Role of C-terminal Extensions of Subunits β2 and β7 in Assembly and Activity of Eukaryotic Proteasomes*

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A close inspection of the crystal structure of the yeast 20 S proteasome revealed that a prominent connection between the two β-rings is mediated by the subunit β7/Pre4. Its C-terminal extension intercalates between the β1/Pre3 and β2/Pup1 subunits on the opposite ring. We show that the interactions promoted by the β7/Pre4 tail are important to facilitate the formation of 20 S particles from two half-proteasome precursor complexes and/or to stabilize mature 20 S proteasomes. The deletion of 19 residues from the β7/Pre4 C terminus leads to an accumulation of half-proteasome precursor complexes containing the maturation factor Ump1. The C-terminal extension of β7/Pre4, which forms several hydrogen bonds with β1/Pre3, is in addition required for the post-acidic activity mediated by the latter subunit. Deletion of the C-terminal tail of β7/Pre4 results in an inhibition of β7/Pre3 propeptide processing and abrogation of post-acidic activity. Our data obtained with yeast strains that express the mature form of Pre4 lacking its propeptide suggest that interactions between the Pre4 C terminus and Pre3 stabilize a conformation of its active site, which is essential for post-acidic activity. Deletion of the C-terminal extension of β2/Pup1, which wraps around β3/Pup3 within the same β-ring, is lethal, indicating that this extension serves an essential function in proteasome assembly or stability.

Ubiquitin-mediated proteolysis is the main pathway for ATP-dependent non-lysosomal degradation of intracellular proteins in eukaryotes (1, 2). Polyubiquitylated substrates are recognized and degraded by small peptides by a multisuubunit protease termed 26 S proteasome. This large proteolytic complex is composed of a 20 S barrel-shaped complex (the 20 S proteasome) and a 19 S regulatory complex. The latter harbor isopeptidase and ATPase activities as well as binding sites for polyubiquitin chains (3–5).

20 S proteasomes are present in all eukaryotes, as well as in some archaea and eubacteria. In 1995, the structure of the 20 S proteasome of the archaean Thermoplasma acidophilum solved by x-ray crystallography was reported. This proteasome is composed of two distinct types of subunits, α and β, that are organized in four heptameric stacks with the arrangement α3β7α3. The β-rings form a central cavity that contains 14 active sites located on the inner surface (5). Two years later the crystal structure of the eukaryotic Saccharomyces cerevisiae 20 S proteasome was determined revealing a similar organization. However, the two identical halves contain seven different α-type subunits and seven distinct β-type subunits occupying unique locations within the structure. Only three of the seven different β-subunits are active (6). Based on mutational and structural studies it was found that subunits β1/Pre3, β2/Pup1, and β5/Pre2 harbor the peptidase sites, which mediate activities classified as post-acidic, trypsinic, and chymotryptic, respectively (6–9). In mammalian proteasomes, the constitutive active subunits β1, β2, and β5, upon immune stress, can be replaced by the interferon-γ-induced subunits β-chain, β2i, and β5i, respectively, resulting in the formation of so-called “immunoproteasomes” (10). More recently, the crystal structure of a constitutive mammalian 20 S proteasome was determined showing that the overall structure of eukaryotic proteasomes is highly conserved (11).

In all proteasomes studied, α-type and β-type subunits share a common “β-sandwich” fold, in which two antiparallel β-sheets are sandwiched in between two layers of α-helices (3). Major structural differences exhibited by the individual subunits, such as insertions or C- and N-terminal extensions, are important features that determine the specific subunit interactions within the α- or β-rings (cis contacts) and between the rings (α-trans-β and β-trans-β contacts). These interactions target each of the 14 subunits to unique locations within the structure of the eukaryotic 20 S proteasome (6, 11).

In Thermoplasma, the β-subunits are synthesized as precursors that require posttranslational processing for activation. Similarly, all active eukaryotic β-type subunits are synthesized as inactive precursors with N-terminal propeptides. These propeptides are cleaved off by intrasubunit autolysis yielding the mature forms with N-terminal threonine residues that act as nucleophiles attacking peptide bonds of substrates (7, 12–14). This property places proteasomes into the family of “Ntn hydrolases” that is characterized by an N-terminal nucleophile (15, 16).

Another structural aspect of several β-subunits is their C-terminal extensions that are involved in interactions within or across the rings. Subunit β2 has the longest C-terminal extension (37 residues) with which it embraces β3 within the same ring. The C-terminal extension of β7 resembles a clamp that projects from one half of the proteasome to reach out to the opposing half entering into its β-ring between β1 and β2. In the present study, we have investigated the role of the C-terminal extensions of β2 and β7. We present evidence showing that, in yeast, the β7/Pre4 C-terminal domain has a role in mediating...
efficient generation of 20 S proteasome from its precursors. In addition, we show that specific interactions between this domain and β1/Pre3 are essential for post-acidic peptidase activity. The C-terminal extension of β2/Pup1 is essential for yeast cell viability indicating that functional proteasomes cannot form in its absence.

EXPERIMENTAL PROCEDURES

Yeast Media—Yeast-rich (YPD) and synthetic (SD) minimal media with 2% dextrose were prepared as described previously (17).

Construction of Yeast Strains and Plasmids—The yeast strains used in this study are listed in Table I. Construction of chromosomal open reading frames that expressed C-terminally truncated versions of β7/Pre4 was performed as follows. Different 3′-portions of PRE4 were generated by PCR using primers containing flanking EcoRI and XbaI sites. The 3′-portions derived from JD281 (Table I) with the indicated genotypes were spotted in serial dilutions onto YPD medium and incubated at 30 °C for 2 days. wild type, E, analysis of the level of two proteasomal subunits, β7/Pre4 (top) and α7/Pre10 (bottom), in the same strains (a–d) as in D with anti-Pre4 and anti-Pre10 antibodies, respectively. Strain JD183 expressing a slower migrating Pre10-ha (wt) was included as a control to demonstrate specificity of the anti-Pre10 antibodies. 30 μg of crude extract proteins were loaded/lane. The propeptide-containing precursor forms of Pre4 and Pre4-Dc19 detected in the ump1-α background are indicated by arrowheads. For the wild-type UMP1 strains, these forms were detectable only upon long exposure of the immunoblot (data not shown).

Fractionation of Whole-cell Extracts by Gel Filtration, Electrophoresis, and Immunoblotting—S. cerevisiae cells were grown at 30 °C in SD medium to an A600 of 1.2 ± 0.2 and treated as described by Ramos et al. (19). Protein extraction and fractionation on a Superose 6 column coupled to an AKTA PPLC (Amersham Biosciences) as well as SDS-PAGE and immunoblots were performed as described previously (19). Native PAGE was carried out according to published protocols (22). Polyclonal rabbit antibodies raised against β7/Pre4 were a kind gift from the laboratory of Dieter Wolf. Polyclonal anti-α7/Pre10 antibodies were raised against Pre10-His6 expressed in Escherichia coli.

Proteasomal Peptidase Activity Assays—Determination of proteasomal activities in crude extracts was performed as follows. Yeast cells

FIG. 1. Structural aspects of the β7/Pre4 C terminus tail. A, structure of S. cerevisiae 20 S proteasome emphasizing the β7/Pre4 β-trans-β interactions with β1/Pre3 and β2/Pup1. This figure was prepared with RasMol 2.6. β7/Pre4 is shown in the spacefill representation; all other subunits are displayed as wireframe. B, detail of the contact sites between the β7/Pre4 C-terminal extension (orange and red) and β1/Pre3 (various shades of blue). The prediction of hydrogen bonds (shown as dotted lines) and the graphic representation were done using SETOR (25). Side chains of relevant residues are highlighted as balls and sticks. C, amino acid sequence alignments of the C-terminal regions of β7 subunits of the indicated species. Residues identical between β7/Pre4 and other species are boxed. D, synthetic growth inhibition by ump1-α and pre4-Dc19. Spore clones (a–d) derived from JD281 (Table I) with the indicated genotypes were spotted in serial dilutions onto YPD medium and incubated at 30 °C for 2 days. wt, wild type. E, analysis of the level of two proteasomal subunits, β7/Pre4 (top) and α7/Pre10 (bottom), in the same strains (a–d) as in D with anti-Pre4 and anti-Pre10 antibodies, respectively. Strain JD183 expressing a slower migrating Pre10-ha (wt) was included as a control to demonstrate specificity of the anti-Pre10 antibodies. 30 μg of crude extract proteins were loaded/lane. The propeptide-containing precursor forms of Pre4 and Pre4-Dc19 detected in the ump1-α background are indicated by arrowheads. For the wild-type UMP1 strains, these forms were detectable only upon long exposure of the immunoblot (data not shown).

The abbreviations used are: Ub, ubiquitin; CP, core particle; R, regulatory particle.
from exponentially growing cultures (A
sub = 0.8–1.2) were harvested at 3000 × g, washed with cold water, frozen in liquid nitrogen, and stored at −80 °C. The cells were lysed with acid-washed glass beads (20 0.4–0.6 mm, Sigma) in extraction buffer (50 mM Tris-HCl, pH 7.5, 2 mM ATP, 5 mM MgCl₂, 1 mM dithiothreitol, 15% glycerol) by vortexing. Cell debris was removed by centrifugation at 15,800 × g at 4 °C. The protein content in the supernatant was determined using the Bio-Rad Bradford assay. For assaying chymotrypsin-like activity, reactions were set up with 5 μg of extract protein and 5 μg of succinyl-Leu-Leu-Val-Tyr 7-amido-4-methylcoumarin (Bachem) as substrate. The trypsin-like activity and the post-acidic activity were measured using 20 μg of protein and 5 μg of t-butoxycarbonyl-Leu-Arg-Arg 7-amido-4-methylcoumarin (Affinity) and Ac-Gly-Pro-Leu-Asp 7-amido-4-methylcoumarin (Bachem) as substrates, respectively. The assay conditions were described previously (19).

RESULTS

Structure of the βP/Pre4 C-terminal Tail and Its Interactions with βP/Pre3—The overall architecture of the 20 S proteasome and the general structures of the α- and β-subunits are conserved between T. acidophillum (5), S. cerevisiae (6), and mammals (11). One striking structural difference between the bacterial and the eukaryotic proteasome is confined to the β7 subunit. Inspection of the crystal structure of the yeast proteasome reveals a long C-terminal domain of βP/Pre4. This tail is composed of 19 amino acid residues and extends from one half of the proteasome to the other where it ends between the subunits β1/Pre3 and β2/Pup1. The latter of which carries the active site for post-acidic peptide activity (Fig. 1A). Both the carboxyl group of the last residue Ile225 and the side chain of Gin223 of βP/Pre4 form hydrogen bonds with the side chain of Arg185 of β1/Pre3 (residue numbers refer to the processed subunits according to Ref. 6). Arg185 together with Pre3 in turn interacts with Gly219 of β7/Pre4 form hydrogen bonds stabilizing Asp32 of Pre3, which itself forms a hydrogen bond with Leu34. The latter two residues are direct neighbors of Lys33, which is part of the post-acidic catalytic site, suggesting that this network of hydrogen bonds may be important for post-acidic activity (Fig. 1B). A comparison of the C-terminal sequence of β7 subunits from various species (Fig. 1C) revealed that its extension is only moderately conserved among eukaryotes.

The C-terminal Extension of β7/Pre4 Facilitates the Formation of 20 S Particles from Two Precursor Complexes—During our studies on the ump4-1/Pre4-3 mutant, in which a residue of the β7/Pre4 subunit was exchanged that is close to the site where its C-terminal extension emanates from one β-ring to reach out to the other, we found that proteasome precursor complexes accumulate. Prompted by this finding we asked whether a defined deletion of the C-terminal tail of β7/Pre4 would be inhibitory to the formation of 20 S proteasomes from two 15 S half-proteasome precursor complexes. These precursor complexes are characterized by the presence of the propeptide-bearing precursor forms of the active β-type subunits, β1/Pre3, β2/Pup1, and β5/Pre2, and the proteasome maturation factor Ump1 (19). We generated a strain in which the C-terminal 19 amino acid residues of β7/Pre4 were deleted (pre4-ΔC19). In the same strain, a double ha epitope tag was linked to the C terminus of β2/Pup1. To examine whether the C-terminal truncation of Pre4 caused changes in the intracellular ratios of proteasomal precursor complexes and mature proteasomes, we analyzed cell extracts of the pre4-ΔC19 mutant by gel filtration on a Superose 6 column. As for the wild type, the half-proteasome precursor complex of the pre4-ΔC19 eluted with a peak in fractions 22 and 23 (Fig. 2). The ratio of this precursor complex to mature proteasomes, however, is significantly higher in the mutant (Fig. 2, middle panel) resulting also in a spreading of the immunoblot signal from fractions 21 to 25. As described previously (19), the mature form of β2/Pup1 is present in earlier fractions containing proteasomal peptidase activity due to the presence of assembled and active 20 S and 26 S proteasomes. Noteworthy is also the observation that the mature form of β2/Pup1 is in addition detected as a free subunit in fractions 29 and 30 of pre4-ΔC19 mutant extracts (Fig. 2). In contrast, no free mature β2/Pup1 was detected in these fractions of wild-type extracts. These findings suggest that the free processed subunits observed in the pre4-ΔC19 are derived from matured 20 S proteasomes that fell apart, either in vivo or in vitro.

Similar experiments were performed with wild-type and pre4-ΔC19 strains expressing β1/Pre3 tagged at the C terminus with two ha epitopes. Proteins extracted from these strains were fractionated by gel filtration on Superose 6. Again, we observed a strikingly increased amount of β1/Pre3 precursor form in fractions 22 and 23 in the pre4-ΔC19 mutant in comparison with the wild type (Fig. 3). In contrast to the β2/Pup1 precursor, however, the β1/Pre3 precursor form is in addition present in a wide range of fractions ranging from those containing the free subunit to those with 20 S and 26 S proteasomes. Particularly interesting was the simultaneous presence of β1/Pre3 mature and precursor forms in the fractions containing 20 S and 26 S proteasome suggesting a defect in β1/Pre3 maturation.

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The \( \beta7/\)Pre4 C-terminal Tail Is Essential for \( \beta7/\)Pre3-mediated Post-acidic Peptidase Activity—Previous work by others (23) had shown that a mutation in the \( PRE4 \) gene that led to a C-terminal shortening of \( \beta7/\)Pre4 by 15 residues resulted in a loss of the post-acidic peptidase activity, which is mediated by the \( \beta7/\)Pre3 subunit. Consistent with that report, we observed a significant accumulation of \( \beta7/\)Pre3 precursor forms in assembled proteasomes in our mutant \( \text{pre4}^{-} \Delta C19 \) (Fig. 3) together with a complete loss of post-acidic activity (Fig. 4A). We therefore asked whether the defect in post-acidic activity in this mutant was due to a defect in \( \beta7/\)Pre3 processing. To answer this question, we generated a congenic set of strains carrying on one side either the \( \Delta C19 \) allele or its wild-type counterpart, and on the other side a gene expressing a mature form of \( \beta7/\)Pre3 (\( \text{Pre3}^{-}\Delta pro \)) or wild-type \( \text{Pre3} \). The \( \beta7/\)Pre3 propeptide is dispensable for proteasome assembly in vitro (20, 24), but its deletion inactivates the post-acidic activity since its N terminus is subject to N-acetylation (20). Inactivation of the \( \text{NAT1} \) gene encoding a subunit of \( \text{Nat1-Ard1} \) No-acetyltransferase, however, restores near wild-type levels of post-acidic activity to the \( \text{pre3}^{-}\Delta pro \) strain (20). Therefore all strains used in the experiment shown in Fig. 4 in addition lacked the \( \text{NAT1} \) gene. As shown in Fig. 4D, none of the mutations affected the stability of \( \beta7/\)Pre4 as its ratio to subunit \( a7/\text{Pre10} \) was not affected. Indistinguishably from the \( \text{pre4}^{-} \Delta C19 \) single mutant, the \( \text{pre4}^{-} \Delta C19 \text{pre3}^{-}\Delta pro \) double mutant did not display any post-acidic peptidase activity (Fig. 4A). 

In extracts of the \( \text{pre3}^{-}\Delta pro \) single mutant, in contrast, wild-type activity levels were detected (Fig. 4A). We conclude that the C-terminal extension of \( \beta7/\)Pre4 is essential for post-acidic activity of the proteasome independent of an apparent role in mediating efficient autocatalytic processing of \( \beta7/\)Pre4-

In contrast to its effect on post-acidic activity, the \( \text{pre4}^{-} \Delta C19 \) mutation resulted only in a slight reduction of proteasomal chymotryptic and tryptic activity (Fig. 4, B and C). These data suggest that the C-terminal extension of \( \beta7/\)Pre4, aside of its role in the dimerization of half-proteasome precursors, has a specific role in the formation of the post-acidic peptidase site. Consistent with previous studies (8, 9, 24), the post-acidic activity does not appear to be required for the activation of the chymotryptic and tryptic peptidase sites.

Dissection of the Role of the \( \beta7/\)Pre4 C-terminal Extension in Post-acidic Activity—To further understand the involvement of the C-terminal extension of \( \beta7/\)Pre4 in the activity of the post-acidic peptidase site, we prepared a set of mutants expressing \( \beta7/\)Pre4 variants with truncated C termini (Fig. 5). We asked in particular whether truncations that included the residues Ile\(^{225}\) and Gly\(^{219}\) affected post-acidic activity of the proteasome. These two residues are involved in a network of hydrogen bonds formed with residues flanking the Lys\(^{219}\) in the catalytic center of \( \beta7/\)Pre3 (see above; Fig. 1B). A deletion of only the last amino acid residue Ile\(^{225}\) (\( \text{pre4}^{-} \Delta C19 \)) resulted in a drop of post-acidic activity in crude extracts by 20% in comparison with the wild type consistent with a significant role of this residue in the post-acidic activity (Fig. 5A). A further deletion into the C terminus that included residue Gly\(^{219}\) resulted in a loss of about 90% of the post-acidic activity. None of the deletions affected the stability of the \( \beta7/\)Pre4 subunit as its ratio to \( a7/\text{Pre10} \) was unchanged (Fig. 5B). Taken together these data are consistent with a model (depicted in Fig. 1B) in which the C-terminal extension of \( \beta7/\)Pre4 engages in hydrogen bonds that are essential to stabilize an active conformation of the catalytic site in \( \beta7/\)Pre3. This function of the \( \beta7/\)Pre4 C-terminal extension can be separated from its role in half-proteasome precursor dimerization (see below).

Activation of the Post-acidic Site and Its Role in Proteasome Assembly Are Not Correlating Functions of the \( \beta7/\)Pre4 Tail—Because the deletion of only seven residues of the \( \beta7/\)Pre4 tail resulted in a drop of the post-acidic activity to less than 10%, we asked whether the formation or stability of the assembled 20 S proteasome was also affected by this shorter tail truncation. To answer this question, we analyzed the distribution of an epitope-tagged version of \( \text{Pre2} \)-Pup1 (Pup1-ha) in crude extracts of \( \text{pre4}^{-} \Delta C7 \) mutant cells fractionated by gel filtration. In this mutant, the amount of precursor complexes containing unprocessed proPup1-ha was far less strikingly increased than in the \( \text{pre4}^{-} \Delta C19 \) mutant (Fig. 2). Only a slight increase of such precursor complexes was detected in \( \text{pre4}^{-} \Delta C7 \) in comparison to wild type. We conclude that a truncation of the \( \beta7/\)Pre4 C-terminal extension that nearly abolishes post-acidic activity only moderately affects proteasome assembly supporting a model assigning two distinct functions to this domain.

Truncation of \( \beta7/\)Pre4 C Terminus and Deletion of UMP1 Result in a Synthetic Growth Defect—Despite the observed defects in proteasome assembly (see above), the \( \text{pre4}^{-} \Delta C19 \) mutant cells showed no significant growth defects at 30 or 37 °C when compared with congenic wild-type cells (Fig. 1D and data not shown). This observation is in accordance with the finding that the overall amount of active proteasomes in the cell, as judged by the chymotryptic and tryptic activities in this mutant, was very similar to that of wild type (see above). These data suggested that the reduced efficiency of assembly of 20 S
proteasomes from its precursors is compensated by a moderate increase in the expression of proteasome subunit genes. We have shown previously that maturation factor Ump1 is required for efficient assembly of 20 S proteasomes from two half-proteasome precursors (19), similar to what is described above for the C-terminal extension of β7/Pre4. To test whether the ump1-Δ mutation and the pre4-ΔC19 mutation have a synthetic effect, we crossed strains with these two mutations and analyzed the progeny by tetrad dissections. Consistent with the observation that both mutations affect the dimerization of half-proteasome precursor complexes, the double mutant showed a strong synthetic growth defect (Fig. 1C). To test whether the observed synthetic growth inhibition by the pre4-ΔC19 and ump1-Δ mutations is due to an imbalance of proteasome subunits, e.g., due to a more rapid turnover of the mutant Pre4-ΔC19 subunit, we compared the levels of subunits β7/Pre4 and α7/Pre10 by immunoblotting. The results shown in Fig. 1E demonstrate that the overall levels of these subunits are similar in the single mutants and in the double mutants supporting the notion that the synthetic phenotype is due to a strong inhibition of proteasome assembly. Similar to what we observed previously for the processing of the active subunits β1, β2, and β5 (19), the ump1-Δ mutation also inhibited the processing of β7/Pre4 (Fig. 1E, lanes c and d).

The Deletion of the β2/Pup1 C-terminal Extension Is Lethal—Another prominent feature that distinguishes eukaryotic β-subunits from those in the Thermophillus “Upr” proteasome is a 30-residue C-terminal extension of the β2 subunit (Fig. 6C). Analysis of the crystal structure of the yeast proteasome (Fig. 6A) revealed that the extension of β2/Pup1 wraps around the β3/Pup3 subunit within the same ring of β-subunits. To study the relevance of this extension to the biogenesis of functional proteasomes, we replaced one copy of the PUP1 allele encodes a Pup1 protein that is truncated at the C terminus by 30 residues. To test whether the C-terminal extension of β2/Pup1 serves an essential function in proteasome biogenesis, we generated a diploid strain that expressed an epitope-tagged version of the truncated Pup1 (Pup1-ΔC30-ha). Extracts from the resulting strain JD325 (see Table I) were analyzed by gel filtration and immunoblotting and compared with an otherwise identical wild-type strain expressing Pup1-ha. As shown in Fig. 6D, unprocessed Pup1-ΔC30-ha was detectable in increased amounts in fractions 22 and 23 that typically contain the Ump1-containing half-proteasome precursor complex (19) and moreover was also present in the fractions 19–21 comprising larger assemblies similar in size to the 20 S particles. When compared with a wild-type strain, however, only minute quantities of processed Pup1-ΔC30-ha could be detected in fractions 15–19 that typically contain 26 S proteasome (CPR3) and the 20 S particle (CP) with one regulatory particle (CP-R) (Fig. 6D). Similarly, we observed a striking reduction of Pup1-ΔC30-ha-containing CPR or CPR3 complexes when we analyzed crude extracts from the mutant strain by native PAGE (Fig. 6E). Note that the experiments with the Pup1-ΔC30-ha variant were performed with a diploid strain carrying also a wild-type PUP1 gene copy. The small amounts of larger assemblies containing processed forms of Pup1-ΔC30-ha therefore may depend on the presence of a functional Pup1 subunit in the other half of the complex. We conclude that Pup1-ΔC30 assemblies into proteasomal complexes but that formation of 26 S proteasomes containing this truncated subunit is impaired.

DISCUSSION

Several proteasomal β-subunits of eukaryotic proteasomes are distinguished from their counterparts in bacteria by striking C-terminal extensions. In this report we show that the C-terminal extension of the yeast β7/Pre4 subunit serves two distinct functions in proteasome biogenesis and activity. First, it functions as a molecular clamp mediating and stabilizing the interaction of two half-proteasome precursor complexes. The second function that can be assigned to the seven C-terminal residues of Pre4 is to keep the post-acidic site in an active conformation. Another finding of this study is that the C-terminal extension of β2/Pup1 serves an essential function in proteasome biogenesis.

The C-terminal Tail of β7/Pre4 Acts as a Molecular Clamp Stabilizing the 20 S Proteasome—The C-terminal tail of β7/Pre4, which extends from one half of the proteasome to the other (Fig. 1A), facilitates the formation of 20 S particles from two half-proteasome precursor complexes. In the pre4-ΔC19 mutant lacking this extension, we detected an accumulation of half-proteasome precursor complexes (Figs. 2 and 3). The overall proteasomal chymotryptic or tryptic activities detected in this mutant, however, were only slightly reduced (less than 5 and 9%, respectively) when compared with wild type (Fig. 4B and C). Similar to effects observed previously in mutants with
FIG. 6. The C-terminal domain of β2/Pup1 is essential for the formation of functional proteasomes. (A) structure of S. cerevisiae 20 S proteasome (6) emphasizing the interaction of β2/Pup1 and β3/Pup3. This figure was prepared with RasMol 2.6. β2/Pup1 and β3/Pup3 are shown in the spacefill representation; all other subunits are displayed as wireframe. (B) deletion of 30 amino acid residues from the C terminus (C30) of β2/Pup1 is lethal. Shown is a tetrad analysis of strain JD329 (Table I) heterozygous for pup1-β2C30. C, amino acid sequence alignments of the C-terminal regions of β2 subunits of the indicated species. Residues identical between β2/Pup1 from S. cerevisiae and other species are boxed. The C-terminal 30 residues that form the extension shown in A are highlighted in red. D, crude extracts of strain JD139 expressing Pup1-ha and of a heterozygous diploid strain containing one gene copy expressing the truncated β2/Pup1-ΔC30-ha (JD325; Table I) were fractionated by gel filtration. Top, to confirm reproducibility of the fractionations, the chymotryptic activities in the fractions obtained from both strains are compared. Thyroglobulin (670 kDa) and ferritin (440 kDa) were run on the same column. The positions of the peaks of these molecular weight standards are indicated on the top. Middle and bottom, the same fractions were analyzed by SDS-PAGE and immunoblotting. E, crude extracts from the same strains as in D were analyzed by native PAGE and immunoblotting.
proteasome defects (19), the assembly defect in the pre4-ΔC19 mutant therefore seems to be partly compensated by a slightly increased expression of proteasome subunits (Fig. 4D). As a result, the pre4-ΔC19 mutant strain does not display any obvious growth defects or phenotypes. When we combined the pre4-ΔC19 with the ump1-Δ mutation, however, we observed a striking synthetic inhibition of growth (Fig. 1D). Similar to the data presented here for pre4-ΔC19, the ump1-Δ mutation was shown previously to impair the assembly of 20 S proteasomes from two half-proteasome precursor complexes (19).

Why do eukaryotic proteasomes require a C-terminal extension of β7 to mediate efficient assembly of proteasomes while T. acidophilum β-subunits lack such a feature? In this archaea, the interaction between half-proteasome precursor complexes is apparently stabilized by other features. One such feature of T. acidophilum β-subunits at the interface of the two β-rings could be a small extension in form of the side chain of Arg 165. Because we also observed a defect in the processing of proβ1/Pre3 in Fig. 4), and β7/Pre4 lacking its C-terminal extension. We conclude that the β7/Pre4 C-terminal extension is an essential component of the post-acidic site by providing a network of hydrogen bonds that stabilizes an active conformation of β1/Pre3 as illustrated in Fig. 1B. Although one of the shorter truncations removing only seven residues (pre4-ΔC7) resulted in a severe reduction in post-acidic activity (by ~90%; Fig. 5), it had relatively little effect on the dimerization of two half-proteasome precursor complexes (Fig. 2). We interpret this result as evidence that these two functions of the C-terminal extension of β7/Pre4 are distinct and separable. A comparison of the C-terminal extensions of the orthologous β7 subunits of S. cerevisiae and other species revealed that it is not highly conserved in sequence (Fig. 1C). This observation suggests that this domain in the S. cerevisiae β7/Pre4 is specifically adapted to its function in post-acidic activity in the context of the budding yeast 20 S proteasome.

The C-terminal Extension of β2/Pup1 Is Essential for Proteasome Biogenesis—In comparison with β-subunits of the T. acidophilum proteasome, subunit β2 of eukaryotic proteasomes is characterized by a C-terminal extension of ~30 amino acid residues (Fig. 6C). As shown in Fig. 6A, this extension wraps around the neighboring subunit β3/Pre3 in the same β-ring of the S. cerevisiae proteasome (6). A similar structural arrangement was found for the subunits β2 and β3 in the bovine proteasome (11). We show that a deletion of this extension is lethal to yeast cells (Fig. 6B). Our experiments with a diploid strain that expressed wild-type β2/Pup1 in addition to an epitope-tagged variant of the truncated Pup1 (pup1-ΔC30-ha) showed that the latter is incorporated into proteasomal complexes. Prepeptide processing of the Pup1-ΔC30-ha variant and the formation of 26 S proteasomes containing this subunit, however, were very inefficient (Fig. 6). One possible explanation is that subunit β3/Pre3, which seems to be held in place by the β2/Pup1 C-terminal tail, cannot be incorporated properly in the absence of this tail leading to an inhibition of the assembly and maturation of 26 S proteasomes.

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