Deubiquitination, a New Player in Golgi to Endoplasmic Reticulum Retrograde Transport

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Modification by ubiquitin plays a major role in a broad array of cellular functions. Although reversal of this process, deubiquitination, likely represents an important regulatory step contributing to cellular homeostasis, functions of deubiquitination enzymes still remain poorly characterized. We have previously shown that the ubiquitin protease Ubp3p requires a co-factor, Bre5p, to specifically deubiquitinate the coat protein complex II (COPII) subunit Sec23p, which is involved in anterograde transport between endoplasmic reticulum and Golgi compartments. In the present report, we show that disruption of BRE5 gene also led to a defect in the retrograde transport from the Golgi to the endoplasmic reticulum. Further analysis indicate that the COPI subunit β’-COP represents another substrate of the Ubp3p-Bre5p complex. All together, our results indicate that the Ubp3p-Bre5p deubiquitination complex co-regulates anterograde and retrograde transports between endoplasmic reticulum and Golgi compartments.

Modification of target proteins by ubiquitin participates in a wide array of biological functions. Proteins destined for degradation or processing via the 26 S proteasome are coupled to multiple copies of ubiquitin. However, attachment of ubiquitin or ubiquitin-related molecules may also result in changes in subcellular distribution or modification of protein activity (1, 2). Understanding of mechanisms and regulation of ubiquitin conjugation considerably improved over the past 10 years and recent studies indicate that reversal of this modification, namely deubiquitination, represents an additional level of regulation. The deubiquitination process is catalyzed by proteases called deubiquitinating enzymes, which fall into four distinct families (3), ubiquitin C-terminal hydrolases, ubiquitin-specific processing proteases (USPs or UBP’s)4, OTU-domain ubiquitin-aldehyde-binding proteins, and Jab1/Pad1/MPN-domain-containing metallo-enzymes. Among these four families, USPs represent the most widespread and represented deubiquitinating enzymes across evolution. In particular, the Saccharomyces cerevisiae genome encodes for 16 UBP’s and only one UCH, but none of these enzymes is essential for yeast viability. USPs tend to release ubiquitin from a conjugated protein. They display similar catalytic domains containing conserved Cys and His boxes but divergent N-terminal and occasionally C-terminal extensions (4, 5), which are thought to function in substrate recognition, subcellular localization, and protein-protein interactions. The molecular basis for substrate recognition by UBP has been described poorly. Indeed human HAUSP has been shown to be necessary and sufficient to deubiquitinate its specific substrate, the p53 tumor suppressor (6), but no additional substrate for this Ubp has been identified so far. The tumor suppressor CYLD has been shown to negatively regulate NF-κB signaling by deubiquitinating NEMO and TRAF2 (7–9).

We recently reported that the yeast Ubp3p forms a complex with Bre5p, which specifically deubiquititates Sec23p, a component of the COPII complex essential for anterograde transport between the endoplasmic reticulum (ER) and the Golgi apparatus (10). Ubp3p is the only yeast Ubp able to catalyze Sec23p deubiquitination indicating that Ubps can exert their activity on specific substrates and are probably not redundant. Ubp3p directly interacts with Sec23p, and its catalytic cysteine residue is essential for the deubiquitinating activity. In contrast Bre5p does not participate to Sec23p recognition nor complements a catalytically inactive Ubp3p but rather acts as an essential positive regulator of Ubp3p. Bre5p displays an N-terminal domain related to the nuclear transport factor 2 (NTP2) and responsible for the interaction with Ubp3p and a C-terminal domain presenting putative RNA-binding sites. Mammalian cells display two proteins homologous to Bre5p, G3BP1 and G3BP2. Interestingly, G3BP1 has been shown to interact with USP10 (11), a human ubiquitin protease sharing 46% similarity and 27% identity with Ubp3p. The interaction between USP10 and G3BP1 suggests that the Ubp3p-Bre5p complex might have been conserved during evolution. However, there is no clue whether these yeast and human complexes are implicated in similar functions.

In the present report, we analyzed whether the yeast Ubp3p-Bre5p complex could have other substrates than Sec23p and whether the human USP10-G3BP1 complex could recognize these substrates.

EXPERIMENTAL PROCEDURES

Yeast Strains and Antibodies—The S. cerevisiae strains and plasmids used in this study are listed in the Supplementary Table and Refs. 33–36. When indicated, the BRE5 and UBP3 genes were disrupted as described (12). Rabbit polyclonal antibodies to yeast Gcs1p (1:1000 dilution) and Glo3p (1:1000) were kindly provided by G. Johnston, α-COP (1:800), β’-COP (1:200), and δ-COP (1:500) by F. Letourneur and

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FIG. 1. Genetic interaction between BRE5 and GLO3 involves the NTF2-like domain of Bre5p. bre5Δ–glo3 strain transformed with the pCH1122–BRE5 plasmid was transformed with pLAC111 empty vector (Vector), pBRE5 (BRE5), or pGLO3 (GLO3) (A) or with p146.515 (BRE5 (1–400)), or pBRE5–146.515 (BRE5 (146–515)) (B). Transformants were subsequently streaked on 5-fluoroorotic acid medium to induce loss of pCH1122–BRE5.

FIG. 2. Bre5p regulates retrograde transport between ER and Golgi compartments. A, cells disrupted for BRE5 display an ER retrieval defect. MATa WT (FLY264), ret 1.1 (FLY306), or bre5Δ (FLY264 bre5Δ) cells were mated with MATa RH311-3D cells, and diploids were selected on appropriate minimal medium. B, purified recombinant fusion proteins between GST and WBP1 peptides presenting dilyse (GST-WBP1) or dierein motifs (GST-WBP1-SS) were incubated with lysates from wild-type cells (BRE5) or cells disrupted for BRE5 (bre5Δ) and pulled down with glutathione beads. Bound proteins were revealed by immunoblotting with antibodies to β'-COP and δ-COP. Loading was controlled using a nonspecific band (*).

antibodies to β'-COP#8381 (1:600), β'-COP#8562 (1:1000), γ-COP (1:1000), and δ-COP (1:800) were generous gifts from R. Duden.

ER Retrieval Defect Assay of Dilyseine-tagged Ste2p—The in vivo assay to monitor ER retrieval of dilyseine-tagged Ste2p in yeast was performed essentially as described previously (13). Briefly, MATα cells (WT, ret1.1, and bre5Δ) deleted of the endogenous STE2 gene and expressing the Ste2-WBP1 fusion protein, were mated with MATα cells (RH311-3D). Resulting diploids were selected on growth medium lacking uracil and histidine.

Yeast Extracts and GST Pull-down Assay—Cells grown in yeast/peptone/dextrose were collected during the exponential growth phase (Δ600 = 2). Total protein extracts were prepared by the NaOH-trichloroacetic acid lysis method (14). Alternatively cells were lysed at 4°C with glass beads in IP buffer (50 mM Hepes, pH 7.5, 150 mM NaCl, 1 mM dithiothreitol, 0.5% Triton, 1% glycerol, supplemented with a mixture of protease inhibitors, 10 μg/ml aprotinin, pepstatine, leupeptine, 1 mM phenylmethylsulfonyl fluoride). The resulting lysate was centrifuged for 30 min at 13,000 × g. 3 μg of GST-WBP1 or GST-WBP1-SS fusion proteins expressed from plasmids generously provided by F. Letourneur were incubated with lysates of BRE5 or bre5Δ cells for 1h at 4°C and an additional hour at 4°C in the presence of glutathione-Sepharose beads. Beads were washed in IP buffer and heated in sample buffer before SDS-PAGE and immunoblotting.

Two-hybrid Assay—A bait fusion protein between USP10 and the LexA DNA binding domain was expressed from the pBTM116 plasmid (11). COP1 bovine subunits fused to the Gal4 activation domain were expressed from the pAD-Gal4 strains. COP1 forms a complex with the ubiquitin protease Ubp3p that deubiquitates the COPII protein Sec23p (10). To identify additional substrates of the Ubp3p–Bre5p complex, we characterized another mutant strain from this screen and found that the viability of these cells could be rescued by the GLO3 gene (Fig. 1A). Both BRE5 and a truncated version of BRE5 encoding a protein deleted of its RNA-binding domain (BRE5 (1–400)) could complement the bre5Δ–glo3 mutant. In contrast, deletion of the NTF2-like domain (BRE5 (146–515)) responsible for the interaction with Ubp3p abolished the ability of Bre5p to rescue the viability of bre5Δ–glo3 mutant (Fig. 1B).

FIG. 3. β'-COP is a substrate of the Ubp3p–Bre5p complex. A, yeast extracts were prepared from wild-type cells, bre5Δ and ubp3Δ deletion mutants, and analyzed by SDS-PAGE and by Western blotting using specific antibodies against the indicated proteins. B, β'-COP degradation is proteasome-dependent. Wild-type CIM3 (YPH 499), cim3-1 (CMY 763), bre5Δ, and cim3-1 bre5Δ strains were grown in YPD at 23°C (Δ600 = 0.5) and then shifted to 37°C for 120 min (+) in the presence of 100 μg/ml cycloheximide. Yeast extracts were prepared and analyzed by SDS-PAGE and immunoblotting using an anti-β'-COP antibody. Loading was controlled using a nonspecific band (not shown). Arrow, nonspecific; *, monoubiquitinated β'-COP.

RESULTS AND DISCUSSION

BRE5 Genetically Interacts with the GLO3 Gene—Screening for mutations that induce synthetic lethality upon disruption of the BRE5 gene recently led to the identification of a genetic interaction between BRE5 and SFB3, a gene encoding for a Sec24-like component of the COPII complex. Indeed Bre5p forms a complex with the ubiquitin protease Ubp3p that deubiquitates the COPII protein Sec23p (10). To identify additional substrates of the Ubp3p–Bre5p complex, we characterized another mutant strain from this screen and found that the viability of these cells could be rescued by the GLO3 gene (Fig. 1A). Both BRE5 and a truncated version of BRE5 encoding a protein deleted of its RNA-binding domain (BRE5 (1–400)) could complement the bre5Δ–glo3 mutant. In contrast, deletion of the NTF2-like domain (BRE5 (146–515)) responsible for the interaction with Ubp3p abolished the ability of Bre5p to rescue the viability of bre5Δ–glo3 mutant (Fig. 1B).
ER Retrieval Defect in Cells Disrupted for BRE5—Glo3p is one of the GTPase-activating proteins (GAP) for the small GTPase ADP-ribosylation factor 1 (Arf1p). Arf1p and the heptameric coatomer complex (α, β, β′, γ, δ, ε, ε′-COP) form the coat protein complex I (COP) involved in retrograde transport within the Golgi apparatus (16) and from the Golgi to the ER (13, 17). The GDP-bound form of Arf1p is likely recruited on Golgi membranes by the transmembrane protein p23 prior to budding, Arf1p-dependent GTP hydrolysis initiates vesicle uncoating (21, 22), and Arf1p-GDP as well as p23/p24 proteins are incorporated into the COPI complex and therefore affects coatomer assembly and ER retrieval.

The genetic interaction between GLO3 and BRE5 led us to analyze whether deletion of BRE5 could induce defects in retrograde transport from the Golgi to the ER. For this purpose, we used a previously described in vivo assay to monitor ER retrieval of dilsyline-tagged proteins in yeast (13). Indeed, it has been clearly shown that dilsyline signals confer a COPI-mediated localization to the ER (13). This assay is based on the use of MATa cells deleted from the endogenous α-factor receptor STE2 gene but expressing a fusion protein between Ste2p and WBP1. The dilsyline retrieval motif of WBP1 confers an ER retrieval defect when retrograde transport from Golgi to ER is defective, Ste2p-WBP1 (WT) cells mate (Fig. 2A). However, when retrograde transport from Golgi to ER is defective, Ste2p-WBP1 is mislocalized to the cell surface, allowing mating to occur. In particular, a ts mutation in the α subunit of the coatomer (ret1.1) was able to restore mating (Fig. 2A) as described previously (13). Deletion of BRE5 led to a partial rescue of the ability to mate indicating that lack of Bre5p causes a defect in ER retrieval (Fig. 2A).

Mutations affecting ER retrieval of dilsyline-tagged proteins fall into two classes distinguished by their ability to alter, or not, binding of coatomer to dilsyline motifs in vitro (13). To better characterize the effects of BRE5 deletion on retrograde transport, lysates from BRE5 and bre5Δ cells were incubated with purified recombinant fusion proteins between GST and WBP1 peptides presenting dilsyline (GST-WBP1) or diserine motifs (GST-WBP1-SS) and pulled down with glutathione beads as described previously (17). Bound proteins were revealed by immunoblotting with antibodies to β′-COP and δ-COP, two subunits of COPI coatomer (Fig. 2B). Neither β′-COP nor δ-COP were retained on GST-WBP1-SS beads. Although both subunits were bound to GST-WBP1 beads, the efficiency of recruitment was affected upon BRE5 deletion. A precise quantification performed on three independent experiments indicated that the amount of bound β′-COP and δ-COP from bre5Δ cells extracts corresponded to 50 and 65%, respectively, of material bound using WT extracts. This result suggests that coatomer assembly is likely altered in bre5Δ cells thus inducing an ER retrieval defect. Consistently, cells disrupted for BRE5 encoding gene have been shown to be hyper-sensitive to brefeldin A (25), an inhibitor of nucleotide exchange on the ADP-ribosylation factor, which blocks coatomer binding to membranes (26).
We recently reported that absence of the Bre5p-Ubp3p complex induces an accumulation of monoubiquitinated Sec23p that facilitates its subsequent polyubiquitination and degradation by the 26S proteasome (10). To address a possible role of the Bre5p-Ubp3p complex in β'-COP turnover, we first measured β'-COP stability in the presence of cycloheximide and found that the half-life of this protein is shorter in bre5Δ cells than in WT cells (data not shown). Since polyubiquitination rather than modification with a single ubiquitin triggers proteins for recognition and degradation by the 26S proteasome, modification of β'-COP was analyzed in cin3-1 thermosensitive mutants defective for the proteasome activity (28). A 2-h shift from 23 to 37 °C in the presence of cycloheximide led to a rapid degradation of β'-COP in wild-type cells (CIM3) and bre5Δ cells, whereas it was stabilized in cin3-1 strains (Fig. 3B) indicating that degradation of β'-COP depends on the proteasome activity. Interestingly, deletion of BRE5 in cin3-1 cells led to an accumulation at 37 °C of both unmodified and high molecular weight forms of β'-COP likely corresponding to polyubiquitinated species of β'-COP (Fig. 3B). These results indicate that absence of a functional Ubp3p-Bre5p complex led to a faster polyubiquitination and degradation of β'-COP by the proteasome.

Mammalian β'-COP Interacts with USP10—G3BP1, one of the two human proteins similar to Bre5p, has been previously shown to interact with USP10 (11), a human ubiquitin protease sharing 46% similarity and 27% identity with Ubp3p mainly concentrated in the catalytic domain (Fig. 4A). Using a two-hybrid assay, we found that USP10 not only interacts with G3BP1 but also with G3BP2, the second human homologue of Bre5p (data not shown). Moreover, similarly to the Ubp3p-Bre5p complex, the interaction between USP10 and G3BP1 or G3BP2 is mediated by the NTF2-like domain of G3BPs (data not shown).

Yeast β'-COP is very similar to bovine β'-COP (43% identity, 64% similarity). To test whether Ubp3p-Bre5p and USP10-G3BP complexes could exert their deubiquitinating activity on similar substrates, we tested whether human USP10 could regulate Golgi to ER transport by acting on the function of both unmodified and high ubiquitinated G3BPs (data not shown).

Deubiquitination of β'-COP by the Ubp3p-Bre5p Complex

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