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Identification and characterization of the gene for *Drosophila* L3 ribosomal protein

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Abstract

A cDNA clone that encodes a *Drosophila* homologue of ribosomal protein L3 was isolated from a *Drosophila* ovary gridded cDNA library. The *Drosophila* ribosomal protein L3 gene (*RpL3*) is highly conserved with ribosomal protein L3 genes in other organisms. It is a single copy gene and maps to position 86D5–10 on polytene chromosomes. A *Minute* gene in this region, *M(3)86D*, is a possible candidate to encode RPL3. *RPL3* message is expressed ubiquitously. A partial *RPL8* cDNA clone was also isolated and mapped to 62F.

Keywords: *Minute*; cDNA cloning; *P1* genomic mapping; Intron/exon mapping; *RpL3*; *RpL8*; mRNA

1. Introduction

Eukaryotic ribosomes are the sites of protein synthesis. Each ribosome is composed of two subunits, the 60S and the 40S subunit. Each subunit consists of ribosomal proteins and ribosomal RNA molecules (Wool et al., 1996).

Most *Drosophila* ribosomal protein genes are single-copy genes (Kay and Jacobs-Lorena, 1987; Baumgartner et al., 1993; McNabb and Ashburner, 1993). At present, approximately 40 ribosomal protein genes in *Drosophila* have been sequenced (The FlyBase Consortium, 1997). However, most of them have not been genetically characterized in detail (The FlyBase Consortium, 1997). More than 10 ribosomal protein loci are associated with a dominant *Minute* bristle phenotype and recessive lethality (e.g. Kongsuwan et al., 1985; Andersson et al., 1994; Schmidt et al., 1996; The FlyBase Consortium, 1997); two of them give rise to a tissue overgrowth phenotype [summarized by Chan et al. (1997)]. However, not all mutations in ribosomal protein genes cause a Minute phenotype (e.g. *RpS14*, Dorer et al., 1991).

Ribosomal protein L3 (*RpL3*) is a component of the 60S ribosome subunit. Some mutations in the yeast *RpL3* gene give rise to resistance to trichodermin, an inhibitor of ribosome peptidyl transferase activity, implying that RPL3 protein is situated in the vicinity of the peptide bond-forming site in the ribosome (Fried and Warner, 1981; Schultz and Friesen, 1983). Here, we report the cloning of a novel *Drosophila RpL3* gene, which is conserved between eukaryotes and prokaryotes. The cytological location of *Drosophila RpL3* is in the vicinity of a candidate *Minute* mutation, *M(3)86D*.

2. Materials and methods

2.1. Isolation of a RPL3 cDNA clone

A eukaryotic translational initiation factor 5A (*eIF5A*) cDNA fragment (Koettnitz et al., 1994) was labelled with [α-^{32}P]dATP using random priming and

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Abbreviations: BLASTN, Basic Local Alignment Search Tool for a nucleotide query against a nucleotide sequence database; BLASTX, Basic Local Alignment Search Tool for a nucleotide query against a protein sequence database; bp, base pairs; cDNA, complementary DNA; CSPD™, Disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2′-(5′-chloro)tricyclo[3.3.1.1^{3,7}]decan}-4-yl)phenyl phosphate; dUTP, deoxyuridine triphosphate; eIF5A, eukaryotic initiation factor 5A; EST, Expressed Sequence Tag; GCG, Genetics Computer Group; kb, kilobase(s); mRNA, messenger RNA; ORF, open reading frame; PCR, polymerase chain reaction; STS, Sequence Tagged Site.

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then used to screen two gridded filters carrying clones from a 0–8 h *Drosophila* embryonic cDNA library (Brown and Kafatos, 1988; Hoheisel et al., 1991) at low stringency. Hybridization was carried out at 68°C overnight in hybridization buffer [6× SSC; 0.5% SDS; 200 mg/ml salmon sperm DNA; 0.1% *N*-lauroylsarcosine and 1% Southern blocking solution (Boehringer Mannheim)]. Filters were then washed with 4× SSC, 0.1% SDS at room temperature for 2 h before autoradiographic exposure.

2.2. Sequence analysis

Cycle DNA sequencing reactions and sequence analysis were performed as described in Chan et al. (1997). Universal primers SP6 and T7, which annealed to sequences in the pNB40 vector (Brown and Kafatos, 1988), were used to sequence the 5′ and 3′ ends of the cDNA insert, respectively. Internal sequencing primers of *RPL3* used were: a, 5′ CTC CCT TCG GTC TGC GTG 3′; b, 5′ CAG CTG AAC GGC GGC TCC 3′; c, 5′ CTT CGG CCA GGA CGA GAT G 3′; d, 5′ CAA GGG CTG CTG CAT CGG C 3′; e, 5′ CTT GTC GGC AGG GGT CTG 3′; f, 5′ CCA GCG CCG ATG CGG TAG 3′; g, 5′ GCG GAT CTG CGA ATG GGC 3′; h, 5′ GCA GGT CAG ATG GAC TGG C 3′; i, 5′ GTA CTC GGT GGA GGC GTT G 3′ (Fig. 1A).

2.3. In-situ hybridization to polytene chromosomes

Double-stranded *RPL3* cDNA probe was labelled with biotin-16-dUTP and prepared by nick translation (Sambrook et al., 1989). *In situ* hybridization to polytene chromosomes was performed according to Engels et al. (1986) with minor modifications.

2.4. Genomic PCR analysis

Genomic PCR reactions were performed by using either a single colony of each relevant bacterial *P1* genomic clone or single fly genomic DNA (Gloor et al., 1993) as template, and different combinations of oligonucleotides as primers (Fig. 1A). *P1* bacterial cells were picked from kanamycin-containing (25 μg/ml) agar plates and transferred to 0.5-ml microcentrifuge tubes containing PCR reaction mix [1× PCR buffer (Promega); 1.25 mM $MgCl_2$; 400 μM dNTP mix and 1 unit of *Taq* DNA polymerase (Promega)] before amplification. One fiftieth of a single fly DNA preparation or an entire single colony preparation was used for each PCR reaction. PCR reactions were performed as follows: 15 s at 98°C, and then 30 s at 58°C, 1.5 min at 72°C, 30 s at 95°C for 30 cycles.

2.5. Southern blot analysis

A *RPL3* cDNA fragment was generated by restriction digestion of the pNB40 *RPL3* plasmid with *Hin*dIII and *Eco*RI enzymes. The fragment was then purified and labelled with [α-^{32}P]dATP using random priming (Sambrook et al., 1989).

Drosophila genomic DNA was prepared from a Canton S strain essentially according to Ashburner (1989). Genomic DNA was digested with *Bgl*II, *Eco*RI, *Hin*dIII, *Pst*I and *Xba*I separately and then blotted, hybridized and detected according to Sambrook et al. (1989).

2.6. Northern blot analysis

Total RNA was prepared from 0–16-h-old Canton S strain embryos according to Andres and Thummel (1994). The same [α-^{32}P]dATP labelled *Hin*dIII–*Eco*RI cDNA fragment (as described in Section 2.5) was used as probe. Northern hybridization was carried out according to Sambrook et al. (1989).

2.7. In-situ hybridization to whole-mount embryos

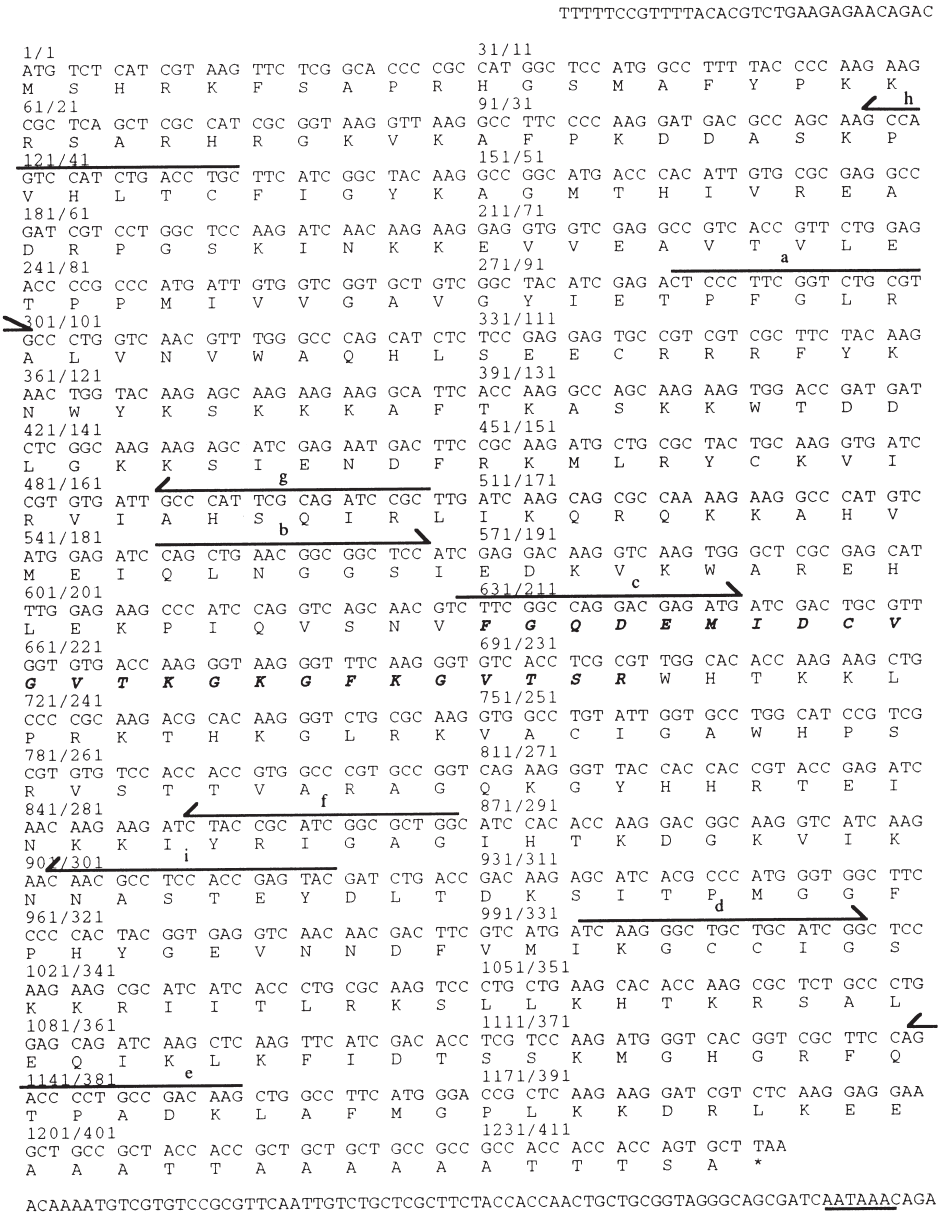
Single-stranded DNA probes labelled with digoxygenin-11-dUTP (Boehringer Mannheim) were prepared by PCR using a *RPL3* cDNA fragment as template and either SP6 primer to label the sense strand or T7 primer to label the anti-sense strand as described by Tautz et al. (1992). Under our labelling and detection conditions, both sense and anti-sense probes were equally sensitive, and a 10-μl spot of a 1:100 000 dilution could be detected using CSPD™ chemiluminescence (Tropix). In-situ hybridization was performed essentially according to Tautz and Pfeifle (1989).

3. Results and discussion

3.1. Isolation of an RPL3 cDNA clone

A low-stringency screen was set up to search for a homologue of eukaryotic translational initiation factor *eIF5A* in *Drosophila melanogaster*. Seven putative positive clones were found. 5′ and 3′ ends of all seven putative positive clones were sequenced using SP6 and T7 primers, respectively. None of them showed any sequence similarity to *eIF5A*. Nevertheless, one of the false positive cDNA clones was found to encode an L3 *Drosophila* ribosomal protein (RPL3) (see below). In the same screening exercise, a partial cDNA clone of ribosomal protein gene L8 (*RpL8*) was also isolated and mapped to 62F (data not shown); the *RPL8* cDNA clone was not investigated further here.

A



B

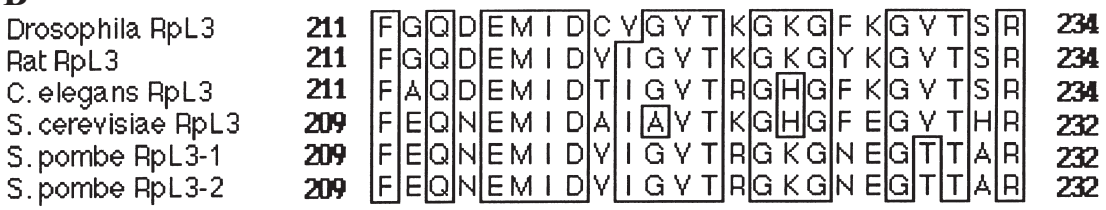


Fig. 1. (**A**) Nucleotide sequence of *RPL3* cDNA clone and its deduced amino acid sequence. Nucleotide position of the *RPL3* open reading frame (counted from the first nucleotide of the first ATG) is indicated above the nucleotide sequence and followed by the amino acid position; the RPL3 signature is italicized and in bold. The stop codon is designated as '*'. The poly A signal is underlined, and the final (a) indicates the position of the poly A tail. Positions and 5′–3′ directions of primers are represented by arrows above the sequence. (**B**) Comparison of the ribosomal protein L3 signature of *Drosophila* RPL3 protein with the rat (X62166); *C. elegans* (Z69337); *Saccharomyces cerevisiae* (J01351); *Schizosaccharomyces pombe* RPL3-1 (U00798) and RPL3-2 (X57734) ribosomal protein L3 homologues. Amino acid residues that are identical in at least four of these sequences are boxed. The Genbank Accession No. for the *Drosophila RPL3* cDNA is AF016835.

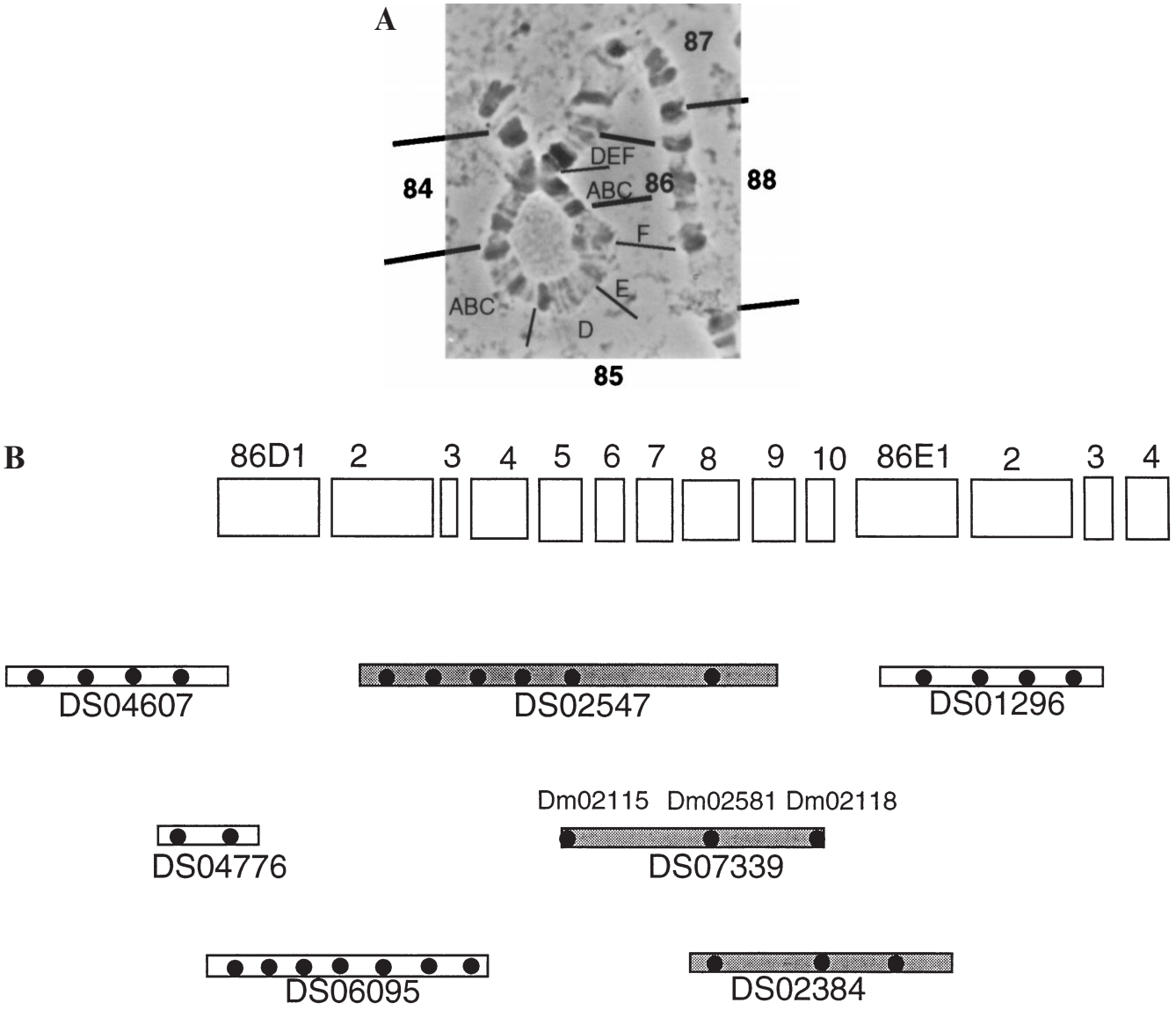


Fig. 2. (**A**) In-situ hybridization of *RPL3* cDNA to polytene chromosomes. Polytene map divisions (Lindsley and Zimm, 1992) are shown. (**B**) PCR-based *P1* mapping of *RpL3*. *RpL3*-negative *P1* clones are represented by open boxes. *RpL3*-positive *P1* clones are represented by shaded boxes. Black dots represent sequence-tagged sites (STSs). The three labelled STSs and the P1 clones shown are documented in Encyclopaedia of Drosophila Release 3.1 (1996).

3.2. Sequence analysis of RPL3 *cDNA and RPL3 protein*

The *RPL3* cDNA clone was sequenced completely in both strands. It contains 1381 bp, including a 5′ UTR of 33 bp, a coding region of 1251 bp, and a 3′ UTR of 97 bp (see Section 3.4). The predicted initiation codon occurs in the context AGACATG which is similar to the consensus sequence at initiation codon in *Drosophila* (Cavener, 1987). The *RPL3* open reading frame (ORF) starts with an ATG codon and stops with a TAA codon. A putative polyadenylation signal is situated 70 bp downstream of the ORF (Fig. 1A).

The predicted RPL3 protein consists of 416 amino acid residues (Fig. 1A). The deduced molecular mass of the protein is 46 886 Da, and the protein is basic with a calculated isoelectric point of 10.68.

BLASTX and ALIGN searches showed that RPL3 has 74.4% identity to its rat homologue (Kuwano and Wool, 1992), 70.9% to its *Caenorhabditis elegans* homologue (Zhu et al., 1996), 64.3% to its *Saccharomyces cerevisiae* homologue (Schultz and Friesen, 1983), 65.3% to its *Schizosaccharomyces pombe* homologue RpL3-1 (Liebich et al., 1994) and 65.1% to its *Schizosaccharomyces pombe* homologue RpL3–2 (Liebich et al., 1994). A ribosomal protein L3 signature (Bairoch, 1993) is also present in the middle of the RpL3 protein at residues 211–234 (Fig. 1B).

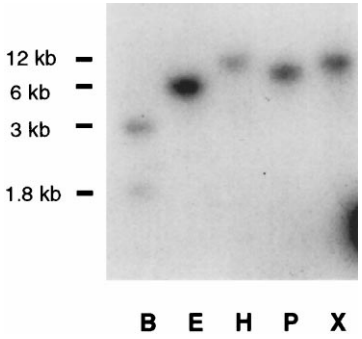


Fig. 3. Genomic Southern Hybridization of *RPL3* cDNA probe to digests of Canton S genomic DNA. B, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I and X, *Xba*I.

3.3. *Cytological location and genomic structure of* RpL3

RpL3 was mapped to the region including the right of 86D and the left of 86E on polytene chromosomes (Fig. 2A). Genomic DNA isolated from a Canton S strain was digested with five different restriction enzymes and hybridized with the a *RPL3* cDNA probe. In most digests, only one genomic fragment was detected (Fig. 3). The only exception was that two *Bgl*II fragments were detected (Fig. 3). This is consistent with the sequence data of the cDNA clone that there is a *Bgl*II site at nucleotides 848–853. These data suggest that *RpL3* is a single copy gene.

To map *RpL3* in more detail, PCR-based mapping was performed using nine *P1* clones in this region as template and oligonucleotides b and f (Fig. 1A) as primers. A genomic fragment with a size of about 300 bp was amplified from three *P1* clones, DS02547, DS07339 and DS02384 (Fig. 2B). The other *P1* templates used (Fig. 2B) failed to give any PCR product. DS07339 has been mapped cytologically to 86D5–10 (Encyclopaedia of Drosophila Release 3.1, 1996). By combining the results of cytological mapping and PCR-based mapping, the cytological location of *RpL3* is refined to 86D5–10.

To determine whether introns are present in *RpL3*, different oligonucleotides were used as PCR primers on genomic and cDNA templates (Table 1). These results suggest: (1) 1.2 kb of intronic sequence located somewhere between nucleotides 301 and 1139; (2) 0.9 kb of intronic sequence between nucleotides 301 and 852; (3) 0.6 kb of intronic sequence between nucleotides 301 and 490; and (4) 0.25 kb of intronic sequence between nucleotides 648 and 903. This demonstrates at least three introns in the *RPL3* cDNA clone: 0.6 kb of intron sequence between nucleotides 301 and 490; 0.3 kb of intron sequence between nucleotides 490 and 567; and 0.25 kb of intron sequence between nucleotides 852 and 903 (Fig. 1A).

A *Minute* mutation, *M(3)86D*, has been localized in region 86D (Encyclopaedia of Drosophila Release 3.1, 1996; The FlyBase Consortium, 1997). *RpL3* is, at present, the only ribosomal protein gene that has been found in this region. This suggests that *RpL3* may correspond to *M(3)86D*. Apart from *M(3)86D*, there are at least eight more genes in this region that have not been studied molecularly (Encyclopaedia of Drosophila Release 3.1, 1996; The FlyBase Consortium, 1997). They are *E(var)3–14*, *E(var)113*, *fs(3)6284*, *l(3)04629*, *l(3)09656*, *l(3)10419*, *mgr* and *tho*. Moreover, five more lethal *P*-element insertions in this region have not been assigned to complementation groups (Encyclopaedia of Drosophila Release 3.1, 1996; The FlyBase Consortium, 1997). They are *l(3)03445*, *l(3)rM060*, *l(3)rK137*, *l(3)rM007 and P{ry1}R311*.1. *RpL3* could also potentially correspond to any of these mutations.

3.4. *Analysis of* RPL3 *transcript*

A single band of 1.6 kb was detected on a Northern blot probed with *RPL3* cDNA (data not shown). The size of the *RPL3* cDNA clone is 1381 bp excluding the poly(A) tail. A poly(A) tail (260–300 nucleotides) is also present in most mature mammalian mRNAs (Birnstiel et al., 1985). Since the size of the band on the Northern blot and is of a similar length of the sum of the cDNA clone and a typical poly(A) tail, it is possible that the existing cDNA clone encodes a full-length *RPL3* cDNA. To detect longer *RPL3* cDNA clones, a BLASTN search of *Drosophila* nucleotide sequences (Berkeley *Drosophila* Genome Project, pers. commun.) was performed, using the existing *RPL3* cDNA sequence as query. Fifty-three *Drosophila* EST clones with a score

Table 1
PCR-based intron/exon mapping of *RpL3*

Primers (position of 3′ base)	Size of genomic product (kb)	Size of cDNA product (kb)	Size difference (kb)
a(301)+e(1139)	2.0[a]	0.8	1.2
a(301)+f(852)	1.6[a]	0.7	0.9
a(301)+g(490)	0.8[a,b]	0.3	0
b(567)+f(852)	0.3[ab]	0.3	0
c(648)+i(903)	0.5[ab]	0.25	0.25

[a]Canton S single fly genomic DNA template.
[b]*P1* clone DS02384 single colony template.

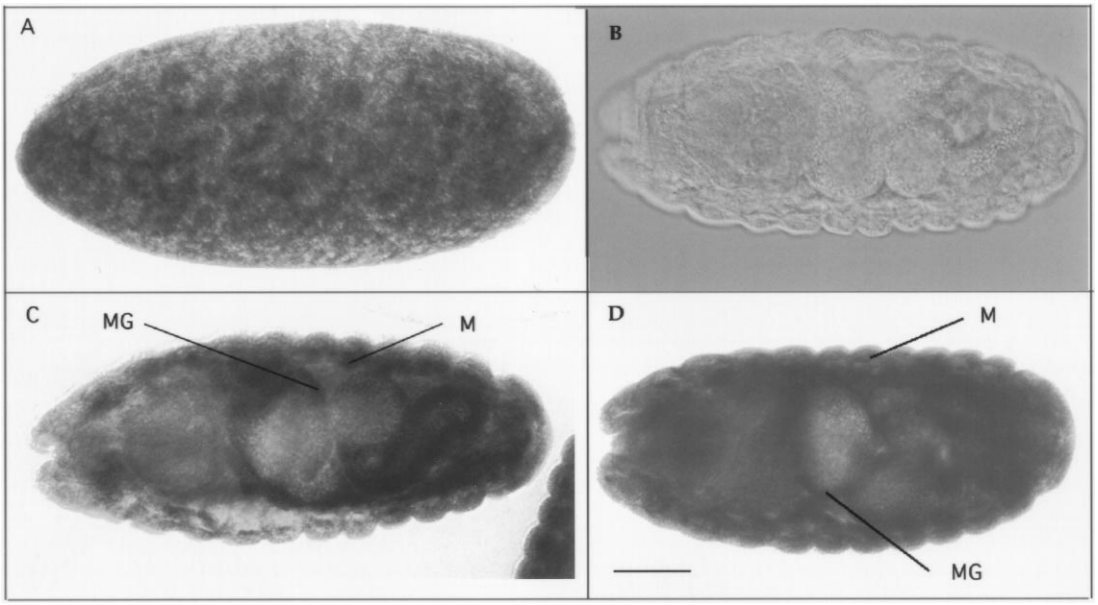


Fig. 4. Expression of *RPL3* in *Drosophila* embryos. (**A**) A preblastoderm embryo hybridized with the *RpL3* antisense probe and developed for 1 h. (**B**) A stage 17 embryo hybridized with the *RpL3* sense probe and developed for 1 h. Stage 16 embryos hybridized with the antisense probe and developed for (**C**) 15 min and (**D**) 1 h. MG, midgut; M, muscle. The scale bar represents 50 μm.

of at least 95% sequence identity were found, but none of them contained a 5′ end longer than that of the existing cDNA clone.

3.5. In-situ hybridization to whole-mount embryos

RPL3 messenger RNA was expressed ubiquitously throughout embryogenesis. Expression was first detected as a maternal component at preblastoderm stages (Fig. 4A). Later in embryogenesis, *RPL3* was also expressed ubiquitously but at higher levels in the midgut, hindgut and in muscle (Fig. 4C and D). No signal was detected using a control sense probe (Fig. 4B).

4. Conclusions

A *Drosophila RPL3* cDNA clone has been isolated in this study and is highly conserved with *RpL3* genes in other organisms. Like most *Drosophila* ribosomal protein genes, *RpL3* is a single-copy gene. The ubiquitous expression of *RPL3* message is consistent with its role in general protein translation.

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