ELSEVIER

Gene 201 (1997) 99–105

GENE
AN INTERNATIONAL JOURNAL ON GENES AND GENOMES

Molecular cloning of the *Drosophila melanogaster* gene a*5_dm* encoding a 20S proteasome a-type subunit

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Received 12 June 1997; received in revised form 10 July 1997; accepted 14 July 1997; Received by S. Yokoyama

Abstract

Proteasomes are large, multisubunit particles that act as the proteolytic machinery for most regulated intracellular protein breakdown in eukaryotic cells. The core proteinase of this complex, known as the 20S proteasome, is a hollow barrel-shaped structure made up of four stacked rings of seven subunits each, with the outer two rings each being made up of seven distinct a-type subunits, and the two inner rings composed of seven different b-type subunits. Here we present the cloning, sequencing and genetic mapping of a *Drosophila melanogaster* gene, a*5_dm*, encoding one of the proteasome a subunits. This gene, which is homologous to the yeast *PUP2* and the human *Zeta* genes, maps to chromosome 2 at position 54B3-5. The map positions of the previously cloned proteasome genes *Pros25* and *Pros29* were also determined, and found to lie at positions 87B and 57B, respectively. A search for other *D. melanogaster* a*5_dm*-like genes encoding potential isoforms of this subunit failed to identify any closely related genes. © 1997 Elsevier Science B.V.

Keywords: Anchored PCR; Degenerate primers; *PUP2*; *Zeta*

1. Introduction

The proteolytic pathway responsible for degrading most cellular proteins in eukaryotes is the ubiquitin/proteasome pathway (for reviews, see Ciechanover, 1994; Hochstrasser, 1995; Jentsch and Schlenker, 1995). In this system, targetted proteins are tagged by the covalent attachment of chains of ubiquitin, marking them as substrates for a large complex called the 26S proteasome (Hilt and Wolf, 1996; Coux et al., 1996). The core particle of this complex, known as the 20S proteasome, is highly conserved in its structure, and is found in all eukaryotes and at least some archaebacteria and eubacteria. Eukaryotic 20S proteasomes are hollow cylindrical structures made up of four stacked rings: the two inner rings are identical and each comprises seven different b-type subunits, while the two identical outer rings each contain seven distinct a-type subunits (Groll et al., 1997). Some of the b-type subunits are known to contain the catalytically active sites for proteolysis which are situated within the central chamber, while the a-type subunits play a role in the assembly of the proteasome and are involved in interactions with components that cap the 20S proteasome at each end to yield the 26S complex. To clarify the relationship among the 20S proteasome subunits from different organisms, the nomenclature presented in Table 1 has been suggested (Groll et al., 1997), and will be followed here. Under this system, which is based on the solved crystal structure of the yeast proteasome, the different a-type and b-type subunits are numbered sequentially within each ring, with adjacent a and b subunits having the same numbers.

In order to learn more about the biological functions of proteasomes in a higher eukaryotic system, a genetic and molecular study of proteasomes in *Drosophila melanogaster* has been undertaken. One part of that study is to clone and genetically map all of the 20S proteasome subunit genes. To date, genes encoding four a-type and one b-type subunits have been cloned (Haass et al., 1989, 1990a,b; Frentzel et al., 1992; Seelig et al., 1993; Saville and Belote, 1993). In this report, we describe the

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Abbreviations: PCR, polymerase chain reaction; S, sedimentation constant; dNTP, deoxynucleotide; UTR, untranslated region; kDa, kilodalton(s); bp, base pair(s); kb, kilobase pair(s).

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PII S0378-1119(97)00432-0

Table 1
Standard nomenclature proposed by Groll et al. (1997) for the a- and b-type subunits corresponding to the cloned yeast, human and Drosophila proteasome subunit genes

Standard name[a]	GDB symbol[b]	Yeast (sc)	Human (hs)	Drosophila (dm)
a*1*	PSMA6	C7/PRS2	IOTA	
a*2*	PSMA2	Y7	HC3	PROS25
a3	PSMA4	Y13	HC9	PROS29
a*4*	PSMA7	PRE6	XAPC7	PROS28.1[d]
a*5*	PSMA5	PUP2	ZETA	
a*6*	PSMA1	PRE5	HC2	PROS35
a*7*	PSMA3	C1/PRS1	HC8	
b*1*	PSMB6	PRE3	DELTA (Y)[c]	
b*2*	PSMB7	PUP1	Z[c]	
b*3*	PSMB3	PUP3	HC10-II	
b*4*	PSMB2	C13/PRE1	HC7	
b*5*	PSMB5	PRE2	EPSILON (X)[c]	
b*6*	PSMB1	C5/PRS3	HC5	l(3)73Ai
b*7*	PSMB4	PRE4	HN3	

[a]Nomenclature proposed by Groll et al. (1997). The subunit symbol is followed by a dash and two letters indicating the species (e.g., a*1_sc*, a*1_hs* and a*1_dm* for the yeast, human and fly genes, respectively).
[b]Human Genome Database symbol [see Coux et al. (1996)].
[c]The EPSILON (X), DELTA (Y), and Z subunits can be replaced by the c-interferon-inducible subunits LMP7, LMP2 and MECL1, which play a role in antigen presentation (Akiyama et al., 1994; Goldberg et al., 1995; Hisamatsu et al., 1996).
[d]There are two closely related subunits PROS28.1A and PROS28.1B that are testes-specific in their expression (Yuan et al., 1996).

cloning and genetic mapping of a sixth *Drosophila* proteasome subunit gene, a*5_dm*. We also report the chromosomal localization of two other cloned subunit genes that had not previously been mapped.

2. Materials and methods

2.1. General procedures

All standard techniques (e.g., library screening, DNA extraction, restriction digestion, DNA sequencing, Southern blots, etc.) were done using published protocols (Sambrook et al., 1989; Ashburner, 1989).

2.2. PCR amplification using degenerate primers

The following oligonucleotides, obtained from Gibco/BRL, were used as degenerate primers in PCR reactions: PSMA5-D5′, 5′ AGGATCCAYATIGGITGYGCIATG 3′; PSMA5-D3N, 5′ TGGATCCARIGCIACICCRAAIGG 3′; PSMA5-D3′, 5′ CGGATCCARYTTYTCYTCCATIACYTG 3′, where I = deoxyinosine, R = a mixture of A and G, and Y = a mixture of C and T. The following primers, corresponding to sequences within the pJG4-5 plasmid vector, were used in combination with the appropriate degenerate primer for the anchored PCR procedure: JG45-5′, 5′ CCTACCCTTATGATGTGC 3′ and JG45-3′, 5′ GTTAGTTCCAACAGCCGA 3′ (located 5′ and 3′, respectively, of the cDNA cloning site in pJG4-5). Approximately 20 ng of template DNA (either genomic DNA or an aliquot of a embryonic cDNA library cloned in pJG4-5) was mixed with 125 nM each primer, 10 mM Tris–HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 200 mM dNTP. After denaturing the template by heating to 94°C for 5 min, 1 unit AmpliTaq DNA polymerase (Perkin Elmer Cetus) was added and 35 cycles of the following regime were performed: 1.5 min at 94°C, 45 s at 46°C and 1.5 min at 72°C, followed by 8.5 min at 72°C. The samples were electrophoresed through a 2% agarose gel and the DNA fragments excised and purified using the GeneClean procedure (Bio101).

2.3. Recombinant library screening and subcloning

Seventy-five thousand plaques of a *D. melanogaster* genomic library and 150 000 plaques of a *D. melanogaster* embryonic cDNA library were transferred to nitrocellulose filters and processed for hybridization (Sambrook et al., 1989). Hybridization was carried out using either high (50% formamide) or reduced (37% formamide) stringency conditions, as described in O'Neil and Belote (1992). The probe consisted of the gel purified PCR product radiolabeled with ^{32}P-dATP using the Random Primed Labeling Kit (Boehringer-Mannheim) according to the manufacturer's instructions. After plaque-purified recombinant phage had been isolated, DNA was prepared and restriction fragments that hybridized to the probe were identified by Southern blotting. These were subcloned into the plasmid vector pGEM3 (Promega) for further analysis.

2.4. Polytene chromosome in situ hybridization

Biotinylated DNA in situ hybridization to larval salivary gland polytene chromosomes was performed as described in Ashburner (1989). Probes were prepared by random primed labeling of recombinant phage DNA. The modified deoxynucleotide used was biotinylated dUTP from Gibco-BRL, and signal detection was done using a Detek-I-hrp Kit (ENZO).

3. Experimental and discussion

3.1. PCR amplification of Drosophila a5_dm sequences using degenerate primers

The a5 proteasome subunits of humans and the yeast *Saccharomyces cerevisiae* have previously been characterized at the molecular level. DeMartino et al. (1991)

isolated sequences encoding the human a5 subunit from a cDNA library using degenerate oligonucleotide probes corresponding to peptide sequences obtained from an isolated proteasome subunit given the name zeta. The yeast gene was identified fortuitously as an open reading frame adjacent to the *GCN5* gene that showed high sequence similarity to several other previously cloned a subunits, and was given the name *PUP2* (for *pu*tative *p*roteasome subunit) (Georgatsou et al., 1992). Subsequently, the *PUP2* gene was shown to be identical to the *DOA5* (*d*egradation *o*f *a*lpha) locus, which was identified on the basis of a mutant allele that affects the in vivo degradation of the mating factor MATa2 (Hochstrasser et al., 1995; Chen and Hochstrasser, 1995).

As an initial approach toward cloning the *Drosophila* gene encoding the a5 proteasome subunit, we attempted to PCR amplify genomic DNA using degenerate primers corresponding to conserved regions of the a5 protein. In order to identify such regions, the deduced amino acid sequences of the yeast and human a5 subunits were retrieved from the GenBank database and compared. One degenerate oligonucleotide corresponding to the conserved amino acid sequence HIGCAM (amino acid position 73–78, using the human sequence as a reference) was designed for use as a 5′ primer and another corresponding to the conserved sequence QVMEEKL (amino acid position 204–211) was chosen for use as a 3′ primer. An additional 3′ primer was constructed corresponding to the conserved sequence PFGVAL that lies between the other two primer regions (position 136–141).

Unfortunately, when the 5′ degenerate primer was used in combination with either of the 3′ primers, no PCR product was obtained using either genomic DNA or cDNA as template. Because this approach demands that both degenerate primers be functional in order to successfully amplify the sequences of interest, we next tried using an anchored PCR strategy in which the template was a cDNA library cloned in the plasmid vector pJG4-5. For these reactions, each of the degenerate oligonucleotide primers described above was paired with a specific primer corresponding to a known sequence within the pJG4-5 vector. While two of the degenerate primers failed to yield a PCR product, the 3′ degenerate primer corresponding to the QVMEEKL region did PCR amplify a 730 bp product when used in combination with the 5′ JG4-5 specific primer. This DNA fragment was gel purified and partially sequenced using the degenerate oligonucleotide as a sequencing primer. Comparison of the sequence that was obtained with the known coding regions of the *PUP2* and *zeta* genes confirmed that this PCR product encodes a protein that shares significant amino acid identity to these yeast and human a5 subunits.

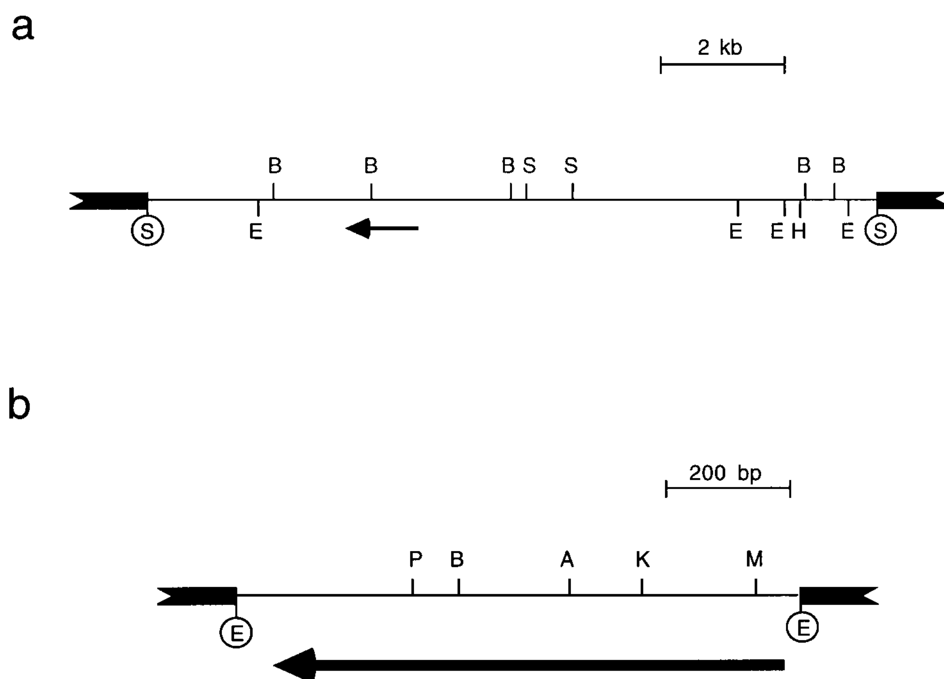

Fig. 1. Restriction maps of a*5_dm* genomic and cDNA clones. **(a)** Restriction map of the genomic clone l DmPSMA5-14. The thin line represents the *Drosophila* genomic DNA insert, and the incomplete thick bars are the l EMBL3 phage arms. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sal*I. The arrow represents the site and orientation of the a*5_dm* gene. **(b)** Restriction map of the cDNA clone pGEM/PSMA5-0.9R. The thin line represents the a*5_dm* cDNA insert, and the incomplete thick bars are the vector sequences. A, *Acc*I; B, *Bam*HI; E, *Eco*RI; K, *Kpn*I; M, *Mlu*I; P, *Pst*I. The arrow indicates the orientation of the cDNA with the arrowhead corresponding to the 3′ end. For both maps, the circled letters represent vector sites flanking the inserted DNA.

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atcagacgcacttATCGCAAGTCATTTCAGCGTCAAGTCATCCGGCAATTTGTTTGCGATTTTGTTAAGAAAAgtaagcc   -66

ctttgcaagtgttattgtcaaccagcgtgcataacacttaactaattacagaattatagCCCACCATGTTTCTCACGCGT    15
                                                                  M  F  L  T  R        5

TCCGAGTACGACAGAGGCGTGAACACCTTCTCGCCGGAGGGCCGCCTCTTCCAAGTGGAATATGCCATTGAGGCCATCAA    95
 S  E  Y  D  R  G  V  N  T  F  S  P  E  G  R  L  F  Q  V  E  Y  A  I  E  A  I  K    32

ATTGGGCTCCACAGCAATTGGAATTTGCACGCCAGAAGgtatgttctttgctggcataagtacacgcggagatctaatgg   175
 L  G  S  T  A  I  G  I  C  T  P  E                                                  44

taaaattgcattccagGAGTGGTTTTGGCGGTGGAGAAGCGCATCACTTCGCCGCTAATGGTACCCAGTACTGTGGAGAA   255
                 G  V  V  L  A  V  E  K  R  I  T  S  P  L  M  V  P  S  T  V  E  K    66

GATTGTGGAGGTGGACAAGCACATTGGTTGCGCCACCTCCGGCTTGATGGCCGATGCCAGGACTCTGATCGAGAGGGCTC   335
 I  V  E  V  D  K  H  I  G  C  A  T  S  G  L  M  A  D  A  R  T  L  I  E  R  A       92

GCGTGGAGTGCCAGAACCACTGGTTCGTCTACAACGAGCGCATGTCCATCGAGTCCTGCGCCCAGGCTGTGTCCACTTTG   415
R  V  E  C  Q  N  H  W  F  V  Y  N  E  R  M  S  I  E  S  C  A  Q  A  V  S  T  L     119

GCCATCCAGTTCGGTGATAGTGGCGACAGCGATGGTGCCGCCGCCATGAGTCGTCCCTTTGGTGTGGCCATTCTATTTGC   495
 A  I  Q  F  G  D  S  G  D  S  D  G  A  A  A  M  S  R  P  F  G  V  A  I  L  F  A   146

CGGCATCGAGGCGGGACAACCCCAGTTGTGGCACATGGATCCTTCCGGCACATTCGTCGGCCACGGAGCCAAGGCCATTG   575
 G  I  E  A  G  Q  P  Q  L  W  H  M  D  P  S  G  T  F  V  G  H  G  A  K  A  I      172

GCTCGGGCAGCGAAGGTGCTCAGCAGAATCTGCAGGACTTATTTAGACCCGATTTGACTCTCGATGAGGCTATCGACATT   655
G  S  G  S  E  G  A  Q  Q  N  L  Q  D  L  F  R  P  D  L  T  L  D  E  A  I  D  I     199

TCGCTCAACACACTTAAACAGGTTATGGAGGAGAAACTAAACTCCACCAATGTGGAGGTGATGACCATGACGAAAGAAAG   735
 S  L  N  T  L  K  Q  V  M  E  E  K  L  N  S  T  N  V  E  V  M  T  M  T  K  E  R   226

AGAGTTCTACATGTTCACCAAGGAGGAGGTGGAGCAGCACATTAAGAACATTGCGTAAGCGGCCAGGTTGGTTTTAAATT   815
 E  F  Y  M  F  T  K  E  E  V  E  Q  H  I  K  N  I  A  *                            244

GGATCTAATTTCAAATGTAATGTGAATAAAGAAAGGGGGATTCTAAATCGAACTAATGtaatagaaggtatactgttgat   895
                                                         ↑
caagtgattggtacattagtaaa                                                             918
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Fig. 2. Nucleotide and amino acid sequences of the a*5_dm* gene of *D. melanogaster*. Upper case letters correspond to the cDNA sequence, and lower case letters represent flanking sequence and introns. The numbers refer to the nucleotide and amino acid positions at the end of each line. The underlined sequence is the polyadenylation signal. The arrow points to a G that is present in the cDNA at a position immediately preceding the polyA tail but that is missing from the genomic sequence. The GenBank accession number for this sequence is U64721.

3.2. Isolation and characterization of a5_dm *genomic and cDNA clones*

Genomic Southern blot analysis using a radiolabeled probe prepared from this PCR product indicated that the amplified sequence is present in the *Drosophila* genome and suggested that it was a single copy gene (data not shown). This probe was therefore used to screen *D. melanogaster* genomic and embryonic cDNA libraries under high stringency conditions, resulting in the isolation of 23 genomic and six cDNA clones. The cDNA clones all contained an insert of 0.9 kb. Of the 23 genomic clones, eight were chosen for further analysis. Restriction maps of representative genomic and cDNA clones are shown in Fig. 1. The a*5_dm* cDNA was sequenced in its entirety, and the two *Bam*HI fragments from the genomic clones were partially sequenced, and the results are given in Fig. 2. Comparison of the genomic and cDNA sequences reveal that the a*5_dm* gene has two small introns, one within the 5′ UTR just six nucleotides upstream of the translation start site, and one within the glycine codon at amino acid position 45. While the transcription start site has not been mapped, the cDNA sequence indicates that it lies at least 132 bp upstream of the translation start site. There is a conventional polyadenylation signal

27 nucleotides upstream of the polyadenylation site. Interestingly, the cDNA sequence has an extra G nucleotide immediately preceding the run of A's that correspond to the poly-A tail. The origin of this G, which is clearly missing in the genomic sequence, is unknown. It may represent a sequence polymorphism that exists between the strains used to make the two different libraries, or it might be a cloning artifact.

The a*5_dm* inferred open reading frame encodes a protein of 244 amino acids and a molecular mass of 26 739 kDa that is 59% and 71% identical to the amino acid sequence of the a5 subunits encoded by the yeast *PUP2* and human *zeta* genes, respectively (Fig. 3). The N-terminal 40 amino acids of the protein are particularly well-conserved, with only one amino acid difference between the fly and human sequences and only three between the fly and yeast.

3.3. Chromosomal localization of the a5_dm *gene and search for mutant alleles*

As the initial step toward genetic characterization of a*5_dm*, its chromosomal map position was determined by *in situ* hybridization to polytene chromosomes. As shown in Fig. 4, the a*5_dm* probe hybridizes to chromosome region 54B3-5, on the right arm of chromosome *2*. No other sites of hybridization are evident, consistent with the Southern blot results, suggesting that this is a single copy gene. This chromosomal site is not near any of the previously mapped proteasome subunit genes. Since two of the previously cloned proteasome subunit genes, *Pros25* (a*2_dm*) and *Pros29* (a*3_dm*), had not been mapped, we also determined their chromosomal positions, and found them to lie at 87B (chromosome *3*) and 57B (chromosome *2*), respectively. Thus, the Drosophila proteasome subunit genes that have been mapped to date are widely dispersed in the genome, with no two genes mapping to the same region (Table 2).

Fig. 4. *In situ* hybridization of a biotin-labeled a*5_dm* probe to larval salivary gland chromosomes. The probe hybridizes to a single band at chromosome position 54B3-5 (arrow) on the right arm of chromosome 2.

Since the yeast *PUP2* gene has been shown to be essential for cell viability (Georgatsou et al., 1992), it is expected that a*5_dm* mutants will be lethal alleles. A search of the Flybase database (FlyBase, 1996) indicated that four recessive lethal complementation groups have been identified in the 54B region. Each of these four genes, named *l(2)01038*, *l(2)06373*, *l(2)07129*, and *l(2)10491*, is defined by a mutant allele in which a single P-element transposon has inserted into 54B and disrupted an essential gene. To see if any of these mutants represented insertions within a*5_dm*, genomic Southern

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Dm   M F L T R S E Y D R G V N T F S P E G R L F Q V E Y A I E A I K L G S T A I G I C T P E G V V L A V   50
Hs   M F L T R S E Y D R G V N T F S P E G R L F Q V E Y D I E A I K L G S T A I G I Q T S E G V C L A V   50
Sc   M F L T R S E Y D R G V S T F S P E G R L F Q V E Y S L E A I K L G S T A I G I A T K E G V V L G V   50

Dm   E K R I T S P L M V P S T V E K I V E V D K H I G C A T S G L M A D A R T L I E R A R V E C Q N H W  100
Hs   E K R I T S P L M E P S S I E K I V E I D A H I G C A M S G L I A D A K T L I D K A R V E T Q N H W  100
Sc   E K R A T S P L L E S D S I E K I V E I D R H I G C A M S G L T A D A R S M I E H A R T A A V T H N  100

Dm   F V Y N E R M S I E S C A Q A V S T L A I Q F G D S G D S D G A A A M S R P F G V A I L F A G I E A  150
Hs   F T Y N E T M T V E S V T Q A V S N L A L Q F G E E D A D P G A - - M S R P F G V A L L F G G V D E  148
Sc   L Y Y D E D I N V E S L T Q S V C D L A L R F G E - G A S G E E R L M S R P F G V A L L I A G H D A  149

Dm   G Q - P Q L W H M D P S G T F V G H G A K A I G S G S E G A Q Q N L Q D L F R P D L T L D E A I D I  199
Hs   K G - P Q L F H M D P S G T F V Q C D A R A I G S A S E G A Q S S L Q E L Y H K S M T L K E A I K S  197
Sc   D D G Y Q L F H A E P S G T F Y R Y N A K A I G S G S E G A Q A E L L N E W H S S L S L K E A E L L  199

Dm   S L N T L K Q V M E E K L N S T N V E V M T M T K E R E F Y M F T K E E V E Q H I K N I A            244
Hs   S L I I L K Q V M E E K L N A T N I E L A T V Q P G Q N F H M F T K E E L E E V I K D I              241
Sc   V L K I L K Q V M E E K L D E N N A Q L S C I T K Q D G F K I Y D N E K T A E L I K E L K E K E A A  249

Sc   E S P E E A D V E M S                                                                                260
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Fig. 3. Amino acid sequence comparison of the Drosophila, human and yeast a5_dm proteasome subunits. Shaded areas represent amino acid identity between at least two of the proteins. The human and yeast sequences were retrieved from the GenBank database (accession numbers X61970 and X64918). Dm, *D. melanogaster*; Hs, *H. sapiens*; Sc, *S. cerevisiae*. The numbers refer to the amino acid position at the end of each line.

Table 2
Chromosomal map positions of 20S proteasome subunit genes of *Drosophila melanogaster*

Gene	Linkage group	Salivary chromosome map position	Reference
Pros28.1 (a*4_dm*)	X	14B	Frentzel et al., 1992
PSMA5 (a*5_dm*)	2	54B	this report
Pros29 (a*3_dm*)	2	57B	this report
Pros28.1B (a*4t2_dm*)	2	60D	Yuan et al., 1996
l(3) 73Ai (b*6_dm*)	3	73A	Saville and Belote, 1993
Pros25 (a*2_dm*)	3	87B	this report
Pros35 (a*_dm*)	3	89F	Frentzel et al., 1992
Pros28.1A (a*4t1_dm*)	3	92F	Yuan et al., 1996

blot analysis was carried out on DNA extracted from flies carrying these mutations using the a*5_dm* cDNA clone as a probe. The results showed that none of the four mutants contains a P-element insertion (or any other detectable rearrangement) within at least 8 kb of the a*5_dm* gene region (data not shown). Given this result, it is unlikely that any of these four lethal alleles correspond to a*5_dm* mutants.

3.4. Search for related genes encoding a5_dm *isoforms*

In *Drosophila*, at least one of the a-type subunits, a*4_dm*, exists as more than one isoform. One form, the 'housekeeping' form, is expressed at a high level in most, if not all, tissues during all stages of development in both sexes. Two additional isoforms, encoded by genes that are closely related but distinct from those encoding the 'housekeeping' gene, are expressed exclusively in the male germ line during later stages of spermatogenesis (Yuan et al., 1996). It has not yet been determined if any other proteasome subunits also have testes-specific isoforms, nor has the functional significance of these testes-specific proteasome subunits been elucidated. To see if the a*5_dm* gene has closely related sequences in the genome that might encode alternative isoforms, we screened a genomic library under the same reduced stringency conditions used to identify the a*4_dm* testes-specific genes, using the a*5_dm* cDNA as a probe, and isolated 14 additional clones. PCR amplification and sequencing analyses of these clones indicated that they all represented the same locus as that corresponding to a*5_dm* itself. Thus, unlike the situation seen with the a*4_dm* subunit gene, this experiment suggests that there is only a single type of a*5_dm* subunit gene in the *Drosophila* genome.

4. Conclusions

(1) The a*5_dm* gene of *D. melanogaster* encodes an a-type proteasome subunit with high sequence similarity to the yeast *PUP2* (59% amino acid identity) and human *zeta* (71% amino acid identity) genes, both of which encode a5 subunits.
(2) The a*5_dm* gene maps to chromosome position 54B3-5 on the right arm of chromosome *2*. Of the Drosophila proteasome genes mapped to date, no two have mapped to closely linked sites. Of four identified lethal complementation groups in this region, none appears to be a mutation in the a*5_dm* gene.
(3) a*5_dm* is a single copy gene, and there appear to be no closely related genes encoding a5_dm isoforms.
(4) The strategy used here to isolate the a*5_dm* gene should, in principle, be useful for the isolation of all other 20S subunit genes that have yet to be cloned from *Drosophila*. It is hoped that the molecular characterization and mapping of these genes will make it feasible to manipulate proteasome function in vivo, using mutational approaches, in order to address the importance of proteasome-mediated protein degradation in various developmental processes in this well-studied experimental organism.

Acknowledgement

We would like to thank Dr Russ Finley for the pJG4-5 cDNA library and Dr Kerrie-Ann Smyth for providing the salivary gland chromosome squashes for the *in situ* hybridization experiment, Evan Katz for carrying out the reduced stringency hybridization screen, and Xiaoqing Yuan for mapping *Pros29* (*a3_dm*). We would also like to thank Jing Ma, Mary Miller and Kerrie-Ann Smyth for their helpful comments on the manuscript. This material is based upon work supported by the National Science Foundation under Grant No. MCB-9506885. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

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