Nucleotide sequence of *PUP1* encoding a putative proteasome subunit in *Saccharomyces cerevisiae*

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Proteasomes are ring-shaped 20 S particles that exhibit multiple proteolytic activities and are composed of several protein subunits and small RNAs (1–4). They have been found in archaeobacteria and a variety of eukaryotes ranging from man to yeast (1, 5). Possible *in vivo* functions include control of mRNA translation, post-translational modification of newly synthesized proteins and protein degradation (6–9). Three *S.cerevisiae* genes (*YCI*, *YC7-α* and *PRE1*) encoding proteasome subunits have been described (10, 11). All three were essential for growth. The amino acid sequences of these three yeast proteasome subunits were similar to each other and to proteasome subunits of *Drosophila* and rat.

We have fortuitously identified another gene in this family. Upstream of the gene *PET123*, recently characterized in this laboratory (12), we found an open reading frame encoding a protein of M_r 32,916 with marked similarity to amino acid sequences of other proteasome subunits (Table I). Thus, this gene, termed *PUP1* (putative proteasome subunit), likely encodes another component of the yeast proteasome.

PUP1 was disrupted by inserting the *LEU2* gene on a *SalI*–*XhoI* restriction fragment from YEp13 (13) into its *XhoI* site. Sporulation of a diploid strain (PTH258) heterozygous for this disruption yielded tetrads containing only two viable spores, all of which were Leu[–], indicating that *PUP1* is essential for growth. We also inserted the *PUP1* open reading frame behind the galactose inducible promoter of plasmid pFL39 (14). A strain containing this plasmid (pPHY97) and the *pup1::LEU2* chromosomal disruption grew on medium containing galactose, but not on glucose. This system should allow characterization of the terminal phenotype resulting from Pup1 depletion.

Gene order on chromosome XV was determined by a three point cross in which random meiotic spores resulting from recombination between *pet123::URA3* and *pup1::LEU2* were selected and scored for the flanking marker *his3*. The order was *CEN* – *PET123* – *PUP1* – *HIS3*.

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Table I. Similarity between various proteasome subunits

Proteasome subunit	(Yeast)				(Drosophila)			(Rat)	
	Pup1	Pre1	YCI	YC7-α	D-281	D-35	RK	RC3	RC5
Pup1	100								
Pre1	26	100							
YCI	23	22	100						
YC7-α	12	19	31	100					
D-281	25	25	31	28	100				
D-35	22	26	29	28	31	100			
RK	17	21	51	31	30	27	100		
RC3	21	27	34	30	36	32	29	100	
RC5	21	24	20	24	19	21	14	20	100
RC9	21	23	31	28	33	28	30	38	23

Numbers represent percent amino acid identity

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