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Direct recognition of the C-terminal polylysine residues of nonstop protein by Ltn1, an E3 ubiquitin ligase

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ABSTRACT

When mRNAs lack stop codons, errors in gene expression and coding of aberrant proteins that are harmful in cells can result. In *Saccharomyces cerevisiae*, a 180-kDa E3-ubiquitin ligase, Ltn1 has been known to associate with ribosomes and marks translationally-arrested aberrant nascent polypeptides for proteasomal degradation. Here, we demonstrate the Ltn1 E3-ubiquitin ligase directly binds to the nonstop proteins and efficiently ubiquitylates them. The middle domain of Ltn1 is responsible for recognizing the polylysine residues of the nonstop protein with an affinity of 2–3 μM. This biochemical characterization of Ltn1 expands our knowledge regarding the fundamental process that removes aberrant nascent polypeptides in eukaryotes.

1. Introduction

Proteins are continuously monitored by quality control processes from the time they emerge from ribosomes until they are finally targeted for degradation [1–4]. Across all organisms, messenger RNA lacking stop codons, "nonstop mRNA," can generate errors in gene expression and subsequently encodes aberrant proteins [5]. If the aberrant proteins cannot be cleared properly by the chaperons or proteases inside the cell, the gene expression quality control turns to deleterious mechanisms [3,6]. In prokaryotes, these aberrant proteins are tagged by ssrA/tmRNA, which conducts its degradation by a subset of ATP-dependent proteases [7]. The ssrA-tagged substrates are recognized by the ClpAP protease machinery or delivered to ClpXP by the SspB (Stringent starvation protein B) for efficient degradation [8–11]. Specifically, the C-terminus of "nonstop proteins" is tagged with the ssrA sequence, AANDENYALAA [5,12]. Then, the SspB protein enhances the delivery of ssrA-tagged substrates to the ClpXP complex, which performs the final degradation of the protein [12,13].

Unlike the well-established tmRNA/ssrA mechanism in bacteria [11,14–16], the degradation of nonstop proteins in the eukaryotic system has been mysterious for long time. The translation of a poly(A)-tail encodes polylysine residues at the C-terminus of aberrant proteins [17], and that causes ribosomes to stall and subsequently results in proteasomal degradation [18–22]. Only recently, a 180-kDa E3 ubiquitin ligase, Ltn1 in *Saccharomyces cerevisiae*, has been shown to associate with ribosomes and mark translationally-arrested aberrant nascent polypeptides for proteasomal degradation [19]. The Ltn1 from yeast consists of 1562 amino acid residues and shares sequence similarity with only 13–15% of Ltn1 homologs from other species [19,23]. Nevertheless, the N-terminal region (residues 18–322), which selectively binds to the 60S ribosomal subunit, and the C-terminal region (residues 1266–1562), which contains the catalytic RING domain, are evolutionarily well-conserved [19,23]. Ribosome stalling during mRNA processing occurs when a nonstop protein containing polylysine residues, encoded by the poly(A)-tail, leads to Ltn1 recruitment to the ribosome exit site [6]. However, how the aberrant polypeptides are ubiquitylated is currently unknown due to the lack of biochemical characterization of the Ltn1 E3 enzyme. Recently, the overall architecture of Ltn1 in yeast was reported using the electron microscopic technique [23] and it was determined that the Ltn1 is a highly flexible molecule with three distinct domains; a large central domain, hereafter the M-domain (residues 477–1265) plus the aforementioned N- and C-domains. The exact role for the M-domain is not clear, although it has been proposed that its HEAT/ARM repeat structure [23] may be involved in protein–protein interactions.

Abbreviations: DLS, dynamic light scattering; GFP, green fluorescent protein; IPTG, isopropyl β-D-thiogalactoside; K12, twelve lysine residue repeat; MBP, maltose binding protein; M-domain, middle domain of Ltn1 (residues 477–1265); NΔLtn1, N-terminal (residues 1–476) of deleted Ltn1; PCR, polymerase chain reaction; SEC-MALS, size-exclusion chromatography with multi-angle light scattering; SPR, surface plasmon resonance; SspB, Stringent starvation protein B; Ub, ubiquitin.

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To elucidate the molecular mechanism of Ltn1, we overexpressed and purified the full-length Ltn1 or its fragment (NΔLtn1: N-terminal residue with residues 1–476 removed) in *S. cerevisiae* by flag tagging its N-terminus. Here, we demonstrate that the Ltn1 E3 ubiquitin ligase directly binds *via* its M-domain to a model nonstop protein containing a stretch of more than twelve sequential lysines at the C-terminus and efficiently ubiquitylates them. Based on this finding, we are able to propose that the polylysine tail of aberrant polypeptide chains plays a critical role not only in stalling the ribosome, but also through its recognition by Ltn1 *via* its M-domain. This finding expands our knowledge regarding the fundamental process that removes aberrant nascent polypeptides in eukaryotes.

2. Materials and methods

2.1. Cloning, expression, and purification of Ltn1

The DNAs coding for full length Ltn1 or NΔLtn1 were amplified by a polymerase chain reaction (PCR), with forward and reverse primers containing the restriction sites BglII and XhoI, and genomic DNA from *S. cerevisiae* S288C used as a template. The PCR product was cloned into a modified YEpTOP2PGAL1 vector and the resultant plasmid for expressing N-terminal TLAG-tagged Ltn1 was transformed into the haploid (type a) yeast strain BCY123. After transformation, yeasts were plated on uracil dropout synthetic media agar plates [0.81 g/L CSM-URA with 40 mg/L ADE (MP), 1.7 g/L YNB without ammonium sulfate (MP), ammonium sulfate (Sigma)] supplemented with 2% agar (USB) and 2% glucose (Sigma). Colonies took 2–3 days to appear, were continuously inoculated in uracil dropout synthetic media containing 2% glucose. The cells were then switched to a uracil dropout synthetic media containing 2% glycerol (USB) and 1.5% lactic acid (Sigma). When the OD_{600} reached approximately 0.4, the media were changed to the uracil dropout synthetic media containing 2% galactose (Sigma) which activated transcription of the N-terminal flag-tagged Ltn1 gene from the GAL1 promoter. The yeast cells were further cultured at 30 °C for 14 h. Cells were harvested by centrifugation, resuspended in PBS buffer containing 10% glycerol, 1% Triton X-100, 0.1% Nonidet P-40, and 2 mM TCEP, and subsequently disrupted by liquid nitrogen grinding. The cell lysate was centrifuged and then the supernatant was applied to flag-tag affinity beads (Sigma). Further purification was performed by size exclusion chromatography (Superdex 200 10/300 GL, GE Healthcare) with a PBS buffer containing 0.05% Nonidet P-40, and 1 mM TCEP. Eluents from the columns were analyzed by SDS–PAGE and the protein was visualized by staining the gel with Coomassie brilliant blue R-250.

2.2. Dynamic light scattering and size-exclusion chromatography with multi-angle light scattering

Dynamic light scattering (DLS) data was obtained using a DynaPro Titan (Wyatt), which operates as a "batch" instrument using a micro cuvette. Before it is loaded into the cuvette, the Ltn1 protein sample was filtered through 0.22 μm centrifuge tube filters (Costar). Data was acquired and analyzed using DYNAMICS software. Size-exclusion chromatography with multi-angle light scattering (SEC-MALS) experiments were performed using a FPLC system (GE Healthcare) connected to a Wyatt MiniDAWN TREOS MALS instrument and a Wyatt Optilab rEX differential refractometer. A Superdex 200 10/300 GL (GE Healthcare) gel-filtration column pre-equilibrated with PBS buffer containing 0.05% Nonidet P-40 and 1 mM TCEP was calibrated using a BSA protein standard. Prepared Ltn1 protein was injected at a flow rate of 0.5 ml/min. Data were evaluated using the Zimm model for static light-scattering data fitting and graphed using EASI Graph with a UV peak in the ASTRA V software (Wyatt).

2.3. Preparation of nonstop protein substrates

To prepare the nonstop proteins, which have C-terminal polylysine residues, firstly, a C-terminal coding 12 lysine residues was synthesized and cloned into a modified pMAL-C2X (NEB) expression vector, and then transformed into *Escherichia coli* DH5α cells. The transformed cells were cultured in LB medium containing 50 μm/ml ampicillin at 37 °C until they reached an $OD600_{nm}$ of 0.5. Expression was induced by adding isopropyl β-D-thiogalactoside (IPTG) to a final concentration of 0.2 mM at 37 °C for 3 h. Cells over-expressing the nonstop protein, N-terminal maltose binding protein (MBP) fused with C-terminal 12 lysine residues (MBP-K12), were harvested by centrifugation. The resultant pellet was resuspended in PBS buffer and subsequently disrupted by ultrasonication. The cell lysate was centrifuged, and then supernatant was applied to an amylose resin (NEB). The MBP-K12 was eluted with a PBS buffer containing 10 mM maltose. Further purification was carried out by a successive anion exchange (mono Q, GE Healthcare) and size exclusion (Superdex 75 10/300 GL, GE Healthcare) chromatography. For green fluorescent protein (GFP) fused with C-terminal 12 lysine residues (GFP-K12), the DNA coding the polylysine residues was cloned into a pET-GFP vector with an N-terminal six-histidine tag. The remaining procedures were essentially the same with the preparation of MBP-K12, except a nickel-chelating column was used instead of amylose affinity chromatography.

2.4. Assays for interaction between Ltn1 and nonstop protein

The MBP pull down assay and the surface plasmon resonance (SPR) were used to observe the interaction between Ltn1 and MBP-K12. For the MBP-pull down assay, the amylose resin was rinsed with PBS buffer and then the purified Ltn1 protein and MBP-K12 were incubated with the equilibrated amylose resin for 1 h with gentle mixing at 4 °C. After capturing the protein, the unbound protein was removed by washing several times with the PBS buffer, and complex was eluted with 10 mM maltose in PBS buffer.

The SPR was performed using the SR7500DC system (Reichert). The Ltn1 protein in PBS buffer was immobilized on a gold slide PEG chip (Reichert) by the amine coupling method. Nonstop protein in injection buffer (20 mM HEPES, pH 7.7, 150 mM NaCl, 0.5 mM TCEP, 0.007% NP40) was injected to be trapped on the chip through the immobilized Ltn1 protein. Evaluation and calculation of the binding parameters were carried out according to the Scrubber2 software.

2.5. Preparation of ubiquitin, Uba1 and UbcH5 for ubiquitylation assay

Human ubiquitin (Ub) was cloned into a modified pET-28a expression vector (Novagen). Transformed BL21 (DE3) *E. coli* cells were cultured in LB medium containing 50 μm/ml kanamycin at 37 °C until reaching an $OD600_{nm}$ of 0.5. Expression was induced by adding IPTG to a final concentration of 0.3 mM at 22 °C for 20 h. The cells were harvested by centrifugation, the pellet was resuspended in 50 mM ammonium acetate (pH 4.5), and subsequently disrupted by ultrasonication and then centrifuged once again. The supernatant was incubated at 70 °C for 7 min to denature all *E. coli* proteins and then centrifuged. The supernatant applied to a cation exchange (Hitrap SP, GE Healthcare) and size-exclusion chromatography (HiLoad Superdex 75 16/60 pg, GE Healthcare) was performed with 50 mM Tris–HCl pH 8.0, 100 mM NaCl, and 1 mM DTT. The virus that results in a His-tagged

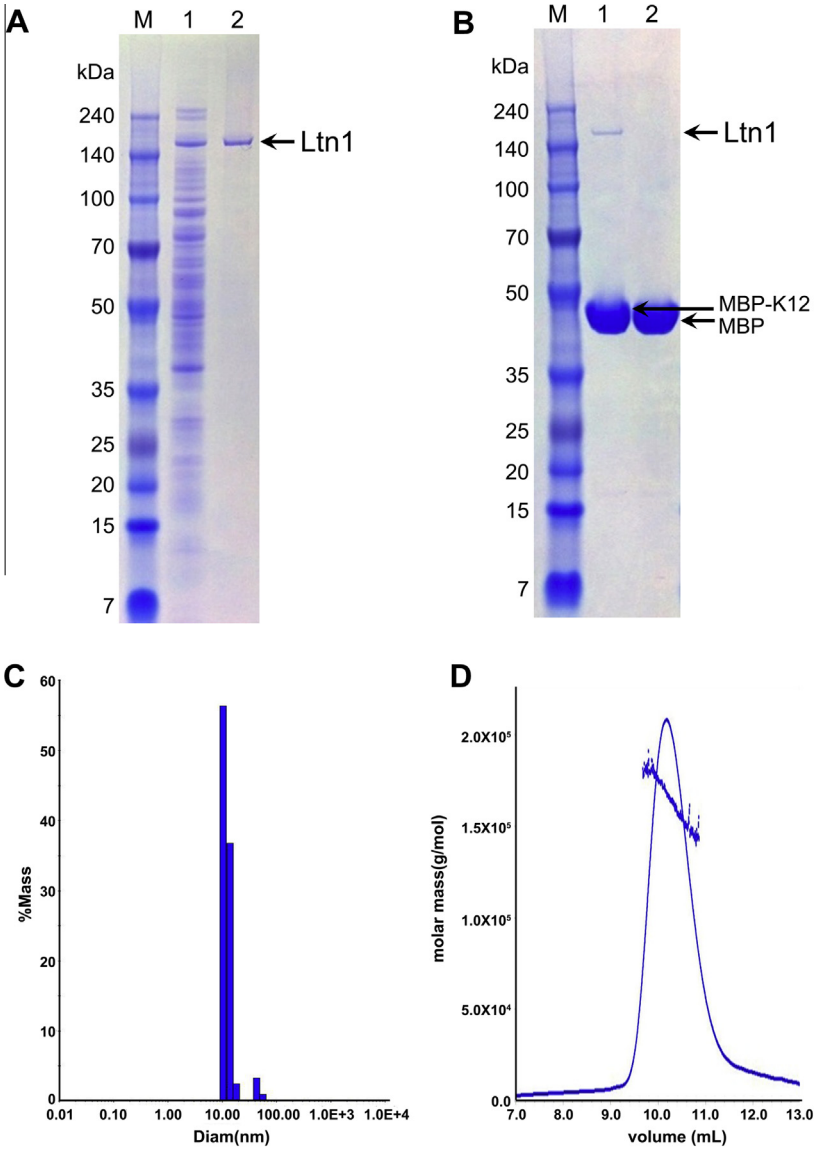

Fig. 1. Biochemical characterization of Ltn1. (A) Overexpression and purification of Ltn1. Lane M is LandMark™ Broad Range Prestained Protein Marker (dual color). It is a mixture of 10 purified and prestained polypeptides whose molecular weights are well-adjusted, ranging from 7 to 240 kDa. Lane 1 is the soluble fraction, which shows the expression after galactose induction. Lane 2 is the purified N-terminal flag-tagged Ltn1 with a molecular weight of 180 kDa. (B) The MBP pull down assay between Ltn1 and the nonstop protein MBP-K12 or MBP protein only. Lane M is a protein size marker, the same as in panel (A). The Ltn1 protein was successfully pulled down by MBP-K12 containing 12 C-terminal lysine residues (Lane 1). Lane 2 is the negative control showing that MBP protein by itself cannot pull down the Ltn1 protein. The images show 4–12% (w/v) Bis–Tris polyacrylamide gels electrophoresis stained with Coomassie blue. (C) Dynamic light scattering data of Ltn1 acquired using DYNMICS software. The analyzed diameter was 12.3 nm, which is in the range of the actual size of Ltn1 [23]. (D) Size exclusion chromatography with multi-angle light scattering data. The elution position at 10.3 mL, using Superdex 200 10/30 GL (GE Healthcare) and the absolute molecular weight of Ltn1 (180 kDa) prove that it is a monomer in solution. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mouse, Uba1, was obtained from Prof. Chin Ha Chung at Seoul National University, Korea. Harvested cells were resuspended in 50 mM Tris–HCl pH 8.0, 100 mM NaCl, and 5 mM β-mercaptoethanol, and then disrupted by ultrasonication. The cell lysate was centrifuged and then the supernatant was applied to a nickel-chelating Sepharose (GE Healthcare) column. Further purification was carried out by successive anion exchange chromatography (Hitrap Q, GE Healthcare). Human UbcH5, a homolog protein of yeast Ubc4, which is a specific E2 enzyme of Ltn1, was purchased (BostonBiochem, Inc.)

2.6. In vitro ubiquitylation assay

Purified human Ub, purified mouse Uba1 protein as an E1 activating enzyme, UbcH5 protein as an E2 conjugating enzyme, and purified yeast Ltn1 protein as an E3 ligating enzyme were used for the *in vitro* ubiquitylation assay. MBP-K12 or GFP-K12, two nonstop proteins were used as substrates for the *in vitro* ubiquitylation assay. Approximately 20 ng of E1, 40 ng of E2, and 5 μg of Ub were added to the reaction mixture with variable amounts of E3 ligase and substrate. Reactions were carried out at 20 °C for 2 h. The reactions were stopped by adding SDS sample buffer, boiling for 5 min, and then probed by Western blotting.

3. Results

3.1. Biochemical characterization of Ltn1

Initial attempts to produce full-length yeast Ltn1 using *E. coli* were not successful, and it was overexpressed as a soluble form

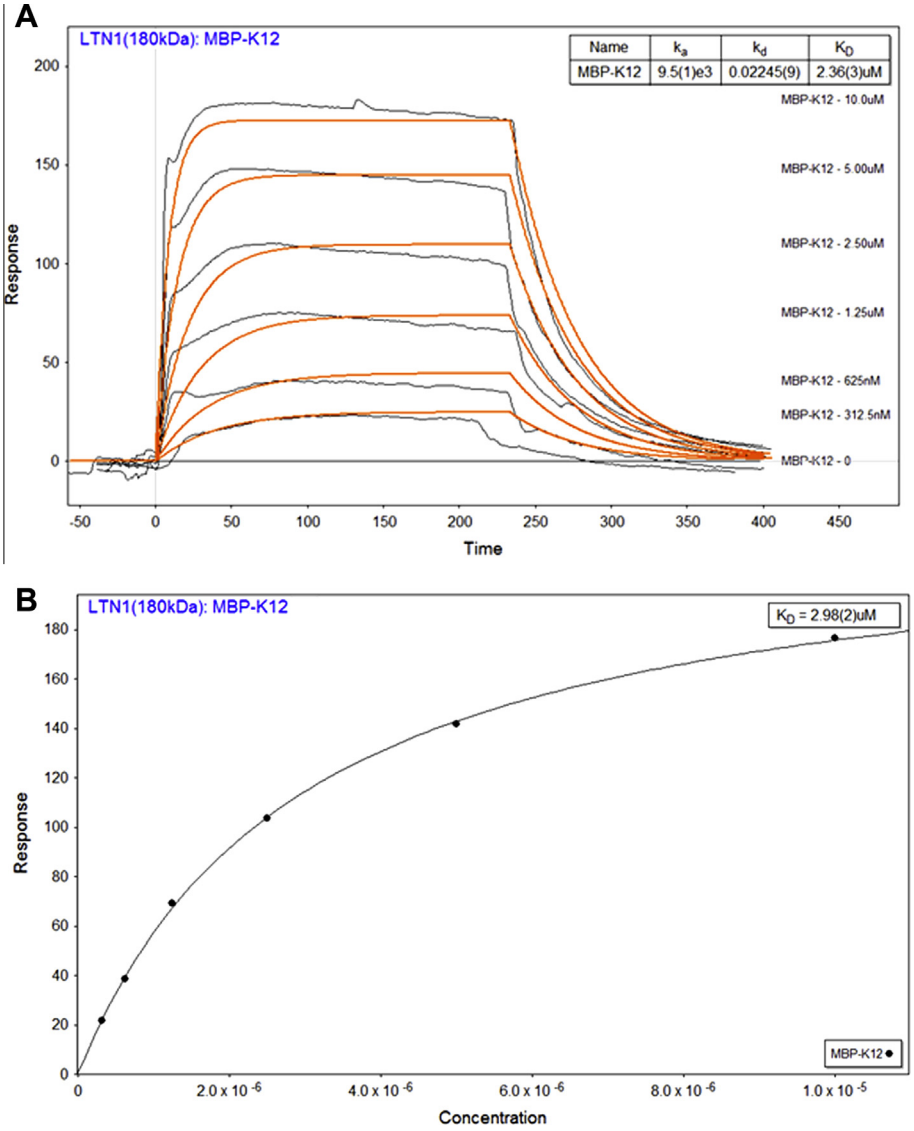

Fig. 2. The surface plasmon resonance (SPR) assay. (A) Kinetic analysis between Ltn1 and nonstop MBP-K12 protein. The binding affinity was measured using Reichert SR7500DC system with PEG chip (Cat#: 13206061). The concentration of MBP-K12 varies from 0.3125 to 10 μM. The flow rate of the analyte was 30 μl/min, and the association and dissociation times were 4 and 3 min, respectively. The k_a and k_d values were 9.5×10^3 and 0.02, respectively and thus, K_D was calculated to be 2.36 μM. (B) Equilibrium analysis between Ltn1 and nonstop protein regarding (A). The value of K_D was 2.96 μM under this process.

using the same host, *S. cerevisiae*. The affinity purification using flag beads removed most of the intrinsic yeast proteins in a single step and then they were further purified by size-exclusion chromatography (Fig. 1A). The Ltn1 from Baker's yeast possesses an exact molecular weight of 180,186 Da by calculating its amino acid residues. The expected elution volume of a 180-kDa protein in size-exclusion chromatography using Superdex 200 10/300 GL column is around 13 ml, however it eluted at 10.3 ml, suggesting that Ltn1 exists as an oligomer in solution. In order to determine its exact oligomeric state, we performed the DLS and SEC-MALS experiments. The results of the diameter of Ltn1 protein from the DLS data is around 15 nm (Fig. 1C) and the absolute molecular weight of the Ltn1 protein by SEC-MALS was approximately 180,000 Da (Fig. 1D), confirming that the Ltn1 protein is a monomer in solution. Ltn1 is known as an E3 ubiquitin ligase for nonstop proteins containing polylysine residues at the C-terminus [19] and therefore, it was reasonable to determine whether it interacts with the substrate directly.

3.2. Direct interaction between Ltn1 and nonstop protein

To check the *in vitro* interaction between Ltn1 and its substrate, we prepared a MBP-K12 model nonstop protein. Using the MBP pull down assay, we checked whether the nonstop protein, MBP-K12, and Ltn1 co-precipitated with amylose beads. Indeed, we found that Ltn1 was pulled down with the amylose bead, and were even able to visualize it with Coomassie blue staining (Fig. 1B). To conduct a negative control experiment, we used MBP without its C-terminal polylysine residues, and we found that the Ltn1 protein did not pull down at all. To confirm the direct interaction further, the SPR experiment was applied. Ltn1 was immobilized on a gold slide PEG chip, where it only trapped the nonstop protein, not the maltose binding protein, a similar result to the MBP-pull down assay. The association constant (k_a) of MBP-K12 on the immobilized Ltn1 was 9510 and the dissociation constant (k_d) of the immobilized Ltn1 was 0.02245(9) (Fig. 2A). Therefore, the equilibrium dissociation constant ($K_D = k_d/k_a$) is 2.36(3) μM, and the value

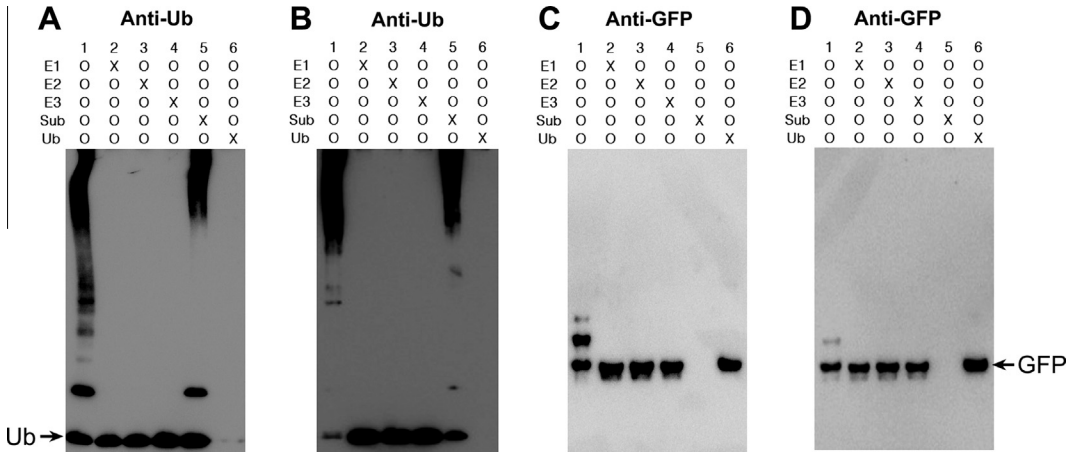

Fig. 3. The Ltn1 ubiquitylates GFP-K12 *in vitro*. (A) Ubiquitylation was detected using a Western blot with an anti-ubiquitin (anti-Ub) antibody. Lane 1 includes the E1, E2, and E3 enzymes, GFP-K12 as a substrate, and Ub. Lanes 2–6 had the same conditions as lane 1, except E1, E2, E3, the substrate and Ub were each omitted, respectively. Lanes 1 and 5 showed the ubiquitylated products. (B) The same reaction as in panel (A), except the NΔLtn1 mutant was used as the E3 ubiquitin ligase. The NΔLtn1 mutant shows almost the same results as the full-length Ltn1. (C) The same reaction as in panel (A), except detection was with an anti-GFP antibody. Only lane 1 showed the ubiquitylation of GFP-K12 substrate, suggesting that the Ltn1 or NΔLtn1 auto-ubiquitylate themselves. (D) The same reaction as in panel (B), except detection was with an anti-GFP antibody. The ubiquitylated GFP-K12 substrate was detected, although the ubiquitylation activity of NΔLtn1 might be lower than that of full-length Ltn1.

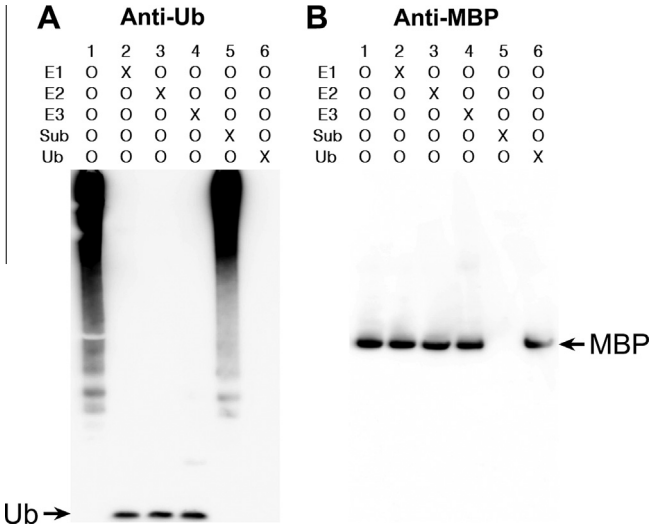

Fig. 4. The Ltn1 cannot ubiquitylate MBP-K12 *in vitro*. (A) Ubiquitylation was detected using a Western blot with an anti-Ub antibody. Lane 1 includes the E1, E2, and E3 enzymes, MBP-K12 as the substrate, and Ub. Lanes 2-6 are the same as lane 1, except one of E1, E2, E3, substrate, and Ub, was omitted in each lane, respectively. Lanes 1 and 5 show the ubiquitylated products, suggesting that Ltn1 or NΔLtn1 auto-ubiquitylate themselves. (B) The same reaction as in panel (A), except detection was performed using an anti-MBP antibody. There is no ubiquitylated product of MBP-K12, suggesting that the spatial orientation of the substrate against the RING domain of Ltn1 might also be critical.

obtained from the equilibrium analysis, 2.98(2) μM, is also very similar (Fig. 2B). The binding affinity is within the sub-micromolar range, which is not strong.

3.3. Ubiquitylation of nonstop protein by Ltn1

Ubiquitylation is a post-translational modification carried out by E1-activing, E2-conjugating, and E3-ligating enzymes. As previously described, Ltn1, a RING-domain-type E3 ubiquitin ligase, confers Ub to the nonstop protein containing C-terminal polylysine residues, marking it for proteasomal degradation. We performed an *in vitro* ubiquitylation assay using Uba1 as E1, UbcH5 as E2, Ltn1 (or NΔLtn1) as E3, and nonstop proteins as substrates. The result of the assay confirmed that Ltn1 as an E3 ligase enzyme mediates the transfer of Ub to nonstop proteins through recognizing the C-terminal polylysine residues. As found in many E3 ligases, Ltn1 also ubiquitylates itself. As shown in lane 5 of Fig. 3A, Ltn1 protein is self-ubiquitylated clearly, and even the NΔLtn1 mutant ubiquitylates itself, as shown in lane 5 of Fig. 3B. The same ubiquitylation pattern of Ltn1 was also obtained in presence of different substrate, MBP-K12 (Fig. 4A). To confirm that the nonstop substrate is specifically ubiquitylated, we blotted the reaction mixtures with an antibody of the substrate, anti-GFP (Fig. 3C). Clearly, the GFP-K12 substrate is ubiquitylated, but not heavily. It should be noted that we performed the ubiquitylation assay with two different model substrates, MBP-K12 and GFP-K12, but we found that only GFP-K12 works, whereas MBP-K12 was not ubiquitylated by the Ltn1 E3 ligase (Fig. 4B).

4. Discussion

The 180 kDa Ltn1 protein can be divided into three functional domains; the N-terminal ribosome-binding domain, the uncharacterized large middle M-domain, and the C-terminal RING domain [19,23]. The monomeric behavior of Ltn1 in solution was confirmed by SEC-MALS experiments (Fig. 1D). The expectation of oligomer formation due to the faster migration in size exclusion chromatography might be due to its flexible and elongated shape, which was recently visualized by EM data [23]. The role of the M-domain was not clear, but the HEAT/ARM repeats of this domain suggest that it may be the protein-protein interaction region. Indeed, our experiments show that a region within this middle domain specifically recognizes the twelve lysine chain attached at the C-terminus of nonstop proteins, which was encoded by the translation of a poly(A)-tail sequence [17]. The MBP-pull down and the SPR assays fully showed the direct interactions between Ltn1 and nonstop proteins (Figs. 1B and 2). In addition, an *in vitro* ubiquitylation assay in the presence of E1 and E2 enzymes confirmed that our purified Ltn1 properly acts as an E3 ligase enzyme that ubiquitylates the nonstop proteins, marking their proteolysis. Two model substrates, MBP-K12 and GFP-K12, were used for the ubiquitylation assay, but only GFP-K12 acted as a substrate. It is intriguing that although the 12 lysine residues in both proteins were recognized by the M-domain of Ltn1, only GFP-K12 was modified. This result suggests that not only the recognition of the C-ter-

minal polylysine residues, but also the spatial location of the target lysine residues for poly-ubiquitylation must be critical in nascent polypeptides. Most likely, this is governed by the spatial orientation between the middle substrate recognition domain and the C-terminal RING domain in Ltn1.

Previously, it was shown that the C-terminal polylysine residues of nonstop proteins play a critical role in stalling the ribosome [17,24]. Then, Ltn1 E3 ligase is recruited to the 60S ribosome, most likely through its N-terminal domain [23]. Subsequently, the C-terminal RING domain of Ltn1 actually ubiquitylates the properly oriented lysine residue(s) on the surface of aberrant polypeptides. During the process, the 80S ribosome dissociates into the 40S and 60S subunit, and a synthesizing polypeptide chain is released from the ribosome exit site [6]. Based on our biochemical data, the polylysine residues at the C-terminal region of an aberrant polypeptide probably play another role in the interaction with the M-domain of Ltn1. Therefore, our finding provides an additional step in a mechanism of aberrant nascent polypeptide removal, which is the actual recognition of a translation product of the poly(A)-tail by the M-domain of Ltn1. The atomic details regarding how Ltn1 recognizes polylysine residues will require a high-resolution complex structure analysis between Ltn1 and nonstop proteins containing polylysine tails. However, the data presented here expands our knowledge regarding the fundamental process that removes aberrant nascent polypeptides in eukaryotes.

Acknowledgments

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References

[1] I. Amm, T. Sommer, D.H. Wolf, Protein quality control and elimination of protein waste: the role of the ubiquitin–proteasome system, Biochim. Biophys. Acta (BBA) – Mol. Cell Res. 2014 (1843) 182–196.
[2] X. Wang, J.S. Pattison, H. Su, Posttranslational modification and quality control, Circ. Res. 112 (2013) 367–381.
[3] F. Kriegenburg, L. Ellgaard, R. Hartmann-Petersen, Molecular chaperones in targeting misfolded proteins for ubiquitin-dependent degradation, FEBS J. 279 (2012) 532–542.
[4] M.A. Theodoraki, N.B. Nillegoda, J. Saini, A.J. Caplan, A network of ubiquitin ligases is important for the dynamics of misfolded protein aggregates in yeast, J. Biol. Chem. 287 (2012) 23911–23922.
[5] S.D. Moore, R.T. Sauer, The tmRNA system for translational surveillance and ribosome rescue, Annu. Rev. Biochem. 76 (2007) 101–124.
[6] S. Shao, K. von der Malsburg, Ramanujan S. Hegde, Listerin-dependent nascent protein ubiquitination relies on ribosome subunit dissociation, Mol. Cell 50 (2013) 637–648.
[7] D. Dulebohn, J. Choy, T. Sundermeier, N. Okan, A.W. Karzai, Trans-translation: the tmRNA-mediated surveillance mechanism for ribosome rescue, directed protein degradation, and nonstop mRNA decay†, Biochemistry 46 (2007) 4681–4693.
[8] I. Levchenko, M. Seidel, R.T. Sauer, T.A. Baker, A specificity-enhancing factor for the ClpXP degradation machine, Science 289 (2000) 2354–2356.
[9] E.Y. Park, B.G. Lee, S.B. Hong, H.W. Kim, H. Jeon, H.K. Song, Structural basis of SspB-tail recognition by the zinc binding domain of ClpX, J. Mol. Biol. 367 (2007) 514–526.
[10] E.Y. Park, H.K. Song, A degradation signal recognition in prokaryotes, J. Synchrotron Radiat. 15 (2008) 246–249.
[11] D.N. Bolon, D.A. Wah, G.L. Hersch, T.A. Baker, R.T. Sauer, Bivalent tethering of SspB to ClpXP is required for efficient substrate delivery: a protein-design study, Mol. Cell 13 (2004) 443–449.
[12] H.K. Song, M.J. Eck, Structural basis of degradation signal recognition by SspB, a specificity-enhancing factor for the ClpXP proteolytic machine, Mol. Cell 12 (2003) 75–86.
[13] M. Lies, M.R. Maurizi, Turnover of endogenous SsrA-tagged proteins mediated by ATP-dependent proteases in *Escherichia coli*, J. Biol. Chem. 283 (2008) 22918–22929.
[14] G.-F. Tu, G.E. Reid, J.-G. Zhang, R.L. Moritz, R.J. Simpson, C-terminal extension of truncated recombinant proteins in *Escherichia coli* with a 10Sa RNA decapeptide, J. Biol. Chem. 270 (1995) 9322–9326.
[15] B.K. Ray, D. Apirion, Characterization of 10S RNA: a new stable RNA molecule from *Escherichia coli*, Mol. Gen. Genet. 174 (1979) 25–32.
[16] D.N. Bolon, R.A. Grant, T.A. Baker, R.T. Sauer, Nucleotide-dependent substrate handoff from the SspB adaptor to the AAA+ ClpXP protease, Mol. Cell 16 (2004) 343–350.
[17] S. Ito-Harashima, K. Kuroha, T. Tatematsu, T. Inada, Translation of the poly(A) tail plays crucial roles in nonstop mRNA surveillance via translation repression and protein destabilization by proteasome in yeast, Genes Dev. 21 (2007) 519–524.
[18] R. Verma, R.S. Oania, N.J. Kolawa, R.J. Deshaies, Cdc48/p97 promotes degradation of aberrant nascent polypeptides bound to the ribosome, Elife (Cambridge) 2 (2013) e00308.
[19] M.H. Bengtson, C.A.P. Joazeiro, Role of a ribosome-associated E3 ubiquitin ligase in protein quality control, Nature 467 (2010) 470–473.
[20] Q. Defenouillere, Y. Yao, J. Mouaikel, et al., Cdc48-associated complex bound to 60S particles is required for the clearance of aberrant translation products, Proc. Natl. Acad. Sci. U.S.A. 110 (2013) 5046–5051.
[21] O. Brandman, J. Stewart-Ornstein, D. Wong, et al., A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress, Cell 151 (2012) 1042–1054.
[22] K. Ito, S. Chiba, Arrest peptides: cis-acting modulators of translation, Annu. Rev. Biochem. 82 (2013) 171–202.
[23] D. Lyumkis, S.K. Doamekpor, M.H. Bengtson, et al., Single-particle EM reveals extensive conformational variability of the Ltn1 E3 ligase, Proc. Natl. Acad. Sci. 110 (2013) 1702–1707.
[24] L.N. Dimitrova, K. Kuroha, T. Tatematsu, T. Inada, Nascent peptide-dependent translation arrest leads to Not4p-mediated protein degradation by the proteasome, J. Biol. Chem. 284 (2009) 10343–10352.