Abstract. The cDNA encoding ε-COP, the 36-kD subunit of coatomer, was cloned from a bovine liver cDNA library and sequenced. Immunoblotting with an anti-ε-COP antibody showed that ε-COP exists in COP-coated vesicles as well as in the cytosolic coatomer. Using the cloned cDNA, recombinant His
tagged ε-COP was overexpressed in cultured Chinese hamster ovary (CHO) cells, from which metabolically radiolabeled coatomer was purified by taking advantage of the His
tag. Radiolabeled coatomer was employed to establish that all the subunits of the coatomer enter coated vesicles as an intact unit.

Intracellular traffic between the membrane compartments of eukaryotic cells relies on the movement of vesicular carriers (Jamieson and Palade, 1967; Palade, 1975; Rothman et al., 1984a,b). Golgi-derived (non-clathrin or COP) coated vesicles can be produced in a cell-free system that reconstitutes intercisternal protein transport (Balch et al., 1983, 1984; Balch and Rothman, 1985; Orci et al., 1986, 1989) and purified (Malhotra et al., 1989; Serafini et al., 1991). This led to the identification of four of the subunits of the coat, or COPs, termed α-COP (160 kD), β-COP (110 kD), γ-COP (98 kD), and δ-COP (61 kD).

Independently, a cytosolic complex that acts as the coat protomer (termed 'coatomer') containing the same four coat proteins was purified (Waters et al., 1991, 1992) and separated by preparative 15% polyacrylamide SDS-PAGE. Gels were stained with Coomassie blue NTA affinity resin. Using this radiolabeled coatomer, we could follow coat assembly on Golgi membranes, and also determine the stoichiometry of each subunit at every stage.

Materials
EXPRE355S52P protein labeling mix, [α-32P]dCTP and [α-35S]bio-dATP were from DuPont New England Nuclear (Boston, MA). [γ-32P]ATP and [125I]protein A were purchased from ICN Biomedical Inc. (Costa Mesa, CA). FBS was from GIBCO BRL (Gaithersburg, MD). Peroxidase-conjugated secondary antibodies and affinity purified rabbit anti-mouse IgG were purchased from Bio-Rad Labs. (Hercules, CA). A monoclonal antibody to β-COP (M3A5) was kindly donated by Dr. T. E. Kreis. A monoclonal antibody CMIA10 (Palmer et al., 1993) was provided by Dr. D. J. Palmer. A polyclonal anti-γ-COP antibody was prepared as described (Kuge et al., 1993). Recombinant myristoylated ADP ribosylation factor (ARF)1 was provided by Drs. J. B. Helms and G. Tanigawa (Helms et al., 1993).

DNA Manipulations
DNA manipulations, including restriction enzyme digestion, ligation, plasmid isolation, subcloning, E. coli transformation, 32P-labeling of DNA, and oligonucleotide probes for filter hybridization, were carried out by the standard methods, unless otherwise stated. DNA nucleotide sequences were determined by the dideoxy chain termination methods with Sequenase, using walking primers.

Purification of ε-COP
Coatomer was prepared from bovine liver as described (Waters et al., 1991, 1992) and the subunits of coatomer, COPs, were separated by preparative 15% polyacrylamide SDS-PAGE. Gels were stained with Coomassie blue NTA affinity resin. Using this radiolabeled coatomer, we could follow coat assembly on Golgi membranes, and also determine the stoichiometry of each subunit at every stage.

1. Abbreviations used in this paper: ARF, ADP ribosylation factor; GTPγS, guanosine 5′-O-(3-thiotriphosphate); FVDF, polyvinylidene difluoride.
Preparation of Cytosol and Golgi Membranes

Approximately 100 pmols of p35 and p36 were run on a 15% polyacrylamide SDS-PAGE, transferred to polyvinylidene difluoride (PVDF) or nitrocellulose filter and stained with Ponceau S. The proteins on PVDF membrane were subjected to NH₂-terminal amino acid sequence analysis. The proteins on nitrocellulose membrane were digested in situ by trypsin and the resulting peptides were fractionated by reverse phase HPLC (Tempest et al., 1990) and subjected to amino acid sequence analysis (Tempe and Riviere, 1989). The analyzed sequences were used to design primers for a two stage polymerase chain reaction (PCR). The primers used for amplification were TT(TC)GA(TC)GT(ACT)AA(AA)AA(TC)GC (codons Pro155-Ala251, sense) and GGG(AAT)TG(TC)GA(TC)GT(AA)AA(AA)AA (codons Phe125-Pro131, antisense). The bovine liver ZAP II cDNA library (Stratagene, LaJolla, CA) was used as the template for the first round of PCR. The amplification reactions were performed for five cycles with a denaturing temperature of 94°C for 1 min, annealing at 55°C for 1 min, and elongation at 72°C for 2 min. The products of the amplification were analyzed by Southern blot with an internal probe, AA(AA)AA(TC)GC-(AGTC)AA(AA)(GS)AA (codons Lys72-Ile78, sense) and 0.3-kb product was found to hybridize with the probe. For Southern blot analysis, DNA fragments were electrophoresed through a 2% agarose gel, transferred to nylon filters, and hybridized with the 32P-labeled probe for 48 h at 48°C in 3 M sodium phosphate, pH 6.8. A final wash was performed in 1 x SSPE, 0.1% SDS at 50°C for 1 h.

Preparation of Recombinant Hist(e)-e-COP

The coding region of e-COP cDNA was engineered by PCR to add a BamHI site upstream of the first ATG and a HindIII site downstream of the termination codon. The PCR product was digested with BamHI and HindIII and cloned into a pGEM vector (Qiagen), which allows the production of recombinant protein containing an NH₂-terminal affinity tag consisting of six adjacent histidine residues. The resulting plasmid was introduced into E. coli (M15 harboring plasmid pREP4, Qiagen). The transformant was grown to a density of A600 = 0.9 in Super medium (25 g Bacto-trypone, 15 g yeast extract and 5 g NaCl per liter) with 100 µg/ml ampicillin, 50 µg/ml kanamycin, and further cultured at 37°C, and further cultured for 2 h in the culture medium. The pellets were collected by centrifugation at 10,000 g for 30 min, and the supernatant was loaded on a Ni-NTA-agarose column (8 ml bed volume) previously equilibrated with Sonication buffer.

Preparation of Affinity Purified Antibodies against e-COP

A rabbit was injected with 100 µg purified His6-e-COP emulsified in Freund's complete adjuvant. Three weeks later the rabbit was injected with 100 µg purified His6-e-COP emulsified in Freund's incomplete adjuvant and this booster injection was repeated every 3 wk. After the antiserum was precipitated with 60% saturated ammonium sulfate and dialyzed against PBS, the anti-e-COP antibody was purified by adsorption to His6-e-COP conjugated to AminoLink® coupling gel (Pierce, Rockford, IL) as described (Harlow and Lane, 1988) and dialyzed against 10 mM MOPS/KOH, pH 7.5 and 150 mM KCl.

Western Blot Analysis

Proteins were fractionated by SDS-PAGE under reducing conditions (Laemmli, 1970) and electroblotted onto nitrocellulose in 25 mM Tris, 192 mM glycine and 20% methanol and 0.05% SDS at 22 V/cm for 1 h. e-COP was detected using the mouse monoclonal M3A5 (0.4 mg/ml) and peroxidase-conjugated anti-mouse IgG (diluted 1:1,000). e-COP and γ'-COP were detected using the affinity purified antibodies against e-COP (50 ng/ml) and γ-COP (20 ng/ml), respectively, and peroxidase-conjugated anti-rabbit IgG (diluted 1:1,000). Peroxidase labeling was detected by chemiluminescence using ECL reagent (Amersham Corp., Arlington Heights, IL). Analysis of samples for b-COP, e-COP, and γ-COP was achieved by fractionation using 12.5% polyacrylamide gels and immunodetection of b-COP, e-COP, and γ'-COP on the top, middle, and bottom portions of nitrocellulose blots, respectively. For quantitation, the top portion (b-COP) of nitrocellulose blots was incubated in affinity purified rabbit anti-mouse IgG (1:3,000), and then the top and the middle (e-COP) portions were incubated in 0.2 µCi/ml [125I]protein A, dried, and exposed to Kodak X-AR 5 film at −80°C for 15 h. The blot was quantitated by excision of the 110-kD and 35/36-kD regions from the nitrocellulose and counting in a γ counter.

Gel Filtration of Bovine Brain Cytosol

Bovine brain cytosol (0.2 ml, 33.8 mg/ml of protein) was fractionated using a 24-ml Superose 6 gel filtration column (1-cm ID, Pharmacia LKB Biotechnology, Piscataway, NJ) previously equilibrated with 150 mM KCl, 10% (wt/vol) glycerol, 25 mM Tris-HCl, pH 7.4 and 1 mM diithiothreitol at 4°C. The column was eluted in this buffer at 0.3 ml/min (0.4 ml/ fraction).

Immunoelectron Microscopy

Rabbit liver Golgi membranes were incubated in the presence of coatomer, coatomer-depleted cytosol, guanosine 5'-O-(3-thiotriphosphate) (GTPγS), ATP and ATP generation system, according to the cell free transport assay (Baich et al., 1984). Also CHO Golgi membranes were incubated in AIFs. The membranes were collected by centrifugation and fixed with glutaraldehyde. The membrane pellet was cryosectioned as described by Tokuyasu (1980). The protein A-gold method (Roth et al., 1978) was used to visualize antibodies. The affinity purified anti-e-COP antibody (4.11 mg/ml) was diluted 1:100.

Construction of Plasmids Encoding e-COP Containing Six Histidine Residues to be Expressed in Mammalian Cells

The coding region of e-COP cDNA was engineered by PCR to add the region encoding six histidine residues and a KpnI site immediately upstream of the first ATG and a XbaI site immediately downstream of the termination codon. The PCR product was digested with KpnI and XbaI, and cloned into the pSVSport-1 vector (GIBCO BRL).

Introduction of Plasmid into CHO Cells

Transfection of CHO cells was performed by the calcium phosphate coprecipitation method as described (Lewis et al., 1987). One ml of calcium phosphate/DNA precipitate containing 22 µg of pSVSport-1 carrying His6-tagged e-COP cDNA and 2.2 µg of pSVneo was added to a 100-mm diam dish containing 2 x 10⁶ cells. After exposure to the DNA for...
from ClIO Transformants plates, and then cultured in the medium containing 500 µg/ml of Geneticin 7 h, the cells were treated with dimethyl sulfoxide (10% for 20 min), grown at 0.3 cell/well in 2.5 ml medium containing 100 µg G-418, using 24-well plates. After one week, 15 independent colonies were purified and further disrupted by sonication at 4°C. 40 µg lysates of 15 independent G-418 resistant colonies were checked for expression of e-COP by immunoblotting.

Purification of Radiolabeled Coatomer
from CHO Transformants

The CHO transformants were cultured in 100-mm plates containing MEMa/10% FBS/500 µg/ml G-418 and passed routinely. For purification of labeled coatomer, the CHO transformants from four confluent culture plates were harvested, suspended in 100 µl of 25 mM Tris/HCl, pH 7.4, 250 mM sucrose and 1 mM PMSE, and disrupted by sonication at 4°C. 40 µg lysates of 15 independent G-418 resistant colonies were checked for expression of e-COP by immunoblotting. The transformation with the highest level of e-COP expression was selected and used for purification of coatomer.

Figure 1. The sequence of the e-COP gene. The predicted amino acid sequence is shown in single-letter code. All protein sequences determined by Edman degradation are indicated with an underline. Double-underline shows a polyadenylation signal and (***) shows a termination codon. These sequence data are available from EMBL under accession number X76980.
0.4 μg of labeled coatamer in the presence of 20 μM GTP or GTPγS per 50 μl reaction mixture.

Isolation of Radiolabeled Coated Vesicles

A 2-ml reaction mixture to isolate non-clathrin coated vesicles derived from rabbit liver Golgi membranes was prepared by the same methods as described (Serafini et al., 1991), using 17 μg of labeled coatamer and 10 μg of coatamer-depleted cytosol instead of cytosol in the presence of 20 μM GTP or GTPγS. Also the same 2-ml reaction mixture with 0.5 mg soybean trypsin inhibitor was prepared with or without 20 μg myristoylated ARF in the absence of coatamer-depleted cytosol. The complete reaction mixture was immediately pipetted into two 1.5-ml Eppendorf tubes and incubated for 15 min at 37°C. After this incubation the tubes were chilled for 10 min in an ice/water bath (all subsequent steps were carried out on ice), and the membranes were collected by microcentrifugation for 5 min. The reaction mixture was removed by aspiration, and the membrane pellet was resuspended in 0.6 ml of 50 mM KCl, 25 mM Hepes/KOH, pH 7.2, 2.5 mM magnesium acetate, 0.2 M sucrose and 0.1 mg/ml BSA using a Pipetman P-1000. Resuspended membranes were incubated on ice for 15 min and then collected by microcentrifugation for 5 min. Supernatant was removed by aspiration and discarded, and the membrane pellet was resuspended as before into 0.6 ml of 250 mM KCl, 25 mM Hepes/KOH, pH 7.2, 2.5 mM magnesium acetate, 0.2 M sucrose and 0.1 mg/ml BSA. After incubation for 15 min on ice, the larger contaminating membranes were removed by microcentrifugation as before. The supernatant was recovered, transferred to a new tube, and microcentrifuged for 10 min. 545 μl of recovered supernatant was placed into a new tube, and adjusted to 20% (wt/wt) sucrose by addition of 55% stripping buffer* (SB*; 250 mM KCl, 25 mM Hepes/KOH, pH 7.2, 2.5 mM magnesium acetate, 0.2 M sucrose and 0.1 mg/ml BSA, and at the indicated sucrose concentration [wt/wt]. The crude coated vesicles were overlayed with 714 μl each of the following in an SW55 tube: 50% SB*, 45% SB*, 40% SB*, 35% SB*, 30% SB*, and 25% SB*. The gra-
Figure 5. Immunoelectron microscopic localization of ε-COP to the coat of non-clathrin-coated vesicles. (a) Rabbit liver Golgi membranes were incubated in the presence of coatomer, cytosol depleted of coatomer, GTPγS, ATP and ATP-regenerating system. (b) CHO Golgi membranes were incubated in AIF₄⁻ to accumulate coated vesicles. Immunolabeling was performed on ultrathin cryosections using affinity purified anti-ε-COP antibody (dilution 1:100). Protein A-gold was used to localize bound antibodies. Magnifications: (a) 102,700×; (b) 118,500×.
Figure 6. Overexpression of ε-COP in CHO cells. (Lane 1) CHO transfec-
tant (ε-COP containing His6 tag at NH2-terminus) cytosol (40 μg); and (lane 2) CHO cytosol (40 μg) were analyzed by immu-
noblot using M3A5, anti-ε-COP polyclonal antibody, and anti-ζ-
COP polyclonal antibody.

Results

Cloning and Sequencing ε-COP

Coatomer was purified from bovine liver (Waters et al., 1991, 1992) and run on an SDS-PAGE gel; Coomassie-
staining bands of 35 kD and 36 kD (p35 and p36) were cut out separately. The peptide maps of both proteins after tryp-
sin digestion were similar except for an additional peak in p36 (data not shown). The amino acid sequence of this peak was identical to the NH2-terminal amino acid sequence of p36 but the NH2 terminus of p35 lacks the first five amino acids of p36. Therefore the doublet band in coatomer prepara-
tions is derived from a single protein: p35 is a proteolytic product of p36, which we call the ε-COP subunit of coatomer.

The protein sequences of four additional peptides were de-
termined from the tryptic digest of ε-COP (underlined in Fig. 1). Mixed oligonucleotide primers were synthesized according to the first (the NH2-terminal) and third peptide se-
quenences and used to amplify a 0.3-kb product from a bovine liver cDNA library. The specificity of this band was con-
firmed by hybridization with another mixed oligonucleotide probe synthesized according to an internal peptide sequence. This PCR product was subcloned, sequenced, and used as a probe against the same cDNA library to select a full-length clone of ε-COP. Nineteen positively hybridizing plaques were purified and mapped with restriction endonucleases and eight different cDNAs were identified which share a common restriction map. An open reading frame of 924 bp was identified with a termination codon 12 bp upstream to ATG, a predicted molecular weight of 34.5 kD, a polyadenyl-
ation signal (AATAAA; double underlining in Fig. 1), and a poly(A) tail following the 3' untranslated region (Fig. 1). All protein sequences determined by Edman degradation were contained within this open reading frame (underlined in Fig. 1).

Genomic Southern and Northern blot analyses using the full-length clone of ε-COP as a probe also suggested that p35 and p36 are encoded by the same single-copy gene (data not shown).

No protein sequences significantly similar to ε-COP were found by the BLASTP program (Altschul et al., 1990).

Localization of ε-COP

The full-length cDNA encoding ε-COP was cloned into a pQE10 expression vector to generate a plasmid construct which, when transformed into E. coli, produced an ε-COP recombinant protein containing six histidine residues at its NH2-terminus. The protein was expressed at a high level and purified by affinity chromatography on Ni-NTA agarose. This protein was injected into rabbits and antiserum was purified by affinity chromatography on AminoLink® Gel coupled to His-ε-COP.

Western blot analysis shows that affinity purified anti-ε-
COP antibody reacts against ε-COP specifically in cytosol as well as in purified coatomer preparations (Fig. 2).

In whole cytosol, ε-COP is present almost exclusively in a large complex, most likely the coatomer, as is the case for β-COP (Fig. 3). ε-COP, as well as β-COP, was also present in COP-coated vesicles (Figs. 4 and 5). Fig. 4 shows a su-
crose gradient of crude Golgi-derived COP-coated vesicles formed during an incubation of rabbit liver Golgi mem-
bretes, bovine brain cytosol, and GTPyS. Both ε-COP and β-COP were found together in fractions 5–7 (Fig. 4) at ca. 41% (wt/wt) sucrose (1.18 g/ml-1); Fig. 4b), which is the density expected for COP-coated vesicles. Immunoelectron
Figure 7. Purification of labeled coatomer. (a) Coomassie-stained SDS gel. (12%) (Lane 1) bovine brain cytosol (18 μg); (lane 2) mono-Q pool (8 μg) from labeled CHO transfectants; (lane 3) Ni-NTA pool (6 μg) from same preparation; (lane 4) coatomer purified from bovine liver (4 μg). (b) Autoradiogram of a.

Figure 8. Ni-NTA chromatography of labeled coatomer. (a) Fractions of the Ni-NTA column were analyzed by immunoblot using M3A5, anti-ε-COP polyclonal antibody, and anti-β'-COP polyclonal antibody. (b) Radioactivity of 1 μl of each fraction in a.

Expression of His-tagged ε-COP in CHO Cells and Isolation of Radiolabeled Coatomer

cDNAs of ε-COP containing an additional six histidine residues at its amino terminus was cloned into pSVSport-1, a mammalian expression vector. The resulting plasmids were cotransfected with pSV2neo into CHO cells in order to select transfectants in medium containing G418. ε-COP expression in fifteen cloned transfectants was analyzed by Western blot and the cell line with the highest level of expression was used in subsequent studies (Fig. 6).

A suspension culture of CHO transfectants was uniformly labeled with [35S]methionine. Labeled cytosol from the CHO transfectants (Fig. 7, a and b, lane 1) was used as starting material and radiolabeled coatomer was purified by the two steps of Mono Q chromatography (Fig. 7, a and b, lane 2) and affinity chromatography on Ni-NTA agarose (Fig. 7, a and b, lane 3). Unlike E. coli, mammalian cells contain many proteins which bind to Ni-NTA agarose. For this reason, an additional step of Mono Q chromatography was needed before Ni-NTA affinity chromatography. The Western blot analysis using a monoclonal antibody (M3A5) to β-COP and the anti-ε-COP antibody showed that the Mono Q flow-through fraction contains small amount of ε-COP but not β-COP (data not shown); the coatomer fraction of course contains both proteins. The ε-COP in the flow-through fraction presumably is due to excess, overexpressed ε-COP not combined into coatomer. Western blot analysis of the Ni-NTA chromatography fractions showed that β-COP and β'-COP (p20) were co-eluted with ε-COP by 50 mM imidazole (Fig. 8). These data and especially the absence of β-COP and β'-COP (p20) from the flow-through fraction suggest that the His-tagged ε-COP is incorporated as a subunit of coatomer in the CHO transfectant, and has replaced virtually all of the native ε-COP in coatomer. Since the CHO transfectant grew as well as wild type, apparently the His-tagged coatomer is functional.

Analysis of the final material by SDS-PAGE revealed that the major Coomassie-staining bands corresponded to the mobility of the subunits of coatomer purified from bovine liver, except for the bands corresponding to δ-COP and ε-COP (Fig. 7 a, lanes 3 and 4). The slightly higher Mr of ε-COP is pre-
Figure 9. ARF and GTPγS-dependent coatamer binding to Golgi membrane. Golgi membranes were incubated as indicated. After incubation, the membranes were recovered and analyzed by immunoblot using M3A5.

sumably due to the six additional histidine residues; this observation independently confirms the high degree of substitution for native ε-COP. A direct comparison of ε-COP from native CHO coatamer with *E. coli* expressed His₆-ε-COP confirms that this mobility difference is due to His₆. The increased abundance of ζ-COP in CHO coatamer purified by Ni-NTA may be caused by the decreased number of purification steps which might minimize proteolytic degradation or dissociation. The ζ-COP subunit in liver coatamer is in fact substoichiometric (Waters et al., 1991). A typical preparation yields ca. 120 μg (6.0 × 10⁴ cpm/μg) coatamer containing histidine at the NH₂-terminus from a two-liter confluent culture.

Coat Assembly

Purified His₆-tagged coatamer from the CHO transfectant could bind to Golgi membranes in an ARF and GTPγS-dependent fashion (Fig. 9), as is the case for native coatamer (Donaldson et al., 1992; Palmer et al., 1993).

The radiolabeled His₆-coatamer is also assembled into coats on Golgi-derived transport vesicles. Coated vesicles were purified after incubation of Golgi membranes with labeled coatamer and coatamer-immuno-depleted bovine brain cytosol (Fig. 10 a) in the presence of GTPγS. All of the added radiolabeled coatamer subunits were recovered in an isopycnic gradient in fractions 6-8 (Fig. 10 b), which is the density expected for non-clathrin-coated vesicles (ca. 41% (wt/wt) sucrose; Fig. 10 c). When GTP replaces GTPγS under this condition, coated vesicles do not accumulate (Malhotra et al., 1989); now the coatamer proteins are not present in the density gradient at the position expected for coated vesicles (Fig. 10 d).

We also examined radiolabeled coated vesicles formed with only myristoylated ARF and labeled coatamer as cytosolic factors instead of crude cytosol fractions. Again, all of the subunits of coatamer were recovered in fractions 6-8 (Fig. 11 c), as expected for COP-coated vesicles. However, now vesicles can accumulate when GTP replaces GTPγS (Fig. 11 a), perhaps because a cytosolic GTPase-activating protein (GAP) is absent. The appearance of coatamer subunits in these fractions required myristoylated ARFI protein (Fig. 11 d).

To determine the ratio of coatamer subunit at various stages, purified labeled coatamer, labeled coatamer bound to Golgi membranes, and coatamer in coated vesicles formed with either coatamer-depleted cytosol or whole cytosol were subjected to SDS-PAGE, and then the [³⁵S]methionine label associated with each coatamer subunit was determined by a phosphorimager. As seen in Table I, the ratios of [³⁵S]-counts among subunits is essentially invariant under every condition, suggesting that coatamer exists as a stoichiometric complex containing a distinct complement of proteins as if cycles on and off membranes (The mole ratios in Table I are only approximate, as [³⁵S]-counts are corrected for MW, not Met content. This is because the Met content of several COPs is not yet known). However there may be a significant enrichment of ζ-COP as coatamer assembles into coats (Table I),

![Figure 1a](image1)

![Figure 1b](image2)

![Figure 1c](image3)

![Figure 1d](image4)

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Figure II. Assembly of coatomer to coated vesicles. Labeled coated vesicles were prepared by incubation of Golgi membranes and labeled coatomer with (a) myristoylated ARF1 and GTP, (c) myristoylated ARFI and GTPγS, or (d) GTP in the absence of myristoylated ARFI. (b) Percent sucrose (wt/wt) composition of the fraction in a as determined by refractometry.

Raising the possibility that coatomer complexes deficient in this subunit cannot assemble. In fact, antibody directed against ε-COP inhibits coat assembly (Kuge et al., 1993).

Discussion

The standard coatomer purification procedure requires several steps and is time consuming. This has made it difficult to prepare radiolabeled coatomer in active form, limiting the kinds of experiments that can be done in the cell-free transport system. To circumvent this problem, we prepared a CHO transfectant containing His6-tagged coatomer. The transfectant enables us to purify native radiolabeled coatomer by a relatively simple method in short order. The isolated His6-tagged coatomer seems to be functional because the growth of transfectants is identical to that of wild-type cells though the CHO transfectants contain exclusively His6-tagged coatomer (Fig. 7 a). Furthermore, His6-tagged coatomer shows the same activity as purified coatomer from bovine liver cytosol; for example, coatomer binding to Golgi membranes is dependent on myristoylated ARF and GTPγS (Fig. 9) (Palmer et al., 1993). The His6-tagged coatomer will be an important tool for further investigations to study the role of coatomer in vesicular transport.

We were also able to prepare functional coatomer with the His6-tag at the COOH-terminus of ε-COP, suggesting that ε-COP is functional when it is substituted at either end. The functionality of the NH2-terminal tagged ε-COP confirms that the p35 form (clipped at the NH2-terminus) is not necessary for coatomer function.

Labeled coatomer could be used to determine the ratio of coatomer subunits at different stages during coated vesicle assembly. The ratio was invariant except for ε-COP, which is enriched in assembled coats. This is the first demonstration that coatomer complex is incorporated en bloc, as hypothesized (Waters et al., 1991).

Table I. Approximate Molar Ratios of Coatomer Subunits in Coatomer and Coated Vesicles

| Stage                                              | α-COP* | β-COP plus β'-COP | γ-COP | δ-COP | ε-COP | γ'-COP |
|----------------------------------------------------|--------|-------------------|-------|-------|-------|--------|
| 1. Purified coatomer                               | [1]    | 3.1               | 1.5   | 2.5   | 3.0   | 2.6    |
| 2. Coatomer bound to Golgi membranes               | [1]    | 2.6               | 1.3   | 2.4   | 2.6   | 2.8    |
| 3. Coatomer in coated vesicles (prepared with coatomer-depleted cytosol) | [1] | 2.5 | 1.6 | 3.5 | 3.9 | 5.9 |
| 4. Coatomer in coated vesicles (prepared with whole cytosol) | [1] | 2.6 | 1.7 | 3.3 | 3.9 | 5.8 |

* Approximate molar amounts of each COP (intensity in phosphorimager divided by MW) normalized to α-COP at each stage.
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