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Genetics

Identification of an essential gene, *l(3)73Ai*, with a dominant temperature-sensitive lethal allele, encoding a *Drosophila* proteasome subunit

(multicatalytic proteinase/antimorph/dominant negative mutation)

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ABSTRACT **Proteasomes are multicatalytic proteinase complexes that function as a major nonlysosomal proteolytic system in all eukaryotes. These particles are made up of 13–15 nonidentical subunits, and they exhibit multiple endopeptidase activities that promote the intracellular turnover of abnormal polypeptides and short-lived regulatory proteins. Although the biochemical characterization of proteasomes has been quite extensive, and although a number of the genes encoding proteasome subunits have been cloned from various organisms, there is still much to be learned about their function *in vivo* and what role(s) they might play during development. Here, we report the identification of the *l(3)73Ai*^{1} allele of *Drosophila melanogaster* as a dominant temperature-sensitive lethal mutation in a gene encoding a component of the proteasome, thus opening the way for future genetic and developmental studies on this important proteolytic system in a higher eukaryote.**

Proteasomes are highly conserved proteinase complexes that have been found in archaebacteria and in all eukaryotes examined (for reviews, see refs. 1 and 2). In eukaryotes, these complexes can be found in the cytosol and the nucleus as large (20S, ≈700 kDa) particles consisting of a family of subunits arranged in a barrel-shaped configuration. Biochemical studies have shown that proteasomes exhibit multiple endopeptidase activities and that they can exist in active and latent forms. Further *in vitro* analyses indicate that the 20S proteasome particles can assemble with two other factors to form 26S particles that are responsible for degrading ubiquitin-tagged proteins (3, 4). The role of proteasomes in the ubiquitin proteolytic pathway is supported by genetic observations in yeast, where it has been found that a temperature-sensitive mutation in a proteasome subunit gene causes ubiquitin–protein conjugates to accumulate at the nonpermissive temperature (5). Thus, the proteasome is an integral component of an important nonlysosomal proteolytic system responsible for removing unnecessary and abnormal proteins from eukaryotic cells.

During the last few years, there has been a great deal of information gathered on the biochemical and physical properties of proteasomes, and, recently, molecular studies have been initiated (see ref. 1). Despite the impressive body of experimental data, however, the *in vivo* function of proteasomes remains obscure. In *Drosophila*, different developmental stages exhibit different patterns of proteasome subunit proteins, as seen in two-dimensional gels (6), and immunostaining experiments reveal region-specific changes in proteasome abundance during early embryogenesis (7). Similarly, in chicken embryonic muscle cells, the proteolytic activities of proteasomes change during development, and this is correlated with a developmental- and tissue-specific alteration in proteasome subunit composition (8). These observations suggest that proteasome function is under developmental control and that proteasomes might be involved in controlling specific processes related to development, such as cell division or morphogenesis.

To better understand the biological function(s) of proteasome-regulated protein degradation, genetic studies would be of considerable value. Although such studies have been initiated in yeast (5, 9–12), no proteasome mutants have yet been described for any other organism. In this paper we report the identification and initial characterization of mutant alleles of a gene, *l(3)73Ai*, encoding a *Drosophila* proteasome subunit. One allele, *l(3)73Ai*^{1}, is a previously discovered dominant temperature-sensitive lethal mutation (13) that behaves genetically as an antimorphic mutation and that exhibits an intriguing lethal phenotype at the restrictive temperature. Two other alleles, *l(3)73Ai*^{2} and *l(3)73Ai*^{3}, are nonconditional recessive lethals (14). The identification of *l(3)73Ai* as a gene encoding a proteasome subunit, and the availability of a dominant conditional mutant allele, have allowed us to initiate a genetic study of the biological function of proteasomes in the *Drosophila* model system.‡

MATERIALS AND METHODS

Standard Techniques. All standard molecular techniques were done as described (15). Flies were reared on cornmeal/dextrose/sucrose/yeast/agar medium containing propionic acid as a mold inhibitor. Genetic variants of *Drosophila melanogaster* are described in ref. 16.

P-Element-Mediated Germ-line Transformation. Microinjection of *Drosophila* embryos was done using standard methods (17). Restriction fragments to be introduced into the genome were subcloned into the P-element transformation vector pUChsneo (18) or into pW8 (19).

Cloning of the *l(3)73Ai*^{1} Mutant Allele. Genomic DNA was isolated from *l(3)73Ai*^{1}/*Df(3L)st-j7* flies that had been reared at 18°C. Size-selected *Eco*RI fragments (3–4 kb) were ligated into λgt11 and, after packaging, the resultant bacteriophage were plated and screened with a 3.5-kb *Eco*RI fragment probe containing the *l(3)73Ai* gene. The 3.5-kb *Eco*RI insert of a representative phage clone was subcloned into the plasmid pIBI76 (International Biotechnologies) for sequencing. This insert was also cloned into pW8 to yield *P[3.5R,DTS]*, which was used for germ-line transformation experiments.

The *P[Actin-1.2BR,DTS]* construct, containing the *l(3)73Ai*^{1} gene driven by the actin 5C promoter, was made by replacing the 2.4-kb *Bam*HI/*Eco*RI fragment of *P[3.5R,DTS]* with the

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actin 5C promoter region from the plasmid pUC18ΔR1-actin5CSRS (20).

Proteasome Purification and Immunoblot Analysis. Proteasomes were isolated from adult flies (Oregon R strain) using the sucrose density gradient centrifugation method as described (6, 21).

For the generation of anti-*l(3)73Ai* protein antibodies, a *trpE–l(3)73Ai* fusion gene was produced by ligating a cDNA restriction fragment containing the entire *l(3)73Ai* coding region into the expression vector pATH21 (22). The fusion protein was gel purified as described (23) and used to immunize rabbits (Pocono Rabbit Farm, Canadensis, PA). Proteins from the sucrose gradient fractions were separated by SDS/PAGE and electrophoretically transferred to poly(vinylidene difluoride) membrane according to the method of the supplier (Bio-Rad). Filters were incubated for 2 hr with purified anti-*trpE–l(3)73Ai* IgG in incubation solution (phosphate-buffered saline/5% Carnation dry milk/0.3% Tween 20) and then treated with secondary antibody [alkaline phosphatase-conjugated goat anti-rabbit IgG (Bio-Rad)]. Antibody binding was detected using the Bio-Rad AP conjugate staining kit. Immunoblot controls were done with IgG purified from preimmune serum.

RESULTS

Genetic Properties of *l(3)73Ai*. As part of a comprehensive study on temperature-sensitive mutations in *D. melanogaster*, Holden and Suzuki (13) carried out a mutagenesis experiment designed to identify dominant temperature-sensitive (DTS) lethal mutations on chromosome 3. This screen resulted in the isolation of 10 DTS mutants representing at least eight separate loci. These mutants were characterized by the property that heterozygotes fail to survive to adulthood when raised at 29°C, whereas at culture temperatures of 25°C or less, viability is normal. One of these mutants, originally called *DTS5*, was mapped by recombination to a position just proximal to the eye color gene scarlet. Cytogenetic analysis placed *DTS5* in salivary gland chromosome region 73A9-10 (14). The *DTS5* locus was subsequently renamed *l(3)73Ai* to conform to standard nomenclature (14), and the DTS mutant allele is now referred to as $l(3)73Ai^1$.

In $l(3)73Ai^1$/+ flies raised at the restrictive temperature of 29°C, embryonic and larval development appears to proceed normally, but metamorphosis is abnormal and death occurs at the late pupal stage (Table 1, A and B). The most conspicuous defect is a failure of the adult abdominal segments (i.e., the tergites and sternites) to develop. At marginally restrictive temperatures, the anterior structures of the adult fly appear to develop normally, while the abdomen region fails to differentiate and histolyzes, resulting in a dead pharate adult that lacks abdominal structures. At slightly higher temperatures, the anterior structures also show gross defects, such as reduced size and a frequent failure of head eversion. In addition to its dominant conditional phenotype, $l(3)73Ai^1$ also acts as a recessive lethal—that is, $l(3)73Ai^1$ homozygotes or hemizygotes [e.g., $l(3)73Ai^1/Df(3L)st\text{-}j7$], raised at 25°C, die during the first larval instar stage (Table 1, C). The lethal phenotype of hemizygotes can be suppressed by rearing the flies at 18°C, although surviving adults often exhibit abdominal tergite defects and are sterile (Table 1, D). The $l(3)73Ai^1$ allele can be classified as a "gain-of-function" mutant, since a deletion of the chromosomal region containing the *l(3)73Ai* locus has no dominant phenotypic effect (Table 1, E). An additional observation concerning $l(3)73Ai^1$ is that its dominant conditional lethal phenotype can be rescued by an additional wild-type copy of the locus (Table 1, F).

Two other mutant alleles of this locus, $l(3)73Ai^2$ and $l(3)73Ai^3$, behave as nonconditional recessive lethal alleles, having lethal periods during the first larval instar (14). The fact that these alleles are completely recessive, and the observation that homozygous and hemizygous mutant individuals exhibit similar lethal phenotypes, suggest that these represent loss-of-function mutations.

Identification of the *l(3)73Ai* Transcription Unit. Cytogenetic analysis demonstrates that *l(3)73Ai* lies just proximal to the transformer gene, within the chromosomal walk described previously (14, 24). The results of deficiency mapping and Northern blot analyses, and the preliminary characterization of cDNA clones from this region, defined three transcription units within the interval containing the *l(3)73Ai* locus (Fig. 1). We have used P-element-mediated germ-line transformation methods to test transduced DNA fragments for their ability to provide $l(3)73Ai^+$ function to transgenic

Table 1. Genetic properties of *l(3)73Ai* mutations and transduced DNA fragments

	Genotype	Culture temperature, °C	Phenotype
A	*DTS5*/+	18–25	Viable
B	*DTS5*/+	29	Late pupal lethal
C	*DTS5*/$DTS5^-$	25	Early larval lethal
D	*DTS5*/$DTS5^-$	18	Viable but sterile
E	$DTS5^-$/+	18–29	Viable
F	*DTS5*/*Dp(+,+)*	18–29	Viable
G	*P[3.5R,DTS5$^+$], +/DTS5*	18–29	Viable
H	*P[3.5R,DTS5$^+$], $DTS5^-$/DTS5*	18–29	Viable
I	*P[7.9B,DTS5$^+$];+/DTS5*	29	Late pupal lethal
J	*P[7.9B,DTS5$^+$];$DTS5^-$/DTS5*	25	Late pupal lethal
K	*P[3.5R,DTS5];$DTS5^-$/+*	18–25	Viable
L	*P[3.5R,DTS5];$DTS5^-$/+*	29	Late pupal lethal
M	*P[Actin5C-1.2BR,DTS5];$DTS5^-$/+*	18–25	Viable
N	*P[Actin5C-1.2BR,DTS5];$DTS5^-$/+*	29	Late pupal lethal

See text and legend to Fig. 1 for descriptions of the fragments used. + = $l(3)73Ai^+$; *DTS5* = $l(3)73Ai^1$; $DTS5^-$ = *Df(3L)st-j7*, a deletion of the chromosome region containing the *l(3)73Ai* locus; *Dp(+,+)* = *Dp(3;3)st$^+$-g18*, a tandem duplication of the chromosome region containing the *l(3)73Ai* locus; *P[3.5R,DTS5$^+$]* = P-element transposon carrying a 3.5-kb *Eco*RI fragment from wild-type flies; *P[7.9B,DTS5$^+$]* = P-element transposon carrying a 7.9-kb *Bam*HI fragment from wild-type flies; *P[3.5R,DTS5]* = P-element transposon carrying a 3.5-kb *Eco*RI fragment from $l(3)73Ai^1/Df(3L)st\text{-}j7$ flies; *P[Actin5C-1.2BR,DTS5]* = P-element transposon carrying a 1.2-kb *Eco*RI/*Bam*HI fragment isolated from $l(3)73Ai^1/Df(3L)st\text{-}j7$ flies and placed downstream of the actin 5C promoter.

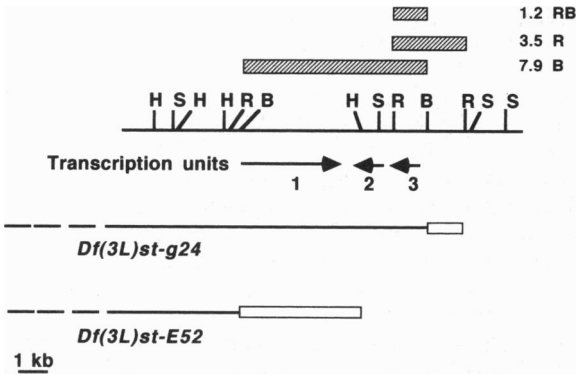

FIG. 1. Molecular map of the *l(3)73Ai* genomic region. The restriction map of the insert from the recombinant phage λ*DmOTF4A* containing DNA from chromosome region 73A9-10 is shown. The restriction sites are H, *Hin*dIII; S, *Sal* I; R, *Eco*RI; B, *Bam*HI. Above the restriction map are the three fragments (hatched bars) that were used in transformation experiments to define the *l(3)73Ai* locus. The arrows below the restriction map represent the three transcription units that map to this region. *Df(3L)st-E52* is a deleted chromosome that retains *l(3)73Ai*$^{+}$ function, and *Df(3L)st-g24* is one that is *l(3)73Ai*$^{-}$. The lines indicate the sequences that are missing in the two deficiency chromosomes (the dashed line means that the deficiencies extend beyond the region shown), and the boxes represent the approximate positions of the deficiency breakpoints, as determined by whole genome Southern analysis (24, 25).

flies, in order to correlate the *l(3)73Ai* gene with one of these transcription units. In these experiments it was found that the 3.5-kb *Eco*RI fragment, labeled 3.5R in Fig. 1, completely rescues the dominant temperature-sensitive lethal and the recessive lethal phenotypes associated with *l(3)73Ai*^{1} (Table 1, G and H). Moreover, the analogous fragment isolated from individuals hemizygous for *l(3)73Ai*^{1} (see below) is able to confer the dominant temperature-sensitive lethal phenotype on flies carrying one wild-type copy of this gene (Table 1, K and L). These results prove that the *l(3)73Ai* locus lies within this 3.5-kb *Eco*RI fragment.

Although the 7.9-kb *Bam*HI fragment does not rescue the dominant temperature-sensitive lethal effect of *l(3)73Ai*^{1} in transgenic flies (Table 1, I), it is able to supply some wild-type function, as evidenced by its partial suppression of the recessive early larval lethal phenotype associated with *l(3)73Ai* mutant alleles (Table 1, C and J). Since the rightmost *Bam*HI site of this fragment is within 25 bp of the start of transcription unit 3 (see below) it is likely that some of the 5′ regulatory sequences necessary for wild-type levels of expression of that transcription unit are missing from the 7.9-kb *Bam*HI fragment. This might explain its inability to completely rescue *l(3)73Ai*^{1}. In another set of experiments, the 1.2-kb *Eco*RI/*Bam*HI restriction fragment from *l(3)73Ai*^{1} mutant individuals was placed downstream of the actin 5C promoter to allow constitutive expression of transcription unit 3. When this construct is introduced into flies carrying one wild-type *l(3)73Ai* gene it acts as a dominant temperature-sensitive lethal gene (Table 1, M and N). Taken together, these results establish the correspondence between the *l(3)73Ai* gene and transcription unit 3.

Characterization of the *l(3)73Ai* Transcription Unit. Northern blot analysis indicates that *l(3)73Ai* specifies a transcript of about 1.0 kb, which is present from the early embryo through the adult stages. The steady-state levels of *l(3)73Ai* RNA show moderate fluctuations during development, with the levels declining during the larval stages and then reaching a peak during the pupal stage (data not shown). Six *l(3)73Ai* cDNA clones of about 1.0 kb in size were isolated from a wild-type pupal cDNA library (24), and all appeared to have similar structure. To further characterize the *l(3)73Ai* transcription unit, we sequenced the wild-type genomic 1.2-kb *Eco*RI/*Bam*HI restriction fragment and one of the cDNA clones (Fig. 2). Additional upstream sequences were obtained by sequencing a portion of the adjacent 2.3-kb *Bam*HI/*Eco*RI restriction fragment. The *l(3)73Ai* transcript contains an open reading frame (ORF) of 235 codons, potentially encoding a polypeptide of about 26 kDa. A search of the available data bases revealed that this ORF shows a high degree of amino acid sequence similarity to the proteasome subunit C5, isolated from rat (26), and to the homologous proteasome subunit from yeast (12). The amino acid sequence comparison of these three proteins shows that the *Drosophila* protein is 45% identical to the rat, and 37% identical to the yeast, proteasome subunits (Fig. 3). Though some of the previously characterized proteasome subunits are thought to be associated with specific proteolytic activities within the intact complex (1, 5), the role of the C5 subunit in proteasome function or regulation is unknown.

Copurification of the *l(3)73Ai* Gene Product with *Drosophila* 20S Proteasomes. Proteasomes can be isolated in sucrose density gradients as a 20S particle that can be resolved by

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-264   tatacatttaatcaaatctagttgccgttc gtttcgttttatggtatctgaatgtatata tttggtcaaaaccgtaaggagtgcaagttt atggtacaaattggaattaattttaggttt
-144   ttatattgttattttatttatatgttaaat taaaaatgtttactaaataaatacctcctg ctaataaaaatatcccaaaccgacgtatgt atacctatcgataagcgtttaagcaggctg
-24    gatccgatgctcaacaacggtcacATTGAT TTTGTGATTTTGGTTAGCCGCTTGCAAACT TGCAAATCATTTCGTTGGTTTTTTACTTGG CGAGTTAATTGGTGAATTTCAGCACAAAAC

+97    ATGAGCAGATTGGGCTTTGAGCAATTCCCG GACTACCAGGTGCCCGGCATGAAGCATCCT GATTTCTCGCCCTACGAGTCCAATGGCGGg tgagttgctatttcccagcggaatcgagat
       MetSerArgLeuGlyPheGlnPhePro AspTyrGlnValProGlyMetLysHisPro AspPheSerProTyrGluSerAsnGlyGl                                    30

+217   gtggaagaatgtctccatctaaccggagtc ttccctgtttacagCTCCATTGTGGCCATC GCCGGAGATGACTTTGCCGTAATTGCAGCG GACACCCGCCTGAGCAGCGGCTACAACATT
                                      ySerIleValAlaIle AlaGlyAspAspPheAlaValIleAlaAla AspThrArgLeuSerSerGlyTyrAsnIle    55
                                                                                        T
                                                                                        Ile
+337   CACTCGCGAACGCAGAGTAAACTCTTTAAA CTCTCGCCCCAGACAGTGTTGGGTTCCGCA GGCTGCTGGGCGGACACGCTCTCGTTGACC GGATCGATTAAGGTGCGCATGCAGAGCTAC
       HisSerArgThrGlnSerLysLeuPheLys LeuSerProGlnThrValLeuGlySerAla GlyCysTrpAlaAspTheLeuSerLeuThr GlySerIleLysValArgMetGlnSerTyr    95

+457   GAGCATACCCATCTGCGCACCATGACCACT GAGGCCGTGGCCCAGATGCTCTCCATCGCC ATGTACAATCGCCGCTTCTTCCCGTACTAC GTGTCGAACATTCTGGCTGGAATTGACAAC
       GluHisThrHisLeuArgThrMetThrThr GluAlaValAlaGlnMetLeuSerIleAla MetTyrAsnArgArgPhePheProTyrTyr ValSerAsnIleLeuAlaGlyIleAspAsn   135

+577   GAGGGCAAGGGCGTCGTCTACTCCTACGAT CCCATCGGTCACTGCGAGAAGGCTACATAC CGCGCCGGCGGCACTGCCGGCACCCTGCTG CAACCGGTGCTGGACAACCAGATTGGTCAC
       GluGlyLysGlyValValTyrSerTyrAsp ProIleGlyHisCysGluLysAlaThrTyr ArgAlaGlyGlyThrAlaGlyThrLeuLeu GlnProValLeuAspAsnGlnIleGlyHis   175
                                                  C                                                 -
+697   AAGAACATGAATTTGGAAGACGCCGACAAG ATCAAGTTAACCAAGGAGTGGGCCGTGAGC GTTGCCTCCGACACCTTCATCTCTGCCGCT GAGCGCGACATCTACACCGGCGACTCTGTG
       LysAsnMetAsnLeuGluAspAlaAspLys IleLysLeuThrLysGluTrpAlaValSer ValAlaSerAspThrPheIleSerAlaAla GluArgAspIleTyrThrGlyAspSerVal   215
                                                  Arg                                               T
+817   CTGATCAACATCATAACCAAAGATGGAATT GAAGTACGAACTCTGACGCTGCGTCAGGAC TAGCCTGTGGCGAGGATGTTGCCGTCTCTT TTGGTTAGGGGCATTCGGTGGAAACCAGGC
       LeuIleAsnIleIleThrLysAspGlyIle GluValArgThrLeuThrLeuArgGlnAsp End                                                           235

+937   ATTCTAAGTTAGTTGGCTTCAAATTTTTGT ATTTACCCAAAACAACAAAACATCGTAATA AACCGAATCTTTGAGATTTAAAATATGCGG CCTAGTGTGaccagctggtcctagaaataa
+1057  atcgttcccaagcagtgctcacagaagagg ctgtgcgaatagtacagctttccccgtacc aacaaatccattttgctagctaagtttgtg cccgctggtgtgcgtaacaaaatcatttct
+1177  tagtgttcgtctctcagagaa
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FIG. 2. Nucleotide and amino-acid sequences of *l(3)73Ai*. Uppercase letters correspond to the cDNA sequence. Numbers on the left represent nucleotide position, where +1 is the putative transcription initiation site, and numbers on the right refer to the amino acid position. The boxed amino acids at position 47 indicate the *l(3)73Ai*^{1} mutation (Thr → Ile). At amino acid position 193 there is a polymorphism (Trp/Arg) that occurs between the wild-type strains used to generate the genomic and cDNA libraries (see ref. 24 for the source of these libraries).

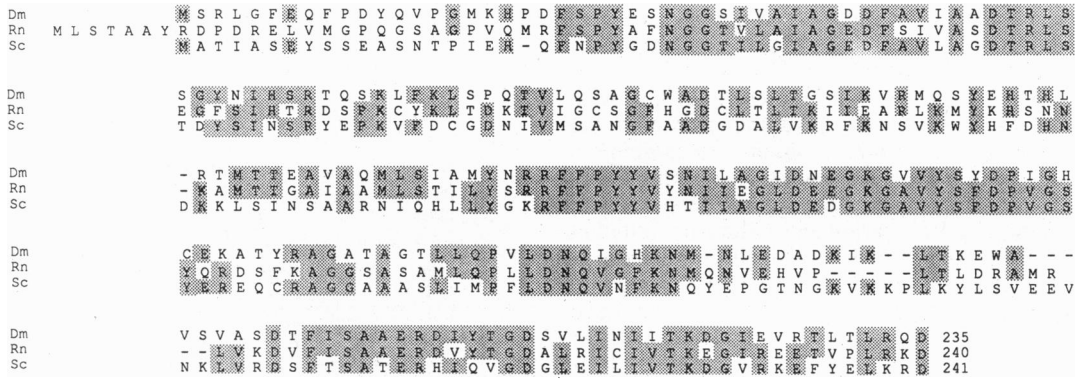

FIG. 3. Amino acid sequence comparison among the *l(3)73Ai* protein, the rat C5 proteasome subunit (26), and the homologous proteasome subunit from yeast (12). Identical amino acids are shaded. Dashes represent positions where the sequences have been shifted in order to maintain the best alignment among all three proteins. Dm, *D. melanogaster*; Rn, *Rattus norvegicus*; Sc, *Saccharomyces cerevisiae*.

one-dimensional SDS/PAGE into six to eight distinct subunits of 22–30 kDa (6, 21). To confirm that the protein encoded by the *l(3)73Ai* locus is a component of *Drosophila* proteasomes, we isolated proteasomes from adult flies and tested them for the presence of the l(3)73Ai polypeptide. Antiserum raised against a TRP-E–l(3)73Ai fusion protein was used to immunostain Western blots of proteasome preparations, and the results showed that the l(3)73Ai protein does indeed cofractionate with proteasomes (Fig. 4). The size of the cross-reactive polypeptide, estimated to be 26.5 kDa, fits almost exactly the size of the *l(3)73Ai* translation product inferred from the DNA sequence.

Cloning and Sequencing of the *l(3)73Ai*^{1} Mutant Allele. The genetic properties of the *l(3)73Ai*^{1} allele can best be explained as resulting from a mutation in which an abnormal, thermolabile polypeptide is produced. As shown in Fig. 2, there is a single difference in the coding regions of the wild-type and mutant genes, resulting in a threonine to isoleucine substitution at amino acid position 47. That this threonine is critical for the wild-type function of the encoded proteasome subunit is supported by the fact it is invariant among all species examined. Thus, it is highly probable that this mutation, replacing a polar uncharged amino acid with a hydrophobic nonpolar residue, is responsible for the dominant temperature-sensitive effect of *l(3)73Ai*^{1}.

In addition to this missense mutation, there is a single nucleotide difference, C → T, at position +902 when the *l(3)73Ai*^{1} allele and the wild-type gene are compared. We do not believe that this is related to the *l(3)73Ai*^{1} mutant effect, however, since it occurs in the 3′ untranslated portion of the gene, and since a sequence comparison between the *l(3)73Ai* gene of *D. melanogaster* and the homologous gene from *Drosophila virilis* shows that this region is not well conserved within the genus (ref. 25; unpublished data). Unfortunately, the original chromosome on which the *l(3)73Ai*^{1} mutation was induced is no longer available for comparison.

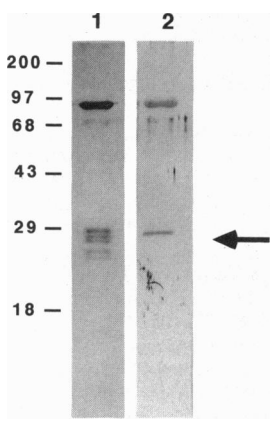

FIG. 4. Identification of the l(3)73Ai protein as a subunit of the *Drosophila* proteasome. Lane 1, SDS/PAGE gel lane of the fraction (≈20S) of a sucrose gradient containing *Drosophila* proteasomes. The gel has been stained with Coomassie blue to visualize total protein. The proteasome subunits are seen in this gel as five or six bands of between 22 and 29 kDa in size. Lane 2, immunoblot of a similar gel using polyclonal antiserum raised against a TRP-E–l(3)73Ai fusion protein. This antiserum stains a polypeptide of 26.5 kDa (arrow). Other fractions, not containing the proteasome subunits, do not show this band. The staining of the higher molecular mass proteins is nonspecific, as it is also seen when preimmune antiserum is used. The numbers refer to the molecular masses (in kDa) of protein standards.

DISCUSSION

Two *Drosophila* loci, *Pros35* (chromosomal map position, 89F-90A) and *Pros28.1* (map position, 14B4), encoding proteasome subunits have been previously isolated as genomic and cDNA clones (27). The molecular characterization of these has provided insights into the important features of proteasome subunit gene structure and regulation in this organism. For neither locus, however, have mutants been identified that permit one to investigate, *in vivo*, the biological role of proteasomes using genetic analysis. The present report represents one case in which mutants affecting proteasome subunit function have been defined for a metazoan.

Biochemical and immunocytological studies on *Drosophila* proteasomes have provided circumstantial evidence that proteasome function is under developmental control (6, 7), and other studies (28, 29) have suggested that proteasomes are involved in controlling the progression of the cell cycle. Given this, it is reasonable to expect that mutations in genes encoding proteasome subunits would exhibit lethal phenotypes. The lethal effects of the mutations reported here indicate that in flies, as has been previously shown in yeast, proteasome function is essential for viability. What was not anticipated, however, was the particular array of developmental defects exhibited by the individuals carrying the *l(3)73Ai*^{1} allele. Though many interpretations of the *l(3)73Ai*^{1} late-pupal lethal phenotype are possible, the observed defect is suggestive of one in which cells are unable to carry out the rapid burst of cell division that normally occurs during adult abdominal tergite differentiation.

It is likely that proteasomes are maternally inherited (6), and so the survival of homozygotes carrying the loss-of-function *l(3)73Ai* alleles, such as *l(3)73Ai*^{2} and *l(3)73Ai*^{3}, through the embryonic stage does not necessarily imply that proteasomes are not essential for embryogenesis. In fact, immunostaining experiments suggest that proteasomes may

be involved in cell-specific events responsible for cell proliferation and morphogenesis during early *Drosophila* development (ref. 7; unpublished results).

The identification of *l(3)73Ai* as a gene encoding a proteasome subunit, and the availability of the dominant conditional allele, *l(3)73Ai*^{1}, should now make it possible to examine in more detail the function of proteasomes, using mutational analysis and the powerful tools of *Drosophila* genetics. For example, as reported by Velissariou and Ashburner (cited in ref. 30), new alleles of the *l(3)73Ai* locus, including deletions, should be readily obtainable by mutagenizing *l(3)73Ai*^{1}/+ heterozygotes and screening at 29°C for mutations that have lost the dominant temperature-sensitive lethal effect. Similar screens could be used to isolate dominant second-site suppressors of *l(3)73Ai*^{1}. Genes identified in such an experiment might include those encoding proteins that interact with the *l(3)73Ai* protein (e.g., other proteasome subunits). A preliminary mutant screen has already resulted in the identification of one second-site dominant suppressor mutation (ref. 25; unpublished data).

The genetic properties of *l(3)73Ai*^{1} indicate that it is an antimorph, or dominant-negative mutation. Operationally, an antimorph is recognized by the fact that its dominant mutant phenotype is suppressed by increasing the dosage, and is made more severe by decreasing the dosage, of the wild-type gene (31). It has been proposed that if a protein normally acts as part of a multimeric complex, then an abnormal variant that is capable of interacting with the wild-type polypeptide subunits, but is otherwise defective, might act as a "poison subunit" rendering the whole complex nonfunctional and that such a mutant would behave genetically as an antimorph (32). Since proteasomes act as multicatalytic proteases containing multiple copies of several different subunits, our finding that the *l(3)73Ai*^{1} mutant allele is a missense mutation is consistent with this hypothesis.

In addition to its use for the study of proteasome function in a genetically manipulable higher eukaryote, a practical use of the cloned dominant temperature-sensitive gene concerns its potential application as a genetic tool. As mentioned above, we have shown that the mutant *l(3)73Ai*^{1} gene, when reintroduced into the genome by germ-line transformation, is able to confer dominant temperature sensitivity to transformed flies, at least when it is heterozygous over one copy of the wild-type gene. By introducing multiple copies of the cloned *l(3)73Ai*^{1} gene onto specific *Drosophila* chromosomes (e.g., balancer chromosomes, sex chromosomes, etc.) by germ-line transformation, it should be possible to construct strains in which unwanted classes of progeny could be selectively eliminated simply by shifting the culture to 29°C. These stocks might be useful for a number of crossing schemes and mutagenesis experiments (for examples, see ref. 33).

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