Impaired Proteasome Function Rescues Thermosensitivity of Yeast Cells Lacking the Coatomer Subunit \(\epsilon\)-COP*

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‡‡The abbreviations used are: COP(\(\alpha\)), coat protein(\(\alpha\)); ER, endoplasmic reticulum; CPY, carboxypeptidase Y; NLS, nuclear localization sequence; PAGE, polyacrylamide gel electrophoresis; Leu-\(\beta\)-gal, leucine-\(\beta\)-galactosidase; NLS-\(\beta\)-gal, SV40 T antigen NLS-tagged \(\beta\)-galactosidase; SP, secretory protein.

Formation of COPI-coated transport vesicles requires a cytosolic protein complex consisting of seven subunits: \(\alpha\), \(\beta\)-, \(\beta^\prime\)-, \(\gamma\), \(\delta\), \(\epsilon\)- and \(\zeta\)-COP, collectively designated coatomer. The yeast *Saccharomyces cerevisiae* gene encoding the \(\epsilon\)-COP subunit is known as SEC28/ANU2. \(\alpha\)nu2 null mutant cells (\(\alpha\)nu2Δ) are temperature-sensitive, and \(\alpha\)-COP is rapidly degraded in these cells when they are shifted to the restrictive temperature. We isolated extragenic suppressors that rescue the temperature-sensitive defects of \(\alpha\)nu2Δ cells. Genetic analysis revealed that one of the suppressors is allelic to *PRES8* (PRS4), which encodes a 20 S proteasome subunit. In the presence of a proteasome inhibitor, MG132, \(\alpha\)nu2Δ cells did not cease growth even at the restrictive temperature. Furthermore, MG132 inhibited the rapid decrease of \(\alpha\)-COP levels in \(\alpha\)nu2Δ cells shifted to the restrictive temperature. However, secretion of certain proteins by these cells was impaired even in the presence of MG132. In conclusion, impairment of proteasome-dependent proteinolysis rescued some, but not all, temperature-sensitive defects of \(\alpha\)nu2Δ cells. These results are discussed in terms of evidence that \(\epsilon\)-COP plays a critical role in maintaining the structural integrity of \(\alpha\)-COP.

Intracellular protein transport between membrane-bound organelles of the secretory and endocytic pathways is carried out through budding and fusion of several types of coated vesicles. One of the non-clathrin coats, COPI, which was first identified in mammalian cells, consists of coatomer and ADP-ribosylation factor (1, 2). Coatomer is a cytosolic ~800-kDa complex composed of seven coat proteins (COPIs): \(\alpha\)-COP (160 kDa), \(\beta\)-COP (110 kDa), \(\beta^\prime\)-COP (102 kDa), \(\gamma\)-COP (98 kDa), \(\delta\)-COP (61 kDa), \(\epsilon\)-COP (35 kDa), and \(\zeta\)-COP (20 kDa). The structure of coatomer is evolutionarily conserved, because all of the coatomer subunits have been identified not only in mammals but also in the yeast *Saccharomyces cerevisiae* (3–11). The yeast gene encoding \(\epsilon\)-COP is named SEC28 by Duden et al. (10) or ANU2 by us (11). \(\alpha\)nu2Δ mutant cells grow well at temperatures up to 34 °C and cease growth only at a higher temperature of 37 °C.

COPI function is, directly or indirectly, essential for anterograde protein transport from the endoplasmic reticulum (ER) to the Golgi. Indeed, several mutants of coatomer subunit genes accumulate ER precursor forms of some secretory proteins under restrictive conditions (3, 5, 8, 12). In the case of \(\alpha\)nu2Δ cells, ER-to-Golgi anterograde transport of carboxypeptidase Y (CPY) is blocked at the restrictive temperature of 37 °C (10, 11). Moreover, COPI is believed to directly mediate retrograde protein transport from the Golgi to the ER. Indeed, most of the coatomer subunit mutants studied thus far exhibit defects in retrieval of ER resident proteins to the ER even under permissive or semipermissive conditions (6, 8, 9). For some of these mutants, retrograde inhibition is most obvious at semipermissive temperatures, because inhibition of anterograde transport at restrictive temperatures often makes it difficult to score retrieval efficiency. However, retrieval of an ER-resident test protein is normal both at 30 and 32 °C in \(\alpha\)nu2Δ cells (10, 11). This seems reasonable if, as discussed in Duden et al. (10) and in this study, the primary defect of \(\alpha\)nu2Δ cells is rapid degradation of \(\alpha\)-COP occurring only at the restrictive temperature.

What is the functional role of \(\epsilon\)-COP? A mammalian Chinese hamster ovary mutant cell line, \(idl^F\), contains a single point mutation in the \(\epsilon\)-COP gene conferring temperature sensitivity (13). When cultured at the restrictive temperature, \(idl^F\) cells exhibit rapid degradation of \(\epsilon\)-COP and various defects in both the secretory and endocytic pathways (13–16). These observations imply that \(\epsilon\)-COP plays essential roles in those cellular functions. However, studies using yeast cells presented a different view, because, as described above, ANU2 was not essential for cell growth at low temperatures. In contrast, all of the coatomer genes apart from ANU2 are essential for cell viability at all temperatures (3, 5–9). These observations suggest that \(\epsilon\)-COP is not essential for coatomer function, at least at low temperatures.

Duden et al. (10) provided several lines of evidence suggesting that one role of \(\epsilon\)-COP is to stabilize another coatomer subunit, \(\epsilon\)-COP. First, temperature-sensitive defects of \(\alpha\)nu2Δ cells are suppressed by overexpression of the \(\alpha\)-COP gene, *RET1*. Second, overexpression of ANU2 suppresses defects of an \(\epsilon\)-COP mutant, *ret1-3*, in which \(\epsilon\)-COP is unstable at the restrictive temperature. Third and most importantly, \(\alpha\)-COP, but none of the other coatomer subunits, is rapidly degraded in \(\alpha\)nu2Δ cells shifted to the restrictive temperature of 37 °C. In their view, the primary defect of \(\alpha\)nu2Δ cells should be the rapid degradation of \(\alpha\)-COP, and thus they may grow even at the restrictive temperature provided that this degradation is blocked. However, no data concerning this have been published.
As a step toward understanding further the role(s) of e-COP, we tried to find conditions in which temperature-sensitive defects of anu2Δ cells are rescued. Here we show that in anu2Δ cells shifted to 37 °C, impairment in proteasome-dependent protein breakdown rescues the growth defect as well as preventing the rapid diminution of α-COP levels otherwise observed. Our results provide evidence that the primary defect of anu2Δ cells is rapid degradation of α-COP and thus that a function of e-COP is to stabilize α-COP. It is likely that e-COP acts to maintain structural integrity of α-COP.

EXPERIMENTAL PROCEDURES

Plasmids—Plasmids pRS316 and pRS306 are yeast centromeric and integrative vectors, respectively, containing the URA3 selectable marker (17). A centromeric plasmid containing the ANU2 gene, pYIL076w (11), was renamed pRS316-ANU2. The isolation of plasmids YCp50–072.1 and YCp50–072.2 from a centromere-based genomic library (18) and subsequent construction of plasmids pRS316-PRE8 and pRS306-pRE8 are described below. Plasmid pUb-Leu-lacZ was used for GAL promoter-controlled expression of a ubiquitin-leucine-β-galactosidase fusion protein (19). Plasmid pNLS-lacZ, a generous gift of Dr. M. Oka (Washington University, St. Louis, MO) was used for GAL promoter-controlled expression of β-galactosidase tagged with the nuclear localization sequence (NLS) of the SV40 large T antigen (20). Both pUb-Leu-lacZ and pNLS-lacZ are 2-μm plasmids containing the URA3 selectable marker.

Yeast Media and Strains—YPD is a rich medium containing 2% dextrose, 2% bactopeptone (Difco, Detroit, MI), and 1% yeast extract. SD is a synthetic minimal medium containing 2% dextrose, 0.67% yeast nitrogen base, and growth supplements described in Ref. 21. SC-Met is the same synthetic complete medium containing 2% dextrose, 0.67% yeast nitrogen base, 2% bactopeptone (Difco, Detroit, MI), and 1% yeast extract. SC is a synthetic minimal medium containing 2% dextrose, 0.67% yeast nitrogen base, and 2% bactopeptone (Difco, Detroit, MI), and 1% yeast extract.

Strains Relevant genotype Source

| Name  | Relevant genotype | Source |
|-------|------------------|--------|
| FY8   | MATa ura3–52 lys2Δ92 | F. Winston |
| FY24  | MATa ura3–52 rpl1Δ63 leu2Δ1 | F. Winston |
| FY78  | MATa his3Δ200 | F. Winston |
| FY79  | MATa his3Δ200 | F. Winston |
| YKIM11 | MATa ura3–52 lys2Δ92 | this study |
| YKIM21 | MATa ura3–52 leu2Δ1 lys2Δ92 anu2Δ::LEU2 | this study |
| YKIM22 | MATa his3Δ200 leu2Δ1 anu2Δ::LEU2 | this study |
| YKIM31 | MATa ura3–52 leu2Δ1 lys2Δ92 anu2Δ::LEU2 tan1–1 | this study |
| YKIM32 | MATa his3Δ200 leu2Δ1 anu2Δ::LEU2 tan1–1 | this study |
| YKIM41 | MATa ura3–52 lys2Δ92 tan1–1 | this study |
| YKIM42 | MATa his3Δ200 tan1–1 | this study |
| YKIM50 | MATa ura3–52 rpl1Δ63 leu2Δ1 PRE8::pRS306-pRE8 | this study |

**Table I**

Yeast strains

Proteasome Dysfunction Suppresses e-COP Gene Disruption

DNA fragment (containing PRE8) from YCp50–072.1 was subcloned into the HindIII and BamHI sites of pRS316. 

The tan1–1 mutation was inserted into PRE8 locus by integrative mapping. To create plasmid pRS306-PRE8, the PRE8-containing DNA fragment described above was subcloned into the HindIII and BamHI sites of pRS306. To obtain strain YKIM50, pRS306-PRE8 was linearized within the PRE8 sequence with BamHI and transformed into a wild-type strain (FY24). Integration of the URA3 gene at the PRE8 locus of YKIM50 was confirmed by genomic Southern analysis. YKIM50 was then crossed with a tan1–1 strain (YKIM41), and the resultant diploid strain was subjected to meiotic analysis (30 tetrads). For every tetrad analyzed, the URA3 marker was constantly found to segregate away from the weakly temperature-sensitive tan1–1 allele.

Antibodies—A rabbit antiserum against purified yeast carboxytoxin was generously provided by Dr. R. Duden (University of Cambridge, Cambridge, UK; Ref. 5). Antisera recognizing yeast β-COP were produced by immunizing rabbits with recombinant yeast β-COP (further details are available upon request). A rabbit polyclonal antibody against CPY was generously provided by Dr. A. Nakano (RIKEN, Saitama, Japan). A rabbit polyclonal antibody against β-galactosidase was provided by Cappel (West Chester, PA).

**Assay for in Vivo Degradation of β-Galactosidase Derivatives—**For pulse labeling of cells, [35S]methionine/cysteine (Express protein labeling mix; NEN Life Science Products) was added to the cultures (1.6 MBq/ml), and incubation was continued for 5 min. Chase was initiated by adding cycloheximide (final concentration, 0.5 mg/ml) to the cultures and terminated by adding trichloroacetic acid (final concentration, 10%). Cells were harvested from 1 ml of cultures, and immunoprecipitation was carried out as described in Klionsky et al. (22), except that the antibody used was against β-galactosidase.

**Treatment of Cells with MG132**—MG132 (carbobenzoxyl-leucyl-leucyl-arginylene) was provided by Peptide Institute, Inc. (Osaka, Japan). Before treatment with MG132, cells were grown to 0.1 (for measurement of the growth curve) or 1.0 (for other assays) of OD600/ml in SC-Met at 23 °C. MG132 was prepared as a 50 mM stock solution in Me2SO. This stock solution was added to the cultures to obtain a 50 μM final concentration of MG132. An equal volume of Me2SO (without MG132) was added to the treatment control cultures. These cultures were shifted to 37 °C just after addition of MG132 or Me2SO, and incubation was continued for 2 h for the assays described below.

**Assay for General Secretion Competence—**Bovine serum albumin (final concentration, 500 μg/ml) and α2-macroglobulin (final concentration, 100 μg/ml) were added to the MG132-treated and control cultures 10 min before pulse labeling. For pulse labeling of cells, [35S]methionine/cysteine was added to the cultures (4 MBq/ml), and incubation was continued for 10 min at 37 °C. Chase was initiated by adding excess amounts of unlabeled methionine (final concentration, 1 mM) and cysteine (final concentration, 0.8 mM) to the cultures and continued for 30 min at 37 °C. After the pulse-chase, the cultures were centrifuged at 5,000 × g for 5 min. Then media fractions were precipitated by adding trichloroacetic acid to a final concentration of 10%. After washing the pellets twice in acetone, proteins were solubilized in Laemmli sample buffer, and then proteins were added to 16,000 × g for 15 min, and 0.25 A600 equivalents were loaded for 8% SDS-PAGE. Gels were analyzed with a BAS-5000 Bio-Image Analyzer (Fujiﬁlm, Tokyo, Japan).

**Other Methods—**Measurement of β-galactosidase activity in yeast cells using 3-nitrophenyl-β-D-galactopyranoside as the substrate was performed as described in Ref. 21. For CPY transport assays, pulse and chase experiments were carried out as described by Yamazaki et al. (9),
The gene corresponding to this open reading frame is PRE8 (PRS4). As shown in Fig. 1, the weak temperature sensitivity of tan1-1 cells was complemented by a plasmid carrying a genomic DNA fragment containing only one open reading frame of PRE8. This plasmid also complemented the ability of anu2Δ tan1-1 double mutant cells to grow at 37 °C. Integrative mapping confirmed that the tan1-1 mutation mapped to PRE8.

Proteasome-dependent Protein Degradation Is Impaired in tan1-1 Cells—PRE8 encodes an α-type subunit of the 20 S proteasome (23), which is the “catalytic core” of a larger 26 S complex that degrades proteins by an ATP-dependent process (24, 25). Because tan1-1 is allelic to PRE8, we tested whether proteasome function is impaired in tan1-1 cells. To check the ability of the proteasome to degrade proteins in vivo, we used an N-end rule substrate Leu-β-galactosidase (Leu-β-gal), which bears N-terminal Leu, a destabilizing residue (19, 26). When Leu-β-gal is expressed as a ubiquitin-tagged form in yeast cells, it is rapidly cleaved into an untagged form and degraded. SV40 T antigen NLS-tagged β-galactosidase (NSL-β-gal) was used as a stable protein control that was barely degraded by the proteasome.

In wild-type and tan1-1 cells, Leu-β-gal (initially tagged with ubiquitin) or NLS-β-gal was expressed under GAL promoter control. When cultured at the semipermissive temperature of 36 °C, tan1-1 cells had a 4-fold greater steady-state level of enzymatically active Leu-β-gal than wild-type cells (Fig. 2A). In contrast, the steady-state level of enzymatically active NLS-β-gal was slightly lower in tan1-1 cells than in wild-type cells (Fig. 2B). Furthermore, a pulse-chase experiment confirmed that degradation of Leu-β-gal is severely retarded in tan1-1 cells (Fig. 2C). These results indicate, as expected, that proteasome-dependent proteolysis is impaired in tan1-1 cells.

A Proteasome Inhibitor, MG132, Rescues the Temperature-sensitive Growth of anu2Δ Cells—To demonstrate that temperature sensitivity of anu2Δ cells is rescued in a general way by impaired proteasome function rather than by some specific activity of the tan1-1 mutation, we used a pharmacological inhibitor of the proteasome. Some peptide aldehydes are known to act as substrate analogues of the 20 S proteasome (27). We chose carboxbenzoxyl-leucinyl-leucinyl-leucinal, named MG132, as a proteasome inhibitor, because it inhibits proteasome-dependent proteolysis not only in mammalian cells but also in yeast cells (28). In contrast to a report by Lee and Goldberg (28), MG132 was effective in our strains, which were wild-type for ISE1/GAL (data not shown), mutation of which enhances permeability to many drugs (29).

As shown in Fig. 3, wild-type and anu2Δ cells were shifted to 37 °C with or without MG132. Growth of wild-type cells was slightly retarded by MG132. In contrast, MG132 clearly restored growth of anu2Δ cells. Taken together with the observations on the tan1-1 mutation, this result shows that impaired proteasome function rescues temperature sensitivity of anu2Δ cells.

MG132 Inhibits Rapid Decreases of α-COP Levels in anu2Δ Cells Shifted to the Restrictive Temperature—Duden et al. (10) reported that α-COP, but none of the other coatomer subunits, is rapidly degraded in anu2Δ cells shifted to the restrictive temperature of 37 °C. We therefore tested whether MG132 inhibits this degradation. α-COP levels in anu2Δ cells apparently diminished within 2 h after the temperature shift to 37 °C in the absence of MG132, whereas they remained roughly constant in the presence of MG132 (Fig. 4). In wild-type cells, α-COP levels were almost unaffected by temperature shift or by addition of MG132. In contrast, levels of another coatomer subunit, β-COP, in anu2Δ cells did not differ significantly from those in wild-type cells under any of these conditions.

except that cells were cultured as described above under “Treatment of Cells with MG132.” Cell extracts for Western blot analysis were prepared as described in Duden et al. (10), and Western blot analysis was performed as described previously (11).

RESULTS

A pre8 Mutant Allele, tan1-1, Suppresses Temperature-sensitive Growth of anu2Δ Cells—To gain further understanding of the role(s) of e-COP, we isolated spontaneous suppressors that rescue the temperature-sensitive growth defect of anu2Δ cells. Six mutants that contained mutations allowing growth of anu2Δ cells at 37 °C were characterized. All of them contained single mutations, termed tan (confer temperature resistance to anu2Δ cells) mutations, that showed no genetic linkage to each other or ANU2.

One of the suppressors, tan1-1, was further characterized, as shown in Fig. 1. As described above, anu2Δ tan1-1 double mutant cells grew even at 37 °C. The tan1-1 mutation itself confers weak temperature sensitivity, because tan1-1 single mutant cells showed retarded growth at the higher temperature of 38.5 °C. At this temperature, anu2Δ tan1-1 double mutant cells did not grow at all.

The gene affected in tan1-1 single mutant cells was cloned by complementation of their weak temperature sensitivity after transformation with a centromeric library. Genomic DNA inserts of two library plasmids complementing tan1-1 shared only one open reading frame. The gene corresponding to this
MG132 Only Partially Restores Protein Secretion of anu2Δ Cells at the Restrictive Temperature—To analyze general protein secretion rather than that of specific test proteins, Gaynor and Emr (12) pulse-chase labeled various sec mutant cells with [35S]methionine/cysteine and collected total media proteins to visualize them by SDS-PAGE and autoradiography. They showed that no media proteins were secreted from either sec18Δ, sec23Δ, or sec1Δ mutant cells under their restrictive conditions. This is reasonable, because, for example, Sec23p is an essential subunit of COPII (30), which is believed to govern anterograde vesicle formation from the ER. In contrast, some of the media proteins were still secreted from a α-COP mutant, sec21-3. We performed this general secretion assay using anu2Δ cells.

Wild-type and anu2Δ cells were cultured at 37 °C in the presence or absence of MG132 for 2 h and then pulse-chase labeled with [35S]methionine/cysteine, and their media proteins were analyzed. At least nine protein bands were apparent in media from wild-type cells, and they were almost unaffected by MG132 (Fig. 5A, compare lanes 1 and 2). We focused on three major bands, which were tentatively named secretory proteins (SP) 1, 2, and 3, and quantified them using a BAS-5000 image analyzer (Fig. 5B). In the absence of MG132, secretion of SP1 and SP2 was significantly reduced by the anu2Δ mutation, whereas that of SP3 was not. MG132 restored secretion of SP2 from anu2Δ cells to the normal extent. However, secretion of SP1 from anu2Δ cells was impaired even in the presence of MG132.

We also assayed intracellular transport of an endogenous vacuolar protein, CPY. CPY is commonly used as a test protein to evaluate efficiency of anterograde secretory transport in yeast cells. CPY is synthesized initially as a proenzyme that is first modified with core oligosaccharides in the ER, generating the p1 form, and next in the Golgi complex, where the core sugars are elongated, generating the p2 form. After delivery to the vacuole, the pro region is cleaved to yield the mature...
Proteasome Dysfunction Suppresses e-COP Gene Disruption

In this study, we show that impairment of proteasome-dependent proteolysis rescues the temperature sensitivity of anu2Δ cells by two means. First, this temperature sensitivity was suppressed by a mutation in the PRE8 gene (Fig. 1), which encodes a 20 S proteasome subunit. Impairment of proteasome-dependent proteolysis in this pre8 mutant was confirmed by accumulation of a test substrate of the proteasome in vivo (Fig. 2). Second, a proteasome inhibitor, MG132, restored growth of anu2Δ cells at the restrictive temperature (Fig. 3). Duden et al. (10) indicated that α-COP, but none of the other coatomer subunits, is rapidly degraded in anu2Δ cells shifted to the restrictive temperature. Here we also show that this rapid degradation of α-COP is inhibited by MG132 (Fig. 4). α-COP levels in wild-type cells were almost unaffected by the presence of MG132. The most likely explanation for these results is that the primary defect of anu2Δ cells is inappropriate degradation of α-COP by the proteasome and thus that anu2Δ cells can grow even at the restrictive temperature, provided that this degradation is blocked. Therefore, we interpret our findings as direct evidence that a function of e-COP is to stabilize α-COP as initially proposed by Duden et al. (Ref. 10; see “Introduction”).

In this view, e-COP should not be a key element in coatomer. Indeed, all of the coatomer subunit genes apart from ANU2 are essential for cell viability (3, 5–9). Moreover, to our knowledge, there are no reports suggesting direct interaction of e-COP with cellular components except other coatomer subunits. Lowe and Kreis (32) investigated the assembly of the coatomer subunits in mammalian cells using native immunoprecipitations from pulse-chase labeled cells. They showed that e-COP is the last subunit to be added to the coatomer complex during its biosynthetic assembly, suggesting that e-COP is not a core subunit in this complex.

The study by Lowe and Kreis (32) also showed that biosynthetic assembly of the coatomer complex takes 1–2 h to complete and that once assembled, this complex is stable with a half-life of about 28 h. In addition, they reported that no significant amounts of partial coatomer complexes could be detected and that the only subunit to exist in a steady-state outside of the coatomer complex is ζ-COP. According to Harakuge et al. (33), coatomer is incorporated en bloc into the COPI coat during vesicle formation. Hence, it is likely that the coatomer complex is hardly disassembled at all under physio-

**Fig. 5.** Effect of MG132 on the secretory pathway of anu2Δ cells. Wild-type (YKIM11) and anu2Δ (YKIM21) cells cultured in SC-Met medium at 23 °C were treated with control vehicle (Me2SO; indicated as Control) or 50 μM MG132 and shifted to 37 °C. 2 h later, these cultures were subjected to an assay for general secretion competence. A, assay for general secretion competence. As described under “Experimental Procedures,” cells were pulse-chase labeled with [35S]methionine/cysteine (pulse for 10 min and chase for 30 min). Proteins secreted into the medium during the pulse-chase were visualized by SDS-PAGE and autoradiography. anu2Δ cells (YKIM21) transformed with pRS316-ANU2 were also subjected to this assay (treated with Me2SO and indicated as Control; lane 5). The positions of three major protein bands tentatively named SP 1, 2 and 3 are indicated. MW, molecular mass marker ([14C]methylated proteins; Amersham). B, radioactivity of each protein band in A was quantified by a BAS-5000 image analyzer. Values are expressed relative to the level of wild-type cells treated with Me2SO (corresponds to lane 1 in A; set at 100%) and are the means of three independent determinations with standard deviation shown by the error bars. C, CPY transport assay. Cells were pulse-chase labeled with [35S]methionine/cysteine (pulse for 8 min and chase for 20 min). CPY was recovered by immunoprecipitation, subjected to SDS-PAGE, and visualized by autoradiography. The positions of precursor (p1, ER; p2, Golgi) and mature (m, vacuolar) forms of CPY are indicated. Unidentified protein cross-reactive with the anti-CPY antibody is marked by an asterisk.

BAS-5000 image analyzer. Values are expressed relative to the level of wild-type cells treated with Me2SO (corresponds to lane 1 in A; set at 100%) and are the means of three independent determinations with standard deviation shown by the error bars. C, CPY transport assay. Cells were pulse-chase labeled with [35S]methionine/cysteine (pulse for 8 min and chase for 20 min). CPY was recovered by immunoprecipitation, subjected to SDS-PAGE, and visualized by autoradiography. The positions of precursor (p1, ER; p2, Golgi) and mature (m, vacuolar) forms of CPY are indicated. Unidentified protein cross-reactive with the anti-CPY antibody is marked by an asterisk.
logical conditions. e-COP probably interacts directly with α-COP in this complex, as supported by the following three lines of evidence. The first is the results from yeast two-hybrid analysis of the coatomer subunits (34). The second is the interaction of in vitro translated e-COP and α-COP (34), and the third is in vitro disassembly assays of coatomer showing that at high salt concentrations, the coatomer complex is disassembled to generate a partial complex comprising α-, β′-, and e-COP (35).

Based on our results and the literature reports discussed above, we speculate that e-COP is constantly bound to α-COP as a kind of additional element to maintain α-COP structural integrity and thus the coatomer complex, especially at high temperatures. One of the critical functions of the proteasome-dependent protein degradation machinery is the rapid breakdown of abnormal proteins. Hence, it is likely that the α-COP that is not bound to e-COP is recognized as “abnormal” protein by this machinery.

In this study, we also examined whether MG132 restores the secretory pathway in anu2Δ cells (Fig. 5). Similar to a γ-COP mutant described by Gaynor and Emr (12), only some of the media proteins exhibited reduced secretion in anu2Δ cells shifted to the restrictive temperature. MG132 restored secretion of only one of two major media proteins the secretion of which was apparently reduced by the anu2Δ mutation. This observation suggests that MG132 only partially rescues defects that under such conditions, loss of structural integrity of other components. In this case, it might be possible to compensate loss of such components by impaired proteasome function. Therefore, the approach used in this study may be adapted to dissect functions of proteins contained in cytosolic protein complexes other than coatomer.

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