data presented so far has relied upon inferences about p24 complex formation drawn from the stabilization of Erv25p. We wished to obtain direct biochemical evidence that Emv146p is present in stable protein complexes that contain Erv25p. Others have detected the existence of p24 protein complexes from the behavior of the proteins in rate zonal centrifugation of cholate-solubilized material, and directly by co-immunoprecipitation. In our experiments, we could not use immunoprecipitation because our polyclonal antisera for Emp24p and Erv25p were both able to recognize the Emv chimeras. We therefore turned to a tagging approach, exploiting the fact that the proteolytic cleavage that removes the Emp24p signal sequence occurs between residues Ala-20 and His-21. We introduced seven extra histidine codons into the EMP24 sequence so that signal cleavage should generate a form of Emp24p carrying eight N-terminal histidine residues. As shown in Fig. 5A, cells containing this modified gene contained an Emp24p immunoreactive protein with reduced electrophoretic mobility, as expected if the protein carries the N-terminal extension. Furthermore this His-tagged Emp24p protein restored normal Erv25p levels (Fig. 5A) and also fully complemented the Kar2p localization defect in a strain lacking Emp24p (not shown). We were able to purify the His-tagged Emp24p from these cells following a protocol employing complete denaturation (6 M urea and 1% Triton X-100), whereupon the material recovered on Ni-NTA-agarose contained no detectable Erv25p, as expected (data not shown). In order to recover protein complexes containing His-tagged Emp24p, we used Ni-NTA chromatography to analyze a Triton X-100 solubilized extract prepared from yeast membranes. As shown in Fig. 5B, when the detergent extract from cells containing His-tagged Emp24p was treated with Ni-NTA agarose, there was significant depletion of the protein from the extract, and the bound protein survived extensive washing with buffer containing 20 mM imidazole but was eluted from the column by exposure to 250 mM imidazole. Furthermore, immunoblot analysis revealed that Erv25p was also depleted from the detergent extract by Ni-NTA agarose and was present in the eluted fraction. Two controls illustrate the specificity of this result. First, a control membrane protein, the ER protein Sec61p, was not present in the eluted material. Second, when the detergent extract was prepared from wild-type cells, neither Emp24p nor Erv25p was recovered in the affinity-purified material, and indeed neither protein was detectably depleted by the initial binding reaction; thus, the binding of both proteins to Ni-NTA agarose depends upon the presence of the N-terminal His tag on Emp24p. We conclude that p24 complexes containing His-tagged Emp24p and Erv25p can be recovered by nickel affinity chromatography. We repeated this experiment with extracts prepared from cells containing an analogous His-tagged form of Emp24p and found that this also led to the recovery of Erv25p. This result provides clear confirmation that the Emv146p chimera can replace Emp24p in p24 complexes.

**DISCUSSION**

We have presented evidence that a region of the lumenal domain of Emp24p consisting of some 50 amino acids is necessary to direct its assembly into hetero-oligomeric complexes with other p24 family members. We investigated which part of Emp24p is responsible for its assembly into p24 complexes by
exploiting the observation that the absence of Emp24p leads to the degradation of Erv25p, presumably because it fails to be stabilized by being assembled into a complex. Our initial hypothesis was that the assembly of p24 heteromers might be driven by interactions between the transmembrane domains of the proteins that contain polar residues suggestive of interactions within the plane of the lipid bilayer. We tested this idea by investigating the properties of chimeric p24 proteins.

In the chimeras Emv164p and Emv146p, the C-terminal portions of Emp24p encompassing the transmembrane domain, comprising 34 and 52 residues, respectively, were replaced with the corresponding sequences from Erv25p. Both of these chimeras were able to function as replacements for Emp24p: in cells containing either chimera, we found that Erv25p levels were restored to normal, suggesting that the chimeric proteins were able to enter p24 complexes in place of Emp24p. We used a His-tagged form of Emp24p to isolate complexes containing Erv25p, and because Erv25p was also isolated in association with a His-tagged form of Emv146p, we conclude that sequences downstream of Emp24p Lys-147 are not essential for its assembly into p24 complexes.

Replacement of a further 47 residues of Emp24p resulted in the chimera, Emv98p, which was unable to replace Emp24p, indicating that sequences necessary for the protein to assemble as Emp24p into protein complexes have been lost. This suggests that a region of Emp24p between Lys-147 and the second conserved cysteine residue (Cys-99) specifies the assembly property of Emp24p. A corollary of this conclusion would be that Emv98p, containing the corresponding region of Erv25p, might be assembled into p24 complexes in place of Erv25p. Evidence in support of this came from the finding that Emv98p was more stable in cells containing Emp24p, just like its Erv25p parent. We conclude from these data that the region of p24 proteins corresponding to the Emp24p region Lys-147 to Cys-99 specifies their assembly into p24 complexes.

The finding that the cytosolic domain and transmembrane domain of Emp24p can be replaced with the corresponding region from Erv25p also indicates that the unusual C-terminal sequence of Emp24p is not essential for function. The cytosolic portion of Emp24p is unique among p24 family members in that it lacks a C-terminal KXXK motif, implicated in COPI binding, although it does contain the FF motif that is also thought to be important for COPI association (14, 15). The absence of the C-terminal motif from Emp24p is apparently of no functional significance because the p24 complexes that are formed in the presence of Emv146p, which carries the cytosolic domain of Erv25p, were found to be distributed normally between the ER and Golgi and provided normal function as assessed by restoration of Kar2p localization. Studies of the interactions between p24 C-terminal peptides and γ-COP have revealed a range of affinities, pointing to possible differences in the tendency of the various p24 proteins to partition between the ER and Golgi and to a possible dynamic equilibrium between unassembled p24 molecules and heteromeric oligomers (20). However, the apparent lack of functional significance of the unusual C terminus of Emp24p suggests rather that the interactions of p24s with COPI occur predominantly when the proteins are assembled, explaining how the loss of a COPI signal from a single component can be accommodated.

Our data implicate a relatively short region of p24 proteins as being responsible for defining the assembly properties of the protein. There are two features of this region that could play a role in determining the specificity and stability of complex formation: the presence of a heptad repeat sequence, and the presence of sequence motifs that are specific for p24 subfamilies.

As pointed out by others (4, 15), p24 proteins contain a region with a heptad repeat pattern, consistent with the possibility that coiled-coil interactions are involved in oligomerization. As shown in Fig. 6, this region of Emp24p and Erv25p spans, but lies mainly to the N-terminal side of, the junction point in Emv146p, a position consistent with its involvement in subfamily-specific interactions. Are there sequences in this region that are specific to a particular p24 subfamily, over and above any heptad repeat sequence? To address this question, we examined patterns of subfamily-specific sequence conservation. For Emp24p, this analysis was not particularly fruitful: the sequence of Emp24p is only distantly related to other members of the p24b subfamily, and the region in question is almost devoid of conserved residues, although the conservation of amino acids corresponding to Emp24p positions Leu-132, Leu-139, and Val-147 (the spacing of which corresponds to the heptad repeat) is noteworthy. For the p24b subfamily (which includes Erv25p), the most obvious feature in the region is a conserved sequence, LRXXEVELRR, which lies within the heptad repeat. This single sequence may be important for the assembly of p24 into hetero-oligomeric p24 complexes.

Marzioch et al. (4) have suggested that Emp24p, Erv25p, Erp1p, and Erp2p form a tetramer, and one possibility is that four p24 molecules, one from each subfamily, assemble into a tetramer that is formed, at least in part, through a four-stranded parallel helical bundle. However, the pattern of interdependence seen in yeast raises the possibility that the postulated p24 tetramer is a dimer of dimers. Thus, the loss of...
either Erp1p or Erp2p causes only a modest reduction in the levels of Emp24p andERV25p, and in the absence of all three yeast members of the p24α subfamily (Erp1p, Erp5p, and Erp6p), the Emp24p and ERV25p molecules that remain behave as a dimer in gel filtration. Perhaps coiled-coil interactions are responsible for p24β-p24β associations and for p24α-p24α associations, with the formation of the tetrameric complex resulting from a different type of interaction between the dimers.

![Image](image-url)

The picture of p24 oligomer structure that begins to emerge is one of a quasi-structural role for these proteins in vesicle trafficking. Recent findings indicate that secretory cargo is selected for anterograde transport in vesicular-tubular clusters lying between the ER and Golgi, where COPI-coated buds form in a manner that excludes secretory cargo (28, 29). The major transmembrane components of these COPI-coated buds are likely to be the p24 proteins (17). Interactions between the polar faces of p24 transmembrane helical domains are consistent with the existence in COPI-coated vesicles of an inner lining of p24 molecules projecting well into the vesicle lumen, as is the existence of a luminal rod-like structure formed as a result of the subfamily-specific interactions that we have detected in our experiments. This raises the possibility that the p24 proteins may act to prevent secretory cargo returning in COPI vesicles by masking the inner charged surface of the vesicles and by passively filling much of the space within them. In this way, only membrane proteins with retrieval motifs (e.g. Erd2p) and their ligands would be able to make the return trip to the ER: there would be no room for anything else. Further studies of structure-function relationships in p24 molecules will help to define their role in vesicle traffic.

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