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Cytoplasmic proteasomes are not indispensable for cell growth in *Saccharomyces cerevisiae*

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ABSTRACT

The 26S proteasome is an essential protease complex responsible for the degradation of ubiquitinated proteins in eukaryotic cells. In rapidly proliferating yeast cells, proteasomes are mainly localized in the nucleus, but the biological significance of the proteasome localization is still unclear. In this study, we investigated the relationship between the proteasome localization and the functions by the anchor-away technique, a ligand-dependent sequestration of a target protein into specific compartment(s). Anchoring of the proteasome to the plasma membrane or the ribosome resulted in conditional depletion of the nuclear proteasomes, whereas anchoring to histone resulted in the proteasome sequestration into the nucleus. We observed that the accumulation of ubiquitinated proteins in all the proteasome-targeted cells, suggesting that both the nuclear and cytoplasmic proteasomes have proteolytic functions and that the ubiquitinated proteins are produced and degraded in each compartment. Consistent with previous studies, the nuclear proteasome-depleted cells exhibited a lethal phenotype. In contrast, the nuclear sequestration of the proteasome resulted only in a mild growth defect, suggesting that the cytoplasmic proteasomes are not basically indispensable for cell growth in rapidly growing yeast cells.

1. Introduction

The 26S proteasome is a 2.5 MDa multi-subunit protease responsible for degradation of polyubiquitinated proteins in eukaryotic cells [1,2]. The ubiquitin–proteasome system is involved in a wide variety of cellular processes and its malfunction leads to a variety of diseases [3,4]. The 26S proteasome is composed of at least 33 different subunits and is arranged into two particles: a highly conserved 20S core particle (CP) containing a central catalytic cavity and one or two 19S regulatory particles (RP). The CP is a barrel-shaped structure of ~730 kDa, consisting of four heptameric rings, whereas the RP is a ~930 kDa complex consisting of 19 different subunits. The RP mediates multiple aspects of proteasome function such as substrate recognition, deubiquitination, unfolding and translocation of substrates into the CP. The RP can be separated into two major subcomplexes, the base and the lid. The base includes six different AAA + ATPase subunits (Rpt1–Rpt6) and three non-ATPase subunits (Rpn1, Rpn2, Rpn13), while the lid consists of nine non-ATPase subunits (Rpn3, 5–9, 11, 12, and Rpn15). Recent structural studies have shown that the lid partly surrounds the base ATPase ring and two ubiquitin receptors, Rpn10 and Rpn13, are located on the periphery of the 19S RP [5–8]. Recent studies have suggested that the assembly process of the proteasome is a highly ordered and multistep mechanism [9–12]. All the subcomplexes of the proteasome, the CP, the base, and the lid, seemed to be formed independently and the former two require multiple proteasome-dedicated chaperones for efficient and correct assembly.

The proteasomes are localized within both the cytoplasm and the nucleus. In rapidly proliferating yeast, proteasomes are enriched in the nucleus, presumably to ensure degradation of nuclear proteins [13–15]. Upon quiescence entry, yeast proteasomes relocalize from the nucleus into the cytoplasm to form cytoplasmic foci, named proteasome storage granules [16]. The proteasome granules are rapidly disappeared by addition of glucose, a trigger of quiescence exit, and are localized again to the nucleus, suggesting that the nucleocytoplasmic transport of the 26S proteasome occur rapidly and bidirectionally. Although whether the proteasome biogenesis directly couples to the nuclear transport is yet understood, previous studies have showed that importin α/β and an adaptor protein Sts1/Cut8 are required for the nuclear localization [17–19]. In a temperature-sensitive mutant of importin α, *srp1–49*, proteasomes specifically accumulated at outer membrane of the nuclear envelope at restrictive temperature [17,20,21]. Sts1, Cut8 in *Schizosaccharomyces pombe*, is an essential NLS-containing protein that can interact with the proteasome [18,19,22]. Both Sts1 and Cut8 are proteasome substrate themselves, therefore, it was

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proposed that a negative feedback loop of the proteasome activities and the nuclear localization. Because selective degradation of securin is absolutely required for correct segregation of chromosomes, the proteasome activities in the nucleus are thought to be essential [23]. Consistently, these proteasome transporter mutants exhibit a growth defect under the non-permissive conditions. However, how much degree of protein degradation in the nucleus is still unclear. In contrast, biological significance of cytoplasmic proteasomes has not been well documented, although protein quality-control systems such as ER-associated degradation (ERAD) exist in the cytoplasm [24,25].

In this study, we investigated the functional relationship between the proteasome localization and the biological significance by anchor-away technique, a ligand-dependent sequestration of a target protein into specific compartments [26]. We found that cytoplasmic sequestration of the proteasome caused a lethal phenotype. In contrast, although significant amount of the ubiquitinated proteins accumulated, the sequestration of the proteasome to the nucleus resulted in only a mild growth defect. These results directly demonstrated that the nuclear proteasomes are essential and that the cytoplasmic proteasomes are not indispensable at least in proliferating yeast cells.

2. Materials and methods

2.1. Yeast strains and media

Saccharomyces cerevisiae strains used in this study are listed in Table 1. All the strains are isogenic to W303 strain. Standard genetic techniques were used to manipulate yeast strains [27]. C-terminal FRB-GFP-tagging was carried using standard PCR-based gene insertion method with a plasmid pFA6a-FRB-GFP-KanMX6 [26,28]. Collect integration was confirmed by genomic PCR. For spotting test, yeast cells were grown in YPD medium (1% yeast extracts, 2% peptone, 2% glucose, 400 mg/l adenine sulfate, and 20 mg/l uracil) at 28 °C. Then, serial dilutions (1:10) of overnight cultures were spotted onto YPD agar supplemented with 1 μg/ml rapamycin (Sigma).

2.2. Fluorescence microscopy

The cells grown in YPD medium supplemented with 1 μg/ml rapamycin or on the rapamycin plate were mounted on a glass slide and observed with a BX52 fluorescence microscope (Olympus) equipped with a UPlanApo 100×, 1.45 NA objective (Olympus), a confocal scanner unit CSU21 (Yokogawa), and an ORCA-ER CCD camera (Hamamatsu Photonics), as described previously [29]. Images were processed using the IPLab software (Scanalytics Inc.).

2.3. Western blotting

Yeast cells were grown in YPD medium in the absence and presence of 1 μg/ml rapamycin to an OD_{600} of 0.7–1.0. Total cell extracts were prepared by mild-alkali method [30]. The proteins were separated by SDS–PAGE on 4–12% NuPAGE Bis-Tris gels with MES buffer (Life Technologies) and blotted onto a PVDF membrane using the NuPAGE immunoblotting system (Life Technologies) [31]. The membranes were probed with anti-ubiquitin Lys48-specific (Apu2, Millipore) or anti-PGK (22C5, Molecular Probes) monoclonal antibodies. Horseradish peroxidase-conjugated antibodies (Jackson ImmunoResearch) were used as secondary antibodies. Chemiluminescence signals produced by an ECL Prime reagent (GE Healthcare) were detected using a CCD camera-based imager LAS4000 (Fujifilm).

3. Results and discussion

3.1. The proteasome targeting by the anchor-away technique

To investigate the compartment-specific functions of the proteasomes, we used the anchor-away technique in *S. cerevisiae* [26]. The technique depends on the heterodimerization of the human FKBP12 (the human 12 kDa FK506 binding protein) to the FRB (FKBP12-rapamycin-binding) domain of human mTOR that occurs in the presence of rapamycin [26,32,33]. Because rapamycin is toxic to wild-type yeast, rapamycin-resistant strains containing a mutated *TOR1* (*tor1–1*) and deleted *FPR1* (*Δfpr1*) gene encoding formyl peptide receptor 1 were used as background strains throughout the study. Among 33 proteasome subunits, we inserted the FRB-tag into an essential lid subunit Rpn11 by homologous recombination. To investigate the cellular distribution of proteasomes, green fluorescent protein (GFP) tag was also inserted. The proteasome distributions and growth rates were indistinguishable among wild-type, *RPN11-GFP* [29], and the *RPN11-FRB-GFP* strains, suggesting that the FRB-tagging does not affect proteasome functions (Figs. 1 and 2, data not shown). Pma1-FKBP is used for the proteasome targeting to the plasma membrane (Fig. 1A), whereas Rpl13a- and Htb2-FKBP are used for the targeting to the cytoplasm and the nucleus, respectively. We generated a series of anchor-away strains of the proteasome by genetic crosses (Table 1).

As reported originally by Haruki et al. [26], the yeast importin β, Kap95, was rapidly targeted to the plasma membrane by the Pma1-anchoring after the addition of rapamycin within 30 min (Fig. 1B). We next analyzed the Pma1-anchoring proteasome strain (Fig. 1C). After addition of rapamycin, cytosolic proteasomes were rapidly targeted to the plasma membrane within 10 min, then, the nuclear proteasomes were gradually relocalized to the cytoplasm and were targeted to the Pma1-anchor within 4 h, a time period

Table 1
Yeast strains used in this study.

Strain name	Genotype	Reference
W303–1A	*MATa ura3–1 trp1–1 leu2–3,112 his3–11,15 ade2–1 can1–100*	Our stock
K14708	*MATα tor1–1 fpr1::NAT*	[26]
HHY51	*MATα tor1–1 fpr1::NAT HTB2-FKBP12::HIS3 KAP95-FRB::kanMX6*	[26]
HHY168	*MATα tor1–1 fpr1::NAT RPL13A-2 × FKBP12::TRP1*	[26]
HHY225	*MATa tor1–1 fpr1::NAT PMA1–2 × FKBP12::TRP1 KAP95-FRB-GFP::KanMX6*	[26]
YYS1199	*MATa Δpdr5::KanMX4*	[29]
YYS1981	*MATa tor1–1 fpr1::NAT*	This study
YYS1983	*MATa tor1–1 fpr1::NAT PMA1–2 × FKBP12::TRP1*	This study
YYS1985	*MATa tor1–1 fpr1::NAT RPL13A-2 × FKBP12::TRP1*	This study
YYS1989	*MATa tor1–1 fpr1::NAT HTB2-FKB12::HIS3*	This study
YYS1991	*MATα tor1–1 fpr1::NAT RPN11-FRB-GFP::KanMX6*	This study
YYS1993	*MATa tor1–1 fpr1::NAT PMA1–2 × FKBP12::TRP1 RPN11-FRB-GFP::KanMX6*	This study
YYS1995	*MATa tor1–1 fpr1::NAT RPL13A-2 × FKBP12::TRP1 RPN11-FRB-GFP::KanMX6*	This study
YYS1999	*MATa tor1–1 fpr1::NAT HTB2-FKB12::HIS3 RPN11-FRB-GFP::KanMX6*	This study

corresponding roughly to three doubling time of the cells (data not shown). After 24 h, the proteasome signals were only detected in the Pma1-resident compartments, the plasma membrane, multivesicular bodies, and vacuole (Fig. 1C, plasma membrane). In the ribosome-anchor cells, the nuclear proteasome signals were disappeared in the same kinetics of the Pma1-anchor cells and distributed throughout the cytoplasm (Fig. 1C, ribosome). These observations suggested that the proteasomes are highly mobile molecules in the cytoplasm, consistent with a previous study in mammalian cells [34], and that the nuclear export occurs slowly. In the histone-anchor cells, apparently normal distribution of the proteasome was observed, but the signal intensity of the cytoplasmic proteasomes decreased to near background level (Fig. 1C, histone). Although the anchor-away technique was developed for cytoplasmic sequestration of nuclear proteins, nuclear sequestration by the Htb2-anchor appeared to occur effectively. Thus, the cellular distributions of the proteasome can be manipulated by the simple anchor-away technique.

3.2. The cytoplasmic localization of the proteasomes is not essential in yeast

We next analyzed cell growth of the anchor-away strains. Serial dilutions of the indicated strains were spotted onto rapamycin-containing or control YPD plate (Fig. 2). The cells expressing Kap95-FRB-GFP and Pma1-FKBP were used again as a positive control. The spot-test suggested that the proteasome targeting to the plasma membrane and the ribosome resulted in lethality on the plate containing rapamycin, while wild-type growth was observed on the control plate (Fig. 2). We also determined the growth rate and found that both the *RPN11-FRB-GFP PMA1-FKBP* and the *RPN11-FRB-GFP RPL13A-FKBP* cells arrested their growth during three to four doubling times in the presence of rapamycin (data not shown). Concomitantly with the growth arrest, the nuclear-depletions of the proteasomes were observed by fluorescent

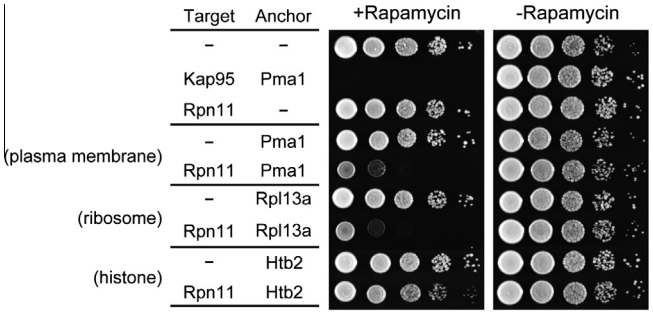

Fig. 2. Conditional depletion of the proteasome from the nucleus is lethal in yeast cells. Serial dilutions (1:10) of the yeast cells were grown on YPD + 1 μg/ml rapamycin at 28 °C. The cells expressing Kap95-FRB-GFP and Pma1-FKBP (HHY225) were used as a positive control.

microscopic analysis (data not shown). Thus, these results clearly suggested that the essential function of the proteasome is that in the nucleus. In contrast, strikingly, the nuclear sequestration of the proteasome resulted in only a mild growth defect, suggesting that the cytoplasmic proteasomes are not indispensable for the cells growth (Fig. 2).

3.3. Accumulations of polyubiquitinated proteins in the proteasome-sequestrating cells

Does proteasomes have compartment-specific functions other than the protein degradation? In the nucleus, the ubiquitin–proteasome system has been shown to interact with chromatin and regulates multiple steps in gene transcription, from controlling activators to mRNA exports. Complicatedly, both proteolytic and/or non-proteolytic roles have been proposed in these processes (reviewed in Geng et al. [35]). In addition, the nuclear compartmentalized protein quality control has emerged with the

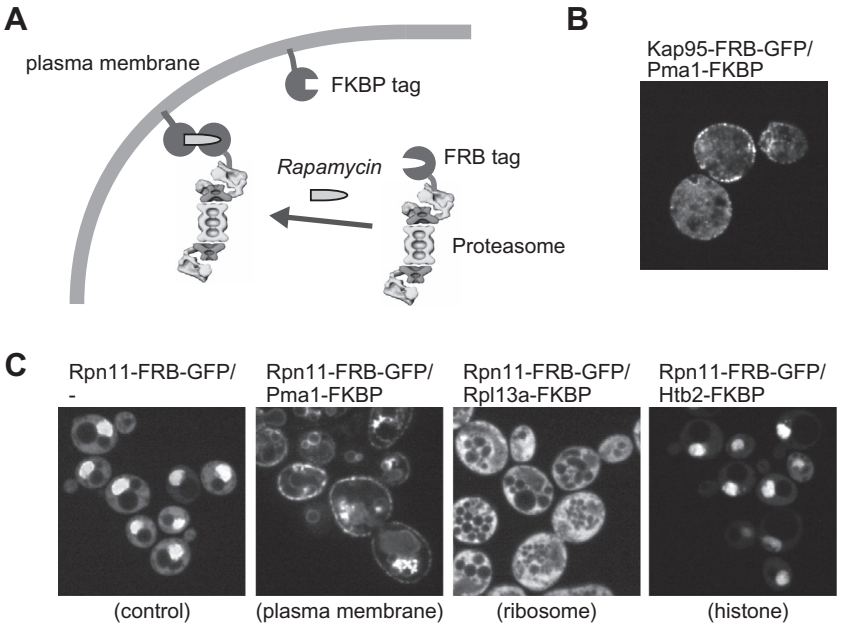

Fig. 1. Compartment-specific sequestrations of the 26S proteasome in yeast cells. (A) A scheme of the anchor-away technique. FRB tag was fused to an essential proteasome subunit Rpn11 as the target molecule. For the plasma membrane anchoring, FKBP12 tag was fused the abundant plasma membrane protein H^+-ATPase Pma1 as an anchor. Upon addition of rapamycin, the FRB-tagged proteasomes and the FKBP-tagged Pma1 were formed the ternary complex on the plasma membrane. (B) Fluorescent microscopic image of the plasma membrane anchored Kap95, a yeast importin β. The cells expressing Kap95-FRB-GFP and Pma1-FKBP (HHY225) were treated with 1 μg/ml rapamycin for 2 h and GFP signals were imaged with a confocal fluorescent microscopy. (C) Fluorescent microscopic images of the proteasome targeted cells. The cells expressing Rpn11-FRB-GFP and the anchor proteins, Pma1-FKBP (YYS1993 for the plasma membrane), Rpl13a-FKBP (YYS1995 for the ribosome), Htb2-FKBP (YYS1999 for histone H2B), or control (YYS1991), were treated with 1 μg/ml rapamycin for 24 h and GFP signals were imaged with confocal fluorescent microscopy.

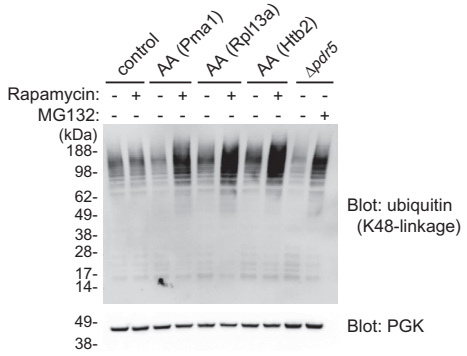

Fig. 3. Accumulation of ubiquitinated proteins in the proteasome targeted cells. The proteasome targeted cells as in Fig. 1(C), the anchor-away cells were indicated by AA, were treated with 1 μg/ml rapamycin for 6 h and were analyzed by Western-blot with anti-ubiquitin K48-linkage specific antibody. As a control, the *Δpdr5* cells cultured with or without MG132, a proteasome inhibitor, were analyzed in parallel. Anti-PGK was used to confirm the equal loading.

identification of a yeast ubiquitin ligase San1 [36]. In the cytoplasm, proteasomes participate in two major protein quality control systems and degrade newly synthesized polypeptides at the ribosome and ERAD substrates [37]. Interestingly, a recent study has identified a specific compartment called JUNQ, juxta-nuclear quality control compartment, for proteasomal degradations of cytoplasmic misfolded proteins in both yeast and mammalian cells [38,39]. If the nuclear ubiquitinated substrates are also shuttled to the JUNQ, cytoplasmic sequestration of the proteasome might not cause the accumulation of the ubiquitinated proteins.

To address this issue, we analyzed accumulation of ubiquitinated proteins in the anchor-away cells. Exponentially growing cells were treated with rapamycin for 6 h and total cell extracts were analyzed by Western-blot with Lys48-linked ubiquitin chain-specific antibody (Fig. 3). As a control, a proteasome inhibitor (MG132)-treated cells were also analyzed. Strikingly, significant accumulations of the polyubiquitinated proteins were observed in all the proteasome-targeted cells as well as in the MG132-treated cells. Noteworthy, the similar accumulation levels between the plasma membrane- and the ribosome-anchoring cells suggested that the membrane-tethered proteasomes are competent to degrade cytosolic ubiquitinated proteins probably by extrinsic ubiquitin receptor proteins [2]. Thus, the result suggests that both the nuclear and the cytoplasmic proteasomes facilitate the degradation of ubiquitinated proteins. The accumulation in each compartment also suggests that most ubiquitinated proteins might not shuttle between the nucleus and the cytoplasm. If so, the cytoplasmic accumulation of the ubiquitinated proteins might not cause cell death at least in rapidly dividing yeast cells. It would be interesting to identify the accumulated substrates in future study. Also, use of anchor-away technique described here might be a good approach to determine the transcriptional roles in the nuclear proteasomes.

Collectively, we demonstrated that the nuclear proteasome is essential for cell growth by the anchor-away technique. Unexpectedly, we found that the cytoplasmic proteasomes are not indispensable for cell growth in yeast.

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References

[1] W. Baumeister, J. Walz, F. Zuhl, E. Seemuller, The proteasome: paradigm of a self-compartmentalizing protease, Cell 92 (1998) 367–380.
[2] D. Finley, Recognition and processing of ubiquitin-protein conjugates by the proteasome, Ann. Rev. Biochem. 78 (2009) 477–513.
[3] A.L. Schwartz, A. Ciechanover, Targeting proteins for destruction by the ubiquitin system: implications for human pathobiology, Ann. Rev. Pharmacol. Toxicol. 49 (2009) 73–96.
[4] K. Tanaka, T. Mizushima, Y. Saeki, The proteasome: molecular machinery and pathophysiological roles, Biol. Chem. 393 (2012) 217–234.
[5] G.C. Lander, E. Estrin, M.E. Matyskiela, C. Bashore, E. Nogales, A. Martin, Complete subunit architecture of the proteasome regulatory particle, Nature 482 (2012) 186–191.
[6] P.C. da Fonseca, J. He, E.P. Morris, Molecular model of the human 26S proteasome, Mol. Cell 46 (2012) 54–66.
[7] K. Lasker, F. Forster, S. Bohn, T. Walzthoeni, E. Villa, P. Unverdorben, F. Beck, R. Aebersold, A. Sali, W. Baumeister, Molecular architecture of the 26S proteasome holocomplex determined by an integrative approach, Proc. Natl. Acad. Sci. USA 109 (2012) 1380–1387.
[8] F. Beck, P. Unverdorben, S. Bohn, A. Schweitzer, G. Pfeifer, E. Sakata, S. Nickell, J.M. Plitzko, E. Villa, W. Baumeister, F. Forster, Near-atomic resolution structural model of the yeast 26S proteasome, Proc. Natl. Acad. Sci. USA 109 (2012) 14870–14875.
[9] L. Bedford, S. Paine, P.W. Sheppard, R.J. Mayer, J. Roelofs, Assembly, structure, and function of the 26S proteasome, Trends Cell Biol. 20 (2010) 391–401.
[10] S. Murata, H. Yashiroda, K. Tanaka, Molecular mechanisms of proteasome assembly, Nat. Rev. Mol. Cell Biol. 10 (2009) 104–115.
[11] R.J. Tomko Jr., M. Hochstrasser, Order of the proteasomal ATPases and eukaryotic proteasome assembly, Cell Biochem. Biophys. 60 (2011) 13–20.
[12] Y. Saeki, K. Tanaka, Assembly and function of the proteasome, Meth. Mol. Biol. 832 (2012) 315–337.
[13] C. Enenkel, A. Lehmann, P.M. Kloetzel, Subcellular distribution of proteasomes implicates a major location of protein degradation in the nuclear envelope-ER network in yeast, EMBO J. 17 (1998) 6144–6154.
[14] C.R. Wilkinson, M. Wallace, M. Morphew, P. Perry, R. Allshire, J.P. Javerzat, J.R. McIntosh, C. Gordon, Localization of the 26S proteasome during mitosis and meiosis in fission yeast, EMBO J. 17 (1998) 6465–6476.
[15] A. Amsterdam, F. Pitzer, W. Baumeister, Changes in intracellular localization of proteasomes in immortalized ovarian granulosa cells during mitosis associated with a role in cell cycle control, Proc. Natl. Acad. Sci. USA 90 (1993) 99–103.
[16] D. Laporte, B. Salin, B. Daignan-Fornier, I. Sagot, Reversible cytoplasmic localization of the proteasome in quiescent yeast cells, J. Cell Biol. 181 (2008) 737–745.
[17] E. Isono, K. Nishihara, Y. Saeki, H. Yashiroda, N. Kamata, L. Ge, T. Ueda, Y. Kikuchi, K. Tanaka, A. Nakano, A. Toh-e, The assembly pathway of the 19S regulatory particle of the yeast 26S proteasome, Mol. Biol. Cell 18 (2007) 569–580.
[18] K. Takeda, N.K. Tonthat, T. Glover, W. Xu, E.V. Koonin, M. Yanagida, M.A. Schumacher, Implications for proteasome nuclear localization revealed by the structure of the nuclear proteasome tether protein Cut8, Proc. Natl. Acad. Sci. USA 108 (2011) 16950–16955.
[19] K. Takeda, M. Yanagida, Regulation of nuclear proteasome by Rhp6/Ubc2 through ubiquitination and destruction of the sensor and anchor Cut8, Cell 122 (2005) 393–405.
[20] P. Wendler, A. Lehmann, K. Janek, S. Baumgart, C. Enenkel, The bipartite nuclear localization sequence of Rpn2 is required for nuclear import of proteasomal base complexes via karyopherin alphabeta and proteasome functions, J. Biol. Chem. 279 (2004) 37751–37762.
[21] M.M. Tabb, P. Tongaonkar, L. Vu, M. Nomura, Evidence for separable functions of Srp1p, the yeast homolog of importin alpha (Karyopherin alpha): role for Srp1p and Sts1p in protein degradation, Mol. Cell Biol. 20 (2000) 6062–6073.
[22] L. Chen, L. Romero, S.M. Chuang, V. Tournier, K.K. Joshi, J.A. Lee, G. Kovvali, K. Madura, Sts1 plays a key role in targeting proteasomes to the nucleus, J. Biol. Chem. 286 (2011) 3104–3118.
[23] M. Yanagida, Cell cycle mechanisms of sister chromatid separation; roles of Cut1/separin and Cut2/securin, Genes Cells 5 (2000) 1–8.
[24] B. Meusser, C. Hirsch, E. Jarosch, T. Sommer, ERAD: the long road to destruction, Nat. Cell Biol. 7 (2005) 766–772.
[25] J.L. Brodsky, Cleaning up: ER-associated degradation to the rescue, Cell 151 (2012) 1163–1167.
[26] H. Haruki, J. Nishikawa, U.K. Laemmli, The anchor-away technique: rapid, conditional establishment of yeast mutant phenotypes, Mol. Cell 31 (2008) 925–932.
[27] F. Sherman, Getting started with yeast, Methods Enzymol. 194 (1991) 3–21.
[28] M.S. Longtine, A. McKenzie 3rd, D.J. Demarini, N.G. Shah, A. Wach, A. Brachat, P. Philippsen, J.R. Pringle, Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*, Yeast 14 (1998) 953–961.
[29] Y. Saeki, A. Toh-e, T. Kudo, H. Kawamura, K. Tanaka, Multiple proteasome-interacting proteins assist the assembly of the yeast 19S regulatory particle, Cell 137 (2009) 900–913.
[30] V.V. Kushnirov, Rapid and reliable protein extraction from yeast, Yeast 16 (2000) 857–860.

[31] Y. Saeki, T. Kudo, T. Sone, Y. Kikuchi, H. Yokosawa, A. Toh-e, K. Tanaka, Lysine 63-linked polyubiquitin chain may serve as a targeting signal for the 26S proteasome, EMBO J. 28 (2009) 359–371.
[32] P.J. Belshaw, S.N. Ho, G.R. Crabtree, S.L. Schreiber, Controlling protein association and subcellular localization with a synthetic ligand that induces heterodimerization of proteins, Proc. Natl. Acad. Sci. USA 93 (1996) 4604–4607.
[33] J. Chen, X.F. Zheng, E.J. Brown, S.L. Schreiber, Identification of an 11-kDa FKBP12-rapamycin-binding domain within the 289-kDa FKBP12-rapamycin-associated protein and characterization of a critical serine residue, Proc. Natl. Acad. Sci. USA 92 (1995) 4947–4951.
[34] E.A. Reits, A.M. Benham, B. Plougastel, J. Neefjes, J. Trowsdale, Dynamics of proteasome distribution in living cells, EMBO J. 16 (1997) 6087–6094.
[35] F. Geng, S. Wenzel, W.P. Tansey, Ubiquitin and proteasomes in transcription, Ann. Rev. Biochem. 81 (2012) 177–201.
[36] R.G. Gardner, Z.W. Nelson, D.E. Gottschling, Degradation-mediated protein quality control in the nucleus, Cell 120 (2005) 803–815.
[37] E.K. Fredrickson, R.G. Gardner, Selective destruction of abnormal proteins by ubiquitin-mediated protein quality control degradation, Semin. Cell Dev. Biol. 23 (2012) 530–537.
[38] D. Kaganovich, R. Kopito, J. Frydman, Misfolded proteins partition between two distinct quality control compartments, Nature 454 (2008) 1088–1095.
[39] T. Ben-Gedalya, R. Lyakhovetsky, Y. Yedidia, M. Bejerano-Sagie, N.M. Kogan, M.V. Karpuj, D. Kaganovich, E. Cohen, Cyclosporin-A-induced prion protein aggresomes are dynamic quality-control cellular compartments, J. Cell Sci. 124 (2011) 1891–1902.