MOLECULAR AND CELLULAR BIOLOGY, Aug. 1994, p. 5412–5424
0270-7306/94/$04.00+0

Vol. 14, No. 8

La Proteins from *Drosophila melanogaster* and *Saccharomyces cerevisiae*: a Yeast Homolog of the La Autoantigen Is Dispensable for Growth

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Received 31 March 1994/Returned for modification 9 May 1994/Accepted 16 May 1994

The human autoantigen La is a 50-kDa protein which binds to the 3′ termini of virtually all nascent polymerase III transcripts. Experiments with mammalian transcription extracts have led to the proposal that the La protein is required for multiple rounds of transcription by RNA polymerase III (E. Gottlieb and J. A. Steitz, EMBO J. 8:851–861, 1989; R. J. Maraia, D. J. Kenan, and J. D. Keene, Mol. Cell. Biol. 14:2147–2158, 1994). Although La protein homologs have been identified in a variety of vertebrate species, the protein has not been identified in invertebrates. In order to begin a genetic analysis of La protein function, we have characterized homologs of the La protein in the fruit fly *Drosophila melanogaster* and the yeast *Saccharomyces cerevisiae*. We show that both the *Drosophila* and yeast La proteins are bound to precursors of polymerase III RNAs in vivo. The *Drosophila* and yeast proteins resemble the human La protein in their biochemical properties, as both proteins can be partially purified from cells by a procedure previously devised to purify the human protein. Similarly to vertebrate La proteins, the *Drosophila* and yeast homologs preferentially bind RNAs that terminate with a 3′ hydroxyl. Despite the fact that the La protein is conserved between humans and *Saccharomyces cerevisiae*, yeast cells containing a null allele of the gene encoding the La protein are viable, suggesting that another protein(s) plays a functionally redundant role.

Patients suffering from the autoimmune disorders systemic lupus erythematosus and Sjogren's syndrome often develop antibodies against the La antigen, a 50-kDa nuclear protein. The La protein binds to newly synthesized RNA polymerase III transcripts to form small ribonucleoprotein particles (RNPs). These La RNPs contain precursors of 5S rRNA and tRNA, newly synthesized 7SL RNA and U6 RNA, and the Y class of small cytoplasmic RNAs (9, 19, 39, 40). The La protein also forms RNPs with certain viral RNAs synthesized by RNA polymerase III. These viral RNAs include the adenovirus-encoded VAI and VAII RNAs and the Epstein-Barr virus EBER1 and EBER2 RNAs (24, 25, 41). The La protein is able to bind all these distinct RNAs because it prefers RNAs that terminate with the sequence UUU_{OH}, which is at the 3′ terminus of the vast majority of newly made RNA polymerase III transcripts (28, 38, 44).

The La protein has been proposed to function in the termination of transcription by RNA polymerase III (16, 17). In these experiments, transcription extracts depleted of the La protein lost the ability to synthesize polymerase III products (16). The residual RNAs synthesized in the absence of the La protein were slightly shorter at the 3′ end and appeared to be generated by stalled transcription complexes (17). These findings were incorporated into a model in which the La protein was required for the synthesis and release of full-length polymerase III transcripts. In a recent study, Maraia et al. (27) examined the transcription of polymerase III RNAs from immobilized DNA templates. They found that the addition of purified La protein increased release of these RNAs from the templates and stimulated multiple rounds of transcription on the immobilized DNAs. However, other groups have found that purified RNA polymerase III is capable of accurate transcription termination in the absence of other proteins (7, 11, 48) and that polymerase recycling and transcript release are efficient in the absence of the La protein (7).

The La protein has also been implicated in other processes. Terns et al. (46) described a small RNP consisting of the La protein and U6 snRNA in *Xenopus* oocytes and speculated that La protein binding might modulate the assembly of the U6 small nuclear RNA into splicing complexes. The La protein has also been found to enhance the fidelity of poliovirus translation in reticulocyte lysates and has been proposed to facilitate cap-independent translation of this mRNA (29).

It is our goal to undertake a genetic analysis of La protein function. A genetic approach will allow us to assay La protein function in vivo and to identify interacting molecules. As a first step in this analysis, we have characterized potential La protein homologs from the fruit fly *Drosophila melanogaster* and the yeast *Saccharomyces cerevisiae*. We show that both the fruit fly and yeast La proteins bind newly synthesized transcripts of RNA polymerase III and are similar to the mammalian La protein in their biochemical characteristics and RNA-binding properties. In addition, we report that the La protein homolog from *S. cerevisiae* is dispensable for growth, suggesting that other gene products are able to compensate for the loss of this protein.

MATERIALS AND METHODS

Isolation of a *Drosophila* La cDNA. Degenerate oligonucleotide primers corresponding to the RNP1 and RNP2 elements of the human La protein RNA recognition motif (8) were used to amplify a 144-bp fragment from an S2 cell cDNA library in λzapII (a gift of K. Matsuno and S. Artavanis, Yale Universi-

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ty). The primers had the sequences 5′-GTNTA(C/T)AT(A/C/T)AA(A/G)GGNTT(C/T)CC-3′ and 5′-(A/G)AA(A/G/T)ATNGANCC(C/T)TT(A/G)AA-3′. The 144-bp fragment was cloned into pCR II (Stratagene) and sequenced. The sequence of the PCR product was used to design a primer that, in conjunction with a library-specific primer, was used to amplify a cDNA fragment containing nucleotides 16 to 575 of the *Drosophila* cDNA (Fig. 1). The larger PCR product was used to probe the S2 cDNA library (10^6 plaques), and 82 positive clones were identified. Six of the positive plaques were purified and characterized. The clone with the largest cDNA insert, pDL77, was completely sequenced. As this cDNA did not contain an in-frame stop codon upstream of the first AUG, we obtained additional 5′ sequence by using an internal primer and a library-specific primer to amplify the 5′ ends of several of the original positive clones.

Fusion proteins and antisera. To prepare antibodies directed against the *Drosophila* La protein, pDL77 was digested with *Eco*RI and *Xho*I, and the insert was cloned into the *Eco*RI and *Xho*I sites of pTrcHis (Invitrogen). Recombinant fusion protein was induced and purified from *Escherichia coli* lysates under nondenaturing conditions with a ProBond column (Invitrogen) as described by the manufacturer. The purified protein was concentrated by ultrafiltration through Centriprep-10 units (Amicon) and used to immunize rabbits. The resulting antibodies were purified by affinity chromatography as described previously (12).

To prepare antibodies against the *LHP1* gene product, the oligonucleotides 5′-AGTGGATCCATGTCTGAAAAACCACAACAAGAGG-3′ and 5′-AACTGTAGTAGATCTCTACTTCTTATTTTTGGGGAATTTAGGC-3′ were used to amplify a DNA fragment encoding amino acids 1 to 252 of the predicted protein. After digestion with *Bam*HI and *Xho*I, the PCR product was inserted into the corresponding sites of the pTrcHis vector. Recombinant fusion protein was purified as described above and used to immunize rabbits.

Monoclonal anti-2,2,7-trimethylguanosine antibodies were purchased from Oncogene Sciences. Patient anti-La sera were gifts of J. Craft (Yale University).

Cells and strains. S2 cells were grown at 25°C in Schneider's *Drosophila* medium (Gibco) supplemented with 15% heat-inactivated fetal calf serum (Irvine Scientific), 100 U of penicillin per ml, and 100 μg of streptomycin (Gibco) per ml. HeLa cells were provided by S. DeGregorio (Yale University). The wild-type *Saccharomyces cerevisiae* was strain YPH501 (*MAT***a**/*MAT*α *ura3/ura3 lys2/lys2 ade2/ade2 trp1/trp1 his3/his3 leu2/leu2*) (43). The *S. cerevisiae* strain p46 carrying the *rna82-1* mutation (*MAT***a** *ade2 leu1 rna82-1*) was kindly provided by P. Piper (33).

Immunoprecipitation and immunoblotting. HeLa cells were washed three times in phosphate-buffered saline (PBS; 10 mM Na_2HPO_4 [pH 7.4], 1.76 mM KH_2PO_4, 2.7 mM KCl, and 136 mM NaCl) and resuspended in NET-2 (40 mM Tris HCl [pH 7.4], 150 mM NaCl, 0.05% Nonidet P-40). After sonication (three times for 15 s each with a Branson sonifier at setting 3), the extract was sedimented at 100,000 × *g* in a Beckman TLA100.3 rotor for 20 min. Drosophila S2 cell lysates were prepared from cells washed once in PBS, resuspended to a concentration of 5×10^7 cells per ml in NET-2 containing 10 mM vanadyl ribonucleoside complexes, and sonicated as described above. Yeast cells were grown in YPD (1% Bacto yeast extract, 2% Bacto Peptone, 2% dextrose) at 30°C to an optical density at 600 nm of 0.3, washed once in H_2O, and disrupted with glass beads in 100 μl of NET-2 plus 0.5 mM phenylmethylsulfonyl fluoride and 125 ng each of leupeptin, chymostatin, pepstatin, and antipain per ml. Following disruption, 900 μl of NET-2 was added and the lysates were sedimented at 14,000 × *g* for 30 min. Immunoprecipitations were performed as described previously (49). RNAs extracted from each sample were labeled at the 3′ end with [^{32}P]pCp and

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-61   TTTTCACACGTGTTTCAGTGAGCGCACATTTCAGTTCTTTGCGAAATTTTCACAGCTAAA
1     ATGGCCGAAGTTGCTGAAACCCCGAGTGTGGAAGCCCAGGAAGAGGTAGCACAGCCCGCC
      M  A  E  V  A  E  T  P  S  V  E  A  Q  E  E  V  A  Q  P  A   20
61    GAGGCGCAGGTGCTGGAGGCGAAGAACGGCGACGCCAAGAAGGACCCGGCTCCCGCAGCA
      E  A  Q  V  L  E  A  K  N  G  D  A  K  K  D  P  A  P  A  A   40
121   GAGGAAGCCGCTGGCGGCTTCACCAAACAGGAGCGCGCCATAATCCGGCAGGTAGAGTAC
      E  E  A  A  G  G  F  T  K  Q  E  R  A  I  I  R  Q  V  E  Y   60
181   TACTTCGGCGATGCCAATCTGAATCGGGACAAGTTTCTGCGCGAGCAGATCGGCAAGAAC
      Y  F  G  D  A  N  L  N  R  D  K  F  L  R  E  Q  I  G  K  N   80
241   GAGGACGGCTGGGTGCCCCTGTCGGTGCTCGTTACCTTCAAGAGATTGGCCTCCCTGTCC
      E  D  G  W  V  P  L  S  V  L  V  T  F  K  R  L  A  S  L  S  100
301   ACGGACTTATCTGAGATCGTGGCTGCCCTGAACAAATCCGAGGAGGGCCTCGTGGAGATT
      T  D  L  S  E  I  V  A  A  L  N  K  S  E  E  G  L  V  E  I  120
361   AGCGAAGACAAACTTAGCCTGCGTCGCCATCCGGAGCGTCCTATTCCAGAGCACAACGAA
      S  E  D  K  L  S  L  R  R  H  P  E  R  P  I  P  E  H  N  E  140
421   GAGCGGCGCAAGGAGATCCAGGAGCGGACCGCATACGCCAAGGGCTTCCCCCTAGATTCG
      E  R  R  K  E  I  Q  E  R  T  A  I  A  H  G  F  P  L  D  S  160
481   CAGATTAGCGAACTGCTCGACTTTGCTGCCAACTACGACAAAGTGGTCAACCTGACCATG
      Q  I  S  E  L  L  D  F  A  A  N  Y  D  K  V  V  N  L  T  M  180
541   CGCAAGCACTATGACAAGCCCACCAAGTCGTACAAGTTCAAGGGCAGCATCTTCCTTACT
      R  K  H  Y  D  K  P  T  K  S  Y  K  F  K  G  S  I  F  L  T  200
601   TTCGAGACCAAGGACCAGGCAAAGGCCTTCCTCGAACAGGAGAAGATCGTTTATAAGGAG
      F  E  T  K  D  Q  A  K  A  F  L  E  Q  E  K  I  V  Y  K  E  220
661   CGCGAGCTGCTGCGTAAATGGCAGGTGGACTACCTTAAGGAGAAGCAGGAGGAATACGCC
      R  E  L  L  R  K  W  Q  V  D  Y  L  K  E  K  Q  E  E  Y  A  240
721   CAGAAGAACGAGAAGCGCAAAAACAAAAAGGAGGCCAAGCCTGAGCCTGCCTTCGAGCTG
      Q  K  N  E  K  R  K  N  K  K  E  A  K  P  E  P  A  F  E  L  260
781   CCCAAGAACGCCATTGTTGTCTTCGAGGGCGCTCCGGAGACATCTAGCCGCGAGGAAATT
      P  K  N  A  I  V  V  F  E  G  A  P  E  T  S  S  R  E  E  I  280
841   CGCGAAGCCTTCGAGAAGATCAAGGACTTCGAGGTGGCCTACATTGAGTTCGCCAAGGGC
      R  E  A  F  E  K  I  K  D  F  E  V  A  Y  I  E  F  A  K  G  300
901   GAAACCAAGGGCTCTGTGCGGCTGACGGAGGCCGATGCGGCCGAAAAGTACATTGCCAAG
      E  T  K  G  S  V  R  L  T  E  A  D  A  A  E  K  Y  I  A  K  320
961   GTGGAGGAAGGCAAGCTCAAGTTCAAAGATGAGGTCTCTCTGTCGCTGCGAAAGGCCACC
      V  E  E  G  K  L  K  F  K  D  E  V  S  L  S  L  R  K  A  T  340
1021  GAGGAGGAGGAGAAGGAGTTCATCGACAAGGCCATAGAATTCATGAAGAAGCGACGCGAT
      E  E  E  E  K  E  F  I  D  K  A  I  E  F  M  K  K  R  R  D  360
1081  TTCACCAGGAACAAGGGCAAGCGCTTCAATCGCAAGCGCCACGGCGGAAACGACCACAAA
      F  T  R  N  K  G  K  R  F  N  R  K  R  H  G  G  N  D  H  K  380
1141  CATGGCGGTGGTAAGAAGGCGCGTGGGGATTAACTCATCCACCTATATTCCGCCACCCGA
      H  G  G  G  K  K  A  R  G  D  *                              391
1201  GTCTTTTGATCGCAGTAGACCAAGCTTAATTGTTACTCGGTAGATCTAGTTGACCTAGCT
1261  TTAAGGCCTTACATATATAATACTTGCTTTCTCTTAGTTTTCAAAATAATCGTTGCAGGT
1321  ACAGAATATTCTTCAAATATGCATGTATAGTCTGTTACCTAAATAACGAGGTGGGCTCTA
1381  ATTTATTTTACATAGAAGACTATGAATCCAATTGTTTCCCTCAGCAGAAAGCTACAAATA
1441  AAGACCAGTGTGTAAAACCTGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA  1491
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FIG. 1. Nucleotide sequence of a *Drosophila* La cDNA and predicted amino acid sequence of the *Drosophila* La protein. Nucleotides are numbered to the left and amino acids are numbered to the right of each line. An upstream in-frame stop codon before the first methionine is underlined, and the 81 amino acids comprising the RRM (3, 37) are boxed. Two conserved amino acid sequence motifs, RNP1 and RNP2, are enclosed by shaded boxes.

T4 RNA ligase (13) and fractionated in 5% polyacrylamide–8 M urea gels.

For immunoblotting, cell extracts were fractionated in sodium dodecyl sulfate (SDS)-polyacrylamide gels and transferred to nitrocellulose. The membranes were blocked for 1 h in 5% dried milk in TBS-T (10 mM Tris-HCl [pH 7.5], 150 mM NaCl, 0.1% Tween 20) and incubated with antisera (diluted 1:1,000 in blocking buffer) for 1 h. After three washes in TBS-T, the filter was incubated for 1 h with a horseradish peroxidase-linked secondary antibody (Amersham) diluted 1:30,000 in blocking buffer. After three washes, the membranes were incubated in ECL (Amersham) detection reagents as described by the manufacturer.

Purification of the *Drosophila* La protein from S2 cells. S2 cells (1.6×10^9 cells) were resuspended at 10^8 cells per ml in buffer A (25 mM Tris HCl [pH 8.0], 0.1 mM EDTA, 0.5 mM dithiothreitol, 3 mM $MgCl_2$)–0.1 M NaCl–125 ng each of leupeptin, chymostatin, pepstatin, and antipain per ml–0.5 mM phenylmethylsulfonyl fluoride. After sonication, the cell extract was sedimented at $15,000 \times g$ in a Sorvall SS34 rotor for 1 h at 4°C. A 1-ml aliquot of the supernatant was incubated with 0.5 ml of insoluble pancreatic RNase (Sigma) for 2 h at 4°C and applied to a 1-ml column of DEAE Sepharose (Pharmacia). The column was washed with 3 ml of buffer A–0.1 M NaCl. The flowthrough and wash fractions were collected and applied to a 1-ml column of poly(U)-Sepharose (Pharmacia). After washing with buffer A–0.1 M NaCl, the column was eluted in steps (3 ml each) with buffer A containing either 0.5, 1.0, or 2.0 M NaCl. After analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), fractions from the poly(U)-Sepharose column which contained nearly pure *Drosophila* La protein were dialyzed against buffer A–0.1 M NaCl and concentrated with a Centricon 10 unit (Amicon) for the UV cross-linking experiments.

Fractionation of yeast extracts. One-liter cultures of haploid YPH501 cells or haploid YPH501 cells carrying the *lhp1*::*LEU2* allele were grown in YPD at 30°C to an optical density at 600 nm of 1.0. Cells were harvested, washed twice in 50 ml of 0.1 M Tris HCl (pH 7.5), and resuspended in 25 ml of zymolyase buffer (1.2 M Sorbitol, 0.1 M Tris-HCl [pH 7.5], 10 mM EDTA, 10 mM dithiothreitol). After a 10-min incubation on ice, 10 mg of zymolyase 100T (ICN) was added and the incubation was continued for an additional 20 min at 30°C with gentle agitation. The spheroplasts were pelleted at $1,500 \times g$ in a Sorvall SS34 rotor and washed twice with 25 ml of zymolyase buffer (lacking dithiothreitol). The spheroplasts were then resuspended in 25 ml of buffer A plus protease inhibitors and phenylmethylsulfonyl fluoride as described above and sonicated. The spheroplast lysate was sedimented at $15,000 \times g$ in a Sorvall SS34 rotor for 1 h at 4°C, the lipid layer was discarded, and the supernatant was collected. Aliquots were frozen in liquid nitrogen and stored at −70°C. Fractionation of the haploid cell extract was carried out as described for the *Drosophila* La protein.

UV cross-linking. A construct in which the mouse U6 RNA coding sequence was placed behind a T7 promoter (5) was a gift of D. Wassarman. ^{32}P-labeled U6 RNA was synthesized in vitro with T7 RNA polymerase as described elsewhere (50), except that transcription was carried out with 2 μg of linearized template, 1 mM each ATP, GTP, and CTP, and 50 μCi of [α-^{32}P]rUTP (Amersham; 400 Ci/mmol) in place of rUTP. Competitor U6 RNA was synthesized by use of 1 mM (each) all four nucleoside triphosphates. Synthesis of competitor U6 RNA was monitored by addition of 5 μCi of [α-^{32}P]rUTP to the reaction. The efficiency of transcription was monitored by trichloracetic acid precipitation of RNA and liquid scintillation counting. The transcript used for cross-linking was labeled to a specific activity of 88,000 to 100,000 cpm/ng, while the competitor RNA had a specific activity of 100 cpm/ng. Control experiments, in which the low-specific-activity competitor RNA was used in the initial cross-linking experiment, resulted in no detectable label transfer to any proteins. Whitfield degradation of competitor U6 RNA, which removes the last nucleotide of the RNA and leaves a terminal phosphate by an oxidation–β-elimination reaction, was performed as described previously (44), except that released terminal nucleotides were removed by centrifugation through G25 Sephadex columns (Boehringer Mannheim) prior to extraction with phenol–chloroform–isoamyl alcohol (50:49:1) and ethanol precipitation. Following Whitfield degradation, the competitor RNA was subjected to gel electrophoresis to verify that it remained intact.

For cross-linking, U6 RNA (60,000 to 100,000 cpm) was mixed together with 10 μg of *E. coli* tRNA and incubated with either S2 or yeast cell extracts (5 μl), purified *Drosophila* La protein (50 to 100 ng), or yeast column fractions (~5 μg of total protein) for 10 min on ice. When necessary, a 100-fold molar excess of competitor U6 RNA was added to the reaction mixture. Final volumes of 20 μl for all reactions were maintained with the addition of buffer A–0.1 M NaCl. After incubation, samples were irradiated with UV light (254 nm) at a distance of 5 to 10 cm for 10 min. Cross-linked samples were digested and analyzed as described elsewhere (30).

Indirect immunofluorescence. S2 cells were dispersed on coverslips and allowed to attach overnight in Schneider's medium supplemented as described above. After being washed with PBS, cells were fixed in PBS containing 2% paraformaldehyde at 25°C for 10 min. Cells were then washed three times in PBS, and autofluorescence was quenched by incubating the coverslips in 50 mM NH_4Cl for 15 min. Cells were permeabilized and blocked in 0.5% Triton X-100–1% goat serum (Jackson Immunochemicals). Primary antibodies were diluted 1:1,000 in 0.5% Triton X-100–1% goat serum and incubated with the cells for 30 min to 1 h at 25°C. After being washed with PBS, fluorescein isothiocyanate-conjugated goat anti-rabbit or goat anti-mouse antibodies were diluted 1:100 in 0.5% Triton X-100–1% goat serum and incubated with the cells for 30 min to 1 h at 25°C. After being washed, cells were mounted in 10 mM Tris (pH 8.0)–0.5% *n*-propyl gallate–90% glycerol. Cells were examined with a Bio-Rad MRC-600 scanning laser confocal microscope. Actin filaments were visualized by staining with rhodamine-conjugated phalloidin (Molecular Probes) as described by the manufacturer.

In situ hybridization. Salivary gland chromosomes from Oregon R larvae were prepared for in situ hybridization as described elsewhere (2). The pDL77 plasmid was labeled with biotin-11-dUTP (Boehringer Mannheim), hybridized to the chromosomes, and detected as described elsewhere (2).

***LHP1* gene disruption.** Subclone pN57 (31) was a gift of M. Nagiec (University of Kentucky). The 2.7-kb insert was excised with *Sal*I and *Bgl*II, cloned into the *Sal*I and *Bam*HI sites of pBluescript KSII (Stratagene), and sequenced from nucleotide 1118 to the *Sal*I site (see Fig. 2 in reference 31).

To create a null allele of *LHP1*, we digested our plasmid subclone with *Pfl*MI and *Cla*I to excise the open reading frame encoded by *LHP1*. After all staggered ends were filled in with T4 DNA polymerase, the Bluescript vector containing the sequences flanking the *LHP1* gene was ligated to a *Aat*II-*Pvu*II fragment containing the *LEU2* gene from pRS305 (43). The resulting plasmid, pKOIII, was digested with *Sal*I and *Xba*I, and the 4.6-kb insert was isolated and transformed into competent *S. cerevisiae* YPH501 (43). Individual transformants

were selected on minimal medium lacking leucine. To identify homologous recombination events at the *LHP1* locus, DNA was prepared from Leu$^+$ transformants, digested with *Bgl*II and *Sal*I, and analyzed by Southern blotting. Genomic Southern blots were probed with an antisense transcript derived from transcription of the insert from pN57 in pBluescript KSII by use of the T7 promoter. Haploid segregants were obtained by sporulation and dissection of the resulting tetrads.

Nucleotide sequence accession numbers. The sequence of the *Drosophila* La cDNA has been assigned GenBank accession number L32988. The complete sequence of the *LHP1* gene has been assigned accession number L33023.

RESULTS

Cloning of the *Drosophila* La protein. The La protein is a member of the family of RNA-binding proteins that contain a conserved 80-amino-acid sequence known as the RNA recognition motif (RRM) or RNA-binding domain (3, 37). To identify a homolog of the mammalian La protein from *D. melanogaster*, we designed degenerate primers for use in PCR which corresponded to the two highly conserved elements of this domain, RNP1 and RNP2. As the exact sequence of the RNP1 and RNP2 elements varies between individual members of the RRM family of proteins, we reasoned that primers corresponding to the mammalian La RNP1 and RNP2 sequences might specifically amplify a cDNA encoding the *Drosophila* La protein. A fragment of the predicted size (144 nucleotides) was amplified from a *Drosophila* S2 cDNA library and sequenced. Although an open reading frame encoded by this fragment showed significant homology to the mammalian and *Xenopus* La proteins (8, 10, 42), the most-conserved regions corresponded to the primer sequences. We therefore verified that we had identified a cDNA encoding the *Drosophila* La protein by using a primer derived from the PCR product, in conjunction with a library-specific primer, to amplify a DNA fragment encoding the N terminus of the protein. Partial sequencing of the N-terminal fragment revealed extended homology between the human La protein and the *Drosophila* sequence. We then used the N-terminal PCR product as a probe to isolate a full-length cDNA clone from the same library. The primary sequence of this clone, shown in Fig. 1, encodes a protein of 390 amino acids with a predicted molecular mass of 45 kDa.

An initial search of the database with the *Drosophila* sequence revealed extensive homology to La protein sequences from *Xenopus laevis* and several mammalian species (8, 10, 42, 47). Figure 2A shows an alignment of the *Drosophila* protein with the human and one of the two *Xenopus* La proteins (two *Xenopus* cDNAs that are 91% identical have been described [42]). Although the three proteins are 26% identical overall, the conservation is especially striking in the N-terminal portion of the proteins (*Drosophila* La residues 54 to 137). In this region, the proteins are 52% identical. On the basis of sequence conservation, the La protein can be divided into three regions: a highly conserved N-terminal portion, a less well conserved RNA recognition motif (indicated by the hatched bar in Fig. 2), and a highly charged C-terminal portion.

The cytological location of the gene encoding the *Drosophila* La protein homolog was determined by in situ hybridization of the cDNA clone to polytene chromosomes (Fig. 3). A single band of hybridization was detected on the left arm of chromosome 2, at locus 38C (arrows in Fig. 3A and B). We confirmed that the La protein was indeed encoded by a single-copy gene by performing Southern blot analysis on *Drosophila* genomic DNA (data not shown). Several P element insertions, as well as a number of mutations, are located in the same region as the gene encoding *Drosophila* La (21, 26). Further work will be required to determine if any of these mutations are in the gene encoding the *Drosophila* La protein.

The *Drosophila* La protein binds a precursor to 5S rRNA in vivo and is located in the nucleus. To establish that the protein encoded by the *Drosophila* cDNA was a genuine homolog of the mammalian La protein, we raised rabbit antibodies against a fusion protein containing the *Drosophila* La protein linked to polyhistidine. To characterize these antibodies, protein immunoblots were performed (Fig. 4B). These antibodies recognized a single polypeptide in *Drosophila* S2 cell lysates (Fig. 4B, lane 3) that migrated slightly more slowly than the human La protein (lane 6). We also noted that our antibodies against the *Drosophila* La protein do not recognize the human La protein (Fig. 4, lane 4) and that patient antibodies do not cross-react with the *Drosophila* La protein (lane 5), suggesting that the antigenic epitopes recognized by both sera are not conserved between humans and flies.

Because the protein detected on immunoblots migrated at an apparent molecular mass (52 kDa) that was somewhat larger than the predicted molecular mass, we examined the migration of the predicted protein after in vitro translation. As our cDNA was inserted into a pBluescript SKII vector, we prepared a synthetic mRNA by transcription with T3 RNA polymerase. When the in vitro-synthesized mRNA was translated in a rabbit reticulocyte lysate, the major translation product comigrated with the *Drosophila* protein detected on immunoblots (data not shown).

To identify the small RNAs bound by the *Drosophila* protein in vivo, we performed immunoprecipitations from S2 cells and examined the RNAs contained within the immunoprecipitate by labeling with [^{32}P]pCp (Fig. 4A, lane 6). We compared these RNAs with the RNAs present in an anti-La immunoprecipitate from human HeLa cells (Fig. 4A, lane 3). The heterogeneous mixture of small RNAs present in anti-La immunoprecipitates from HeLa cells includes tRNA precursors (migrating faster than 5S rRNA in lane 3) and precursors of 5S rRNA containing additional U residues at the 3′ end (39). The affinity-purified rabbit antibodies directed against the *Drosophila* protein also immunoprecipitate RNPs containing a heterogeneous spectrum of small RNAs (Fig. 4A, lane 6). The majority of RNAs in the immunoprecipitate migrate between 5S rRNA and mature tRNA on the gel, consistent with the migration of precursor tRNAs. To establish the identity of some of the RNAs, we excised three of these RNAs from the gel and subjected them to enzymatic RNA sequencing. One of these species (arrow, Fig. 4A) was identified as the 135-nucleotide precursor of *Drosophila* 5S rRNA (20), which terminates in the sequence $CUUUU_{OH}$. The other two species that were partially sequenced (indicated by asterisks in Fig. 4A) terminate in a stretch of uridine residues and appear to be derived from a single RNA, as the 3′ sequences were identical (51). As the partial sequences of these two RNAs did not match any sequences in the nucleic acid data base, they appear to be derived from a previously uncharacterized small RNA.

We examined the cellular location of the *Drosophila* La protein in S2 cells by indirect immunofluorescence. The protein appeared confined to the nucleus, and significant cytoplasmic staining was not detected (Fig. 5C). As a control, the same cells were stained with rhodamine-conjugated phalloidin, which stains filamentous actin. In this case, cytoplasmic staining was evident (Fig. 5D).

Purification and RNA-binding properties of the *Drosophila* La protein. Because the human and *Drosophila* proteins are

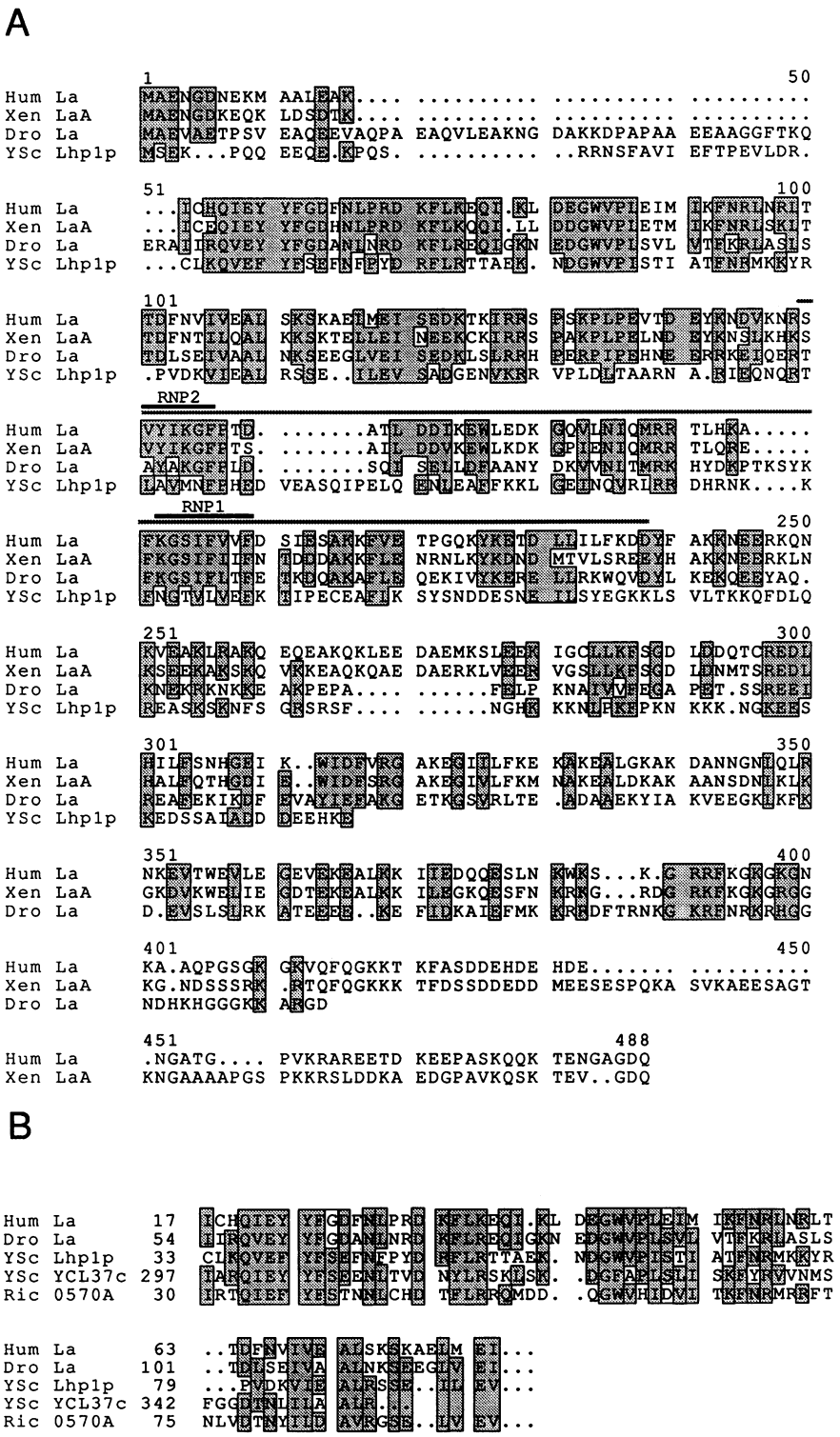

FIG. 2. Alignment of La protein homologs. (A) Comparison of human (8), *X. laevis* (42), *D. melanogaster*, and *S. cerevisiae* (YSc Lhp1p) La protein homologs. Comparisons were created with the Genetics Computer Group software Pileup program and then further aligned manually. Alignment gaps are indicated by dots. Similar or identical amino acids which are conserved among at least three of the four proteins are shaded and boxed, according to the following similarity rules: A = G, L = I = V, F = Y = W, H = R = K, and D = E (45). The RNA recognition motif (3, 37) is indicated by a hatched bar above the aligned sequences. The RNP1 and RNP2 motifs are denoted by black bars above the sequences. (B) Alignment of a highly conserved region in the N terminus of the La proteins encoded by two putative open reading frames in yeast genomic DNA and a rice cDNA. The conserved N-terminal regions of the human, *Drosophila*, and *S. cerevisiae* La proteins were aligned manually with part of YCL37c, a hypothetical protein encoded by an open reading frame on chromosome III of *S. cerevisiae* (32). A similar region from a partially sequenced rice cDNA (Ric 0570A) (23) is also aligned above. Amino acids which are similar in at least three sequences are boxed and shaded.

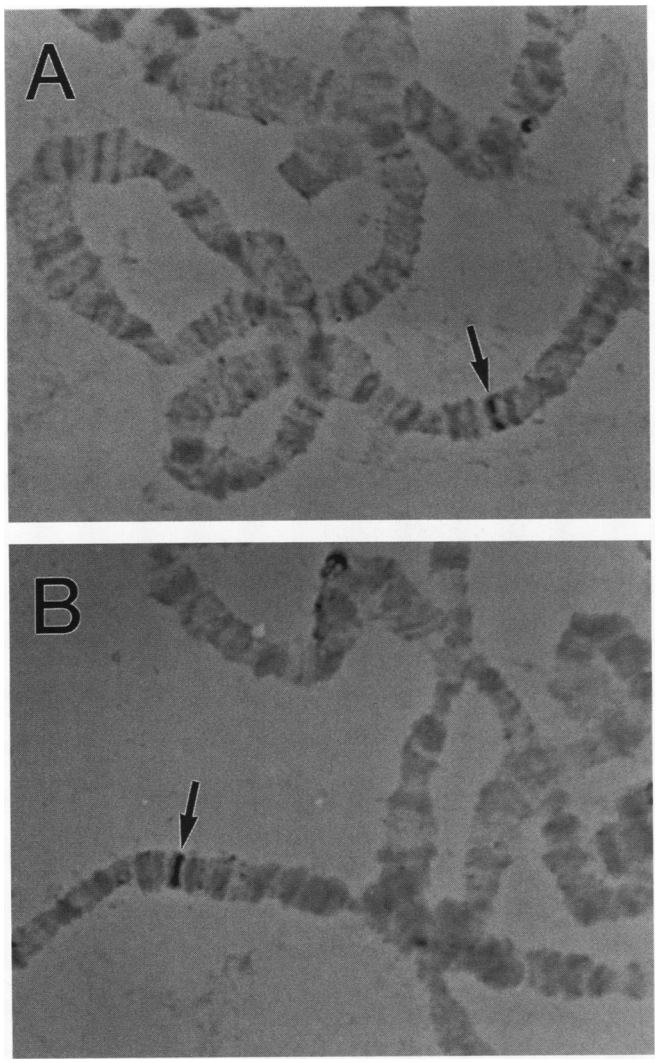

FIG. 3. Cytological localization of the *Drosophila* La cDNA. A biotinylated *Drosophila* La cDNA was hybridized to polytene chromosomes and detected by peroxidase staining with horseradish peroxidase-streptavidin. Hybridization signals are indicated by arrows. Panels A and B are examples of two different chromosome squashes. The positive signals map to position 38C on the left arm of chromosome 2.

only 27% identical, we wondered if the two proteins would exhibit similar biochemical and RNA-binding characteristics. We therefore attempted to purify the *Drosophila* protein by a procedure similar to that devised by Stefano (44) to purify the La protein from HeLa cells. We assayed for the *Drosophila* La protein by protein immunoblotting (Fig. 6B). The *Drosophila* La protein fractionates into two peaks on DEAE Sepharose (Fig. 6B, lanes 2 and 3), as has been described for the human protein. (The fraction of the human protein that binds to the DEAE Sepharose column is complexed with RNA [44]). When the DEAE flowthrough fraction is applied to a poly(U)-Sepharose column, the major protein species eluting between 1.0 and 2.0 M NaCl is the *Drosophila* La protein (Fig. 6A and B, lane 7).

The vertebrate La protein prefers to bind RNAs that contain a uridylate stretch at the 3′ end (28, 38, 44). Converting the 3′ hydroxyl of a substrate RNA to a 3′ phosphate dramatically decreases binding by the vertebrate La protein (44, 46). We therefore examined the ability of the *Drosophila* protein to distinguish between RNAs containing 3′ hydroxyls and 3′ phosphates in an assay similar to that described by Terns et al. (46). In these experiments, we synthesized ^{32}P-labeled mouse U6 RNA in vitro using T7 RNA polymerase and examined the proteins that could be cross-linked with UV light to the labeled RNA. Proteins in close contact with the RNA substrate were visualized by label transfer (Fig. 6C). Although a number of proteins in the *Drosophila* S2 cell extract were cross-linked to the radiolabeled U6 RNA, the major cross-linked product migrated at 52 kDa, as would be expected for the *Drosophila* La protein (Fig. 6C, lane 1). When the cross-linking was performed with a 100-fold excess of competitor U6 RNA in which the 3′ hydroxyl had been converted to a 3′ phosphate, only the 52-kDa protein bound the labeled RNA (Fig. 6C, lane 2). Thus, the 52-kDa protein was the only protein in the cell extract that preferentially bound U6 RNA containing a 3′ hydroxyl. To determine if the 52-kDa protein corresponded to the *Drosophila* La protein, we subjected the partially purified protein to the same cross-linking assay (Fig. 6C, lanes 3 to 5). Similarly to the 52-kDa band detected in the cell extracts, the partially purified La protein can be cross-linked to the labeled U6 small nuclear RNA (lane 3). The addition of competitor U6 RNA containing a 3′ hydroxyl (Fig. 6C, lane 4) but not U6 RNA containing a 3′ phosphate (lane 5) largely eliminated binding of the labeled RNA by the *Drosophila* La protein. Thus, the *Drosophila* homolog exhibits RNA-binding properties very similar to those of the previously characterized vertebrate La proteins.

Two *S. cerevisiae* proteins and a rice protein have homology with La proteins. During our characterization of the *Drosophila* La protein, we became aware of several predicted proteins in the databases that appeared to be related to La proteins. We and others (23) have noted that a potential protein, YCL37c, encoded by an open reading frame in chromosome III of the yeast *S. cerevisiae*, contains significant homology to the highly conserved N-terminal region of the *Drosophila* and vertebrate La proteins (shown in Fig. 2B). This presumably reflects a shared sequence motif, as the conserved region is located towards the C-terminus of YCL37c, while it is located at the N-terminus of the known La proteins. YCL37c does not exhibit other sequence homologies with La proteins and lacks an RRM. Another predicted polypeptide that appears related to La proteins is encoded by a rice cDNA-expressed sequence tag (23). The 381-nucleotide sequence deposited in the database could encode a rice protein with an N-terminus related to that of the known La proteins (Fig. 2B).

A third sequence in the database was a promising candidate for a La protein homolog in the yeast *S. cerevisiae*. A partially sequenced open reading frame upstream of the *SLC1* gene, designated *ORFX*, had been described as having no homology to previously sequenced proteins (31). This potential polypeptide appeared to be related to La proteins. We completed the sequence of the open reading frame and found that it could encode a protein of 275 amino acids and 32-kDa molecular mass that was 23% identical and 34% similar to the *Drosophila* La protein (shown in Fig. 2A). We have therefore renamed this gene *LHP1* (La-homologous protein). While the predicted protein appears to be most related to the other La proteins in the N-terminal domain, the homology extends through the RRM and into the C-terminus. Although the C-terminal domain of the yeast protein is truncated, compared with the known La proteins, it is also highly charged. For both the yeast and the *Drosophila* La proteins, nearly 50% of the amino acids carboxy-terminal to the RRM are charged (Fig. 2A).

To identify the product of the *LHP1* gene (Lhp1p), antibod-

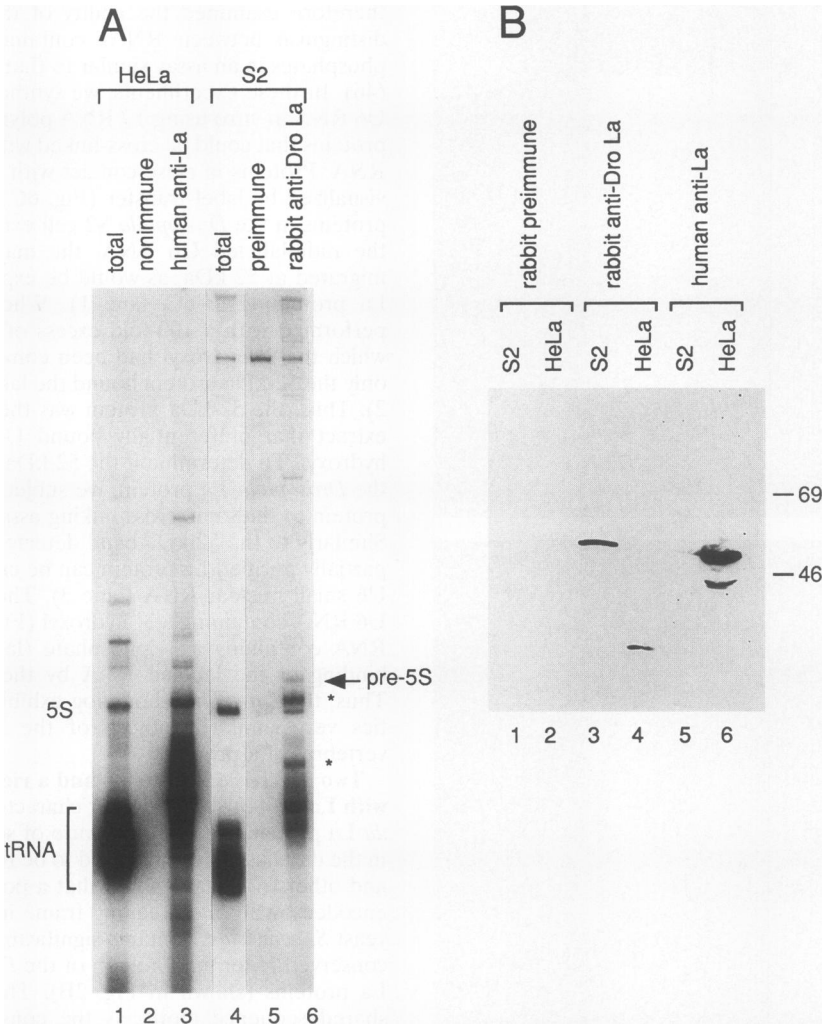

FIG. 4. Antibodies directed against the *Drosophila* La protein immunoprecipitate a precursor to 5S rRNA and recognize a 52-kDa protein in S2 cell extracts. (A) A HeLa cell extract was divided into equal aliquots and either extracted with phenol (lane 1) or mixed with human nonimmune (lane 2) or human anti-La (lane 3) serum. A *Drosophila* S2 cell extract was also divided equally and either extracted with phenol (lane 4) or mixed with either rabbit preimmune serum (lane 5) or rabbit anti-*Drosophila* La antibodies (lane 6). RNAs contained within immunoprecipitates (lanes 2, 3, 5, and 6) and 2% of total RNA (lanes 1 and 4) were end labeled with [^{32}P]pCp and fractionated in 5% polyacrylamide–8 M urea gels. The arrow indicates the precursor to *Drosophila* 5S rRNA. The asterisks on the right denote two additional RNAs which were partially sequenced. (B) *Drosophila* S2 cell extract (lanes 1, 3, and 5) and HeLa cell extract (lanes 2, 4, and 6) were subjected to immunoblot analysis as described in Materials and Methods. The blots were probed with either rabbit preimmune (lanes 1 and 2), rabbit anti-*Drosophila* La (lanes 3 and 4), or human anti-La (lanes 5 and 6) serum. The faster-migrating band recognized by the human anti-La serum in HeLa extracts (lane 6) is a degradation product of the human La protein (44). The identity of the band in lane 4 is unknown.

ies were generated against a fusion protein containing a portion of Lhp1p linked to polyhistidine (see Materials and Methods). Although immunoblots of yeast cell extracts probed with antisera from three different rabbits revealed a number of immunoreactive polypeptides, the major protein recognized by all antisera and not by preimmune sera was a polypeptide that migrated on gels with a molecular size of approximately 38 kDa (data not shown, but see Fig. 7C, lanes 2 and 3, arrow). Although the yeast protein migrates with a larger apparent molecular weight than predicted, this was true for the *Drosophila* La protein as well. We believe that this protein is the product of the *LHP1* gene, as it is absent from haploid strains lacking this gene (see below).

***LHP1* is a dispensable gene.** To determine if *LHP1* is an essential gene, we constructed a null allele of the gene in vitro, in which the gene was completely replaced with *LEU2* (Fig. 7A). The null allele was used to displace one chromosomal copy of the gene in diploid strain YPH501 (43). We confirmed that one of the two wild-type *LHP1* alleles had been replaced by the *lhp1*::*LEU2* allele by subjecting Leu$^+$ transformants to genomic Southern blot analysis (Fig. 7B). After sporulation of the diploid strain, 10 tetrads were dissected, all of which contained four viable spores. To confirm that the Leu$^+$ spores lacked Lhp1p, we prepared cell extracts from the haploid strains and analyzed them for the presence of Lhp1p by protein immunoblotting. As shown in Fig. 7C, haploid strains containing the *lhp1*::*LEU2* allele did not contain the 38-kDa protein (compare lanes 1 and 4 with lanes 2 and 3).

Lhp1p resembles other La proteins in its biochemical characteristics and RNA-binding properties. The result that

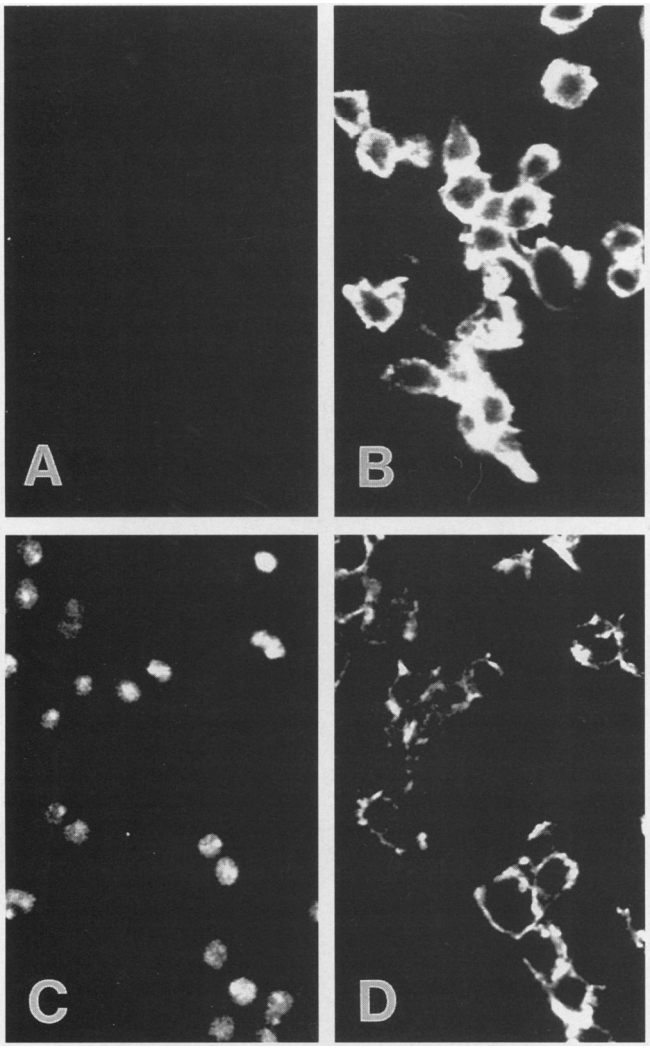

FIG. 5. Indirect immunofluorescence of S2 cells. S2 cells grown on coverslips were stained with either rabbit preimmune sera (A) or rabbit anti-*Drosophila* La antibodies (C). Both sets of coverslips were then stained with rhodamine-conjugated phalloidin (B and D, respectively) to detect filamentous actin.

cells lacking *LHP1* are viable was unexpected. We therefore characterized Lhp1p to determine if this protein is similar to known La proteins by examining its RNA-binding characteristics. First, we subjected yeast extracts to the same fractionation procedure that we used to partially purify the *Drosophila* La protein from S2 cells and assayed for Lhp1p by immunoblotting. As shown in Fig. 8A, Lhp1p behaves remarkably similarly to the *Drosophila* and human La proteins in this procedure. Lhp1p separates into two fractions on DEAE Sepharose and binds tightly to poly(U)-Sepharose. However, SDS gel analysis of the proteins eluted with 1.0 and 2.0 M NaCl from the poly(U)-Sepharose column revealed that these fractions contained numerous protein species in addition to Lhp1p (data not shown).

We next used our UV cross-linking assay to determine if Lhp1p preferentially binds RNAs containing 3′ hydroxyls. When we performed the cross-linking analysis on total yeast extracts, the major protein that cross-linked to the ^{32}P-labeled U6 RNA had a mass of approximately 55 kDa and we were unable to detect any cross-linking to Lhp1p (Fig. 8B, lanes 1 to 3). However, when the poly(U)-Sepharose fractions were subjected to the UV cross-linking assay, a 38-kDa protein in the 1.0 M NaCl eluate cross-linked to the labeled U6 RNA (Fig. 8B, lanes 4 to 6). This cross-linked protein was most likely Lhp1p, as it was not detected when the experiment was performed with the yeast strain lacking the *LHP1* gene (data not shown). Cross-linking of the 38-kDa protein was abolished by the addition of excess competitor U6 RNA containing a 3′ hydroxyl (Fig. 8B, lane 6), but not when the competitor U6 RNA contained a 3′ phosphate (lane 5). Although other proteins in the fraction were also cross-linked to the labeled U6 RNA, binding of these proteins was not inhibited by the addition of excess competitor RNA (Fig. 8B, compare lanes 4 and 6). Taken together, these results indicated that Lhp1p is most likely a yeast homolog of the La autoantigen.

Lhp1p binds nascent polymerase III RNAs in vivo. To verify that Lhp1p was indeed a yeast La protein, we performed immunoprecipitations from cell extracts and examined the small RNAs associated with Lhp1p. We examined both wild-type yeast cells and an *rna82-1* mutant strain, which is defective in the processing of 5S rRNA precursors (33). A complex pattern of small RNAs was contained within the immunoprecipitates from both the wild-type and *rna82-1* mutant extracts (Fig. 9, lanes 2 and 5). The majority of these RNAs were not present when immunoprecipitations were performed from cells lacking Lhp1p (Fig. 9, lane 8). Many of the immunoprecipitated RNAs migrated between 5S rRNA and mature tRNA, as would be expected for precursor tRNAs. While the patterns of immunoprecipitated RNAs from the wild-type and *rna82-1* mutant extracts were similar, a band migrating just above 5S rRNA was enriched in the immunoprecipitates from the mutant cells (Fig. 9, lane 2). Enzymatic sequencing revealed that this species was the 132- to 134-nucleotide precursor to yeast 5S rRNA (34). The comigrating RNA in the immunoprecipitates from wild-type cells (Fig. 9, lane 5) was also identified as the 5S rRNA precursor. In addition, the prominent RNA migrating near the top of the gel was found to be scR1, the RNA component of the yeast signal recognition particle (14, 18). This RNA is the *S. cerevisiae* homolog of the mammalian 7SL RNA and is believed to be transcribed by RNA polymerase III (14). Identification of the many other RNAs contained within the immunoprecipitates is the subject of ongoing studies.

DISCUSSION

The La protein associates with virtually all newly synthesized polymerase III transcripts in vertebrate cells. Experiments in which the La protein was depleted from transcription extracts (16) or added to isolated transcription complexes (27) have led to the proposal that the La protein functions in the termination of transcription by RNA polymerase III. To allow us to undertake a genetic analysis of La protein function, we identified and characterized homologs of the vertebrate La protein from the fruit fly *D. melanogaster* and the yeast *S. cerevisiae*. This analysis has revealed that the La protein is a conserved and ubiquitous component of eukaryotic cells. Surprisingly, yeast cells containing a disruption of the gene encoding the La protein homolog are fully viable, suggesting that in *S. cerevisiae* another gene product may compensate for the loss of this protein.

Although La protein homologs were not previously cloned from invertebrates, the presence of La-like proteins in flies and lower eukaryotes has been inferred from biochemical experiments (6, 15, 35). Francoeur et al. (15) identified a 46-kDa

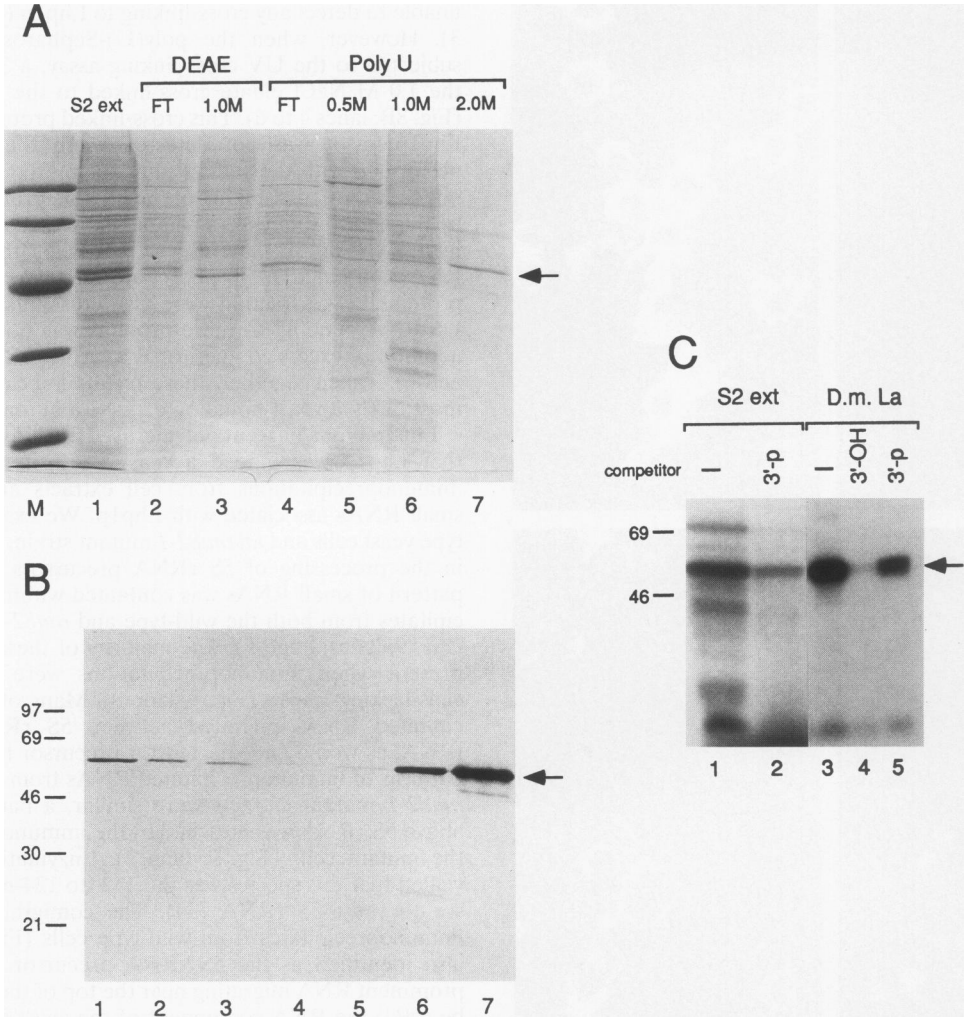

FIG. 6. Purification and RNA-binding characteristics of the *Drosophila* La protein. (A) An S2 cell extract was fractionated by chromatography on DEAE-Sepharose and poly(U)-Sepharose. Fractions from the various purification steps were stained with Coomassie blue. FT, flowthrough fractions. Approximately equal amounts of protein were loaded in lanes 2 to 6. Lane M, molecular mass standards. (B) A gel run in parallel to that shown in panel A was subjected to immunoblotting with the antisera directed against the *Drosophila* La protein. Molecular masses (in kilodaltons) are indicated at the left. Numbered lanes correspond in panels A and B. (C) UV light-induced cross-linking of the *Drosophila* La protein to U6 small nuclear RNA. ^{32}P-labeled U6 RNA was synthesized by use of T7 RNA polymerase, mixed with either S2 cell extract (lanes 1 and 2) or partially purified *Drosophila* La protein (lanes 3 to 5), and cross-linked as described in Materials and Methods. In addition to the ^{32}P-labeled U6 RNA substrate, a 100-fold excess of competitor U6 RNA containing either a 3′ hydroxyl (lane 4) or a 3′ phosphate (lanes 2 and 5) was added. In lanes 1 and 3, no competitor RNA was added. Although U6 RNA containing a 3′ phosphate weakly competes for binding to *Drosophila* La (compare lanes 1 and 3 with lanes 2 and 5), this may be due to incomplete conversion of a small fraction of the 3′ hydroxyls to 3′ phosphates during Whitfield degradation. Molecular masses (in kilodaltons) are indicated at the left. The arrow indicates the *Drosophila* La protein (see the text).

protein in the protozoan *Plasmodium falciparum* that was immunoprecipitated by a patient anti-La serum. Brow (6) detected a protein in a yeast cell extract that bound to a slowly processed variant of yeast 5S rRNA. Characteristically for the La protein, formation of the yeast RNA-protein complex was disrupted when the oligoribonucleotide $(Up)_4U_{OH}$ was added in competition experiments. In *Drosophila* cell extracts, an in vitro-synthesized 5S rRNA precursor was cross-linked with UV light to a 50-kDa protein (35). Because binding of the 50-kDa protein to the 5S rRNA precursor decreased when poly(U) homopolymers were added to the extract, the protein was suggested to be the *Drosophila* La protein. By use of our antibodies directed against the *Drosophila* and yeast La proteins, it should be possible to determine if the proteins that we have identified correspond to the previously detected activities.

Structure of the La protein. Our study of *Drosophila* and yeast La protein homolog has allowed us to identify conserved regions of the La protein. The sequence similarity between La proteins from yeast to humans is greatest in the region that is N-terminal to the RRM (Fig. 2A). Many, if not most, RRM-containing proteins require sequences in addition to the RRM for specific RNA binding (22). The highly conserved region of the La protein that lies adjacent to the RRM is within the region shown to be required, in addition to the RRM, for binding to precursors of polymerase III RNAs (22, 36). Thus, the conserved amino acids in this region may provide important contacts with bound RNAs. Since an otherwise unrelated protein predicted to be encoded by the YCL37c open reading frame contains a highly related sequence towards the C terminus, this conserved region apparently constitutes a new

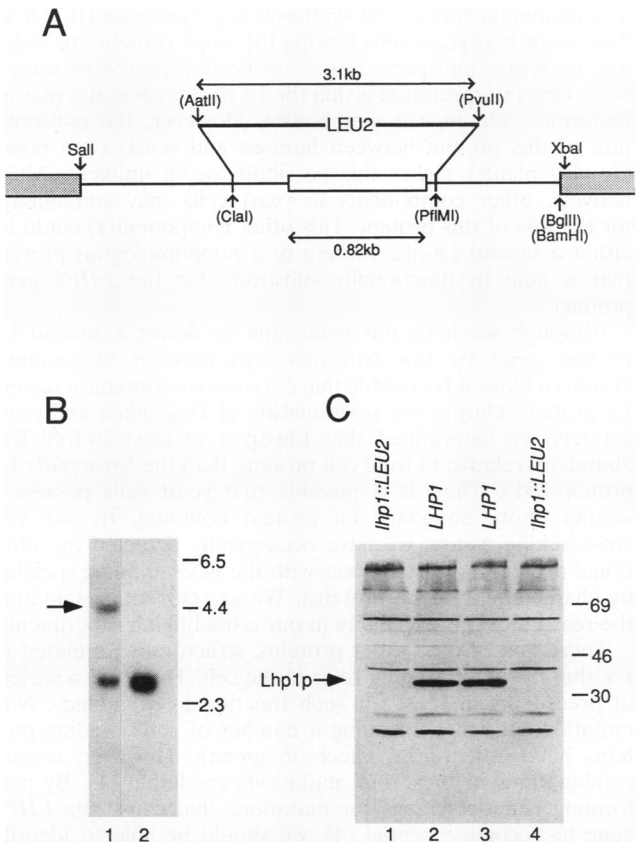

FIG. 7. Disruption of the *LHP1* gene. (A) The strategy used to disrupt *LHP1* is depicted (see Materials and Methods). The 0.82-kb open reading frame of *LHP1* is represented by a clear rectangle, and flanking sequences are drawn as thin lines. Plasmid sequences are represented by shaded boxes. Restriction sites are indicated with arrows, and sites which were destroyed by blunt-end or compatible end ligation are in parentheses. (B) Southern blot analysis of genomic DNA isolated from diploid strains carrying the *lhp1*::*LEU2* allele. Genomic DNA from a Leu$^+$ transformant (lane 1) or from nontransformed YPH501 cells (lane 2) was digested with *Sal*I and *Bgl*II and probed with an antisense RNA probe covering the 2.7-kb insert of subclone pN57. Molecular mass standards (in kilodaltons) are indicated to the right of the gel. An arrow points to the integrated null allele, *lhp1*::*LEU2* (lane 1). (C) Protein immunoblot of extracts from haploid cells derived from a dissected tetrad. Antibodies raised against the *LHP1* fusion protein recognize a 38-kDa polypeptide, Lhp1p (lanes 2 and 3; arrow), which is absent in cells derived from the two haploid spores which contained the *lhp1*::*LEU2* allele (lanes 1 and 4).

sequence motif. It will be interesting to determine if the putative YCL37c protein (which lacks an RRM) is capable of specific RNA binding. As several errors have already been detected in the assignment of open reading frames on chromosome III (23), it will be necessary first to establish that the predicted protein actually exists.

While less conserved, the C terminus of the La protein is highly charged in all the La proteins. Since this part of the protein can be deleted without affecting specific RNA binding (22, 36), it presumably has a function that is distinct from RNA binding. It is possible that the aberrant migration of the *Drosophila* and yeast La proteins in SDS-PAGE is due to the highly charged domain. The aberrant migration of the U1 small nuclear RNP 70-kDa protein was demonstrated to be due to the highly charged C terminus (37).

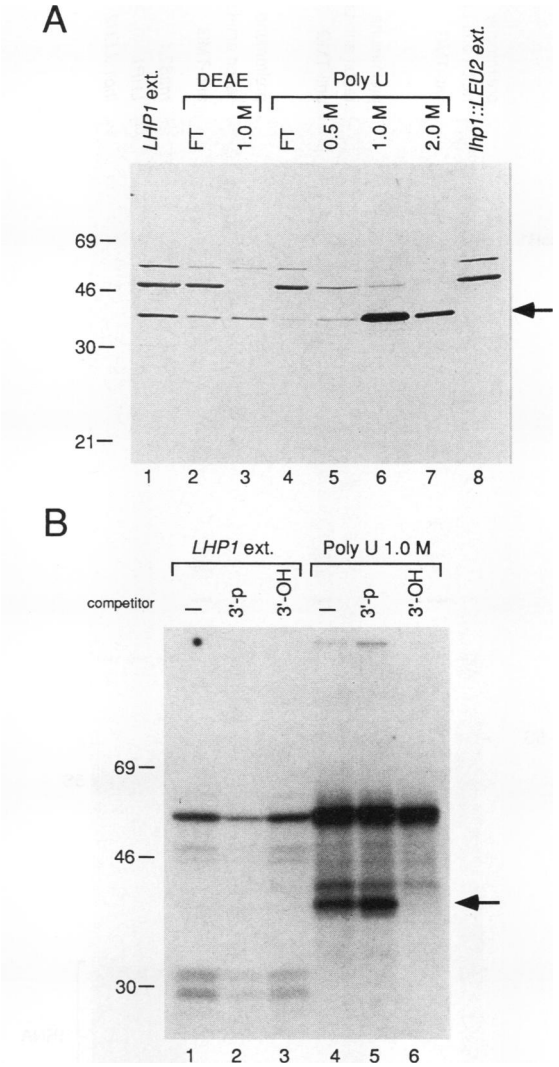

FIG. 8. Partial purification of Lhp1p and UV light-induced cross-linking of U6 RNA to Lhp1p. (A) An extract of haploid cells containing the *LHP1* allele was fractionated as described in Materials and Methods. Fractions from the various purification steps were assayed by protein immunoblotting. Equivalent protein amounts were loaded into each lane with the exception of the 2.0 M NaCl eluate (lane 7), which contained less protein. Antibodies against Lhp1p identify a 38-kDa protein (arrow) in the *LHP1* cell extract (lane 1) which is absent in extracts of *lhp1*::*LEU2* cells (lane 8). The anti-Lhp1p serum used in this experiment and that used in Fig. 7C were obtained from different rabbits. Although we do not know the identity of the other two protein bands recognized by this serum, neither of these proteins binds tightly to poly(U)-Sepharose, and they are thus unlikely to represent additional La-like proteins. FT, flowthrough fractions. (B) UV cross-linking of ^{32}P-labeled U6 RNA to Lhp1p. An extract derived from haploid cells containing the *LHP1* allele (lanes 1 to 3) or the fraction eluting from the poly(U)-Sepharose column with 1.0 M NaCl (lanes 4 to 6) was incubated with in vitro-transcribed ^{32}P-labeled U6 RNA and subjected to UV cross-linking analysis. In addition to the labeled U6 RNA, reaction mixtures contained either no competitor RNA (lanes 1 and 4) or a 100-fold molar excess of U6 RNA terminating with either a 3′ phosphate (lanes 2 and 5) or a 3′ hydroxyl (lanes 3 and 6). Molecular mass (in kilodaltons) is indicated at the left of each panel.

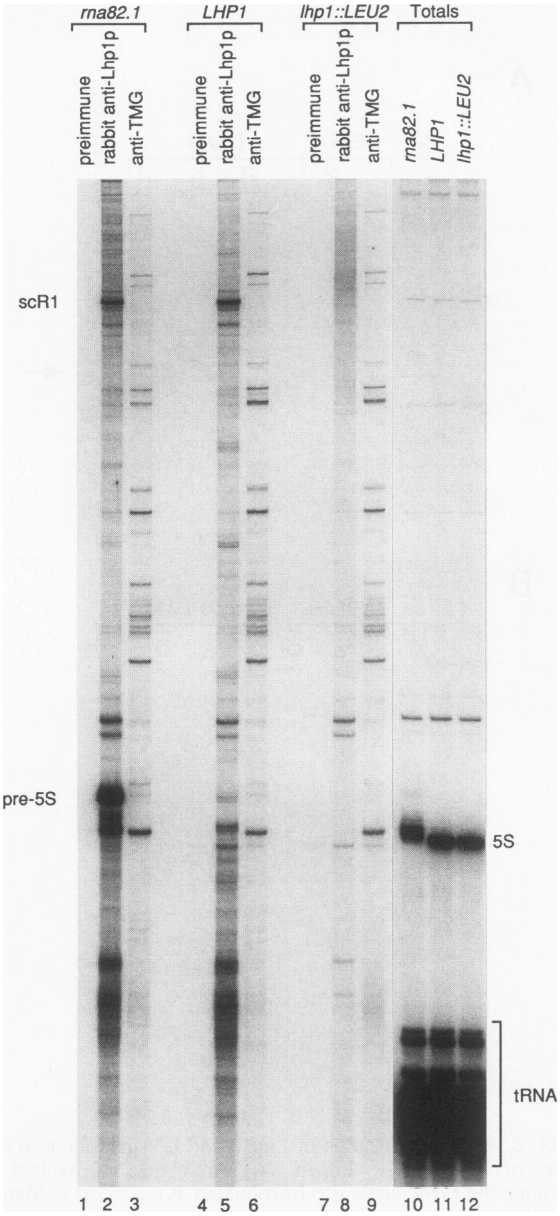

FIG. 9. Anti-Lhp1p antibodies immunoprecipitate nascent polymerase III RNAs from yeast cell extracts. Extracts were prepared from *rna82-1* cells (lanes 1 to 3 and 10), wild-type cells (lanes 4 to 6 and 11), or *lhp1*::*LEU2* cells (lanes 7 to 9 and 12) as described in Materials and Methods. Equal aliquots of extracts were either phenol extracted (lanes 10 to 12) or incubated with rabbit preimmune (lanes 1, 4, and 7), rabbit anti-Lhp1p (lanes 2, 5, and 8), or mouse anti-trimethylguanosine antibodies (lanes 3, 6, and 9). RNAs contained within immunoprecipitates (lanes 1 to 9) and 2% of total RNA (lanes 10 to 12) were end labeled with [^{32}P]pCp. RNAs identified as pre-5S rRNA and scR1 are indicated. The mature 5S rRNA from the *rna82-1* mutant cells migrates slightly more slowly than wild-type 5S rRNA; this is due to the presence of several additional nucleotides at the 3′ end (33).

Dispensability of the yeast La protein homolog. It has been proposed that the La protein is required for multiple rounds of polymerase III transcription (16, 17, 27). We have demonstrated that the La protein is a highly conserved component of eukaryotic cells, as would be expected for a protein that plays a fundamental role in the synthesis of polymerase III RNAs. The result that yeast cells lacking the Lhp1 protein are viable was therefore unexpected and can be interpreted in several ways. One interpretation is that the La protein does not play an important role in cell metabolism. However, the conservation of this protein between humans and yeasts (and possibly also plants) makes this possibility seem unlikely. Alternatively, other components in yeast cells may compensate for the loss of this protein. This other component(s) could be either a second La-like protein or a nonhomologous protein that is able to functionally substitute for the *LHP1* gene product.

Although we have not been able to detect a second La protein gene by low-stringency hybridization of genomic Southern blots, it is possible that *S. cerevisiae* contains a second La protein. During our fractionation of *Drosophila* and yeast extracts, we determined that Lhp1p is at least 10-fold less abundant, relative to total cell protein, than the *Drosophila* La protein (51). Thus, it is possible that yeast cells possess a second, more abundant La protein homolog. In our UV cross-linking assays, we have occasionally detected an additional protein in yeast extracts with the RNA-binding specificity characteristic of La proteins. We are currently examining the reason for this variability in our cross-linking experiments.

There may also be other proteins, structurally unrelated to La, that have overlapping roles in the cell. There are a variety of precedents in yeast for such functional redundancy. Null mutations of genes encoding a number of actin-binding proteins have little or no effect on growth. However, certain combinations of these null mutations are lethal (1). By performing genetic screens for mutations that cause the *LHP1* gene to become essential (4), we should be able to identify other proteins that are involved in the same process as Lhp1p. Thus, despite the fact that yeast cells containing a null allele of the *LHP1* gene show no discernible growth defects, we should be able to use available genetic strategies to elucidate the in vivo function of the La protein.

ACKNOWLEDGMENTS

We are grateful to J. Keene and D. Kenan for suggesting the PCR strategy that we used to isolate the *Drosophila* La cDNA and to C. Hashimoto, C. Hong, and L. Cooley for assisting us with the in situ hybridization experiments. We thank R. Maraia and B. Baum for alerting us to the homology between the yeast *ORFX* and mammalian La proteins, M. Nagiec for sending us pN57, P. Piper for the *rna82-1* mutant strain, and S. Kim and A. Horwich for assisting us in the genetic analysis of *LHP1*. We thank D. Van Horn for expert technical assistance and C. Green, C. Hashimoto, C. O'Brien, and E. Ullu for their comments on the manuscript. We are also grateful to K. Matsuno, S. Artavanis-Tsakonas, R. Diederich, J. Craft, S. DeGregorio, and D. Wassarman for gifts of materials used in this work and to P. Male for photography.

This work was supported by grant GM48410 from the National Institutes of Health.

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