EC
Journals.ASM.org

The Ubiquitin Ligase Ubr11 Is Essential for Oligopeptide Utilization in the Fission Yeast *Schizosaccharomyces pombe*

Kenji Kitamura,[a] Mai Nakase,[b] Hideki Tohda,[c] and Kaoru Takegawa[b]

Center for Gene Science, Hiroshima University, Higashi-Hiroshima, Japan[a]; Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Fukuoka, Japan[b]; and ASPEX Division, Research Center, Asahi Glass Co., Ltd., Kanagawa-ku, Yokohama, Japan[c]

Uptake of extracellular oligopeptides in yeast is mediated mainly by specific transporters of the peptide transporter (PTR) and oligopeptide transporter (OPT) families. Here, we investigated the role of potential peptide transporters in the yeast *Schizosaccharomyces pombe*. Utilization of naturally occurring dipeptides required only Ptr2/SPBC13A2.04c and none of the other 3 OPT proteins (Isp4, Pgt1, and Opt3), whereas only Isp4 was indispensable for tetrapeptide utilization. Both Ptr2 and Isp4 localized to the cell surface, but under rich nutrient conditions Isp4 localized in the Golgi apparatus through the function of the ubiquitin ligase Pub1. Furthermore, the ubiquitin ligase Ubr11 played a significant role in oligopeptide utilization. The mRNA levels of both the *ptr2* and *isp4* genes were significantly reduced in *ubr11*Δ cells, and the dipeptide utilization defect in the *ubr11*Δ mutant was rescued by the forced expression of Ptr2. Consistent with its role in transcriptional regulation of peptide transporter genes, the Ubr11 protein was accumulated in the nucleus. Unlike the situation in *Saccharomyces cerevisiae*, the oligopeptide utilization defect in the *S. pombe ubr11*Δ mutant was not rescued by inactivation of the Tup11/12 transcriptional corepressors, suggesting that the requirement for the Ubr ubiquitin ligase in the upregulation of peptide transporter mRNA levels is conserved in both yeasts; however, the actual mechanism underlying the control appears to be different. We also found that the peptidomimetic proteasome inhibitor MG132 was still operative in a strain lacking all known PTR and OPT peptide transporters. Therefore, irrespective of its peptide-like structure, MG132 is carried into cells independently of the representative peptide transporters.

Most organisms utilize oligopeptides, which are hydrolyzed and recycled as amino acids or used as a source of nitrogen or carbon. Extracellular oligopeptides are imported into cells by specific plasma membrane transporters, which are distinct from amino acid transporters. Proteins of the highly conserved peptide transporter (PTR) family or SLC15 family, which are found in eukaryotes and some bacteria, import di- and tripeptides in a proton-coupled manner (49). In the budding yeast *Saccharomyces cerevisiae*, Ptr2 is a unique member of this PTR family and is responsible for the import of di- and tripeptides (23, 25, 44). Interestingly, PepT1 and PepT2, mammalian representatives of the SLC15 family, import a wide range of substrates from naturally occurring small peptides to various peptidomimetics or even unrelated drugs (7, 14, 46). The fission yeast *Schizosaccharomyces pombe* has a unique homolog of this family, SPBC13A2.04c/Ptr2, which was investigated in this study. In addition to these widely conserved PTR proteins, fungi and plants express an oligopeptide transporter (OPT) family of proteins (21, 29). In *S. pombe*, the OPT family protein Isp4 is responsible for the uptake of tetra- and pentapeptides (34). Two other genes encoding OPTs were identified in the *S. pombe* genome: *pgt1/opt1* (glutathione transporter) and the uncharacterized SPCC1840.12 (referred to as *opt3* in this study) (16, 52). The human opportunistic pathogen *Candida albicans* has 8 *OPT* genes, and some of their encoded proteins can transport longer peptides, up to at least 8 amino acids in length (35, 45). Additionally, the allantoate/ureidosuccinate permease Dal5 can transport several dipeptides in *S. cerevisiae* (9, 23).

Apart from the biochemical nature of these transporters, the molecular mechanisms regulating their expression have been characterized in detail, especially in *S. cerevisiae*. Transcription of these transporter genes, and hence utilization of peptides, is repressed in the presence of a preferable rich nitrogen source such as ammonium but is induced by relatively poor nitrogen sources such as allantoin and urea (6, 20). In addition to nitrogen catabolite repression, extracellular dipeptides induce *PTR2* expression by accelerating the ubiquitin-dependent degradation of Cup9 protein (8, 53). Cup9 forms a corepressor complex with Tup1-Ssn6 and inhibits the transcription of *PTR2* (56). Ubiquitination of Cup9 is mediated by the ubiquitin ligase Ubr1, which is involved in the N-end rule protein degradation pathway (8, 15, 48, 54). The imported dipeptide directly binds and activates Ubr1 by relieving it from autoinhibition through a conformational change and promotes the interaction between Cup9 and Ubr1 (15, 57). Thus, di/tripeptides effectively stimulate their own uptake by increasing the protein levels of the corresponding transporter Ptr2. Furthermore, the presence of amino acids influences peptide utilization via upregulation of peptide transporter expression (5, 24, 44, 55, 56). This amino acid-induced stimulation requires the Ssy1-Ptr3-Ssy5 (SPS) amino acid-sensing system and also depends on the function of Ubr1 (10, 12, 23, 33, 55, 56). Interestingly, amino acids accelerate the Ubr1-mediated degradation of Cup9 but do not stimulate the degradation of the N-end rule substrates themselves.

The ubiquitin ligase Ubr is widely conserved in eukaryotes (48, 54), and a deficiency in the human *UBR1* gene leads to the hereditary disease Johanson-Blizzard syndrome (58). We have reported the characterization of the Ubr ubiquitin ligases in *S. pombe*. Two

Received 29 September 2011 **Accepted** 28 December 2011
Published ahead of print 6 January 2012
Address correspondence to Kenji Kitamura, kkita@hiroshima-u.ac.jp.
Supplemental material for this article may be found at http://ec.asm.org/.

doi:10.1128/EC.05253-11

TABLE 1 *S. pombe* strains used in this study

Strain	Genotype	Source and/or reference
KSP2	*h*$^-$	Our stock
KSP634	*h*$^+$ *leu1-32*	Our stock
KSP635	*h*$^-$ *leu1-32*	Our stock
KSP1058	*h*$^-$ *ura4-D18 leu1-32*	Our stock
KSP1923	*h*$^-$ *ubr1*::*ura4*$^+$ *ura4-D18 leu1-32*	27
KSP2222	*h*$^-$ *ubr11*::*ura4*$^+$ *ura4-D18 leu1-32*	27
KSP2263	*h*$^+$ *ubr11*::*hphMX6 leu1-32*	This study
KSP2321	*h*$^-$ *ubr11*::*ura4*$^+$ *ura4-D18*	27
KSP2335a	*h*$^+$ *ubr11*::*hphMX6 leu1*$^+$-[*pDUAL-HFG41*]int *his2*	This study
KSP2335b	*h*$^+$ *ubr11*::*hphMX6 leu1*$^+$-[*Pnmt41-HFG-ptr2*$^+$]int *his2*	this study
KSP2335c	*h*$^+$ *ubr11*::*hphMX6 leu1*$^+$-[*Pnmt41-*^{6His}*Ub-Flag-halo7-ubr1*$^+$]int *his2*	This study; 28
KSP2422	*h*$^+$ *ptr2*::*ura4*$^+$ *ura4-C190T*	This study
KSP2472	*h*$^-$ *ubr11*::*hphMX6*	This study
KSP2698	*h*$^+$ *ptr2*::*ura4*$^+$ *ura4-D18 his7-366 leu1*$^+$-[*pDUAL-HFG41*]int	This study
KSP2699	*h*$^+$ *ptr2*::*ura4*+ *ura4-D18 his7-366 leu1*$^+$-[*Pnmt41-HFG-ptr2*$^+$]*int*	This study
KSP2817	*h*$^-$ *ubr11*::*hphMX6 ura4-D18 leu1-32* [*pDUAL*(*Pnmt41-*^{6His}*Ub-Flag-halo7-ubr11*$^+$)-*ura4*$^+$]multicopy	This study; 28
KSP2863	*h*$^+$ *leu1*$^+$-[*Pnmt41-Ub*^{G76V}*-GFP*]int *mei2*$^+$*-3HA-kanMX6 ura4-D18*	This study
KSP2874	*h*$^+$ *ubr11*::*hphMX6 leu1*$^+$-[*Pnmt41-*^{6His}*Ub-Flag-halo7-ubr11*$^+$]int *his2*	This study; 28
KSP2882	*h*$^-$ *isp4*::*hphMX6 ura4-D18 leu1-32*	This study
KSP2884	*h*$^-$ *ptr2*::*ura4*$^+$ *ura4-D18 leu1-32*	this study
KSP2886	*h*$^-$ *tup11*::*ura4*$^+$ *tup12*::*ura4*$^+$ *ura4-C190T leu1-32*	This study; 37
KSP2887	*h*$^-$ *tup11*::*ura4*$^+$ *tup12*::*ura4*$^+$ *ura4-C190T leu1-32*	This study; 37
KSP2888	*h*$^-$ *ubr11*::*hphMX6 tup11*::*ura4*$^+$ *tup12*::*ura4*$^+$ *ura4-C190T leu1-32*	This study
KSP2889	*h*$^-$ *ubr11*::*hphMX6 tup11*::*ura4*$^+$ *tup12*::*ura4*$^+$ *ura4-C190T leu1-32*	This study
KSP2920	*h*$^+$ *ptr2*::*ura4*$^+$ *isp4*::*hphMX6 pgt1*::*natMX6 opt3*::*pSVEM-bsd leu1*$^+$-[*Pnmt41-Ub*^{G76V}*-GFP*]int *mei2*$^+$*-3HA-kanMX6 ura4-D18*	This study
KSP2924	*h*$^-$ *opt3/SPCC1840.12*::*kanMX6 ura4-D18 leu1-32*	This study
KSP2925	*h*$^-$ *pgt1*::*natMX6 ura4-D18 leu1-32*	This study
KSP2934	*h*$^-$ *isp4*::*hphMX6 pgt1*::*natMX6 opt3*::*kanMX6 ura4-D18 leu1-32*	This study
KSP2971	*h*$^-$ *isp4*::*hphMX6 ura4-D18 leu1*$^+$-[*Pnmt41-HFG-isp4*$^+$]int	This study
KSP2972	*h*$^-$ *isp4*::*hphMX6 ura4-D18 leu1*$^+$-[*Pnmt41-HFG-isp4*$^+$]int p(*gms1-RFP; ura4*$^+$)	This study
KSP2996	*h*$^-$ *pub1*::*ura4*$^+$ *isp4*::*hphMX6 leu1*$^+$-[*Pnmt41-HFG-isp4*$^+$]int *ura4-D18*	This study; YGRC[a]
KSP2999	*h*$^-$ *isp4*::*hphMX6*	This study
591-1C	*h*$^+$ *isp4*::*hphMX6 lys1-131 ura4-D18 leu1-32*	This study
597-1C	*h*$^+$ *isp4*::*hphMX6 leu1-32*	This study
598-1A	*h*$^+$ *ptr2*::*ura4*$^+$ *dal5h1*::*hphMX6 dal5h2*::*natMX6 leu1-32*	This study
598-1C	*h*$^-$ *isp4*::*kanMX6 leu1-32*	This study
598-13CL	*h*$^-$ *ptr2*::*ura4*$^+$ *isp4*::*kanMX6 dal5h1*::*hphMX6 dal5h2*::*natMX6*	This study
2885L	*h*$^-$ *isp4*::*hphMX6 ptr2*::*ura4*$^+$ *ura4-D18*	This study
2928L	*h*$^-$ *ptr2*::*ura4*$^+$ *isp4*::*hphMX6 pgt1*::*natMX6 opt3*::*kanMX6 ura4-D18*	This study

[a] YGRC, Yeast Genetic Resource Center of Japan (YGRC/NBRP) (http://yeast.lab.nig.ac.jp/nig/).

distinct but homologous Ubr proteins, Ubr1 and Ubr11, exist in this yeast. Both Ubr1 and Ubr11 are dispensable for growth, and strains in which both genes are simultaneously deleted from the genome are viable. The *ubr1* strain exhibits pleiotropic phenotypes, including the accumulation of the Mei2 meiosis initiator protein (27), an altered oxidative stress response and drug resistance partly due to the inefficient degradation of the Pap1 transcription factor (19, 28), a localization defect of the nuclear proteasome (51), a defect in invasive growth (13), and a morphology defect and aberrant meiosis (our unpublished results). Inactivation of Ubr11 does not exacerbate the defects in the *ubr1* strain. Furthermore, forced expression of Ubr11 from the heterologous *nmt* promoter fails to rescue the defects, suggesting that Ubr1 and Ubr11 perform unique, nonoverlapping functions. We found that only Ubr11 is responsible for the N-end rule degradation pathway in *S. pombe* (our unpublished results). Nonetheless, no apparent phenotype has been identified so far in the *ubr11* mutant.

In this study, all the known possible peptide transporter proteins in *S. pombe* were examined for their involvement in oligopeptide utilization. We also investigated the role of Ubr ubiquitin ligase in the regulation of oligopeptide transporters. Our data showed that Ubr11 is important for the expression of Ptr2 and Isp4, which are responsible for transporting di- and tetrapeptides, respectively. To the best of our knowledge, other than for *S. cerevisiae*, this is the first study to demonstrate the conserved requirement for the Ubr ubiquitin ligase in peptide utilization.

MATERIALS AND METHODS

Yeast strains, media, and culture conditions. The *S. pombe* strains used in this study are listed in Table 1. Gene disruption was carried out using the PCR products of the 5′ and 3′ untranslated regions of the corresponding gene with *ura4*$^+$ or drug resistance genes as selection markers (4, 17, 30, 47). Detailed information on strain construction methods is in the supplemental material. The complete medium YES and minimal medium EMM were used for growth (40). In some experiments, the ammonium chloride (nitrogen source) in EMM was replaced with 0.1 to 0.15% (wt/vol) proline or was completely omitted for nitrogen-free medium. Expres-

sion from the *nmt* promoter was repressed by the addition of 5 μg/ml thiamine (+B_1) (38). Oligopeptides were purchased from Bachem AG (Bubendorf, Switzerland), Sigma-Aldrich (St. Louis, MO), Peptide Institute (Osaka, Japan), or Wako Pure Chemical Industries (Osaka, Japan). Most oligopeptides were dissolved in water, sterilized by filtration, and added to the autoclaved medium at 0.2 mM. Z-Ile-Leu (Sigma-Aldrich) was dissolved in dimethyl sulfoxide (DMSO) and added at 0.4 mM. Soy peptides (Hi-Nute AM; Fuji Oil, Osaka, Japan) were dissolved in medium at 0.1% (wt/vol) and autoclaved. *S. pombe* cells were grown at 28°C in liquid medium or at 31°C on agar solid medium. To inactivate the temperature-sensitive proteasome activity in the *mts2* strain, cells were cultured at 36°C for 3.5 h.

Plasmid construction. All pDUAL vector-derived plasmids were digested with NotI and integrated into the *leu1-32* loci of the host strains or used as an episomal multicopy plasmid (*ura4*$^+$ selection) if indicated (36). These are distinguished by the suffix int or multicopy in the genotypes in Table 1. To express the N-terminally green fluorescent protein (GFP)-tagged Ptr2 protein, the coding region of *ptr2* was amplified by PCR using oligonucleotides ptr2N and ptr2C (see Table S2 in the supplemental material for the nucleotide sequence) and inserted into the BglII site of the pDUAL-HFG41 vector using the "in fusion" reaction (Clontech, CA). The GFP-Isp4-expressing plasmid was made in similar way using oligonucleotides isp4N and isp4C. For plasmids expressing Dal5-like genes, coding regions of each gene were amplified with dal5h1N and dal5h1C for *dal5h1*/SPCC417.10 and with dal5h2N and dal5h2C for *dal5h2*/SPBC1773.15 and then inserted between the NdeI and BglII sites in the pDUAL-HFG41 vector.

To construct a plasmid encoding a proteasome activity reporter substrate, the ubiquitin^{G76V}-GFP fusion gene (including the stop codon) was excised from the Ub-G67V-GFP plasmid (plasmid 11941, obtained from Addgene) (11, 36) by digestion with NheI and XbaI and inserted into the NheI site of the pDUAL-HFF41 vector to yield pDUAL(nmt41-Ub^{G76V}-GFP). Due to the mutation of the last (76th) amino acid from glycine to valine in the ubiquitin moiety, the peptide bond between ubiquitin^{G76V} and GFP is resistant to cleavage by deubiquitinase. As a result, Ub^{G76V}-GFP is expressed as a fusion protein, ubiquitinated by the ubiquitin ligase Ufd4, and degraded by the proteasome (UFD degradation pathway) (22, 26).

Plasmids expressing Gms1-red fluorescent protein (RFP) and Halo7-tagged Ubr proteins have been described previously (28, 42). Plasmids harboring the genomic *ubr11*, *ptr2*, or *isp4* gene in the episomal multicopy vector pAL-SK were obtained from the Yeast Genetic Resource Center of Japan (YGRC/NBRP) (http://yeast.lab.nig.ac.jp/nig/).

RT-PCR. To monitor the mRNA levels of the *ubr11* gene or peptide transporter genes *ptr2* and *isp4*, total RNA was extracted and analyzed by reverse transcription-PCR (RT-PCR), as described previously (28). The *cdc2* and *nda3* (β-tubulin) genes were also analyzed as a control for RNA input levels.

Ub^{G76V}-GFP fusion protein expression, flow cytometry, and Western blotting. Cells were treated with the proteasomal inhibitor MG132 (50 μM; Peptide Institute, Osaka, Japan) or its solvent DMSO (1%, vol/vol) for 3.5 h where indicated. To monitor the intensity of Ub^{G76V}-GFP fluorescence, live cells were directly analyzed with a FACSCalibur (Becton Dickinson, NJ). Ub^{G76V}-GFP protein levels were also examined by Western blotting using anti-GFP antibody GF200 (Nakalai Tesque, Kyoto, Japan) and anti-Cdc2 antibody sc-53 (Santa Cruz Biotechnology, Santa Cruz, CA), as described previously (28).

Fluorescence microscopy. Expression of GFP-Ptr2, GFP-Isp4, and Flag-Halo7-Ubr11 proteins from the *nmt41* promoter was induced in the absence of thiamine (−B_1). The Flag-Halo7-Ubr11 protein (Halo-Ubr11) was labeled with the red fluorescent TMR ligand (Promega, Madison, WI), as described previously (28). The nuclear chromatin region was stained with Hoechst 33342 at 1 μg/ml. Cells were observed under a fluorescence microscope (model TE2U; Nikon, Tokyo, Japan), and fluorescent signals were captured by a charge-coupled-device (CCD) camera (ORCA-ER) with AQUA-Lite software (Hamamatsu Photonics, Shizuoka, Japan).

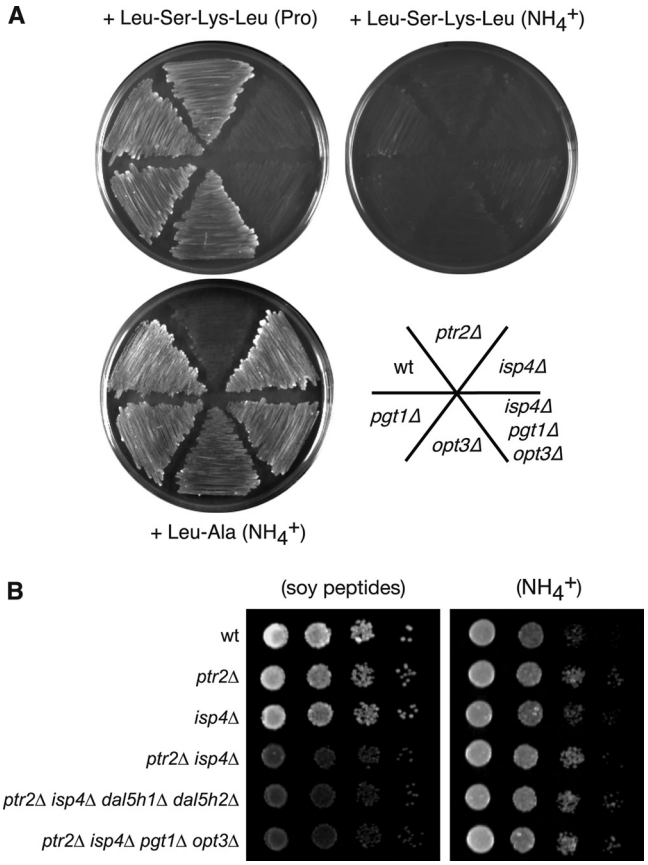

FIG 1 Ptr2 and Isp4 are major transporters for oligopeptide utilization. (A) Leucine-auxotrophic *leu1* strains lacking the possible peptide transporter(s) were grown in minimal medium containing the indicated di- or tetrapeptides as a source of leucine. The nitrogen source in each medium is shown in parentheses: Pro, proline; NH_4^+, ammonium chloride. Strains: wild type (wt), KSP1058; *ptr2*Δ, KSP2884; *isp4*Δ, KSP2882; *pgt1*Δ, KSP2925; *opt3*Δ, KSP2924; *isp4*Δ *pgt1*Δ *opt3*Δ, KSP2934. (B) Utilization of soy peptides by transporter mutants. Prototrophic strains lacking the indicated gene(s) were serially diluted and spotted to medium containing soy peptides or NH_4^+ as a sole nitrogen source. Strains: wt, KSP2; *ptr2*Δ, KSP2422; *isp4*Δ, KSP2999; *ptr2*Δ *isp4*Δ, 2885L; *ptr2*Δ *isp4*Δ *pgt1*Δ *opt3*Δ, 2928L; *ptr2*Δ *isp4*Δ *dal5h1*Δ *dal5h2*Δ, 598-13CL.

RESULTS

Roles of the PTR and OPT family transporters in oligopeptide utilization in *S. pombe*. First, we investigated the roles of PTR and OPT proteins in oligopeptide utilization. *S. pombe* has a single PTR-type transporter gene, SPBC13A2.04c (referred to as *ptr2* based on its homology to *PTR2* in *S. cerevisiae*), that has not yet been characterized. Strains carrying individual deletions of the entire *ptr2* gene or one of the three OPT family genes (*isp4*, *pgt1*, and *opt3*/SPCC1840.12) were prepared. To monitor oligopeptide utilization in the above-described transporter mutants, di- and tetrapeptides containing leucine were used to determine if they could support the growth of peptide transporter mutants harboring the leucine-auxotrophic *leu1* mutation (Fig. 1A). As previously reported (34), strains lacking the *isp4* gene failed to grow in minimal medium containing tetrapeptide (Leu-Ser-Lys-Leu) as a

source of leucine. Other strains grew well under the same condition. For dipeptide utilization, only the *ptr2*Δ strain failed to grow in the dipeptide (Leu-Ala) minimal medium. Therefore, Ptr2 has an essential role in dipeptide- but not tetrapeptide-dependent growth. None of the 3 OPT transporters has a role in dipeptide (Leu-Ala) utilization, because even the triple OPT mutant *isp4*Δ *pgt1*Δ *opt3*Δ strain grew as efficiently as the control wild-type strain. Neither the *pgt1*Δ nor the *opt3*Δ mutation affected growth in di- or tetrapeptide medium. Given that peptide utilization is under the control of nitrogen catabolite repression, we examined the effects of different nitrogen sources. Although dipeptide utilization was observed even in nitrogen-rich (ammonium [NH_4^+]) medium, tetrapeptides was imported only when a poor nitrogen source (proline) was used (Fig. 1A; see Fig. 3B). We also examined the roles of these transporters by monitoring the utilization of soy peptides, a mixture of various oligopeptides derived from soybeans. When soy peptides were used as a sole nitrogen source, cells lacking either *ptr2* or *isp4* could grow well, but simultaneous inactivation of both *ptr2* and *isp4* significantly slowed the growth under this condition (Fig. 1B). By Blast search using the *S. cerevisiae* Dal5 protein as a query, we found two Dal5-like proteins from the *S. pombe* genome database (referred to as Dal5h1/SPCC417.10 and Dal5h2/SPBC1773.15). The retarded growth in the *ptr2*Δ *isp4*Δ strain in soy peptide medium was not further exacerbated by additionally deleting both *DAL5*-like genes (*dal5h1* and *dal5h2*) or by deleting the remaining two OPT genes (*pgt1* and *opt3*) (Fig. 1B), indicating that Ptr2 and Isp4 are major peptide transporters in *S. pombe* within the peptide substrates tested in this study.

The Opt2 protein in *S. cerevisiae* is essential for the detoxification of certain drugs (2). In the presence of $ZnSO_4$ (0.75 to 6 mM) or spermine (0.25 to 2 mM), which are reported to retard the growth of the *S. cerevisiae opt2* strain, growth-inhibitory profiles of the two drugs were indistinguishable in the wild type and each of the *opt* mutant strains (data not shown). Therefore, it is unclear whether the OPT family proteins have a similar role in drug resistance in *S. pombe*.

Cellular localization of Ptr2 and Isp4. Cellular localization of Ptr2 and Isp4 proteins was monitored by fusing GFP to the N terminus of the corresponding transporters. Functionality of these GFP-fused transporters was confirmed by their rescue of the corresponding mutants (Fig. 2B; see Fig. 4B). GFP-Ptr2 was localized at the plasma membrane, particularly at both ends of the cell and also at the center in dividing cells (Fig. 2A). In contrast, GFP-Isp4 signals were observed as cytoplasmic punctate dots which largely overlapped with those of Gms1, a UDP-galactose transporter protein localized in the Golgi apparatus (42), in growing cells when NH_4^+ was used as a nitrogen source (Fig. 2C; see Fig. S1 in the supplemental material). This localization is consistent with the fact that utilization of tetrapeptide via Isp4 was not operative in the NH_4^+ medium (Fig. 1A). Upon nitrogen starvation, however, GFP-Isp4 moved to the plasma membrane (Fig. 2D, left panel). Interestingly, GFP-Isp4 localized at the plasma membrane even under a rich nitrogen condition in the *pub1*Δ ubiquitin ligase mutant (Fig. 2D, right panel). These localization behaviors of Isp4 are quite similar to those of permeases Aat1 and Cat1 (3, 43). Localization of both Ptr2 and Isp4 on the cell surface is consistent with their role in the uptake of extracellular oligopeptides.

Ubr11 is essential for oligopeptide utilization. Next we examined whether the Ubr ubiquitin ligases had a role in peptide utilization in *S. pombe*, as reported for *S. cerevisiae* (1, 8). Leucine-auxotrophic *leu1* strains were tested for their ability to grow in medium containing the dipeptide Leu-Ala as a source of leucine. Only the *ubr11*Δ mutant, and not the wild-type and *ubr1*Δ strains, failed to grow in the Leu-Ala dipeptide medium (Fig. 3A). The same *ubr11*Δ strain grew well in the presence of monomeric leucine. We also tested the other leucine-containing dipeptides, Ala-Leu, His-Leu, Lys-Leu, and Tyr-Leu, and obtained similar results (Fig. 3B and data not shown). Furthermore, both the Ala-Leu and Leu-Ala dipeptides did not inhibit the growth of the prototrophic *ubr11*Δ strain (Fig. 3B and data not shown), indicating that the

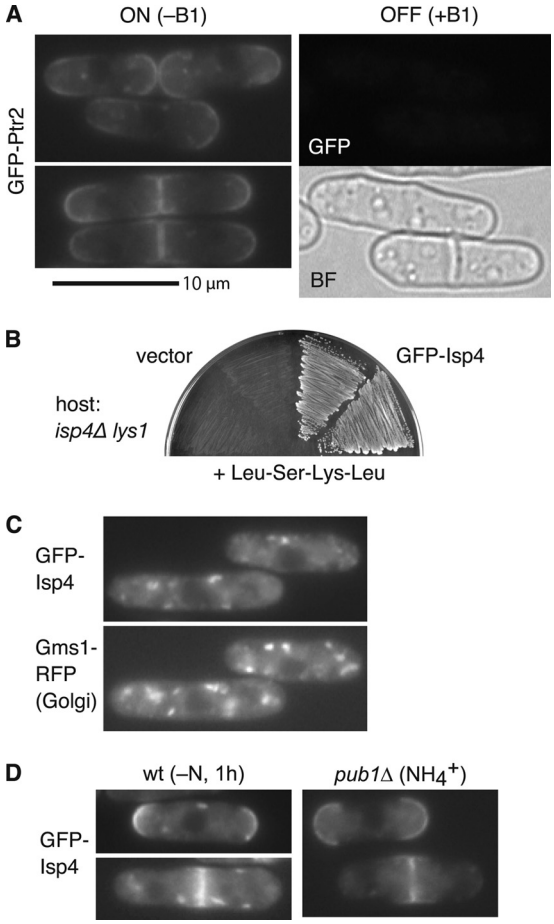

FIG 2 Cell surface localization of Ptr2 and Isp4. (A) GFP-Ptr2 localizes to the cell surface at both ends and also to the central region of dividing cells. Left panels, expression of GFP-Ptr2 was induced in the absence of thiamine (ON, $-B_1$), and the GFP signals were observed. Right panels, GFP and bright-field (BF) images of the same cells in the uninduced condition (OFF, $+B_1$). Strain, KSP2699. For the functionality of the GFP-Ptr2, see Fig. 4B. (B) The N-terminally GFP-tagged Isp4 is functional. A lysine-auxotrophic *isp4*Δ strain (591-1C) was transformed with a control GFP vector or the GFP-Isp4-expressing plasmid. Utilization of the Leu-Ser-Lys-Leu tetrapeptide as lysine via the GFP-Isp4 was monitored. (C) Isp4 localizes in the Golgi apparatus under rich nitrogen (NH_4^+) conditions. Localization of the functional GFP-Isp4 overlapped with that of the Golgi-localized Gms1-RFP protein. Strain, KSP2972. (D) Membrane localization of GFP-Isp4 is regulated by nutrients through the function of the ubiquitin ligase Pub1. (Left panel) GFP-Isp4 was localized at the plasma membrane after 1 h in nitrogen-free medium (−N). Strain, KSP2971. (Right panel) In the *pub1*Δ strain (KSP2996), GFP-Isp4 membrane localization was detected even in the presence of the rich nitrogen source NH_4^+.

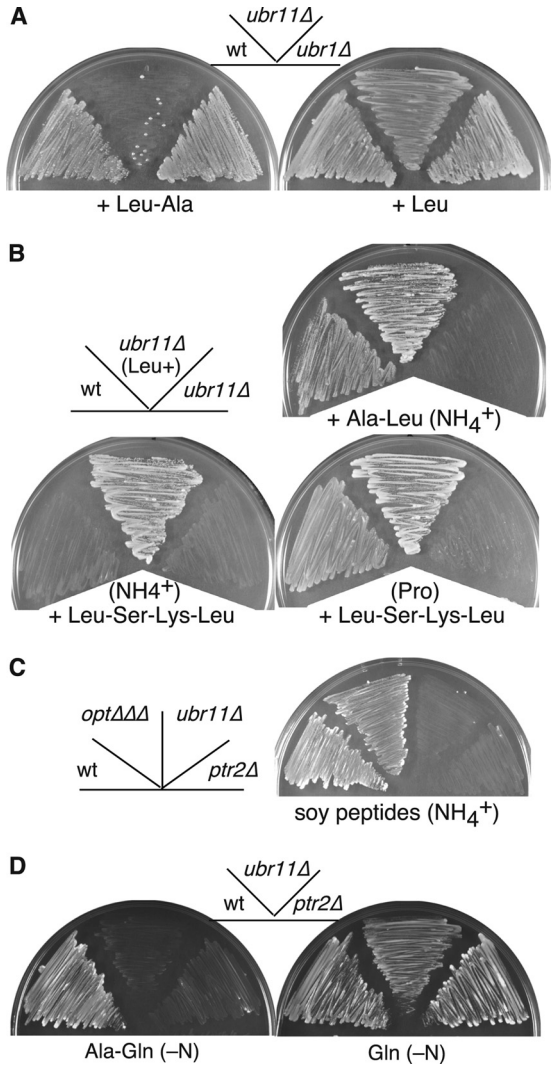

FIG 3 Ubr11 is essential for oligopeptide utilization. (A) Wild-type (KSP634), *ubr11*Δ (KSP2222), and *ubr1*Δ (KSP1923) strains, which are auxotrophic for leucine, were grown in minimal medium containing Leu-Ala dipeptide (left) or leucine (right). Colonies of the *ubr11*Δ strain in the Leu-Ala dipeptide medium are spontaneous suppressor mutants. (B) The same wild-type or *ubr11*Δ strains used for panel A were grown in the presence of Ala-Leu dipeptide or Leu-Ser-Lys-Leu tetrapeptide. The nitrogen source used in each plate is indicated in parentheses. To obtain prototrophic (Leu^+) cells, the same *ubr11*Δ strain (KSP2222) was transformed with a vector that rescued the auxotrophy for leucine. (C) The indicated strains were grown in minimal medium containing soy peptides as a source of leucine. The *opt*ΔΔΔ strain lacks all the genes of the OPT family (*isp4*Δ *pgt1*Δ *opt3*Δ). Strains: wt (KSP1058), *ptr2*Δ (KSP2884), *ubr11*Δ (KSP2263), *opt*ΔΔΔ (KSP2934). Only Ptr2, and none of the OPTs, is responsible for the uptake of soy peptides containing leucine under this condition. (D) The indicated prototrophic strains were grown in a medium containing the Ala-Gln dipeptide (left) or monomeric glutamine (right) as the sole nitrogen source. Strains; wt, KSP2; *ubr11*Δ, KSP2472; *ptr2*Δ, KSP2422. The *ubr11*Δ and *ptr2*Δ strains failed to grow in the Ala-Gln dipeptide medium, but their growth was not affected if glutamine was used instead of the dipeptide.

*ubr11*Δ strain was unable to utilize leucine-containing dipeptides. Similarly, the *ubr11*Δ *leu1* cells also failed to grow in the Leu-Ser-Lys-Leu tetrapeptide medium (Fig. 3B). We also tested the effect of soy peptides and found that soy peptides supported the growth of leucine-auxotrophic (but otherwise wild-type) strains. In contrast, the *ubr11*Δ *leu1* mutant failed to grow in the same medium (Fig. 3C), indicating that the soy peptides did not contain enough monomeric leucine as amino acid to support the growth of the *ubr11*Δ *leu1* mutant and, more importantly, that Ubr11 was essential to utilize soy peptides in which various different kinds of leucine-containing oligopeptides should exist. Similar to that of the *ubr11*Δ strain, growth of the *ptr2*Δ *leu1* mutant, but not that of the *isp4*Δ *pgt1*Δ *opt3*Δ *leu1* mutant, was severely retarded in the soy peptide medium (Fig. 3C), indicating that under this condition Ptr2, but not the OPT proteins, plays a major role.

We confirmed the defects in a different way by examining whether cells were competent to utilize dipeptides as a nitrogen source. For this purpose, growth of prototrophic strains was monitored in minimal medium containing dipeptides (Ala-Gln) as the sole nitrogen source. A wild-type strain was able to grow in this dipeptide medium (Fig. 3D). In marked contrast, the *ptr2*Δ peptide transporter and, importantly, the *ubr11*Δ mutant failed to grow in the same medium. All the strains grew well when the amino acid glutamine, one of the constituents of the Ala-Gln dipeptide, was used as a nitrogen source. Collectively, Ubr11 is indispensable for the utilization of both di- and tetrapeptides.

Ubr11 regulates the mRNA levels of *ptr2* and *isp4*. As described above, peptide utilization depends on specific transporters, Ptr2 and Isp4. It is likely that the expression or function of these peptide transporters is affected in the *ubr11*Δ strain. Indeed, RT-PCR analyses clearly revealed that the mRNA levels of *ptr2* and *isp4* were significantly reduced in *ubr11*Δ cells (Fig. 4A). In contrast to the case for these two transporters, expression of the *pgt1* and *opt3* genes was not affected by the lack of the *ubr11* gene (data not shown).

We found that forced expression of the *ptr2* gene from the heterologous *nmt* promoter suppressed the dipeptide utilization defect in *ubr11*Δ cells (Fig. 4B), supporting the notion that the *ubr11*Δ mutant was defective for the expression of peptide transporters mostly at the transcriptional level. This indicates that insufficient expression of these peptide transporters leads to the peptide utilization defect in the *ubr11*Δ mutant.

Considering the observation that Ubr11 regulates the transcription of *ptr2* and *isp4*, we hypothesized that Ubr11 may be a nuclear protein. We monitored the cellular localization of Ubr11 that was functionally marked by a Halo7 tag at the N terminus (28). Labeling of the Halo-Ubr11 fusion protein by the fluorescent ligand confirmed that Ubr11 was enriched in the nucleus, especially in the Hoechst-stained chromatin region (Fig. 4C).

Tup11/12 corepressors are required for efficient peptide utilization. In *S. cerevisiae*, the Tup1-Ssn6 corepressor system complexed with Cup9 inhibits *PTR2* gene expression, and the peptide uptake defect in the *ubr1* strain is rescued by inactivating Tup1 or Ssn6 (56). *S. pombe* has two *TUP1*-like genes, *tup11* and *tup12* (41). We examined whether the phenotype of the *ubr11*Δ cells was also suppressed by inactivation of both the *tup* genes, as in *S. cerevisiae*. For reasons that are unclear, inactivation of both *tup11* and *tup12* resulted in an inability of the cells to use the Leu-Ala dipeptide (Fig. 5A). This growth defect was not observed when soy peptides were used as a source of leucine (Fig. 5B). In contrast to the parental strain, the *ubr11*Δ *tup11*Δ *tup12*Δ triple mutant failed to grow in soy peptide medium containing ammonium (Fig. 5B) or proline (data not shown) as a nitrogen source, suggesting that inactivation of the Tup11/12 complex does not suppress the defects in the *ubr11*Δ strain. Because of the inviability of *ssn6*Δ cells

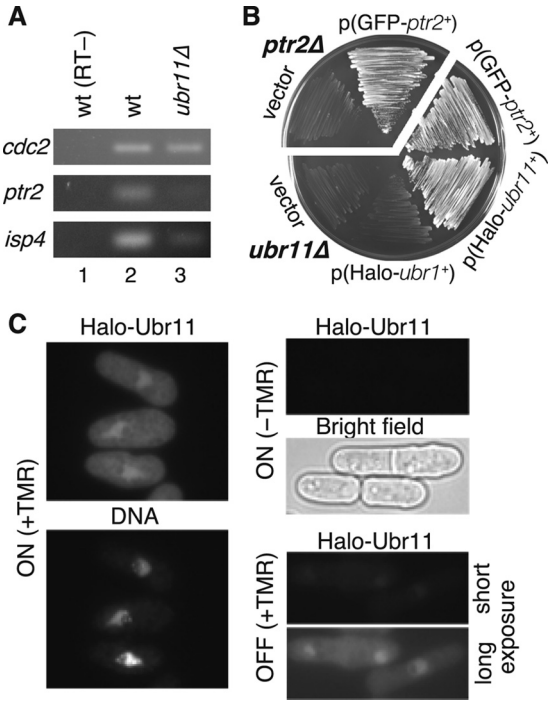

FIG 4 Ubr11 is required for the expression of *ptr2* and *isp4*. (A) The mRNA levels of the peptide transporter genes in each strain were compared by RT-PCR. Cells were cultured in EMM-N (proline) medium. The *cdc2* gene was used as an internal control for the input RNA levels. Lane 1, the same total RNA used in lane 2 was similarly processed but without reverse transcription, as a control. Lane 2, wild-type cells (KSP2); lane 3, *ubr11*Δ cells (KSP2321). (B) Ectopic expression of the dipeptide transporter Ptr2 rescues the peptide utilization defect in the *ubr11*Δ strain. The *ptr2*Δ and *ubr11*Δ strains, both auxotrophic for histidine, were transformed with the indicated plasmids or a control vector. Growth of each strain in minimal medium containing the His-Leu dipeptide as a source of histidine was examined. The GFP-tagged Ptr2 was functional because it could rescue the peptide uptake defect in a *ptr2*Δ strain (KSP2698 or KSP2699). Only the *ubr11*Δ mutant expressing ectopic Halo-tagged Ubr11 (KSP2874) or GFP-Ptr2 (KSP2335b), but not control GFP only (KSP2335a) or Halo-Ubr1 (KSP2335c), grew under this condition. (C) Ubr11 accumulates in the nuclear chromatin region. The Halo-Ubr11 protein was induced in a *ubr11*Δ strain (KSP2817) and labeled with a fluorescent TMR ligand for the halo tag (ON, +TMR). Fluorescence specificity was verified by observing induced cells without labeling (ON, −TMR) or uninduced cells (OFF, +TMR). Residual weak expression from the *nmt* promoter occurred even under the uninduced condition; therefore, the Halo-Ubr11 signal was detected after a longer exposure time.

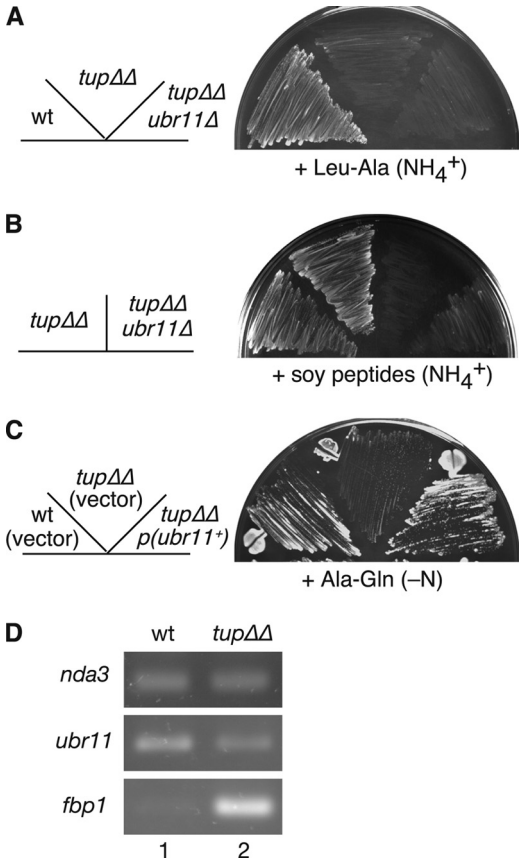

FIG 5 The Tup corepressor proteins are required for efficient peptide utilization. (A and B) The indicated leucine-auxotrophic strains were grown in minimal medium containing Leu-Ala dipeptide (A) or soy peptides (B) as a source of leucine. *tup*ΔΔ, *tup11*Δ *tup12*Δ double disruptant. Strains: wt, KSP635; *tup*ΔΔ, KSP2886 and -2887; *tup*ΔΔ *ubr11*Δ, KSP2888 and -2889. (C) Bypass of Tup protein function by increasing Ubr11 protein expression. The same strains used for panels A and B (KSP635 and KSP2886) were transformed with a plasmid harboring the genomic *ubr11*$^+$ gene or a control vector, and grown in medium containing Ala-Gln dipeptide as the sole source of nitrogen. (D) *ubr11* mRNA levels in the wild-type (KSP635, lane 1) and the *tup11*Δ *tup12*Δ (KSP2886, lane 2) strains. The *nda3* (β-tubulin) gene was used as an internal control. Expression levels of the *fbp1* gene, whose transcription is derepressed in the *tup11*Δ *tup12*Δ strain, are also provided.

of *S. pombe* (18), we did not test the effect of Ssn6 inactivation on the suppression of defects in the *ubr11*Δ strain.

A prototrophic *tup11*Δ *tup12*Δ strain grew very weakly in medium containing the dipeptide Ala-Gln as the sole nitrogen source. Interestingly, this slow growth was significantly improved by increasing the expression of Ubr11 from a multicopy plasmid (Fig. 5C). Although the endogenous *ubr11* mRNA levels were slightly decreased in the *tup11*Δ *tup12*Δ strain (0.6- to 0.7-fold compared to those in the wild-type strain), sufficient levels of mRNA could be still detected (Fig. 5D).

MG132 is imported independently of PTR and OPT proteins. Since the human peptide transporters have broad substrate specificity, we examined the effect of nonnative oligopeptides in *S. pombe* and found that extracellular Ile-Leu dipeptide that is benzyloxycarbonylated at the N terminus (Z-Ile-Leu) could support the growth of leucine-auxotrophic strain only if the Isp4 was functional (Fig. 6A). The peptide aldehyde MG132 [(benzyloxycarbonyl)leucyl-leucyl-leucine aldehyde], which is widely used as a proteasome inhibitor (31), has the same chemical modification with Z-Ile-Leu at its N terminus. Given their structural similarities and the role of Isp4 in the utilization of Z-Ile-Leu, we examined whether the peptidomimetic MG132 was also carried into cells via peptide transporters. Ub^{G76V}-GFP was used as a reporter substrate for monitoring proteasomal activity (11). We confirmed that Ub^{G76V}-GFP was stabilized in the *mts2* proteasome mutant (see Fig. S2A in the supplemental material). The fluorescence intensity of Ub^{G76V}-GFP determined by flow cytometry correlated well with its protein levels detected by Western blotting (see Fig. S2C in the supplemental material). As expected, instability of this monitoring substrate depended on the ubiquitin ligase Ufd4, one of the central players in the UFD degradation pathway (see Fig. S2B in the supplemental material) (22, 26).

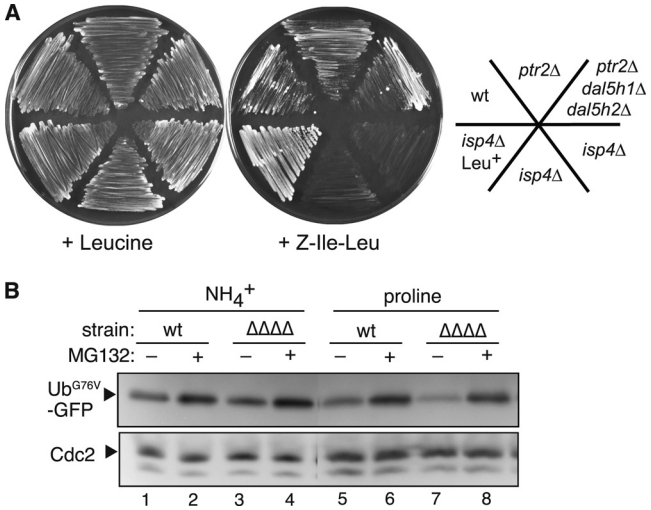

FIG 6 MG132 is imported independently of the PTR and OPT proteins. (A) Isp4-dependent utilization of the N-terminally modified nonnative dipeptide Z-Ile-Leu [(benzyloxycarbonyl)isoleucyl-leucine]. Z-Ile-Leu could support the growth of the leucine-auxotrophic strain and thus was used as a source of leucine only if the Isp4 was functional. Consistent with the use of Isp4, Z-Ile-Leu could support growth under the poor (proline) but not rich (NH_4^+) nitrogen condition (data not shown). The prototrophic (Leu^+) *isp4*Δ strain could grow under this condition, suggesting that Z-Ile-Leu did not inhibit the growth of the *isp4*Δ mutant. Strains: wt, KSP635; *ptr2*Δ, KSP2884; *ptr2*Δ *dal5h1*Δ *dal5h2*Δ, 598-1A), *isp4*Δ Leu^+, KSP2999; *isp4*Δ, 597-1C and 598-1C. (B) MG132 is operative in the peptide transporter-deficient strain. Ub^{G76V}-GFP was induced in the wild-type strain (KSP2863) and a quadruple transporter mutant (ΔΔΔΔ, *ptr2*Δ *isp4*Δ *pgt1*Δ *opt3*Δ) (KSP2920) under rich (NH_4^+) or poor (proline) nitrogen conditions. Cells were treated with MG132 (lanes 2, 4, 6, and 8) or DMSO (lanes 1, 3, 5, and 7) during the last 3.5 h before the extracts were prepared. Ub^{G76V}-GFP protein levels were analyzed by immunoblotting with the anti-GFP antibody.

In the wild-type strain, MG132 prevented the degradation of Ub^{G76V}-GFP (Fig. 6B; see Fig. S2A and C in the supplemental material). We tested the effect of MG132 in a strain lacking all the known PTR and OPT proteins (ΔΔΔΔ [*ptr2*Δ *isp4*Δ *pgt1*Δ *opt3*Δ]). Interestingly, a similar degree of inhibition by MG132 was also observed in this quadruple transporter mutant strain (Fig. 6B). The effect of MG132 was observed under both rich (NH_4^+) and poor (proline) nitrogen conditions, suggesting that PTR and OPTs are not required for the import of MG132. We also examined the effect of MG132 in a quadruple mutant simultaneously lacking Ptr2, Isp4, and two Dal5-like proteins (Dal5h1 and Dal5h2). However, MG132 still effectively inhibited the degradation of Ub^{G76V}-GFP in this mutant (see Fig. S2D in the supplemental material).

DISCUSSION

The fission yeast *S. pombe* has 1 *PTR* and 3 *OPT* peptide transporter family genes. In this study, we investigated the necessity of these transporters and confirmed that Ptr2 (PTR family) and Isp4 (OPT family) are essential for dipeptide and tetrapeptide (Leu-Ser-Lys-Leu) utilization, respectively (Fig. 1A). For Dal5-related transporters (Dal5h1 and Dal5h2), we have no evidence that either protein has a role in peptide utilization in *S. pombe*. First, inactivation of Ptr2 completely abrogated, but simultaneous inactivation of both Dal5h1 and Dal5h2 did not affect, the utilization of three dipeptides (see Fig S3A and B in the supplemental material). Second, forced expression of Dal5h1 or Dal5h2 in the *ptr2*Δ strain did not suppress the utilization defect of Ala-Gln dipeptide, a preferred substrate of Dal5 in *S. cerevisiae* (see Fig. S3C in the supplemental material). Although only a limited number of defined oligopeptides were used in this study, we also examined the utilization of soy peptides, a mixture of various peptides (see Fig. S4 in the supplemental material). When soy peptides were used as a sole nitrogen source, growth of the *ptr2*Δ *isp4*Δ double mutant was significantly retarded, though each parental single mutant grew normally (Fig. 1B). Besides, slow growth in the *ptr2*Δ *isp4*Δ strain was not further aggravated by additional inactivation of other possible transporters. Collectively, these data indicate that Ptr2 and Isp4 play a major role in general peptide transport in *S. pombe*, at least with the peptide substrates used in this study. Other transporters may be specialized for specific peptides such as glutathione (Pgt1) (16, 52) or may have a role in different environmental situations not examined in this study. Since the *ptr2*Δ *isp4*Δ strain still grows in the soy peptide medium (Fig. 1B), one attractive idea is that an uncharacterized transporter is involved in the peptide uptake under this condition. Alternatively, this residual growth may rely on the utilization of amino acids that could be included in the soy peptides used in this study. However, the amount of free amino acids in the soy peptides seems to be small, since they are insufficient to support the growth of *ptr2*Δ and *ubr11*Δ strains that are auxotrophic for several different amino acids (Fig. 2A; see Fig. S4 in the supplemental material).

The most important finding in this study is that the ubiquitin ligase Ubr11 is required for the expression of Ptr2 and Isp4 in *S. pombe* (Fig. 4A). The defect in peptide utilization is the first apparent phenotype of the *ubr11*Δ mutant. Involvement of the Ubr protein in peptide utilization was first described for *S. cerevisiae*, and our finding in *S. pombe* is the second example of such a role. We found that Ubr11, but not Ubr1, is the ubiquitin ligase (N-recognin) in the N-end rule pathway in *S. pombe* (our unpublished data), strongly suggesting that peptide utilization is an N-recognin-specific function. Regardless of the conservation of Ubr proteins in eukaryotes, studies on their physiological roles are still limited. The *ubr* mutants exhibit various phenotypes in both *S. cerevisiae* and *S. pombe*, but curiously, only the peptide utilization defect is common to both yeasts. It will be interesting to see if Ubr proteins also play a role in peptide utilization in other organisms. Prior to utilization, oligopeptides are digested to amino acid monomers by an intracellular peptidase(s) (6, 39); this process may be defective in the *ubr11*Δ mutant. However, the mRNA levels of the *ptr2* and *isp4* transporter genes are apparently reduced in the *ubr11*Δ mutant (Fig. 4A), and peptide utilization was rescued by the ectopic expression of Ptr2 from the heterologous *nmt* promoter (Fig. 4B). Although we have not directly measured peptide uptake in the *ubr11*Δ strain, it is likely that the peptide utilization defect in the *ubr11*Δ mutant is caused by the deficiency of peptide import due to inefficient expression of the transporters and that the action of peptidases is irrelevant.

Consistent with the fact that Ubr11 regulates peptide transporter gene expression, the Ubr11 protein is enriched in the nucleus, especially in the chromatin region (Fig. 4C). In *S. cerevisiae*, Ubr1 facilitates peptide uptake by ubiquitination, thereby promoting the proteasome-dependent degradation of Cup9, a transcriptional repressor of the *PTR2* gene (8, 53). The Tup1-Ssn6 corepressor complex, together with Cup9, represses transcription of the *PTR2* gene (56). In *S. pombe*, the peptide uptake defect is not

rescued by the inactivation of the Tup11/12 complex (Fig. 5B), unlike in *S. cerevisiae*. Furthermore, there is no apparent Cup9 homolog in the *S. pombe* genome. Therefore, although the requirement for the Ubr ubiquitin ligase in peptide utilization is conserved between the two evolutionarily distant yeast species, the target substrate for the Ubr protein and the actual mechanism of transporter gene derepression are different. We identified a suppressor mutation that completely restored *ptr2* and *isp4* mRNA levels, and consequently peptide utilization, in the *S. pombe ubr11*Δ mutant (our unpublished results). Our attempts to clone the suppressor gene have been unsuccessful so far. Although the identity of this suppressor gene remains unknown, it is expected to play a very important role in peptide transporter gene expression that is antagonistic to that of Ubr11. Unexpectedly, the *tup* mutant of *S. pombe* was inefficient in peptide utilization. Interestingly, increasing the expression of Ubr11 substantially cured the peptide utilization defect in the *tup11*Δ *tup12*Δ strain. The fact that the requirement for the Tup11/12 corepressor complex in peptide utilization can be bypassed by a high dosage of Ubr11 reinforces the importance of Ubr11's role in peptide utilization.

Finally, we addressed the question of whether import of the peptide-like proteasome inhibitor MG132 required peptide transporters. Despite the utilization of (benzyloxycarbonyl)isoleucylleucine (Z-Ile-Leu) via Isp4 and its structural resemblance to MG132, none of the possible peptide transporters tested in this study was involved; MG132 inhibited proteasome activity even in the strain that lacked all the PTR and OPT transporters (Fig. 6B) and also in a quadruple mutant lacking two *dal5*-like genes together with being *ptr2*Δ and *isp4*Δ (see Fig. S2D in the supplemental material). Since MG132 is a modified trileucine, its amino acid length or aldehyde modification of the third leucine may interfere with its recognition by PTR or OPT transporters. MG132 is generally ineffective in wild-type cells of *S. cerevisiae*, but it is active if a rich nitrogen source, such as ammonium, in the growth medium is replaced by proline together with the addition of a small amount of sodium dodecyl sulfate (32). Given that the nature of the nitrogen source greatly affects the expression profiles of many genes, this presumably implies that MG132 is actively imported by a specific carrier that is expressed only under the proline condition and not by simple passive diffusion. Most of the proteasomal subunit genes are essential for growth. Unlike the proteasome mutations, MG132 treatment does not cause cell cycle arrest in *S. pombe* (50). Unraveling the MG132 transport system is an important challenge to more effectively block proteasome activity without using special membrane ergosterol synthesis or efflux pump mutants, which may greatly affect the cellular physiology.

ACKNOWLEDGMENTS

We thank S. Fukutome for technical support, S. Kitagawa for the kind gift of soy peptides, and A. Erler, A. Matsuyama, M. Yoshida, and the Yeast Genetic Resource Center of Japan (YGRC/NBRP) for plasmids and strains. We also thank I. Yamashita and N. Tanaka for discussion and support.

This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

1. **Alagramam K, Naider F, Becker JM.** 1995. A recognition component of the ubiquitin system is required for peptide transport in *Saccharomyces cerevisiae.* Mol. Microbiol. **15**:225–234.
2. **Aouida M, Khodami-Pour A, Ramotar D.** 2009. Novel role for the *Saccharomyces cerevisiae* oligopeptide transporter Opt2 in drug detoxification. Biochem. Cell Biol. **87**:653–661.
3. **Aspuria PJ, Tamanoi F.** 2008. The Tsc/Rheb signaling pathway controls basic amino acid uptake via the Cat1 permease in fission yeast. Mol. Genet. Genomics **279**:441–450.
4. **Bähler J, et al.** 1998. Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe.* Yeast **14**:943–951.
5. **Basrai MA, Zhang H-L, Miller D, Naider F, Becker JM.** 1992. Toxicity of oxalysine and oxalysine-containing peptides against *Candida albicans*: regulation of peptide transport by amino acids. J. Gen. Microbiol. **138**: 2353–2362.
6. **Becker JM, Naider F.** 1977. Peptide transport in yeast: uptake of radioactive trimethionine in *Saccharomyces cerevisiae.* Arch. Biochem. Biophys. **178**:245–255.
7. **Brandsch M.** 2009. Transport of drugs by proton-coupled peptide transporters: pearls and pitfalls. Expert Opin. Drug Metab. Toxicol. **5**:887–905.
8. **Byrd C, Turner GC, Varshavsky A.** 1998. The N-end rule pathway controls the import of peptides through degradation of a transcriptional repressor. EMBO J. **17**:269–277.
9. **Cai H, Hauser M, Naider F, Becker JM.** 2007. Differential regulation and substrate preferences in two peptide transporters of *Saccharomyces cerevisiae.* Eukaryot. Cell **6**:1805–1813.
10. **Cai H, Kauffman S, Naider F, Becker JM.** 2006. Genomewide screen reveals a wide regulatory network for di/tripeptide utilization in *Saccharomyces cerevisiae.* Genetics **172**:1459–1476.
11. **Dantuma NP, Lindsten K, Glas R, Jellne M, Masucci MG.** 2000. Short-lived green fluorescent proteins for quantifying ubiquitin/proteasome-dependent proteolysis in living cells. Nat. Biotechnol. **18**:538–543.
12. **Didion T, Regenberg B, Jørgensen MU, Kielland-Brandt MC, Andersen HA.** 1998. The permease homologue Ssy1p controls the expression of amino acid and peptide transporter genes in *Saccharomyces cerevisiae.* Mol. Microbiol. **27**:643–650.
13. **Dodgson J, et al.** 2009. Functional genomics of adhesion, invasion, and mycelial formation in *Schizosaccharomyces pombe.* Eukaryot. Cell **8**:1298–1306.
14. **Döring F, et al.** 1998. Delta-aminolevulinic acid transport by intestinal and renal peptide transporters and its physiological and clinical implications. J. Clin. Invest. **101**:2761–2767.
15. **Du F, Navarro-Garcia F, Xia Z, Tasaki T, Varshavsky A.** 2002. Pairs of dipeptides synergistically activate the binding of substrate by ubiquitin ligase through dissociation of its autoinhibitory domain. Proc. Natl. Acad. Sci. U. S. A. **99**:14110–14115.
16. **Dworeck T, Wolf K, Zimmermann M.** 2009. SpOPT1, a member of the oligopeptide family (OPT) of the fission yeast *Schizosaccharomyces pombe*, is involved in the transport of glutathione through the outer membrane of the cell. Yeast **26**:67–73.
17. **Erler A, Maresca M, Fu J, Stewart AF.** 2006. Recombineering reagents for improved inducible expression and selection marker re-use in *Schizosaccharomyces pombe.* Yeast **23**:813–823.
18. **Fagerström-Billai F, Durand-Dubief M, Ekwall K, Wright AP.** 2007. Individual subunits of the Ssn6-Tup11/12 corepressor are selectively required for repression of different target genes. Mol. Cell. Biol. **27**:1069–1082.
19. **Gatti L, et al.** 2011. Ubiquitin-proteasome genes as targets for modulation of cisplatin sensitivity in fission yeast. BMC Genomics **12**:1–11.
20. **Godard P, et al.** 2007. Effect of 21 different nitrogen sources on global gene expression in the yeast *Saccharomyces cerevisiae.* Mol. Cell. Biol. **27**: 3065–3086.
21. **Hauser M, Narita V, Donhardt AM, Naider F, Becker JM.** 2001. Multiplicity and regulation of genes encoding peptide transporters in *Saccharomyces cerevisiae.* Mol. Membr. Biol. **18**:105–112.
22. **Heessen S, Dantuma N, Tessarz P, Jellne M, Masucci MG.** 2003. Inhibition of ubiquitin/proteasome-dependent proteolysis in *Saccharomyces cerevisiae* by a Gly-Ala repeat. FEBS Lett. **555**:397–404.
23. **Homann OR, Cai H, Becker JM, Lindquist SL.** 2005. Harnessing natural diversity to probe metabolic pathways. PLoS Genet. **1**:715–729.
24. **Island MD, Naider F, Becker JM.** 1987. Regulation of dipeptide transport in *Saccharomyces cerevisiae* by micromolar amino acid concentrations. J. Bacteriol. **169**:2132–2136.
25. **Island MD, Perry JR, Naider F, Becker JM.** 1991. Isolation and characterization of *S. cerevisiae* mutants deficient in amino acid-inducible peptide transport. Curr. Genet. **20**:457–463.

26. **Johnson ES, Ma PC, Ota IM, Varshavsky A.** 1995. A proteolytic pathway that recognizes ubiquitin as a degradation signal. J. Biol. Chem. **270**: 17442–17456.
27. **Kitamura K, et al.** 2001. Phosphorylation of Mei2 and Ste11 by Pat1 kinase inhibits sexual differentiation via ubiquitin proteolysis and 14-3-3 protein in fission yeast. Dev. Cell **1**:389–399.
28. **Kitamura K, Taki M, Tanaka N, Yamashita I.** 2011. Fission yeast Ubr1 ubiquitin ligase influences the oxidative stress response via degradation of active Pap1 bZIP transcription factor in the nucleus. Mol. Microbiol. **80**: 739–755.
29. **Koh S, et al.** 2002. An oligopeptide transporter gene family in *Arabidopsis.* Plant Physiol. **128**:21–29.
30. **Krawchuk MD, Wahls WP.** 1999. High-efficiency gene targeting in *Schizosaccharomyces pombe* using a modular, PCR-based approach with long tracts of flanking homology. Yeast. **15**:1419–1427.
31. **Lee DH, Goldberg AL.** 1998. Proteasome inhibitors: valuable new tools for cell biologists. Trends Cell Biol. **8**:397–403.
32. **Liu C, Apodaca J, Davis LE, Rao H.** 2007. Proteasome inhibition in wild-type yeast *Saccharomyces cerevisiae* cells. Biotechniques **42**:158–162.
33. **Ljungdahl PO.** 2009. Amino-acid-inducing signalling via the SPS-sensing pathway in yeast. Biochem. Soc. Trans. **37**:242–247.
34. **Lubkowitz MA, et al.** 1998. *Schizosaccharomyces pombe isp4* gene encodes a transporter representing a novel family of oligopeptide transporters. Mol. Microbiol. **28**:729–741.
35. **Lubkowitz MA, Hauser L, Breslav M, Naider F, Becker JM.** 1997. An oligopeptide transport gene from *Candida albicans.* Microbiology **143**: 387–396.
36. **Matsuyama A, et al.** 2004. pDUAL, a multipurpose, multicopy vector capable of chromosomal integration in fission yeast. Yeast **21**:1289–1305.
37. **Matsuzawa T, et al.** 2010. The *gld1*$^{+}$ gene encoding glycerol dehydrogenese is required for glycerol metabolism in *Schizosaccharomyces pombe.* Appl. Microbiol. Biotechnol. **87**:715–727.
38. **Maundrell K.** 1993. Thiamine-repressible expression vectors pREP and pRIP for fission yeast. Gene **123**:127–130.
39. **Moneton P, Sarthou P, Le Goffic F.** 1986. Transport and hydrolysis of peptides in *Saccharomyces cerevisiae.* J. Gen. Microbiol. **132**:2147–2153.
40. **Moreno S, Klar A, Nurse P.** 1991. Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe.* Methods Enzymol. **194**:795–823.
41. **Mukai Y, Matsuo E, Roth SY, Harashima S.** 1999. Conservation of histone binding and transcriptional repressor functions in a *Schizosaccharomyces pombe* Tup1p homolog. Mol. Cell. Biol. **19**:8461–8468.
42. **Nakase M, et al.** 2010. Mannosylinositol phosphorylceamide is a major sphingolipid component and is required for proper localization of plasmamembrane proteins in *Schizosaccharomyces pombe.* J. Cell Sci. **123**: 1578–1587.
43. **Nakase M, et al.** Intracellular trafficking and ubiquitination of the *Schizosaccharomyces pombe* amino acid permease Aat1p. Microbiology, in press.
44. **Perry JR, Basrai MA, Steiner HY, Naider F, Becker JM.** 1994. Isolation and characterization of a *Saccharomyces* peptide transport gene. Mol. Cell. Biol. **14**:104–115.
45. **Reuss O, Morschhäuser J.** 2006. A family of oligopeptide transporters in required for growth of *Candida albicans* on proteins. Mol. Microbiol. **60**:795–812.
46. **Rubio-Allaga I, Daniel H.** 2008. Peptide transporters and their roles in physiological processes and drug disposition. Xonobiotica **38**:1022–1042.
47. **Sato M, Dhut S, Toda T.** 2005. New drug-resistant cassettes for gene disruption and epitope tagging in *Schizosaccharomyces pombe.* Yeast **22**: 583–591.
48. **Sriram SM, Kim BY, Kwon YT.** 2011. The N-end rule pathway: emerging functions and molecular principles of substrate recognition. Nat. Rev. Mol. Cell Biol. **21**:735–747.
49. **Steiner H-Y, Naider F, Becker JM.** 1995. The PTR family: a new group of peptide transporter. Mol. Microbiol. **16**:825–834.
50. **Takeda K, Mori A, Yanagida M.** 2011. Identification of genes affecting the toxicity of anti-cancer drug Bortezomib by genome-wide screening in *S. pombe.* PLoS One **6**:e22021.
51. **Takeda K, Yanagida M.** 2005. Regulation of nuclear proteasome by Rhp6/Ubc2 through ubiquitination and destruction of the sensor and anchor Cut8. Cell **122**:393–405.
52. **Thakur A, Kaur J, Bachhawat AK.** 2008. Pgt1, a glutathione transporter from the fission yeast *Schizosaccharomyces pombe.* FEMS Yeast Res. **8**:916–929.
53. **Turner GC, Du F, Varshavsky A.** 2000. Peptides accelerate their uptake by activating a ubiquitin-dependent proteolytic pathway. Nature **405**: 579–583.
54. **Varshavsky A.** 2011. The N-end rule pathway and regulation by proteolysis. Protein Sci. **20**:1298–1345.
55. **Wiles AM, Cai H, Naider F, Becker JM.** 2006. Nutrient regulation of oligopeptide transport in *Saccharomyces cerevisiae.* Microbiology **152**: 3133–3145.
56. **Xia Z, Turner GC, Hwang CS, Varshavsky A.** 2008. Amino acids induce peptide uptake via accelerated degradation of CUP9, the transcriptional repressor of the PTR2 peptide transporter. J. Biol. Chem. **283**:28958–28968.
57. **Xia Z, et al.** 2008. Substrate-binding sites of UBR1, the ubiquitin ligase of the N-end rule pathway. J. Biol. Chem. **283**:24011–24028.
58. **Zenker M, et al.** 2005. Deficiency of UBR1, a ubiquitin ligase of the N-end rule pathway, causes pancreatic dysfunction, malformations and mental retardation (Johanson-Blizzard syndrome). Nat. Genet. **37**:1345–1350.