Sem1p Is a Novel Subunit of the 26 S Proteasome from *Saccharomyces cerevisiae*

The 26 S proteasome, which catalyzes degradation of polyubiquitinated proteins, is composed of the 20 S proteasome and the 19 S regulatory particle (RP). The RP is composed of the lid and base subcomplexes and regulates the catalytic activity of the 20 S proteasome. In this study, we carried out affinity purification of the lid subcomplexes from the tagged strains of *Saccharomyces cerevisiae*, and we found that the lid contains a small molecular mass protein, Sem1. The Sem1 protein binds with the 26 S proteasome isolated from a mutant with deletion of SEM1 but not with the 26 S proteasome from the wild type. The lid lacking Sem1 is unstable at a high salt concentration. The 19 S RP was immunoprecipitated together with Sem1 by immunoprecipitation using hemagglutinin epitope-tagged Sem1 as bait. Degradation of polyubiquitinated proteins in vitro is impaired in the Sem1-deficient 26 S proteasome. In addition, genetic interaction between SEM1 and RPN10 was detected. The human Sem1 homologue hDSS1 was found to be a functional homologue of Sem1 and capable of interacting with the human 26 S proteasome. The results suggest that Sem1, possibly hDSS1, is a novel subunit of the 26 S proteasome and plays a role in ubiquitin-dependent proteolysis.

In eukaryotic cells, the ubiquitin-proteasome system regulates various cellular processes (1–3). In this pathway, target proteins are polyubiquitinated by E1/E2/E3 enzymes, and the thus formed polyubiquitin chain is recognized by the 20 S proteasome, and the protein portion is degraded in an ATP-dependent manner. The 26 S proteasome is composed of a core particle (CP, also known as the 20 S proteasome), which contains proteolytic active sites in its cavity, and the 19 S regulatory particle (RP), which regulates the catalytic activity of the CP (2–4). The CP, built of two copies each of seven di-
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With the exception of RPN9, RPN10, and RPN13 genes, all of the regulatory subunit genes are essential. Rpn10 binds the polyubiquitin chain both in its free form and when incorporated into the 26 S proteasome (10–13). Rpn10 positions at the interface between the lid and base and strengthens the lid-base interaction (6). The base is thought to promote translocation of substrates into the CP (14). Binding of the RP to the CP is thought to open a narrow pore at the ends of the CP. Rpt2 functions as a gating device for the CP channel (15, 16). Rpt5/6’ have been reported to be additional intrinsic polyubiquitin chain-binding subunits (17). Rpn1 acts as a receptor for ubiquitin-like proteins such as Rad23 (12, 13). Among the lid subunits, Rpn3, Rpn6, Rpn9, Rpn11, and Rpn12 have been characterized (18–21): Rpn11 has been reported to be a novel metalloprotease that has a deubiquitinating enzyme activity, releasing polyubiquitin chains from substrates (22–24).

In this study, we purified the base and lid subcomplexes of the 26 S proteasome from the yeast *Saccharomyces cerevisiae*, and we found that the respective subcomplexes have additional small molecular mass subunits. Rpn13 was identified as an additional subunit of the base, whereas Sem1 was identified as an additional subunit of the lid. Rpn13 has been reported to be one of the RP subunits (8). Sem1 was originally identified as a suppressor of the sec15-1 temperature-sensitive mutation and a negative regulator of pseudohyphal differentiation (25). We have obtained several lines of evidence that indicate that Sem1 is a novel lid subunit of the 26 S proteasome and is necessary for enzymatic activity of the 26 S proteasome. First, the recombinant Sem1 is capable of binding with the 26 S proteasome purified from a mutant with deletion of SEM1 (∆sem1) but not with that from the wild type in vitro. Second, the purified 26 S proteasome from the ∆sem1 strain seems to be structurally equivalent to the wild type 26 S proteasome, but the 26 S proteasome and the lid from ∆sem1 became unstable under high salt conditions. Third, the 19 S RP can be immunoprecipitated with Sem1 as bait. Fourth, the ∆sem1 strain shows a temperature-sensitive phenotype, and polyubiquitinated proteins were accumulated in this mutant at the restrictive temperature. Fifth, there was little degradation of polyubiquiti-

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The abbreviations used are: E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin ligase; CP, core particle; RP, regulatory particle; SHFM, split hand/split foot malformation; GST, glutathione S-transferase; ORF, open reading frame; MS, mass spectrometry; Suc, succinyl; MCA, 4-methylcoumaryl-7-amide; PACE, proteasome-associated control element; HA, hemagglutinin.

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Received for publication, March 22, 2004
Published, JBC Papers in Press, April 26, 2004, DOI 10.1074/jbc.M403165200

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This paper is available on line at http://www.jbc.org

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nated Scl1 by the 26 S proteasome from the Δsem1 strain in vitro. Finally, genetic interaction between RP10 and SEM1 genes was observed. The human DSS1 (hDSS1) gene, a homologue of SEM1, is a candidate gene responsible for an autosomal dominant form of split hand/split foot malformation (SHFM) disorder (26), and the hDSS1 protein has been reported to interact with the breast cancer susceptibility antigen BRCA2 (27). We demonstrated that hDSS1 complements the phenotype of the S. cerevisiae Δsem1 mutant and that hDSS1 is capable of interacting with the human 26 S proteasome in mammalian cells, suggesting that hDSS1 is functionally conserved among eukaryotes. Thus, these biochemical and genetic findings suggest that Sem1p is a component of the 26 S proteasome and functions in ubiquitin-dependent proteolysis.

EXPERIMENTAL PROCEDURES

Yeast Strains, Media, and Genetic Methods—The S. cerevisiae strains used in this study are listed in Table 1. All strains are isogenic to W303. The Escherichia coli strain DH5α was used as a host for propagation and construction of plasmids. E. coli strain Rosetta (DE3) (Novagen) were used for expression of GST fusion proteins. Synthetic media (SD) were prepared as described previously (28). Synthetic media lacking appropriate nutrient(s) were used to select strains containing specific plasmids. Yeast nutrient-rich medium (YPD) consisted of 2% glucose, 2% polypeptide, 1% yeast extract (Difco), 400 mg/ml uracil, and 20 μg/ml uracil. Transformation of yeast was carried out by the method described by Ca. Yeast cultures were grown to an absorbance of 600 nm (A600) at 2.0 in YPD medium at 30 °C. Cells were harvested and washed twice with ice-cold water and once with buffer A (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 10% glycerol). The pellet was stored at −80 °C until use. Cells (4 g) were thawed, suspended in 4 ml of buffer B (buffer A containing 4 mM ATP, 10 mM MgCl2, and 2 mM ATP regeneration system), and lysed by glass beads using a bead-beater (Biospec Products). An equal volume of buffer B was added to the lysate, and the mixture was centrifuged at 20,000 × g for 30 min at 4 °C. The beads were transferred to a 2-ml disposable column (Bio-Rad) and incubated with 100 μl of 3× FLAG peptide (100 μg/ml) (Sigma) in buffer C for 30 min at 4 °C to elute the 26 S proteasome. The purified 26 S proteasome was stored at −80 °C. The base, lid, and CP were isolated using YYS98 (Δrpn10 Δrpn11Δ4FLAG) and YYS37 (Δpre1Δ3FLAG) strains, respectively.

### Table 1

| Strain    | Genotype | Source                  |
|-----------|----------|-------------------------|
| W303-1A   | MATa leu2 his3 trp1 ura3 ade2 can1 | Our stock               |
| YYS37     | MATa pre1::PRE1Δ3FLAG, HIS3 | This study               |
| YYS39     | MATa rpn1::RP1Δ4FLAG, HIS3 | This study               |
| YYS40     | MATa Δrpn11::RP11Δ4FLAG, HIS3 | This study               |
| YYS80     | MATa Δrpn10::kan’ | This study               |
| YYS98     | MATa Δrpn10::kan’ Δrpn1::RP11Δ4FLAG-HIS3 | This study               |
| YYS99     | MATa Δrpn10::kan’ Δrpn11::RP11Δ4FLAG-HIS3 | This study               |
| YYS143    | MATa Δpre1::TRP1 | This study               |
| YTS63     | MATa Δsem1::URA3 | This study               |
| YTS67     | MATa Δsem1::URA3 Δrpn10::kan’ | This study               |
| YTS68     | MATa Δsem1::URA3 Δrpn11::RP11Δ4FLAG-HIS3 | This study               |

**Plasmid Characteristics**

| Plasmid | Characteristics | Source                  |
|---------|-----------------|-------------------------|
| pKT10   | P2oHIS3, 2 μm, ori | Our stock               |
| pRS303  | HIS3            | Our stock               |
| pRS304  | TRP1            | Our stock               |
| pRS306  | URA3            | Our stock               |
| pRS314  | ARS-CEN, TRP1   | Our stock               |
| pTS901CT| ARS-CEN, TRP1, 5× HA epitope | This study               |
| YCP-SEM13HA | ARS-CEN, SEM1, SEM13HA (pTS901CT) | This study               |
tively, as follows. Anti-FLAG M2 beads, to which the 26 S proteasome from each of the above strains had been bound, were incubated with buffer D (50 mM Tris-HCl, pH 7.5, 500 mM NaCl, and 10% glycerol) for 1 h at 4 °C to dissociate the 26 S proteasome to the lid, base, and CP. The beads were then washed four times with 10 ml of buffer D, incubated with buffer D containing 0.2% Triton X-100 for 5 min at 25 °C, and washed twice with the same buffer and twice with buffer A. The base, lid, or CP, bound to the beads, was then eluted with 3× FLAG peptide as described above.

**SDS-PAGE and Peptide Sequence Analysis**—The isolated base and lid were resolved by 12.5% SDS-PAGE, and protein bands were excised from the SDS-polyacrylamide gel. The gels containing the respective bands were excised and washed twice with the same buffer and twice with buffer A. The proteins were then eluted with 40 μl of 0.1 M Tris-HCl, pH 3.0, which immediately neutralized by addition of 1.5 μl of 1.5 M Tris-HCl, pH 8.8.

**Accumulation of Polyubiquitinated Proteins**—The wild type and mutant strains of *S. cerevisiae* were cultured in YPD medium at 25 °C to the logarithmic phase and then cultured at 37 °C. Cells corresponding to an A660 at 1.0 unit were periodically harvested and lysed by the mild alkali method (34). The extract was subjected to SDS-PAGE and then to Western blotting with an FK2 antibody that specially recognizes polyubiquitin chains (32) and also with an anti-actin antibody (Sigma) as a control.

**Preparation of Polyubiquitinated Cdc34 and Gel Shift Assay for Its Binding with the 26 S Proteasome**—Polyubiquitinated Cdc34 was produced using Cdc34/ubc3 (E2) and Uba1 (E1). (The details of isolation procedures of Cdc34 and Uba1 will be published elsewhere.) A reaction mixture consisting of 25 mM Tris-HCl, pH 7.5, 50 mM NaCl, 10 mM dithiothreitol, 10 mM MgCl2, 10 mM ATP, 2.5 μg/ml ubiquitin (Sigma), 40 ng/ml Uba1, and 200 ng/ml Cdc34 was incubated for 12 h at 37 °C. The level of polyubiquitination was estimated by Western blotting with an FK2 antibody. The resultant polyubiquitinated Cdc34 in the indicated amount was mixed with 3 pmol of the affinity-purified 26 S proteasomes in a final volume of 4 μl, and the mixture was incubated for 15 min at room temperature and then subjected to nondenaturing PAGE, followed by in-gel peptideasy assay for the proteasome.

**Preparation of Polyubiquitinated Sic1** and in Vitro Degradation Assay—to prepare polyubiquitinated Sic1, we developed a novel method using Rsp5 as an E3 ligase. (The details of this method will be published elsewhere.) In brief, the PY motif was introduced into the N-terminal region of Sic1, designated as Sic1PY, to allow Rsp5 to bind to Sic1. The resultant Sic1PY and the original Sic1 were expressed as fusion proteins with T7 tags at the N termini and HAT tags (Clontech) at the C termini, designated as TT-Sic1PY-HAT and TT-Sic1-HAT, respectively, constructed by the pET system (Novagen). T7-Sic1PY-HAT and T7-Sic1-HAT proteins were purified according to the method described by Verma et al. (23) and were then incubated with Uba1 (E1), Ub4c (E2), and Rsp5 (E3). (The details of isolation procedures of Ub4c and Rsp5 will be published elsewhere.) The resultant polyubiquitinated Sic1PY (400 nm) was incubated with the affinity-purified 26 S proteasome (200 pmol) from the wild type and 26 S proteasome reaction was terminated by the addition of SDS-loading buffer, and the reaction mixture was then subjected to SDS-PAGE, followed by Western blotting with an anti-T7 antibody to detect the degree of degradation.

**Growth Assay**—The wild type and mutant strains of *S. cerevisiae* were cultured in YPD medium at 25 °C to A660 of 1.0–2.0, and their densities were adjusted to A660 of 0.5 by adding YPD medium. A 10-fold serial dilution was prepared in deionized water, and the serially diluted cells (2 μl each) were spotted on YPD agar plates and incubated at 25 or 37 °C for 2 days. For suppression analysis, low copy plasmids, pRS314-SEM1 and pRS314-SS1, were transformed into the Δsem1 strain (YT963). The transformants were spotted onto SD-Trap plates and incubated at 25 or 37 °C for 7 days.

**Mammalian Cell Culture, Transfection, and Immunoprecipitation**—Human embryonic kidney (HEK) 293T cells were cultured in Dulbecco’s modified Eagle’s medium (Sigma) supplemented with 10% inactivated fetal bovine serum (Invitrogen) at 37 °C under a 5% CO2 atmosphere. HEK293T cells were transfected with the pCI-neo-hDSS1-FLAG or pCI-neo-MTase1-FLAG plasmid according to the manufacturer’s protocol and cultured for 36 h at 37 °C. The cells were washed with ice-cold phosphate-buffered saline, suspended with buffer A containing 0.2% Nonidet P-40 with or without 2 mM ATP and 5 mM MgCl2, and then lysed using a 23-gauge needle. The lysate was centrifuged at 20,000 × g for 30 min at 2 °C, and the resulting supernatant was incubated with 50 μl of anti-FLAG antibody for 4 h. After the beads had been washed five times with the respective buffers, the materials bound to the beads were eluted with 50 μl of 3× FLAG peptide (100 μg/ml) in buffer C and subjected to SDS-PAGE and to Western blotting. Alternatively, the eluates obtained from the above

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**Immunoprecipitation with Anti-HA Antibody-Immunoblotting Beads**—The *S. cerevisiae* strains were grown to an A660 at 1.0 in SD-Trap media at 30 °C. Cells were pelleted by centrifugation and washed with buffer A. The pellet was suspended in 1 ml of buffer A and lysed by glass beads. An equal volume of buffer A was added to the lysate, and the suspension was centrifuged at 20,000 × g for 30 min at 2 °C. 25 μl of anti-HA affinity matrix (Roche Applied Science) was added to the supernatant, and the mixture was rotated for 1 h at 4 °C. The beads were washed four times with buffer A, and the bound proteins were then treated with 40 μl of 1.5 M Tris-HCl, pH 3.0, which immediately neutralized by addition of 1.5 μl of 1.5 M Tris-HCl, pH 8.8.

**Polyubiquitinated Proteins**—The wild type and mutant strains of *S. cerevisiae* were cultured in YPD medium at 25 °C to the logarithmic phase and then cultured at 37 °C. Cells corresponding to an A660 at 1.0 unit were periodically harvested and lysed by the mild alkali method (34). The extract was subjected to SDS-PAGE and then to Western blotting with an FK2 antibody that specially recognizes polyubiquitin chains (32) and also with an anti-actin antibody (Sigma) as a control.
purified by conventional chromatography (5), and peptide mass fingerprinting revealed that the 26 S proteasomes from the tagged strains contained all of the subunits and some proteasome-interacting proteins that had been reported previously (6–9) (data not shown). Thus, we concluded that 3× FLAG epitope tagging to the respective subunits should not affect the structure and enzymatic activity of the 26 S proteasome and that the respective tagged strains can be used to isolate the 26 S proteasome. Because the lid subcomplex has been reported to be easily dissociated from the 26 S proteasome prepared from a mutant with deletion of RPN10 (Δrpn10) (6), the base and lid subcomplexes were isolated from the respective tagged Δrpn10 strains by affinity chromatography in the presence of 500 mM NaCl (Fig. 1 C, base and lid). The poly peptide pattern of the 26 S proteasome was a close summation of those of the base, lid, and 20 S proteasome (CP) (Fig. 1C).

Small Molecular Mass Proteins Are Co-purified with the Lid and Base Subcomplexes—Closer inspection of Fig. 1C revealed that the affinity-purified lid and base subcomplexes contain two proteins with molecular masses of 19.5 (band 1 in Fig. 1C) and 17.5 kDa (band 2), respectively. Both bands were also detected in the 26 S proteasome but not in the 20 S proteasome. It is possible that these proteins are degradation products of 19 S RP itself, but the fact that these proteins were found to be co-fractionated with the respective subcomplexes by conventional purification procedures, including anion exchange chromatography and gel filtration (7), suggests that these small proteins are components of the 19 S RP or proteasome-interacting proteins.

To identify these proteins, the respective protein bands were excised from the gel and were in-gel digested with V8 protease or lysyl endopeptidase. The resultant peptides were extracted, separated by reverse-phase high performance liquid chromatography, and subjected to amino acid sequence determination. The identified peptides are listed in Table II. The sequence of each peptide completely matched the deduced sequence from the yeast ORF reported in theSaccharomyces Genome Data Base, allowing the assignment of each protein as the product of a specific chromosomal gene. Given that the S. cerevisiae genome is entirely known, the peptide sequence data are sufficient to assign each band as the product of a single gene. As a result, band 1 was identified as a small acidic protein, Sem1, whereas band 2 was identified as Rpn13, a recently identified novel subunit of the budding yeast 19 S RP (8). Thus, the latter result strongly suggests that Rpn13 is a base subunit.

By amino acid sequencing, Sem1 was identified as a protein bound to the affinity-purified lid. Sem1 is a small (89 amino acid residues) acidic protein with a theoretical molecular mass of 10,386.15 Da and 4.16, respectively. The difference between the theoretical molecular mass (10.4 kDa) and apparent mobility in the SDS-polyacrylamide gel (19.5 kDa) of Sem1 may be caused by its acidity, because bacterially expressed Sem1 also migrated to a point about 19 kDa in SDS-PAGE (data not shown). The SEM1 (Suppressor of Exocytosis Mutations) gene is located at YDR363W-A and has been cloned as a multicopy suppressor of the temperature-sensitive mutation sec15-1 (25). The SEM1 gene is not essential, and its deletion strain shows a temperature-sensitive phenotype. Sem1 is highly conserved across evolution. Sem1 and the human homologue are 69% similar and 47% identical, whereas the mouse, rat, and human homologues are 100% identical (25). Thirteen amino acid residues are completely conserved throughout the species, suggesting a crucial role for these residues. The human homologue, DSS1 (GenBank accession number U41515), has been mapped to the locus affected in the autosomal dominant form of SHFM disorder (26). Because Sem1 or its homologue...
stability of the 26 S proteasome and the lid subcomplex at high
structural role of Sem1 in the 26 S proteasome, we tested the
— GST-Sem1 (lane 8) whereas that from the wild type
proteasomes (Fig. 2) rather than a proteasome-interacting protein.
that Sem1 is an intrinsic component of the 26 S proteasome
the affinity-purified 26 S proteasome from the wild type strain
uitin receptors for the 26 S proteasome, are able to bind with
proteasome antibodies (Fig. 2) except for Sem1. The subunit composition of the 26 S proteasome from the
sem1 strain was essentially the same as that from the wild type strain (SEM1) except for Sem1.
To determine whether the lack of Sem1 affects the structure
and/or enzymatic activity of the 26 S proteasome, the affinity-purified 26 S proteasomes from the respective strains were separated by nondenaturing PAGE, followed by in-gel peptidase assay using Suc-Leu-Leu-Val-Tyr-MCA (Fig. 2B). The mobility and peptidase activity of the 26 S proteasome from the
sem1 strain were found to be equivalent to those of the 26 S proteasome from the wild type SEM1 strain. The same result was obtained when peptidase activity was assayed under SDS-free conditions (data not shown). As judged from the data of SDS-PAGE and nondenaturing PAGE, the purified 26 S proteasome seems to be a double-capped form (RP2CP). Thus, these results imply that the global structure of the 26 S proteasome remains unchanged in the absence of Sem1. In addition, it should be noted that the band intensity of Sem1 in the SDS-polyacrylamide gel seems to be almost equivalent to that of Rpn13 (see Figs. 1C and 2A), implying that Sem1 is a stoichiometric component of the 26 S proteasome. To confirm this, we performed a GST pull-down assay using GST-Sem1 fusion protein. GST-Sem1 or GST, expressed and purified from E. coli, was bound to glutathione-immobilized agarose beads and incubated with the affinity-purified 26 S proteasome from the wild type SEM1 or sem1 strain. After washing, the bound proteins were eluted with SDS loading buffer and subjected to SDS-PAGE followed by Western blotting with anti-FLAG (Rpn113HA, lid), and anti-20 S proteasome antibodies. Because 3× FLAG peptides
have not been reported to be a proteasome subunit in either yeast or other species, it should be tested whether Sem1 is a subunit of the 26 S proteasome.

GST-Sem1 Binds with Only the 26 S Proteasome from \( \Delta \)sem1 Strain—It is known that some lid subunit mutations result in a structural defect of the 26 S proteasome. For example, the 26 S proteasome purified from a \( \Delta \)rpn9 mutant lacked some subunits and was easily dissociated into subcomplexes (33), and the lid-less proteasome was affinity-purified from an \( \Delta \)rpn11mut1-2 mutant (23). To characterize the 26 S proteasome from the mutant with deletion of SEM1 (\( \Delta \)sem1), the 26 S proteasomes were affinity-purified from YYS40 (RPN113FLAG) and YTS67 (\( \Delta \)sem1 RPN113FLAG) and subjected to SDS-PAGE analysis (Fig. 2A). The subunit composition of the 26 S proteasome from the \( \Delta \)sem1 strain was essentially the same as that from the wild type strain (SEM1) except for Sem1.

To determine whether the lack of Sem1 affects the structure and/or enzymatic activity of the 26 S proteasome, the affinity-purified 26 S proteasomes from the respective strains were separated by nondenaturing PAGE, followed by Western blotting with anti-FLAG antibody-immobilized beads from cells transformed with the corresponding plasmid (Fig. 3A). The same result was obtained with cells transformed with plasmid (Fig. 3A). The proteasomes were purified from each of the 26 S proteasome purified from the wild type SEM1 strain, YYS40 (RPN113FLAG), and the \( \Delta \)sem1 strain, YTS67 (\( \Delta \)sem1 RPN113FLAG) and had almost equal peptidase activities. For example, the 26 S proteasome purified from the wild type SEM1 strain (b), the 26 S proteasome purified from \( \Delta \)sem1 strain (c), and control sample purified from no-tag strain (a) in the same volume. The materials bound to the beads were eluted with SDS-loading buffer and analyzed by Western blotting with anti-Rp5 (base), anti-FLAG (Rpn113FLAG, lid), and anti-20 S proteasome antibodies.

| Band | Amino acid sequence | Protein identified | Residue no. | Chromosomal locus |
|------|---------------------|--------------------|-------------|------------------|
| 1    | INKKSL         | Sem1               | 24–30       | YDR363W-A        |
| 2    | LDRVYKE        | Rpn13/Daq1         | 81–87       | YLR421C          |

Fig. 2. GST-Sem1 binds with the 26 S proteasome isolated from \( \Delta \)sem1 strain. A, subunit compositions of the 26 S proteasomes purified from the wild type SEM1 strain, YYS40 (RPN113FLAG), and the \( \Delta \)sem1 strain, YTS67 (\( \Delta \)sem1 RPN113FLAG). The proteasomes were resolved by 12% SDS-PAGE, and protein bands were visualized by Coomassie Brilliant Blue (CBB) staining. The no-tag lane refers to a control sample that had been affinity-purified from an untagged parental strain, W303-1A. The position of Sem1 is indicated by an asterisk. M, molecular mass standards. B, nondenaturing PAGE and in-gel peptidase assay of the proteasomes purified from the wild type SEM1 and \( \Delta \)sem1 strains. The proteasomes were visualized by in-gel peptidase assay as in Fig. 1B. The 26 S proteasomes from both strains were detected at the same positions corresponding to the double-capped proteasome (RP2CP) and had almost equal peptidase activities. C, GST pull-down assay using GST-Sem1 as bait. One µg each of GST or GST-Sem1 was bound to glutathione-immobilized agarose beads and was then incubated with 2 µg each of the 26 S proteasome purified from the wild type SEM1 strain (b), the 26 S proteasome purified from \( \Delta \)sem1 strain (c), or control sample purified from no-tag strain (a) in the same volume. The materials bound to the beads were eluted with SDS-loading buffer and analyzed by Western blotting with anti-Rp5 (base), anti-FLAG (Rpn113FLAG, lid), and anti-20 S proteasome antibodies.

salt concentrations (Fig. 3A). The 26 S proteasomes were pulled down by using anti-FLAG antibody-immobilized beads from strains, including untagged control strain (Fig. 3A, I), RPN113FLAG strain (Fig. 3A, II), \( \Delta \)sem1 RPN113FLAG strain (Fig. 3A, III), and \( \Delta \)sem1 RPN113FLAG strain carrying YCP-SEM13HA plasmid (Fig. 3A, IV). The bead-bound 26 S proteasomes were treated with NaCl at various concentrations, and the bead-retained proteins were eluted with 3× FLAG peptides and analyzed by SDS-PAGE and Western blotting with anti-FLAG (Rpn113FLAG, lig), anti-Rp9 (lid), anti-Rp5 (base), and anti-HA (Sem13HA) antibodies. Because 3× FLAG epitope had been tagged to Rp11, the Rpn113FLAG-containing complexes should be pulled down and eluted with 3× FLAG peptides in these experiments. Under control conditions with 0.1 M NaCl, equal amounts of the 26 S proteasomes from the respective strains were detected by both Western blotting and SDS-PAGE analyses (Fig. 3A, lanes 6–8), and under conditions in which the bead-bound 26 S proteasome had been treated pre-
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Results

The 26 S proteasome lacking Sem1 is unstable under the high salt conditions. A, SDS-PAGE analysis of the 26 S proteasomes pretreated with NaCl at various concentrations. The 26 S proteasome was pulled down from YYS40 (RPN11^FLAG) (II), YTS67 (Δsem1 RPN11^FLAG) (III), YTS67 cells carrying YCp-SEM1^HA (IV), or W303-1A (mock purification) (I) by immunoprecipitation (IP) by using anti-FLAG antibody-immobilized agarose beads and washed with 0.1 M NaCl (lanes 5–8). The bead-bound 26 S proteasome was treated with 0.5 M NaCl for 5 min at 25 °C (lanes 9–11) or with 1 M NaCl for 30 min at 25 °C (lanes 12–14) and then washed with NaCl at the respective concentrations. The proteins, bound to the beads, were then eluted with FLAG peptide and analyzed by Western blotting with anti-FLAG (Rpn11^FLAG, bait), anti-Rpn9 (lid), anti-Rpt5 (base), and anti-HA (Sem1^3HA) antibodies (upper panel). Input refers to 6% of the whole cell lysate used for immunoprecipitation (lanes 1–4). The purified proteins were also analyzed by SDS-PAGE followed by protein staining with SYPRO Orange (bottom panel). The positions of Sem1 and Sem1^3HA are indicated by an asterisk and an arrowhead, respectively. M, molecular mass standards. B, densitometric analysis of the 26 S proteasomes pretreated with 1 M NaCl. The protein bands detected in the SDS-polyacrylamide gel in A (lanes 12–14) were analyzed by ImageGauge software. The lid subunits corresponding to the respective peaks are indicated above, and Rpn11^3FLAG is indicated by an arrowhead.

In Fig. 3, The 26 S proteasome lacking Sem1 is unstable under the high salt conditions. A, SDS-PAGE analysis of the 26 S proteasomes pretreated with NaCl at various concentrations. The 26 S proteasome was pulled down from YYS40 (RPN11^FLAG) (II), YTS67 (Δsem1 RPN11^FLAG) (III), YTS67 cells carrying YCp-SEM1^HA (IV), or W303-1A (mock purification) (I) by immunoprecipitation (IP) by using anti-FLAG antibody-immobilized agarose beads and washed with 0.1 M NaCl (lanes 5–8). The bead-bound 26 S proteasome was treated with 0.5 M NaCl for 5 min at 25 °C (lanes 9–11) or with 1 M NaCl for 30 min at 25 °C (lanes 12–14) and then washed with NaCl at the respective concentrations. The proteins, bound to the beads, were then eluted with FLAG peptide and analyzed by Western blotting with anti-FLAG (Rpn11^FLAG, bait), anti-Rpn9 (lid), anti-Rpt5 (base), and anti-HA (Sem1^3HA) antibodies (upper panel). Input refers to 6% of the whole cell lysate used for immunoprecipitation (lanes 1–4). The purified proteins were also analyzed by SDS-PAGE followed by protein staining with SYPRO Orange (bottom panel). The positions of Sem1 and Sem1^3HA are indicated by an asterisk and an arrowhead, respectively. M, molecular mass standards. B, densitometric analysis of the 26 S proteasomes pretreated with 1 M NaCl. The protein bands detected in the SDS-polyacrylamide gel in A (lanes 12–14) were analyzed by ImageGauge software. The lid subunits corresponding to the respective peaks are indicated above, and Rpn11^3FLAG is indicated by an arrowhead.

Viously with 0.5 M NaCl for 5 min at 25 °C (lanes 9–11) or with 1 M NaCl for 30 min at 25 °C (lanes 12–14), dissociation of the 26 S proteasome was detected in all cases, especially in the case of the Δsem1 strain (Fig. 3A, lanes 10 and 13). These results imply that the 26 S proteasome from the Δsem1 strain is more sensitive to NaCl treatment, which causes dissociation of the 26 S proteasome into the base and the 20 S proteasome in comparison with the case of the wild type SEM1 strain (Fig. 3A, compare lane 9 with 10 and lane 12 with 13). Densitometric analysis revealed that all lid subunits were dissociated in the Δsem1 strain under the most severe conditions (Fig. 3B, lane 13), although equal amounts of Rpn11^3FLAG remained bound to the beads in both cases of the wild type and Δsem1 strains (Fig. 3B, lanes 12 and 13). In addition, it should be noted that instability of the lid in the Δsem1 strain was fully suppressed by expressing Sem1^3HA (lanes 11 and 14 in Fig. 3A and lane 14 in Fig. 3B). In this case, Sem1^3HA, as well as Sem1, was found to bind to the lid, as detected by protein staining with SYPRO Orange (Fig. 3A, bottom panel). These results clearly indicate that Sem1 is a component of the lid and is required for assembly of the integral structure of the lid and the 26 S proteasome.

The 19 S RP Is Able to be Co-immunoprecipitated with Sem1—To eliminate the possibility that Sem1 is contained in other complexes, e.g., an exoctic complex, we carried out immunoprecipitation with Sem1^3HA as bait. Cell extracts prepared from two tagged transformants, YTS63 cells carrying YCp-SEM1^3HA and YYS143 (RPT65^3HA) cells (control), were subjected to immunoprecipitation with anti-HA antibody-immobilized beads, and the bead-bound proteins were eluted at low pH. The respective eluates were resolved by SDS-PAGE and analyzed by Western blotting with anti-Rpn9 (lid), anti-Rpt5 (base), and anti-HA (Rpt65^3HA and Sem1^3HA) antibodies (Fig. 4A) and by protein staining (Fig. 4B). Equivalent amounts of almost all 19 S RP subunits were recovered in either case of immunoprecipitation with Rpt65^3HA or Sem1^3HA as bait (Fig. 4B, lanes b and c). These results indicate that Sem1 is a stoichiometric component of the 19 S RP and that the 19 S RP is the only major Sem1-containing complex.

Polyubiquitinated Proteins Are Accumulated in Δsem1 Strain at a Restrictive Temperature—The above biochemical results unambiguously indicate that Sem1 is a component of the lid, but the 26 S proteasome lacking Sem1 seems to have normal peptidase activity (see Fig. 2B). On the other hand, it has been reported that accumulation of polyubiquitinated proteins was detected at a restrictive temperature in several lid subunit mutants such as Δrpn9 and rpn12-1 strains (33). In our background strain W303, the Δsem1 strain showed a temperature-sensitive phenotype as described previously (27) (see Fig. 9A). Therefore, we next carried out an experiment to determine whether polyubiquitinated proteins are accumulated in the Δsem1 strain at a restrictive temperature. The wild type W303-1A and Δsem1 strains were grown at 25 °C to mid-logarithmic phase and then grown at 37 °C. The cells were harvested at the indicated times, and the total cell lysate was prepared and analyzed by Western blotting with an FK2 antibody recognizing polyubiquitin chains. As shown in Fig. 5, large amounts of high molecular mass polyubiquitinated proteins accumulated at the restrictive temperature in the Δsem1 strain in comparison with the case of the wild type, indicating that the function of the 26 S proteasome in the degradation of polyubiquitinated proteins is defective in the Δsem1 strain at the restrictive temperature. Thus, it can be concluded that Sem1 plays a role in ubiquitin-dependent proteolysis.

The 26 S Proteasome Lacking Sem1 Is Unable to Degrade a Polyubiquitinated Protein in Vitro—To obtain definitive in vitro evidence that Sem1 is required for degradation of polyubiquitinated proteins, we next carried out two in vitro experiments including a polyubiquitin chain binding assay and a polyubiquitinated protein degradation assay. A gel shift assay has recently been developed to evaluate polyubiquitin chain binding activity of the 26 S proteasome (12). According to this method, polyubiquitinated Cdc34 was prepared in the presence of ubiquitin, Uba1 (E1), and ATP (Fig. 6A) and was incubated
The 19 S RP is able to be co-immunoprecipitated with Sem1. A, immunoprecipitation with Sem1HA and Rpt6HA as baits. The cell extracts from YYS143 (RPT6HA) (b), YTS63 cells carrying YCp-SEM1HA (c), and W303-1A (mock purification) (a) were immunoprecipitated (IP) by using anti-HA antibody-immobilized agarose beads in the absence of ATP. The protein complexes, bound to the beads, were eluted at low pH and were then subjected to SDS-PAGE and Western blotting with anti-Rpn9 (lid), anti-Rpt5 (base), and anti-HA antibodies. B, proteins separated by SDS-PAGE in A were stained with Coomassie Brilliant Blue (CBB). The protein bands indicated were verified by peptide mass fingerprinting. Note that almost equal amounts of almost all 19 S RP subunits were recovered in both the cases of Sem1HA and Rpt6HA (b and c). M, molecular mass standards.

Polyubiquitinated proteins are accumulated in Δsem1 strain at the restrictive temperature. The wild type (WT) and YTS63 (Δsem1) cells were grown in YPD at 25 °C to the logarithmic phase and were then grown at 37 °C. The cells were collected at the indicated times, and the total cell extracts were prepared and subjected to Western blotting with anti-polyubiquitin (anti-polyUb) (FK2) and anti-actin (control) antibodies.

The 26 S proteasome lacking Sem1 is able to bind with polyubiquitinated Cdc34. A, preparation of polyubiquitinated Cdc34. Ub1 (E1) protein purified from S. cerevisiae and recombinant Cdc34 were incubated with bovine ubiquitin (Ub) for 12 h at 37 °C. Polyubiquitination of Cdc34 was evaluated by Western blotting with anti-polyubiquitin (polyUb) antibody (FK2). B, gel shift assay using polyubiquitinated Cdc34. Polyubiquitinated Cdc34 (Ub-nCdc34) in the indicated amount was incubated with 3 pmol of the 26 S proteasome purified from the wild type SEM1 or Δsem1 strain as in Fig. 2A. The mixture was subjected to nondenaturing PAGE and in-gel peptidase assay as in Fig. 1B. Note that the Ub-nCdc34-bound 26 S proteasomes in both cases were gel-shifted to almost the same positions in comparison with the original ones (RP/CP). CBB, Coomassie Brilliant Blue.

with the 26 S proteasome purified from the wild type SEM1 or Δsem1 strain. The resultant reaction mixture containing the 26 S proteasome and polyubiquitinated Cdc34 was subjected to nondenaturing PAGE and to in-gel peptidase assay (Fig. 6B). In both cases of the SEM1 and Δsem1 strains, the 26 S proteasomes were gel-shifted to similar points in the presence of polyubiquitinated Cdc34, suggesting that the 26 S proteasome retains the ability to recognize/bind polyubiquitin chains even in the absence of Sem1. In a control experiment using original Cdc34, i.e. when ubiquitin or E1 enzyme was omitted, gel shift of the 26 S proteasome was not detected (data not shown).

Next, we tested whether the 26 S proteasome from the Δsem1 strain retains the activity to degrade polyubiquitinated proteins in vitro. Polyubiquitinated Sic1, as a known good substrate for the 26 S proteasome (8, 23), but its preparation using an E3 ligase complex SCFCdc4 in the present study was technically difficult. We therefore prepared polyubiquitinated Sic1IP by our original method using Rsp5 as an E3 ligase. To allow Rsp5 to bind to Sic1, the PY motif (35), a sequence required for Rsp5 binding, was introduced into the N-terminal region of Sic1, designated as Sic1IP. T7 epitope- and HAT epitope-tagged Sic1IP, T7-Sic1IP-HAT, was highly polyubiquitinated by Uba1, Ubc4, and Rsp5 in a PY motif-dependent manner, and the resultant polyubiquitinated-Sic1IP was efficiently degraded by the purified 26 S proteasome (Fig. 7A). By using this polyubiquitinated-Sic1IP as a substrate, the activities of the 26 S proteasomes purified from the wild type SEM1 and Δsem1 strains were measured. As shown in Fig. 7, B and C, ~80% of polyubiquitinated Sic1PY (Ub-n-Sic1PY) was quickly degraded by the 26 S proteasome from the wild type strain within 3 min under the conditions used, whereas the degradation by the 26 S proteasome lacking Sem1 proceeded very slowly and ~50% of polyubiquitinated-Sic1PY remained undigested after 10 min. Thus, the 26 S proteasome lacking Sem1 has a defect in the degradation of the polyubiquitinated protein, strongly supporting the above idea that Sem1 plays a role in ubiquitin-dependent proteolysis.

The Δrpn10 Δsem1 Double-deletion Mutant Displays a Synthetic Phenotype for Vegetative Growth—Because Sem1 was found to be a component of the lid, we next analyzed genetic interactions among SEM1 and other proteasome subunit genes. Because the mutant with deletion of RPN10 that encodes a proteasomal intrinsic ubiquitin receptor has been well characterized and shows a mild phenotype (10, 11), a Δsem1 Δrpn10 double-deletion mutant was constructed to analyze the genetic interaction between SEM1 and RPN10. It was found that this double-deletion mutant is viable but shows slower growth than that of the wild type strain or those of single deletion mutants (Fig. 8). The wild type strain and Δrpn10
from \(/\)H9004 (control) proteins were incubated with ubiquitinating enzymes, Uba1, Ubc4, and Rsp5. The resultant polyubiquitinated Sic1PY was incubated with the antibody. Note that the wild type Sic1 was neither ubiquitinated nor degraded by the 26 S proteasome. The signals on the gels in buffer at the indicated times, and the reaction mixture was subjected to Western blotting with anti-T7 antibody. C in the mutant with \(\Delta sem1\) strain, the PY motif-introduced Sic1, and its degradation by the 26 S proteasome. Recombinant T7-Sic1PY-HAT and T7-Sic1-HAT strain was incubated with polyubiquitinated Sic1PY (400 nM) at 30 °C. The reaction was terminated by the addition of SDS-loading buffer at the indicated times, and the reaction mixture was subjected to Western blotting with anti-T7 antibody. C, time dependence of the 26 S proteasome-mediated degradation of polyubiquitinated Sic1PY. The signals on the gels in B were quantified by ImageGauge software (Fuji) and plotted as a function of incubation time. Note that the half-life \(t_{1/2}\) in the case of the 26 S proteasome from \(SEM1\) strain is \(~\)1 min, whereas that from \(\Delta sem1\) strain is more than 10 min.

The Human Sem1 Homologue, hDSS1, Is Capable of Binding with the Human 26 S Proteasome—It has been reported that the morphology and growth defect at 35 °C in the mutant with deletion of DSS1 ( \(\Delta dss1\) ) in yeast Schizosaccharomyces pombe are significantly rescued by expressing human DSS1 (hDSS1) (27). To determine whether hDSS1 complements the temperature-sensitive phenotype of the \(\Delta sem1\) strain of \(S.\) cerevisiae, the hDSS1 gene was cloned and expressed under the TDI3 promoter in the \(\Delta sem1\) strain. As shown in Fig. 9A, the expression of hDSS1 rescued the growth defect of the \(\Delta sem1\) strain at the restrictive temperature. This result suggests that hDSS1 has the same function as that of \(S.\) cerevisiae Sem1 or a function overlapping that of \(S.\) cerevisiae Sem1.

The above-stated finding that hDSS1 is a functional homologue of Sem1 led us to speculate that hDSS1 is also a component of the human 26 S proteasome. To determine this, FLAG epitope-tagged hDSS1 or POH1 (a human homologue of Rpn11) (control) was transiently overexpressed in HEK293T cells, and the cell extracts were subjected to immunoprecipitation with anti-FLAG antibody-immobilized agarose beads. The resultant immunoprecipitates were analyzed by Western blotting with antibodies against the human 26 S proteasome, i.e. anti-MSS1 (human Rpt1), anti-p45 (human Rpt6), and anti-ζ (human α5 subunit of the 20 S proteasome) antibodies (Fig. 9B). Both the CP subunit ζ and the RP subunits MSS1 and p45 were co-immunoprecipitated with hDSS1 as well as with the 19 S RP subunit POH1. To determine whether the complexes immunoprecipitated using hDSS1\(^{\text{FLAG}}\) and POH1\(^{\text{FLAG}}\) as baits have the abilities to hydrolyze Suc-Leu-Leu-Val-Tyr-MCA, a peptidase assay for the proteasome was carried out in the presence of ATP (Fig. 9C). Approximately equal levels of peptidase activities were detected in both the cases of hDSS1\(^{\text{FLAG}}\) and POH1\(^{\text{FLAG}}\). When immunoprecipitation was carried out in the absence of ATP, the level of peptidase activity decreased to one-third the level in the presence of ATP in either case (data not shown). These results strongly suggest that hDSS1 binds with or is incorporated into the human 26 S proteasome.

**DISCUSSION**

In this study, we found that Sem1 is a novel lid component of the 26 S proteasome of \(S.\) cerevisiae. We carried out affinity purification of the 26 S proteasome and lid and base subcomplexes from tagged strains of \(S.\) cerevisiae, and we found that the highly purified lid has an additional small molecular mass protein (Fig. 1C). Comparison of the intensities of protein bands on SDS-polyacrylamide gel showed that this protein seems stoichiometric to other RP subunits, and we found to our surprise that this protein is Sem1, as identified by amino acid sequencing. Subsequently, we carefully carried out several experiments to determine whether Sem1 is a component of the 26 S proteasome. First, it was found by GST pull-down assays that GST-Sem1 binds with the 26 S proteasome from the \(\Delta sem1\) strain but not with the 26 S proteasome from the wild type strain (Fig. 2C). Second, analysis of the stability of the 26 S proteasome revealed instability of the lid lacking Sem1 under high salt conditions (Fig. 3). Third, immunoprecipitation using HA epitope-tagged Sem1 as bait showed that almost all 19 S RP subunits are able to be immunoprecipitated together.
Sem1p Is a Novel Proteasome Subunit

Fig. 9. Human Sem1 homologue, hDSS1, interacts with the human 26 S proteasome. A, human DSS1 complements the temperature sensitivity of yeast Δsem1 cells. Low copy plasmids (pRS314) containing the SEM1 gene with its own promoter and the hDSS1 gene with the TDH3 promoter were transformed into YTS63 (Δsem1) cells. The resultant transformants were cultured, and 10-fold diluted cells were spotted on SD-Trp plates and incubated at 25 or 37 °C for 2 days. WT, wild type. B, co-immunoprecipitation of the endogenous human 26 S proteasome with overexpressed hDSS1. HEK293T cells were transfected with the pCI-neo (mock) (a), pCI-neo-POH1 (hRPN11)Δ(FLAG) (b), or pCI-neo-hDSS1Δ(FLAG) (c) plasmid, and immunoprecipitation (IP) was performed using anti-FLAG antibody-immobilized beads. The resultant immunoprecipitates in B were subjected to Western blotting with antibodies. C, peptidease assay using Suc-Leu-Leu-Val-Tyr-MCA as a substrate.

with Sem1 (Fig. 4). Fourth, it was found by proteasomal degradation assays that the 26 S proteasome lacking Sem1 has a defect in degradation of polyubiquitinated proteins both in vivo and in vitro (Figs. 5 and 7). Finally, genetic analysis revealed genetic interaction between SEM1 and RPN10 (Fig. 8). Based on the results described above, we conclude that Sem1 is a novel subunit of the 26 S proteasome, and we therefore propose that Sem1 be renamed Rpn15 to strengthen its identity as a component of the lid subcomplex. As the 26 S proteasome lacking Sem1 has the ATP-dependent peptidase activity (Fig. 2) and retains the ability to bind polyubiquitinated proteins (Fig. 6), it can be inferred that this Sem1-deficient 26 S proteasome has a defect in the release of polyubiquitin chains from substrates or the translocation of substrates into the CP. Little accumulation of the original protein Sic1PY in assays of degradation of polyubiquitinated Sic1PY (Fig. 7) prefers the former possibility, i.e. impairment of deubiquitination in the Sem1-deficient 26 S proteasome. In addition, we showed that human DSS1 is a functional homologue of Sem1 (Fig. 9A) and that it interacts with the 26 S proteasome in mammalian HEK293T cells, as revealed by immunoprecipitation (Fig. 9B), suggesting that hDSS1 is also a subunit of the human 26 S proteasome.

It is well known that most of the proteasomal genes are contained in the same cluster that consists of functionally related genes with similar gene expression patterns (36). It is also known that Rpn4 functions as a transcriptional activator of genes encoding proteasomal subunits through its binding to an upstream activating sequence, 5′-GTTGGCAA-3′, a proteasome-associated control element (PACE) (37). Recently, it has been reported that the sequence 5′-AGTGGCAA-3′ is an alternative PACE and that both PACEs exist in the promoters of all 32 proteasomal genes and some proteasome-associated protein genes (9). Our search of the Saccharomyces Genome Data Base revealed that the latter PACE sequence is present upstream of the SEM1 gene (42–35 bp upstream), suggesting that expression of the SEM1 gene is regulated by Rpn4. The presence of the PACE in SEM1 provides support for our proposal that Sem1 is a component of the 26 S proteasome.

The SEM1/DSS1 genes are highly conserved from yeast to mammals. S. cerevisiae SEM1 was originally identified as a multicopy suppressor of the sec15-1 temperature-sensitive mutation (25). SEM1 is not an essential gene, and deletion of the SEM1 gene results in suppression of some exocyst mutations and triggers pseudohyphal growth in diploid cells (25). The fact that the 19 S RP is the only major Sem1-containing complex (Fig. 4) raises the possibility that the 26 S proteasome is involved in exocytosis. It is possible that a putative protein functioning in exocytosis is polyubiquitinated and degraded by the 26 S proteasome. The S. pombe DSS1 gene is also a nonessential gene and shows an elongated phenotype, caused by cell cycle delay at a restrictive temperature (27). It is well known that the 26 S proteasome play key roles in cell cycle progression (1, 3). The hDSS1 gene was identified as one of three candidate genes at 7q21.3–q22.1 involved in an autosomal dominant form of SHFM disorder, a heterogeneous limb developmental disorder, and is expressed during limb development (26). Thus, it can be inferred that Sem1/DSS1 is involved in regulation of exocytosis, cell cycle, and/or development. In addition, the hDSS1 protein is capable of interacting with the product of the breast cancer susceptibility gene BRCA2 (27). The crystal structure of a complex composed of the C-terminal domain (~800 residues) of BRCA2 and hDSS1 has been determined, and the binding site between BRCA2 and hDSS1 is highly conserved across evolution (38). In mammalian cells, BRCA2 binds to RAD51 and plays a role in DNA double-strand break repair (39). Recently, Ustilago maydis DSS1 has been reported to play an essential role in DNA repair, recombination, and genome maintenance (40). The phenotypes of U. maydis mutants with deletion of DSS1 reflect those of U. maydis mutants deficient in Brh2, the U. maydis BRCA2 homologue, and U. maydis RAD51 (40–42). Thus, it is possible that DSS1 is involved in DNA repair and recombination through BRCA2.

Our findings that yeast Sem1 is a subunit of the 26 S proteasome and that hDSS1 is capable of binding with the human 26 S proteasome in mammalian cells, together with the fact that hDSS1 interacts with BRCA2, led us to assume that BRCA2 could interact with hDSS1 bound to the 26 S proteasome. Recently, a complex termed BRCC containing BRCA1, BRCA2, and RAD51 was identified as a ubiquitin E3 ligase complex that enhances cellular survival following DNA damage (43). It is likely that hDSS1 functions as an adaptor between the 26 S proteasome and the E3 ligase complex BRCC through
its direct interaction with BRCA2. Further studies on interaction between the human 26 S proteasome and BRCA2, as well as structural elucidation of the human 26 S proteasome possibly containing hDSS1, are necessary to determine the role of hDSS1 in DNA repair and ubiquitin-dependent proteolysis.

Acknowledgment—We thank Y. Abe of the Center for Instrumental Analysis, Hokkaido University, for help in performing peptide sequence analysis.

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