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Two Yeast La Motif-containing Proteins Are RNA-binding Proteins that Associate with Polyribosomes

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We have characterized two *Saccharomyces cerevisiae* proteins, Sro9p and Slf1p, which contain a highly conserved motif found in all known La proteins. Originally described as an autoantigen in patients with rheumatic disease, the La protein binds to newly synthesized RNA polymerase III transcripts. In yeast, the La protein homologue Lhp1p is required for the normal pathway of tRNA maturation and also stabilizes newly synthesized U6 RNA. We show that deletions in both *SRO9* and *SLF1* are not synthetically lethal with a deletion in *LHP1*, indicating that the three proteins do not function in a single essential process. Indirect immunofluorescence microscopy reveals that although Lhp1p is primarily localized to the nucleus, Sro9p is cytoplasmic. We demonstrate that Sro9p and Slf1p are RNA-binding proteins that associate preferentially with translating ribosomes. Consistent with a role in translation, strains lacking either Sro9p or Slf1p are less sensitive than wild-type strains to certain protein synthesis inhibitors. Thus, Sro9p and Slf1p define a new and possibly evolutionarily conserved class of La motif-containing proteins that may function in the cytoplasm to modulate mRNA translation.

INTRODUCTION

The La protein is an RNA-binding protein that was originally identified as an autoantigen in patients with rheumatic diseases. The La protein has been identified in eukaryotes from yeast to humans (Chambers *et al.*, 1988; Yoo and Wolin, 1994; Lin-Marq and Clarkson, 1995; Van Horn *et al.*, 1997), where it binds nascent RNA polymerase III transcripts, including pre-tRNAs, pre-5S rRNAs, and pre-U6 RNA (Rinke and Steitz, 1982, 1985). Part of the binding site for the La protein on these RNAs is the sequence UUU_{OH}, which is at the 3′ end of all newly synthesized RNA polymerase III transcripts (Stefano, 1984). Experiments performed in vitro have implicated the vertebrate La protein in various processes, including RNA polymerase III transcription (Gottlieb and Steitz, 1989; Maraia, 1996), stabilization of histone mRNAs from degradation (McLaren *et al.*, 1997) and cap-independent mRNA translation (Meerovitch *et al.*, 1993). Whether the La protein functions in all of these processes in vivo is uncertain.

In the budding yeast *Saccharomyces cerevisiae*, genetic and biochemical analyses have revealed that the La protein Lhp1p is necessary for the normal maturation of pre-tRNAs (Yoo and Wolin, 1997). Binding by Lhp1p also stabilizes newly synthesized, unassembled U6 RNA from degradation (Pannone *et al.*, 1998). These studies suggest that the La protein may function as a molecular chaperone to facilitate the correct fate of newly synthesized RNA polymerase III transcripts (Pannone *et al.*, 1998).

Interestingly, two *S. cerevisiae* proteins, Sro9p and Slf1p, share a highly conserved motif with all La proteins (Yoo and Wolin, 1994; Yu *et al.*, 1996; Kagami *et al.*, 1997). Although these proteins are otherwise unrelated to La proteins, Sro9p and Slf1p exhibit similarity throughout their length (29.8% identity) and may result from an ancient gene duplication (Wolfe and Shields, 1997). Genetic experiments have implicated both Sro9p and Slf1p in several processes. High-copy *SRO9* (also called *SYS2*) suppresses the secretory pathway mutants *sec7–1* and *ypt6*Δ (Tsukada and Gallwitz, 1996). Sec7p is a constituent of the secretory vesicle coat that functions in endoplasmic reticulum to Golgi vesicle transport (Kaiser *et al.*, 1997), whereas Ypt6p, a homologue of the mammalian small GTPase Rab6, functions in transport from the Golgi apparatus (Tsukada *et al.*, 1999). High-copy *SRO9* also suppresses the slow growth phenotypes of the following mutants: a deletion of the nonessential gene *RHO3*, which functions in bud formation (Imai *et al.*, 1996), *act1-1*, an actin mutant (Kagami *et al.*, 1997), and a partial deletion of the cytoskeletal protein tropomyosin (Kagami *et al.*, 1997). In addition, a deletion in *SRO9* exacerbates the slow growth of *act1–1* and *rho3–1* mutants and exhibits synthetic lethality with a partial deletion in tropomyosin (Kagami *et al.*, 1997). *SLF1* was first identified as a high-copy suppressor of a mutation that renders yeast cells sensitive to high $CuSO_4$-containing media (Yu *et al.*, 1996). Nevertheless, like *SRO9*,

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Table 1. Yeast strains

Strain	Genotype	Source
CY1	*MATα ura3-52 lys2-801 ade2-101 his3 Δ200 leu2 Δ1 LHP1*	Yoo and Wolin, 1997
CY2	*MATα ura3-52 lys2-801 ade2-101 his3Δ200 leu2Δ1 lhp1::LEU2*	Yoo and Wolin, 1997
NY579	*MATα leu2-3112 ura3-52 pep4::URA3*	Laboratory of Peter Novick
YPH258	*MATα ura3-52 lys2-801 ade2-101 his3Δ200 leu2Δ1*	Sikorski and Hieter, 1989
YPH259	*MATα ura3-52 lys2-801 ade2-101 his3Δ200 leu2Δ1*	Sikorski and Hieter, 1989
YSS203	YSS328 but *sro9::URA3/SRO9*	This study
YSS207	*MATα ura3-52 lys2-801 ade2-101 his3Δ200 leu2Δ1 slf1::HIS3*	This study
YSS212	*MATα ura3-52 lys2-801 ade2-101 his3Δ200 leu2Δ1 sro9::URA3*	This study
YSS220	YSS328 but *slf1::HIS3/SLF1 sro9::URA3/SRO9*	This study
YSS222	YSS328 but *slf1::HIS3/SLF1 lhp1::LEU2/LHP1*	This study
YSS227	*MATα ura3-52 lys2-801 ade2-101 his3Δ200 leu2Δ1 slf1::HIS3 sro9::URA3*	This study
YSS228	*MATα ura3-52 lys2-801 ade2-101 his3Δ200 leu2Δ1 slf1::HIS3 lhp1::LEU2*	This study
YSS227/ YSS228	*MATα/α ura3-52/ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 slf1::HIS3/slf1::HIS3 sro9::URA3/SRO9 lhp1::LEU2/LHP1*	This study
YSS233	YSS328 but *slf1::HIS3/SLF1*	This study
YSS241	*MATα, ura3-52 lys2-801 ade2-101 his3Δ200 leu2Δ1 slf1::HIS3 sro9::URA3*	This study
YSS302	*MATα ura3-52* YEP24-*SLF1*	This study
YSS305	*MATα ura3-52* YEP24-*SRO9*	This study
YSS308	*MATα ura3-52* YEP24	This study
YSS328	*MATα/α ura3-52/ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 his3Δ200/his3Δ200 leu2Δ1/leu2Δ1*	This study

high-copy *SLF1* suppresses a partial deletion of tropomyosin (Kagami *et al.*, 1997).

Overexpression of *SRO9* also suppresses mutations in processes that are unrelated to intracellular transport and the actin cytoskeleton. High-copy *SRO9* suppresses the cold sensitivity of several mutations that affect pre-mRNA splicing (M. Inada, J. P. Staley, and C. Guthrie, personal communication).

Because *SRO9* and *SLF1* are high-copy suppressors of mutations in several processes, the actual function of these proteins is unclear. As the motif that these proteins share with authentic La proteins is important for RNA binding by La proteins (Pruijn *et al.*, 1991), Slf1p and Sro9p may also be RNA-binding proteins. Although this motif [previously called the La domain (Van Horn *et al.*, 1997)] does not by itself bind RNA, small deletions within the motif dramatically affect RNA binding by the La protein (Goodier *et al.*, 1997).

To understand the function of Sro9p and Slf1p, as well as to elucidate the relationship between these proteins and authentic La proteins, we have taken a molecular genetic and biochemical approach. We demonstrate that Sro9p and Slf1p are not functionally redundant with the authentic La protein Lhp1p. Instead, Sro9p and Slf1p are RNA-binding proteins that associate with translating ribosomes. Consistent with a role in mRNA translation, strains lacking either Sro9p or Slf1p exhibit decreased sensitivity to a subset of protein synthesis inhibitors. Thus, these two proteins constitute a second branch of the La family of proteins that may function in mRNA translation.

MATERIALS AND METHODS

Yeast Strains, Media, and Molecular Genetic Techniques

Yeast strains are listed in Table 1. YSS strains and CY strains were derived from the strain YNN216, which is congenic with S288C (Sikorski and Hieter, 1989), with the exception of YSS302, YSS305, and YSS308, which are transformants of NY13 (a gift of P. Novick, Yale University, New Haven, CT). Genetic manipulations and growth media were as described in Sherman *et al.* (1986).

Phylogenetic Analysis

The *Caenorhabditis elegans* and *Homo sapiens* La motif-containing protein sequences (genes R144.7 (U23515), T12F5.5 (AF039718), C44E4.4 (AF003140), KIAA0731 (AB018247)) and *Mus musculus* expressed sequence tags (AA823891, AA396971, AA530305, AA823920, AA681670, AA474319, AA681353, AA510776, and AA413852) were obtained by a BLAST search of the GenBank database using as a query protein either Sro9p or the *H. sapiens* La protein. The *M. musculus* expressed sequence tags were assembled into a contiguous sequence (contig) using the CuraTools Robot sequence assembly program (CuraGen Corp, New Haven, CT). La motifs were aligned by MegAlign using the CLUSTAL method with PAM250 residue weight table, and default parameters (DNASTAR, Madison, WI). La motifs were aligned for the dendrogram by PileUp (Genetics Computer Group, Madison, WI). Dendrograms were generated with the maximum parsimony criterion; bootstrap analysis was performed with a heuristic search, and the maximum parsimony criterion, with 1000 bootstrap replicates by PAUPSearch (Genetics Computer Group). Pairwise alignments were performed by the BCM-launcher pairwise comparison (Human Genome Center, Baylor College of Medicine).

Deletion of SRO9 *and* SLF1

The *sro9::URA3* allele of YSS203 (Table 1) was generated by PCR amplification of the *SRO9* gene using the primers 5′-GATCTGGACTCTCGAGCAAG-3′ and 5′-TATGATGATAATGTACAATGAATTC-3′. This fragment was digested with *Hae*II, filled in and *Bam*HI-digested, and ligated to *Hinc*II/*Bam*HI-digested pBluescriptII-KS$^-$ (Stratagene, La Jolla, CA). This clone was digested with *Pst*I and PflMI, filled in, and ligated to a 1.5-kb filled-in *Cla*I/*Bam*HI fragment containing *URA3*. This plasmid was *Xho*I/*Xba*I-digested and used to transform YSS328. In this allele 46% of *SRO9* was deleted; the La motif was entirely deleted. YSS203 (*sro9*::*URA3*/*SRO9*) was sporulated, and tetrads were dissected. The growth of haploids bearing this allele was identical to that of haploids con-

taining a complete deletion of *SRO9*, as well as a partial deletion of the upstream gene *YCL36c* (our unpublished results).

An *slf1::HIS3* allele was generated by amplification of *HIS3* from pRS313 (Sikorski and Hieter, 1989) using oligos SGS1 (5′-AAACGAGAGAGCCCAAAAATATAACCAAGATAAAGAAAATCAATCATAAAGTGAATTCAAAGCGCGCCTCGTTCAGAATG-3′) and SGS2 (5′-TTATGTTATATTTTTAGAGAGAATCTGCTATTACTTTATACATGTTAACTATATACATAATACTCTTGGCCTCCTCTAGTA-3′). The PCR product was transformed into YSS328, resulting in an allele in which *SLF1* and 2 bp of upstream and 29 bp of downstream sequence were deleted. Transformants were sporulated, and tetrads were dissected. The tetrads analyzed were as follows: 22 tetrads (YSS203), 18 tetrads (YSS233), 14 tetrads (YSS220), 23 tetrads (YSS222), and 38 tetrads (YSS227/YSS228).

Antibody Generation, Immunoblotting, and Immunofluorescence

A fusion of Slf1p to polyhistidine was constructed using oligos SGS15 (5′-ATTAGGATCCTCATCGCAAAACCTCAATGATAATCCAAAA-3′) and SGS16 (5′-ATTAGGTACCTTAATCATTTATTTGTAAGTTTTGTTCAAACTG-3′) to amplify the *SLF1* coding sequence. The amplified DNA was digested with *Bam*HI and *Kpn*I and ligated to these sites in pTrcHisA (Invitrogen, San Diego, CA). Fusion protein was induced as described by the manufacturer, purified from the lysate using a HiTrap chelating column (Amersham Pharmacia Biotech, Arlington Heights, IL), and used to inject rabbits. The Sro9p-6-histidine fusion construct was made by amplifying *SRO9* with oligonucleotides 5′-GCCGGCCTCGAGATGAAGATCTTTTGGGATCC-3′ and 5′-GCCGGCGAATTCTGCAAGTGTGAGAGGCC-3′. This fragment was *Eco*RI/*Xho*I-digested and ligated to *Eco*RI/*Xho*I-digested pTrcHisA.

The rabbit anti-Lhp1p polyclonal antibody has been described (Yoo and Wolin, 1994). Affinity-purified rabbit anti-Sbh1p was a gift of T. Rapoport (Harvard University, Cambridge, MA). The rabbit anti-Rpl5p antibody was a gift of J. Woolford (Carnegie Mellon University, Pittsburgh, PA). Actin was detected by mouse monoclonal antibody clone C4 (Boehringer Mannheim, Indianapolis, IN). Primary signals were visualized by incubation of immunoblots with either horseradish peroxidase-conjugated donkey anti-rabbit Ig or sheep anti-mouse Ig (Amersham Pharmacia Biotech) and enhanced chemiluminescence.

Immunofluorescence was performed largely as described (Pringle *et al.*, 1991). Cells were grown in YPD, harvested at OD_{600} = 0.4–0.7, and fixed in 3.7% formaldehyde at 25°C for 1 h. Cells were then spheroplasted for ~40 min at 37°C with 5 μg/ml zymolyase 100T (ICN Immunobiologicals, Costa Mesa, CA) and 0.02% glusulase (DuPont NEN, Wilmington, DE). After absorption to an *lhp1::LEU2* strain, anti-Lhp1p was used at 1:500 dilution. Anti-Sro9p was used at 1:100 after absorption to an *sro9*Δ *slf1*Δ strain. Antigens were visualized by CY3-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA). Cell outlines were visualized by differential interference contrast optics. In these experiments, CY1 was the wild-type strain, CY2 was the *lhp1*::*LEU2* strain, and YSS212 was the *sro9*::*URA3* strain.

Construction of High-Copy SRO9 *and* SLF1 *Plasmids*

For overexpression studies, an *SRO9*-containing 1.9-kb *Xho*I/*Eco*RI fragment was digested from a genomic clone (a gift of P. Brennwald, Cornell University Medical College, New York, NY) and ligated to *Xho*I/*Eco*RI-digested pRS316 (Sikorski and Hieter, 1989). The *SRO9* gene was then removed as a 2.1-kb *Pvu*II/*Sma*I fragment and ligated to *Pvu*II-digested YEP24 (Carlson and Botstein, 1982). To overexpress *SLF1*, the SpeI/*Sac*I fragment was excised from cosmid 9787 (American Type Culture Collection) and ligated to SpeI/*Sac*I-digested pRS316. The *SLF1* gene was excised via SpeI/*Msp*I digestion, filled in with Klenow, and blunt-ligated to *Nhe*I/*Sma*I-digested YEP24 that had also been filled in.

Differential Centrifugation and Polyribosome Analysis

For cell fractionation experiments, the *pep4::URA3* strain NY579 was used. Cells were grown in YPD at 30°C, harvested in log phase (OD_{600} = 0.6–1.0) by centrifugation at 3000 × *g* for 5 min in an SS34 Sorvall rotor (DuPont), washed once in lysis buffer S (LBS) [40 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1× protease inhibitor cocktail tablets, EDTA free (Boehringer Mannheim), 1 μm pepstatin], and lysed by vortexing with glass beads (425–600 μM). Unbroken cells and large debris were removed by centrifugation at 800 × *g* for 10 min. The cleared lysate was sedimented at 10,000 × *g* for 10 min, and the resulting supernatant was sedimented in a Beckman TLA100 rotor at 100,000 × *g* for 1 h. Pellets were resuspended in a volume of LBS equivalent to the corresponding supernatant. All steps were performed at 4°C. Triton X-100 (0.2, 0.65, and 0.87%) and NaCl (100, 150, 200, and 350 mM), when included, were added after glass bead lysis.

For polyribosome analysis, lysates were prepared as described above using LBS + 2 mM $MgCl_2$ in the presence of protease inhibitors. Where indicated, 100 μg/ml cycloheximide (Sigma, St. Louis, MO) was added to cells immediately before harvesting. Homogenates were sedimented at 800 × *g* for 10 min, and the cleared lysate was sedimented at 10,000 × *g* for 15 min. When micrococcal nuclease or EDTA were included, the 10,000 × *g* supernatants were incubated at 4°C with either 20 mM EDTA (for 15 min) or 5 U/μl micrococcal nuclease (for 30 min in the presence of 3 mM $CaCl_2$). Control lysates were incubated under identical conditions, but without EDTA or nuclease. Supernatant (100 OD_{260} units of 10,000 × *g*) was layered onto 12 ml of 20–47% sucrose gradients in LBS + 2 mM $MgCl_2$, which were sedimented for 3.75 h in an SW40 Beckman rotor at 39,000 rpm (Nelson *et al.*, 1992). Gradients were collected with an ISCO (Lincoln, NE) Model 185 density gradient fractionator.

In Vitro Translation and RNA Homopolymer Binding

The T7 promoter was introduced upstream of *SRO9* by PCR amplification using oligos SGS44 (5′-TTATACCCTCTGAAATGTGTTAATACGACTCACTATAGCTGGTAGGTCAAGAACAAAGAAAG-3′) and SGS45 (5′-GTTTTTTGTGTAAAATGCAATGAACG-3′). The fragment was subcloned into pCR-Blunt (Invitrogen), and the T7-*SRO9* fragment was released by digestion with *Eco*RI. The T7-*LHP1* construct was constructed analogously, but using oligos SGS46 (5′-TTATACCCTCTGAAATGTGTTAATACGACTCACTATAGGGGTTCTATTTGGTTCTACTGGAAC-3′) and SGS47 (5′-GCTATGATAATGAGATACGAGAACC-3′). After sequencing, the T7-*LHP1* fragment was excised using *Eco*RI. T7-*SLF1* was constructed by amplification of the N terminus of *SLF1* using oligos SGS57 (5′-AATACTCGAGGTGAATTCAAAAATGTCATCGCAAAA-3′) and SGS58 (5′-GGAAGGAGATGGCATTATTAGC-3′); this fragment was *Xho*I/*Nco*I-digested. A 2.1-kb *Nco*I/*Sac*I fragment containing the remainder of *SLF1* was ligated with the T7-*SLF1* *Xho*I/*Nco*I fragment and *Xho*I/*Sac*I-digested Bluescript SK$^+$ (Stratagene). This plasmid was used undigested for production of Slf1p. The T7-*CAK1* plasmid was a gift of M. Solomon (Yale University, New Haven, CT).

The homopolymer-binding assay was performed as described (Siomi *et al.*, 1993). ^{35}S-labeled Sro9p, Slf1p, Lhp1p, and Cak1p were generated by an in vitro-coupled transcription/translation kit (Promega, Madison, WI) using [^{35}S]methionine (Amersham Pharmacia Biotech). Each reaction was performed individually. The reactions were then combined and incubated with the indicated homopolymer (40 μg) immobilized on agarose beads [poly(A), poly(C), poly(G) (Sigma), or poly(U) (Amersham Pharmacia Biotech)] or calf thymus single-stranded DNA (ssDNA; 40 μg) immobilized on cel-

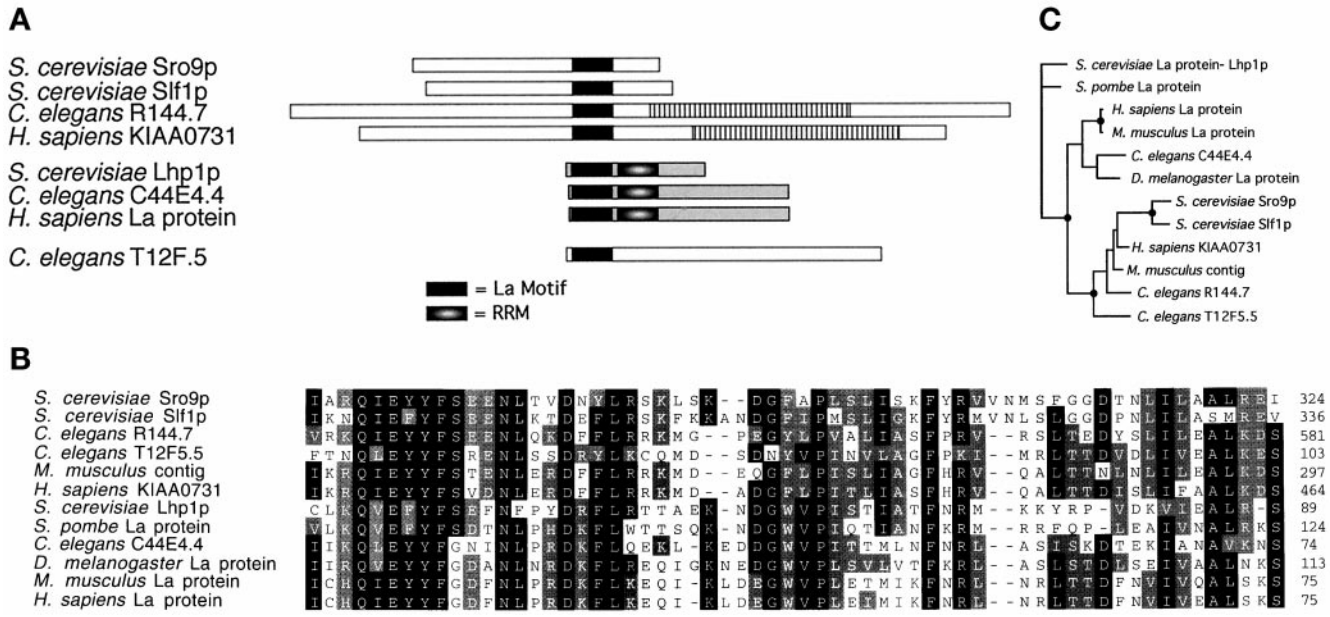

Figure 1. Phylogenetic analysis of La motif-containing protein families. (A) Graphic representation of all La motif-containing proteins (top four, as well as bottom protein) and authentic La proteins (indicated by gray background). The La motif is in black, and the RNA-recognition motif (RRM) is indicated by a gradient box. The human La protein may have a second RRM in the C terminus (Birney *et al.*, 1993). Boxed areas with vertical stripes indicate a region of 38.8% identity between *C. elegans* R144.7 and *H. sapiens* KIAA0731. (B and C) Alignment of the La motifs of selected La [*H. sapiens* (Chambers *et al.*, 1988), *M. musculus* (Topfer *et al.*, 1993), *D. melanogaster* (Yoo and Wolin, 1994), *C. elegans* C44E4.4 (AF003140) (Wilson *et al.*, 1994), *Schizosaccharomyces pombe* (Van Horn *et al.*, 1997), *S. cerevisiae* (Yoo and Wolin, 1994)] and La motif-containing proteins [*S. cerevisiae* Sro9p (Matsui and Toh-E, 1992), Slf1p (Yu *et al.*, 1996), H. *sapiens* KIAA0731 (Nagase *et al.*, 1998), *M. musculus* contig (see MATERIALS AND METHODS), and *C. elegans* R144.7 (U23515) and T12F5.5 (AF039718) (Wilson *et al.*, 1994)]. Black shading indicates identity in the majority of sequences. Gray shading indicates similarity in at least half the sequences. Similar residues were defined as D=E, H=K=R, A=G, I=L=V, F=W=Y, S=T. Dendrogram and bootstrap analysis were generated as described in MATERIALS AND METHODS. One thousand bootstrap replicates were performed, and the percentage of replicates in which nodes were confirmed was determined. Nodes confirmed in >70% of replicates indicate strong bootstrap support and are indicated by a solid circle. Note that the La motifs of Sro9p, Slf1p, and the other nonauthentic La proteins cluster into a single group with strong support (75%), indicating a closer relationship within this group than with the La motifs of authentic La proteins.

lulose beads (Sigma) in binding buffer (10 mM Tris-HCl pH 7.4, 2.5 mM $MgCl_2$, 0.5% Triton X-100) with the stated concentration of NaCl for 30 min at 4°C. The supernatant was removed, and the beads were washed four times with binding buffer. Protein was eluted by boiling the beads in SDS-PAGE sample buffer.

Drug Sensitivity Tests

Drug sensitivity assays were performed as described by Cui *et al.* (1995). After growing cells to saturation in YPD, the cultures were diluted to OD_{600} = 0.4, and 300-μl aliquots were plated on YPD agar. A 0.25-inch sterile filter disk was placed in the center of each plate, and 10 μl of cycloheximide (0.25 μg/μl), paromomycine sulfate (250 μg/μl), anisomycin (10 μg/μl), or hygromycin B (20 μg/μl) were applied to each disk. The plates were incubated at 24.5°C for 2 d. All antibiotics were purchased from Sigma.

RESULTS

Sro9p and Slf1p Belong to a Novel Class of La Motif-containing Proteins

Although both Sro9p and Slf1p share a motif with all known La proteins, both the overall structure of these proteins and the position of the La motif within these proteins differ from *bona fide* La proteins. As described previously (Yoo and Wolin, 1994; Van Horn *et al.*, 1997), authentic La proteins contain a highly conserved amino-terminal La motif, a less well conserved RNP-type RNA-recognition motif (RRM) (Query *et al.*, 1989), and a highly charged C terminus (Figure 1A). In contrast, the La motif is located toward the C terminus of both Sro9p and Slf1p (Figure 1A). Furthermore, although Sro9p and Slf1p are related to each other throughout their length, they do not resemble authentic La proteins outside the La motif (Kagami *et al.*, 1997) (Figure 1A).

To determine whether Sro9p and Slf1p are members of a conserved family of La motif-containing proteins that are distinct from authentic La proteins, we performed sequence analyses. Using BLAST with Sro9p as a query sequence, we found several other ORFs in GenBank that also contained the La motif but were not authentic La proteins. Sequences from *C. elegans* (R144.7) *and H. sapiens* (KIAA0731), as well as an *M. musculus* sequence assembled from expressed sequence tags (see MATERIALS AND METHODS), were detected that possessed greater similarity to Sro9p and/or Slf1p than to any known La protein (Figure 1 and our unpublished results). The *C. elegans* sequence R144.7 was annotated in the database as having weak similarity to La proteins (Wilson *et al.*, 1994).

Each of these proteins (*C. elegans* R144.7, *H. sapiens* KIAA0731, and *S. cerevisiae* Sro9p and Slf1p) are distinct from authentic La proteins by several criteria. First, the La motif is located either centrally or at the C terminus rather than at the amino terminus, as is characteristic of authentic La proteins (Figure 1A). (Because the mouse sequence is a partial sequence, the position of the La motif cannot be determined.) Second, the La motif of these proteins is phylogenetically divergent from that of authentic La proteins (Figure 1, B and C). Last, these proteins lack homology to La proteins outside the La motif (our unpublished results).

Although these La motif-containing proteins do not display sequence identity to authentic La proteins outside the motif, they do exhibit some homology within the group. Sro9p and Slf1p display some sequence identity throughout their length (Kagami *et al.*, 1997); the *C. elegans* R144.7 and the *H. sapiens* KIAA0731 exhibit 23.6% identity overall and 38.8% identity in a C-terminal region downstream of the La motif, a region unique to these two proteins (Figure 1A). Finally, the La motifs of Sro9p, Slf1p, *C. elegans* R144.7, *H. sapiens* KIAA0731, and the *M. musculus* contig are more related to each other than to those of authentic La proteins (Figure 1C). Note that the La motifs of these nonauthentic La proteins fall into a single node confirmed by statistical analysis (1000 bootstrap replicates), indicating greater similarity among this group. By these criteria there are at least two La motif-containing protein families: the authentic La proteins and at least one other class of La motif-containing proteins.

We also scanned the recently completed *C. elegans* genome for La motif-containing proteins. This revealed that, in addition to R144.7, *C. elegans* contains two additional proteins. One of these, C44E4.4, is homologous to authentic La proteins throughout its length and is thus likely to be the *C. elegans* homologue of the La protein. The *C. elegans* C44E4.4 is 32.5% identical with the *H. sapiens* La protein and 28.1% identical to the *Drosophila melanogaster* La protein [for comparison, there is 27% identity between the *H. sapiens* and *D. melanogaster* La proteins (Yoo and Wolin, 1994)]. The other sequence, T12F5.5, contains a La motif located at the amino terminus of the protein. Although the placement of this motif is similar to that of authentic La proteins, the protein is otherwise unrelated in sequence. Furthermore, the La motif of T12F5.5 is more similar to that of R144.7 than to authentic La proteins (Figure 1, B and C). Thus, the protein encoded by this sequence may constitute a third branch of the La motif-containing protein family.

Disruptions of SRO9, SLF1, *and* LHP1 *Are Not Synthetically Lethal*

It has been reported that yeast lacking *LHP1*, *SRO9*, or *SLF1* are viable and have no discernible growth defects (Yoo and Wolin, 1994; Yu *et al.*, 1996; Kagami *et al.*, 1997). To determine if the proteins encoded by the three genes function in a single essential process, we analyzed whether the absence of two or more of these genes would result in either synthetic lethality or a synthetic slow-growth phenotype.

First, *SRO9* and *SLF1* were each deleted in wild-type diploids, tetrads were dissected, and the phenotypes were analyzed. Tetrad analysis of *slf1*::*HIS3*/*SLF1* transformants confirmed that the *slf1*::*HIS3* mutation yields no growth phenotype on YPD (Yu *et al.*, 1996). Tetrad analysis of an *sro9::URA3/SRO9* transformant indicated that deletion of the *SRO9* gene yields a slight slow-growth phenotype (Figure 2A) that segregated with the *URA3* marker. This is in contrast to a report that the growth rate of an *sro9* deletion strain was indistinguishable from that of the wild-type strain (Kagami *et al.*, 1997). Next, *slf1::HIS3*, *sro9::URA3*, and *lhp1::LEU2* segregants were mated to generate combinations of each of these mutations (strains YSS220, YSS222, YSS227/YSS228). Tetrad analysis of YSS222 (*slf1::HIS3/SLF1, lhp1::LEU2/LHP1*) yielded uniformly sized segregants, revealing that cells lacking both *SLF1 and LHP1* grow normally (Figure 2B). Dissection of YSS220 (*slf1::HIS3/SLF1, sro9::URA3/SRO9*) (our unpublished results) and YSS227/YSS228 (*slf1::HIS3/slf1::HIS3, sro9::URA3/SRO9, lhp1::LEU2/LHP1*) yielded the same results as YSS203 (*sro9::URA3/SRO9*) (two large/two small) (Figure 2, compare A and C), indicating that cells lacking all three genes grow indistinguishably from cells lacking only *SRO9*. Thus Sro9p, Slf1p, and Lhp1p do not function in a single essential process.

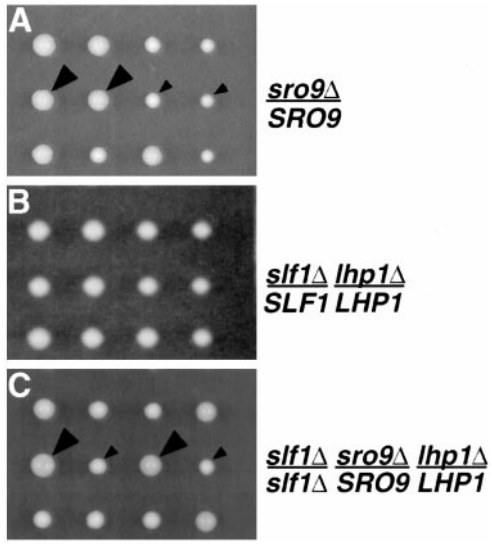

Figure 2. Deletion of *SRO9*, *SLF1*, and *LHP1* does not result in synthetic lethality. (A) An *sro9::URA3/SRO9* diploid (YSS203) was sporulated, and tetrads were dissected. The *sro9::URA3* allele confers a slight slow-growth phenotype, which segregates with the uracil auxotrophy. Two of the smaller *URA3* segregants are indicated with small arrowheads, and two of the larger *ura3* segregants are indicated with large arrowheads. (B) Segregants of the tetrad dissection of an *slf1::HIS3/SLF1*, *lhp1::LEU2/LHP1* diploid (YSS222). The deletion of *SLF1* or *LHP1* or both did not confer a growth defect. (C) Segregants of the tetrad dissection of an *sro9::URA3/SRO9*, *slf1::HIS3/slf1::HIS3*, *lhp1::LEU2/LHP1* diploid (YSS227/YSS228). *sro9::URA3*, *slf1::HIS3*, *lhp1::LEU2* triple deletion segregants grew similarly to *sro9::URA3* haploids, indicating that the additional deletions did not confer synthetic lethality or slow growth. Two large and two small segregants are indicated as in A.

Immunolocalization of Sro9p and Lhp1p

Because the authentic La protein predominantly localizes to the nucleus in higher eukaryotes (Hendrick *et al.*, 1981; Yoo and Wolin, 1994), we compared the subcellular distribution of Sro9p and Slf1p with that of Lhp1p. Rabbit antibodies were raised against recombinant proteins containing Sro9p and Slf1p linked to polyhistidine. Immunoblots of yeast extracts were probed with these antibodies to determine

their specificity for their corresponding antigens. The anti-Sro9p antibody detected an ~60-kDa protein that is absent in the *sro9::URA3* strain and thus corresponds to Sro9p (Figure 3A, lanes 1–3). The anti-Slf1p antibody recognized two bands: an ~57-kDa protein that corresponds to Slf1p because it is absent in the *slf1::HIS3* strain, and a nonspecific band that comigrates with Sro9p but is unrelated to Sro9p because it is present in the *sro9::URA3* strain (Figure 3A, lanes 4–6). Because *SRO9* and *SLF1* are predicted to encode 52- and 51-kDa proteins, respectively, these proteins have a slightly higher mobility on SDS-polyacrylamide gels than expected.

To determine the subcellular location of Sro9p, wild-type and *sro9::URA3* yeast cells were stained with anti-Sro9p antibodies. These experiments confirmed that Sro9p is cytoplasmic [as was reported by Kagami *et al.* (1997)]. Furthermore, Sro9p is present in dot-like structures of nonuniform size (Figure 4A). Because there was no antibody staining in the strain lacking Sro9p, the staining pattern was specific for Sro9p. In contrast, Lhp1p localizes to the yeast nucleus of wild-type cells (Figure 4B), indicating that the localization of wild-type Lhp1p is similar to the localization reported for Lhp1p fused to protein A (Rosenblum *et al.*, 1997). Because there was no staining in cells lacking Lhp1p, the signal was specific for Lhp1p. In contrast, immunofluorescence microscopy using anti-Slf1p antibodies did not yield a signal greater than background staining, indicating that these antibodies were not useful for immunolocalization experiments (our unpublished results).

Sro9p Levels Are Decreased in Yeast Strains Overexpressing Slf1p

Because deletions in *SRO9* and *SLF1* did not result in synthetic growth defects, it was unclear whether these proteins were functional homologues. We therefore determined whether overexpression of one affected the expression of the other. Slf1p and Sro9p were each overexpressed, and the relative amounts of Slf1p and Sro9p in each strain were assayed by immunoblot. In strains bearing an *SLF1* high-copy plasmid, the amount of Sro9p was reduced approximately fivefold relative to a strain containing the plasmid alone (Figure 3B, lanes 2 and 3). In contrast, the amount of Slf1p did not change when *SRO9* was overexpressed (lanes 1 and 3). Neither the levels of the La protein Lhp1p nor a control ribosomal protein (Rpl5p) was affected by overexpression of Sro9p or Slf1p (Figure 3B). It should be noted that although both Sro9p and Slf1p are overexpressed via a 2-μ plasmid, Slf1p is overexpressed to a greater extent. The decrease in Sro9p in the presence of excess Slf1p could be due to a specific feedback mechanism that limits the total amount of the two proteins, consistent with these proteins being functional homologues. Alternatively, the two genes could share a specific transcription factor that is no longer in excess when *SLF1* is present in multiple copies. Either possibility is consistent with a functional redundancy of the two proteins.

Sro9p and Slf1p Each Sediment as Part of a Large Complex

To determine whether the punctate structures observed by immunofluorescence with anti-Sro9p antibodies corre-

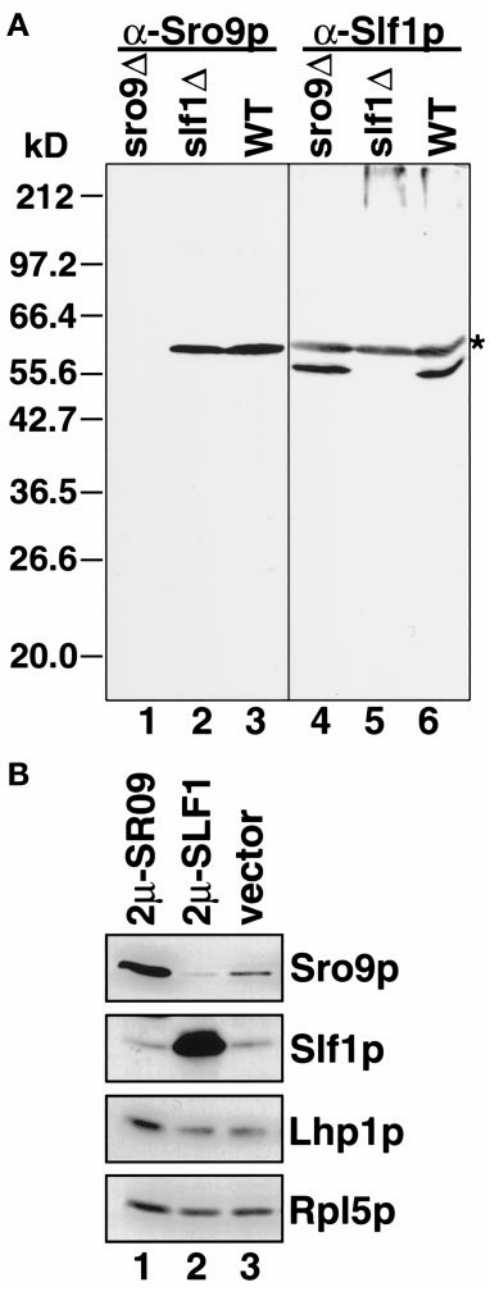

Figure 3. (A) Characterization of anti-Sro9p and anti-Slf1p antibodies. Lysates of *sro9::URA3* (YSS212), *slf1::HIS3 (YSS207)*, or wild-type (WT) (CY1) haploids were subjected to immunoblotting using the indicated antibodies. Note that the anti-Slf1p antibodies also recognize a second band (indicated by an asterisk) that is similar in mobility to Sro9p but is unrelated. Molecular weight standards are indicated. (B) Sro9p may be down-regulated in the presence of high-copy *SLF1*. Lysates from wild-type strains containing either *SRO9* or *SLF1* on a high-copy plasmid, or containing the vector alone, were subjected to immunoblotting and probed sequentially with anti-Sro9p, anti-Slf1p, anti-Lhp1p, and anti-Rpl5p.

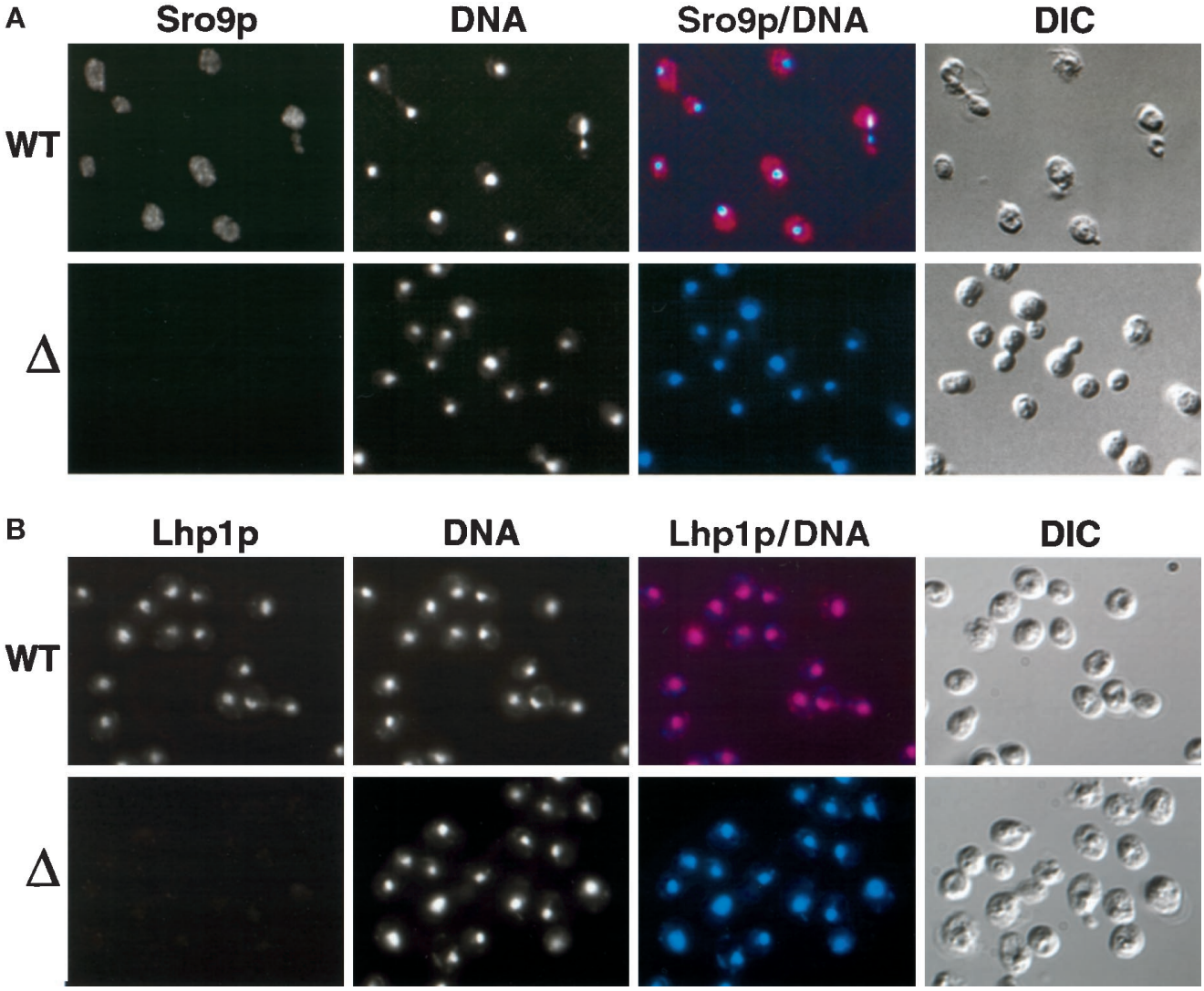

Figure 4. Intracellular localization of Sro9p and Lhp1p. (A) Sro9p localizes to cytoplasmic dots. Anti-Sro9p antibodies were used to stain WT and *sro9::URA3* cells and were visualized by indirect immunofluorescence microscopy. DNA was visualized by epifluorescence with Hoechst 33258. (B) Lhp1p localizes to the nucleus. WT cells and *lhp1::LEU2* cells were stained with anti-Lhp1p antibodies and visualized as in A.

sponded to heavy particles, we performed differential centrifugation and analyzed the distribution of Sro9p and Slf1p. Cells were lysed with glass beads, and unbroken cells and large debris were removed by low-speed centrifugation. The postnuclear supernatant (Figure 5, PNS) was sedimented at 10,000 × *g* for 10 min (P2, S2), and the subsequent supernatant was sedimented at 100,000 × *g* for 1 h (P3, S3).

Immunoblot analysis revealed that Sro9p and Slf1p remained in the supernatant after the 10,000 × *g* sedimentation but pelleted after the 100,000 × *g* centrifugation. As a control, the blot was reprobed to detect Sbh1p, a membrane protein of the endoplasmic reticulum (Panzner *et al.*, 1995). As expected, Sbh1p was found in the 10,000 × *g* pellet (Walworth *et al.*, 1989). These results differ from a report that a substantial portion of Sro9p remains in the 100,000 × *g*

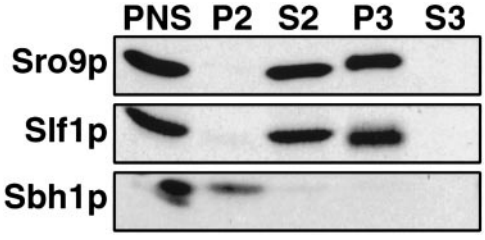

Figure 5. Sro9p and Slf1p are present in the 100,000 × *g* pellet. The postnuclear supernatant (PNS) was spun at 10,000 × *g* for 10 min (P2, S2), and the resulting supernatant (S2) was spun at 100,000 × *g* for 1 h (P3, S3). Supernatants (S) and pellets (P) were separated by SDS-PAGE, immunoblotted, and sequentially probed with anti-Sro9p, anti-Slf1p, and affinity-purified anti-Sbh1p, which detects an endoplasmic reticulum protein.

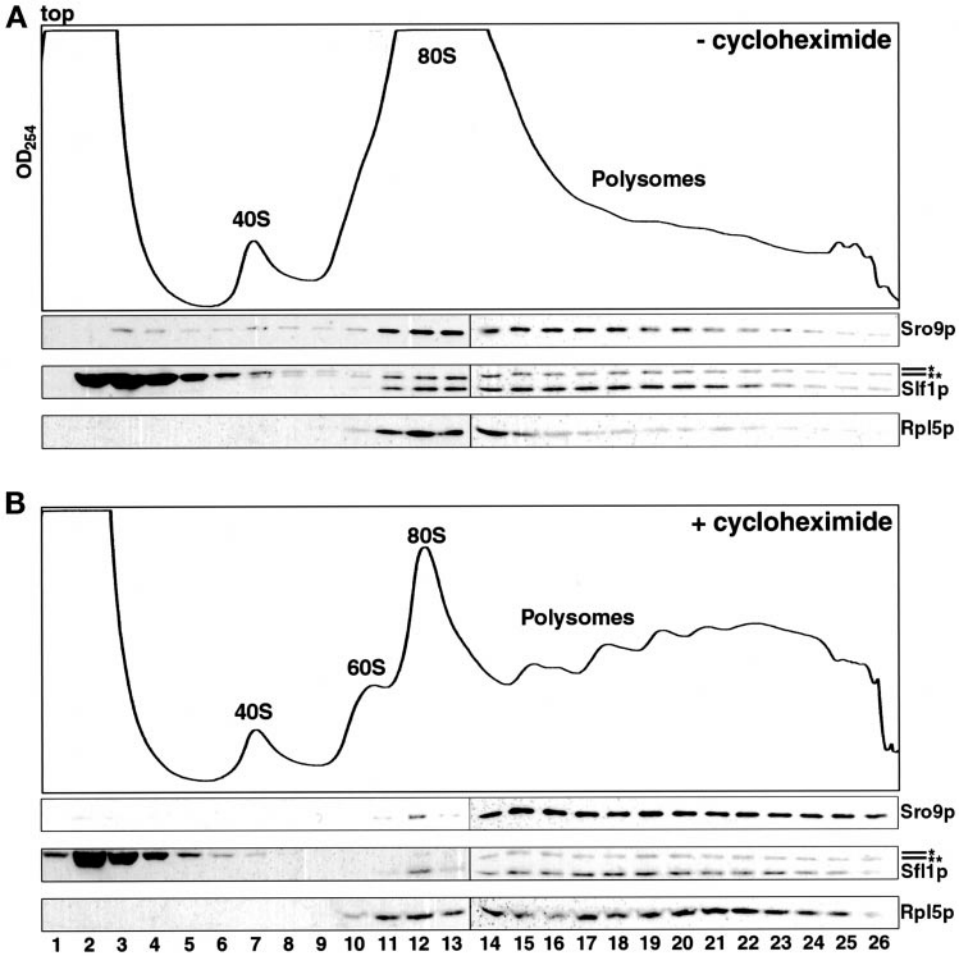

Figure 6. Sro9p and Slf1p cosediment with translating ribosomes. Lysates were prepared in the absence (A) or presence (B) of cycloheximide. Supernatant (100 OD_{260} units of 10,000 × *g*) (see Figure 5) was applied to 20–47% linear sucrose gradients. After sedimentation, gradients were collected as the OD_{254} was monitored. The positions of 40S and 60S ribosomal subunits, 80S monosomes, and polyribosomes are indicated. Fractions were separated by SDS-PAGE, blotted, and probed sequentially with anti-Sro9p, anti-Slf1p, and anti-Rpl5p (which detects a ribosomal protein). Note the coincident shift in sedimentation of the ribosomes and Sro9p and Slf1p (A vs. B). The asterisk in the Slf1p immunoblots (*) denotes the unrelated background band (see Figure 3A). The double asterisk (**) denotes the Sro9p protein, because immunoblots were not stripped before reprobing. The vertical line in each immunoblot strip indicates that the strip was assembled from two separate immunoblots, which were processed and exposed in parallel.

supernatant (Kagami *et al.*, 1997). This discrepancy may result from differences in the cell lysis procedures. We prepared lysates by rapid homogenization of intact yeast cells (see MATERIALS AND METHODS), whereas Kagami *et al.* (1997) lysed spheroplasts, a longer procedure that could result in dissociation of a complex during spheroplasting. Our data indicate that Sro9p and Slf1p are each components of a large complex. Their sedimentation is unaffected by varying NaCl concentrations (50, 100, 150, and 200 mM); at 350 mM NaCl only trace amounts of Sro9p and Slf1p are released from the 100,000 × *g* pellet (our unpublished results). In addition, these proteins are unlikely to be contained within membrane-bound vesicles, because the sedimentation was unaffected by addition of Triton X-100 (0.87%) to the lysate (our unpublished results). Finally, the sedimentation of Sro9p and Slf1p was independent of the actin cytoskeleton because actin remained in the supernatants (S2 and S3) (our unpublished results) and was therefore likely to be monomeric.

Sro9p and Slf1p Are Associated with Polyribosomes

Since Sro9p and Slf1p each associate with a heavy complex and share a domain with a known RNA-binding protein, we hypothesized that these proteins might associate with ribosomes. We therefore fractionated cell extracts on sucrose gradients and compared the distribution of Sro9p and Slf1p with that of polyribosomes. To preserve polyribosomes, the translation elongation inhibitor cycloheximide, which freezes translating ribosomes on the mRNA (Wettstein *et al.*, 1964), was added to half the cells before harvesting. In the absence of cycloheximide, most ribosomes were in the 80S monosome peak, although some polyribosomes remained (Figure 6A). Under these conditions, both Sro9p and Slf1p sedimented with the 80S ribosome (Figure 6A, fractions 11–14) as well as the polysomes (Figure 6A, lanes 15–26).

In the presence of cycloheximide, there was a significant decrease in the amount of 80S monosomes and a corresponding increase in polyribosomes (Figure 6B). As would be expected if Sro9p and Slf1p were polysome-associated, cycloheximide also altered the sedimentation of Sro9p and Slf1p. In the presence of cycloheximide, Sro9p and Slf1p sedimented mainly with polysomes (Figure 6B, fractions 14–26). As a control, the sedimentation of the ribosomal protein Rpl5p (Deshmukh *et al.*, 1993) was monitored. Although Rpl5p was present in both the 80S monosome and polysome fractions, Sro9p and Slf1p sedimented almost ex-

clusively with polysomes (Figure 6B). Furthermore, Sro9p and Slf1p sedimented with polysomes even when most of the ribosomes were in the monosome form (Figure 6A, lanes 11–26). These data suggest that Sro9p and Slf1p associate preferentially with polysomes rather than with monosomes or ribosomal subunits.

Polysome-disrupting Conditions Alter the Sedimentation of Sro9p and Slf1p

To further test our hypothesis that Sro9p and Slf1p associate with translating ribosomes, we analyzed the sedimentation of these proteins under conditions that disrupt polysomes. First, we used micrococcal nuclease to degrade portions of mRNAs that are not protected by ribosomes (Wolin and Walter, 1988). When micrococcal nuclease was added to an extract from cycloheximide-treated cells, the polysomes were digested to monosomes (Figure 7B). Similarly, the majority of Sro9p and Slf1p now migrated with the 80S monosome peak (Figure 7, compare A and B). Interestingly, although Sro9p and Slf1p are putative functional homologues, the sedimentation of these proteins was distinct in the nuclease-treated extracts. Although both Sro9p and Slf1p remained at least ~50% associated with the 80S peak, some Sro9p was released to the top of the gradient. In contrast, some Slf1p cosedimented with the 40S peak and almost no Slf1p was released to the top of the gradient (Figure 7B). Thus, although Sro9p and Slf1p are both polyribosome-associated, their functions and/or binding properties could be slightly different.

Finally, we examined the distribution of Sro9p and Slf1p in the presence of EDTA, which dissociates ribosomes into 40S and 60S subunits (Figure 7, C and D). When cell extracts from cycloheximide-treated cells were incubated with EDTA, both Sro9p and Slf1p shifted upward in the gradient; however, both Sro9p and Slf1p migrated further into the gradient after EDTA treatment than Rpl5p, which is released during EDTA treatment as a complex with 5S rRNA (Blobel, 1971). Thus, upon EDTA treatment, both Sro9p and Slf1p remain associated with other proteins or RNA species, or both.

Because the human La protein has been reported to sediment with 40S subunits (Peek *et al.*, 1996), we also examined the distribution of Lhp1p on sucrose gradients. In contrast to Sro9p and Slf1p, Lhp1p was found at the top of the gradient (Figure 7C). On long exposures of the blot, a very small fraction of Lhp1p was detected that may comigrate with the 40S subunit (Figure 7C, fraction 6). Nonetheless, the vast majority of Lhp1p in yeast cells does not sediment with either ribosomal subunits or ribosomes.

In summary, because three different conditions that alter polyribosome profiles (cycloheximide, micrococcal nuclease, and EDTA) resulted in corresponding shifts in the sedimentation of both Sro9p and Slf1p, we conclude that these proteins are associated with translating ribosomes.

Cells Lacking Sro9p or Slf1p Are Less Sensitive to Certain Inhibitors of Translation

Since Sro9p and Slf1p associate with polyribosomes, we examined the sensitivity of strains lacking these proteins to several protein synthesis inhibitors. Wild-type strains and strains lacking these proteins were incubated on rich media plates in the presence of filter discs containing paromomycin, cycloheximide, hygromycin B, or anisomycin. By comparing the zone of growth inhibition around the disk, we assessed the relative antibiotic sensitivity of the strains. Strains lacking Slf1p or Sro9p or both proteins were less sensitive than isogenic wild-type strains to the aminoglycoside antibiotic paromomycin (Figure 8A), which decreases translational fidelity during elongation (Singh *et al.*, 1979). Interestingly, strains lacking Slf1p were reproducibly less sensitive to paromomycin than strains lacking Sro9p or strains lacking both proteins (Figure 8A). Strains lacking Sro9p, but not strains lacking only

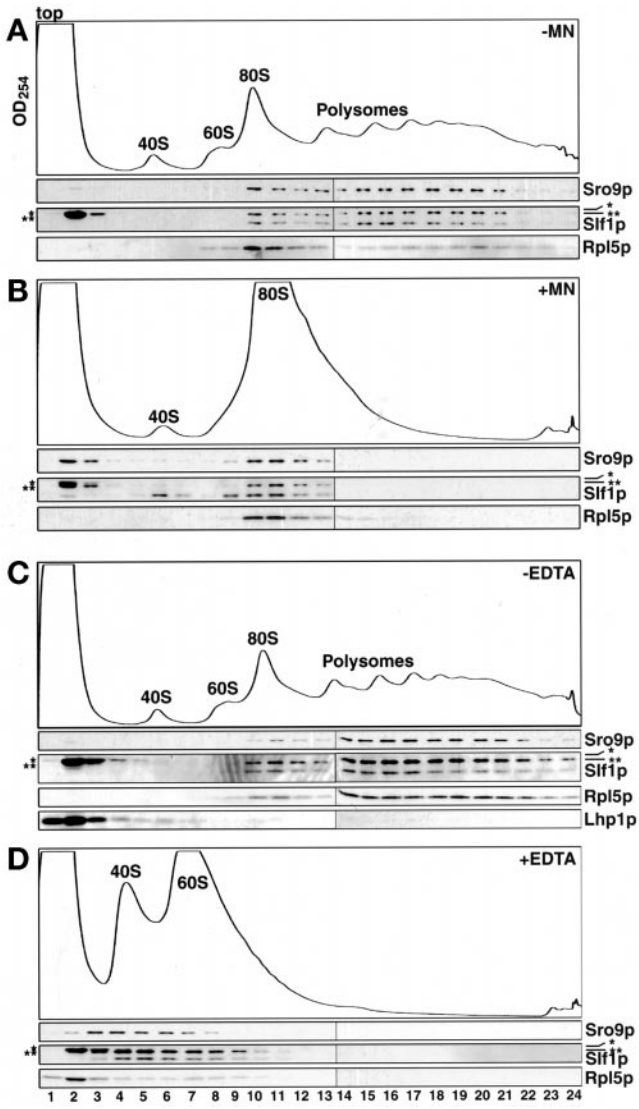

Figure 7. Polysome-disrupting conditions alter the sedimentation of both Sro9p and Slf1p. Cells were harvested in the presence of cycloheximide, and the lysates were subjected to sucrose gradient analysis. (A and B) Lysates were incubated in the absence (A) or presence (B) of 5 U/μl micrococcal nuclease before sedimentation on gradients. (C and D) Lysates were incubated in the absence (C) or presence (D) of 20 mM EDTA.

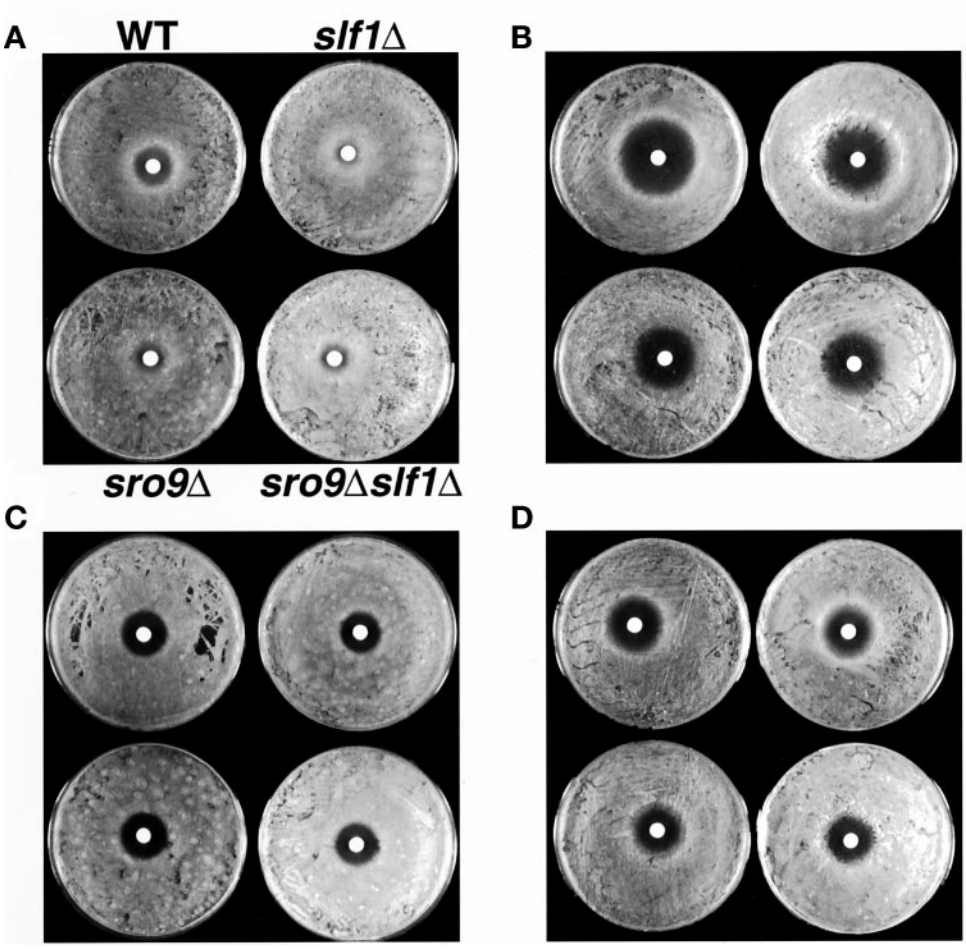

Figure 8. Strains lacking either Slf1p or Sro9p are less sensitive to a subset of protein synthesis inhibitors. Wild-type cells, *slf1::HIS3* cells, *sro9::URA3* cells, and *slf1::HIS3 sro9::URA3* cells were plated on YPD agar in the presence of disks containing 2.5 mg paromomycin (A), 2.5 μg cycloheximide (B), 200 μg hygromycin B (C), or 100 μg anisomycin. Plates were incubated for 2 d at 24.5°C.

Slf1p, were also less sensitive to the elongation inhibitor cycloheximide (Figure 8B); however, these strains, as well as strains lacking both proteins, were similar to wild-type strains in their sensitivities to hygromycin B (Figure 8C), which like paromomycin decreases translational fidelity (Singh *et al.*, 1979). They were also similar in their sensitivity to anisomycin (Figure 8D), which inhibits the peptidyl transferase reaction (Cundliffe, 1990). The differential sensitivity of strains lacking Sro9p or Slf1p to specific translation inhibitors argues against the possibility that these strains are generally less sensitive to inhibitors than wild-type strains. Instead, together with the association of these proteins with polysomes, our results argue that Sro9p and Slf1p, either directly or indirectly, affect ribosome structure and/or function.

Because strains lacking Sro9p or Slf1p displayed decreased sensitivity to certain protein synthesis inhibitors, we determined whether polysome profiles were altered in the strains. To this end, cell extracts from strains lacking either Sro9p or both proteins were fractionated on sucrose gradients. This revealed no significant differences in the polysome profiles between wild-type strains and strains lacking these proteins (our unpublished results).

Sro9p and Slf1p Bind RNA In Vitro

Because Sro9p and Slf1p share a domain with a known RNA-binding protein, we tested whether these proteins exhibited RNA-binding activity in vitro. ^{35}S-labeled Sro9p was produced by in vitro translation and mixed with one of four RNA homopolymers immobilized on agarose beads. As a control, we examined the binding of Sro9p to ssDNA. We compared the RNA-binding activity of Sro9p with that of Lhp1p and, as a negative control, the protein kinase Cak1p (Kaldis *et al.*, 1996) (Figure 9). This revealed that ^{35}S-labeled Sro9p bound poly(U), poly(G), ssDNA, and to a lesser extent poly(A), but did not detectably bind poly(C) (Figure 9A). Experiments in which the RNA-binding activity of Slf1p was assayed revealed that this protein bound a similar spectrum of homopolymers (our unpublished results). Lhp1p displayed nearly the same homopolymer preferences as Sro9p (Figure 9A) and Slf1p (our unpublished results), which is consistent with the specificity of the human La protein for homopolymers (Stefano, 1984).

To determine the specificity and strength of the homopolymer binding, the experiment was repeated in the presence of increasing concentrations of NaCl (Siomi *et al.*, 1993). Although Sro9p binding to poly(A) and ssDNA was

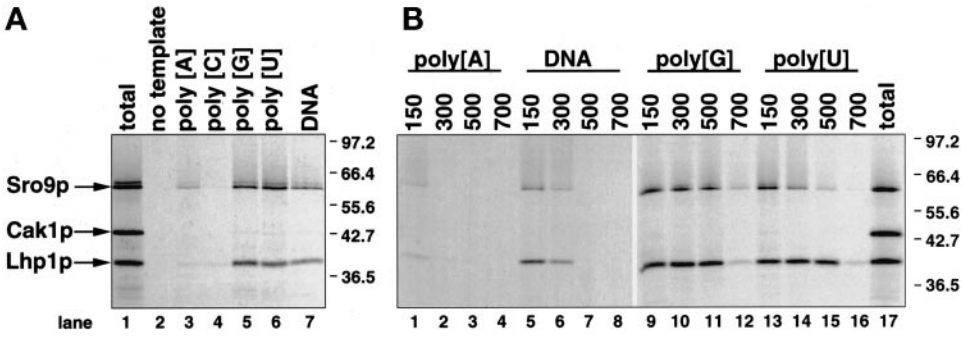

Figure 9. Sro9p binds RNA homopolymers in vitro. (A) ^{35}S-labeled Sro9p, Lhp1p, and Cak1p were synthesized by coupled transcription/translation in reticulocyte lysates and incubated with 40 μg of poly(A), poly(C), poly(G), poly(U), or ssDNA in the presence of 150 mM NaCl. Bound proteins were analyzed by SDS-PAGE. The lane marked "total" contains an amount of translation product equivalent to that used for each binding reaction. Molecular weight markers are indicated. (B) Binding was performed as described for A, but in the presence of the indicated NaCl concentrations.

diminished by higher salt (Figure 9B, lanes 1–8), binding to poly(U) and poly(G) was less affected by the NaCl concentration. Sro9p remained bound to poly(U) in 300 mM NaCl and remained bound to poly(G) even in 500 mM NaCl (lanes 15 and 11). Some Sro9p remained bound to poly(U) at the highest concentrations of NaCl (500 and 700 mM), and Sro9p binding to poly(G) was unaffected until the highest salt concentration (700 mM). A similar experiment in which Slf1p binding to homopolymers was assayed as a function of NaCl concentration revealed that Slf1p was indistinguishable from Sro9p in its homopolymer binding specificity (our unpublished results). Interestingly, both Sro9p and Slf1p could be distinguished from Lhp1p in their preference for homopolymers. Although Sro9p, Slf1p, and Lhp1p all exhibited strong binding to poly(G) and poly(U), Lhp1p bound poly(G) and poly(U) with equal strength at 500 mM NaCl, whereas Sro9p exhibits stronger binding to poly(G) at this salt concentration. Neither protein bound ssDNA at 500 mM NaCl. Thus, both Sro9p and Slf1p are RNA-binding proteins in vitro, and their specificity for RNA is distinct from that of Lhp1p.

DISCUSSION

We have used a combination of genetics and biochemistry to dissect the functions of two yeast proteins, Sro9p and Slf1p, that share a highly conserved motif with the La proteins. Because strains lacking Sro9p, Slf1p, and the La protein Lhp1p grow indistinguishably from cells lacking only Sro9p, the three proteins do not function redundantly in a single essential process. In agreement with others (Kagami *et al.*, 1997; Rosenblum *et al.*, 1997), we found that although Lhp1p is nuclear, Sro9p is predominantly cytoplasmic. Cell fractionation revealed that Sro9p and Slf1p associate with polyribosomes. Consistent with a role in translation, strains lacking either Sro9p or Slf1p have reduced sensitivity to a subset of protein synthesis inhibitors. Both Sro9p and Slf1p bind RNA in vitro and thus may bind RNA in vivo. These observations suggest that these two La motif-containing proteins may function in the cytoplasm to modulate mRNA translation.

A New Class of La Motif-containing Proteins

Our experiments have revealed that yeast contains two functional classes of La motif-containing proteins. One class consists of Lhp1p, the yeast homologue of the human La autoantigen. Lhp1p and other La proteins are nuclear phosphoproteins that bind nascent RNA polymerase III transcripts (Rinke and Steitz, 1982; Yoo and Wolin, 1994; Van Horn *et al.*, 1997). A second class of La motif-containing proteins, consisting of Sro9p and Slf1p, associates with polyribosomes. Because Sro9p and Slf1p have similar RNA homopolymer binding characteristics in vitro and are both ribosome-associated, they may have related functions. Consistent with this idea, Sro9p is down-regulated when Slf1p is overexpressed, and strains lacking either protein have reduced sensitivity to paromomycin; however, because cells lacking Sro9p, but not cells lacking Slf1p, are also less sensitive to cycloheximide, their functions may not be completely overlapping. Because deletion of *SRO9*, but not *SLF1*, results in slow growth, Sro9p may be more important to normal log-phase growth than Slf1p. Furthermore, *SRO9* has a relatively high codon bias (0.38), whereas *SLF1* has a low codon bias (0.06) (Hodges *et al.*, 1999), suggesting that Sro9p is expressed at a higher level than Slf1p. Interestingly, in a genome-wide experiment, it was found that Sro9p is down-regulated as the cell approaches stationary phase, whereas Slf1p is up-regulated (DeRisi *et al.*, 1997). Thus these proteins may have evolved toward specialization for different phases of cell growth.

H. sapiens, *M. musculus*, and *C. elegans* all have La motif-containing proteins that are distinct from the bona fide La protein in each organism. Because the La motifs of these proteins are more similar to Sro9p and Slf1p than to the La motifs of authentic La proteins, these proteins, along with Sro9p and Slf1p, may constitute a new functional class of proteins. Whether all of these higher eukaryotic La motif-containing proteins are cytoplasmic and function in mRNA translation is not known. Nonetheless, our phylogenetic analysis suggests that the functions of these proteins will be distinct from that of authentic La proteins. Furthermore, these proteins may function in processes that involve RNA binding.

Although the La motif has not been identified as containing a recognizable RNA-binding motif in any published compilation of such motifs (Birney *et al.*, 1993; Burd and Dreyfuss, 1994), it has been modeled to resemble an RRM (Kenan, 1995) and has thus been referred to as an RRM in some publications (Goodier *et al.*, 1997); however, an independent modeling of the La motif failed to support the assignment as a canonical RRM (Y. Shamoo, personal communication). Furthermore, although the residues that make up the RRM are highly degenerate, the La motifs of Sro9p, Slf1p, Lhp1p, and the other La motif-containing proteins are highly related at the amino acid level (Figure 1B). Although certain families of proteins, such as the SR family of splicing factors and the ELAV family of proteins, each have highly related RRMs (Birney *et al.*, 1993), the homologies within these protein families are not restricted to the RRM but extend throughout the proteins. The La motif appears to be unique in that it is found, essentially intact, in otherwise unrelated proteins. Thus, until the structure of the La protein has been determined, the assignment of the La motif as an RRM remains uncertain.

At least for the human La protein, the isolated La motif is not sufficient for RNA binding (Goodier *et al.*, 1997). Similarly, we found that a fragment of Sro9p containing the isolated La motif failed to bind RNA homopolymers (our unpublished data). Because several well characterized RNA-binding motifs require sequences flanking the motifs for RNA-binding activity (reviewed by Birney *et al.*, 1993), the failure of the isolated motif to bind RNA does not rule out a role for the La motif in RNA binding. In fact, even small deletions within the La motif of the human La protein dramatically affect RNA binding as well as protein function in vitro (Chang *et al.*, 1994; Goodier *et al.*, 1997). Whether the La motifs in Sro9p and Slf1p are major contributors to specific RNA recognition by these proteins is not yet known. Future experiments, in which we identify the RNA targets of these proteins and dissect the requirements for RNA binding, will be required to answer this question.

Possible Roles for Sro9p and Slf1p

Our data that Sro9p and Slf1p are polysome-associated, coupled with the decreased sensitivity of strains lacking these proteins to specific protein synthesis inhibitors, suggest roles for these proteins in mRNA translation; however, the preferential association of Sro9p and Slf1p with polysomes rather than 80S monosomes or ribosomal subunits makes it unlikely that these proteins are structural components of ribosomes. Furthermore, because yeast lacking both Sro9p and Slf1p are viable, these proteins cannot be required for the translation of any essential proteins. Instead, Sro9p and Slf1p could modulate mRNA translation, either for all mRNAs or for a specific subset. Because strains lacking either Sro9p or Slf1p are less sensitive to paromomycin, which acts on the 40S ribosomal subunit to increase the translational error rate (Cundliffe, 1990), Sro9p and Slf1p could function, either directly or indirectly, to modulate translational accuracy. Such a role would be consistent with the fact that cells lacking these proteins do not display significant changes in polysome profiles, because several other mutations that affect translational fidelity do not result in detectable polysome defects (Atkin *et al.*, 1997; Cui *et al.*, 1998). Furthermore, because mRNA degradation is closely linked to translation and often requires that the mRNA be polysome-associated (reviewed by Jacobson and Peltz, 1996), it is possible that these proteins also function in some aspect of mRNA stability or decay.

Although the human La protein has been reported to interact with 40S ribosomal subunits and to facilitate cap-independent translation, it is unlikely that Sro9p and Slf1p function to promote translation initiation. First, neither Sro9p nor Slf1p interacts with free 40S subunits when cell extracts are fractionated on sucrose gradients under normal conditions (Figures 6 and 7, A and C). Second, the preferential association of Sro9p and Slf1p with polysomes is more consistent with a role in either elongation or termination than in translation initiation. Last, because paromomycin and cycloheximide both act on elongating ribosomes, the decreased sensitivities to these antibiotics that we observed in strains lacking Sro9p or Slf1p are most compatible with a role for these proteins in elongation.

Because *SRO9* exhibits genetic interactions with *RHO3* and tropomyosin, it was proposed that Sro9p stabilizes actin filaments (Kagami *et al.*, 1997). Sro9p did not sediment, however, with actin filaments, and there is no evidence for a physical interaction of Sro9p with actin (Kagami *et al.*, 1997). We also found that Sro9p and Slf1p sediment independently of actin. Furthermore, overexpression of either Sro9p or Slf1p had no effect on actin levels in either wild-type or *act1–3* strains (our unpublished data). Because *SRO9* exhibits wide-ranging genetic interactions (see INTRODUCTION), a genetic argument for an interaction with actin is not strong. In addition, genetic results can be misleading when evaluating a protein like Sro9p that may have global effects on cells. For example, a component of both the SWI/SNF complex and the TFIIF and TFIID transcription complexes was originally identified as having an actin cytoskeletal function (Welch and Drubin, 1994; Cairns *et al.*, 1996). Similarly, a subtle defect in protein synthesis could exacerbate mutations in other pathways, possibly explaining several observations of synthetic lethality; however, it remains possible that Sro9p and Slf1p function in some way to facilitate the specific expression of actin and the other genes with which they exhibit genetic interactions.

Given that Sro9p and Slf1p exhibit homology to La proteins, perhaps they, like the La proteins, are molecular chaperones. Binding by Sro9p and Slf1p could stabilize mRNAs in the correct conformation for translation, modulate tRNA codon/anticodon interactions, or even facilitate rRNA rearrangements that are necessary for optimal translation. If the La motif constitutes part of a specific RNA-binding motif, perhaps Sro9p and Slf1p bind substrate(s) that in some way resembles the RNAs bound by the La protein. An understanding of the precise function of these two La motif-containing proteins will require the identification of their RNA targets, as well as the determination of how these novel RNA-binding proteins interact with ribosomes during the process of mRNA translation.

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