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PRE2, Highly Homologous to the Human Major Histocompatibility Complex-linked *RING10* Gene, Codes for a Yeast Proteasome Subunit Necessary for Chymotryptic Activity and Degradation of Ubiquitinated Proteins*

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We have cloned the yeast *PRE2* gene by complementation of *pre2* mutants, which are defective in the chymotrypsin-like activity of the 20 S proteasome (multicatalytic-multifunctional proteinase complex). The *PRE2* gene, a β-type member of the proteasomal gene family, is essential for life and codes for a 287-amino acid proteasomal subunit with a predicted molecular mass of 31.6 kDa. Missense mutations in two *pre2* mutant alleles were identified. They led to enhanced sensitivity of yeast cells against stress. At the same time, *pre2* mutants accumulated ubiquitinated proteins. The Pre2 protein shows striking homology to the human Ring10 protein (60% identity excluding the 70 amino-terminal residues), which is encoded in the major histocompatibility complex class II region. It represents a component of the low molecular mass polypeptide complex, previously shown to be a special type of the 20 S proteasome. The low molecular mass polypeptide complex is assumed to be involved in antigen presentation, generating peptides from cytosolic protein antigens, which are subsequently presented to cytotoxic T-lymphocytes on the cell surface. The high homology of Pre2 to Ring10 implies the hypothesis that Ring10 is a subunit of the low molecular mass polypeptide complex central in its chymotryptic activity. One might further suggest that replacement of constitutive proteasomal components by functionally related major histocompatibility complex-linked low molecular mass polypeptides, as is Ring10, adapts mammalian proteasomes for functions in the immune response.

Proteasomes are ubiquitous non-lysosomal proteinase complexes of high molecular mass (about 650 kDa) with a characteristic, hollow cylindrical structure. These 20 S particles, highly conserved from yeast to man (1, 2), are composed of a variable set of nonidentical but structurally related subunits of sizes between 21 and 38 kDa and exhibit multiple proteolytic activities (reviewed in Refs. 3–5). *In vitro* analysis of the mammalian 20 S proteasome shows its ability to assemble with additional proteins to a 26 S proteinase complex in an ATP-dependent fashion (6–8), which is able to degrade ubiquitinated protein substrates (reviewed in Refs. 5 and 9).

Recent results from mammals indicate a participation of proteasomes in the pathway of antigen presentation by major histocompatibility complex (MHC)[1] class I molecules (reviewed in 9–11). Here, proteasomes are thought to generate peptide fragments from foreign proteins in the cytosol, which are delivered, after association with MHC class I glycoproteins and β-microglobulin in the endoplasmic reticulum, to the cell surface and scanned by cytotoxic T-lymphocytes. In both the human and the murine MHC class II regions, two γ-interferon-inducible genes were identified that encode components of the low molecular mass polypeptide particle, a multiprotein complex shown to be immunologically closely related to the proteasome (12, 13). The low molecular mass polypeptides encoded by the human MHC genes *RING10* (14) and *RING12* (15) and the murine *RING12* equivalent *LMP-2* (16) indeed revealed significant homology to known proteasome components.

Experiments in yeast are gradually uncovering general functions of the enzyme complex in cellular life (17, 18). Here, the proteasome is found to function in degradation of short-lived, stress-induced, and abnormal proteins. Among the proteins degraded are those of the ubiquitin-mediated proteolytic pathway (17, 18).

These studies were initiated through analysis of mutants defective in proteolytic functions of proteinase yscE, the yeast proteasome. Three isolated mutants define two complementation groups, *PRE1* and *PRE2*, and show a defect exclusively in the chymotrypsin-like activity of the enzyme particle (17, 19). The trypsin-like and the peptidylglutamyl peptide hydrolyzing activities remain unaffected. *PRE1* was cloned and sequenced and was shown to encode an integral proteasomal subunit essential for cell viability (17, 19).

Here, we report on the essential yeast proteasomal gene *PRE2*. The *PRE2* gene product shows striking structural similarities to the Ring10 protein, which imply related functions of both subunits within yeast and human proteasomes, *i.e.* a participation in their chymotryptic activity. As in *pre1* mutants, the defect in the chymotrypsin-like activity of *pre2* mutant proteasomes leads to hypersensitivity of cells to the amino acid analogue canavanine and to stress-induced accumulation of ubiquitinated proteins.

EXPERIMENTAL PROCEDURES

Isolation of Mutants—Mutants of proteasomal chymotrypsin-like activity were isolated on Petri dishes after ethyl methanesulfonate mutagenesis of strain cl3-ABYS-86 (MATα *pra1-1 prb1-1 prc1-1*

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[1] The abbreviations used are: MHC, major histocompatibility complex; Cbz, benzyloxycarbonyl; kbp, kilobase pair(s).

cps1-3 ura3Δ5 leu2-3,112 his⁻) using Cbz-Gly-Gly-Leu-4-nitroanilide as substrate (17). The 4-nitroaniline released by enzymatic hydrolysis was detected by a coupling reaction with *N*-(1-naphthyl)ethylenediamine yielding a pink color. Non-staining or weakly staining colonies were picked and genetically analyzed as described in Ref. 17.

Preparation of Extracts, Enzyme Tests, Immunological Detection, and Quantification of Ubiquitin-Protein Conjugates—For testing proteasomal proteolytic activities, cells were grown in liquid YPD medium (1% yeast extract, 2% peptone, 2% glucose (21)) into stationary phase. Cell extracts were prepared by vortex mixing of 30% (v/v) cell suspensions in 50 mM Tris/HCl, pH 8.2, with equal volumes of washed glass beads for 2 min. The chymotrypsin-like activity and the peptidylglutamyl peptide-splitting activity of the proteasome were tested in the supernatants as described in Ref. 17 using Cbz-Gly-Gly-Leu-4-nitroanilide and Cbz-Leu-Leu-Glu-β-naphthylamide, respectively, as substrates.

For immunological detection of ubiquitin-protein conjugates, strains were grown in liquid YPD medium to early stationary phase at 30 °C and subsequently incubated at 37 °C for 3 h. After harvesting and washing, cells were resuspended in distilled water to yield a 50% (v/v) suspension, and washed glass beads, equivalent to the respective cell volume, were added. Cells were heated for 10 min at 95 °C in Eppendorf tubes and subsequently vortex-mixed 6 times for 30 s with intermittent heating for 30 s. SDS-EDTA solution (4.5% SDS, 2.25 mM EDTA), equivalent to the cell volume, was added, and the samples were heated for an additional 10 min at 95 °C. Samples were centrifuged and the supernatants were used for immunoblotting. For SDS-polyacrylamide gel electrophoresis (10% gels), 50 μg of protein were applied onto each lane. Immunoblotting using ubiquitin-protein conjugate antibody was done as described in Ref. 17. Quantification of the dye produced by the peroxidase linked to the rabbit-immunoglobulin antibody was done using the JAVA 1.3 video analysis system (Jandel, Corte Madera, CA).

Gene Cloning and Analysis—Standard molecular biological (20) and yeast genetic/microbiological techniques (20–22) were used. The *PRE2* gene was cloned by complementation of the defective chymotrypsin-like activity of a *pre2-2* mutant strain using a yeast genomic library in the *CEN4-ARS-URA3* shuttle vector YCp50 (23). One recombinant YCp50 plasmid restored the proteolytic activities of both the *pre2-1* and the *pre2-2* mutant. The complementing portion of its 12-kbp genomic insert was limited to 1.05 kbp after cloning of subfragments into the *CEN14-ARS-URA3* shuttle vector pDP83.[2] For sequence analysis of the *PRE2* locus (see Fig. 1*A*) by the dideoxy chain termination method, pDP83-based plasmids with overlapping inserts from the *PRE2* region served as template.

Construction of plasmids carrying a deletion of the *PRE2* coding region started from a pDP83-based plasmid harboring a 0.9-kbp fragment from the 5′-flanking region of *PRE2*, which had been generated by exonuclease digestions (*left bar* in Fig. 1*A*). A unique polylinker *Bam*HI site was used to introduce a 1.3-kbp *Bgl*II/*Bam*HI fragment from the 3′-flanking region of *PRE2* (*right bar* in Fig. 1*A*). In the resulting plasmid pDP83ΔE2 the 5′- and 3′-non-coding regions are connected, resulting in a 1.0-kbp deletion (see Fig. 1*B*) of the entire *PRE2* coding region. This construct served two purposes. 1) Cloning of the *pre2* mutant alleles by "gap repair" (22) was done as follows. Plasmid pDP83ΔE2 was linearized with *Bam*HI at the 5′-3′-junction and introduced into *pre2-1* and *pre2-2* mutant strains. Plasmids from Ura⁺ transformants were checked for the correct gap repair event by restriction analysis. The complete nucleotide sequences of the cloned *pre2-1* and *pre2-2* alleles were determined and a one-base exchange was detected in each allele (see Fig. 1*B*). Chromosomal introduction by the two-step gene replacement method (22) of each mutant allele into a wild-type strain actually led to loss of wild-type chymotrypsin-like activity. 2) Chromosomal deletion of the *PRE2* gene by one-step gene disruption (22) was done as follows. Plasmid pDP83ΔE2 was cut with *Bam*HI, and a 1.7-kbp *Bam*HI fragment carrying the *HIS3* gene was inserted. The diploid strain YS18/18 (*MATα/MATa his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 ura3Δ5/ura3Δ5 canR/canR*) was transformed with the *pre2Δ::HIS3* deletion allele excised from the vector. His⁺ transformants were selected, and replacement of one chromosomal *PRE2* copy by the deletion allele was verified by Southern blot analysis. Several of these *PRE2/pre2Δ::HIS3* heterozygous diploids were sporulated, and asci were subjected to tetrad dissection. Of each tetrad, viability of only two His⁺ spores was observed.

Construction of Isogenic pre Mutant Strains—Isogenic single-mutant strains were generated by introduction of the cloned *pre1* or *pre2* alleles into wild-type strains WCG4a or WCG4α (*MATa* or *MATα his3-11,15 leu2-3,112 ura3*) by the two-step gene replacement method (22). The resulting mutant strains were used for generation of isogenic double mutants by mating of a *pre1-1* strain with a *pre2-1* or *pre2-2* strain, respectively. The resulting heterozygous diploids were sporulated, and co-segregation of the two uncoupled mutations among the haploid progeny was identified within tetrads comprising two wild-type and two mutant spores with respect to chymotryptic activity. Further proof for mutant spore clones indeed carrying the *pre1-1* allele together with the respective *pre2* mutation was obtained by backcrossing them with wild type and following the independent segregation of the *pre1-1* and *pre2* alleles in the spore progeny of the resulting diploid.

RESULTS AND DISCUSSION

The *PRE2* gene was cloned from a yeast genomic library (23) by complementation of the defective chymotrypsin-like activity in the two *pre2* mutants isolated. Sequence analysis of the complementing DNA region revealed an open reading frame that encodes a 287-amino acid protein with a predicted relative molecular mass of 31.6 kDa (Fig. 1).

Data bank search found the nucleotide sequences situated upstream of the *PRE2* gene to match almost perfectly those of the *RPL16B* gene from *Saccharomyces carlsbergensis* (24). *RPL16A* and *RPL16B* are duplicated genes encoding ribosomal proteins and are found in *S. cerevisiae* as well (25). The distance between the translation initiation codons of the divergently transcribed *PRE2* and *RPL16B* genes amounts to 600 nucleotides (Fig. 1). Whether these two genes share common regulatory sites is unknown.

Standard genetic procedures (22) revealed the two *pre2* mutant genes to be alleles of the isolated *PRE2* gene. The cloned *PRE2* DNA was able to direct integration of a selectable marker gene to the wild-type locus corresponding to the *pre2* mutant alleles (not shown). Furthermore, the *pre2-1* and *pre2-2* mutant alleles were cloned (see "Experimental Procedures") and shown to confer a defective proteasomal chymotryptic activity to wild-type cells. Both mutant genes contain a missense mutation each (Fig. 1*B*).

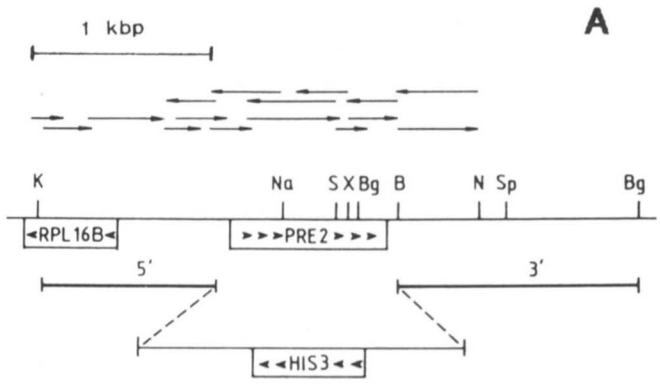
PRE2 is essential for cell proliferation. Sporulation of heterozygous diploids carrying a null allele of *PRE2* (Fig. 1*A*) produces asci of which only the two spores harboring a wild-type *PRE2* gene grow up to visible colonies. Although the *pre2* null mutant spores are capable of germinating, they arrest after two to three cycles of cell division. An identical cell division arrest is seen with spores deleted in the *PRE1* gene (not shown). Seven of eight other hitherto cloned yeast proteasomal genes (26–30)[3] are essential for life as well. Our results with *PRE2* confirm the importance of a complete proteasome particle for cell survival.

Biochemical analysis of *pre* mutants with respect to their defect in the chymotrypsin-like activity revealed a reduction in *pre2-2* mutants of 95%, which is similar to the value found in *pre1-1* mutants (Table I; Ref. 17). *Pre2-1* mutants exhibit a 3-fold higher residual activity compared with *pre2-2* and *pre1-1* mutants (Table I). Introduction of the *pre2-1* mutation into a *pre1-1* mutant strain leads to a slight additional decrease in the chymotrypsin-like activity compared with *pre1-1* single mutants. Combination of the *pre2-2* mutation with the *pre1-1* mutation in a double mutant finally yields a residual activity of only 4% (Table I).

As shown previously (17), the peptidylglutamyl peptide-splitting activity remains in the *pre1* and *pre2* mutants. Interestingly, this activity is even increased in these mutants, most strongly in those carrying the *pre1-1* allele (Table I).

[2] D. Pridmore, unpublished data.

[3] Hilt, W., Enenkel, C., Gruhler, A., Singer, T., and Wolf, D. H. (1993) *J. Biol. Chem.* **268**, 3161–3167.



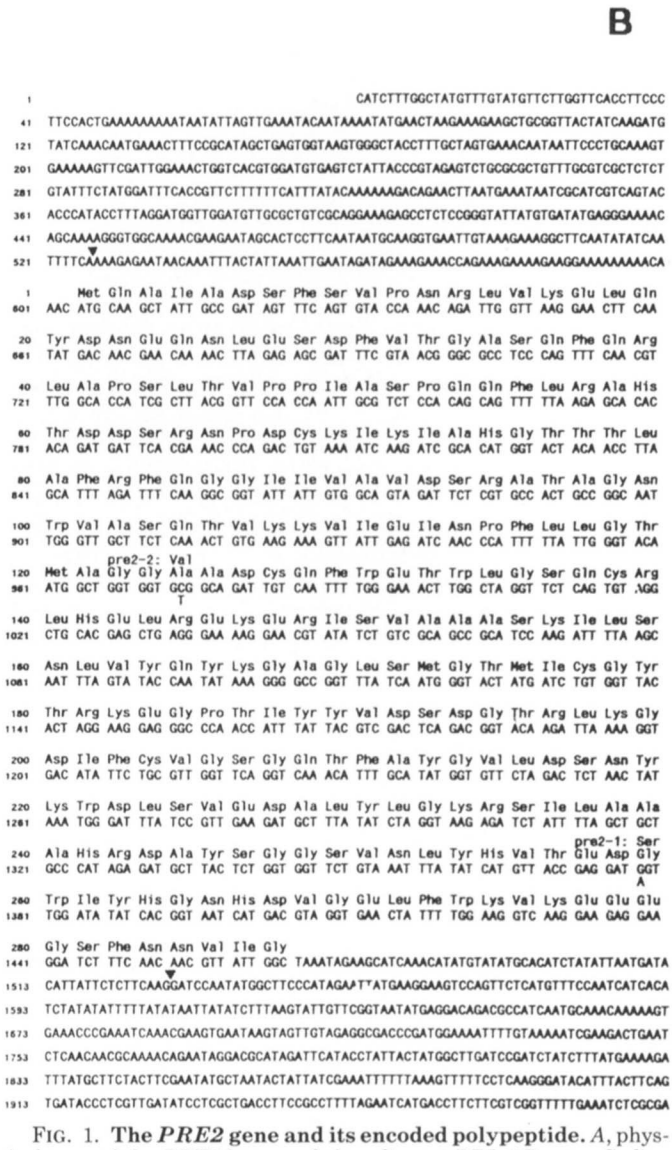


FIG. 1. **The *PRE2* gene and its encoded polypeptide.** *A*, physical map of the *PRE2* locus and the adjacent *RPL16B* gene. Coding regions are shown as *boxes* with *arrowheads* indicating the direction of transcription. *Arrows above* the restriction map represent DNA stretches sequenced on both strands. DNA fragments included in plasmids used for cloning of the two *pre2* mutant alleles and for chromosomal introduction of a *pre2Δ::HIS3* deletion allele (see "Experimental Procedures") are shown *beneath* the *map. Bars* represent fragments from the 5′- and 3′-non-coding regions, separated by a gap marking the extent of the *PRE2* gene deletion (see also *B*). Replacement of the *PRE2* gene by the *HIS3* marker gene is indicated. Restriction enzyme cutting sites: *X, Xba*I, *K, Kpn*I, *Na, Nae*I, *S, Sal*I, *Bg, Bgl*II, *B, Bam*HI, *N, Nru*I, and *Sp, Spe*I. *B*, nucleotide sequence of the *PRE2* gene and predicted amino acid sequence of the encoded protein. The first three nucleotides (CAT) of the DNA sequence shown correspond to the translation initiation codon of the *RPL16B* gene, which is transcribed in the opposite direction relative to the *PRE2* gene (see *A*). Altered nucleotides in the *pre2-1* and *pre2-2* mutant alleles and resulting amino acid exchanges are indicated. *Triangles* mark the end points of the *PRE2* gene deletion.

TABLE I

Proteasomal chymotrypsin-like and peptidylglutamyl peptide hydrolyzing activities in crude extracts of pre mutant cells

Strains investigated are isogenic except for the respective *pre* mutation(s) as indicated. For strain construction, preparation of cell extracts, and enzyme tests see "Experimental Procedures." Values presented are means of at least three independent determinations.

Mutation	Activity against	
	Cbz-Gly-Gly-Leu-4-nitroanilide	Cbz-Leu-Leu-Glu-β-naphthylamide
	%	
Wild type	100	100
pre1-1	5.5 ± 1.5	153 ± 17
pre2-1	15.2 ± 2.3	117 ± 14
pre2-2	5.0 ± 1.1	119 ± 14
pre2-1/pre1-1	4.9 ± 0.9	149 ± 36
pre2-2/pre1-1	4.0 ± 0.6	145 ± 23

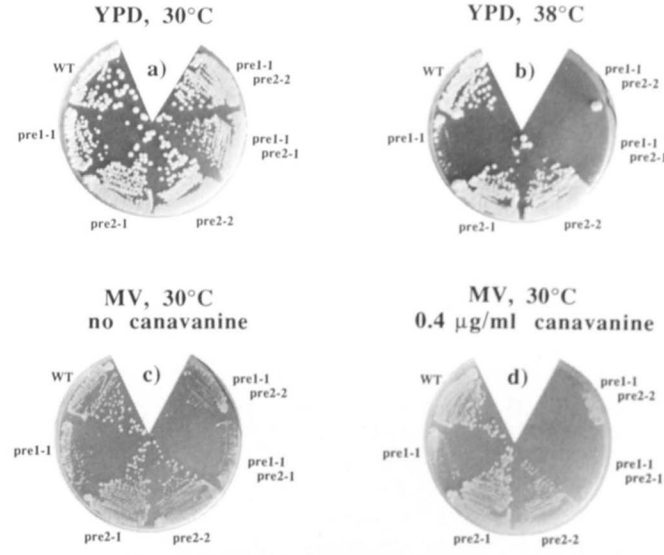


FIG. 2. **Effects of elevated temperature and canavanine application on growth of *pre* mutant cells.** Cells from isogenic strains differing in the *pre* mutations as indicated (see "Experimental Procedures"; *WT*, wild type) were streaked for single colonies onto YPD-agar plates (*a, b*) or onto MV-agar plates (0.68% yeast nitrogen base without amino acids, 2% glucose, 2% agar, and supplements as required (21)) containing no (*c*) or 0.4 μg/ml (*d*) canavanine sulfate. Plates were incubated at 30 °C (*a, c, d*) or 38 °C (*b*) for up to 2 days.

Because of the high activity of other proteases against the substrate of the trypsin-like proteasomal activity (Cbz-Ala-Arg-Arg-4-methoxy-β-naphthylamide) in crude extracts, effects of the *pre1* and *pre2* mutations on this activity could not be determined.

Cells defective in chymotrypsin-like activity caused by the *pre1-1* mutation have been reported to exhibit increased sensitivity to stresses, as are elevated temperature (38 °C) and application of the amino acid analogue canavanine (17). Even growth at 30 °C on YPD and, more pronounced, on MV medium is affected by the *pre1-1* mutation (Fig. 2, *a* and *c*).

The stress conditions mentioned were shown to lead to accumulation of ubiquitinated proteins in *pre1-1* mutant cells (17). In part, similar phenotypes are induced by mutations residing in the *PRE2* gene. With its high residual chymotrypsin-like activity, a *pre2-1* mutant strain does not exhibit heat and canavanine sensitivity (Fig. 2, *b* and *d*). However, when the *pre2-1* mutation is combined with the *pre1-1* mutation, the resulting double mutants show phenotypes that are by far more dramatic than those observed for the *pre1-1* single mutant (Fig. 2, *a–d*). In contrast to *pre2-1* single mutants, *pre2-2* mutants exhibit some canavanine sensitivity (Fig. 2*d*), but as *pre2-1* mutants, they are not heat-sensitive (Fig. 2*b*). Combination of *pre2-2* with *pre1-1* yields double mutants, which also show strengthening of the *pre1-1*-induced phenotypes (Fig. 2, *a–d*). Remarkably, *pre2-1*, which causes a less pronounced phenotype compared with *pre2-2*, induces a somewhat tighter phenotype when combined with *pre1-1* as does *pre2-2*.

Analysis of the accumulation of ubiquitinated proteins in the mutant strains parallels their stress-dependent growth behavior. While no accumulation is seen in *pre2-1* mutant cells upon stress application (not shown), *pre2-2*, *pre1-1*, and *pre1-1/pre2-2* mutants exhibit a 3-, 9-, and 18-fold increase, respectively, in the amount of high molecular weight ubiquitin-protein conjugates compared with wild type (Fig. 3). We consider this accumulation of ubiquitinated proteins in the *pre* mutant cells to result from reduced degradation. This view is supported by the finding that hydrolysis of short-lived N-end rule substrates, which are known to be destined for degradation by attachment of ubiquitin chains, is blocked in these mutants (18).

An additional phenotype of the *pre* mutants rests in their capacity to better survive a heat shock of 52 °C when preincubated at 37 °C (not shown).

The Pre2 protein is shown to be an integral component of the yeast proteasome by two lines of evidence. 1) The reduction of chymotrypsin-like activity is a feature inherent to purified *pre2* mutant proteasomes (17). 2) The Pre2 protein shows structural similarity to proteins from yeast (including Pre1, see Fig. 4) and other organisms, which have been unambiguously identified as components of the proteasome particle. All identified proteasomal genes encode subunits with characteristic similarities and can be classified as a proteasomal gene family of ancient origin (5, 31). *PRE2* clearly represents a member of this family.

An extraordinarily high degree of homology is found between the Pre2 protein and the recently analyzed *RING10* gene product, which is encoded in the human MHC class II region. Not considering the poorly conserved amino termini, a striking 60% identity and 80% similarity can be established in the entire remaining three-quarters of the *PRE2*- and *RING10*-encoded proteins (Fig. 4). Because identity between proteasomal proteins of a given species generally does not exceed values of 40%, stronger homologies between two proteasome subunits of different origin indicate corresponding functions of the respective subunits within the heterologous protease complexes. Thus, the highly conserved structures of the Pre2 and Ring10 proteins from evolutionarily distant organisms, *i.e.* yeast and man, predict some functional equivalence of these two subunits.

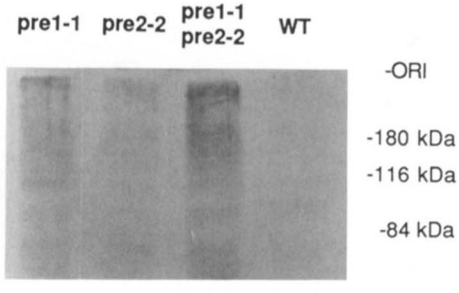


FIG. 3. **Accumulation of high molecular weight ubiquitin-protein conjugates in heat-stressed *pre* mutant cells.** Proteins in cell extracts of heat-treated wild-type (*WT*) and *pre* mutant strains (homozygous diploids with the relevant genotypes as indicated) were separated by SDS-polyacrylamide gel electrophoresis on 10% gels, blotted onto nitrocellulose filters, and reacted with antibody recognizing ubiquitin-protein conjugates. Origin (*ORI*) of the gel and molecular weights of marker proteins are indicated. Quantification of the amount of immunoreactive ubiquitin-protein conjugates was done within the regions containing proteins with molecular masses higher than 110 kDa.

The alignment of the Pre2 and Ring10 protein sequences in Fig. 4 includes other yeast (Pre1 and Pup1 (17, 28)) and human (Ring12 and δ (15, 32)) proteasome subunits as well as the β-subunit of the proteasome from the archaebacterium *Thermoplasma acidophilum* (31). These seven proteins can be grouped into the β-subfamily of proteasome components, of which the archaebacterial β-subunit is regarded as the ancestor (31). The only two types of subunits, α and β, constituting the *Thermoplasma* proteasome, solely confer chymotrypsin-like activity to the complex. This activity is thought to reside in the β-subunit (31). Remarkably, the amino acid exchanges in the *pre2-1* (Gly-259 to Ser-259) and the *pre2-2* (Ala-124 to Val-124) gene products both reside at positions highly conserved among the archaebacterial and eukaryotic proteasomal proteins compared in Fig. 4.

None of the yeast proteasomal subunits involved in chymotrypsin-like activity, Pre1 and Pre2, exhibit any sequence similarity to known proteinases. By assuming some functional homology of Pre2 and Ring10, speculations about a new type of serine proteinase represented by Ring10 (14) cannot be supported by the Pre2 sequence data. No histidine exists in the Pre2 region corresponding to the Ring10 sequence stretch that was found to contain a histidine residue in a similar environment as the one present in the catalytic triad of subtilisin-type proteinases. Two recently cloned genes from the rat and the mouse (33, 34), which encode proteasomal subunits (RC1 and MC13, respectively) highly homologous to Ring10, also lack a histidine at this position. The chymotrypsin-like activity of the proteasome could be brought about by a completely new proteinase type, which might even be formed by interaction of two or more subunits. The fact that mutations in two different subunits of the yeast proteasome result in defective chymotrypsin-like activity may point to a cooperation of distinct proteasomal proteins in active-site formation.

Mutations in yeast proteasomal subunits have uncovered proteasome functions in a variety of cellular events such as growth, differentiation, and stress response (this paper (17)). One molecular basis of action seems to reside in the capacity to degrade ubiquitinated proteins (this paper (17, 18)), which are derived from different degradation pathways (18). In mammalian cells, the proteasome is also expected to exert a variety of different functions, a specialization of which is the hypothetical participation in antigen processing. The striking homology between the human MHC-encoded Ring10 protein and the proteasomal Pre2 subunit, necessary for the chymotrypsin-like activity of the yeast protease complex, supports the involvement of human proteasomes in this latter process. Whether ubiquitin conjugation to proteins is a prerequisite for cleavage and subsequent presentation of peptides as antigens has to be investigated.

In contrast to Pre2, the Ring10 subunit does not seem to

FIG. 4. **Alignment of the Pre2 protein sequence with other β-type proteasomal components from the archaebacterium *T. acidophilum* (*Taß*, Ref. 31), the yeast *Saccharomyces cerevisiae* (*ScPre1* and *ScPup1* (17, 28)), and man (*HsRing10*, *HsRing12*, and *Hsδ* (14, 15, 32)).** Note that the Hsδ sequence is assembled with residues encoded by a cDNA lacking the 5′-end of the coding region and an overlapping amino-terminal sequence derived from amino acid sequencing. Some of the amino-terminal peptides (*NT*) obtained from amino acid sequencing of proteasomal subunits from man (*Hsε* (35)) and rat (*Rn5*, *Rn6*, and *Rn7* (36)) are included (*x*, unidentified residues). Sequences are shown in *single-letter code*; gaps (–) are inserted for optimal alignment. Amino acids are depicted in *upper case letters* if more than half of the residues in a row are conserved (gaps and *x* are regarded as mismatches) and are additionally depicted in *boldface type* if more than half of the residues are identical. Residues identical or conserved at corresponding positions in ScPre2 and HsRing10 are marked with *double* or *single points*, respectively. Similarity groupings are (IVLMAC) (EDQN) (HYWF) (ST) (RK) (PG). *Underlined regions* in HsRing10 represent motifs including the proposed (14) active-site serine, histidine, and asparate (each *doubly underlined*) of a serine protease-type catalytic triad. Amino acid exchanges in the mutant proteins encoded by *pre2-1*, *pre2-2*, and *pre1-1* are indicated. The *pre1-1* allele was cloned analogously to the *pre2* alleles by gap repair. It shows a C to T mutation at nucleotide position 425 of the coding region. (Note that the ScPre1 sequence differs at amino acid positions 183 and 188 from the published data (17), which turned out to be incorrect. The corrected amino acid and nucleotide sequences are available under accession number X56812 in the EMBL, GenBank, and DDBJ data bases.)

be a constitutive proteasomal protein, because the complete absence of this MHC-encoded subunit from human cells does not impair their viability (11). Therefore, it may be assumed that MHC-encoded proteasome subunits substitute for constitutive components of similar structure. These constitutive components may provide the proteasome with similar catalytic specificity but they may change the intracellular biological function of the particle. Incorporation of Ring10 could generate a proteasome that serves the specialized task of antigen processing, whereas an equivalent constitutive component may be contained in proteasomes involved in housekeeping functions, as is the enzyme complex of the unicellular yeast. The strong homology between the human Ring12 and the human δ-component (55% identity, see Fig. 4) favors this model. Subunit δ could represent the constitutive subunit, which is replaced in antigen processing proteasomes by the Ring12 protein. Although until now no constitutive human proteasomal protein with outstanding homology to Ring10 has been identified, the strong similarity of the amino-terminal peptide derived from purified human proteasomal subunit ε (35) with part of the Ring10 sequence (Fig. 4) suggests that other Ring10-related subunits are encoded in the human genome.

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