The homeostatic abundance of the proteasome in *Saccharomyces cerevisiae* is controlled by a feedback circuit in which transcriptional activator Rpn4 up-regulates the proteasome genes and is destroyed by the assembled, active proteasome. Remarkably, the degradation of Rpn4 can be mediated by two independent pathways. One pathway is independent of ubiquitin, whereas the other involves ubiquitination on internal lysines. In the present study, we investigated the mechanism underlying the ubiquitin-dependent degradation of Rpn4. We demonstrated, through *in vivo* and *in vitro* assays, that Rpn4 is a physiological substrate of the Ubr2 ubiquitin ligase, which was originally identified as a sequence homolog of Ubr1, the E3 component of the N-end rule pathway. The ubiquitin-conjugating enzyme Rad6, which directly interacts with Ubr2, is also required for the ubiquitin-dependent degradation of Rpn4. Furthermore, we showed that deletion of *UBR2* exhibited a strong synthetic growth defect with a mutation in the Rpt1 proteasome subunit when Rpn4 was overexpressed. This study not only identified the ubiquitination apparatus for Rpn4 but also unveiled the first physiological substrate of Ubr2. The biological significance of Ubr2-mediated degradation of Rpn4 is also discussed.

The ubiquitin (Ub) proteasome system is the primary intracellular machinery responsible for elimination of abnormal proteins and selective destruction of regulatory proteins involved in a wide variety of cellular processes (1–3). Ubiquitination of a protein substrate is a consecutive process involving multiple enzymes (4). Ub is first activated by the Ub-activating enzyme (E1), forming a thioester between the C-terminal carboxyl group of Ub and a specific cysteine of the E1. The Ub moiety of the E1–Ub thioester is thereafter transferred to one of the Ub-conjugating enzymes (E2). The Ub moiety of the E2–Ub thioester is conjugated via an isopeptide bond to the ε-amino group of a lysine residue of a substrate or a preceding Ub molecule conjugated to the substrate, the latter reaction resulting in a substrate-linked multi-Ub chain. Most E2s function in complex with one of the E3 enzymes or Ub ligases. A Ub ligase also denotes an E2-E3 complex. Ubiquitination of a specific substrate is regulated through modulation of its degradation signal and through control of the activity of a cognate E3 (4–9). The isopeptide bond between Ub and a substrate can be hydrolyzed by deubiquitinating enzymes, which provides yet another layer of regulation for substrate ubiquitination (10).

Most known E3s are grouped into two families (homology to E6-AP C terminus domain E3s and RING E3s) based on their catalytic modules and features of sequence and structure (4, 9). A homology to E6-AP C terminus-domain E3 can accept Ub moiety from an associated E2–Ub thioester, forming an E3–Ub thioester and acting as a proximal Ub donor to the substrate that it selects. By contrast, formation of thioesters between RING E3s and Ub has not been detected. The precise mechanism of RING E3-mediated ubiquitination remains speculative (11–15). The current consensus is that a RING E3 acts as an adaptor to optimize the orientation of the ubiquitination site of a substrate to the active site of a cognate E2, which allows the transfer of the Ub moiety from an E2–Ub thioester to the substrate. The *Saccharomyces cerevisiae* Ubr1, the E3 component of the N-end rule pathway, is one of the most extensively studied RING E3s (15–17). Recent studies showed that Ubr1 is conserved from yeast to humans (18). Moreover, numerous sequence homologs of Ubr1 have been revealed from the data bases, comprising a subfamily of RING E3s (18–20). However, the functions of these UBR E3s other than Ubr1 remain unknown.

Most substrates attached with a multi-ubiquitin chain are degraded by the 26 S proteasome, an ~2500-kDa self-compartmentalized multisubunit protease (21, 22). The 26 S proteasome consists of a barrel-shaped proteolytic core (the 20 S core) with four stacked rings of seven subunits each in an α7β7α7β7α7 configuration. The 20 S core possesses three types of catalytic activities, trypsin-like, chymotrypsin-like, and peptidylglutamyl peptide hydrolyzing activities, which are provided by three of the seven distinct β subunits. The proteolytic active sites are located inside the chamber of the 20 S core, which is capped at one or both ends by the 19 S regulatory particle (also known as cap or PA700). The 19 S regulatory particle is composed of at least 18 different subunits that are formed into two subcomplexes, the base and the lid (23–25). The base is in contact with the 20 S core and possesses six ATPase subunits (Rpt1–Rpt6) and two non-ATPase subunits (Rpn1 and Rpn2). The lid including nine non-ATPase subunits (Rpn3, Rpn5–Rpn9, Rpn11–Rpn13) is linked to the base partly via Rpn10, another non-ATPase subunit. The 19 S regulatory particle mediates the binding and unfolding of ubiquitinated substrates before their translocation into the cavity of the 20 S core for degradation (22). Recent reports demonstrated that the 19 S...
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regulatory particle, specifically the Rpn11 subunit, also possesses a deubiquitinating activity (26, 27).

An important aspect in regard to the regulation of the proteasome is how the proteasome genes are regulated. Biochemical analysis has shown that the proteasome subunits are stoichiometrically present in vitro, suggesting that the proteasome genes are coordinately regulated (23–25, 28). Recent studies revealed that Rpn4 (also named Son1 and Ufd5) is a transcriptional activator required for normal expression of the S. cerevisiae proteasome genes (29, 30). Furthermore, Rpn4 was found to be extremely short lived (t1/2 = 2 min) and degraded by the proteasome (30). These observations and subsequent reports demonstrated that the proteasome abundance is regulated by a negative feedback circuit in which Rpn4 up-regulates the proteasome genes and is destroyed by the proteasome (31, 32).

Regulated degradation of Rpn4 is apparently a key mechanism that controls proteasome homeostasis. Strikingly, the proteasomal degradation of Rpn4 can be mediated by two distinct pathways (33). One pathway involves ubiquitination on internal lysine, whereas the other is Ub-independent. The details of these two pathways, however, remain unexplored.

In the current work, we investigated the mechanism underlying the Ub-dependent degradation of Rpn4. We found that Rpn4 is a physiological substrate of the Ub2 Ub ligase, which was originally identified as a sequence homolog of Ubr1 (19). Furthermore, we demonstrated that the Ubr2-mediated degradation of Rpn4 is critical for cell growth when the proteasome activity is compromised.

EXPERIMENTAL PROCEDURES

Yeast Strains and Plasmids—The E3 deletion mutants derived from BY4741 (MATa his3Δ1 leu2Δ2 met15Δ0 ura3Δ0) were constructed by the Yeast Deletion Consortium (34) and obtained from Invitrogen. rsc5-1, apc11-13, and cdc53-1 mutants were generously provided by Drs. Jon Huibregtse, Tony Hunter, Mike Tyers, and William Tansey (35–37). The other strains used were: JD52 (MATa his3Δ200 leu2–3, 112 lys2–801 trplΔ36 ura3–52); JD53 (MATa his3Δ200 leu2–3, 112 lys2–801 trplΔ63 ura3–52); YXY78 (a rad6Δ Ura3 derivative of JD52); YXY228 (MATa his3Δ200 leu2–3, 112 lys2–801 trplΔ63 ura3–52 PRE1-Flag-6His:Yplac211, a segregant of the diploid produced by mating JD126 with ELY150 (Ref. 31)); YXY220 (MATa his3Δ200 leu2–3, 112 lys2–801 trplΔ63 ura3–52 PRE1-Flag-His6:Yplac211 rpnd4Δ LEU2 (Ref. 31)); YXY224 (a ubr2Δ HIS3 derivative of JD52); YXY267 (a ubr2Δ HIS3 derivative of JD53); YXY254 (a PRE1-Flag-His6:Yplac211 ubr2Δ); YXY255 (YXY254 a PRE1-Flag-His6:Yplac211 ubr2Δ°C); YXY274 (MATa cim5Δ1 his3Δ200 leu2Δ1 ura3–52 (Ref. 38)); YXY258 (MATa cim5Δ1 his3Δ200 leu2Δ1 ura3–52 (Ref. 38)); YXY258 (MATa cim5Δ1 his3Δ200 leu2Δ1 ura3–52 (Ref. 38)); YXY27 (MATa cim5Δ1 his3Δ200 leu2Δ1 ura3–52 (Ref. 38)); YXY27 (MATa cim5Δ1 his3Δ200 leu2Δ1 ura3–52 (Ref. 38)).

The low copy plasmid expressing C terminally FLAG-tagged Rpn411–229 from the FCP1 promoter was described previously (33). RS314CUP1RPN4a expressing C terminally HA-tagged Rpn4 from the FCP1 promoter was derived from RS314CUP1RPN4 (30).

RESULTS

Ub-dependent Degradation of Rpn4 Is Mediated by Ubr2—The contribution of the Ub system in Rpn4 degradation remained unclear until very recently when we discovered that Rpn4 can be degraded by two distinct pathways, either Ub-dependent or Ub-independent (30, 33). We demonstrated that the Ub-dependent degradation of Rpn4 can be inhibited by deletion of its N-terminal 10 amino acids and that the Ub-dependent degradation signal of Rpn4 is located in the N-terminal domain including residues 11–229 (33). To elucidate the mechanism underlying the Ub-dependent degradation of Rpn4, we wished to identify the cognate Ub ligase for Rpn4. We first tested whether the degradation of Rpn411–229 is mediated by one of the known E3s. A plasmid expressing C terminally FLAG-tagged Rpn411–229 from the FCP1 promoter was transformed into yeast mutants that either lack one of the non-essential E3s or are deficient in one of the essential E3s at a restrictive temperature. Immunoblotting with anti-FLAG antibody was used to compare the steady-state levels of Rpn411–229FLAG in these E3 mutant strains. Among the 15 E3 mutants tested, Rpn411–229FLAG was detected only in the ub2Delta mutant (Fig. 1A). Consistent with the immunoblotting results, pulse-chase analysis showed that Rpn411–229FLAG was stabilized in the ub2Delta mutant (Fig. 1B).

We then examined whether the Ub-dependent degradation of Rpn4 is dependent on Ubr2. A low copy plasmid expressing C terminally HA-tagged Rpn4 that lacks the first 10 residues (Rpn411–229HA) from the native RPN4 promoter was introduced into ub2Delta and a congeneric WT strain, respectively. Pulse-chase analysis was used to measure the turnover rates of Rpn411–229HA in these two strains. Although it was rapidly degraded in the WT strain, Rpn411–229HA was stabilized in the ub2Delta mutant (Fig. 1C and D). It is highly unlikely that deletion of the first 10 residues of Rpn4 drastically changed the protein structure and created a cryptic degradation signal for Ubr2, as this small deletion did not affect the transcriptional activity of Rpn4 (Fig. 4C, compare lanes 3 and 2). Moreover, the degradation of intact Rpn4 expressed from the native RPN4 promoter in a low copy vector was also slower in the ub2Delta mutant than in the WT strain (Fig. 1D and E). The incomplete stabilization of Rpn4 in ub2Delta indicates that Ubr2 is not involved in the Ub-independent degradation of Rpn4. Taken-
Fig. 1. Ub-dependent degradation of Rpn4 by Ubr2. A, immunoblotting analysis of C-terminally FLAG-tagged Rpn4<sub>11–229</sub> in E3 mutants. All E3 deletion mutants were derived from BY4741 (wild type). E3 mutants rplp0-1, aei11-13, and cdc53-1 were shifted to 37 °C 30 min before extract preparation. The asterisk marks a cross-reactive band with anti-FLAG antibody, which is just above the position of Rpn4<sub>11–229</sub>FLAG indicated by an arrow. F, FLAG-tagged, B, pulse-chase analysis of Rpn4<sub>11–229</sub>FLAG in WT (lanes 1–3) and ubr2Δ (lanes 4–6). The ubr2Δ mutant used in pulse-chase assays was derived from JD2 (wild type) by replacing the UBR2 open reading frame with HIS3 via a PCR-mediated approach. C, the stability of C-terminally HA-tagged Rpn4<sub>31–10</sub> in WT (lanes 1–3) and ubr2Δ (lanes 4–6) was measured by pulse-chase analysis. D, pulse-chase analysis was carried out to compare the degradation of C-terminally HA-tagged Rpn4 in WT (lanes 1–3) and ubr2Δ (lanes 4–6). E, quantitation of the data from C and D by PhosphorImager to show the decay of Rpn4<sub>31–10</sub>ha in WT (○) and ubr2Δ (●), and the decay of Rpn4ha in WT (□) and ubr2Δ (■).

Together, these results demonstrate that Ubr2 mediates the Ub-dependent degradation of Rpn4.

Rad6 is Required for the Ub-dependent Degradation of Rpn4—Ubr2 was originally identified as a sequence homolog of Ubr1, the RING E3 component of the N-end rule pathway in S. cerevisiae (15, 19). However, the physiological function of Ubr2 remained unknown. Early work using two-hybrid analysis showed that the basic residue-rich domain of Ubr2 interacts with Rad6 (Fig. 2A). These observations suggested that Rad6 may function in complex with Ubr2 in mediating the Ub-dependent degradation of Rpn4. To test this possibility, we compared the stability of Rpn4<sub>31–10</sub>ha in rad6Δ and WT cells by pulse-chase analysis. As shown in Fig. 2B, Rpn4<sub>31–10</sub>ha was indeed stabilized in the rad6Δ mutant, whereas it was rapidly degraded in the congenic WT strain. Thus, Rad6 is the E2 enzyme required for the Ub-dependent degradation of Rpn4.

In Vitro Ubiquitination of Rpn4 by Ubr2—To examine whether Ubr2 directly binds Rpn4, which is expected for a cognate Ub ligase of Rpn4, we performed pulldown assays with Rpn4<sub>11–10</sub> fused to GST. Yeast extracts containing N-terminally FLAG-tagged Ubr2 were incubated with agarose beads preloaded with GST-Rpn4<sub>11–10</sub> (GST-Rpn4<sub>11–10</sub> alone or GST-Rpn4<sub>11–10</sub> alone served as controls). (Rpn2 is a subunit of the 19 S particle.) Proteins retained by the GST fusions were resolved by SDS-PAGE and analyzed by immunoblotting with anti-FLAG antibody. As shown in Fig. 3A, Ubr2 specifically bound to GST-Rpn4<sub>31–10</sub>. Note that GST-Rpn4<sub>31–10</sub> retained Ubr2 as efficiently as GST-Rpn4 in the pulldown assay (data not shown), indicating that deletion of the N-terminal 10 residues of Rpn4 does not affect the Ubr2-Rpn4 interaction.

Taking advantage of the binding of Ubr2 to Rpn4 and the identification of Rad6 as the E2 enzyme for Rpn4<sub>31–10</sub> degradation, we set up a pulldown ubiquitination assay to examine whether Rpn4<sub>31–10</sub> could be ubiquitinated by Ubr2 in vitro (Fig. 3B). Specifically, agarose beads preloaded with GST-Rpn4<sub>31–10</sub> were incubated with extracts from either ubr2Δ cells (lane 2) or ubr2Δ cells overexpressing Ubr2 from the GAL1 promoter in a high copy plasmid (lanes 3 and 4). After a standard pulldown, the beads were further incubated with purified Rad6 and Uba1 in the presence (lanes 2 and 3) or absence (lane 4) of ubiquitin. The GST fusions were then separated by SDS-PAGE and subjected to immunoblotting with anti-GST antibody. Remarkably, a ladder of ubiquitinated species of GST-Rpn4<sub>31–10</sub> was formed in the presence of Ubr2 and ubiquitin (lane 3) but not in the absence of Ubr2 or ubiquitin (lanes 2 and 4). To confirm that the ubiquitination was specific to the lysine(s) on Rpn4<sub>31–10</sub>, we conducted a similar pulldown ubiquitination assay using a GST fusion with Rpn4<sub>31–10</sub>R instead of Rpn4<sub>31–10</sub> (Fig. 3A). It has been shown that the Ub-dependent degradation of Rpn4 requires one or more of the 11 N-terminal lysines of Rpn4. (Ly59 was already deleted in Rpn4<sub>31–10</sub>.) It has been shown that the Ub-dependent degradation of Rpn4 requires one or more of the 11 N-terminal lysines (33). Although its affinity to Ubr2 was not noticeably reduced as compared with Rpn4<sub>31–10</sub> (Fig. 3A, compare lanes 2 and 4), Rpn4<sub>31–10</sub>R was not ubiquitinated by Ubr2 (Fig. 3B, lane 1). These observations confirmed that the ubiquitina-
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**FIG. 3.** In vitro ubiquitination of Rpn4 by Ubr2. A, physical interaction of Ubr2 with Rpn4 through GST-Rpn4 pulldown assays were conducted as described in Fig. 2, except different GST fusions were used as indicated. B, in vitro ubiquitination of Rpn4 by Ubr2. Agarose beads pre-loaded with GST-Rpn4 (lanes 2–4) or GST-Rpn4 (lane 1) were incubated with extracts from ubr2Δ (lane 2) or ubr2Δ overexpressing Ubr2 from the GAL1 promoter on a high copy plasmid (lanes 1, 3, and 4). After a standard pulldown assay, the beads were further incubated with purified Rad6 and Uba1 in the presence (lanes 1–3) or absence of ubiquitin (lane 4) at 30°C for 30 min. The GST fusions were then separated by SDS-PAGE and analyzed by immunoblotting with anti-GST antibody. Non-ubiquitinated GST-Rpn4 or GST-Rpn4 (lanes 1) are indicated by an arrow. Ubiquitinated species of GST-Rpn4 (lanes 2) were marked by a half-square bracket.

nuation of GST-Rpn4 by Ubr2 is specific to the N-terminal lysine(s) of Rpn4. Taken together, these data (Figs. 1–3) allow us to conclude that Ubr2 is the cognate Ub ligase for Rpn4.

**Overexpression of Rpn4 Is Tolerable in ubr2Δ Cells—Deletion of UBR2 produced no noticeable phenotypes under normal conditions.** The finding of Ubr2 as the cognate Ub ligase of Rpn4 prompted us to investigate if the proteasome abundance is higher in ubr2Δ compared with WT cells. We deleted UBR2 from a strain that expressed C-terminally FLAG-His6-tagged Pre1 (Pre1ς), a subunit of the 20 S core, from its chromosomal locus. Immunoblotting analysis with anti-FLAG antibody demonstrated that the steady-state level of Pre1ς was only modestly higher in ubr2Δ than in the WT counterpart (data not shown). This observation is not entirely unexpected as Rpn4 is still degraded even with a lower turnover rate by the Ub-independent pathway in ubr2Δ. Overexpression of Rpn4 from the Pcup1 promoter on a low copy vector, however, clearly produced more Pre1ς in ubr2Δ compared with the WT strain (Fig. 4B, lanes 2 and 3). These observations indicate that the Ubr2-mediated degradation of Rpn4 plays a role in the regulation of proteasome expression.

We then tested whether overexpression of Rpn4 in ubr2Δ would lead to cell growth defects. A low copy plasmid expressing Rpn4 from the Pcup1 promoter was transformed into WT and ubr2Δ. Serial dilution assays were carried out to compare the growth of these transformants in the presence or absence of Cu2+ induction. As shown in Fig. 4A, overexpression of Rpn4 did not affect the growth rates of WT and ubr2Δ cells. The lack of phenotype of Rpn4 overexpression in ubr2Δ was not because of the degradation of Rpn4 by the Ub-independent pathway as ubr2Δ cells overexpressing Rpn4 did not exhibit any growth defects even though Rpn4 was stabilized in ubr2Δ (Fig. 1C) and data not shown). Interestingly, the transcriptional activity of Rpn4 was comparable with that of the full-length Rpn4 (Fig. 4C, compare lanes 2 and 3). Taken together, these results indicate that accumulation of Rpn4 and a higher abundance of proteasome can be tolerated under normal conditions.

**Deletion of UBR2 Exhibits a Synthetic Growth Defect with cim5-1 When Rpn4 Is Overexpressed—Our recent work and that of others demonstrated that deletion of RPN4 produces a synthetic growth defect with proteasome mutants (31, 32). We wondered whether overexpression of Rpn4 is also detrimental to cell growth when the proteasome activity is compromised. Moreover, we wished to examine if deletion of UBR2 could augment the effect of Rpn4 overexpression in this setting. To test this, we transformed a low copy plasmid expressing Rpn4 from the Pcup1, promoter into WT, cim5-1, and a cim5-1 ubr2Δ double mutant. cim5-1 is a temperature-sensitive mutant bearing a mutated Rpt1 subunit of the proteasome (38). Although overexpression of Rpn4 did not affect the WT cells, the growth of cim5-1 cells was slower when Rpn4 was overexpressed (Fig. 4A). Remarkably, the cim5-1 ubr2Δ double mutant was much more sensitive to Rpn4 overexpression than cim5-1. Indeed, a slower growth of the cim5-1 ubr2Δ transformants was readily observed even without Cu2+ induction. Thus, proteasome mutant cells are sensitive to Rpn4 overexpression, and deletions of UBR2 and the cim5-1 mutation produce a strong synthetic growth defect when Rpn4 is overexpressed. The toxicity of Rpn4 overexpression relied on the transcriptional activity of Rpn4 in that overexpression of Rpn4 (Fig. 4A), an Rpn4 mutant the transcriptional activity of which is abolished because of a Cys→Ala mutation at Cys-477 of the C2H2 DNA binding motif (Fig. 4C), did not impair the growth of the cim5-1 ubr2Δ double mutant (Fig. 4A).

It has been shown that Rpn4 is partially stabilized in cim5-1 at a permissive temperature (30°C) (31). To determine whether the synthetic effect of deletion of UBR2 and cim5-1 correlates with a slower degradation of Rpn4 in the cim5-1 ubr2Δ double mutant, we wanted to compare the turnover rates of Rpn4 in cim5-1 and the cim5-1 ubr2Δ double mutant. Because expression of Rpn4 from the induced Pcup1 promoter severely impaired the growth of the cim5-1 ubr2Δ double mutant, and Rpn4 produced a strong synthetic growth defect when Rpn4 is overexpressed. The toxicity of Rpn4 overexpression relied on the transcriptional activity of Rpn4 in that overexpression of Rpn4 (Fig. 4A), an Rpn4 mutant the transcriptional activity of which is abolished because of a Cys→Ala mutation at Cys-477 of the C2H2 DNA binding motif (Fig. 4C), did not impair the growth of the cim5-1 ubr2Δ double mutant (Fig. 4A).

**DISCUSSION**

The identification of Ubr2/Rad6 as the cognate Ub ligase for Rpn4 through in vivo and in vitro assays revealed for the first time the physiological function of Ubr2, which has been considered a member of the UBR E3s family (19). Although the overall sequence homology between Ubr2 and Ubr1 is statistically significant (~22% identity and ~46% similarity), and the featured structures including the RING finger and Rad6 bind-
FIG. 4. Deletion of UBR2 exhibits a synthetic growth defect with cim5-1 in the presence of overexpressed Rpn4. A, a low copy plasmid expressing Rpn4 from the P_CUP1 promoter was transformed into WT, ubr2Δ, cim5-1, and a cim5-1 ubr2Δ double mutant. An otherwise identical vector expressing Rpn4C447A was transformed into the cim5-1 ubr2Δ mutant as control. Serial dilution assays were used to compare the growth of the resulting transformants in the presence and absence of Cu²⁺ induction. B, immunoblotting analysis of C-terminally FLAG-His₆-tagged Pre1 expressed from its native chromosomal locus in a WT strain (lane 2) and a ubr2Δ mutant (lane 3). An untagged WT strain served as a negative control (lane 1). All strains overexpressed Rpn4 from the P_CUP1 promoter on a low copy vector. The upper panel is an immunoblot with anti-FLAG antibody to measure the steady-state levels of Pre1, whereas the lower panel is an immunoblot with anti-tubulin antibody (Serotec, Oxford, UK) serving as a loading control. The same amounts of extracts were used in these two immunoblots. C, Cys → Ala substitution at Cys-447 of the C₂H₂ motif abolished the transcriptional activity of Rpn4. An rpn4Δ strain (YXY210) that expressed C-terminally FLAG-His₆-tagged Pre1 from its chromosomal locus was transformed with a void vector (lane 5) or plasmids expressing Rpn4 (lane 2), Rpn4C447A (lane 3), and Rpn4C447A (lane 4), respectively, from the P_RPN4 native promoter in a low copy vector. The steady-state levels of Pre1 in these transformants were measured by immunoblotting with anti-FLAG antibody (upper panel). An immunoblot with anti-tubulin antibody served as a loading control (lower panel). The same amounts of extracts were used in these two immunoblots. Cell extract from an untagged strain was used as a negative control (lane 1). D, pulse-chase analysis of C-terminally HA-tagged Rpn4 (lanes 1–3) and Rpn4C447A (lanes 4–6) expressed from the P_CUP1 promoter on a low copy vector in JD52 in the presence of 100 μM CuSO₄. E, pulse-chase analysis of C-terminally HA-tagged Rpn4C447A expressed from the P_CUP1 promoter on a low copy vector in cim5-1 (lanes 1–3) and cim5-1 ubr2Δ (lanes 4–6) with 100 μM CuSO₄. Pulse-chase was conducted at a permissive temperature (30 °C). F, quantitation of the data from (E) by PhosphorImager to show the decay curves of Rpn4C447A in cim5-1 (●) and cim5-1 ubr2Δ (○).

FIG. 5. Sequence comparison of the putative type 1 and type 2 substrate binding sites of S. cerevisiae Ubr1 (ScUbr1), Eremothecium gossypii Ubr1 (EgUbr1), Kluyveromyces lactis Ubr1 (KlUbr1), Caenorhabditis elegans Ubr1 (CeUbr1), and Mus musculus Ubr1 (MmUbr1) with the corresponding regions of S. cerevisiae Ubr2 (ScUbr2) and E. gossypii Ubr2 (EgUbr2). The amino acids essential for the degradation of type 1 substrates (*) and type 2 substrates (●) are marked. White-on-black and gray shading highlight the conserved residues with ScUbr1 and ScUbr2, respectively.
Pressing Rpn4/H9004 cell growth (data not shown). Similarly, overexpression of a stable version of Rpn4 in WT did not impair worthy that accumulation of Rpn4 cim5 growth defect. The synthetic growth defect of cim5 some mutant.Interestingly, deletion of UBR2 cell growth when the proteasome activity is compromised. controlling the steady-state level of Rpn4, which is critical for tants; accumulation of such proteins may lead to growth de- short lived in WT cells but stabilized in the proteasome mu- teasome genes) the protein products of which are normally are required to target certain ubiquitinated substrates to the effectively, overproduction of defective proteasomes may sequester of multi-Ub chains from ubiquitinated substrates, which is detrimental to the cell. For instance, excessive amounts of cause of the toxicity of overexpressed Rpn4 in proteasome mu-

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