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Ubiquilin interacts with ubiquitylated proteins and proteasome through its ubiquitin-associated and ubiquitin-like domains

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Abstract **Mammalian cells acquire tolerance against multiple stressors through the high-level expression of stress-responsible genes. We have previously demonstrated that protein-disulfide isomerase (PDI) together with ubiquilin are up-regulated in response to hypoxia/brain ischemia, and play critical roles in resistance to these damages. We show here that ubiquilin interacts preferentially with poly-ubiquitin chains and 19S proteasome subunits. Taken together, these results suggest that ubiquitin could serve as an adaptor protein that both interacts with PDI and mediates the delivery of poly-ubiquitylated proteins to the proteasome in the cytosol in the vicinity of the endoplasmic reticulum membrane.**

Keywords: Protein-disulfide isomerase; Ubiquilin; Endoplasmic reticulum; Up-regulation; Ubiquitin-proteasome system; Protein degradation

1. Introduction

The ubiquitin (Ub)-proteasome system is the major non-lysosomal proteolytic pathway in eukaryotes. Proteins are targeted for destruction by the addition of poly-Ub chains to a lysine residue of the substrate proteins. The ubiquitylation process involves E1 (Ub-activating enzyme), E2 (Ub-conjugating enzyme), and E3 (Ub-ligase) [1–3]. These multi-Ub-tagged substrates are recognized and degraded by the 26S proteasome.

Ubiquilin is a Ub-like (UBL) protein and has an N-terminal UBL domain and a C-terminal Ub-associated (UBA) domain in its structure. UBL proteins are classified into two subclasses, designated as type 1 and type 2 [1]. Type 1 UBL proteins such as SUMO and NEDD8 are covalently linked to target proteins in a manner analogous to that of Ub and are implicated in post-transcriptional protein modification [1,4]. On the other hand, type 2 UBL proteins possess UBL domains at their N-terminus, but do not form conjugates with substrate proteins [1]. However, extensive characterization of type 2 UBL proteins has elucidated that they play critical roles in Ub-dependent proteolysis. The physiological function and interactions among poly-ubiquitylated substrates, the proteasome, and UBL proteins have not been completely elucidated in mammalian cells.

We have previously succeeded in identifying that protein-disulfide isomerase (PDI) is up-regulated in response to hypoxia/brain ischemia and plays an important function in resistance to these damages [5]. Furthermore, PDI associates preferentially with ubiquilin, a mammalian type 2 UBL protein [6]. Interestingly, PDI together with ubiquilin has a protective role against hypoxic stress. In the present study, we indicate that ubiquilin may be associated with not only poly-Ub chains through the UBA domain but also with two subunits of 19S proteasome through the UBL domain. These results suggest that ubiquilin may serve as a shuttle factor that regulates the translocation of proteolytic substrates to the proteasome in concert with PDI under a brain ischemic or hypoxic condition.

2. Materials and methods

2.1. Materials

Anti-FLAG M5 monoclonal antibody (mAb) and anti-HA polyclonal antibody (pAb) were purchased from Sigma (St. Louis, MO) and Santa Cruz Biotech (Santa Cruz, CA), respectively. The yeast two-hybrid system and anti-β-actin mAb were purchased from CLONTECH (Palo Alto, CA). Anti-poly-Ub mAb was purchased from Nippon Bio Test Lab (Tokyo, Japan). Anti-calnexin NT and CT pAbs were purchased from StressGen (Victoria, Canada). All other reagents were obtained from Sigma.

2.2. Plasmids

Recombinant GST-ubiquilin and truncated mutants were generated as previously described [6]. Full-length Ub amino acid 52-residue ribosomal protein fusion (UBA52) and Ub cDNA were isolated from human neuroblastoma RNA by RT-PCR. The cDNAs encoding original human Rpn were kindly provided by Dr. Kawahara and Dr. Tanaka. Human Rpn constructs tagged with an HA epitope at the N-terminal (HA-Rpn1, HA-Rpn2, and HA-Rpn3) and at the C-terminal (Rpn10a-HA and Rpn10e-HA) were subcloned into mammalian expression vector pCR3.1, respectively.

2.3. Yeast two-hybrid assay

Plasmid pAS2-1 harboring cDNA for the UBA domain (Q541-S587) of the ubiquilin was generated as bait for library screening. Yeast two-hybrid screening was performed with a pretransformed cDNA library derived from human adult brain mRNA (CLONTECH) according to the manufacturer's protocol [7].

2.4. In vitro binding assay

GST-fusion proteins were prepared from *Escherichia coli* BL21 (DE3) as described previously and used for pull-down assay [6].

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Abbreviations: UBL, ubiquitin-like; UBA, ubiquitin-associated; NP, asparagine–proline repeats; Ub, ubiquitin; ER, endoplasmic reticulum; UBA52, ubiquitin amino acid 52-residue ribosomal protein fusion; UPR, unfolded protein response; ERAD, ER-associated degradation; mAb, monoclonal antibody; pAb, polyclonal antibody

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Briefly, to examine the interaction between ubiquilin-Ub and ubiquilin-proteasome, SH-SY5Y and HEK 293 cells were lysed in lysis buffer containing 50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM DTT, 5 mM EDTA, 1% Triton X-100, 0.1 μM MG132, 5 mM *N*-ethylmaleimide, and 10% glycerol, with complete protease inhibitors (Roche Diagnostics). For GST-pull down, lysates were incubated with either GST-ubiquilin or GST-truncated mutants immobilized on a glutathione–Sepharose 4B column (Amersham Pharmacia Biotech, Buckinghamshire, UK) at 4 °C for 4 h, followed by five washes with 1 ml of lysis buffer. Ubiquilin-associated UBA52, Ub, and proteasome were detected by SDS–PAGE/Western blot assay using anti-FLAG mAb, anti-poly-Ub mAb, and anti-HA pAb with an enhanced chemiluminescence detection method.

2.5. Immunoprecipitation and Western blotting analysis

Cell extracts were prepared in lysis buffer containing 50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM DTT, 5 mM EDTA, 1% Triton X-100, 0.1 μM MG132, 5 mM *N*-ethylmaleimide, and 10% glycerol, with complete protease inhibitors. For the co-immunoprecipitation assay, immunoprecipitates were prepared using anti-FLAG tag antibody M2 conjugated to agarose. Immune complexes were resolved by SDS–PAGE and then analyzed by Western blotting assay using anti-HA pAb and anti-poly-Ub mAb [8–11].

3. Results

3.1. Ubiquilin associates with poly-Ub

To explore the detailed functions of ubiquilin, we employed a yeast two-hybrid system to screen a human fetal brain cDNA library constructed in a pACT2 plasmid encoding the GAL4 activation domain using the C-terminal UBA domain in ubiquilin as bait (Fig. 1A, *top panel*). From among 1×10^7 transformants, several independent clones were isolated as determined by activation of the *his*, *ade*, and *lac*Z reporter genes. A BLAST search revealed that one of the isolated clones was UBA52, a clone consisting of Ub cDNA fused with ribosomal protein [12–14]. We next attempted to determine the site of the interaction between ubiquilin and UBA52 using a yeast two-hybrid system. As shown in Fig. 1B, the C-terminal of ubiquilin is sufficient for interaction with full-length UBA52. Since UBA52 is a Ub fusion protein, we investigated whether Ub also interacts with the C-terminal of ubiquilin. Interestingly, both Ub and UBA52 could bind to ubiquilin (Fig. 1B). Then, to confirm whether ubiquilin interacts with Ub, we employed an in vitro pull-down assay and co-immunoprecipitation using lysates in mammalian cells transfected with ubiquilin and Ub. Cell lysates transfected with HA-tagged Ub were incubated with GST or GST fused with ubiquilin (GST-ubiquilin). HA-tagged Ubs were detected in a high molecular weight smear eluted from the beads binding to GST-ubiquilin but not from those binding to GST alone (Fig. 1C). To determine whether the interaction between ubiquilin and Ub occurs in mammalian cells, human neuroblastoma SH-SY5Y cells were transiently transfected with FLAG-tagged ubiquilin together with HA-tagged Ub (Fig. 1D). Whole cell lysates were immunoprecipitated with anti-FLAG mAb and then analyzed by Western blotting using anti-HA pAb. The

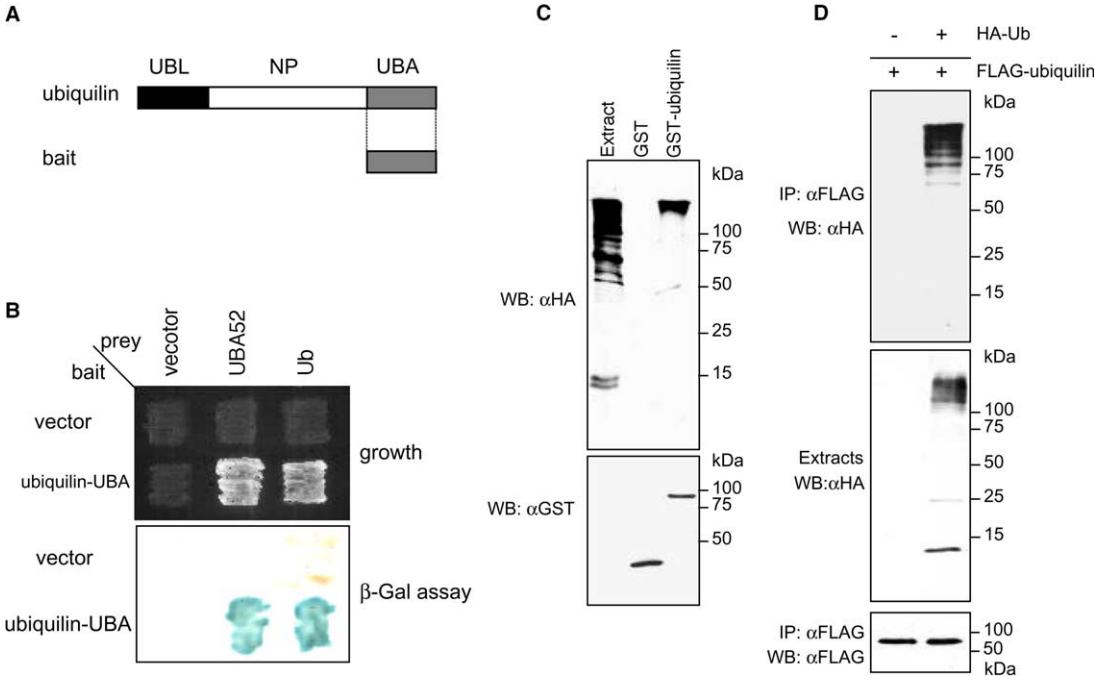

Fig. 1. Association of ubiquilin with UBA52 and Ub. (A) Construction of the UBA domain of ubiquilin as bait (*top panel*). (B) The assay for interaction of ubiquilin with UBA52 and Ub by yeast two-hybrid system. Interaction of ubiquilin with UBA52 and Ub was tested by histidine-prototrophic growth (*top panel*) and β-galactosidase activity (*bottom panel*). The UBA domain of ubiquilin (residues 541–587) was used as bait (*top and bottom panel*). (C) Recombinant GST and GST-ubiquilin were incubated with SH-SY5Y extracts transfected with HA-Ub for 4h, and GST-bound materials were eluted, fractionated by Tris–tricine 15% SDS–PAGE, and immunoblotted with anti-HA pAb or anti-GST mAb. The positions of mono-Ub are indicated at the left of the gel. (D) Co-immunoprecipitation between ubiquilin and Ub was performed in SH-SY5Y cells transfected with plasmid for FLAG-ubiquilin in the presence or absence of HA-Ub cDNA. Equal amounts of cell lysates were immunoprecipitated with anti-FLAG mAb. The immune complex bound to protein G–Sepharose beads was then washed with lysis buffer, resolved by SDS–PAGE, and analyzed by Western blotting using anti-HA pAb (*first panel*) and anti-FLAG mAb (*third panel*). Total cell lysates were analyzed by Western blotting using anti-HA pAb (*second panel*), The positions of mono-Ub are indicated at the left of the gel.

high molecular weight smear was also detected in immunoprecipitates with anti-FLAG antibody from cells co-transfected with HA-Ub (Fig. 1D).

3.2. The interaction domain of ubiquilin with poly-Ub

To determine the domain of ubiquilin required for poly-Ub binding, several truncated mutants of ubiquilin fused to GST were expressed in *E. coli*. GST-fused full-length and truncated mutants of ubiquilin were applied to glutathione–Sepharose and then incubated with SH-SY5Y cell extracts. The interaction abilities were examined by immunoblotting analysis with anti-poly-Ub antibody. As shown in Fig. 2A, the mutants lacking a UBA domain (residues 113–587) failed to interact with poly-Ub, whereas wild-type (residues 1–587) and the mutants containing a UBA domain (residues 113–587 and 541–587) were capable of binding to poly-Ub with high molecular weight smear. Thus, only the UBA domain of ubiquilin has a strong ability to bind with poly-Ub. Because type 2 UBL proteins have been implicated in the accumulation of poly-Ub chains in cells [15,16], we investigated whether overexpression of ubiquilin also induces such accumulation. Ubiquilin and the mutant lacking a UBA domain (residues 1–540) were transfected into human neuroblastoma SH-SY5Y cells, and then the lysates were subjected to Western blotting with the anti-poly-Ub mAb. Overexpression of ubiquilin led to the accumulation of a large number of poly-Ub chains in human neuroblastoma SH-SY5Y cells, whereas expression of the mutant lacking the C terminus (residues 1–540) did not (Fig. 2C). This result suggests that the C-terminal UBA domain of ubiquilin is required for binding and the increased accumulation of poly-Ub chains in mammalian cells.

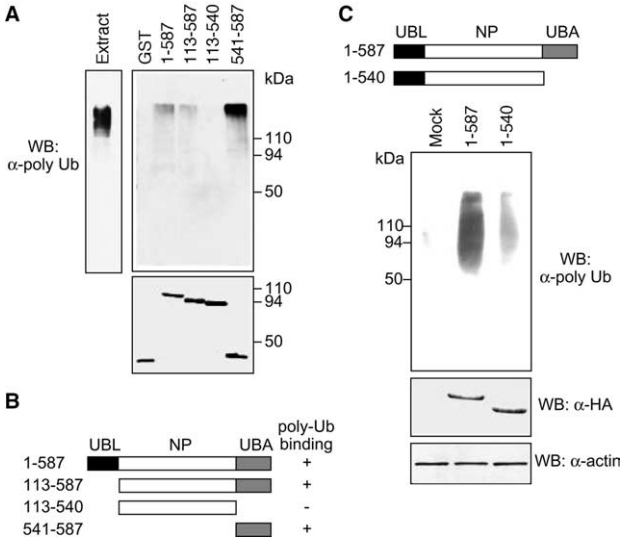

Fig. 2. The UBA domain of ubiquilin is required for its binding. (A) recombinant GST-ubiquilin and GST-truncated mutants were incubated with SH-SY5Y cell extracts for 4 h, and GST-bound materials were analyzed by Western blotting with the anti-poly-Ub mAb (*upper panel*) and anti-GST mAb (*lower panel*) antibodies. (B) A diagram of ubiquilin constructs. Binding abilities of the mutants are shown on the right. (C) HA-tagged ubiquilin (residues 1–587) and HA-tagged deletion UBA of ubiquilin (residues 1–540) were transfected into SH-SY5Y cells, and proteins from the extracts were resolved by SDS–PAGE and analyzed by Western blotting using anti-poly-Ub mAb (*first panel*), anti-HA pAb (*second panel*), and anti-β-actin mAb (*third panel*) antibodies.

3.3. Ubiquilin interacts with human non-ATPase proteasome 19S subunits

Although hPLIC proteins, which are ubiquilin homologs, have been reported to interact with some 20S complex subunits of 26S proteasome [17], little is known about the interaction with 19S components of 26S proteasome. To examine the possibility that the 19S complex subunit(s) is capable of interacting with ubiquilin, a GST-ubiquilin fusion protein was coupled to glutathione–Sepharose beads and incubated with mammalian cell extracts containing transiently expressed HA-tagged Rpn1, Rpn2, Rpn3, Rpn10a, and Rpn10e. The abilities of the subunits to interact with ubiquilin were determined by Western blotting analysis using anti-HA pAb. As shown in Fig. 3A, the GST-pull down assay revealed that each of the subunits Rpn3, Rpn10a, and Rpn10e formed a complex with ubiquilin. However, the truncated mutant ubiquilin lacking a UBL domain did not interact with the Rpn3 and Rpn10a subunits (Fig. 3B). Next, to determine whether ubiquilin binds to the subunit of 19S proteasome in vivo, immunoprecipitation was performed using lysates in HEK 293 cells overexpressing Rpn10a-HA with or without FLAG-ubiquilin. Whole lysates were immunoprecipitated with anti-FLAG mAb and then

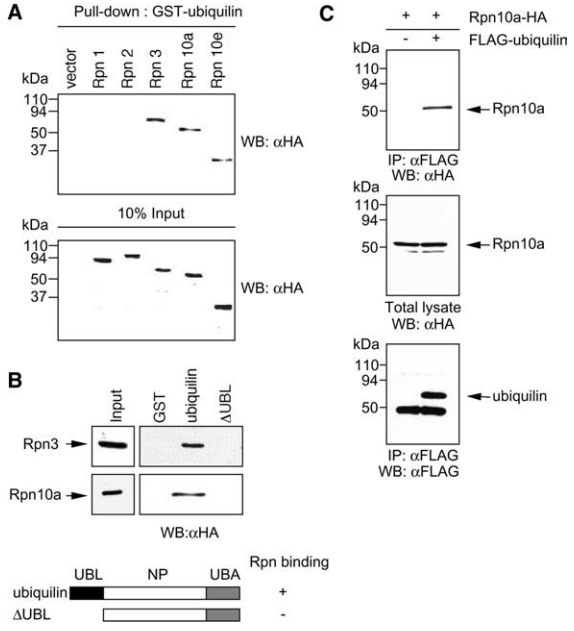

Fig. 3. Ubiquilin binds to Rpn3, Rpn10a, and Rpn10e through its UBL domain. (A) Human 293 cell lysates were transiently transfected with several vectors, HA-tagged Rpn1, Rpn2, Rpn3, RPn10a, and Rpn10e, incubated with glutathione–agarose beads bound with GST-ubiquilin, and then washed, resolved by SDS–PAGE, and analyzed by Western blotting using anti-HA pAb. (B) Human HEK 293 cell lysates were transiently transfected of one of two vectors, HA-tagged Rpn10a, and Rpn3, incubated with glutathione-agarose beads bound with GST-ubiquilin and GST-ΔUBL lacking the UBL domain (residues 113–587). The binding ability was analyzed by Western blotting using anti-HA pAb. Binding abilities of the mutants are shown in the right panel. (C) HEK293 cells transiently transfected with plasmid for HA-tagged Rpn10a in the presence or absence of FLAG-ubiquilin. Equal amounts of cell lysates were immunoprecipitated with anti-FLAG mAb. The immune complex bound to protein G-Sepharose beads was then washed with lysis buffer, resolved by SDS-PAGE, and analyzed by Western blotting using anti-HA pAb (*first panel*) and anti-FLAG mAb (*third panel*). Total cell lysates were analyzed by Western blotting using anti-HA pAb (*second panel*).

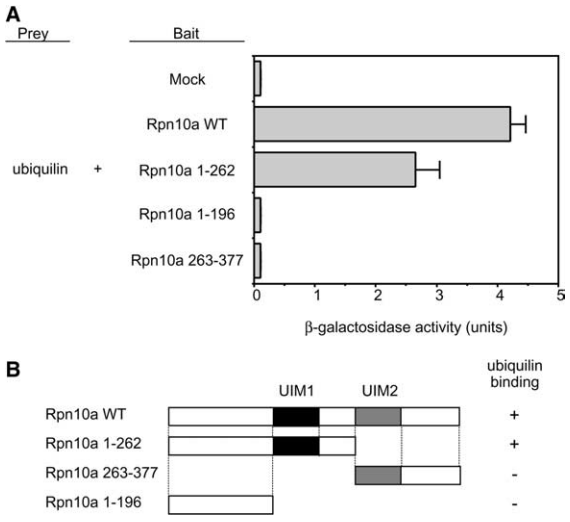

Fig. 4. Determination of the domain of Rpn10a that interacts with ubiquilin. (A) The expression vectors encoding wild-type Rpn10a or various deletion mutants fused with the GAL4 DNA-binding domain were co-transformed with full-length ubiquilin fused with the GAL4 DNA-activating domain in AH109 cells. Each transformant was assayed for β-galactosidase activity. (B) Summary of human Rpn10a proteins. Binding abilities of the mutants are shown.

analyzed by Western blotting using anti-HA pAb. Rpn10a-HA was detected in immunoprecipitates with anti-FLAG from cells co-transfected with Rpn10a-HA and FLAG-ubiquilin (Fig. 3C).

3.4. Determination of the ubiquilin-binding domain in Rpn10a

It has been elucidated that Rpn10a interacts tightly with the poly-Ub chains through a Ub-interacting motif (UIM) [18]. Rpn10a contains at least two independent UIM domains. Hence, we attempted to determine which domain of Rpn10a is required for interaction with ubiquilin using a two-hybrid system. As shown in Fig. 4, the wild-type Rpn10a (1–377) and 1–262 mutants containing a UIM1 domain bound to ubiquilin, whereas the 1–196 and 262–377 mutants lacking a UIM1 domain did not. These results indicate that the ubiquilin-binding region is located within Rpn10a (197–262), which contains the UIM1 domain.

4. Discussion

4.1. Ubiquilin binds to multi-Ub chains

The aim of this study was to isolate and identify proteins capable of interacting with PDI/ubiquilin, because we have reported previously that up-regulation of ubiquilin by hypoxia may be involved in the acquisition of tolerance against this stress [5,6]. In the present study, we carried out yeast two-hybrid screening to isolate proteins, and succeeded in characterizing the Ub-fusion protein made up of UBA52 and Ub as a protein that interacts with ubiquilin. This interaction was mediated via the UBA domain of ubiquilin. The UBA domain is a small domain composed of approximately 40 residues that has thus far been identified in E2, E3, and other proteins linked to ubiquitination. It has been proposed that the general function of UBA domains in type 2 UBL proteins is to interact with poly-Ub chains [19]. In mammalian cells, ubiquilin preferentially interacts with poly-Ub chains, but not with mono-Ub, via its UBA domain (Figs. 1 and 2). In fact, it has been reported that several Ub fusion proteins (UBI1, 2, 3) isolated as the candidate proteins that interact with budding yeast Dsk2p, a type 2 UBL protein in a yeast two-hybrid screening [15]. However, they concluded that the binding between Dsk2p and poly-Ub, but not mono-Ub, was evident. Taken together, these results indicate that there were two reasons for the lack of interaction between mono-Ub and ubiquilin in mammalian cells: (1) ubiquilin specifically interacts with poly-Ub in a yeast two-hybrid system and (2) the binding affinity of poly-Ub is more potent than that of mono-Ub. Thus, we speculated that ubiquilin plays a critical role in the interaction with poly-Ub chains, rather than in the interaction with mono-Ub. In addition, a significant accumulation of poly-Ub chains was evident in ubiquilin-overexpressing cells (Fig. 2C). It has been reported that observation of poly-Ub chains is dependent on the preferential de-ubiquitination enzymatic activities that are involved in the persistence of poly-Ub chains [20–22]. Thus, the high content of ubiquilin in cells may function to stimulate the formation of poly-Ub chains or inhibit the de-ubiquitylation, and bind the poly-Ub proteins via its UBA domain.

4.2. Ubiquilin also associates with subunits of 19S proteasome

Poly-ubiquitylated proteins are first recognized by interaction with 19S regulatory complex and then degraded by the 20S core particle of proteasome. In particular, Rpn10, a subunit of the 19S component, has been shown to bind tightly to poly-Ub chains, but not to mono-Ub, and to be implicated in the recognition of poly-Ub substrates in yeast [20–24]. In addition, the 19S base subunits Rpn1 and Rpn2 have previously been identified as the receptor subunits for UBL domain proteins in yeast [25,26]. On the other hand, hPLIC proteins (ubiquilin-like proteins) have been reported to interact with some 20S core complex subunits [17]. It has since been suggested that not only HR23B but also hPLIC2 (ubiquilin 2) interacts with S5a (Rpn 10a), a 19S component of 26S proteasome [18,27]. Therefore, we here attempted to resolve the question of whether or not ubiquilin associates with 19S proteasome subunits in mammalian cells. Surprisingly, Rpn3, Rpn10a, and Rpn10e possibly interacted with ubiquilin through the UBL domain (Fig. 3). Although Rpn1 and Rpn2 can interact with UBL domains in yeast [25,26], we could not actually observe the interaction between them. Unfortunately, we do not have any clear evidence to demonstrate the differences in interactions as seen in yeast and mammalian cells at present. The differences may depend on a kind of UBL proteins or species. Furthermore, we cannot exclude the possibility that the interaction of ubiquilin with proteasome subunits was not always direct, because a candidate protein isolated by a yeast two-hybrid system sometimes shows the bridged interaction with a target protein through another protein. Recent reports suggest that Rpn10a interacts with a poly-Ub chain-binding protein mediated by the multi-Ub chain-binding sites, UIM1 (196–241) and UIM2 (263–307) [28–30]. UIM2 has been shown to exhibit higher affinity for multi-Ub chains than UIM1 [30]. In this study, we tried to elucidate the possible ubiquilin-binding region in Rpn10a. Our results indicated that the region in Rpn10a responsible for the interaction with ubiquilin is identical to its UIM1, but not its UIM2 domain (Fig. 4A). Hence, the Rpn10a subunit located in the 19S proteasome can interact with not only poly-Ub

chains or poly-ubiquitylated proteins but also with the UBL domain of ubiquilin. Taking these results together, we here propose that ubiquilin may play a crucial function in mediating the protein degradation system via direct interaction with both poly-Ub proteins and some subunits of the 19S proteasome complex composed of base and lid sub-assemblies in mammalian cells.

4.3. Possible involvement of ubiquilin in endoplasmic reticulum (ER) stress-associated protein degradation

The adaptation in response to ER stress constitutes two distinct processes: an unfolded protein response (UPR) and an ER-associated degradation (ERAD). Ubiquilin may be a possible target gene for UPR-induced transcription, since that protein is induced by hypoxia (ER stress) [6]. Ubiquilin has been identified as a protein that may interact with presenilin, which is localized in the ER membrane [31]. These authors demonstrated that the ubiquilin staining pattern co-localized almost perfectly with the presenilin staining pattern, strongly suggesting that ubiquilin is an ER-membrane or ER-associated protein. Furthermore, we have demonstrated that ubiquilin preferentially interacts with PDI in vivo and in vitro [5,6]. From these observations, we speculated that the ability of ubiquilin in ER to interact with both ubiquitylated proteins and the proteasome in cytosol makes them likely adapter molecules for delivering unfolded substrates to the proteasome in the ER.

UPR induces multiple ER and secretory pathway genes, including ERAD-related genes. This system eliminates misfolded ER proteins via degradation through the Ub-proteasome pathway in cytosol [32,33]. We considered that it would be of interest to address the relationship between type 2 UBL proteins, including ubiquilin located in the ER and in the ERAD system, since recent exciting findings have suggested the possibility that type 2 UBL proteins such as Herp and Rad23 are implicated in the ERAD system [34,35]. Therefore, these findings led us to conjecture that ubiquilin may play some role in the ERAD system. We previously found that PDI/ubiquilin up-regulation is involved in cell survival [5,6]. In light of this finding, up-regulated ubiquilin may result in acquisition of tolerance against stresses via control of the ERAD system.

In summary, ubiquilin may be associated with poly-Ub and subunits of the 19S proteasome in mammalian cells. Unfortunately, the mechanism by which ubiquilin promotes the degradation of the misfolded proteins remains unclear. Because the ERAD can be overwhelmed in several neurodegenerative diseases, including Alzheimer's and Parkinson's, further analysis of the cellular and molecular mechanisms of ubiquilin in the ER-stress-associated protein degradation could lead to novel concepts in neurodegenerative diseases.

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References

[1] Hershko, A. and Ciechanover, A. (1998) Annu. Rev. Biochem. 7, 425–479.
[2] Voges, D., Zwickl, P. and Baumeister, W. (1999) Annu. Rev. Biochem. 68, 1015–1068.
[3] Glickman, M.H. and Ciechanover, A. (2002) Physiol. Rev. 82, 373–428.
[4] Melchior, F. (2000) Annu. Rev. Cell Dev. Biol. 16, 591–626.
[5] Tanaka, S., Uehara, T. and Nomura, Y. (2000) J. Biol. Chem. 275, 10388–10393.
[6] Ko, H.S., Uehara, T. and Nomura, Y. (2002) J. Biol. Chem. 277, 35386–35392.
[7] Ito, A., Uehara, T. and Nomura, Y. (2000) FEBS Lett. 470, 360–364.
[8] Araya, R., Uehara, T. and Nomura, Y. (1998) FEBS Lett. 439, 168–172.
[9] Uehara, T., Matsuno, J., Kaneko, M., Nishiya, T., Fujimuro, M., Yokosawa, H. and Nomura, Y. (1999) J. Biol. Chem. 274, 15875–15882.
[10] Uehara, T., Kikuchi, Y. and Nomura, Y. (1999) J. Neurochem. 72, 196–205.
[11] Furuta, Y., Uehara, T. and Nomura, Y. (2003) J. Cereb. Blood Flow Met. 23, 962–971.
[12] Baker, R.T. and Board, P.G. (1991) Nucleic Acids Res. 19, 1035–1040.
[13] Webb, G.C., Baker, R.T., Coggan, M. and Board, P.G. (1994) Genomics 19, 567–569.
[14] Sun, L., Pan, X., Wada, J., Haas, C.S., Wuthrich, R.P., Danesh, F.R., Chugh, S.S. and Kanwar, Y.S. (2002) J. Biol. Chem. 277, 29953–29962.
[15] Funakoshi, M., Sasaki, T., Nishimoto, T. and Kobayashi, H. (2002) Proc. Natl. Acad. Sci. USA 99, 745–750.
[16] Chen, L. and Madura, K. (2002) Mol. Cell. Biol. 22, 4902–4913.
[17] Kleijnen, M.F., Shih, A.H., Zhou, P., Kumar, S., Soccio, R.E., Kedersha, N.L., Gill, G. and Howley, P.M. (2000) Mol. Cell 6, 409–419.
[18] Hiyama, H., Yokoi, M., Masutani, C., Sugasawa, K., Maekawa, T., Tanaka, K., Hoeijmakers, J.H.J. and Hanaoka, F. (1999) J. Biol. Chem. 274, 28019–28025.
[19] Hofmann, K. and Bucher, P. (1996) Trends. Biochem. Sci. 21, 172–173.
[20] Wilkinson, C.R.M., Ferrell, K., Penney, M., Wallace, M., Dubiel, W. and Gordon, C. (2000) J. Biol. Chem. 275, 15182–15192.
[21] Xie, Y. and Varshavsky, A. (2000) Proc. Natl. Acad. Sci. USA 97, 2497–2502.
[22] Takeuchi, J., Fujimuro, M., Yokosawa, H., Tanaka, K. and Toh-e, A. (1999) Mol. Cell. Biol. 19, 6575–6584.
[23] Deveraux, Q., Ustrell, V., Pickart, C. and Rechsteiner, M. (1994) J. Biol. Chem. 269, 7059–7061.
[24] Ferrell, K., Deveraux, Q., van Nocker, S. and Rechsteiner, M. (1996) FEBS Lett. 381, 143–148.
[25] Elasser, S., Gali, R.R., Schwickart, M., Larsen, C.N., Leggett, D.S., Müller, B., Feng, M.T., Tübing, F., Dittmar, G.A.G. and Finley, D. (2002) Nature Cell Biol. 4, 725–730.
[26] Saeki, Y., Sone, T., Toh-e, A. and Yokosawa, H. (2002) Biochem. Biophys. Res. Commun. 296, 813–819.
[27] Walters, K.J., Kleijnen, M.F., Goh, A.M., Wagner, G. and Howley, P.M. (2002) Biochemistry 41, 1767–1777.
[28] Haracska, L. and Udvardy, A. (1995) Eur. J. Biochem. 231, 720–725.
[29] Fu, H., Sadis, S., Rubin, D.M., Glickman, M., van Nocker, S., Finley, D. and Vierstra, R.D. (1998) J. Biol. Chem. 273, 1970–1981.
[30] Young, P., Deveraux, Q., Beal, R.E., Pickart, C.M. and Rechsteiner, M. (1998) J. Biol. Chem. 273, 5461–5467.
[31] Mah, A., Perry, G., Smith, M.A. and Monteiro, M.J. (2000) J. Cell. Biol. 151, 847–862.
[32] Travers, K.J., Patil, C.K., Wodicka, L., Lockhart, D.J., Weissman, J.S. and Walter, P. (2000) Cell 101, 249–258.
[33] Ng, D.T., Spear, E.D. and Walter, P. (2000) J. Cell. Biol. 150, 77–88.
[34] Kokame, K., Agarwala, K.L., Kato, H. and Miyata, T. (2000) J. Biol. Chem. 275, 32846–32853.
[35] Suzuki, T., Park, H., Kwofie, M.A. and Lennarz, W.J. (2001) J. Biol. Chem. 276, 21601–21607.