Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 342 (2006) 253–258
BBRC
www.elsevier.com/locate/ybbrc

UnpEL/Usp4 is ubiquitinated by Ro52 and deubiquitinated by itself

Keiji Wada, Tetsu Kamitani *

Department of Cardiology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Received 18 January 2006
Available online 6 February 2006

Abstract

The autoantigen Ro52 is an E3 ubiquitin ligase that can ubiquitinate itself (self-ubiquitination). Recently, we showed that UnpEL/Usp4 is an isopeptidase that can deconjugate ubiquitin from self-ubiquitinated Ro52. Here, we showed that UnpEL is ubiquitinated by Ro52 in cooperation with UbcH5B in vitro. We also showed that UnpEL is ubiquitinated by Ro52 in HEK293T cells. Interestingly, a catalytically inactive UnpEL mutant was strongly ubiquitinated by Ro52 in HEK293T cells. These results suggest that wild-type UnpEL is ubiquitinated by Ro52 and deubiquitinated by itself (self-deubiquitination), while mutant UnpEL is ubiquitinated by Ro52 but not deubiquitinated by itself. In conclusion, Ro52 and UnpEL transregulate each other by ubiquitination and deubiquitination.

Keywords: Ro52; Sjögren's syndrome; Ubiquitin; TRIM; RBCC; Ubiquitin ligase; UnpEL; Usp4; DUB; Deubiquitinating enzyme

Ubiquitin, a 76-amino-acid polypeptide, plays a key role in the ubiquitin-proteasome system [1]. Initially in ubiquitination, the C-terminus of ubiquitin, which contains a conserved Gly residue, is activated to form a thiol-ester linkage with the Cys residue of the E1 ubiquitin-activating enzyme. Activated ubiquitin is then transferred to the E2 ubiquitin-conjugating enzyme to form another thiol-ester linkage. Subsequently, with the aid of the E3 ubiquitin ligase, ubiquitin becomes covalently attached to the Lys residues of target proteins through the formation of isopeptide bonds [1]. The internal Lys residue of ubiquitin can also form an isopeptide bond with the C-terminal Gly residue of another ubiquitin molecule to create a polyubiquitin chain. This chain serves as a proteasome-targeting signal [1]. In the proteasome, polyubiquitinated proteins are then degraded in an ATP-dependent manner [1]. By targeting ubiquitinated proteins to the proteasome for degradation, ubiquitination plays a critical role in many biological events [1].

Ro52 is a classic autoantigen to which autoantibodies are most commonly found in patients with Sjögren's syndrome [2]. It is a RING-finger protein that belongs to a RBCC (RING-finger/B-box/coiled-coil) family (also known as the TRIM [tripartite motif] family) [3]. Recently, we reported that Ro52 functions as an E3 ubiquitin ligase and ubiquitinates itself in cooperation with E2 ubiquitin-conjugating enzyme UbcH5B [4–6]. Although the self-ubiquitination of Ro52 was shown in our previous studies, the other substrates have been unknown.

UnpEL (also known as Usp4 or Unph) was first identified as a protein with a sequence highly similar to that of the Tre-2 (Usp6) oncogenic protein [7]. The injection of UnpEL-overexpressing NIH3T3 cells into nude mice subsequently showed UnpEL to be an oncogenic protein [8]. In keeping with this, Gray et al. reported that UnpEL mRNA levels are consistently elevated in small-cell tumors and adenocarcinomas of the lung [9]. Structurally, UnpEL possesses both Cys and His boxes, which are essential motifs for the activity of deubiquitinating enzymes [10]. Because of this structure, UnpEL has been thought to function as a deubiquitinating enzyme in mammalian cells.

Recently, some insight into the mechanism of UnpEL's function came from a study conducted by Donato et al. in which they screened the human cDNA library by a yeast two-hybrid assay using Ro52 as bait. This revealed that UnpEL interacts with Ro52 in yeast cells [11], suggesting the functional relationship between Ro52 and UnpEL.

* Corresponding author. Fax: +1 713 563 0424.
E-mail address: tkamitani@mdanderson.org (T. Kamitani).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.01.144

Indeed, very recently, we showed that UnpEL specifically deubiquitinates Ro52 in human cells by its isopeptidase activity [12]. In addition, in our recent study, we detected high-molecular-weight bands of UnpEL when it was coexpressed with Ro52 (unpublished data), implying that UnpEL is ubiquitinated by Ro52. We conducted the present study to test this possibility.

Materials and methods

Cell culture. Human embryonic kidney HEK293T cells (American Type Culture Collection, Manassas, VA) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics.

Antibodies. Mouse anti-FLAG antibody (M5) and rabbit anti-FLAG antibody were purchased from Sigma Chemical Company (St. Louis, MO). Mouse anti-RH antibody (specific for the amino-acid sequences RGSHHHH and GGSHHHH) was purchased from Qiagen (Santa Clara, CA). Mouse anti-HA antibody (16B12) was purchased from Covance (Richmond, CA). Rabbit anti-HA antibody was purchased from Zymed (South San Francisco, CA).

Mammalian expression plasmids and their transfection. To express RH-tagged Mdm2 and Parkin, their cDNAs were amplified by a polymerase chain reaction using appropriate primers from the human brain cDNA library (Clontech, Palo Alto, CA) and subcloned into pcDNA3/RH-N [13]. To express RH-tagged wild-type UnpEL and its mutant C311A, their cDNAs were also subcloned into pcDNA3/RH-N [13]. To express FLAG-tagged Ro52, its cDNA [4] was subcloned into pcDNA3/FLAG-N [14]. All other expression plasmids had been previously prepared. To express FLAG-tagged wild-type UnpEL and its mutant C311A, pcDNA3/FLAG-UnpEL(wt) and pcDNA3/FLAG-UnpEL(C311A) were used, respectively [12]. To express RH-tagged wild-type Ro52, its mutant C16A, HIF1α(Δ*C*), and HA-tagged ubiquitin, we used pcDNA3/Ro52-RH, pcDNA3/Ro52(C16A)-RH, pcDNA3/HIF1α(Δ*C*)-RH, and pcDNA3/HA-Ub, respectively [5]. These plasmids were transfected into HEK293T cells using FuGENE6 (Roche Applied Science, Indianapolis, IN). The transfected cells were then harvested for immunoprecipitation or TALON-bead precipitation 20 h after transfection.

In vivo ubiquitination assay. HEK293T cells were cultured in a 6-cm dish and transfected with total 5 μg of plasmid DNAs to coexpress FLAG-UnpEL (wild-type or its mutant C311A) and HA-ubiquitin with RH-tagged E3 ubiquitin ligase, such as Ro52, Mdm2, or Parkin. Twenty hours after transfection, the transfected cells on the culture dish were washed twice with phosphate-buffered saline (PBS) and incubated in the lysis buffer [50 mM Tris–HCl (pH 7.5), 100 mM NaCl, and 0.1% sodium dodecyl sulfate (SDS)] containing a protease inhibitor cocktail (Roche) for 30 min at room temperature to prepare total cell lysate. The lysate was passed through a 22-gauge needle to shear the DNA and then centrifuged at 100,000*g* at 16 °C for 30 min. The supernatant was incubated with anti-FLAG M2 agarose beads (Sigma) at room temperature for 2 h to immunoprecipitate FLAG-UnpEL. After incubation, the beads were washed three times with Tris-buffered saline (TBS) and treated for 1 h at 50 °C in sample treating solution containing 2% SDS and 5% β-mercaptoethanol. The solubilized FLAG-UnpEL was then analyzed by Western blotting using rabbit anti-FLAG antibody to detect all derivatives of FLAG-UnpEL and rabbit anti-HA antibody to detect ubiquitinated FLAG-UnpEL.

In vitro ubiquitination assay. All eukaryotic expression plasmids for the assay had been previously prepared [5]. An in vitro ubiquitination assay was performed using these plasmids [5]. Briefly, FLAG-tagged UnpEL(C311A) was overexpressed in HEK293T cells by means of plasmid transfection. Twenty hours after transfection, the cells were lysed to prepare total cell lysate, as described above. FLAG-UnpEL(C311A) in the lysate was then immunoprecipitated by anti-FLAG beads (Sigma). Afterwards, FLAG-UnpEL(C311A) immobilized on the beads was incubated in 50 μl of the reaction mixture [50 mM Tris–HCl (pH 7.5), 2 mM $MgCl_2$, and 2 mM DTT] containing RH-tagged ubiquitin (0.1 μg), rabbit E1 enzyme (0.1 μg; Boston Biochem, Cambridge, MA), poly-His-tagged recombinant E2 enzyme (1.0 μl of crude bacterial lysate), maltose-binding protein (MBP)-fused Ro52 (0.1 μg) [5], and 2 mM MgATP (Cat. #B-20; Boston Biochem). After the reaction, FLAG-UnpEL(C311A) immobilized on the beads was washed to remove the reaction mixture and eluted by incubating the beads with 100 μg/ml FLAG peptide (Sigma). The eluted FLAG-UnpEL(C311A) was analyzed by Western blotting using mouse anti-FLAG antibody (M5; Sigma) to detect all derivatives of FLAG-UnpEL(C311A) and mouse anti-RH antibody (Qiagen) to detect ubiquitinated FLAG-UnpEL(C311A).

Treatment with proteasome inhibitor. A proteasome inhibitor, MG132, was purchased from Calbiochem (San Diego, CA) to treat cells, as described previously [15]. In brief, 1×10^6 HEK293T cells were transfected by FuGENE6. After an overnight culture, the culture medium was replaced with fresh medium containing MG132 (40 μM), and the cells were cultured further at 37 °C for 6 h. Then the cells were lysed to prepare total cell lysate, which was used for TALON-bead precipitation.

TALON-bead precipitation of RH-tagged proteins. To investigate the effect of MG132 on the stability of UnpEL or HIF1α(Δ*C*), we purified RH-tagged UnpEL or HIF1α(Δ*C*) by TALON-bead precipitation and analyzed their expression level by Western blotting. Since the sequence of the RH tag is RGSHHHHHH, RH-tagged UnpEL or HIF1α(Δ*C*) can be purified by cobalt-immobilized resin beads (TALON beads, Clontech) [16]. The total cell lysate of the transfectants expressing RH-tagged UnpEL or HIF1α(Δ*C*) was prepared in lysis buffer [20 mM Tris–HCl (pH 8.0), 6 M guanidine–HCl, and 100 mM NaCl]. DNA in the sample was sheared with a 22-gauge needle and then the lysate was centrifuged at 100,000*g* for 30 min at 15 °C. The supernatant was incubated with TALON beads for 1 h at room temperature. The beads were washed once with lysis buffer, followed by two washings with washing buffer [20 mM Tris–HCl (pH 7.0), 15 mM imidazole, 8 M urea, and 100 mM NaCl]. The beads were then washed twice with PBS and treated for 1 h at 50 °C in sample treating solution containing 2% SDS and 5% β-mercaptoethanol. Finally, the solubilized RH-UnpEL or RH-HIF1α(Δ*C*) was analyzed by Western blotting.

Western blotting. Protein samples were treated at 50 °C for 1 h in sample treating solution containing 2% SDS and 5% β-mercaptoethanol. After SDS–polyacrylamide gel electrophoresis, Western blotting was performed according to the protocol provided with the ECL detection system (Amersham Pharmacia Biotech, Piscataway, NJ). As a secondary antibody, horseradish peroxidase (HRP)-conjugated anti-mouse IgG antibody or anti-rabbit IgG antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was used.

Results

UnpEL is ubiquitinated by Ro52 and deubiquitinated by itself in HEK293T cells

In our recent study, we detected the derivatives of UnpEL with higher molecular weights when it was coexpressed with Ro52 (unpublished data), implying that UnpEL is ubiquitinated by Ro52. To test this possibility, we overexpressed HA-tagged ubiquitin and FLAG-tagged UnpEL (wild-type or C311A mutant) with or without RH-tagged Ro52 (wild-type or C16A mutant) (Fig. 1). The mutated UnpEL loses the activity of deubiquitinating enzyme because an active-site Cys-311 is substituted with Ala in the UnpEL (C311A) [12]. Similarly, the mutated Ro52 loses the activity of E3 ubiquitin ligase because an active-site Cys-16 is substituted with Ala in the Ro52 (C16A) [5]. As shown in Fig. 1, the wild-type UnpEL was ubiquitinated (monoubiquitination and

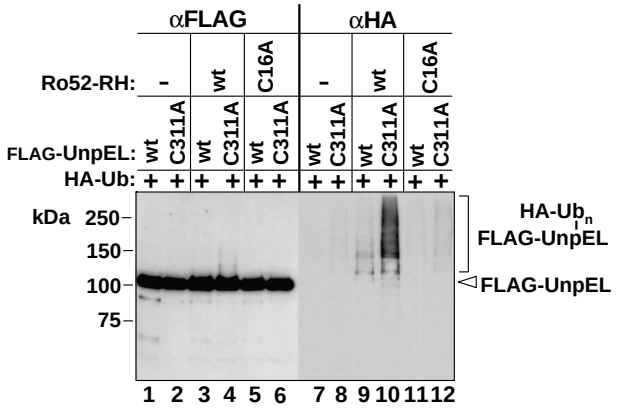

Fig. 1. Ro52-mediated ubiquitination of UnpEL in vivo. FLAG-tagged wild-type UnpEL or its mutant C311A was expressed with HA-tagged ubiquitin (HA-Ub) alone, HA-Ub plus RH-tagged wild-type Ro52 (wt), or HA-Ub plus RH-tagged mutant Ro52 (C16A) in HEK293T cells by means of plasmid transfection. Twenty hours after transfection, the cells were harvested and lysed. FLAG-UnpEL in the lysate was immunoprecipitated by mouse anti-FLAG antibody and solubilized in 2% SDS solution. The solubilized FLAG-UnpEL was then analyzed by Western blotting using rabbit anti-FLAG antibody to detect all derivatives of FLAG-UnpEL (lanes 1–6) and rabbit anti-HA antibody to detect ubiquitinated FLAG-UnpEL (lanes 7–12). Molecular size markers are shown on the left in kilodaltons (kDa).

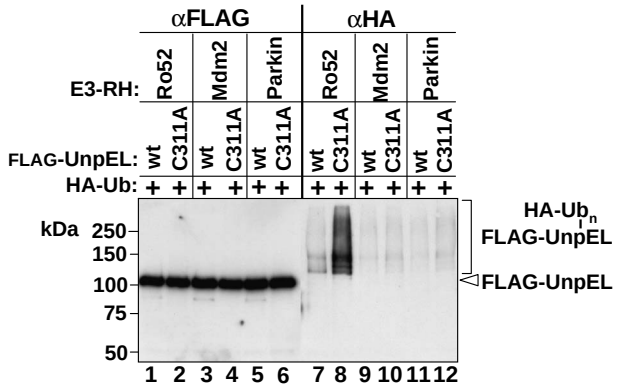

Fig. 2. E3-specific ubiquitination of UnpEL in vivo. FLAG-tagged UnpEL (wild-type or its mutant C311A) and HA-tagged ubiquitin were expressed with different E3 ubiquitin ligases, such as RH-tagged Ro52, Mdm2, and Parkin, in HEK293T cells by means of plasmid transfection. Twenty hours after transfection, the cells were harvested and lysed. FLAG-UnpEL in the lysate was immunoprecipitated by mouse anti-FLAG antibody and solubilized in 2% SDS solution. The solubilized FLAG-UnpEL was then analyzed by Western blotting using rabbit anti-FLAG antibody to detect all derivatives of FLAG-UnpEL (lanes 1–6) and rabbit anti-HA antibody to detect ubiquitinated FLAG-UnpEL (lanes 7–12). Molecular size markers are shown on the left in kilodaltons (kDa).

polyubiquitination) when it was overexpressed with ubiquitin and the wild-type Ro52 (lane 9). Importantly, the ubiquitination was more intense when the mutant UnpEL (C311A) was overexpressed with ubiquitin and the wild-type Ro52 (lane 10). In contrast, the ubiquitination was almost undetectable when the wild-type UnpEL was overexpressed with ubiquitin alone (lane 7) or with ubiquitin plus the mutant Ro52 (C16A) (lane 11). The ubiquitination, however, was weakly detected when the mutant UnpEL (C311A) was overexpressed with ubiquitin alone (lane 8) or with ubiquitin plus the mutant Ro52 (C16A) (lane 12). These results suggest that UnpEL is ubiquitinated by wild-type Ro52 but not by its inactive mutant. The weak ubiquitination shown in lanes 8 and 12 appeared to be mediated by endogenous Ro52 in the HEK293T cells. Interestingly, the inactive mutant of UnpEL is ubiquitinated by Ro52 much more strongly than the wild-type UnpEL (lane 7 vs. lane 8, lane 9 vs. lane 10, and lane 11 vs. lane 12), suggesting that UnpEL is deubiquitinated by itself. In other words, the inactive mutant of UnpEL is strongly ubiquitinated by Ro52 because it cannot be deubiquitinated by itself. The wild-type UnpEL, however, is not strongly ubiquitinated, probably because it is continuously deubiquitinated by itself.

UnpEL is selectively ubiquitinated by Ro52 in HEK293T cells

To examine the E3-specific ubiquitination of the UnpEL, we performed another in vivo ubiquitination assay using the three E3 ubiquitin ligases, Ro52, Mdm2, and Parkin. Mdm2 was previously reported to mediate the ubiquitination of p53 as an E3 ubiquitin ligase [17]. Parkin was also shown to mediate the ubiquitination of synphilin-1 as an E3 ubiquitin ligase [18]. In this in vivo ubiquitination assay, we therefore examined whether Mdm2 and Parkin promote the ubiquitination of UnpEL in HEK293T cells. As shown in Fig. 2, the wild-type UnpEL was ubiquitinated when it was overexpressed with Ro52 (lane 7). Notably, the ubiquitination was much more intense when the mutant UnpEL (C311A) was overexpressed with Ro52 (lane 8). In contrast, the ubiquitination was almost undetectable when the wild-type UnpEL was overexpressed with Mdm2 (lane 9) or Parkin (lane 11). The ubiquitination, however, was weakly detected when the mutant UnpEL (C311A) was overexpressed with Mdm2 (lane 10) or Parkin (lane 12). The weak ubiquitination shown in lanes 10 and 12 seems to be mediated by endogenous Ro52 in HEK293T cells, as described above. Taken together, these results suggest that UnpEL is selectively ubiquitinated by Ro52 in HEK293T cells.

Ro52 monoubiquitinates UnpEL in cooperation with UbcH5B in vitro

We performed an in vitro ubiquitination assay to confirm that UnpEL is ubiquitinated by Ro52. In this assay, we used FLAG-tagged UnpEL mutant as a substrate to avoid its self-deubiquitination. In brief, FLAG-UnpEL (C311A) was overexpressed in HEK293T cells and immunoprecipitated. FLAG-UnpEL (C311A) was then incubated in the reaction mixture containing MBP-fused Ro52 as an E3 ubiquitin ligase, recombinant E1 enzyme, different recombinant E2 enzymes (UbcH2, UbcH5B, UbcH7, UbcH10, and hCDC34 produced in bacteria)

(Fig. 3A), and RH-tagged ubiquitin. After the incubation, FLAG-UnpEL (C311A) was solubilized and analyzed by Western blotting using anti-RH antibody to detect ubiquitinated FLAG-UnpEL (C311A) and anti-FLAG antibody to detect all derivatives of FLAG-UnpEL (C311A). As shown in Fig. 3B, the incubation of FLAG-UnpEL (C311A) in the reaction mixture containing UbcH2, UbcH7, UbcH10, or hCDC34 did not result in the ubiquitination of FLAG-UnpEL (C311A) (lanes 8, 10, 11, and 12), whereas the incubation of FLAG-UnpEL (C311A) in the reaction mixture containing UbcH5B led to the monoubiquitination of FLAG-UnpEL (C311A) (lane 9). These results indicate that UnpEL (C311A) is monoubiquitinated in vitro and that this ubiquitination is catalyzed by UbcH5B but not by the other E2 enzymes. Thus, UbcH5B is utilized not only for the self-ubiquitination of Ro52 [5] but also for the monoubiquitination of UnpEL.

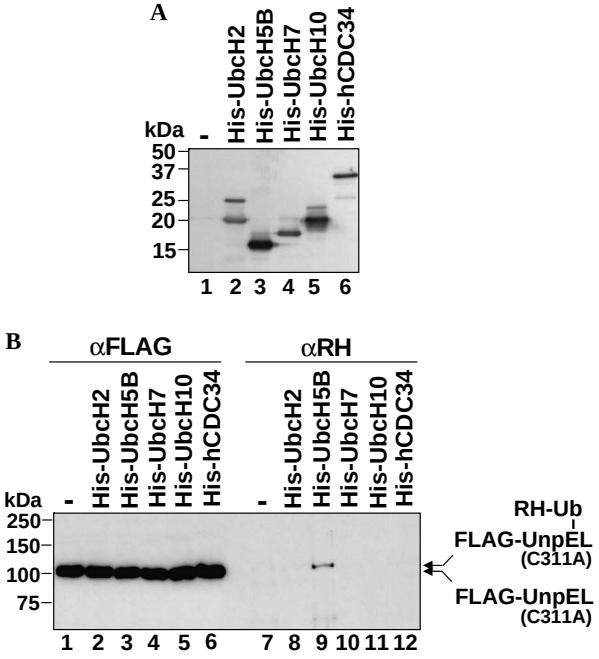

Fig. 3. In vitro ubiquitination of UnpEL catalyzed by UbcH5B and Ro52. (A) Bacterial expression of various E2 ubiquitin-conjugating enzymes. Poly His-tagged E2s were expressed in bacterial cells by transforming the cells with pTrcHis vectors (Invitrogen) and detected by Western blotting using mouse anti-RH antibody. (B) E2-dependent ubiquitination of UnpEL. In vitro ubiquitination of UnpEL was tested using various E2 ubiquitin-conjugating enzymes and Ro52. FLAG-tagged UnpEL(C311A) was overexpressed in HEK293T cells and immunoprecipitated by anti-FLAG beads. FLAG-UnpEL(C311A) immobilized on the beads was then incubated in the reaction mixture containing RH-tagged ubiquitin (RH-Ub), recombinant E1 enzyme, various poly-His-tagged recombinant E2 enzymes (UbcH2, UbcH5B, UbcH7, UbcH10, and hCDC34), and MBP-fused Ro52. After the reaction, FLAG-UnpEL(C311A) immobilized on the beads was washed to remove the reaction mixture and eluted by incubation with FLAG peptide. The eluted FLAG-UnpEL(C311A) was analyzed by Western blotting using mouse anti-FLAG antibody (M5) to detect all derivatives of FLAG-UnpEL(C311A) (lanes 1–6) and mouse anti-RH antibody to detect ubiquitinated FLAG-UnpEL(C311A) (lanes 7–12). Molecular size markers are shown on the left in kilodaltons (kDa).

In this section, we describe that UnpEL is monoubiquitinated by Ro52 in vitro. In the previous section, however, we describe that Ro52 promotes both the monoubiquitination and polyubiquitination of UnpEL in HEK293T cells (Figs. 1 and 2). What could explain the discrepancy between the in vitro and in vivo ubiquitination of UnpEL? We believe that, since there are many other E3 ubiquitin ligases in mammalian cells, Ro52 mediates the monoubiquitination of UnpEL, which triggers the further ubiquitination of UnpEL mediated by other E3 ubiquitin ligases in HEK293T cells.

Ro52-mediated ubiquitination of UnpEL does not target it to the proteasome for degradation

As described above, Ro52 promotes the ubiquitination of UnpEL. Does this ubiquitination target UnpEL to the proteasome for degradation? Generally, a polyubiquitin chain is assembled through the formation of isopeptide bonds involving specific Lys residues in ubiquitin. Substrates with a polyubiquitin chain, linked via Lys-48 or Lys-29, are targeted for degradation by the proteasome [19]. In contrast, the conjugation of a single ubiquitin or the ligation of a K63-linked polyubiquitin chain does not target the substrates for proteasomal degradation [20,21]. To determine whether the ubiquitination of UnpEL leads to its degradation by the proteasome, we performed another in vivo ubiquitination assay. Specifically, using the proteasome inhibitor MG132, we inhibited the proteasomal degradation in HEK293T cells to test whether UnpEL was stabilized and accumulated. Briefly, RH-UnpEL (wild-type or C311A mutant) was coexpressed with HA-ubiquitin and FLAG-Ro52 in HEK293T cells in the presence or absence of the proteasome inhibitor MG132. The cells were then harvested and lysed. Afterwards, RH-UnpEL in the lysate was precipitated by TALON beads, solubilized, and then analyzed by Western blotting using anti-RH antibody to detect all derivatives of RH-UnpEL and anti-HA antibody to detect ubiquitinated RH-UnpEL. As a positive control for the effect of MG132 on the proteasomal degradation of ubiquitinated proteins, we used HIF1α(Δ*C*), an N-terminal fragment (amino acids 1–330) of HIF1α. This is because we had previously detected the clear effect of MG132 on the proteasomal degradation of HIF1α(Δ*C*) [22].

As shown in Fig. 4, treatment with MG132 did not clearly increase the expression of either wild-type UnpEL (lane 1 vs. lane 3) or mutant UnpEL (C311A) (lane 2 vs. lane 4). These results suggest that the ubiquitination of RH-UnpEL does not lead to its proteasomal degradation. In contrast, HIF1α(Δ*C*), the positive control for the effect of MG132, was stabilized by the MG132 (upper panel, lane 5 vs. lane 6), suggesting that the polyubiquitination of HIF1α(Δ*C*) targets it to the proteasome for degradation.

Discussion

Ro52 was previously shown in a yeast two-hybrid assay to interact with UnpEL [11]. It was also shown that Ro52

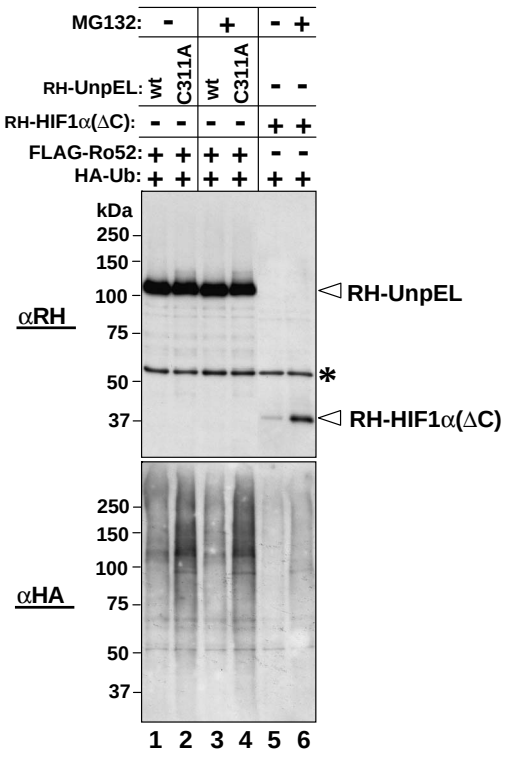

Fig. 4. Effect of MG132 on the in vivo ubiquitination of UnpEL mediated by Ro52. In HEK293T cells, RH-tagged wild-type UnpEL (wt) or its mutant (C311A) was expressed with HA-tagged ubiquitin and FLAG-tagged Ro52 in the absence or presence of the proteasome inhibitor MG132. The cells were harvested and lysed. RH-UnpEL in the lysate was precipitated by TALON beads and solubilized in 2% SDS solution. The solubilized RH-UnpEL was then analyzed by Western blotting using mouse anti-RH antibody to detect all derivatives of RH-UnpEL (upper panel, lanes 1–4) and mouse anti-HA antibody to detect ubiquitinated RH-UnpEL (lower panel, lanes 1–4). As a positive control for MG132 treatment, RH-tagged HIF1α(Δ*C*) was expressed with HA-ubiquitin, precipitated by TALON beads, and analyzed by Western blotting (lanes 5 and 6). A nonspecific band is indicated by an asterisk. Molecular size markers are shown on the left in kilodaltons (kDa).

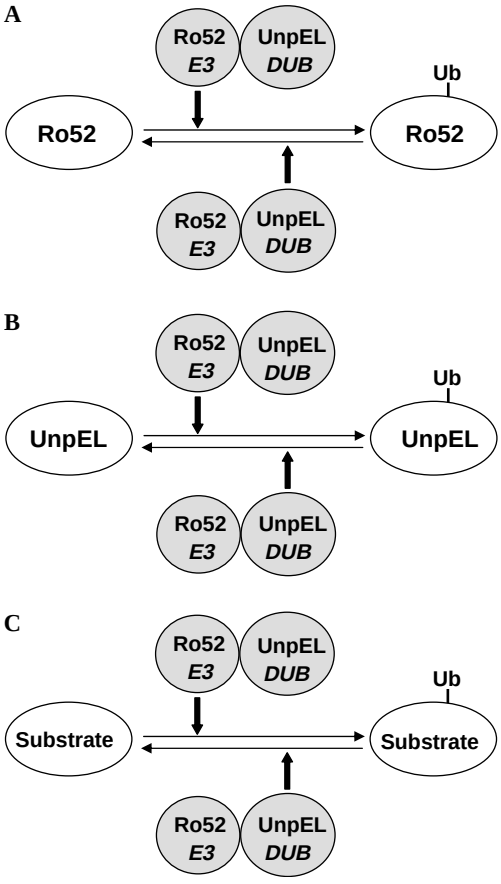

Fig. 5. Dual activities of the hypothetical protein complex consisting of Ro52 and UnpEL. (A) A model for the ubiquitination and deubiquitination of Ro52 mediated by the heterodimeric protein complex. (B) A model for the ubiquitination and deubiquitination of UnpEL mediated by the heterodimeric protein complex. (C) A model for the ubiquitination and deubiquitination of unidentified substrates mediated by the heterodimeric protein complex.

and UnpEL colocalize to the cytoplasmic rod-like structures in HEK293 cells [12]. These observations suggested that Ro52 interacts with UnpEL in mammalian cells to form a heterodimeric protein complex that mediates both the ubiquitination and deubiquitination. This is because Ro52 has the activity of an E3 ubiquitin ligase [5], while UnpEL has the activity of a deubiquitinating enzyme that reverses ubiquitination [12]. Indeed, in our previous studies, we showed that Ro52 is ubiquitinated by itself [5] and deubiquitinated by UnpEL [12], supporting the possibility that the heterodimeric protein complex mediates both the ubiquitination and deubiquitination of Ro52 (Fig. 5A). In addition, in the present study, we showed that UnpEL is ubiquitinated by Ro52 and probably deubiquitinated by itself (see Fig. 1), supporting the possibility that the heterodimeric protein complex mediates both the ubiquitination and deubiquitination of UnpEL (Fig. 5B). Based on these observations, we believe that the heterodimeric protein complex mediates both the ubiquitination and deubiquitination of unidentified substrates other than Ro52 and UnpEL (Fig. 5C).

As described above, the heterodimeric protein complex seems to have dual and opposing activities, but how the activities of the heterodimeric protein complex are regulated is unknown. Although, as our present study showed, the relationship between Ro52 and UnpEL is complicated, it is possible that this regulation occurs through the monoubiquitination of each component of the complex. In other words, the monoubiquitination of each component might determine the total activities of the complex. It will be important to test this possibility in later studies.

Acknowledgment

This work was supported by National Institutes of Health Grant R01 DK56298 (to T.K.).

References

[1] E.T.H. Yeh, L. Gong, T. Kamitani, Ubiquitin-like proteins: new wines in new bottles, Gene 248 (2000) 1–14.

[2] K. Itoh, Y. Itoh, M.B. Frank, Protein heterogeneity in the human Ro/SSA ribonucleoproteins. The 52- and 60-kD Ro/SSA autoantigens are encoded by separate genes, J. Clin. Invest. 87 (1991) 177–186.
[3] M. Torok, L.D. Etkin, Two B or not two B? Overview of the rapidly expanding B-box family of proteins, Differentiation 67 (2001) 63–71.
[4] T. Fukuda-Kamitani, T. Kamitani, Ubiquitination of Ro52 autoantigen, Biochem. Biophys. Res. Commun. 295 (2002) 774–778.
[5] K. Wada, T. Kamitani, Autoantigen Ro52 is an E3 ubiquitin ligase, Biochem. Biophys. Res. Commun. 339 (2006) 415–421.
[6] K. Wada, K. Tanji, T. Kamitani, Function and subcellular location of Ro52β, Biochem. Biophys. Res. Commun. 340 (2006) 872–878.
[7] K. Gupta, N.G. Copeland, D.J. Gilbert, N.A. Jenkins, D.A. Gray, Unp, a mouse gene related to the tre oncogene, Oncogene 8 (1993) 2307–2310.
[8] K. Gupta, M. Chevrette, D.A. Gray, The Unp proto-oncogene encodes a nuclear protein, Oncogene 9 (1994) 1729–1731.
[9] D.A. Gray, J. Inazawa, K. Gupta, A. Wong, R. Ueda, T. Takahashi, Elevated expression of Unph, a proto-oncogene at 3p21.3, in human lung tumors, Oncogene 10 (1995) 2179–2183.
[10] C.A. Gilchrist, D.A. Gray, R.T. Baker, A ubiquitin-specific protease that efficiently cleaves the ubiquitin-proline bond, J. Biol. Chem. 272 (1997) 32280–32285.
[11] F. Di Donato, E.K. Chan, A.D. Askanase, M. Miranda-Carus, J.P. Buyon, Interaction between 52 kDa SSA/Ro and deubiquitinating enzyme UnpEL: a clue to function, Int. J. Biochem. Cell Biol. 33 (2001) 924–934.
[12] K. Wada, K. Tanji, T. Kamitani, Oncogenic protein UnpEL/Usp4 deubiquitinates Ro52 by its isopeptidase activity, Biochem. Biophys. Res. Commun. 339 (2006) 731–736.
[13] T. Kamitani, K. Kito, H.P. Nguyen, E.T.H. Yeh, Characterization of NEDD8, a developmentally down-regulated ubiquitin-like molecule, J. Biol. Chem. 272 (1997) 28557–28562.
[14] T. Tanaka, H. Kawashima, E.T.H. Yeh, T. Kamitani, Regulation of the NEDD8 conjugation system by a splicing variant, NUB1L, J. Biol. Chem. 278 (2003) 32905–32913.
[15] T. Kamitani, K. Kito, T. Fukuda-Kamitani, E.T.H. Yeh, Targeting of NEDD8 and its conjugates for proteasomal degradation by NUB1, J. Biol. Chem. 276 (2001) 46655–46660.
[16] H. Wada, E.T.H. Yeh, T. Kamitani, A dominant negative Ubc12 mutant sequesters NEDD8 and inhibits NEDD8-conjugation in vivo, J. Biol. Chem. 275 (2000) 17008–17015.
[17] T. Iwakuma, G. Lozano, MDM2, an introduction, Mol. Cancer Res. 1 (2003) 993–1000.
[18] K. Tanaka, T. Suzuki, N. Hattori, Y. Mizuno, Ubiquitin, proteasome and parkin, Biochim. Biophys. Acta. 1695 (2004) 235–247.
[19] A. Hershko, A. Ciechanover, The ubiquitin system for protein degradation, Annu. Rev. Biochem. 61 (1992) 761–807.
[20] E.W. Doss-Pepe, L. Chen, K. Madura, Alpha-synuclein and parkin contribute to the assembly of ubiquitin lysine63-linked multiubiquitin chains, J. Biol. Chem. (2005).
[21] L. Sun, Z.J. Chen, The novel functions of ubiquitination in signaling, Curr. Opin. Cell Biol. 16 (2004) 119–126.
[22] K. Kanaya, T. Kamitani, pVHL-independent ubiquitination of HIF1alpha and its stabilization by cobalt ion, Biochem. Biophys. Res. Commun. 306 (2003) 750–755.