Rad23 Provides a Link between the Png1 Deglycosylating Enzyme and the 26 S Proteasome in Yeast*

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In addition to a role in DNA repair events in yeast, several lines of evidence indicate that the Rad23 protein (Rad23p) may regulate the activity of the 26 S proteasome. We report evidence that a de-N-glycosylating enzyme, Png1p, may be involved in the proteasomal degradation pathway via its binding to Rad23p. Interaction of Rad23p and Png1p was first detected by two-hybrid screening, and this interaction in vivo was confirmed by biochemical analyses. The Png1p-Rad23p complex was shown to be distinct from the well established DNA repair complex, Rad4p-Rad23p. We propose a model in which Rad23p functions as an escort protein to link the 26 S proteasome with proteins such as Rad4p or Png1p to regulate their cellular activities.

Proteins that transit through the secretory pathway are subjected to a quality control system (1) in the endoplasmic reticulum (ER) that recognizes aberrantly folded proteins/glycoproteins. It has been shown that in some cases these misfolded and/or unfolded proteins are degraded by ER-associated degradation mechanisms, which involves retrograde transfer of proteins from the ER to the cytosol followed by degradation by the proteasome (2–7). Previously, we described PNG1, a gene encoding a cytoplasmic deglycosylating enzyme, peptide-N-glycanase (PNGase), that is evolutionarily conserved throughout eukaryotes (8). It has been suggested that this enzyme activity is linked to a proteasomal degradation pathway and has a role in efficient degradation of glycoproteins by the proteasome (8–13). This would be achieved by removing bulky N-linked glycans from misfolded glycoproteins that are translocated from the lumen of the ER into the cytosol for degradation. However, a physical link between the proteasome and this deglycosylating enzyme has not yet been described.

Rad23p is known to have a pivotal role in nucleotide excision repair (14–16). Yeast Rad23p stoichiometrically forms a complex with Rad4p to form nucleotide excision repair factor 2 (NEF2). Unlike other NER proteins, the biochemical functions of NEF2 still remain largely unknown. However, NEF2 was recently shown to bind specifically to damaged DNA in an ATP-independent manner (17–19). While the absence of Rad4p is linked to a proteasomal degradation pathway and has a role in efficient degradation of glycoproteins by the proteasome (20–23).

One structural feature of Rad23p is that it contains a ubiquitin-like domain (UBL) at the N terminus that can bind to the 26 S proteasome (24). This association appears to be important for DNA repair (24–26). In addition, Rad23p was shown to have an overlapping function with Rpn10p, a 19 S proteasome subunit that is known to be a multiubiquitin chain-binding receptor for the proteasome (27). It has been suggested that the Rad23p is a negative regulator of multiubiquitin chain assembly (28). However, thus far, the link between DNA repair and proteasome degradation with respect to Rad23p function remains elusive.

We report here a finding that the deglycosylation enzyme, Png1p, exists as a high molecular weight complex with Rad23p. The Png1p-Rad23p complex was found to be distinct from the well established DNA repair complex, Rad4p-Rad23p (NEF2). In addition, we report that the Png1p-Rad23p complex interacts with the 26 S proteasome. These findings led us to hypothesize that Rad23p may function to link the 26 S proteasome with other proteins, such as Rad4p or Png1p. This “escort” property of Rad23p may explain the complex effect of Rad23p-proteasome associations in a variety of cellular processes, including deglycosylation of glycoproteins slated for degradation.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Media**—The yeast strains used in this study were the following: BY4742 (MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0; Ref. 29); Research Genetic strain 10278 (BY4742 rad23Δ::KanMX4); LACO (MATa ade2 his3 leu2 trp1 lys2:lexAop-HIS3 URA3::lexAop-lacZ; Ref. 29); AMR70 (MATa ade2 leu2 his3 trp1 URA3::lexAop-HIS3; Ref. 29); W303–1a (MATa ade2–1 her3–11,15 leu2–3,112 trp1–1 ura3–1 can1–100); TSY190 (W303–1a rad23Δ::URA3 png1Δ::his5Δ [pombe] FOAΔ); and TSY195 (TSY190 RPT1-GFP-HA::URA3::HIS3). TSY190 was prepared by crossing TSY146 (W303–1a rad23Δ::URA3 png1Δ::his5Δ [pombe]; Ref. 8) and MGSC101 (W303–1b [W303–1a MATa] rad23Δ::URA3; Ref. 30; kindly provided by Dr. Jaap Brouwer, Leiden) followed by isolating haploid segregants of the appropriate genotype and selection of 5-fluoroorotic acid (FOA)-resistant cells on FOA plates. TSY195 was prepared by transforming TSY190 with XhoI/NcoI digests of pBS-CIM5-GFP-HA-HU (Ref. 31; kindly provided by Dr. Cordula Enenkel, Humboldt Universität, Berlin) and isolating UraΔ HisΔ transformants. Correct integration of the transformant was confirmed by colony PCR as well as the expression of Rpt1-GFP-HA by Western blotting using mouse anti-HA antibody (12CA5). Standard yeast media and genetic techniques were used (32–34).

**PNGase Activity Assay**—PNGase activity was assayed in yeast sates using fetuin-derived asialoglycopeptide I (1′(4′′CH3)2Leu-Asn[GlcnAc-Man,Gal]3-Aep-Ser-Arg) as described previously (35, 36). Radioactivity was monitored on a PhosphorImager (Molecular Dynamics, Inc.) and quantitated using ImageQuant (version 1.2). One unit was defined as the amount of enzyme that catalyzed hydrolysis of 1 μmol of fetuin-derived asialoglycopeptide I h.

**Construction of Plasmids**—DNA manipulations were performed ac-
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Table I

| Plasmids used in this study | Description | Source |
|----------------------------|-------------|--------|
| pBTM116-PNG1               | PAH1::LexA-PNG1 TRP1 2µ | This study |
| pBTM116-ADE2-PNG1          | PAH1::LexA-PNG1 TRP1 ADE2 2µ | This study |
| pGAD42 RAD23               | PAH1::Gal4BD RAD23 LEU2 2µ | This study |
| pGAD42 RAD23(−77–398)      | PAH1::Gal4BD RAD23−77−398 LEU2 2µ | This study |
| pGAD42 RAD23(−146–398)     | PAH1::Gal4BD RAD23−146−398 LEU2 2µ | This study |
| pGAD42 RAD23(−253–398)     | PAH1::Gal4BD RAD23−253−398 LEU2 2µ | This study |
| pGAD42 RAD23(−1–354)       | PAH1::Gal4BD RAD23−1−354 LEU2 2µ | This study |
| pGAD42 RAD23(−1–317)       | PAH1::Gal4BD RAD23−1−317 LEU2 2µ | This study |
| pGAD42 RAD23(−1–252)       | PAH1::Gal4BD RAD23−1−252 LEU2 2µ | This study |
| pGAD42 RAD23(−77–398)      | PAH1::Gal4BD RAD23−77−398 LEU2 2µ | This study |
| pGAD42 RAD23(−1–354)       | PAH1::Gal4BD RAD23−1−354 LEU2 2µ | This study |
| pRS314                      | TRP1 CEN   | Ref. 38 |
| pRS314 GALI PNG1His6        | TRP1 CEN   | Ref. 40 |
| pRS314 GALI PNG1His6        | LEU2 2µ    | Ref. 56 |
| pESC TRP RAD4(Myc)         | GALI::PNG1 His6 GALI CEN | This study |
|                            | TRP1 CEN   | This study |
|                            | LEU2 2µ    | This study |
|                            | GALI::RAD4-Myc TRP1 2µ | This study |

*All plasmids used contain the Amp® gene.

Plasmids used in this study indicated a gift from Dr. Kiran Madura. The yeast genes used in this study were isolated from the genomic DNA of W303-1a by polymerase chain reaction using Vent DNA polymerase (New England Biolabs). For isolation of the PNG1 gene, the following primers were used: 5′-AAAAAGATTC-ATGGAAGAAGTATAGCAGAAAAA-3′ (5′-primer) and 5′-AAAAACTCGAG-CTATTTACCATCCTCCCCACGC-3′ (3′-primer). The amplified fragments were digested with EcoRI and cloned into pRS314 (38). The pBTM116-PNG1 was subsequently digested with MluI and cloned into MluI sites of pBTM116-ADE2 (39) to give rise to pBTM116-ADE2-PNG1. pRD53-PNG1His6 was prepared by amplifying the PNG1 gene using another 3′-primer, 5′-AAAAACCTGAGCAGTTAGGTGTGTTGTTGTTTACCATCCTCCCCACGC-3′ (3′-primer) and the amplified DNA was digested with XhoI and cloned into the PNG1-containing fragment into NotI sites of pRS314 (40). pESC-TRP-RAD4(Myc) was constructed by co-transformation of pRD53-PNG1His6 with NotI sites of pRS314 and the amplified DNA was digested with EcoRI and the amplified DNA was digested with XhoI and cloned into NotI sites of pRS314. The genomic DNA of W303-1a was used for isolation of the PNG1 gene using another 3′-primer. The Trp+ colonies were isolated, and the transformants bearing plasmids with the correct insert were identified by colony polymerase chain reaction. The expression of the Rad4-Myc protein was further confirmed by Western blotting. The immunoprecipitates were washed twice with buffer A (50 mM Hapes-NaOH, pH 7.5, 150 mM NaCl, 1% Triton X-100), and fractions of 0.9 ml were collected. Fractions were assayed for PNGase activity. For protein determination, 0.3 ml of fractions were precipitated with 10% trichloroacetic acid, and the tagged protein was visualized by Western blotting.

Western Blot Analysis—Western blot analysis was carried out as described (8) using a 1:10 dilution of mouse anti-HA (tissue culture supernatant; 12CA5) or 1:1000 dilution of rabbit or mouse anti-Myc (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) rabbit or mouse anti-His6 (Santa Cruz Biotechnology), or mouse anti-FLAG antibody (M2; Sigma) followed by a 1:2000 dilution with the anti-rabbit or mouse IgG horse-radish peroxidase-conjugated secondary antibody (Roche Molecular Biochemicals). 10% SDS-PAGE gels were used, and gels were visualized using chemiluminescence (KPL) after exposure to medical x-ray film (Fuji).

Immunoprecipitation—Immunoprecipitation experiments were carried out as previously described (27). Briefly, cell extracts were prepared in lysis buffer (20 mM Hepes-KOH, pH 7.5, 100 mM potassium acetate, 5 mM EDTA, 10% glycerol) including various protease inhibitors as described above, and equal amounts of extract (2 mg of total protein) were incubated with 20 µl of protein G-agarose with and without respective anti-Tag antibodies (rabbit anti-Myc (Santa Cruz Biotechnology); rabbit anti-HA (Santa Cruz Biotechnology); rabbit anti-FLAG (Sigma); Sigma), 1,250 in dilution) and incubated overnight at 4 °C. The immunoprecipitates were washed twice with buffer A (50 mM Hepes-NaOH, pH 7.5, 5 mM EDTA, 150 mM NaCl, 1% Triton X-100), dissolved in 20 µl of sample buffer, and analyzed by 10% SDS-PAGE. The results of the sequencing constructs were confirmed.

**Co-immunoprecipitation**—Immunoprecipitation experiments were carried out as previously described (27). Briefly, cell extracts were prepared in lysis buffer (20 mM Hepes-KOH, pH 7.5, 100 mM potassium acetate, 5 mM EDTA, 10% glycerol) including various protease inhibitors as described above, and equal amounts of extract (2 mg of total protein) were incubated with 20 µl of protein G-agarose with and without respective anti-Tag antibodies (rabbit anti-Myc (Santa Cruz Biotechnology); rabbit anti-HA (Santa Cruz Biotechnology); rabbit anti-FLAG (Sigma); Sigma), 1,250 in dilution) and incubated overnight at 4 °C. The immunoprecipitates were washed twice with buffer A (50 mM Hepes-NaOH, pH 7.5, 5 mM EDTA, 150 mM NaCl, 1% Triton X-100), dissolved in 20 µl of sample buffer, and analyzed by 10% SDS-PAGE. The results of the sequencing constructs were confirmed.

**UV Sensitivity Analysis**—For qualitative UV sensitivity analysis, cells were grown in synthetic dextrose (SD) – Leu – Ura + galactose or – Leu – Ura + glucose medium to saturation. Cells were further cultured in the presence of 0.1 mM CuSO4 for another 2 h, and the cell density was normalized to A600 = 1.0 (5 × 106 cells/ml). Cells were taken from the aliquot with Q-tips and streaked as a single line onto the respective plate (– Leu – Ura + galactose + 0.1 mM CuSO4 or – Leu – Ura + glucose + 0.1 mM CuSO4). After these plates were covered with a glass plate, they were placed 20 cm distant from the germicidal UV light (254 nm; Sylvania). Then the glass plate was slid parallel to the line of cells so that the cells were exposed to UV for different times from 0 to 8 s. The plate was then incubated at 25 °C in the dark for 4 days. If a cell line had UV sensitivity, cells would not be expected to grow all the cells were exposed to UV for different times from 0 to 8 s. The plate was then incubated at 25 °C in the dark for 4 days. If a cell line had UV sensitivity, cells would not be expected to grow all the way across the line when exposed to UV. In contrast, wild-type cells would be expected to exhibit growth across the entire line even when they were exposed to the maximum dose of UV.

For quantitative assay for UV treatment, cells were grown in the respective medium (SD – Leu – Ura + glucose or SD – Leu – Ura + galactose) overnight, and after 0.1 mM CuSO4 was added, they were cultured for 2 h, plated on YPAD at appropriate dilutions, and then...
exposed to 254-nm UV light using a UV cross-linker (UV Statalinker model 1800; Stratagene) at given doses. Cells were plated in triplicate and incubated at 25 °C in the dark for 3 days, and the number of surviving colonies were counted.

RESULTS

Png1p Forms a High Molecular Weight Complex in Yeast Cytosol—Our initial assumption that Png1p might be a part of a multiprotein complex in yeast was based on the results of gel filtration analysis of a cell-free yeast extract. As shown in Fig. 1, PNGase activity measurement of the gel filtration fractions showed distinct differences between the elution position of Png1p in the yeast cytosol and that of Png1p expressed in *Escherichia coli*. While the bacterially expressed protein showed the expected molecular mass of a monomer form (~45 kDa), the yeast cytosol protein had a much higher molecular mass (estimated to be ~200 kDa). This result suggested that in the yeast cytosol Png1p may bind to other proteins to form a high molecular weight protein complex.

**Rad23p Specifically Binds to Png1p**—The observation described above led us to carry out two-hybrid library screening with PNG1 as a bait to search for possible Png1p-binding proteins. Upon screening 2.2 × 10^9 transformants in yeast genomic library, we recovered two distinct plasmids that showed a reproducible His“β-galactosidase” phenotype in a yeast genetic screen. These plasmids showed a specific interaction with Png1p through the lexA motif (lexA-PNG1)-specific manner. Sequencing of inserts recovered from these two plasmids showed that they consisted of two different Rad23p fragments (1.5 × 50 cm), and fractions of 0.9 ml were collected and monitored for PNGase activity. When only Rad23p was overexpressed, a higher peak was observed, which corresponded to the higher molecular mass peak. This result suggested that the high molecular weight complex containing Png1p might also contain Rad23p.

To provide direct evidence for interaction of Rad23p and Png1p, FLAG-tagged Rad23p was expressed under an inducible CUP1 promoter. This protein construct was used to show interaction of Rad23p with the 26 S proteasome as well as with Rad4p (24, 27). Png1p is known to be present in extremely low abundance in cells under normal experimental conditions (8), while Rad23p is relatively abundant (17). Therefore, only a minute fraction of Rad23p was expected to be bound to Png1p; this would make it difficult to observe co-migration of Png1p and Rad23p. For this reason, Png1p was overexpressed using an inducible GAL1 promoter in a *pnlΔ rad23Δ* strain for this experiment (TSY190; for details of genotypes of strains, see “Experimental Procedures”). When both proteins were expressed, Png1p exhibited two peaks of activity, one corresponding to the higher molecular mass peak and another to a lower apparent mass. This result suggested that the high molecular weight complex containing Png1p might also contain Rad23p.

When only Png1p was overexpressed in the absence of Rad23p (rad23Δ), the higher molecular weight peak was not detectable (data not shown; see also Fig. 3). When only Rad23p was overexpressed in the absence of Png1p, FLAG-Rad23p was shown to co-migrate with the first peak and one similar to bacterially expressed Png1p (Fig. 4A). FLAG-Rad23p was shown to co-migrate with the first peak and one similar to bacterially expressed Png1p (Fig. 4A).
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Png1p (*png1Δ*), the elution position of Rad23p was shifted to a lower molecular weight (Fig. 4C), suggesting that when Png1p was expressed, all of Rad23p detected co-migrated with Png1p. The C Terminus of Rad23p Is Critical for Its Binding to Png1p—Having biochemical evidence that Png1p binds to Rad23p in yeast cytosol, we determined which domain of Rad23p was involved in interaction with Png1p using the two-hybrid assay. Deletion constructs of GAL4 activation domain-Rad23p were tested for binding against the first peak (fractions 44–50) of PNGase activity. C, yeast cytosol was prepared from TSY190 bearing pCS13 and pRD53 as a control. Western blotting using mouse anti-FLAG antibody to detect FLAG-Rad23p was detected in fractions corresponding to the first peak (fractions 44–50) of PNGase activity. The C-terminal domain of Rad23p, Rpt1p, was carried out. The His6-tagged Png1p was expressed, all of Rad23p detected co-migrated with Png1p. The assumption made was that overexpression of Png1p under the experimental conditions described above caused moderate sensitivity (10-fold decrease in survival). For this experiment, we used *png1Δ* rad23Δ cells. Png1p was expressed overnight using an inducible GALI promoter, and then FLAGRAD23P was transiently expressed for 2 h using the CUP1 promoter. First, a qualitative test was performed to check the UV sensitivity of these cells. As shown in Fig. 7A, under the experimental conditions described above, cells overexpressing Png1p exhibited moderate UV sensitivity (compare sample 2 with sample 1). This level of sensitivity was not seen when the same experiment was carried out without induction of Png1p (compare sample 2C and sample 1C), indicating that this effect was Png1p-dependent. That a comparable amount of Rad23p was expressed in samples 1 and 2 was confirmed by Western blotting, excluding the possibility that the observed UV sensitivity was due to the amount of Rad23p expressed in cells (data not shown). In contrast to the above experiment, when we expressed FLAG-Rad23p overnight, we observed no apparent UV sensitivity in PNG1-overexpressing cells, suggesting that the observed UV sensitivity is a transient effect and cells can make enough Rad4p-Rad23p complex at stationary stage even in the presence of large amount of Png1p (data not shown). With this assay, *png1Δ* cells exhibited no apparent UV sensitivity, implying that PNG1 itself does not have a role in nucleotide excision repair (data not shown).

Using a more quantitative UV sensitivity assay, we confirmed that overexpression of Png1p under the experimental conditions described above caused moderate sensitivity (10-fold decrease in survival) (Fig. 7B). This relatively moderate effect may be caused by a residual amount of NEF2 (Rad4p-Rad23p complex) formation. As expected when Png1p alone (without Rad23p) was induced, the sensitivity was very great (1,000-fold decrease in survival).
Fig. 5. Co-immunoprecipitation of Png1p with 26 S proteasome. TSY195 (W303–1a rad23Δ::URA3 png1Δ::his5 (pombe) FOA<sup>b</sup> RPT1-GFP-HA:URA3::HIS3) bearing pRS314::PG1-His<sub>6</sub> and either pCS13 (P<sub>GAL1</sub>::FLAG-RAD23) or YEp351 (control) was cultured in SD–Trp–Leu–galactose containing 0.1 mM CuSO<sub>4</sub> overnight. Yeast extract was prepared, and equal amounts of protein were incubated with antibody (Ab) against Hist<sub>6</sub> (lanes 1 and 5) or HA (lanes 3 and 7). Then protein G-agarose was added for immunoprecipitation (IP). Following SDS-PAGE, the immunoprecipitated proteins were detected by immunoblotting using anti-HA (lanes 1, 2, 5, and 6) or anti-Hist<sub>6</sub> (lanes 3, 4, 7, and 8). For controls, incubation of protein G-agarose without antibody was also carried out (lanes 2, 4, 6, and 8). In some experiments, a background level of proteins was detected in these control lanes, which is most likely due to antibody-independent precipitation of proteins. For comparison of protein amounts between cells bearing Rad23p (pCS13; lane 9) and control (YEp351; lane 10), one-tenth of the extract used for immunoprecipitation was analyzed by SDS-PAGE followed by Western blotting using anti-Hist<sub>6</sub> as a probe to detect Png1-Hist<sub>6</sub>.p

### Discussion

The de-N-glycosylation process catalyzed by PNGase has been proposed to be involved in proteasomal degradation of misfolded glycoproteins following their transfer from the ER to the cytosol (9–13). Earlier we reported that *S. cerevisiae* has a soluble PNGase activity that is very similar to the soluble PNGase found in higher eukaryotes (36). Subsequently, we isolated the gene encoding this enzyme, PNG1, by isolating a mutant that is defective in PNGase activity and then mapping of the locus responsible for the loss of this activity (8). Comparison of the protein sequence of yeast Png1p with a number of sequences in other eukaryotic data bases revealed that this enzyme is highly conserved, suggesting that it was functionally important in all eukaryotes.

Following the finding that the apparent molecular mass of PNGase detected in the cytosol by an enzyme assay was much greater than its calculated mass of 42.5 kDa, we carried out a two-hybrid analysis to identify yeast proteins that interact with Png1p. By this screening, two distinct candidates were isolated, both of which encoded a part of Rad23p. In a rad23Δ strain, we observed a dramatic decrease in elution position of the yeast Png1p to a position similar to that of bacterially expressed Png1p. These results supported the idea that the formation of a higher molecular weight complex of Png1p involved Rad23p. This was further confirmed by showing that epitope-tagged Rad23p co-migrated with Png1p during gel filtration analysis of the yeast cytosol.

In this experiment, using Png1p-overexpressed cells, the finding of two distinct peaks of PNGase activity may be due to the fact that a fraction of the large amount of Png1p produced under these conditions was bound to Rad23p. The calculated apparent molecular mass of this complex is ~200 kDa, which is approximately twice as large as the expected molecular mass of a 1:1 stoichiometric Rad23p-Png1p complex. This large molecular mass could be due to the presence of more than one molecule of each of the subunits or a change in conformation of the proteins in a complex. Alternatively, other proteins may also be present in the complex; currently, we are investigating this possibility.

Interaction studies of Png1p using deletion constructs of Rad23p provided two important conclusions. First, the N-terminal UbL, which is important for Rad23p to interact with the proteasome (24), was found not to be required for Rad23p binding to Png1p. This finding led us to hypothesize that a physical interaction of Png1p with the 26 S proteasome might be mediated by Rad23p. This hypothesis was confirmed by a co-immunoprecipitation analysis that utilized an antibody to an epitope-tagged subunit of the 26 S proteasome (Rpt1p) and His<sub>6</sub>-tagged Png1p. This interaction between Png1p with the 26 S proteasome was Rad23p-dependent, which is consistent with the idea of Rad23p being an “escort protein” to connect Png1p and the 26 S proteasome. It has been reported that there is a so-called “membrane-associated form” of PNGase, which can be precipitated by ultracentrifugation and has enzymatic

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**TABLE II**

Mapping of interaction domains of the Rad23p to Png1p assessed by yeast two-hybrid assay

| pGAD424 constructs | Growth on SD minus histidine plate | β-Galactosidase assay* |
|--------------------|----------------------------------|-----------------------|
| Rad23p-(1–398) (full-length) | + | + |
| Rad23p-(77–398) (minus N terminus) | + | + |
| Rad23p-(186–398) | + | + |
| Rad23p-(253–398) | + | + |
| Rad23p-(22–317) | + | + |
| Rad23p-(22–354) (minus C terminus) | + | + |

*No color change on β-galactosidase assay; number of plus signs indicates the intensity of color on β-galactosidase assay.

*This plasmid was originally isolated from the pGAD library.
The biological significance of the interaction of the 26 S proteasome with Rad23p still remains to be understood. Now it is evident that there are at least two distinct complexes involving the proteasome and Rad23p (Fig. 8). This finding may to some extent explain the complex role of Rad23p-proteasome interactions. We hope that in the future it will be possible to dissect the specific functions of proteasome or Rad23p in each distinct complex. In this regard, it is of interest that a number of domain-specific Rad23p-binding proteins, other than the nucleotide repair proteins, have been identified in mammalian cells (46–48). These studies, combined with our results, lead us to speculate that such domain-specific interactions may allow Rad23p to connect a proteasome protein through its N terminus to other proteins at its C terminus. This link might be necessary to modulate functions of various binding proteins. Most intriguing is the finding that a human homologue of Dsk2p, a Rad23p-like protein, has recently been shown to provide a link between the proteasome and ubiquitin ligase (49). Thus, a general feature of Ubl-containing proteins would be to link proteins to the proteasome in order to control or modulate the function of these proteins.

While the precise biological function of Png1p is still unclear, it is important to note that this enzyme has been proposed to be involved in proteasome degradation of misfolded glycoproteins in mammalian cells (9, 10). We have previously shown that glycopeptides exported from the ER to the cytosol in yeast are subsequently deglycosylated by the activity of Png1p (36). However, because of the inefficient uptake of proteasome inhibitors (50), it has not been possible to observe an accumulation of de-N-glycosylated protein intermediates in yeast. Interestingly, biochemically purified PNGase from mammalian cells, as well as the purified bacterially expressed yeast Png1p, do not act on intact glycoprotein substrates in vitro (8, 35). As discussed earlier (8), these observations may be relevant to the fact that the 20 S catalytic proteasome subcomplex can only act efficiently on small peptide substrates in vitro, and it has been proposed that the function of the 19 S ATPases is to unwind protein substrates prior to their degradation by the 20 S proteolytic complex (51, 52). Such “protein-unfolding” molecular chaperone activities of 19 S subunits have been recently reported (53, 54). Therefore, the association of Png1p with the 26
S proteasome would produce a complex in which de-N-glycosylation and proteolysis of unwound glycoprotein substrates could be accomplished in an efficient manner. Our findings indicate that the highly conserved enzyme, Png1p, is a part of the proteasomal degradation machinery. Further studies should reveal its precise role in the degradation of misfolded glycoproteins by this pathway.

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