B1 and Alu are sequence-homologous interspersed elements of unknown function that have expanded in the genomes of mice and humans, respectively. A minority of B1 and Alu sequences are expressed as small cytoplasmic RNAs. These RNAs have conserved a secondary structure similar to that present in signal recognition particle (SRP) RNA despite substantial sequence divergence, whereas random B1 and Alu sequences have not. This RNA structure has also been conserved by the source sequences that gave rise to successive transpositions during B1 and Alu evolution. In the present work small cytoplasmic B1 and Alu RNAs synthesized in vitro were found to bind a cellular protein by mobility shift and UV cross-linking analyses. The mouse and human proteins demonstrate the same specificity to a panel of competitor RNAs. Results using mutated B1 RNA indicate that a single strand loop in the conserved Alu motif is essential for binding. Previous work by Strub et al. (Stub, K., Moss, J. B., and Walter, P. (1991) Mol. Cell. Biol. 11, 3949-3959) demonstrated that the Alu-specific protein SRP 9/14 does not footprint to this region of SRP RNA. This observation coupled with the failure of anti-SRP/9 antibodies to identify SRP 9/14 in the B1 RNA-protein complex as well as the apparent mass and other characteristics of the protein described here suggest that it is a novel B1-Alu RNA-binding protein. Conservation of primary and secondary structure by B1 and Alu small cytoplasmic RNAs as well as features of their specific expression and ability to interact with the conserved binding protein indicate that these RNAs are more homologous than previously appreciated.

Short interspersed elements (SINEs) were amplified to high copy number in the genomes of higher eucaryotes and are homologous to regions of transfer and other small cytoplasmic RNAs (reviewed in Refs. 1-5). Sequences known as Alu represent the major family of SINES in humans (1, 2). An Alu repeat is comprised of a ~280-bp dimeric core element followed by a poly(A) tract. The left Alu monomer contains an internal promoter for RNA polymerase III, whereas the right monomer instead contains a stretch of 31 base pairs not found in the left. B1 is a monomeric element which exhibits similar internal promoter structure and 78% overall sequence homology with the left Alu monomer (1). The Alu sequence is evolutionarily related to one of the domains of the 7SL RNA component of signal recognition particle (SRP) (6, 7). The "Alu domain" of SRP modulates translation of mRNAs that encode secretory and membrane proteins (8, 9).

Several workers (10-13) have noted that the Alu domain RNA structure was conserved by founder genes that were the source of successive transpositions during rodent B1 and primate Alu evolution, suggesting that factors involved in retrotransposition might interact with this RNA motif. Specific Alu and B1 transcripts are expressed in vivo as small cytoplasmic RNAs distinct from 7SL, yet their biological role is unknown (14-16). Small cytoplasmic (sc) B1 RNA, but not the great majority of genomic B1 sequences, has conserved a complex secondary structure virtually identical to the Alu domain of 7SL SRP RNA despite substantial divergence in its nucleotide sequence (16). By analogy to SRP function this suggests that scB1 RNA might be involved in the expression of a subset of mRNAs. The expression of scB1 RNA in fetal and germ line-containing tissue (14, 16) suggests a role for this RNA in development.

Analyses of human Alu repeats revealed a highly specific sequence subset which was distinguishable from the majority of "garden variety" Alus (reviewed in Ref. 15). The most abundant Alu transcript detectable in HeLa cells which corresponds to this specific Alu sequence is a small cytoplasmic species of ~120 nucleotides (nt) distinct from 7SL SRP RNA (15). This was a surprising finding, since the highly specific Alu sequence predicts a dimeric transcript of ~300 nt. Cumulatively, the data suggest that the small cytoplasmic Alu (scAlu) and scB1 RNAs are specific cytoplasmic transcripts of conserved structure (15, 16) and that B1 and Alu families consist of specific genes that evolved under selective pressure plus a large number of transposed copies of these genes.

The objectives of the present study were to compare scB1 and scAlu RNAs with respect to size, nucleocytoplasmic distribution, abundance, and ability to specifically interact with a novel cytoplasmic protein in vitro in order to evaluate the degree to which these features of their expression have been conserved. Although characterization of SRP RNA-binding proteins was not our primary goal, we found it useful to include SRP RNA as a competitor in our mobility shift analyses. Information previously known about SRP RNA and its Alu-specific protein binding sites was useful in directing some of our experimental inquiries. The translational control domain of SRP is composed of Alu-homologous RNA and a heterodimeric protein SRP 9/14, whereas the other four SRP polypeptides have been mapped to separate physical and functional domains (Ref. 8 and see "Discussion"). The binding
sites for SRP 9/14 have been footprinted to distinct regions of the Alu portion of SRP RNA (17), whereas a stretch of 17 contiguous bases within the Alu region is excluded from SRP 9/14 contact (17). This 17-base stretch represents a region of 94% sequence identity in scB1 and SRP RNAs, whereas they share less than 75% identity in the remainder of their homologous regions (16). Furthermore, this 17-base stretch comprises a prominent stem-loop motif which in scB1 RNA, and SRP RNA is readily accessible to U2 nuclease (16) and hydroxyl radical (17) cleavage, respectively. Thus the conservation of this motif and its apparent exclusion in SRP RNA from contact with SRP proteins suggests that it might interact with a polypeptide distinct from previously described SRP proteins.

Here we report evidence that such a protein exists. The combined use of RNA mobility shift, UV cross-linking, and ion-exchange chromatographic analyses demonstrated that a polypeptide isolated from the cytoplasm of murine erythroleukemia (MEL) and HeLa cells binds scB1 and scAlu RNAs.

MATERIALS AND METHODS

**RNA Purification and Northern Analysis**—RNA preparation and blotting were as described (16). The oligodeoxynucleotide probe (5'-GGG CCT CCT CTT CTT GAC GA-3'), described elsewhere (16), is 100% complementary to bases 65 through 88 of the conserved and predicted variant Alu consensus sequences as compiled by Matera et al. (15, 18). This oligo-DNA is only 58% complementary to 7SL SRP RNA. Although this probe is nondiscriminatory with respect to the above Alu subfamilies, an oligo-DNA probe that incorporates 2 bases diagnostic of the predicted variant subfamily (15), also known as human-specific (19), also detects the -118-nt RNA as the most abundant discrete cytoplasmic transcript of Alu as well (data not shown). The 22-nt probe of scB1 RNA was described previously (16). Hybridization was at 53°C for B1 and 58°C for Alu. Each was washed at its hybridization temperature.

**RNA Mobility Shift Assays**—[^8P]-Labeled scB1 RNA (0.2-1.0 ng, as indicated in the figure legends) synthesized in vitro was incubated with protein extract in 20-μl reactions containing 10 mM Tris-HCl, pH 7.5, 80 mM KCl, 5 mM MgCl₂, 0.1 mM Triton X-100, 1 mM DTT, 1 mM EDTA, 4 units of RNaseA, 5% glycerol, and 100 ng of poly(rG) (Boehringer Mannheim) (20, 21). After a 40-min incubation at room temperature, samples were loaded onto an 8% nondenaturing polyacrylamide gel containing 45 mM Tris borate, pH 8.3, and 12.5 mM EDTA. Gels were dried prior to autoradiography.

**Synthesis of scB1 cDNA and scAlu**[^32P]-**RNA**—Radioactive scB1 RNA was synthesized as an enzyme-cleavable PCR-derived fragment which was amplified from a plasmid containing scB1 cDNA (pscB1-10), as described (16). The transcription reaction contained 40 mM Tris-HCl, pH 8.1, 8 mM MgCl₂, 5 mM DTT, 1 mM spermidine, 0.01% Triton X-100, 50 μg/ml of bovine serum albumin, 2 mM each of ATP, CTP, and UTP, 0.2 mM GTP, 20 units of RNasin, 50 μM of [α-[^32]P]GTP (3000 Ci/mmol), 25 pmol of DNA fragment, and 200 units of T7 RNA polymerase (Promega). The specific activity of the RNA product was ~3 × 10⁶ cpm/μg and was purified as a single band by PAGE. scAlu RNA was synthesized from the left monomer of an Alu (see Fig. 1) located within the fourth intron of the cloned human α-fetoprotein (AFP) gene fragment, pHAFPSS (22) from a PCR-amplified fragment containing the T7 promoter and a 3' terminal designed to produce an Alu RNA of ~115 nt (16).

**Synthesis of Deleted and Base-substituted B1 and Other RNAs**—[^T7] promoter-containing DNA templates lacking 5, 14, or 40 bp at the 5'-end and those that incorporated site-directed substitutions at positions 35-40 (see Fig. 4A legend) of the scB1 cDNA sequence (16) were constructed by PCR using the appropriate T7-B1 chimeric primers (16). 7SL RNA was synthesized from aplastic RNA (23) to a PCR-generated template in the same way (16) from the 7SL RNA gene pl7SL.1 (23) designed to produce a transcript that corresponds to mature cytoplasmic 7SL RNA of 299 nt. The 7SL, 5'-3', and one site-directed substitution mutants of scB1 RNA are designated 7SL, B1-d5, B1-d14, B1-d40, and B1-Sub in sequence, respectively. They were synthesized as above except that the GTP concentration was 2 mM with the exception of [α-[^32]P]GTP (3000 Ci/mmol), 3000 Ci/mmol was obtained from Boehringer Mannheim. All RNAs were gel-purified prior to use. The size, integrity, and concentration of all RNAs used were verified by ethidium bromide staining and comparison with standard markers after electrophoresis. The authenticity of B1-Sub was verified by restriction analyses which confirmed the loss of an Alu site in the mutated region of the DNA template used to synthesize the mutated scB1 RNA.

**UV cross-linking and subsequent analysis was carried out as described by Vakalopoulou (24) with slight modifications.**[^32P]-Labeled scB1 RNA was incubated with protein extract at room temperature for 40 min. The mixtures were then placed onto a 36-well microtiter plate on ice and irradiated for 10 min, with a Miniscript lamp (model UVG-54; Ultra-Violet Products Inc., San Gabriel, CA) placed 5 cm above the plates. The samples were then mixed with an equal volume of loading buffer containing 50 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, and 2% β-mercaptoethanol, heated to 100°C for 5 min, and analyzed by 8% SDS-polyacrylamide gel electrophoresis. Autoradiography was for 1-5 days.

**Isolation of Cytoplasmic Protein**—MEL and HeLa cells were grown in suspension to 2 × 10⁶ cells/ml and washed twice with cold phosphate-buffered saline. Cells were resuspended in 10 mM HEPES, pH 7.9, 0.75 mM spermidine, 0.15 mM spermine, 0.1 mM EDTA, 0.1 mM KCl, 1 mM DTT, 1 mM phenylmethanesulfonyl fluoride (PMSF), allowed to swell on ice, and disrupted by Dounce homogenization. Nuclei and cytoplasm were separated by centrifugation at 16,000 rpm for 2 min in a Sorvall HB-4 rotor.

**Partial Purification of scB1 RNA-binding Protein**—[^4P]-The cytoplasmic supernatant from MEL cells was further fractionated by centrifugation at 49,000 rpm for 80 min at 4°C in a Ti50 rotor (Beckman). The pellet was resuspended with ice-cold buffer A (20 mM HEPES, pH 7.9, 20% glycerol, 100 mM KCl, 0.2 mM EDTA, 2 mM DTT, 1 mM PMSF, 12.5 mM MgCl₂), adjusted to 0.4 M NaCl, and stirred on ice for 1 h. The material was then resedimented by centrifugation at 49,000 rpm as described above. The supernatant was collected and dialyzed into buffer A and scored at ~8°C. This material was either used directly for RNA binding assays or further purified by anion-exchange chromatography. For further purification the material was diluted with an equal volume of buffer B (10 mM HEPES, pH 7.9, 0.5 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF, and 0.05 M NaCl) and adsorbed onto Q-Sepharose anion-exchange media (Pharmacia LKB Biotechnology Inc.). After washing with 2 column volumes of buffer B, the material was eluted with 0.15-0.5 M NaCl in buffer B. Fractions were collected and assayed by scB1 [γ-^32P]-RNA mobility shift and UV cross-linking. Protein concentration was determined by the Bio-Rad protein assay.

**RESULTS**

**Mouse and Human Cells Contain Alu-related Transcripts of Monomeric Size, Which Are Comparable in Abundance and Nucleocytoplasmic Distribution**—[^28] A highly specific mouse B1 (Alu-equivalent) RNA expressed in fetal and adult germ-line containing tissue can be distinguished as a small cytoplasmic (sc) transcript (14, 16). This scB1 RNA corresponds to a 3'-processed transcript of conserved secondary structure (16). Matera et al. (15) reported that a ~120-nt cytoplasmic RNA is the major human Alu transcript homologous to the highly specific predicted variant sequence. The ~120-nt Alu RNA was unexpected, since the expected full-length transcript of an Alu SINE should be ~300 nt or longer. As proposed by Matera et al. (15) the ~120-nt species does correspond to a 3'-processed transcript of the left monomer of the Alu sequence. However, although B1 and Alu SINES represent monomeric and dimeric structures, respectively, the discrete cytoplasmic transcripts homologous to these sequences are monomeric in structure. The linear structures of these DNA elements and the small cytoplasmic transcripts homologous to them are depicted in Fig. 1.

The presence of human scAlu RNA was confirmed and directly compared with mouse scB1 RNA in Fig. 2 by PAGE/RNA blot analysis using [γ-^32P]-labeled Alu- and B1-specific oligo-DNA probes. Nuclear and cytoplasmic RNAs prepared from MEL and HeLa cells were coelectrophoresed and trans-
whereas the B1 SINE newly inserted elements (15, 18, 19). The right Alu monomer contains a 31-bp sequence not found in the sequences. Thus, Alu and B1 SINE transcripts extend through their 3' direct repeats function. The transcriptional termination signal RNA polymerase RNAs were enriched in the cytoplasm (15, 16) and were demonstrated to be of comparable abundance (Fig. 2). scBl RNA associates with a protein in vitro—An RNA mobility shift assay was used to identify a scBl RNA-binding protein. Incubation of MEL cell cytoplasmic extract with scBl 32P-RNA, synthesized in vitro, lead to the appearance of a complex which could be followed by nondenaturing PAGE (Fig. 3A). The amount of complex formed was dependent on

was also detected in several other human-derived cell lines and normal human blood cells. A. mobility shift of scBl RNA by MEL cytoplasmic protein. scBl 32P-RNA was incubated with no added protein (lane 1) and 0.75 (lane 2) and 1.5 (lane 3) μg of protein as described under "Materials and Methods." Lane 4, the binding reaction included 10 μg of proteinase K. The arrow indicates the RNA-protein complex. B, competition of scBl 32P-RNA-protein complex by unlabeled scBl RNA and 7SL RNA but not tRNA. The binding reactions contained 0.2 ng of scBl 32P-RNA and were supplemented with 0.5, 1, 5, and 10 ng of unlabeled scBl RNA (lanes 7-10), and 0.5, 1, 5, and 10 ng of 7SL RNA (lanes 11-14), Lane 1, no protein was added; lane 2, scBl 32P-RNA plus protein but with no added competitors.

![Diagram](image-url)

**Fig. 1.** Relationship between Alu and B1 interspersed DNA elements and the small cytoplasmic RNAs homologous to these sequences. The Alu DNA (top) SINE is composed of two monomers (designated left and right) which are connected by an A-rich spacer, whereas the B1 SINE (bottom) is monomeric. Both SINEs are flanked by direct repeats (cross-hatched boxes; DR) and are transcribed by RNA polymerase III from internal promoters comprised of A and B boxes (filled rectangles). Poly(A) tails of ~40 bases are associated with newly inserted elements (15, 18, 19). The right Alu monomer contains a 31-bp sequence not found in the left and exhibits no promoter function. The transcriptional termination signal (Term) for RNA polymerase III is a (dT), tract which is not contained within B1 and Alu SINEs. Thus, Alu and B1 SINE transcripts extend through their 3' direct repeats (RNA, broken lines). However, in vivo small cytoplasmic RNAs (RNA, sold lines) homologous to 3'-truncated Alu and B1 transcripts accumulate in the cytoplasm (15, 16).

![Image](image-url)

**Fig. 2.** Comparison of small Alu and B1 RNAs by Northern blot analysis. RNAs isolated from either nuclei (N) or cytoplasms (C) of MEL and HeLa cells were separated by 5% PAGE, transferred to a nylon filter, and probed with oligonucleotides complementary to scBl RNA and Alu homologous sequences which are interspersed in heterogenous nuclear transripts. The ~155-nt cytoplasmic transcript was detected with the B1-specific oligonucleotide in mouse RNA (16) (lane 2) and is shown in Fig. 2 for comparison with the Alu RNA in HeLa cytoplasm which migrated with the 118-nt marker (lane 3). Both of these small RNAs were enriched in the cytoplasm (15, 16) and were demonstrated to be of comparable abundance (Fig. 2). Experiments using in vitro synthesized RNAs showed that MEL cells accumulate approximately 10^6 copies of scBl RNA/cell (data not shown). Alu-homologous RNA was also reproducibly detected as a faint diffuse band in the 350–450-nt size range of HeLa cytoplasmic RNA (Fig. 2) as well. This larger RNA may represent an unprocessed dimeric Alu primary transcript initially identified by Matera et al. (15). The ~118-nt scAlu RNA was also detected in several other human-derived cell lines and normal human blood cells.

![Image](image-url)

**Fig. 3.** A, mobility shift of scBl RNA by MEL cytoplasmic protein. scBl 32P-RNA was incubated with no added protein (lane 1) and 0.75 (lane 2) and 1.5 (lane 3) μg of protein as described under "Materials and Methods." Lane 4, the binding reaction included 10 μg of proteinase K. The arrow indicates the RNA-protein complex. B, competition of scBl 32P-RNA-protein complex by unlabeled scBl RNA and 7SL RNA but not tRNA. The binding reactions contained 0.2 ng of scBl 32P-RNA and were supplemented with 0.5, 1, 5, and 10 ng of unlabeled scBl RNA (lanes 7-10), and 0.5, 1, 5, and 10 ng of 7SL RNA (lanes 11-14). Lane 1, no protein was added; lane 2, scBl 32P-RNA plus protein but with no added competitors.

the amount of protein added (lanes 2 and 3) and was sensitive to proteinase K (lane 4). This indicated that the complex was composed of protein and scB1 \(^{32}\text{P}\)RNA. The binding specificity of this complex was demonstrated by competition with various unlabeled RNAs. The formation of binding complex was significantly reduced by 0.5 ng or more of unlabeled scB1 RNA (Fig. 3B, lanes 3–6), whereas tRNA did not affect scB1 \(^{32}\text{P}\)-RNA complex formation (Fig. 3B, lanes 7–10) even when present at 0 ng/reaction, a 200-fold molar excess.

scB1, Alu, and 7SL SRP RNAs are evolutionarily related transcripts that adopt similar cruciform structures composed of two stem-loop motifs near their 5' ends (12, 13, 16, 25). Unlabeled 7SL RNA competed scB1 \(^{32}\text{P}\)-RNA complex formation (Fig. 3B, lanes 11–14) as efficiently as did unlabeled scB1 RNA (Fig. 3B, lanes 3–6). Competition by unlabeled scB1 and 7SL but not tRNA demonstrated that the binding between scB1 \(^{32}\text{P}\)-RNA and the cytoplasmic protein exhibited some specificity and suggested that the 5' Alu motif structure might be important for binding. Since the first 50 nucleotides of scB1 RNA forms the characteristic Alu cruciform (16) (see Fig. 4A), deletions in this region are predicted to disturb the secondary structure of this RNA. To confirm the importance of the 5'-Alu motif for complex formation, various 5'-deleted as well as site-directed base-substituted scB1 RNAs were synthesized in vitro and used as competitors. B1-d5, B1-d14, and B1-d40 represent 5, 14, and 40 nucleotide 5'-deletions of scB1 RNA (Fig. 4A). Full-length unlabeled scB1 RNA reproducibly competed well with scB1 \(^{32}\text{P}\)-RNA for complex formation when present at 0.1 pmol (10-fold molar excess) or higher (Fig. 4B, lanes 2–5). In contrast, B1-d5 was demonstrably reduced in its ability to compete with full-length scB1 \(^{32}\text{P}\)-RNA (Fig. 4B, lanes 6–9), since it decreased complex formation only when present at 0.25 pmol or more. Removal of either 14 or 40 bases from the 5'-end of scB1 RNA completely abolished its ability to compete in the mobility shift assay (Fig. 4B, lanes 10–16). As expected, a mutant 7SL RNA deleted of its Alu domain reproducibly demonstrated no competition with scB1 \(^{32}\text{P}\)-RNA (data not shown).

We also used full-length scB1 RNA that harbors base substitutions at positions 35–40 which reside in a single strand region of the Alu cruciform (see Fig. 4A) for competition. The results reproducibly showed that changing the wild type sequence 5'-GCACUC-3' to 5'-UGUGAA-3' rendered scB1 RNA unable to compete for complex formation (Fig. 4C). RNA secondary structure prediction using the Zucker minimal free energy program (26) revealed that the above sequence substitution did not affect scB1 RNA structure (not shown). Two other mutants with slightly different base substitutions in the same region were also unable to compete but are not shown for brevity. The size, integrity, and concentration of all RNAs used throughout this study were verified by comparison with standard markers after electrophoresis and staining. These results further demonstrated that the B1 RNA binding activity detected by mobility shift was specific.

\textit{scB1 and scAlu RNAs Bind a Protein Isolated from Either Mouse or Human Cells—Next, we tested the hypothesis that small cytoplasmic Alu and B1 RNAs could associate with a similar protein present in both human and mouse cell cytoplasmic extracts. Fig. 5A demonstrated that scB1 \(^{32}\text{P}\)-RNA could bind with either MEL (lane 1) or HeLa protein (lane 2) in mobility shift assays. \textit{In vitro} synthesized \(^{32}\text{P}\)-RNA designed to mimic the small cytoplasmic Alu RNA in HeLa cells (see “Materials and Methods” and Figs. 1 and 2) was used in mobility shift assays with both HeLa and MEL cell cytoplasmic protein. scAlu \(^{32}\text{P}\)-RNA formed complexes with mouse protein (Fig. 5A, lane 3) and HeLa protein (Fig. 5A, lane 4). Although HeLa and MEL proteins formed complexes of different electrophoretic mobilities in the nondenaturing gels employed for mobility shift analysis (Fig. 5A), UV cross-linking showed that when the binding proteins were separated by denaturing PAGE, they exhibited the same mobility (see below). As expected, the binding between scB1 \(^{32}\text{P}\)-RNA and MEL protein was competed by unlabeled scAlu RNA (Fig. 5B). The binding between scAlu \(^{32}\text{P}\)-RNA and the HeLa protein was competed by either unlabeled scAlu RNA or scB1 RNA but not by 5'-deleted B1 (B1-d40) or tRNA (Fig. 5C). The results demonstrated that the mouse and human proteins interacted with scAlu and scB1 RNAs \textit{in vitro} and exhibited the same apparent specificity to each of the competitors that were tested.}

\textbf{scB1 RNA Mobility Shift and UV Cross-linking Activities Coeluted from Ion-exchange Resin—MEL cell extracts were fractionated by Q-Sepharose anion-exchange chromatography (Fig. 6). The scB1 RNA binding activity bound Q-Sepharose and was subsequently eluted by a NaCl gradient. Mobility shift (Fig. 6A) and UV cross-linking (Fig. 6B) analyses were used to assay the Q-Sepharose fractions. Mobility shift was the most sensitive of these and reproducibly revealed that the scB1 RNA binding activity eluted in fractions 20–22 (Fig. 6A). scB1 \(^{32}\text{P}\)-RNA was UV cross-linked to a protein in the
same fractions and formed a RNA-protein complex that migrated between 97.4 and 200 kDa when compared with standard polypeptide markers (Fig. 6B). However, since we used full-length scB1 RNA (molecular mass, 45 kDa) in these experiments we cannot confidently estimate the mass of the protein component of the cross-linked complex.

We further characterized the specificity of the Q-Sepharose-fractionated UV-cross-linked complex. It was dependent on the addition of extract and UV irradiation (Fig. 7A) and was completely sensitive to proteinase K (data not shown). The formation of this cross-linked complex could be inhibited by unlabeled scB1 RNA but not tRNA (Fig. 7B). Furthermore, a cytoplasmic HeLa protein was UV cross-linked to scB1 RNA and formed a complex of the same electrophoretic mobility as the mouse cell-derived complex (Fig. 7C).

**DISCUSSION**

In this paper we report that B1 and Alu sequences expressed as small cytoplasmic RNAs bind in vitro to a protein extracted from either mouse or human cells. The in vitro binding was specific as demonstrated by mobility shift competition experiments that used full-length, deleted, and substituted scB1 RNAs, as well as 7SL and tRNA. The results suggested that two regions within the Alu motif are involved in the binding: nucleotides within the first 14 residues of the RNA and a sequence-specific single strand region in the conserved second stem-loop motif of the Alu cruciform (Fig. 4A).

Our data argue that the RNA-binding protein identified here is distinct from the Alu domain-specific SRP 9/14 protein and that SRP 9/14 is not responsible for the binding we detected. First, the scB1 RNA sequence differs from the previously determined conserved SRP 9/14 binding site in...
SRP RNAs at a G residue which is 100% conserved from bacteria to human (16, 17). Second, antisera raised against recombinant SRP 9-kDa protein (22) failed to affect the mobility shift of scB1 RNA (data not shown). Furthermore, although this serum specifically precipitated human 7SL SRP RNA, it did not immunoprecipitate scB1 RNA when both were expressed in internally controlled parallel experiments in the microinjected frog oocyte, yet these two RNAs compete for the scB1 3'P-RNA mobility shift activity characterized here with the same efficiency (Fig. 3B). Finally, UV cross-linking analysis suggest a mass for the scB1 RNA-binding protein which appears to be too large to be SRP 9/14. Cumulatively, the data indicate that it is unlikely that scB1 RNA-binding protein is the previously identified heterodimeric protein SRP 9/14.

The binding sites of the Alu domain-specific SRP 9/14 protein on 7SL SRP RNA were determined by Strub et al. (17) to be four stretches of which the largest is 15 bases and contains a sequence that has been highly conserved in evolution. However, a 17-base stretch within the Alu cruciform of 7SL SRP RNA appears not to bind SRP 9/14 (17). It is intriguing to note that this region (i) comprises the most highly conserved stretch (94%) of sequence shared by scB1 and 7SL RNAs (16), (ii) contains a prominent stem loop motif (16, 17) that in 9/14-reconstituted SRP is excluded from SRP 9/14 contact (17), and (iii) contains a single strand loop whose sequence appears to be important for binding to the polypeptide described here. Thus, it is encouraging that the protein we identified interacts with a motif conserved by cellular RNAs.

Our data suggest that 7SL RNA is able to interact with the binding protein identified here. Although this represents a potentially important observation, we must note that our competition experiments used free 7SL RNA, and we therefore do not know if fully assembled SRP, which may account for most of the 7SL RNA in cells, could effectively interact with the RNA-binding protein. Nonetheless, it is conceivable that this protein could functionally interact with SRP RNA but not copurify with SRP.

Andrews and Kole (28) identified the 68-kDa SRP polypeptide as an antigen which binds to a ~575-nt Alu-containing transcript by immunoprecipitation of in vitro transcription products. This was a surprising finding, however, since it had been previously shown that the 68-kDa SRP polypeptide was found tightly bound to the non-Alu region (known as the "S" region) of 7SL SRP RNA (29, 30). Also, the model of SRP structure/function and footprinting onto 7SL SRP RNA assigned 68-kDa SRP to the non-Alu S region of SRP RNA (31, 32). We were unable to demonstrate any specific effect of anti-68kD/SRP antibodies in our assay (data not shown).

Our results suggest that the Alu RNA-binding proteins from human and mouse cells identified here are homologous. First, the binding proteins from human and mouse cells formed complexes with either scAlu or scB1 RNA. Second, the binding between scAlu RNA and HeLa protein demonstrated the same specificity as did the scB1 RNA-MEL protein interaction in all competition experiments tested. Finally, we identified MEL and HeLa scB1 3'P-RNA-binding proteins of the same molecular weight by UV cross-linking SDS-PAGE (Fig. 7B). The cross-linked protein could be specifically competed for by scB1 but not tRNA, and it copurified with the MEL-derived protein responsible for the specific scB1 RNA mobility shift. The results suggest that the protein cross-linked to scB1 RNA is the same protein responsible for the mobility shift and that its binding activity has been conserved from macromolecular human.

Distinctive subsets of human Alu and mouse B1 sequences encode transcripts that appear to represent products of a homologous gene family. First, B1 and Alu elements expanded in their respective genomes through a succession of subfamilies (reviewed in Refs. 15 and 16) most probably through RNA-mediated transposition within germ line tissue (1, 16, 33). Second, Alu and B1 share almost 80% sequence identity and contain similar promoters for RNA polymerase III (1). Finally, only a small fraction of the >106 genomic copies of each of these sequences is expressed as stable cytoplasmic transcripts in human (15) (Fig. 1) and mouse tissue culture cells as well as adult mouse testes (16), which correspond to 3'-processed products of RNA polymerase III (15, 16). Our present results demonstrate that these RNAs have conserved the ability to interact with a specific protein(s). This supports the notion that these cytoplasmic RNAs represent functional transcripts and that they are more homologous than previously appreciated.

The specificity of the protein described here for the Alu domain RNA structure coupled with the evolutionary conservation of this RNA motif in Alu and B1 source genes (10-13) also suggests that this protein may be important for retrotransposition.

Acknowledgments—We thank A. Dugasiczky for providing pHAPPS.5, T. Howard and G. Humphrey for sharing HeLa cells and extracts, K. Strub and P. Walter for antisera to 5KD/SPF, and R. Kole for antisera to 5K/SP. We also thank D. Halle and G. Humphrey for incisive comments on the manuscript, A. Furano for suggesting Fig. 1 and B. Howard and the members of the Laboratory of Molecular Growth Regulation for provocative discussions and support.

REFERENCES