Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 356 (2007) 175–180
BBRC
www.elsevier.com/locate/ybbrc

Mouse homologue of yeast Prp19 interacts with mouse SUG1, the regulatory subunit of 26S proteasome

Choong-Ryoul Sihn [a,b], Si Young Cho [c], Jeong Ho Lee [c], Tae Ryong Lee [c], Sang Hoon Kim [a,b,*]

[a] Department of Biology, Kyung Hee University, Seoul 130-701, Republic of Korea
[b] Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, Seoul 130-701, Republic of Korea
[c] R&D Center, AmorePacific Corporation, Yongin-si, Gyeonggi-do 446-729, Republic of Korea

Received 16 February 2007
Available online 2 March 2007

Abstract

Yeast Prp19 has been shown to involve in pre-mRNA splicing and DNA repair as well as being an ubiquitin ligase. Mammalian homologue of yeast Prp19 also plays on similar functional activities in cells. In the present study, we isolated mouse SUG1 (mSUG1) as binding partner of mouse Prp19 (mPrp19) by the yeast two-hybrid system. We confirmed the interaction of mPrp9 with mSUG1 by GST pull-down assay and co-immunoprecipitation assay. The N-terminus of mPrp19 including U-box domain was associated with the C-terminus of mSUG1. Although, mSUG1 is a regulatory subunit of 26S proteasome, mPrp19 was not degraded in the proteasome-dependent pathway. Interestingly, GFP-mPrp19 fusion protein was co-localized with mSUG1 protein in cytoplasm as the formation of the speckle-like structures in the presence of a proteasome inhibitor MG132. In addition, the activity of proteasome was increased in cells transfected with mPrp19. Taken together, these results suggest that mPrp19 involves the regulation of protein turnover and may transport its substrates to 26S proteasome through mSUG1 protein.
© 2007 Elsevier Inc. All rights reserved.

Keywords: Prp19; SUG1; 26S proteasome; U-box domain; MG132

The 26S proteasome is the major non-lysosomal ATP-dependent proteolytic machinery to degrade polyubiquitinated proteins [1]. The proteasome is assembled from two large macromolecule complexes; a core 20S proteolytic particle and a 19S regulatory complex. The 19S complex is located at the end of the 20S core and provides the components necessary for unfolding and translocation of the ubiquitinated substrates into the 20S particle and regulation of the assembly of the 26S proteasome [2,3]. SUG1 is a component of the 19S regulatory particle of the proteasome [4,5]. In yeast, a mutant SUG1 accumulates ubiquitinated proteins normally degraded by the 26S proteasome [6]. In addition, SUG1 has also been shown to interact directly with various nuclear receptors in ligand-dependent fashion for the gene regulation by proteasome degradation [7,8].

Prp19 has been isolated from two independent groups by genetic screening to identify novel genes involved in DNA repair and pre-mRNA splicing, respectively [9,10]. Prp19 has conserved domain called the U-box as the active site of a novel classified E3 ubiquitin ligase [11] and coiled-coil domain as an oligomerization for the function of pre-mRNA splicing factor [12–14]. Mammalian Prp19 plays diverse roles in animal cells such as the lipid droplet biogenesis in adipocytes and the anti-aging activity in neuronal/astroglial cells as well as DNA repair and RNA splicing [15–17]. Mammalian homologue of yeast Prp19 interacts with terminal deoxynucleotidyl transferase and WRN for DNA repair [18,19].

In the present study, we report that mouse SUG1 (mSUG1), a component of the 19S regulatory complex of

* Corresponding author. Fax: +82 2 964 1079.
E-mail address: shkim@khu.ac.kr (S.H. Kim).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.02.134

the 26S proteasome, interacts with the N-terminal region of mouse Prp19 (mPrp19) to recruit their target proteins to proteasome.

Materials and methods

Cell culture and transfection. Human osteosarcoma U2OS cells were cultured with Dulbecco's modified medium supplemented with 10% fetal bovine serum. U2OS cells were grown to approximately 70–80% confluence on 35 mm dishes with coverslip for microscopy or on 100 mm dishes for other applications such as Western blot and co-immunoprecipitation. Cells were transfected by using ExGen500 reagent (MBI Fermentas, Hanover, USA) following the manufacturer's instructions. At 24 h post-transfection, cells were harvested. To block proteasome activity, cells were treated with 25 μM MG132 (Calbiochem, Darmstadt, Germany) for 6 h.

Plasmid construction. Mouse Prp19 cDNA was cloned as described before [15]. Mouse SUG1 cDNA was isolated from yeast colonies selected from yeast two-hybrid assay. Both cDNAs amplified by PCR were digested by restriction enzymes to insert suitable vectors such as pGBKT7, pcDNA3.1(+) myc (Invitrogen, Inc., USA), pGEX2T (Amersham Biosciences, Uppsala, Sweden), and pEGFPC1 (BD Biosciences, USA). Constructed plasmids were verified by sequence analysis.

Co-immunoprecipitation assay. U2OS cells co-expressing GFP-mPrp19 and mSUG1-myc, or each deletion mutants were lysed in RIPA buffer [50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% NP-40, 50 mM NaF, 200 μM Na_3VO_4, 1 mM EDTA, 0.5% sodium deoxicolated, 0.1% SDS, and protease inhibitor cocktail tablets (Roche, Meylan, France)]. After pre-clearing with protein A–Sepharose beads (Roche Diagnostics, Vienna, Austria), equal amounts of protein lysate were immunoprecipitated with anti-mPrp19 antibody for 2 h at 4 °C. Immune complexes were washed four times with RIPA buffer and were processed by Western blotting as described below.

Western blots. Immunoprecipitated proteins or proteins from whole cell extracts were separated by SDS–PAGE and blotted onto nitrocellulose filters. Blocking was performed at room temperature for 2 h in TBS with 5% nonfat milk, followed by incubation with anti-Prp19 (1:500 dilutions), anti-SUG1 (1:250 dilutions), anti-GFP (1:400 dilutions), anti-p53 (1:1000 dilutions) or anti-β-actin (1:1000 dilutions) antibodies in TBS. After washing with PBS, the membranes were incubated with horseradish-peroxidase conjugated secondary antibodies. Proteins were visualized using an enhanced chemiluminescence kit (Santa Cruz Inc., USA).

GST pull-down assay. GST fusion constructs of full-length mPrp19 were expressed in *Escherichia coli* and the fusion proteins were isolated with glutathione–Sepharose beads (Peptron Inc., Korea). U2OS cells were transiently transfected with pcDNA3-mSUG1-myc vector or pcDNA3-myc vector and were lysed in RIPA buffer at 48 h later. For GST pull-down, cell extracts were incubated with GST fusion proteins for 30 min. GST alone was used as control. After sedimentation by centrifugation, samples were washed with NETN buffer (150 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 20 mM Tris, pH 7.9, 15% glycerol) and resuspended in SDS–PAGE sample buffer. Samples were processed by Western blotting as described above.

Immunofluorescence assay. U2OS cells expressing GFP-mPrp19 and mSUG1-myc proteins were fixed with 4% paraformaldehyde for 15 min and washed with PBS. After blocking with 10% horse serum, coverslips were incubated with anti-myc antibody (1:150 dilution) for 1 h, followed by reacting with TRITC conjugated antibody at a 1:500 dilution in a humidified chamber for 45 min. After washing with PBS containing 0.1% NP-40, cells were stained by DAPI (0.1 μg/ml) for 5 min to detect nuclei. Samples were visualized using a LSM 510 microscope (Carl Zeiss, Oberkochen, Germany).

Analysis of proteasome activity. The activity of 26S proteasome was estimated in U2OS cells transfected with the pZsProSensor-1 proteasome sensor vector (Clontech Lab. Inc., USA). The pZsProSensor-1 vector encodes a destabilized GFP (ZsGreen) fused to the degradation domain of mouse ornithine decarboxylase, a specific substrate for 26S proteasome. This fusion protein is rapidly degraded by the proteasome. For normalization, pcDNA-LacZ vector was also transfected into cells. As a negative control, cells were cultured in the presence MG132 (25 μM) for 6 h. After harvesting cells, the fluorescence intensity of ZsGreen was measured by fluorophotometer with excitation at 496 nm and emission at 505 nm.

Results

The interaction of mPrp19 protein with mSUG1 protein

Initially, mSUG1 was isolated from yeast two-hybrid assay to determine the interacting proteins with mouse ortholog of yeast Prp19 (mPrp19). In order to confirm the interaction of both proteins, *in vitro* and *in vivo* binding assay were conducted. For GST pull-down assay, bacterial expressed GST-mPrp19 fusion protein or GST alone immobilized on glutathione–Sepharose beads was incubated with cell lysates from U2OS overexpressing the myc-tagged mSUG1 protein. The mSUG1 protein was detected in GST-mPrp19 protein-binding extracts, but not in GST alone (Fig. 1A). Next, U2OS cells were co-transfected with GFP-mPrp19 fusion protein and myc-tagged mSUG1 vectors to determine their interactions by co-immunoprecipitation. By immunoblotting with anti-GFP antibody, we confirmed that the myc-tagged mSUG1 protein was interacted with the GFP-mPrp19 fusion protein (Fig. 1B). In addition, endogenous mPrp19 was also associated with mSUG1 in cells expressing a myc-tagged mSUG1 protein (Fig. 1C). These results indicate that mPrp19 protein associates with mSUG1 protein directly.

The binding region of both mPrp19 protein and mSUG1 protein

We then attempted to locate the key fragment that mediates the interaction between mPrp19 and mSUG1. The N-terminus (1–186) and the C-terminus (186–406) of mSUG1 protein were tagged to the myc peptide, respectively (Fig. 2A). GST pull-down assay was performed to delineate the regions of mSUG1 required for interaction with mPrp19. As shown in Fig. 2B, GST fusion protein encoding mPrp19 was associated with the C-terminus of mSUG1 (mSUGB), suggesting that the AAA domain of mSUG1 is required for interacting with mPrp19. To determine the region of mPrp19 binding to mSUG1, several deletion mutants were constructed in pEGFP vector (Fig. 2A). U2OS cells expressing myc-tagged mSUG1 protein were transfected with either pEGFP-mPrp19 vector or a control pEGFP vector. The mPrp19 mutant containing only U box (2–68) was not interacted with mSUG1 protein. Deletion of the 223–503 amino acid fragment in mPrp19 (mPrp19C) had also weak-binding affinity with mSUG1 (Fig. 2C), whereas a construct containing the fragment of mPrp19 (1–223 aa) (mPrp19B) was sufficient for mSUG1 binding. These results clearly demonstrate that the C-terminus of mSUG1 protein including AAA domain and the N-terminus (68–223 aa) of mPrp19 protein are crucial for the interaction between both proteins.

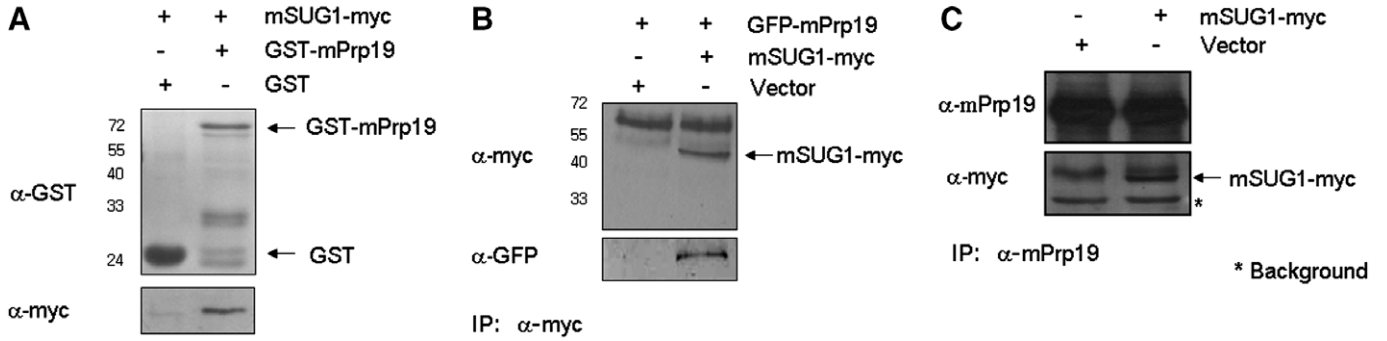

Fig. 1. The interaction of mPrp19 with mSUG1. (A) Purified GST-mPrp19 protein or GST protein was pull-downed with cell lysates from U2OS cells overexpressing mSUG1-myc. The myc-tagged mSUG1 protein was detected by immunoblotting with anti-myc antibody. (B) U2OS cells were co-transfected with both GFP fused mPrp19 vector and mSUG1-myc vector as indicated. Cell lysates were immunoprecipitated (IP) with anti-myc antibody and the signal of both proteins was detected by immunoblotting. (C) Lysates from U2OS cells transfected with mSUG1-myc vector were immunoprecipitated with anti-Prp19 antibody. Precipitates were detected by anti-myc and anti-Prp19 antibodies. *Indicates background signal.

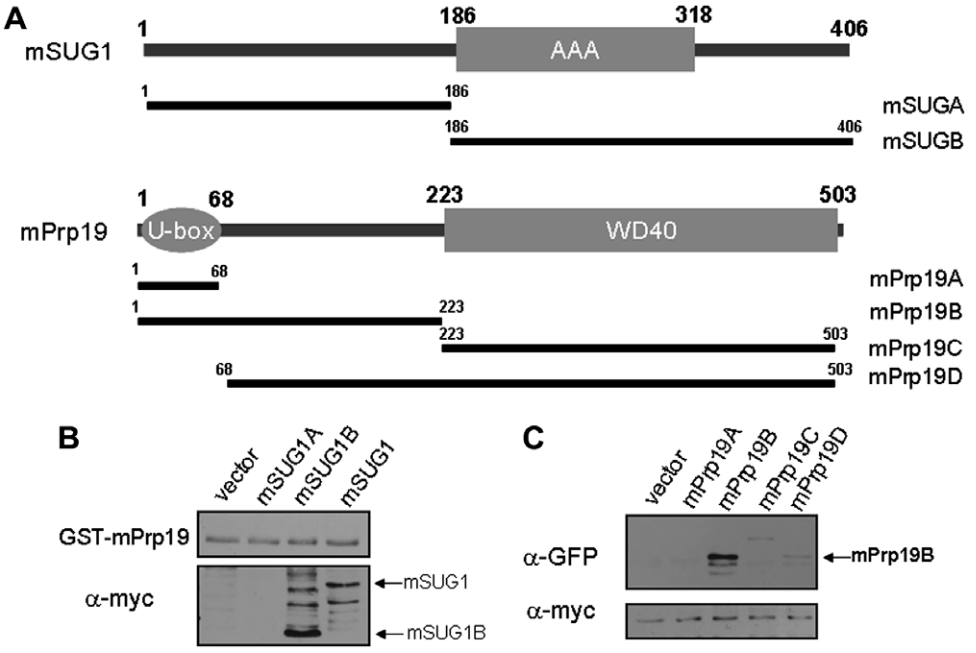

Fig. 2. Determination of binding region within mPrp19 and mSUG1 proteins. (A) Deletion constructs were designed by district domains of mPrp19 and mSUG1 as shown above. (B) For GST pull-down assay, purified GST-mPrp19 was mixed with cell lysates expressing deletion mutants of mSUG1. Samples were analysed by SDS–PAGE and were immunoblotted by using anti-myc antibody. (C) Cells were transfected with mSUG1-myc vector and truncated GFP-mPrp19 vectors. Immunocomplex was precipitated with anti-myc antibody and loaded at SDS–PAGE for immunoblotting.

The stability of mPrp19 protein and its proteasomal activity

The ubiquitin-proteasome pathway is the major system for degradation of short lived proteins. Therefore, in the present study, the possible involvement of this pathway was evaluated with the proteasome inhibitor MG132. U2OS cells were incubated with MG132 for 6 h after transfection with mSUG1 vector. Analysis by Western blotting revealed that MG132 had no significant effect on the mPrp19 protein level (Fig. 3A). In cells overexpressing mPrp19, the endogenous level of mPrp19 was also not affected by MG132 treatment, whereas the expression of p53 as a positive control was strongly induced in U2OS cells exposed to MG132. In addition, since mPrp19 has U-box domain as an ubiquitin ligase, mPrp19 may stimulate the ubiquitination of mSUG1. However, the stability of mSUG1 was not affected by mPrp19 (Fig. 3A). We could not also observe polyubiquitination pattern of mSUG1 protein by *in vivo* ubiquitination assay (data not shown). Taken together, these data imply that mPrp19 is not a substrate for mSUG1-mediated proteasome complex.

We next analysed the subcellular localization of both mPrp19 and mSUG1 in mammalian cells. The mammalian expression vectors encoding a GFP fused mPrp19 protein and a myc-tagged mSUG1 protein were transfected into cells. In U2OS cells, GFP-mPrp19 protein was

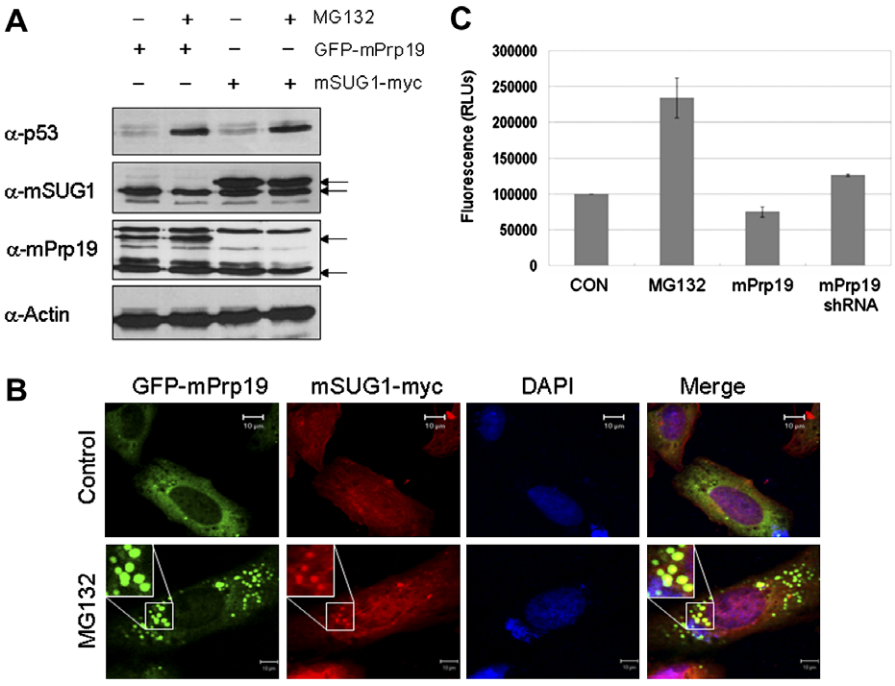

Fig. 3. The functional relationship between both mPrp19 and mSUG proteins. (A) Stability of mPrp19 and mSUG1 proteins in cells. The vectors consisting of GFP-mPrp19 and mSUG1-myc was transfected into U2OS cells. Endogenous and exogenous expressions of both proteins were detected by immunoblotting. Cells were exposed to MG-132, a proteasome inhibitor for 6 h. Arrows show exogenous and endogenous proteins indicated. (B) Co-localization of GFP-mPrp19 with mSUG1. U2OS cells co-expressed GFP-mPrp19 and mSUG1-myc proteins were immunostained by TRITC conjugated-myc antibody to observe the signal of mSUG1 protein. Prp19 was detected by the fluorescence intensity of GFP. MG132 was administered into U2OS cells. (C) U2OS cells expressing mPrp19 or mPrp19 shRNA were transiently transfected with pZsProSensor-1 vector (a reporter for proteasome activity). The mPrp19 shRNA vector was used to knock down its RNA expression in cells. 26S proteasome activity was measured by fluorescence photometer (ex, 496 nm; em, 505 nm). The data were normalized by β-galactosidase activity.

ubiquitously localized at the cytoplasm, whereas mSUG1-myc protein was distributed throughout a cell (Fig. 3B). The co-localization of both proteins was rare in cells. However, in the MG132-treated cells mPrp19 protein was clearly co-localized with mSUG1 protein. Both proteins were formed the speckle-like foci in the cytoplasm in the presence of MG132. The co-localization of mSUG1 with mPrp19 led to the suggestion that mSUG1 and mPrp19 might play a role in the regulation of protein turnover.

Finally, to assess the impact of mPrp19 on proteasome activity, a proteasome reporter construct, pZsProSensor-1, was transiently co-transfected with mPrp19 vector or mPrp19 shRNA vector into U2OS cells. As shown in Fig. 3C, the overexpression of mPrp19 in cells caused the enhancement of 26S proteasome activity, while the knock-down of mPrp19 by using shRNA expressing vector leaded to decrease proteasome activity. These results indicate that mPrp19 play a role on protein degradation process and may transport its substrate to the proteasome complex for quick protein turnover.

Discussion

Most of proteins isolated as binding partner of Prp19 protein have been known to involve the process of DNA repair, chromatin assembly, and RNA splicing [20–22]. In the present study, we isolated mSUG1 through a yeast two-hybrid screening with mPrp19 as bait. SUG1 is one of six ATPase in the 19S regulatory particle of the 26S proteasome which plays a role in proteasome-mediated degradation of transcription factor and nuclear receptor such as Sp1, estrogen receptors, vitamin D receptor or thyroid hormone receptor [8,23].

Based on previous studies, the 19S regulatory particle of the proteasome has been known to bind components of the ubiquitin system to regulate the ubiqutin-proteasome degradation pathway [24,25]. The human RNF2, an E3 ubiquitin ligase, associates with S6 ATPase, a subunit of the 19S proteasomal complex leading to increase the ATP hydrolysis activity [25]. In the present study, mPrp19 and mSUG1 proteins were not served as substrates for their enzymatic activity. However, in the presence of MG132, a proteasome inhibitor, both proteins were concentrated into clastosome foci as a typical pattern for proteasome [26]. In particular, cells transfected with mPrp19 were induced to increase the 26S proteasomal activity. These results together with our results suggest that mPrp19 plays a role in the SUG1-mediated substrate degradation for the 26S proteasome and it may recruit ubiquitinated substrates into proteasome.

Interestingly, there is a report to show that SNEV, an another term of Prp19, interacts with PSMB4, the β7 subunit of the 20S proteasome, accumulating as speckle-like

structures within cells [27]. The report insists that N-terminus of SNEV lacking WD40 domain is essential for binding to PSMB4. In the present study, the same region of mPrp19 having coiled-coil domain was also required for mSUG1 interaction. The N-terminal coiled-coils region is known to be responsible for the interaction of proteasome subunits [28,29]. Since coiled-coils are built from α-helices which mediate the oligomerization of various proteins [30], Prp19 forms a tetramer via its N-terminal coiled-coil domain [14]. Thus, the N-terminus of mPrp19 containing coiled-coil domain is important for the association with proteasome complex directly, which might be coupled for degradation of their substrates.

In most cases, targeting to the 26S proteasome is dependent on tagging of the substrate with a polyubiquitin chain which is mediated by E3 ligase [31]. Therefore, polyubiquitinated proteins by mPrp19 could be targeted to the proteasome through the interaction of both 19S particle and 20S core. However, although Prp19 has an E3 ubiquitin ligase activity to conjugate ubiquitin *in vitro* [32], its substrates are not identified yet. Once specific substrates of Prp19 would be identified, the accurate mechanism of E3 ligase with proteasome complex will get closer to be solved. In conclusion, these findings shed new light on our understanding of Prp19 function related to proteasome activity and it may be putative anchor protein to recruit the substrate of proteasome.

Acknowledgment

This work was supported by a grant from BAERI of National Nuclear R&D program, Korean Ministry of Science and Technology.

References

[1] A. Ciechanover, The ubiquitin-proteasome pathway: on protein death and cell life, EMBO J. 17 (1998) 7151–7160.
[2] Q. Deveraux, V. Ustrell, C. Pickart, M. Rechsteiner, A 26S protease subunit that binds ubiquitin conjugates, J. Biol. Chem. 269 (1994) 7059–7061.
[3] R. Hartmann-Petersen, C. Gordon, Protein degradation: recognition of ubiquitinylated substrates, Curr. Biol. 14 (2004) R754–R756.
[4] D.M. Rubin, O. Coux, I. Wefes, C. Hengartner, R.A. Young, A.I. Goldberg, D. Finley, Identification of the gal4 suppressor Sug1 as a subunit of the yeast 26S proteasome, Nature 379 (1996) 655–657.
[5] M.H. Glickman, D.M. Rubin, V.A. Fried, D. Finley, The regulatory particle of the *Saccharomyces cerevisiae* proteasome, Mol. Cell. Biol. 18 (1998) 3149–3162.
[6] M. Ghislain, A. Udvardy, C. Mann, *S. Cerevisiae* 26S protease mutants arrest cell division in G2/metaphase, Nature 379 (1996) 655–657.
[7] W. Wang, P.M. Chevray, D. Nathans, Mammalian Sug1 and c-Fos in the nuclear 26S proteasome, Proc. Natl. Acad. Sci. USA 93 (1996) 8236–8240.
[8] K. Su, X. Yang, M.D. Roos, A.J. Paterson, J.E. Kudlow, Human Sug1/p45 is involved in the proteasome-dependent degradation of Sp1, Biochem. J. 348 (2000) 281–289.
[9] S.C. Cheng, W.Y. Tarn, T.Y. Tsao, J. Abelson, PRP19: a novel spliceosomal component, Mol. Cell. Biol. 13 (1993) 1876–1882.
[10] J.A. Henriques, E.J. Vicente, K.V. Leandro da Silva, A.C. Schenberg, PSO_4: a novel gene involved in error-prone repair in *Saccharomyces cerevisiae*, Mutat. Res. 218 (1989) 111–124.
[11] S. Hatakeyama, K.I. Nakayama, U-box proteins as a new family of ubiquitin ligases, Biochem. Biophys. Res. Commun. 302 (2003) 635–645.
[12] M.D. Ohi, C.W. Vander Kooi, J.A. Rosenberg, L. Ren, J.P. Hirsch, W.J. Chazin, T. Walz, K.L. Gould, Structural and functional analysis of essential pre-mRNA splicing factor Prp19p, Mol. Cell. Biol. 25 (2005) 451–460.
[13] C.W. Vander Kooi, M.D. Ohi, J.A. Rosenberg, M.L. Oldham, M.E. Newcomer, K.L. Gould, W.J. Chazin, The Prp19 U-box crystal structure suggests a common dimeric architecture for a class of oligomeric E3 ubiquitin ligases, Biochemistry 45 (2006) 121–130.
[14] J. Grillari, P. Ajuh, G. Stadler, M. Loscher, R. Voglauer, W. Ernst, J. Chusainow, F. Eisenhaber, M. Pokar, K. Fortschegger, M. Grey, A.I. Lamond, H. Katinger, SNEV is an evolutionarily conserved splicing factor whose oligomerization is necessary for spliceosome assembly, Nucleic Acids Res. 33 (2005) 6868–6883.
[15] S.Y. Cho, E.S. Shin, P.J. Park, D.W. Shin, H.K. Chang, D. Kim, H.H. Lee, J.H. Lee, S.H. Kim, M.J. Song, I.S. Chag, O.S. Lee, T.R. Lee, Identification of mouse Prp19p as a lipid droplet-associated protein and its possible involvement in the biogenesis of lipid droplets, J. Biol. Chem. 282 (2007) 2456–2465.
[16] Y. Urano, M. Iiduka, A. Sugiyama, H. Akiyama, K. Uzawa, G. Matsumoto, Y. Kawasaki, F. Tashiro, Involvement of the mouse Prp19 gene in neuronal/astroglial cell fate decisions, J. Biol. Chem. 281 (2006) 7498–7514.
[17] R. Voglauer, M.W. Chang, B. Dampier, M. Wieser, K. Baumann, T. Sterovsky, M. Schreiber, H. Katinger, J. Grillari, SNEV overexpression extends the life span of human endothelial cells, Exp. Cell Res. 312 (2006) 746–759.
[18] K.N. Mahajan, B.S. Mitchell, Role of human Pso4 in mammalian DNA repair and association with terminal deoxynucleotidyl transferase, Proc. Natl. Acad. Sci. USA 100 (2003) 10746–10751.
[19] N. Zhang, R. Kaur, X. Lu, X. Shen, L. Li, R.J. Legerski, The Pso4 mRNA splicing and DNA repair complex interacts with WRN for processing of DNA interstrand cross-links, J. Biol. Chem. 280 (2005) 40559–40567.
[20] L.F. Revers, J.M. Cardone, D. Bonatto, J. Saffi, M. Grey, H. Feldmann, M. Brendel, J.A. Henriques, Thermoconditional modulation of the pleiotropic sensitivity phenotype by the Saccharomyces cerevisiae PRP19 mutant allele pso4-1, Nucleic Acids Res. 30 (2002) 4993–5003.
[21] M.D. Ohi, K.L. Gould, Characterization of interactions among the Cef1p-Prp19p-associated splicing complex, RNA 8 (2002) 798–815.
[22] C.H. Chen, W.C. Yu, T.Y. Tsao, L.Y. Wang, H.R. Chen, J.Y. Lin, W.Y. Tsai, S.C. Cheng, Functional and physical interactions between components of the Prp19p-associated complex. Nucleic Acids Res. 30 (2002) 1029–1037.
[23] H. Masuyama, Y. Hiramatsu, Involvement of suppressor for Gal 1 in the ubiquitin/proteasome-mediated degradation of estrogen receptors, J. Biol. Chem. 279 (2004) 12020–12026.
[24] P.G. Corn, E.R. McDonald III, J.G. Herman, W.S. El-Deiry, Tat-binding protein-1, a component of the 26S proteasome, contributes to the E3 ubiquitin ligase function of the von Hippel-Lindau protein, Nature Genetics 35 (2003) 229–237.
[25] S.J. Lee, D. Choi, H. Rhim, S. Kang, E3 ubiquitin ligase RNF2 interacts with the S6' proteasomal ATPase subunit and increases the ATP hydrolysis activity of S6', Biochem. J. 389 (2005) 457–463.
[26] M. Lafarga, M.R. Berciano, E. Pena, I. Mayo, J.G. Castano, D. Bohmann, J.P. Rodrigues, J.P. Tavanez, M. Carmo-Fonseca, Clastosome: a subtype of nuclear body enriched in 19S and 20S proteasome, ubiquitin, and protein substrates of proteasome, Mol. Biol. Cell 13 (2002) 2771–2782.
[27] M. Loscher, K. Fortschegger, G. Ritter, M. Wostry, R. Voglauer, J.A. Schmid, S. Watters, A.J. Rivett, P. Ajuh, A.I. Lamond, H. Katinger, J. Grillari, Interaction of U-box E3 ligase SNEV with

PSMB4, the beta7 subunit of the 20S proteasome, Biochem. J. 388 (2005) 593–603.
[28] C. Gorbea, D. Taillandier, M. Rechsteiner, Assembly of the regulatory complex of the 26S proteasome, Mol. Biol. Rep. 26 (1999) 15–19.
[29] C. Richmond, C. Gorbea, M. Rechsteriner, Specific interactions between ATPase subunits of the 26S protease, J. Biol. Chem. 272 (1997) 13403–13411.
[30] A. Lupas, Coiled coils: new structures and new functions, Trends Biochem. Sci. 21 (1996) 375–382.
[31] Y.A. Lam, T.G. Lawson, M. Velayutham, J.L. Zweier, C.M. Pickart, A proteasomal ATPase subunit recognizes the polyubiquitin degradation signal, Nature 416 (2002) 763–767.
[32] S. Hatakeyama, M. Yada, M. Matsumoto, N. Ishida, K.I. Nakayama, U box proteins as a new family of ubiquitin-protein ligases, J. Biol. Chem. 276 (2001) 33111–33120.