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Yeast coatomer contains a subunit homologous to mammalian β′-COP

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The homologue of the mammalian coatomer complex was isolated from yeast cytosol and separated on a modified urea-containing SDS-polyacrylamide gel system. An additional band in the 100 kDa molecular weight range appeared when compared to the protein pattern obtained in conventional Laemmli gels, exactly as observed for mammalian coatomer. Cross-reactivity with an anti-peptide antibody raised against the C-terminus of β′-COP from bovine, and N-terminal sequence analysis, revealed that this protein from yeast is related to β′-COP from mammals.

Secretory pathway; Transport vesicle; Coatomer; Yeast homologue

1. INTRODUCTION

Protein transport along the secretory pathway is mediated by vesicular carriers that bud from the parental membrane and fuse with the target membrane [1]. Transport vesicles of different cell types have been isolated and shown to contain a protein coat composed of a set of seven distinct subunits: α-, β-, β′-, γ-, δ-, ε- and ζ-COP (coat proteins) [2–4]. COP's exist in the cytosol as a pre-formed complex, the coatomer, that binds to membranes as an intact unit [5]. This coatomer–membrane interaction requires the preceding binding of myristylated ARF-GTP and results in the formation of coated vesicles [6,7].

Reconstitution of vesicular transport in a cell-free system, as well as microinjection experiments using anti-β-COP antibodies, have shown that coatomer is involved in both transport from the endoplasmic reticulum (ER) to the Golgi complex and intra-Golgi transport [8–10]. Furthermore, γ-COP is related to Sec21p, a protein encoded by a yeast gene required for ER-to-Golgi transport [11,12]. Sec21p has been shown to be present in a complex of about 700–800 kDa with a subunit composition similar to that of the mammalian coatomer upon separation in normal SDS-polyacrylamide gels [3,12].

Until recently, it was generally accepted that β- and γ-COP are the sole coatomer subunits in the 100 kDa molecular weight range [3]. However, during our investigations to determine the actual coatomer composition we discovered an additional subunit by applying a modified urea-containing gel system. This protein, termed β′-COP, is a stoichiometric component of both the coatomer complex and Golgi-derived transport vesicles. It is composed of 905 amino acids corresponding to a molecular weight of 102 kDa. The cDNA-derived N-terminal third of β′-COP is made up of five repeats typical for the β-subunits of trimeric G proteins (WD-40 motif; [4,13]).

In order to investigate whether the Sec21p-containing protein complex isolated from yeast cytosol contains a subunit related to β′-COP from mammals, we separated the yeast complex in our modified gel system. Based on N-terminal sequence analysis and immunological cross-reactivity of anti-peptide antibodies directed against mammalian β′-COP, we show that a β′-COP homologue is present in yeast (*Saccharomyces cerevisiae*).

2. MATERIALS AND METHODS

2.1. *Isolation of coatomer*

Mammalian coatomer, the cytosolic protein complex containing subunits of COP-coated vesicles, was purified from bovine brain by the procedure of Waters et al. [3]. Fractions containing coatomer were initially identified with the anti-β-COP antibody, M3A5, kindly provided by Thomas Kreis. β′-COP-containing complex from wild-type yeast, *Saccharomyces cerevisiae* BJ926 was isolated exactly as described by Hosobuchi et al. [12]. Fractions were pooled based on their immunoreactivity with an anti- peptide antibody raised against a peptide from mammalian γ-COP that specifically cross-reacts with Sec21p.

2.2. *Antibodies*

Antibodies anti-γ-800 and anti-β′-891 were produced by immunizing rabbits with the corresponding peptides (800, KRCVMDDDNEVRDRAT, amino acid 163–178 of mammalian γ-COP; 891, LDEDILDD, C-terminus of mammalian β′-COP) coupled to keyhole limpet hemocyanin by glutaraldehyde [14] followed by affinity purification using the peptide coupled to epoxy-activated Sepharose 6B (Pharmacia). The specificity of the anti-peptide antibodies was verified by elimination of the signal after preincubating the antibodies with a 2 millimolar solution of the peptides.

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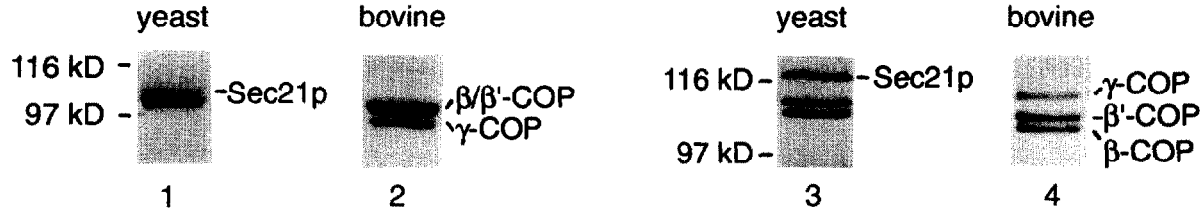

Fig. 1. Analysis of the 100 kDa COP family by SDS-polyacrylamide gel electrophoresis and Coomassie blue staining. Lane 1, proteins in the 100 kDa molecular weight range of purified Sec21p containing protein complex from yeast separated in a 6% acrylamide gel according to Laemmli [15]; lane 2, 100 kDa COP family of purified coatomer from bovine brain separated as described for lane 1; lane 3, same sample as in lane 1 separated in a 6% acrylamide gel with an acrylamide to *N,N'*-methylene-bisacrylamide ratio of 100:1, and additional 6 M urea in the separating gel [16]; lane 4, same sample as in lane 2 separated as described for lane 3.

2.3. *SDS-polyacrylamide gel electrophoresis, immunoblotting and protein sequencing*

Coatomer complex from bovine brain or samples obtained during the purification of the corresponding complex from yeast were separated on 6 or 7.5% SDS-polyacrylamide gels under reducing conditions according to Laemmli [15] or using a modified separating gel [16]. The ratio of acrylamide to *N,N'*-bisacrylamide was increased to 100:1 and 6 M urea was added. For analytical purposes about 5 μg of protein was loaded on mini-gels and directly stained with Coomassie blue. Immunoblotting was performed on Immobilon membranes (Millipore) according to Kyhse-Andersen [17]. The membrane was pre-blocked with 5% (w/v) non-fat milk in PBS and incubated with the antibodies at dilutions as indicated in the figure legends. Peroxidase-conjugated secondary antibodies at a dilution of 1/5,000 and diaminobenzidine/nickelchloride were used as the detection system. For protein sequencing about 500 pmol of protein was electroblotted onto Glassybond membranes (Biometra) in a Bio-Rad semi-dry apparatus. The Coomassie-stained band corresponding to the putative β'-COP homologue was excised and sequenced using an Applied Biosystems gas-phase sequencer with on-line HPLC detection [18].

3. RESULTS AND DISCUSSION

The Sec21p-containing protein complex from yeast cytosol was separated on a SDS-polyacrylamide gel system containing urea and an increased ratio of acrylamide to bisacrylamide (100:1) [16]. A triplet of bands in the 100 kDa molecular weight range was obtained (Fig. 1, lane 3) compared to a dublet in conventional Laemmli gels (Fig. 1, lane 1). A similar pattern was observed for the 100 kDa family of mammalian coatomers (Fig. 1, lanes 2 and 4), although these proteins migrate with a reduced mobility. In a previous study, we assigned the bands of the mammalian coatomer triplet to their corresponding COP proteins using specific antibodies directed against β-, β'- and γ-COP [4,11]. In Laemmli gels mammalian β- and β'-COP comigrate, whereas γ-COP migrates slightly faster (Fig. 1, lane 2). In urea-containing gels, however, γ-COP shows an inversed mobility, thus migrating more slowly than β- and β'-COP which are clearly resolved in this system (Fig. 1, lane 4). β'-COP migrates with an apparent molecular weight of about 110 kDa, β-COP at about 105 kDa.

In order to identify putative homologues of mammalian COP's in yeast, we used antibodies directed against synthetic peptides derived from the cDNA sequence of coatomer isolated from bovine brain. Fig. 2 shows an immunoblot of a cytosolic fraction from yeast (lanes 1 and 2) and mammalian coatomer (lanes 3 and 4) stained with an anti-peptide antibody raised against the sequence, KRCVMDDDNEVRDRAT, derived from mammalian γ-COP. The slowest migrating band of the triplet reacted with this antibody in yeast and, as a control, in the mammalian sample. Addition of the peptide to which the antibody was raised eliminated all cross-reactivity with either yeast or bovine (Fig. 2, lanes

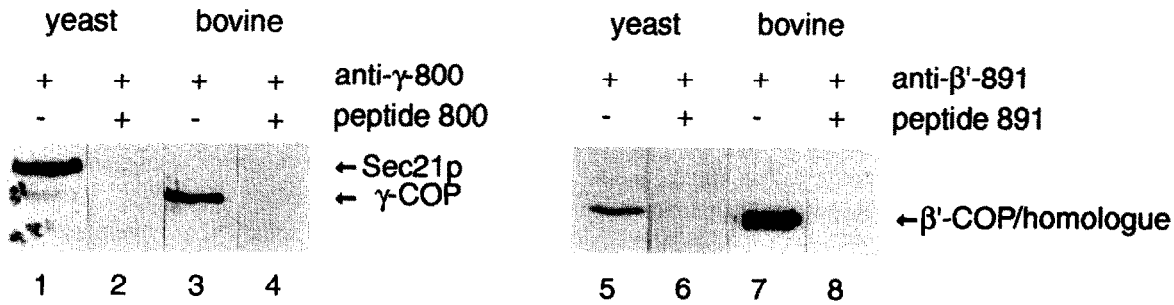

Fig. 2. Immunoblot analysis of the 100 kDa COP family. Lane 1 and 2, yeast coatomer (separated as described in Fig. 1, lane 3) blotted and immunostained with the anti-γ-800 antibody in the absence (−) and presence (+) of the corresponding peptide; lane 3 and 4, bovine coatomer (separated as described in Fig. 1, lane 4) blotted and immunostained with the anti-γ-800 antibody in the absence (−) and presence (+) of the corresponding peptide; lane 5 and 6, yeast coatomer as in lane 1 immunostained with the anti-β'-891 antibody in the absence (−) and presence (+) of peptide; lane 7 and 8, bovine coatomer as in lane 3 immunostained with the anti-β'-891 antibody in the absence (−) and presence (+) of peptide. Anti-γ-800 was used at a dilution of 1:200, anti-β'-891 was diluted 1:1000.

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mammalian   PLRLDIKRKLTARSDRVKSVDLHPTEPW
              :|||||: :::|||||||  :|:|||||
yeast       -MKLDIKKTFSNRSDRVKGIDFHPTEPW
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Fig. 3. Sequence comparison of the 27 N-terminal amino acid residues of β′-COP from mammals with its yeast homologue. Identities are indicated as vertical bars and conservative replacements as colons.

2 and 4). An anti-peptide antibody directed against the C-terminal peptide LDEDILDD, of mammalian β′-COP cross-reacted with the middle band of the yeast triplet (Fig. 2, lane 5), exactly as observed for mammaliani coatomer (Fig. 2, lane 7). Preincubation of the antibody with the corresponding antigenic peptide resulted in loss of the signal in both the yeast and bovine sample (Fig. 2, lanes 6 and 8).

The protein pattern of the 100 kDa family of the cytosolic yeast complex in the urea-containing gel system, together with the cross-reactivity of an antibody raised against the C-terminus of mammalian β′-COP, strongly suggests that a β′-COP-related protein exists in yeast. To corroborate this finding, we subjected the putative protein band to N-terminal protein sequence analysis. In Fig. 3 the N-terminal 27 amino acids of bovine and yeast are compared, revealing a striking identity of 63% and a homology of 89%. Thus, a homologue of mammalian β′-COP is present in yeast coatomer.

The high degree of conservation of the N-terminal amino acid sequence, the immunological cross-reactivity of a C-terminal anti-peptide antibody in mammals and yeast, and the presence of repeated motifs of β-subunits of trimeric G proteins, point to a pivotal role of β′-COP in coatomer function. Typically, β-subunits of trimeric G proteins contain 340 amino acid residues and are almost entirely composed of 7–8 repeated motifs of about 40 residues (WD-40 motif) [19]. These repeated motifs have also been found in a large variety of proteins that do not share any obvious functional properties [13]. However, the members of this protein family have in common that, in addition of their repeated WD-40 motif domain, they contain additional structural domains in their polypeptide chain [20]. Several partners that interact with the WD-40 repeat have been reported that are not related to G proteins [21]. In signal transduction mediated by trimeric G proteins, one function assumed for G_β in association with G_γ is probably to control the interaction of G_α with receptors. Recently, an additional function of G_β subunits has been suggested in a direct regulation of effector molecules [22,23].

For β′-COP it is completely open what the molecular target could be and at present we can only speculate about the function of the β-repeat domain in a protein of the size of β′-COP. One possibility is an interaction with another coatomer subunit, in a similar way as, for example, has been suggested for the β-motif-containing 50 kDa subunit of the human cleavage stimulating factor, a multimeric protein complex involved in polyadenylation of pre-mRNA [24]. The similarity with G_β-subunits might allow for a regulated interaction of coatomer subunits and thus represent the molecular basis for the regulation of coating and budding of transport vesicles [10]. Alternatively, β′-COP might serve to mediate the membrane association of coatomer via a receptor-like and/or a G_α-like molecule, one possible candidate being ARF-GTP.

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