

The Emp24 Complex Recruits a Specific Cargo Molecule into Endoplasmic Reticulum–derived Vesicles

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Abstract. Members of the yeast p24 family, including Emp24p and Erv25p, form a heteromeric complex required for the efficient transport of selected proteins from the endoplasmic reticulum (ER) to the Golgi apparatus. The specific functions and sites of action of this complex are unknown. We show that Emp24p is directly required for efficient packaging of a luminal cargo protein, Gas1p, into ER-derived vesicles.

Emp24p and Erv25p can be directly cross-linked to Gas1p in ER-derived vesicles. Gap1p, which was not affected by *emp24* mutation, was not cross-linked. These results suggest that the Emp24 complex acts as a cargo receptor in vesicle biogenesis from the ER.

Key words: COPII-coated vesicles • ER • Erv25p • *Saccharomyces cerevisiae* • protein sorting

Introduction

In eukaryotic cells, protein transport between the organelles of the secretory pathway is mediated by vesicles that bud from a donor compartment and fuse with an appropriate acceptor compartment (Palade, 1975). The starting point of the exocytic route is the ER. There, correctly folded and assembled cargo molecules can enter COPII-coated vesicles for transport to the *cis*-Golgi compartment (Schekman and Orci, 1996). Many cytosolic and transmembrane proteins have been identified that mediate this vesicular transport step (Rothman and Wieland, 1996; Schekman and Orci, 1996).

p24 proteins are present in heteromeric complexes that have been proposed to cycle between ER and Golgi compartments because they are found in both COPII and COPI vesicles in addition to ER and Golgi membranes (Schimmöller et al., 1995; Stamnes et al., 1995; Belden and Barlowe, 1996; Sohn et al., 1996; Rojo et al., 1997; Dominguez et al., 1998; Füllekrug et al., 1999; Marzioch et al., 1999). In mammals, functional data suggest a role for these proteins in the formation of ER exit sites (Lavoie et al., 1999). In yeast, eight genes encode p24 family members: *EMP24*, *ERV25*, and *ERP1-ERP6* (Marzioch et al., 1999). Mutants in several of these genes show selective protein transport defects. Strains with a single deletion of *EMP24*, *ERV25*, *ERP1*, or *ERP2* show a delay in the ER to Golgi transport of Gas1p (Schimmöller et al., 1995; Belden and Barlowe, 1996; Marzioch et al., 1999), a glycosylphosphatidylinositol (GPI)¹-anchored protein. The

emp24 and *erv25* mutants also show a transport defect for invertase, a soluble secreted protein. Several other cargo molecules, including α factor, a secreted pheromone, do not require p24 proteins for efficient transport. Mutation of several yeast p24 genes results in secretion of the ER luminal protein, Kar2p (Elrod-Erickson and Kaiser, 1996; Marzioch et al., 1999).

The Emp24 complex in yeast comprises Emp24p, Erv25p, and most likely Erp1p and Erp2p. Deletion of *EMP24* causes a strong reduction in the levels of the other three proteins of the complex (Belden and Barlowe, 1996; Marzioch et al., 1999). In this study we provide evidence that the Emp24 complex is directly required for efficient packaging of Gas1p into ER-derived vesicles. Two subunits of this complex can be directly cross-linked to the cargo protein in purified ER-derived vesicles, consistent with the hypothesis that the Emp24 complex plays a role as a cargo receptor in ER to Golgi transport.

Materials and Methods

Strains

An myc epitope was introduced at the NH₂ terminus of mature Emp24p. The myc-tagged *emp24-E178A* mutant was constructed by substituting an appropriate fragment of *EMP24* with the sequenced mutant version obtained by PCR techniques. RH4443 (*MAT α* , *ura3*, *leu2*, *his4*, *bar1*, *emp24::KanMx*) was obtained by replacing the entire *EMP24* coding sequence of RH1959 (*MAT α* , *ura3*, *leu2*, *his4*, *bar1*) with a *KanMx* cassette. *EMP24* alleles were cloned into a YCplac111 (CEN/ARS) plasmid. The *S. cerevisiae* strain RH696-2B (*MAT α* , *sec18-20 gap1 Δ ::LEU2 ura3 ade2 leu2 lys2 Δ 201 pPL269*) was obtained by crossing PLY129 (pPL269) (*MAT α* *gap1 Δ ::LEU2 ura3 ade2 leu2 lys2 Δ 201 pPL269*) (Kuehn et al., 1996), with RH478 (*MAT α* *sec18-20 leu2 his4*). Cytosols were prepared from RH732 (*MAT α* *his4 leu2 ura3 lys2 pep4::URA3 bar1*) and RH2043 (*MAT α* *sec18-20 his4 leu2 ura3 pep4::URA3 bar1*).

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¹Abbreviations used in this paper: DSG, disuccinimidyl glutarate; DSP, dithiobis(succinimidylpropionate); gp α F, glycosylated pro α factor; GPI, glycosylphosphatidylinositol.

Protein Techniques

Protein levels were determined by extraction of log phase cultures and Western blotting (Sütterlin et al., 1997) using antibodies raised against the Emp24p cytosolic tail or the myc epitope. Analysis of Gas1p transport in vivo was by a pulse-chase protocol (Sütterlin et al., 1997). Gas1p maturation was analyzed using a 4-min pulse and subsequent chase at 30°C, followed by immunoprecipitation, SDS-PAGE, and fluorography. Immature (105 kD) and mature (125 kD) forms of Gas1p were quantified using a densitometer. The percentage of mature Gas1p was used as an indication of transport to the Golgi apparatus. Determination of partitioning of Gas1p between aqueous and detergent phases of Triton X-114 was performed as described (Nuoffer et al., 1993), except that the media fractions were not analyzed. After separation into detergent and aqueous phases, Gas1p was denatured, immunoprecipitated, resolved by SDS-PAGE, visualized, and quantified using a PhosphorImager.

In Vitro ER-budding Assay

The permeabilized, cell-based, ER cargo packaging assay was performed as described (Kuehn et al., 1996) with a few modifications. Strains RH1959, RH4438, and RH696-2B were transformed with pCNYG1 to overexpress Gas1p (approximately fivefold) (Nuoffer et al., 1991). Overexpression did not affect Gas1p transport kinetics or dependence on *EMP24*. 10 mM 2-mercaptoethanesulfonic acid replaced 10 mM dithiothreitol. Budding reactions contained 30 μ l of membranes (from 12×10^7 cells), 300 μ g crude cytosol, 3 μ g Sar1p, $1 \times$ ATP mix, and 0.2 mM GTP in a 150- μ l volume. After 2 h at 25°C, a portion of the sample was removed for analysis (total), the remaining aliquot was sedimented (14,000 rpm, 2 min, 4°C), and 125 μ l of the supernatant collected and subjected to flotation on a Nycodenz[®] step gradient (Barlowe et al., 1994). 100 μ l from the top was discarded and the next 800 μ l transferred to a new tube. 400 μ l was diluted threefold with B88 and centrifuged (100,000 *g*, 1 h, 4°C). The membrane pellet was dissolved in 1% SDS in TEPI buffer (Sütterlin et al., 1997) for 10 min at 55°C, subjected to immunoprecipitation, and analyzed by SDS-PAGE with subsequent exposure and quantitation using a PhosphorImager.

Vesicle Immunoisolation

Vesicles were produced in a budding reaction using *sec18* membranes and cytosol. The *sec18* membranes were prepared as above, except that the last 5 min of depletion and the pulse-labeling were at 32°C. The *sec18* cytosol was preincubated (32°C, 10 min) before use. Vesicles were immunoisolated with or without Emp24p anti-tail antibody and processed according to Kuehn et al. (1996).

Cross-Linking

Nycodenz-purified vesicles produced in a budding reaction were adjusted to 2.5 M urea in B88 and incubated with 1 mM dithiobis(succinimidylpropionate) (DSP) or various amounts of disuccinimidyl glutarate (DSG; Pierce) (20°C, 20 min). The cross-linking reaction was quenched by addition of glycine (50 mM final, 5 min, 20°C). Vesicles were sedimented at 100,000 *g* (1 h, 4°C), dissolved with 1% SDS in TEPI (5 min, 95°C for Gas1p and glycosylated pro α factor [gp α F], or 55°C for Gap1p), and immunoprecipitated with Emp24p anti-tail antibody or Erv25 antibody (Belden and Barlowe, 1996) and protein A-Sepharose. Precipitated material was eluted from the Sepharose beads by incubation with 1% SDS in TEPI (5 min, 95 or 55°C) and reimmunoprecipitated with anti-Gas1p or anti-Gap1p antibody.

Results

Emp24p Is Required for Efficient Recruitment of Gas1p into ER-derived Vesicles

To investigate whether Emp24p plays a role in cargo exit from the ER we quantified the packaging of different secretory proteins into vesicles that were generated from wild-type and *emp24* mutant ER membranes in vitro. Budding of Gas1p was much less efficient (>70% less) from *emp24* membranes than from wild-type membranes,

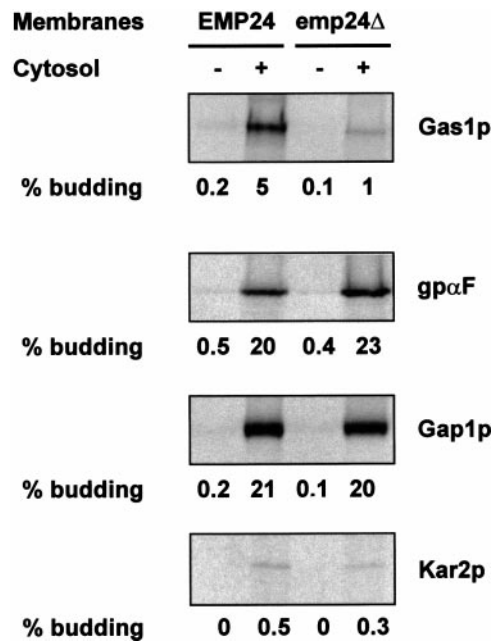


Figure 1. In vitro budding of ER-derived vesicles from wild-type and *emp24* Δ membranes. Spheroplasts from wild-type (EMP24) and mutant (*emp24* Δ) cells were pulse-labeled (3 min), permeabilized, and incubated with an energy source with or without cytosol (2 h, 25°C). Vesicles were purified by differential centrifugation and flotation on Nycodenz[®] gradients. Budding of indicated proteins was assayed by immunoprecipitation, SDS-PAGE, visualization, and quantification using a PhosphorImager. The budding efficiency for a given protein refers to the percentage of the total radiolabeled protein that was recovered in the purified vesicular fraction.

whereas the budding efficiencies of gp α F and an amino acid permease (Gap1p) were similar from the two membrane sources (Fig. 1). gp α F is an established control for selectivity, because its transport from the ER to the Golgi is not affected in *emp24* Δ cells (Schimmöller et al., 1995). Gap1p was also investigated because it has been shown to be efficiently incorporated into COPII-coated vesicles in vitro (Kuehn et al., 1996). Therefore, Emp24p is required for efficient packaging of at least one secretory protein into COPII-coated vesicles. The magnitude of the defect in Gas1p budding from the ER is consistent with the ER to Golgi transport defect seen in vivo (Schimmöller et al., 1995) and could, therefore, be the entire explanation for the transport delay.

It has been proposed that Emp24p may be a negative regulator of vesicle formation (Elrod-Erickson and Kaiser, 1996) and that its absence would result in increased Kar2p exit from the ER. To address this point, we compared the incorporation of Kar2p into vesicles using wild-type and *emp24* membranes. Kar2p was poorly packaged into ER-derived vesicles using either membrane source (Fig. 1). Therefore, it does not seem that Emp24p is required for excluding Kar2p from ER-derived vesicles. This suggests, but does not prove, that the reason for Kar2p secretion in the *emp24* mutant is a defect in retrograde transport.

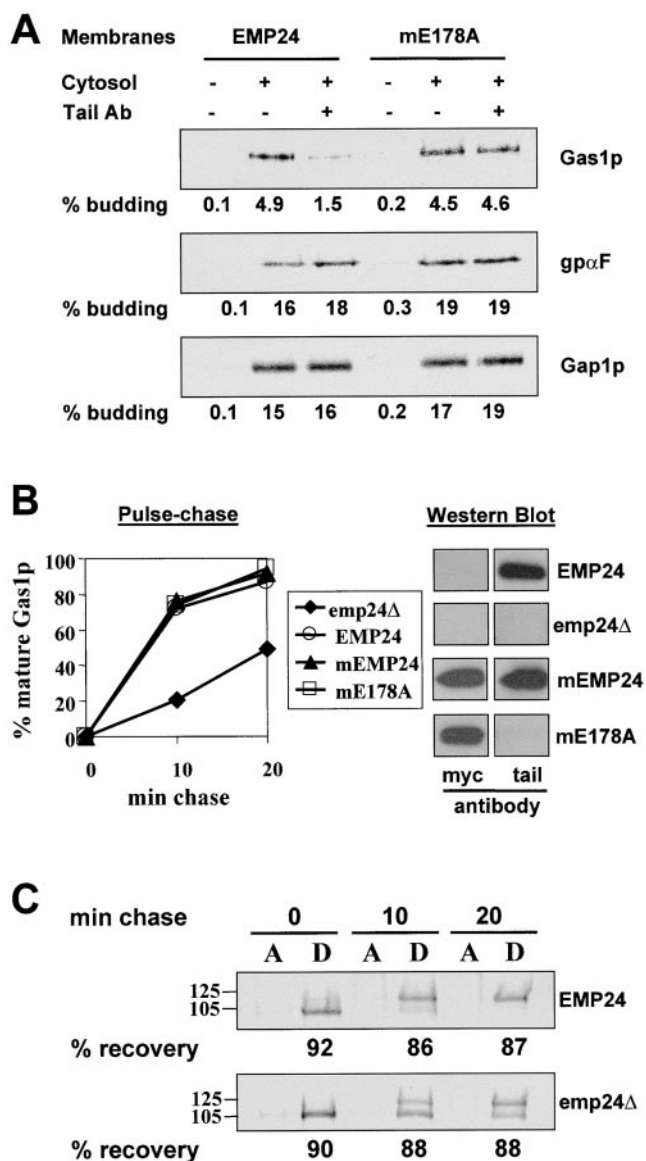


Figure 2. Inhibition of Gas1p budding by anti-Emp24p tail antibodies. (A) Permeabilized spheroplasts were prepared from wild-type (EMP24) and *emp24-E178A* (mE178A) cells as in Fig. 1. They were preincubated in the presence or absence of antibodies against the cytosolic domain of Emp24p (1 h, 4°C). Budding reactions were performed and analyzed as in Fig. 1. (B) The rate of Gas1p maturation in wild-type and *emp24* mutants was analyzed after a pulse-chase immunoprecipitation protocol. Emp24p was visualized by Western blotting using antibodies against the c-myc epitope or against the cytosolic tail of Emp24p. Mutant (*emp24Δ*) cells were transformed with empty vector (*emp24Δ*), the wild-type allele (EMP24), myc-tagged Emp24p (mEMP24), or the myc-tagged *emp24-E178A* (mE178A) allele. (C) After pulse-chase labeling of wild-type (EMP24) and mutant (*emp24Δ*) strains, cells were lysed and extracted with Triton X-114 followed by detergent phase separation. Aqueous (A) and detergent (D) phases were subjected to immunoprecipitation and SDS-PAGE. Gas1p (both forms combined) was quantified using a Phosphor-Imager. Immature Gas1p (105) was converted to the mature form (125) with delayed kinetics in the *emp24Δ* mutant, but was efficiently extracted into the detergent phase.

Emp24p Is Directly Involved in the Selective Packaging of Cargo Molecules

Since Emp24p could play a role in retrograde transport it is possible that the inefficient budding of Gas1p from *emp24Δ* membranes is an indirect consequence of a retrograde transport defect. To address this point, we used antibodies against the cytosolic tail of Emp24p to inhibit the function of Emp24p in wild-type membranes. Preincubation of membranes with antibodies on ice reduced the budding efficiency of Gas1p greater than threefold (Fig. 2 A). This inhibition was specific for Gas1p, because the packaging of Gap1p and gpαF was not affected. As a control for the specificity of our anti-tail antibodies, we used an *emp24* mutant with an E178A substitution in the cytosolic tail. This point mutation does not affect the function of Emp24p (Fig. 2 B), but the anti-tail antibodies no longer recognize the mutant protein (Fig. 2 B). Preincubation of membranes derived from *emp24-E178A* cells with anti-tail antibodies had no effect on budding efficiencies of Gas1p, gpαF, or Gap1p (Fig. 2 A). Therefore, we conclude that Emp24p is required directly for the efficient incorporation of Gas1p into ER-derived vesicles.

One requirement for Gas1p exit from the ER is the attachment of the GPI anchor (Doering and Schekman, 1996). To test whether the GPI anchor is efficiently attached to Gas1p in the *emp24Δ* mutant, we performed a pulse-chase analysis followed by separation of the protein extracts into detergent and aqueous phases using Triton X-114. GPI-anchored Gas1p partitions efficiently into the detergent phase, whereas the unanchored protein partitions approximately equally between detergent and aqueous phases (Nuoffer et al., 1993). After the 4-min pulse-labeling, ~90% of the Gas1p was already found in the detergent phase in both wild-type and *emp24Δ* mutant strains (Fig. 2 C), demonstrating that the GPI anchor is efficiently attached to Gas1p in the *emp24Δ* mutant. Therefore, a defect in GPI anchoring cannot explain the transport defect of Gas1p in the *emp24Δ* mutant.

Emp24p and Gas1p Can be Found in the Same Protein Complex in ER-derived Vesicles

Since Emp24p is efficiently incorporated into ER-derived vesicles (Schimmöller et al., 1995) and is directly required for Gas1p recruitment, we investigated if Gas1p could be found in a protein complex with Emp24p in ER-derived vesicles. ER-derived vesicles were generated in vitro and uncoated vesicles were exposed to the cleavable membrane-permeable cross-linker DSP. After lysis and denaturation, the samples were immunoprecipitated using anti-Emp24p tail antibodies. The precipitates were denatured and subjected to a second immunoprecipitation using antibodies against Gas1p. The cross-linker was cleaved and the samples were analyzed by SDS-PAGE. Gas1p was cross-linked to Emp24p with good efficiency (Fig. 3). No Gas1p was recovered if cross-linker was omitted. Moreover, Gas1p could not be detected when the cross-linking was performed on vesicles generated from *emp24-E178A* membranes (Fig. 3), proving that Gas1p was recovered as part of a complex containing Emp24p. This complex is specific because another cargo molecule, Gap1p, whose budding was not reduced from *emp24* mutant membranes,

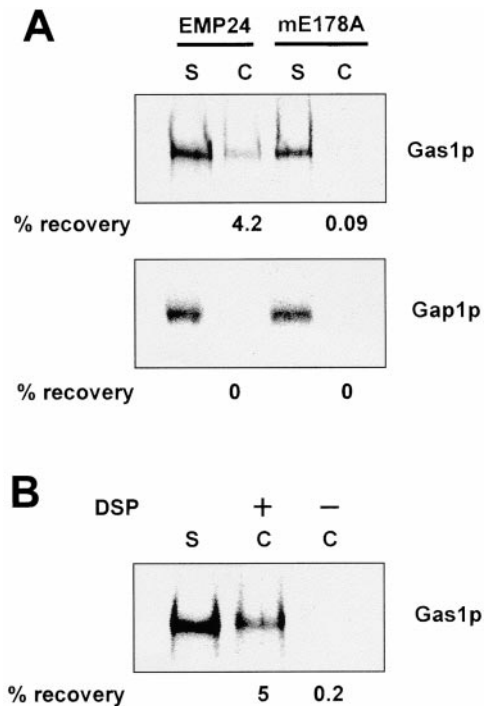


Figure 3. Cross-linking of Emp24p and Gas1p. (A) ER-derived vesicles generated in vitro from wild-type (EMP24) and *emp24-E178A* mutant (mE178A) membranes were incubated with DSP. The samples were denatured and immunoprecipitated with antibodies against the tail of Emp24p. The precipitates were denatured and reprecipitated with antibody against Gas1p or Gap1p (C). 10 or 20% of the vesicle preparation was immunoprecipitated directly with antibodies against Gas1p or Gap1p, without addition of DSP, to use as a standard (S) for recovery. In control experiments, cross-linking with DSP did not affect recovery of Gap1p by immunoprecipitation. Samples were incubated with 5% 2-mercaptoethanol, analyzed by SDS-PAGE, and visualized using a PhosphorImager. (B) Vesicles generated from wild-type membranes were incubated with or without DSP and processed as above.

was not cross-linked to Emp24p. These data clearly demonstrate that Emp24p and Gas1p can be found in the same protein complex in ER-derived vesicles and suggest that this complex is specific.

An alternative explanation for the absence of a role of Emp24p in Gap1p budding and of Gap1p from the cross-linked Emp24 complex would be if Emp24p and Gap1p were found in different ER-derived vesicles. To test this we produced vesicles using membranes and cytosol from a *sec18* mutant. *SEC18* encodes yeast *N*-ethylmaleimide-sensitive fusion protein, and its inactivation under the conditions of this experiment blocks fusion of ER-derived vesicles with the Golgi apparatus (Muñiz, M., and H. Riezman, manuscript in preparation). These conditions ensure that we are analyzing primary ER-derived vesicles. The vesicles were immunisolated using antibody against the cytosolic tail of Emp24p. 83% of the Gas1p and 96% of the Gap1p copurified with the immunisolated vesicles (Fig. 4). Virtually no cargo proteins were isolated when Emp24p tail antibody was omitted. Therefore, Emp24p is found in the same vesicles as Gap1p and Gas1p. The lack

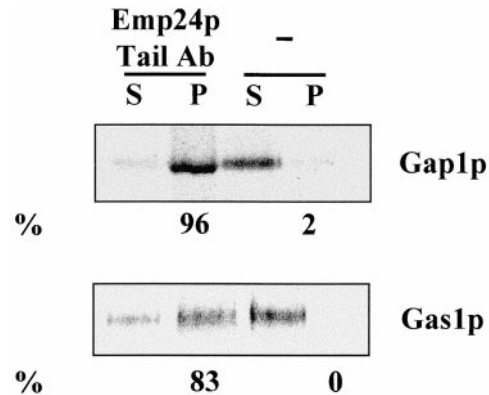


Figure 4. Immunoprecipitation of Emp24p containing vesicles. ER-derived vesicles generated in vitro from *sec18* membranes and cytosol were immunisolated with or without Emp24p anti-tail antibody. The supernatants (S) and pellets (P) were processed for immunoprecipitation.

of effect of the *emp24* mutation on Gap1p budding and Gap1p absence from the cross-linked Emp24 complex cannot be due their presence in distinct vesicles.

Emp24p and Erv25p Can be Directly Cross-Linked to Gas1p in ER-derived Vesicles

The cross-linking results presented above suggest that Emp24p may be part of a cargo receptor that improves the efficiency of cargo entry into ER-derived vesicles. However, they do not show that Emp24p binds the cargo molecule directly. To address this issue we used a noncleavable cross-linker, DSG, and performed similar cross-linking studies on ER-derived vesicles. Emp24p was cross-linked to Gas1p as seen by a smear starting at around 25,000 higher apparent molecular mass than the ER form of Gas1p (Fig. 5 A). Additional bands were seen at higher molecular weights. The band at 130 kD must represent a directly cross-linked product between Emp24p (24 kD) and Gas1p (105 kD). All of the precipitated material was specific because it was not seen when the vesicles were generated from *emp24-E178A* mutant membranes.

Next, we immunoprecipitated the cross-linked material with antibodies against Erv25p, followed by antibodies against Gas1p. A strong band appeared at the position expected for a directly cross-linked product between the two molecules. Therefore, we conclude that Erv25p can also be directly and efficiently cross-linked to Gas1p in isolated ER-derived vesicles. We could also detect this cross-linked product in vesicles derived from *emp24-E178A* mutant (data not shown), confirming that vesicle budding occurred using these membranes.

Finally, we performed a titration of DSG on the isolated vesicles. When cross-linker was omitted, no Erv25p-Gas1p product could be detected (Fig. 5 B). At low concentrations of cross-linker the directly cross-linked product was by far the main product seen. At higher concentrations of cross-linker more cross-linked products were seen and the bands at higher molecular weight became more apparent. The cross-linking studies shown above demonstrate that Gas1p can be specifically and directly cross-linked to the

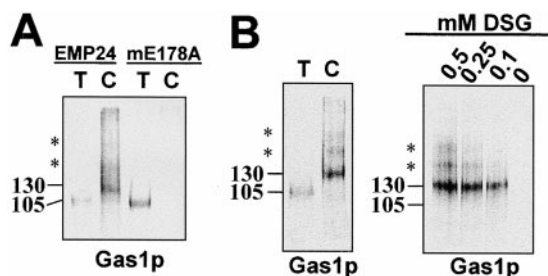


Figure 5. Direct cross-linking of Emp24p and Erv25p with Gas1p. (A) Vesicles generated in vitro from wild-type (EMP24) and *emp24-E178A* mutant (mE178A) membranes were incubated with 0.25 mM of DSG and processed as in Fig. 3. (B) Vesicles generated from wild-type membranes were incubated with the indicated amounts of DSG, denatured, immunoprecipitated with antibody against Erv25p, then reprecipitated with antibody against Gas1p and processed as in Fig. 3.

Emp24 complex. These results are consistent with the hypothesis that the Emp24 complex is a cargo receptor required for the efficient incorporation of Gas1p into ER-derived vesicles.

Discussion

A major finding of this study is that Emp24p is directly required for efficient incorporation of Gas1p into ER-derived vesicles in vitro. Two independent assays were used to show this. One used mutant membranes devoid of Emp24p. The other used antibodies to inhibit Emp24p function directly in wild-type membranes. Both assays showed that Gas1p budding depended strongly on Emp24p, whereas budding of other proteins did not. This striking specificity suggests that this complex has a highly specific role in packaging of Gas1p into ER-derived vesicles.

This direct role of Emp24p in selective budding from the ER can be explained in three ways. First, Emp24p could be required for efficient anchor attachment to Gas1p. We have shown that this is not the case. Second, Emp24p could be required for the formation of ER-derived vesicles. It has been suggested recently that α_2 p24, a mammalian member of the p24 family, plays a role in the formation of ER cargo exit sites (Lavoie et al., 1999). However, in yeast, the absence of Emp24p or the inhibition of its function by the tail antibody does not lead to a general defect in vesicle formation because budding of other cargo proteins was not affected. Also, mutant membranes efficiently package the vSNARE Sec22p (Belden and Barlowe, 1996). Moreover, since Emp24p is found in vesicles with Gas1p as well as with Gap1p, it is highly unlikely that Emp24p is required only for formation of vesicles containing Gas1p.

The third explanation for the direct role of Emp24p in selective budding from the ER is to consider Emp24p as part of a cargo receptor that is required for the packaging of a very small subset of cargo proteins. This cargo receptor would recruit specific proteins into the ER-derived vesicles, increasing the efficiency of packaging. A second major finding of our study, that Emp24p and Erv25p can

be directly cross-linked to Gas1p, supports this hypothesis. This model raises two issues. Why does mutation of *EMP24* or other genes encoding members of the p24 family affect the transport of so few proteins? And why is transport of these proteins not completely defective in *emp24* mutant cells?

One possibility to explain the small number of proteins affected is that there could be a large and heterogeneous family of cargo receptors with overlapping function. Evidence exists for another type of cargo receptor acting in ER to Golgi transport, namely ERGIC 53, a lectin that also apparently facilitates transport of some glycoproteins (Appenzeller et al., 1999). It is interesting to note that mutation of either receptor only affects a small number of cargo molecules in a highly specific manner. There is a large number of proteins that cycle between ER and Golgi compartments. Many of these could have cargo receptor functions in direct or indirect ways.

Another way to explain the small number of proteins affected in a cargo receptor mutant would be that only a small subset of proteins requires a receptor at this step. It seems clear that some very abundant proteins in specialized secretory cells are not concentrated in COPII-coated vesicles (Martínez-Menárguez et al., 1999). Therefore, not all secretory proteins require a receptor for efficient ER exit. So why would some proteins require receptors for this transport step? Some proteins may bind with low affinity to other proteins that are retained in the ER lumen. These cargo molecules would require a higher affinity interaction to liberate them from the ER. Alternatively, some proteins may require high rates of secretion, for example, a protein with an activity that would be detrimental in the ER. Gas1p probably has a function in cross-linking β 1,3 glucans with β 1,6 glucans and chitin in the cell wall (Popolo and Vai, 1999). Such an activity may have negative consequences in the ER.

Previously, a bulk flow mechanism (Wieland et al., 1987) where proteins leave the ER simply by being available for entry into the forming vesicle, similar to the process of fluid-phase pinocytosis, has been proposed. This could explain why mutation of genes encoding members of the p24 family affects transport of some cargo proteins strongly, but not completely. Without a receptor, the cargo protein would still be able to enter ER-derived vesicles by bulk flow. When the function of other cargo receptors, such as the mannose-6-phosphate receptor (Kornfeld, 1992), or yeast Vps10p (Marcusson et al., 1994) is blocked, the lysosomal or vacuolar proteins that are affected still exit the Golgi. However, they are secreted rather than targeted to the lysosome or vacuole. Since, as far as we know, there is only one destination for ER-derived vesicles, namely the Golgi apparatus, protein mis-sorting would not occur in the absence of receptors in the ER. This may also help to explain why only a small subset of proteins actually needs a receptor. As long as they can exit the ER they will go to the Golgi compartment.

A role in specific cargo recruitment is not likely to be the only role of the Emp24 complex. Secretion of Kar2p caused by mutations in subunits of this complex implies a role in the retrograde transport from the Golgi compartment to the ER (Elrod-Erickson and Kaiser, 1996; Marzioch et al., 1999). Consistent with this, p24 tails from animal

cells can bind COPI proteins (Fiedler et al., 1996; Sohn et al., 1996; Dominguez et al., 1998) and when displayed on liposomes they can stimulate the formation of COPI-coated vesicles (Bremser et al., 1999). Quality control in the ER involves recycling from the Golgi compartment to the ER of misfolded proteins (Hammond and Helenius, 1994). Interestingly, a mutation in a p24 protein from *C. elegans*, SEL-9, allows some misfolded proteins to escape the quality control mechanisms in the early secretory pathway (Wen and Greenwald, 1999) which could perhaps be explained by a retrograde defect. Studies that can directly address the retrograde or other putative functions of p24 proteins in vesicular traffic would be desirable.

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