SUPPLEMENTAL INFORMATION

EXTENDED EXPERIMENTAL PROCEDURES

Vectors, Cell Lines, and Materials

To express the N-terminally HA-tagged human PA200, the HA tag was engineered into the pcDNA-6B plasmid, and the full-length PA200 was then inserted with a free C-terminus at sites of KpnI and NotI. To express histones in yeast ectopically, the vector pHHF1-Gal-10/1-FLAG-HHT1 expressing the N-terminally FLAG-tagged H3 was obtained from Dr. O. Rando, and the H3 sequence was replaced with the corresponding sequence to express H2B or H4. Histone H4 was also subcloned in a pET3a plasmid for bacterial expression, and its mutant H4K16R was constructed using primers: 5'-GGGTAAAGGTGGTGCTAGACGTCACCGTAAAGTTC-3' and 5'-GAAC TTTACGGTGACG TCTAGCACCACCTTTACCC-3'. Ub-R-GFP with a C-terminal His tag was subcloned in pYES2/CT-His vector. His-tagged yeast Gcn5 HAT domain (aa98-262) was subcloned at BamHI and XhoI sites in pET28a, and His-tagged TIP60 (aa1-513) was in pET-28b at NdeI and XhoI restriction sites. BRD-like regions of yeast Blm10 (aa1648-1732) and human PA200 (aa 1650-1738) were each inserted at EcoRI and XhoI sites in pGex-4T-2 to generate GST-fusion proteins. Their control regions, Blm10 (aa1980-2073) and PA200 (aa1296-1377), were subcloned similarly. Mutant BRD-like region of Blm10 (Y1663H/N1664D) was constructed using primers: 5'-CTCTTTGAGCTATGTGAATCCTACCATGACAAAGACG-3' and 5'- CGTCTTTGTCATG GTAGGATTCACATAGCTCAAAGAG-3'. Mutant BRD-like region of PA200 (N1716T/F1717S)

was obtained using primers: 5'- AGCGGTCTGCTACAGTGTACCTCTCTTACCATGGACAGT -3' and 5'- ACTGTCCATGGTAAGAGAGGTACACTGTAGCAGACCGCT -3'.

Human embryonic kidney 293T cells, mouse GC1-spg type B spermatogonia, GC-2spd(ts) spermatocytes, TM3 leydig cells, TM4 sertoli cells, and C2C12 myoblast cells were obtained from the American Type Culture Collection and maintained in DMEM, supplemented with 10% fetal bovine serum and antibiotics in 5% CO_2. Fresh tissues from bull and rabbit were purchased from Fucheng slaughterhouse (Hebei, China). Following the sacrifice of the animals, the tissues were immediately removed, immersed in liquid nitrogen, and stored at -80°C until use. Male mice and rats were obtained from the Laboratory Animal Center, Chinese Academy of Medical Sciences (Beijing, China). The animals' care was in accordance with institutional guidelines.

Construction of PA200-Deficient Mice

The PA200-deficient C57BL/6 strain was constructed by gene-targeting technologies. In brief, the genomic region encompassing exons 25 and 26 of PA200/PSME4 gene, which encodes conserved domain in three presumptive PA200 subspecies, was replaced by the cassette containing the neomycin-resistant gene. MEF cells were obtained from wild-type or the PA200-deficient mouse embryos, and immortalized by stably transfecting with human large T-antigen (kindly provided by Dr. Y. Cong).

Yeast strains used in this study

Unless stated elsewhere, yeast mutant strains were constructed using PCR-based protocols (Janke et

al., 2004; Toulmay and Schneiter, 2006), and each Blm10 construct was integrated into the genome. All these Blm10 proteins were expressed at similar levels as examined by western blot using anti-HA antibody. GPD promoter was used for examining the effect of Blm10 overexpression on histone degradation. Since overexpression of HA-tagged wild-type Blm10 had no effect on histone degradation (Fig. 6A), all the point or truncation mutants were constructed under the GPD promoter.

BY4741	MATa *his3⊿1 leu2⊿0 met15⊿0 ura3⊿0*	Wei Li
WT FLAG-H3	MATa BY4741 +*pHHf1-Gal1/10-Flag-HHT1(hph)*	This study
Rpn4-KO FLAG-H3	MATa *lys2-801 leu2-3,2-112 ura3-52 his3-⊿200 trp1-1 rpn4⊿::Nat + pHHf1-Gal1/10-Flag-HHT1(hph)*	This study
Rpn13-KO FLAG-H3	MATa *lys2-801 leu2-3,2-112 ura3-52 his3-⊿200 trp1-1 rpn13⊿::Nat + pHHf1-Gal1/10-Flag-HHT1(hph)*	This study
Blm10-O/E FLAG-H3	MATa BY 4741 *blm10::Nat GPD-HA-Blm10 + pHHf1-Gal1/10-Flag-HHT1(hph)*	This study
Blm10- KO FLAG-H3	MATa BY 4741 *yak1-GFP::Kan blm10⊿::Nat + pHHf1-Gal1/10-Flag-HHT1(hph)*	This study
WT FLAG-H2B	MATa BY4741 + *pHHf1-Gal1/10-Flag-HTB1(hph)*	This study
Blm10-KO FLAG-H2B	MATa BY 4741 *yak1-GFP::Kan blm10⊿::Nat + pHHf1-Gal1/10-Flag-HTB1(hph)*	This study
WT FLAG-H4	MATa BY4741 + *pHHf1-Gal1/10-Flag-HHF1(hph)*	This study
Blm10-KO FLAG-H4	MATa BY 4741 *yak1-GFP::Kan blm10⊿::Nat + pHHf1-Gal1/10-Flag-HHF1(hph)*	This study
WT Ub-R-GFP	MATa BY4741 + *pYES2-Gal-Ub-R-GFP(URA3)*	This study
Blm10-KO Ub-R-GFP	MATa BY 4741 *yak1-GFP::Kan blm10⊿::Nat+ pYES2-Gal-Ub-R-GFP(URA3)*	This study
Rpn4-KO Ub-R-GFP	MATa BY4741 *rpn4⊿::Nat+ pYES2-Gal-Ub-R-GFP(URA3)*	This study
Rpn13-KO Ub-R-GFP	MATa *lys2-801 leu2-3,2-112 ura3-52 his3-⊿200 trp1-1 rpn13⊿::Nat + pYES2-Gal-Ub-R-GFP(URA3)*	This study
YHS539	MATa *his3D1 leuD0 met15D0 ura3D0 (BY4741) yak1-GFP (kan), blm10::Nat hat9*	Daniel Finley
SY1022a	MATa *lys2-801 leu2-3,2-112 ura3-52 his3-⊿200 trp1-1 rpn13⊿::Nat*	Daniel Finley
HA-Blm10	MATa BY 4741 *blm10::Nat-GPD-HA-BLM10(Y1663H,*	This study

Y1663H/1664D	*N1664D)-Kan + pHHf1-Gal1/10-Flag-HHT1(hph)*	
Blm10 F2125S/2126D	MAT**a** BY 4741 *blm10::Nat-GPD-HA-BLM10(F2125S, N2126D)-Kan + pHHf1-Gal1/10-Flag-HHT1(hph)*	This study
Blm10 aa1-1662	MAT**a** BY 4741 *blm10::Nat-GPD-HA-BLM10ΔC-Kan + pHHf1-Gal1/10-Flag-HHT1(hph)*	This study
WT diploid	MATa/α *his3Δ1/his3Δ1 leu2Δ0 /leu2Δ0 lys2Δ0/LYS2 MET15/met15Δ0 ura3Δ0 /ura3Δ0 (4741/4742)*	This study
Blm10-KO diploid	MATa/α *his3Δ1/his3Δ1 leu2Δ0 /leu2Δ0 lys2Δ0/LYS2 MET15/met15Δ0 ura3Δ0 /ura3Δ0 yak1-GFP::Kan/ yak1-GFP::Kan blm10Δ::Nat/ blm10Δ::Nat*	This study

Immunohistochemistry and Apoptosis Detection in Testis

Mouse testis and epididymis were fixed with 4% formaldehyde in PBS overnight. Slices (8 μm) were incubated with antibodies and detected by IHC kits (Zhongshan Golden Bridge Biotechnology, Beijing). The positive cells were then visualized using 3,3-diaminobenzidine tetrahydrochloride (DAB) (brown). Following counterstaining with nuclear haematoxylin (blue), images were captured under a microscope. Apoptosis in testis was analyzed using DeadEnd^{TM} Fluorometric TUNEL System according to standard paraffin-embedded tissue section protocol (Promega).

In Situ Hybridization

Frozen tissues were prepared from mice, and sections at 15 μm were mounted onto slides. The sections were incubated with probes in a humid chamber, and then sequentially incubated with DIG/biotin-conjugated antibody, HRP-streptavidin, and 3,3-diaminobenzidine tetrahydrochloride (DAB) (brown). Following counterstaining with hematoxylin (blue), the slides were observed under the microscope. The hybridization probes for α4 were 5'- CGCCC ATTGC TCTGT GTATA ACGCT GCTTC A-3' (antisense) and 5'- TGAAG CAGCG TTATA CACAG AGCAA TGGGC G-3'

(sense), and the probes for α4s were 5'- ATAGC CAGTT CAATG TTCTT TCCGC CAG-3' (antisense) and 5'- CTGGC GGAAA GAACA TTGAA CTGGC TAT-3' (sense).

Real-Time PCR

mRNAs were prepared from mouse tissues with the Qiagen RNeasy kit in accordance with the manufacturer's instructions. Complementary DNA was then prepared with Reverse Transcription system (Promega) and analysed on an ABI 7500 real-time PCR machine, using power SYBR green PCR master mix in accordance with the manufacturer's instructions. The following primers were then used in the cDNA analysis: α4s, 5'-TGTATCAGACAGATCCCTCCGG-3' (sense) and 5'-CTGGACAACTTCGAGCAAAGC-3' (antisense); α4, 5'-TCACCGCTGATGCAAGGATAG-3' (sense) and 5'-TAGGGCCGAGATACCAAATGG-3' (antisense).

Antibodies and Western Blot Analysis

Except where noted, proteins were extracted in a buffer containing 20 mM HEPES, pH 7.5, 150 mM NaCl, 5 mM $MgCl_2$, 1 mM ATP, 1 mM EDTA, 1 mM DTT, and 0.1 mM Phenylmethylsulphonyl fluoride (PMSF). Soluble histones were extracted in the regular buffer with 25 mM Tris, pH 7.5, 150 mM NaCl, 10% glycerol, 5 mM $MgCl_2$, 1 mM PMSF, and 5 mM ATP. The levels of proteins were analyzed by immunoblotting using horseradish peroxidase-conjugated secondary antibodies. The antibodies against β1, β1i, β2, β2i, β5, β5i, Rpt2, PA200, PA28, Rpn7, and Rpt4 were obtained from BioMol, H1 and H2B from Abcam, H2A, H3, γ-H2AX (Ser 139), H4K16ac, and H4 from Millipore, acetyl-Lys from Cell Signalling, β-actin from Sigma, and GAPDH from Santa Cruz Biotechnologies. The rat anti-α4 and anti-α4s polyclonal antibodies were raised against purified His-tagged α4

(aa219-248) and α4s (aa221-250) fragments, respectively. The mouse anti-H2B5ac antiserum was raised against the acetylated H2B peptide (PEPAK^{ac}SAPAPKKGSKKAVTKA-biotin), which was synthesized by GenScript (Nanjing, China).

Protein Degradation Assay in Budding Yeast

Wild-type or mutant yeast bearing the FLAG-tagged histones or His-tagged Ub-R-GFP plasmid was incubated with galactose for 3 h to induce the FLAG-tagged histone or His-tagged Ub-R-GFP. It is known that Ub-R-GFP is degraded by the 26S proteasome in a polyubiquitin-dependent manner (Wojcik et al., 2006). Then, galactose was replaced with glucose to repress the GAL promotor. Cells were collected at various time points for immunoblotting with an anti-FLAG, anti-His, and/or anti-GAPDH antibody.

Proteasome Purification and Activity Assays

Purification of proteasomes from bovine or rabbit tissues was carried out as described (Qiu et al., 2006). The proteasome's peptidase activity was assayed using the fluorogenic peptide substrates, as described previously, and the activity was defined as the amount of released amc in $min^{-1}mg^{-1}$ proteasomes. The rate of degradation of β-casein by the proteasomes was determined using the fluorescamine assay (Kisselev et al., 1998). The degradation of acetylated histones was assayed in the buffer containing 20 mM Tris, pH 7.5, 0.5 mM EDTA, 1 mM DTT, and 1 mM $MgCl_2$ at 37° C, and a 70-μl reaction mix was supplemented with 280 ng of the 20S proteasome, 3 μg of acetylated or non-acetylated histones, and/or 1 μg PA200. Except for RNF5-$(Ub)_n$ (prepared as in Figure S4F), ATP was not supplemented in the reactions. MG132 was used at 1 μM in the indicated reactions.

Purification of PA200

Purification of PA200 was adapted from established protocols (Qiu et al., 2006; Ustrell et al., 2005)(. In brief, the supernatant from homogenized bovine testis (200 g) was incubated with DE52 DEAE cellulose (100 ml). The resin was then washed with the buffer containing 20 mM Tris-HCl, pH7.5, 10% glycerol, 50 mM NaCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 1 mM DTT, and 2 mM ATP, and was eluted with 250 ml of the above buffer, but containing a 50-300 mM NaCl gradient. The pooled fractions with PA200 were diluted with an equal volume of TSDG pH 8.5 (10 mM Tris, pH 8.5, 25 mM KCl, 10 mM NaCl, 5.5 mM MgCl2, 0.1 mM EDTA, 1 mM DTT, and 10% glycerol), and were loaded to Q Sepharose ion-exchange Fast Flow column. The column was rinsed with 20 ml of TSDG pH 8.5, eluted with 50 ml of 750 mM KCl in TSDG pH 8.5, and directly applied to the equilibrated Superdex 200 26/60 column. The fractions with PA200 from the Superdex column were loaded on a Uno Q column (6 ml, Bio-Rad), washed with 20 ml of TSDG (pH8.5) containing 125 mM KCl, and eluted with a 100-ml linear gradient of 125 to 500 mM KCl in TSDG (pH8.5). The PA200 pooled from the Uno Q step was finally purified by ultracentrifugation (85,000 × g for 19 h) on a 5-20% glycerol gradient.

Histone purification and acetylation

Histones from rabbit thymus or HeLa cells were purified according to standard acid extraction protocols (Shechter et al., 2007). Histone H4 was expressed in *E. coli*, and purified from inclusion bodies as described (Luger et al., 1999). The full length TIP60 with an N-terminal His-tag was expressed in *E. coli*, and purified on a nickle-chelating HisTrap column and followed by gel filtration

chromatography using Superdex 10/300 SD200 column. Histones from thymus were acetylated by His-tagged Gcn5 HAT domain (aa98-262) in the buffer containing 50 mM HEPES, pH 8.0, 10% glycerol, 1 mM dithiothreitol (DTT), 10 mM sodium butyrate, and 0.3 mM of acetyl-CoA, and the reaction was terminated by TCA precipitation. The full-length H4 was acetylated by TIP60 under similar conditions.

GST pull-down assay

BRD-like regions were fused to GST, expressed in bacteria, and purified using standard protocols. The GSH-beads were incubated with the GST-BRD-like regions and acetylated histones in the buffer containing 10 mM Na-Hepes, pH7.5, 150mM NaCl, 0.005% Tween-20, and 2 mM DTT, and then were washed in the buffers containing 10 mM Na-Hepes, pH7.5, 150-300 mM KCl, 0.5-1.0 % Tween-20, and 2 mM DTT. Unless stated elsewhere, 5% of the total protein served as input.

Mass Spectrometry

Protein samples were analyzed by MALDI-TOF using an Applied Biosystems Voyager-DE-STR.

Structural analysis of bromodomain-like regions

The secondary structure of Blm10/PA200 was predicted by Net SurfP (Petersen et al., 2009). The structures of the BRD-like region of yeast Blm10 and human CBP were taken from the crystal structures of Blm10 (PDB code: 3L5Q) and CBP (PDB code:2RNY), respectively. As the crystal structure of PA200 is unavailable, its BRD-like region was modeled after the homologous region in Blm10.

Electron Microscopy and Image Processing

Samples were negatively stained by uranyl formate following the established protocol (Ohi et al., 2004) and observed in a Tecnai T20 microscope operated at 120kV at a magnification of 50,000X. Images were recorded on a Gatan 4Kx4K UltraScan CCD camera with a defocus of 1.5 μm. All images were binned by a factor of 2 to a final pixel size of 5 Å /pixel at specimen level. Regulatory particles of proteasomes were manually selected using WEB, and image processing was performed with SPIDER (Frank et al., 1996). Proteasomes were windowed into 90 x 90 pixel images and treated by standard multi-reference alignment and classification protocol specifying 25 classes. Classes of the same type of particles were then merged to produce final 5 classes. Only side views of the 20S particles associated with regulatory particles were selected for image processing, and isolated 20S particles without any regulatory particles were not selected for multi-reference alignment and classification. Most proteasomes with any kind of regulatory particles are most likely to be on-side views. Thus, the number of proteasomes within each class represents the relative ratio of this type of proteasome in the sample.

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LEGENDS FOR SUPPLEMENTAL FIGURES

Figure S1. Characterization of Proteasomes in Mammalian Testes, Related to Figure 1

(**A**) Native-PAGE analysis of proteasomes from extracts of rat. The extracts from rat spleen, skeletal muscle, and testes were separated on native-PAGE, incubated with LLVY-amc, and visualized under UV light. The large testis-specific proteasome is labeled as Lg.

(**B**) A hybrid proteasome from testes (19S-20S-PA200) under EM.

(**C**) Comparison of the small regulatory particle from the testis proteasomes with PA200, Blm10, and PA28 in complexes by electron microscopy. The images for PA200-20S complexes were reproduced from ref. (Ortega et al., 2005), and those for the Blm10-20S and PA28-20S complexes

were kindly provided by T. Walz as in refs. (Cascio et al., 2002; Schmidt et al., 2005). Two small particles at the ends of the 20S from testes look slightly different, probably because they were observed under different angles.

(**D**) Two pools of proteasomes. Following glycerol gradient fractionation of the UnoQ column elutes, testis samples from rabbits were analyzed by LLVY-amc hydrolysis, and the fractions were combined in pools a and b as divided by the dashed line.

(**E**) Coomassie staining of the purified proteasomes from the pools a and b following native PAGE. The 20S and 26S proteasomes in addition to the proteasomes Lg and Sm were cut out as framed for analysis by mass spectrometry.

(**F**) PA200 is detectable by immunoblotting in the Lg and Sm proteasomes, but not in the 20S and 26S proteasomes. Purified proteasomes from pools a and b in (**D**) were separated by native PAGE, and incubated with LLVY-amc in the absence or the presence of SDS. The 26S and the 20S proteasomes from rabbit skeletal muscle were included as references.

Figure S2. Subunit Identification of Testis-Specific Proteasomes, Related to Figure 1

(**A**) The amino acid sequences of human α4s (PSMA8, GenBank accession No.: NM_144662) and α4 (PSMA7) share 82% identity and 90% similarity. The human α4 gene is located at 20q13.33, whereas α4s is at 18q11.2. Homologs of α4s were found in all the NCBI databases available for mammals, but not for other vertebrates.

(**B**) α4s mRNA is highly expressed in testis. The levels of α4s mRNA in mouse testes were about 100-fold higher than those in other tissues analyzed by real-time PCR.

(**C**) α4s mRNA is specifically detected in spermatids by in situ hybridization analyses, while α4

mRNA is in all the cell types. mRNAs for α4s and α4 from mouse testes were detected using their antisense probes (brown), and their sense probes were used as negative controls. Nuclei were stained with haematoxylin (blue). The filled arrow (spermatocyte), the open arrow (round spermatid), and the open triangle (elongated spermatid) point to the corresponding cells.

(**D**) α4s protein is specifically expressed in the testis and epididymis. α4s protein in various mouse tissues was analyzed by immunoblotting following SDS-PAGE.

Figure S3. PA200, β1i, and β5i Are Present in Proteasome Complex in Testes, but PA28α Is Largely Present in Non-Proteasomal Forms, Related to Figure 2

(**A**) Fractionation of the lysates of GC-2spd cells by glycerol gradient ultracentrifugation. The proteasome subunits in each fraction were analyzed by immunoblotting following SDS-PAGE. Proteasome activity in each fraction was monitored by LLVY-amc hydrolysis.

(**B**) Fractionation of the homogenates of bovine testes by glycerol gradient ultracentrifugation. The proteasome subunits in each fraction were analyzed as in (**A**).

Figure S4. Activities of Testis-Specific Proteasomes, Related to Figure 2

(**A-C**) Peptidase activities and specificities of different proteasomes. Proteasome peptidase activities were assayed using the fluorogenic peptides, LLVY-amc for the chymotrypsin-like activity, LRR-amc for the trypsin-like activity, and LLE-amc for the caspase-like activity. Activities of purified proteasomes (**A & B**) or 20S particles (**C**) (0.5 μg/ml) were assayed using 50 μM of LLVY-amc, LRR-amc, or LLE-amc as a substrate in the absence (**A**) or the presence (**B & C**) of SDS. The peptidase activity at 38 nmol/min/mg protein was assigned to 100. The data were presented as

mean ± SD.

The chymotrypsin-like activity of muscle proteasomes was at least 3-7 fold greater than that from the testis or spleen ($P<0.01$). The trypsin-like activities of the muscle and testis particles were similar ($P>0.05$) and about 3-fold that of spleen ($P<0.01$). The caspase-like activities of the proteasomes from all three tissues were low, but muscle proteasomes had the highest activity. Incubation of these proteasomes with 0.02% SDS dramatically inhibited the chymotrypsin-like, but surprisingly increased markedly the caspase-like, activities of proteasomes from all three tissues ($P<0.01$). SDS had a limited effect on the trypsin-like activities of both muscle and testis, but it enhanced those from spleen at least 5-fold ($P<0.01$). The basal 20S activities from all three tissues were very low (data not shown), and the addition of 0.02% SDS markedly stimulated the trypsin-like and caspase-like activities of these particles ($P<0.01$), but only slightly increased their chymotrypsin-like activities.

(**D**) Inhibition of chymotrypsin-like activity of proteasomes by Velcade. Proteasomes at 0.5 μg/ml were incubated with 50 μM LLVY-amc in the presence of Velcade. Percentages of LLVY-amc hydrolysis inhibition by various concentrations of Velcade were calculated.

(E) Degradation of β-casein by different proteasomes. β-casein (20 μM) was incubated with proteasomes (5 μg/ml) in the absence or the presence of Velcade (50 nM), and the degradation products were determined using fluorescamine.

(**F**) Purification and ubiquitination of RNF5. 293T cells were transfected with the FLAG-tagged RNF5. Then, FLAG-RNF5 was immunoprecipitated with the anti-FLAG antibody, and eluted with the FLAG peptide. In order to obtain the poly-ubiquitin conjugates of RNF5 [RNF5-$(Ub)_n$], in vitro auto-ubiquitination reaction was performed in the presence of ubiquitin, E1, UbcH5, and RNF5.

293T cell lysates and the purified RNF5 or its ubiquitin conjugates were analyzed with the anti-FLAG or anti-ubiquitin antibody as indicated.

(**G**) The testis-specific proteasomes were much less efficient in degrading RNF5 than those from muscle or spleen. RNF5 and its ubiquitinated species were purified from 293T cells, and incubated with proteasomes (0.4 μg/ml) for indicated periods of time. The levels of RNF5 were analyzed by immunoblotting with the anti-FLAG antibody, quantified by densitometry, and relative levels were shown under the bands. Arrows and asterisks denote the non-ubiquitinated RNF5 (18 kDa) and mono-ubiquitinated RNF5, respectively.

(**H**) Purification and ubiquitination of Nrdp1. 293T cells were transfected with the FLAG-tagged Nrdp1. Then, FLAG-Nrdp1 was immune-purified and ubiquitinated as in (**F**). 293T cell lysates and the purified Nrdp1 or its ubiquitin conjugates were analyzed with the anti-FLAG or anti-ubiquitin antibody as indicated.

(**I**) Spermatoproteasomes are not efficient in degrading ubiquitinated Nrdp1. The polyubiquinated species of Nrdp1 [Nrdp1-$(Ub)_n$] prepared *in vitro* as in (**H**) were incubated with proteasomes (0.8 μg/ml) for indicated periods of time. Ubiquitin conjugates were analyzed by immunoblotting with an anti-ubiquitin antibody. Similar results were obtained from at least two independent experiments.

Figure S5. Deletion of PA200 Selectively Delays Core Histone Loss in Elongated Spermatids, Related to Figure 3

(A) PA200 deficiency leads to dramatic reduction of proteasomal peptidase activity. Testis homogenates from the wild-type or PA200-deficient mice were separated by native PAGE. In gel-peptidase activity was assayed by incubating the gel in LLVY-amc and 0.02% SDS.

Proteasome subunits were analyzed by immunoblotting.

(B) -(**D**) Deletion of PA200 retains the core histones H3 (**B**) and H2B (**C**), but not the linker histone H1 (**D**), in elongated spermatids of mice. Histone proteins in testis paraffin sections of the 15-week-old wild-type or PA200-deficient mice were detected by immunohistochemistry (brown), and nuclei were stained with haematoxylin (blue). The step of spermatogenesis was shown at the left of each pair of panels. The filled arrow (spermatocyte), the open arrow (round spermatid), and the open triangle (elongated spermatid) point to the corresponding cells.

Figure S6. PA200/Blm10 Is Required for Acetylation-Associated Degradation of Core Histones during Somatic DNA Damage, Related to Figure 4.

(A) Treatment with both irradiation and TSA reduces the levels of the core histones in wild-type MEF cells. The levels of H2B and H4 were analyzed as in **Figure 4C** (left panel), and quantified by densitometry (normalized to β-actin).

(B) Irradiation and TSA cannot reduce the levels of the core histones in PA200-deficient MEF cells. The levels of H2B and H4 were analyzed as in **Figure 4C** (right panel), and quantified by densitometry (normalized to β-actin).

(C) Acetylation- and Blm10-dependent degradation of the core histones in diploid yeast treated with MMS. The levels of H2B in wild-type or the Blm10-deficient diploid yeast were analyzed as in **Figure 4D**, and quantified by densitometry (normalized to GAPDH).

Figure S7. PA200/Blm10-Containing Proteasomes Selectively Degrade Acetylated Core Histones, Related to Figures 5 and 6.

(A) The BRD-like region of PA200 specifically binds acetylated histones (related to **Figure 5E**). His-tagged HAT domain of Gcn5 was incubated in the acetylation buffer in the presence or absence of purified histones from rabbit thymus. Then, the reaction mix was incubated with GST-fused BRD-like (BRDL) region of PA200 or its mutant (N1716T/F1717S). Following a pull-down assay using GSH-beads, acetylated histones and His-tagged Gcn5 were analyzed by western blot with an anti-acetyllysine antibody and an anti-His antibody, respectively. GST fusion proteins were stained by Coomassie blue. The anti-His antibody did not detect any band from the pull-down complexes (data not shown). Thus, the wild-type BRD-like region of PA200, but not its mutant, specifically pulled down acetylated histones, but not Gcn5.

(B) BRD-like region can specifically bind acetylated histones after removal of Gcn5 (related to **Figure 5E**). Following acetylation of histones, His-Gcn5 HAT was incubated with Ni^{2+} beads, and ~90% His-Gcn5 had been removed. Following the pulldown assay as in (A), the acetyl-histones could still be pulled down by the WT BRDL region of PA200, but not its mutant, further supporting that Gcn5 HAT does not mediate the interaction between GST-BRDL and acetyl-histones.

(C-D) Native PAGE analysis of proteasomes in mutant yeast. Proteasomes were visualized by incubating with LLVY-amc and 0.02% SDS, and HA-tagged Blm10 was analyzed by immunoblotting (related to **Figure 6A**). Deletion of Blm10 led to the disappearance of the proteasomal peptidase activities caused by Blm10-20S and Blm10-20S-19S, which were elevated by the overexpression of HA-Blm10. Deletion of Rpn4 greatly reduced the activity of 26S proteasomes (RP_2-20S and RP-20S), but had little, if any, effect on Blm10-20S. The levels of 20S were also markedly reduced, but were still enough for assembling with Blm10, probably

because there was little competition from RP. Deletion of Rpn13 markedly reduced activity of RP_2-20S, but had little effect on the activity of the RP-20S.

(E) Purities of 20S and the 20S+PA200 complex. Asterisk indicates probably-irrelevant impurities in the 20S preparation (related to **Figure 6D**).