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A Human *Alu* RNA-Binding Protein Whose Expression Is Associated with Accumulation of Small Cytoplasmic *Alu* RNA

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Human *Alu* sequences are short interspersed DNA elements which have been greatly amplified by retrotransposition. Although initially derived from the 7SL RNA component of signal recognition particle (SRP), the Alu sequence has evolved into a dominant transposon while retaining a specific secondary structure found in 7SL RNA. We previously characterized a set of *Alu* sequences which are expressed as small cytoplasmic RNAs and isolated a protein that binds to these transcripts. Here we report that biochemical purification of this protein revealed it as the human homolog of the SRP 14 polypeptide which binds the *Alu*-homologous region of 7SL RNA. The human cDNA predicts an alanine-rich C-terminal tail translated from a trinucleotide repeat not found in the rodent homolog, which accounts for why the human protein-RNA complex migrates more slowly than its rodent counterpart in RNA mobility shift assays. The human *Alu* RNA-binding protein (RBP) is expressed after transfection of this cDNA into mouse cells. Expression of human RBP in rodent × human somatic cell hybrids is associated with substantial increase in endogenous small cytoplasmic *Alu* and scB1 transcripts but not other small RNAs. These studies provide evidence that this RBP associates with *Alu* transcripts in vivo and affects their metabolism and suggests a role for *Alu* transcripts in translation in an SRP-like manner. Analysis of hybrid lines indicated that the *Alu* RBP gene maps to human chromosome 15q22, which was confirmed by Southern blotting. The possibility that the primate-specific structure of this protein may have contributed to *Alu* evolution is considered.

Alu sequences are short transposed elements endogenous to the human genome (24, 55). These elements have accumulated to nearly 1 million copies comprising approximately 5% of human DNA. *Alu* repeats have sequence homology with and are believed to have been derived from the *Alu*-homologous region of the 7SL RNA component of signal recognition particle (SRP) (64, 69, 70, 71). The current model of *Alu* evolution suggests that two independently derived *Alu*-homologous domains of 7SL dimerized to form the more efficient transposing element known as *Alu* (26, 28, 45, 46, 47). Since then, *Alu* repeats have continued to evolve within primate genomes. A major advance in understanding *Alu* mobility was the result of work by researchers in several laboratories who classified *Alu* repeats into subfamilies according to their evolutionary relatedness as determined by sequence homology (8, 27, 45, 59, 72). This, together with recent phylogenetic evidence, indicates that multiple *Alu* source genes continue to spawn new transposed elements (22, 25, 31, 39, 42, 67; reviewed in reference 49).

Why genetic elements with disruptive potential have been allowed to proliferate to high copy number in human DNA remains enigmatic. Perhaps *Alu* mobility improves adaptability of the host genome, or perhaps *Alu* DNA elements or their RNA products have adopted a function(s) which more directly benefits their host cells (9, 19, 51, 74). Given their short length and lack of open reading frame, it is likely that *Alu* elements are not autonomous transposons and that they depend on cellular factors for expression. It was suggested that products of endogenous long interspersed elements meditate retroinsertion of *Alu* transcripts (13). In any case, the cellular factors which support expression of *Alu*s and processing of their transcripts remain unidentified (51).

The *Alu*-homologous region of the 7SL RNA component of SRP forms a tRNA-like secondary structure (17, 52, 75). This RNA secondary structure has been conserved in each of the active *Alu* and murine B1 (*Alu*-equivalent) subfamily founder sequences despite divergence of their primary structures (26, 29, 34, 56). These phylogenetic comparative data provide powerful evidence that this RNA secondary structure is important if not required for Alu transposition (29, 49, 56). In addition, other structural evidence indicate that Alu elements are retrotransposed via RNA intermediaries synthesized by RNA polymerase III (pol III) (49, 50, 71). Pol III transcription starts at the 5′ end of an *Alu* element and continues through its 3′ A-rich region before stopping at a downstream pol III termination site. This produces a primary transcript containing an A-rich tract and ending in oligo(U) (15, 24). Current models of *Alu* transposition suggest that the A-rich tract of *Alu* primary transcripts serves as a template from which *Alu* cDNA is synthesized prior to de novo insertion (23, 33, 49, 65). Thus, it may be relevant that some *Alu* and B1 primary transcripts undergo 3′ processing since this would inactivate the potential transposition intermediaries by conversion to the small cytoplasmic (monomeric) poly(A)-minus RNAs found in vivo (1, 11, 37, 40). Such processing would reduce the potential of *Alu* primary transcripts for retroposition by decreasing the likelihood of generating a full-length reverse cDNA (34, 37).

The cytoplasmic partitioning and accumulation of processed *Alu* and B1 RNAs in vivo suggests that they interact with cellular proteins (1, 32, 34, 35, 37, 40, 57). The monomeric structure of small cytoplasmic *Alu* (sc*Alu*) RNA indicates that it is not a transposition substrate and that it may be involved in

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a distinct cytoplasmic activity. Our laboratory is studying factors which associate with *Alu* and B1 transcripts to affect their metabolism (11, 35, 36, 38). The preservation of the *Alu* RNA secondary structure by sc*Alu* and scB1 suggests that these RNAs might interact with the 9- and 14-kDa *Alu*-specific polypeptide subunits of SRP known as SRP 9/14 (1, 11, 34, 37, 60). Although SRP 9/14 can bind to the isolated *Alu*-homologous region of 7SL RNA (18, 52, 60, 62), it was unknown whether this heterodimeric protein could associate with cellular sc*Alu* and scB1 RNAs. We recently characterized a protein which binds sc*Alu* RNA by in vitro mobility shift analysis (11). However, the identity of this *Alu* RNA-binding protein (RBP) had not been determined.

Rodent × human somatic cell hybrids (SCHs) have been used to study human *Alu* elements (5, 37, 67). It was previously noted that hybrid cell lines which contained human chromosome 15 as the only human chromosome expressed a substantially higher level of sc*Alu* RNA compared with other hybrids but less of the *Alu* primary transcript intermediate (37). In the present study, this observation was pursued and extended. By taking advantage of the different mobilities of the human and rodent RBPs in RNA mobility shift assays (11), the gene encoding *Alu* RBP was mapped to human chromosome 15 by using rodent × human hybrid cell extracts. The results led to the conclusion that the *Alu* RBP gene resides on human chromosome 15q22 and its expressin leads to elevation of sc*Alu* and scB1 RNA levels in vivo. On the basis of this novel activity, we chose to purify and further characterize this protein.

In this study, this *Alu* RBP was purified to apparent homogeneity from HeLa cells. The resulting 18-kDa Alu RBP is highly homologous to the previously cloned mouse 14-kDa subunit of the SRP 9/14 protein. The human cDNA contains a CAG trinucleotide expansion in the 3′ coding region which accounts for the larger size and slower mobility of the human protein-RNA complex noted in gel retardation assays (11). The evidence indicates that the 18-kDa *Alu* RBP is the human SRP 14-equivalent protein.

MATERIALS AND METHODS

Protein purification. Twenty liters of HeLa cells was harvested, washed, and resuspended in buffer containing 10 mM morpholine ethanesulfonic acid (MES; pH 6.5), 10 mM NaCl, 1.5 mM $MgCl_2$, 1 mM dithiothreitol (DTT), and 0.1 mM phenylmethanesulfonyl fluoride (PMSF). Cells were disrupted by Dounce homogenization using a B pestle. After centrifugation at 1,000 × *g* for 10 min, the cytoplasmic fraction was precipitated in 65% NH_4SO_4. The precipitate was dialyzed against buffer A (50 mM Tris-HCl [pH 7.4], 0.1 mM EDTA, 0.5 mM DTT, 0.1 mM PMSF, 20% glycerol) containing 0.05 M NaCl and applied to Q Sepharose (Pharmacia) equilibrated with buffer A containing 0.05 M NaCl. Protein was eluted with a 0.05 to 0.5 M gradient of NaCl in buffer A. Fractions containing sc*Alu* RNA mobility shift activity were pooled and applied to S Sepharose (Pharmacia). This was eluted with a 0.25 to 1.0 M gradient of NaCl in buffer A. Fractions containing activity were again pooled and loaded onto Blue Sepharose (Pharmacia). Elution was with a 0.05 to 3.0 M gradient of NaCl in buffer A. Fractions containing activity were dialyzed against buffer B (0.01 M KCl, 0.5 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF, 20% glycerol, 0.05 M KPO_4 [pH 7.4]) and loaded onto hydroxylapatite (Bio-Rad) equilibrated with buffer B. After washing, the column was eluted with a gradient of KPO_4 in buffer B. The fractions containing activity were dialyzed against buffer A containing 0.05 M NaCl and applied to heparin agarose (Bethesda Research Laboratories [BRL]) equilibrated with buffer A containing 0.05 M NaCl. The column was eluted with a 0.05 to 2.0 M gradient of NaCl in buffer A. The fractions with activity were pooled and concentrated. Two major polypeptides migrating at 18 and 10 kDa in the final pool of activity were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose. After staining with Ponceau S, these bands were cut out and submitted to W. Lane at the Harvard University Microchemistry Facility for in situ tryptic digestion, high-pressure liquid chromatography (HPLC) purification, and amino acid sequencing.

***Alu* RBP cDNA.** We designed oligodeoxynucleotide primers based on the amino acid sequence EVNKFQMAYSNLLR determined from a tryptic peptide derived from purified human 18-kDa *Alu* RBP. This sequence is a perfect match to a region in mouse SRP 14 (61). Thus, our primers were derived from the mouse SRP 14 cDNA (61). To obtain the 5′ region of the corresponding cDNA, we used the method of Frohman et al. (14). First-strand cDNA was synthesized from total poly(A)$^+$ RNA by using the mouse SRP 14 gene-specific antisense primer 5′TTTGAATAGGCCATCTGAAA3′. The cDNA was then poly(dA) tailed by using terminal deoxynucleotide transferase (BRL) and ATP (14). This was PCR amplified by using a second SRP 14 gene-specific antisense primer, 5′GGCCATCTGAAACTTGTTCAC3′ (which is nested with respect to the primer used for first-strand cDNA synthesis), and a dT_{17} adapter primer (14). To obtain the 3′ region of *Alu* RBP cDNA, we first made oligo(dT)-primed cDNA by using total poly(A)$^+$ RNA as template. This total cDNA was used for PCR amplification with the mouse SRP 14 sense primer 5′GAAGTGAACAAGTTTCAGATG3′ and the dT_{17} adapter primer as described previously (14). Both the 5′ and 3′ PCR products were sequenced directly, using the PCR-mediated *fmol* sequencing kit (Promega). Primers corresponding to both the 5′- and 3′-terminal sequences (and containing cloning adapters) were used for direct PCR amplification of *Alu* RBP cDNA, using total oligo(dT)-primed cDNA as the template. This full-length cDNA was then cloned into the *Hin*dIII-*Xba*I sites of pGem-4Z (Promega) and designated pG-18k. The sequence of the full-length insert of this construct was confirmed by dideoxy sequencing and provided the full-length human-specific cDNA sequence, including the region coding for the purified tryptic peptide.

Transient transfection. The full-length cDNA *Hin*dIII-*Xba*I insert of pG-18k was subcloned into the *Hin*dIII-*Xba*I restriction sites of pCMV.3 (a gift from B. Howard). Twelve micrograms of the resulting recombinant plasmid pCMV.3-18k and 60 μl of Lipofectamine (BRL) were mixed with 1 ml of OptiMEM medium (BRL) and incubated at room temperature for 45 min. An additional 5 ml of OptiMEM was added just prior to transfection. NIH 3T3 cells (10^6 per transfection) were incubated with the medium containing plasmid and Lipofectamine at 37°C for 4 h. Transfection medium was then replaced with Dulbecco modified Eagle medium supplemented with 10% calf serum. After an additional 24-h incubation, cells were rinsed twice with phosphate-buffered saline and scraped into a solution of 50 mM Tris-HCl (pH 8), 0.1 mM EDTA, 1 mM DTT, 12.5 mM $MgCl_2$, 0.1 M KCl, 1% Triton X-100, and 20% glycerol. This material was vortexed for 30 s and centrifuged for 10 min in a microcentrifuge at 4°C. After being transferred to clean tubes, the whole cell extracts were frozen and stored at −80°C. An RNA mobility shift assay using T7 RNA polymerase-synthesized scB1 [^{32}P]RNA was performed as described previously (11). scB1 [^{32}P]RNA was incubated

with whole cell extract in 20-μl reactions and analyzed by 8% nondenaturing PAGE (11).

Somatic cell hybrids. Rodent × human SCHs GM10501, GM11418, GM10664, GM10659, GM06317, GM10926B, GM10611, GM10791, GM10498, GM10500, GM10567, GM10479, GM10478, GM07299, GM11010, GM10114, GM10115, GM10449, GM10629, GM10888, and GM10898 were obtained from Coriell Institute for Medical Research (Camden, N.J.) (21). The mouse cell-derived hybrids from this mapping panel are GM10479, GM10567, GM10498, GM10478, GM10323, and GM10659. Additional mouse-derived hybrids A9+2, A9+3, A9+12, and A9+15 were a kind gift of O. Pereira-Smith, as was GM10481 (43). The remaining hybrids were derived from Chinese hamster cells (21). Our independent verification of the human chromosomes in the hybrid cell lines was done by Giemsa-11 staining, which differentially stains rodent and human chromosomes (6, 37), and in some cases also by fluorescence in situ hybridization. At least 15 metaphases were analyzed for each hybrid cell line with the exception of GM11010 and GM10478, in which cases 12 and 11, respectively, were analyzed. The majority of cell lines contained the expected single human chromosome at a frequency within 10% of that published. In addition, GM10659 was examined by fluorescence in situ hybridization using a chromosome 15-specific probe which identified a chromosome 15 fragment translocated to a (der)17 as described previously (21); as expected, no additional chromosome 15 material was observed. For RNA mobility shift analysis, whole cell extracts were prepared as described above.

Southern blotting. The hybrid panel blot shown in Fig. 6A was obtained from Bios Laboratories Inc. (New Haven, Conn.). The blot shown in Fig. 6B was prepared by digesting previously characterized purified DNA (Coriell Institute for Medical Research) (21) with *Pst*I overnight. The DNA was fractionated and transferred to a nylon membrane (GeneScreen Plus; Dupont) by standard methods. The membranes were incubated with hybridization solution containing 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA [pH 7.7]), 0.1% SDS, 5× Denhardt's solution, and 100 μg of salmon sperm DNA per ml. After a 2-h prehybridization period, boiled probe was added and hybridization proceeded at 60°C for 20 h. The blots were washed with 1× SSPE–0.1% SDS for 3 h at 60°C and then 0.5× SSPE–0.1% SDS for 30 min at 60°C. Probe was generated by random priming in the presence of [α-^{32}P]dCTP of the first 305 bp of the sc*Alu*-RBP cDNA, using standard procedures.

Northern (RNA) blotting. Methods for RNA isolation, blotting from 8 M urea–6% polyacrylamide gels, and oligonucleotide hybridization were as described previously (34, 37). The probes for sc*Alu* and scB1 were *Alu*-24 and B1-22, respectively (11, 34). Although the B1-22 probe detects scB1 RNA in mouse-derived cells, it does not cross-react with a discrete hamster RNA under the hybridization conditions used.

The probe for mRNA was derived from sc*Alu*-RBP cDNA as described above. The human multiple-tissue Northern blot was obtained from Clontech (Palo Alto, Calif.). Hybridization was carried out in 5× SSPE–10× Denhardt's solution–50% deionized formamide–100 μg of salmon sperm DNA per ml–2% SDS at 42°C. The final two washes were in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS at 50°C for 20 min each.

Nucleotide sequence accession number. The GenBank accession number for *Alu* RBP retrieval from the sequence data base is U07857.

TABLE 1. Specific activity of *Alu* RNA binding activity recovered after sequential stages of purification

Pooled fraction	Sp act (U[a])	Recovery (%)
Ammonium sulfate	8.7	100
Q Sepharose	31.4	95.3
S Sepharose	211.9	7.8
Blue Sepharose	725.3	7.5
Hydroxylapatite	2,678.0	1.1
Heparin agarose	9,888.8	0.14

[a] Expressed as femtomoles of sc*Alu* RNA shifted per microgram of protein.

RESULTS

Purification of sc*Alu* RBP. The RBP previously described was purified from a cytoplasmic extract of HeLa cells by monitoring its activity in the B1-*Alu* RNA mobility shift assay (11). The activity was fractionated sequentially by Q Sepharose, S Sepharose, Blue Sepharose, hydroxylapatite, and heparin agarose chromatography (Table 1). The final chromatographic elution yielded only two major polypeptides with apparent masses of 18 and 10 kDa which were detectable by SDS-PAGE and silver staining (Fig. 1A, arrows). As can be seen from the elution profiles in the final chromatographic step, these polypeptides copurified with each other and with the RNA mobility shift activity (Fig. 1B and C). The specific activity of RNA binding in the mobility shift assay was increased approximately 1,000-fold by this purification scheme (Table 1).

The purified polypeptides were resolved by SDS-PAGE, transferred to nitrocellulose, and digested with trypsin, and the resulting peptides were separated by HPLC using standard methods (see Materials and Methods). The sequence of a well-resolved peptide derived from the 18-kDa band was determined with high confidence to be EVNKFQMAYS NLLR. This sequence was found to be 100% identical to amino acids 75 to 88 of mouse SRP 14 and the corresponding region of canine SRP 14 (61). The 10-kDa polypeptide did not yield resolvable tryptic peptides and was not further characterized; however, its size and other considerations suggest that it is SRP 9 (see Discussion).

On the basis of the mouse SRP 14 cDNA sequence (61) corresponding to the human tryptic peptide, oligodeoxynucleotides were designed to prime cDNA synthesis from the nonredundant codon positions by using HeLa poly(A)$^+$ RNA as the template. The PCR-based RACE (rapid amplification of cDNA ends) methods of Frohman et al. (14) were used on poly(A)$^+$ RNA and oligo(dT)-primed cDNA to generate 5′ and 3′ ends, respectively, of the cDNA. Primers corresponding to the sequences determined from the 5′ and 3′ termini were used to generate a single contiguous full-length fragment from oligo(dT)-primed cDNA (Fig. 2). The cDNA obtained spans 725 nucleotides and includes 295 nucleotides of 3′ untranslated region. An open reading frame of 136 amino acids predicts the entire tryptic peptide fragment obtained from the purified 18-kDa polypeptide (Fig. 2, underlined). A poly(A) addition signal AUUAAA (44) is found 22 nucleotides upstream from the poly(A) tail. The 28-residue tract comprising the C terminus of the translated sequence $(Ala)_9Pro(Ala)_4ThrAla$ $Pro(Thr)_2(Ala)_2(Thr)_2(Ala)_2Thr(Ala)_2Gln$ is not found in mouse SRP 14 (61).

The human-specific C-terminal tail is derived from an array of trinucleotide repeats in the human cDNA which are not found in the mouse sequence (Fig. 2). Excluding the C-terminal tail, *Alu* RBP and mouse SRP 14 are both 110

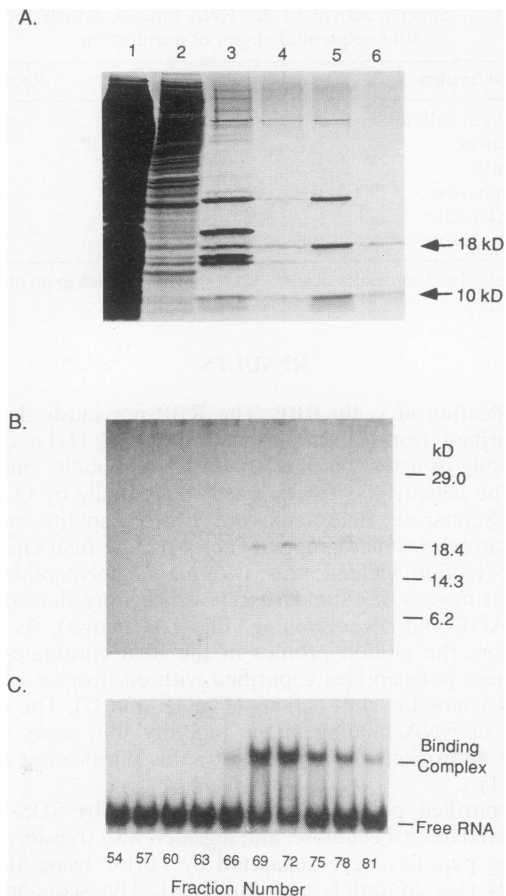


FIG. 1. Purification of sc*Alu* RBP. Two polypeptides of 10 and 18 kDa copurify with sc*Alu* RNA binding activity. (A) Fractions containing *Alu* RBP as determined by RNA mobility shift analysis (11) were pooled after each chromatographic elution, separated by SDS-PAGE (18% polyacrylamide gel) and stained with silver. Lanes: 1, ammonium sulfate precipitate of HeLa cell cytoplasm; 2 to 6, fractions eluted from Q Sepharose (lane 2), S Sepharose (lane 3), Blue Sepharose (lane 4), hydroxylapatite (lane 5), and heparin agarose (lane 6). Positions of the 18- and 10-kDa polypeptides are indicated on the right. (B and C) Fractions eluted from the final heparin column were separated by SDS-PAGE (15% polyacrylamide gel) and stained with silver in (B) or assayed for sc*Alu* RNA mobility shift activity (C). Numbers below the lanes indicate fraction numbers. Positions of molecular weight markers are indicated on the right.

residues long and exhibit 89 and 86% identity in their amino acid and nucleotide sequences, respectively. Of the 10 amino acid residues different between human *Alu* RBP and mouse SRP 14, 7 are conservative substitutions; 4 are serine-to-threonine substitutions, 2 are lysine-to-arginine substitutions, and 1 is a tyrosine-to-phenylalanine substitution. The extreme carboxy ends of human *Alu* RBP and mouse SRP 14 terminate in alanine-glutamine. This observation led to the speculation that the GCA codon used to encode the penultimate alanine of SRP 14 underwent expansion and mutation to produce 18 additional GCA codons and several ACA codons in the human sequence to encode the alanine+threonine-rich C-terminal tail. Similar trinucleotide expansions have apparently occurred during evolution of the cDNAs encoding the TATA-binding protein and other proteins of several species (16). These observations coupled with the high degree of homology between the RBP and SRP 14 sequences strongly support the contention that the *Alu* RBP characterized here is the human equivalent of SRP 14 (see Discussion).

To assess expression of the corresponding mRNA, a high-resolution (urea-PAGE) Northern blot was hybridized with a probe derived from the *Alu* RBP cDNA. This revealed a poly(A)$^+$ RNA species of approximately 900 nucleotides, consistent with the size expected of polyadenylated transcripts corresponding to the cDNA (Fig. 3A). Northern analysis of poly(A)$^+$ RNA isolated from human tissue samples and fractionated by 1.5% agarose indicated ubiquitous expression (Fig. 3B).

Expression of human *Alu* RBP by transient transfection. It was previously demonstrated that both the human and mouse *Alu* RBPs could each bind either sc*Alu* or scB1 RNA interchangeably (11). However, it was noted that the human *Alu* RBP migrated more slowly than its rodent counterpart in RNA mobility shift assays (11). The presence of the alanine+threonine-rich C-terminal tail in the human protein presumably accounts for this difference (see Discussion). To determine whether the isolated cDNA could produce RNA binding activity with the human-specific mobility, it was subcloned downstream of a cytomegalovirus promoter and transiently transfected into mouse NIH 3T3 cells by polycation liposome-mediated gene transfer. Whole cell lysates were then prepared and used for in vitro sc*Alu* RNA mobility shift (Fig. 4). In this assay, HeLa cell lysate produces a single major mobility shift (11) and is shown in lane 2 as a positive control. Mouse cells transfected with the *Alu* RBP cDNA-containing plasmid produced both human and mouse RNA binding complexes (Fig. 4, lanes 3 and 4). Cells transfected with plasmid lacking the cDNA insert (lane 5) expressed only the mouse RNA binding complex, as did untransfected NIH 3T3 cells (lane 6). These results demonstrated that the cDNA isolated led to the production of human *Alu* RBP.

An active *Alu* RBP gene is expressed from human chromosome 15q22. A well-characterized panel of mouse × human SCHs which retain single human chromosomes was used to map the gene for *Alu* RBP (21, 37, 43). Whole cell lysates were made from SCHs and used in RNA mobility shift assays. In Fig. 5A, lanes 8, 10, 18, and 24 display the human-specific mobility shift produced from HeLa extract as positive controls. SCH lines A9+15 (lane 2) and GM10500 (lane 12) expressed the human-specific *Alu* RBP. The remaining SCHs, which collectively contained all human chromosomes other than 15, did not express the human-specific complex (Fig. 5A and data not shown). The two SCHs that expressed human *Alu* RBP each contained human chromosome 15. Cell line A9+15 contains chromosome 15 as its only human chromosome (21, 43). GM10500 contains a single rearranged chromosome in which a portion of the q arm of chromosome 17 has been translocated to a fragment of chromosome 15 (21). The resulting derivative 15 chromosome in GM10500 lacks the distal portion of its q arm but retains the region from q22 through the p terminus and is the only human chromosome present in this cell line (21) (Fig. 5C). SCH GM10498 contains an intact chromosome 17 as its only human chromosome and did not express the *Alu* RBP (Fig. 5A, lane 13). Control experiments using a panel of competitor RNAs (11) demonstrated that the chromosome 15-containing cells expressed authentic *Alu* RBP (not shown). A high percentage of the cells in hybrid lines GM10500 and A9+15 contain a single human chromosome 15 (21). Although it was reproducibly noted that more of the RNA probe associated with the human RBP than with the rodent RBP when the two proteins were coexpressed in these hybrids (Fig. 5B), we do not know the reason for this (see below). The results indicated that the gene encoding the

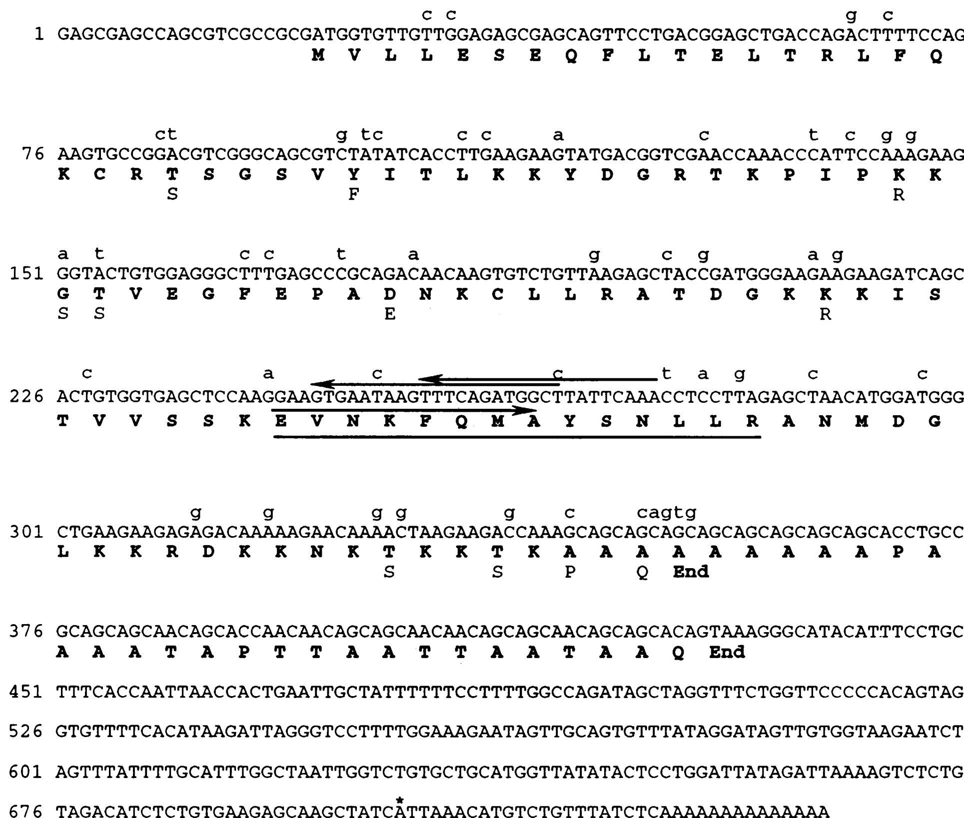


FIG. 2. Complete nucleotide and predicted amino acid sequences of human *Alu* RBP (SRP 14-equivalent) cDNA. Nucleotide positions are numbered at the left. The one-letter amino acid code is indicated below the nucleotide sequence in boldface. The amino acid sequence determined from the tryptic peptide derived from the purified 18-kDa *Alu* RBP is underlined. Comparison with mouse SRP 14 is limited to the coding region: lowercase letters above the human cDNA indicate nucleotide differences found in mouse SRP 14 cDNA (61); letters below the human protein sequence indicate amino acid differences found in mouse SRP 14 (61). The asterisk denotes the position of the poly(A) addition signal (44). Arrows above the nucleotide sequence indicate the positions of antisense primers used for gene-specific cDNA synthesis and PCR amplification of the 5′ cDNA end (14) (see Materials and Methods). The arrow below the sequence indicates the sense primer used to PCR amplify the 3′ cDNA end (14). Primers corresponding to the 5′- and 3′-terminal sequences directed PCR amplification of the contiguous full-length cDNA which is shown.

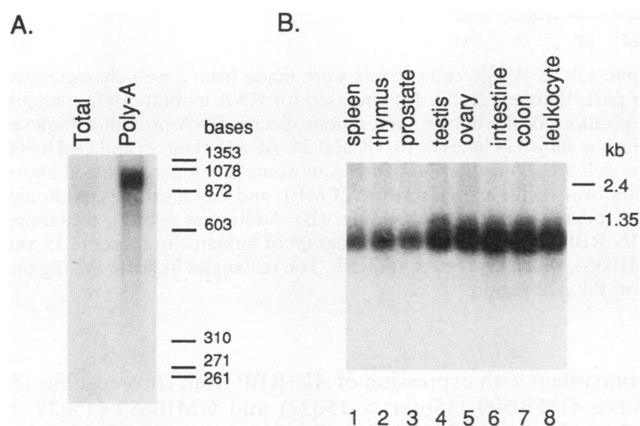


FIG. 3. RNA blot analysis of *Alu* RBP mRNA. A fragment of *Alu* RBP cDNA representing the first 305 bp was used as a probe (blot hybridizations with a more 3′-extended probe generated high background presumably due to the trinucleotide repeat). (A) Total RNA (65 μg) or poly(A)$^+$ RNA (20 μg) was fractionated by PAGE (8 M urea–6% polyacrylamide gel) and blotted along with denatured size markers (indicated on the right). (B) Human multiple tissue Northern blot II (Clontech) containing poly(A)$^+$ RNA fractionated by 1.5% agarose. RNA size markers are indicated on the right.

Alu RBP that we characterized was expressed from human chromosome 15.

The results presented above were confirmed and extended by examining the additional hybrid cell lines GM11418,

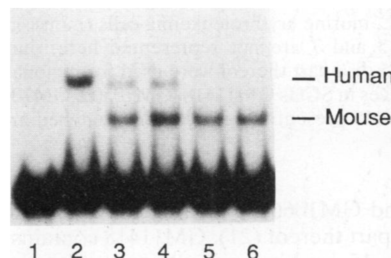


FIG. 4. Expression of *Alu* RBP by cDNA-mediated transient transfection of mouse cells. Shown are sc*Alu* RNA mobility shift analyses of whole cell extracts prepared from HeLa cells (lane 2; positive control), NIH 3T3 cells transfected with *Alu* RBP cDNA insert in pCMV.3-18k (duplicate transfections; lanes 3 and 4), NIH 3T3 cells transfected with vector pCMV.3 alone (lane 5), and nontransfected NIH 3T3 cells (lane 5). Lane 1 contains RNA probe but no extract. The positions of the human- and mouse-specific RNA-protein complexes are indicated on the right.

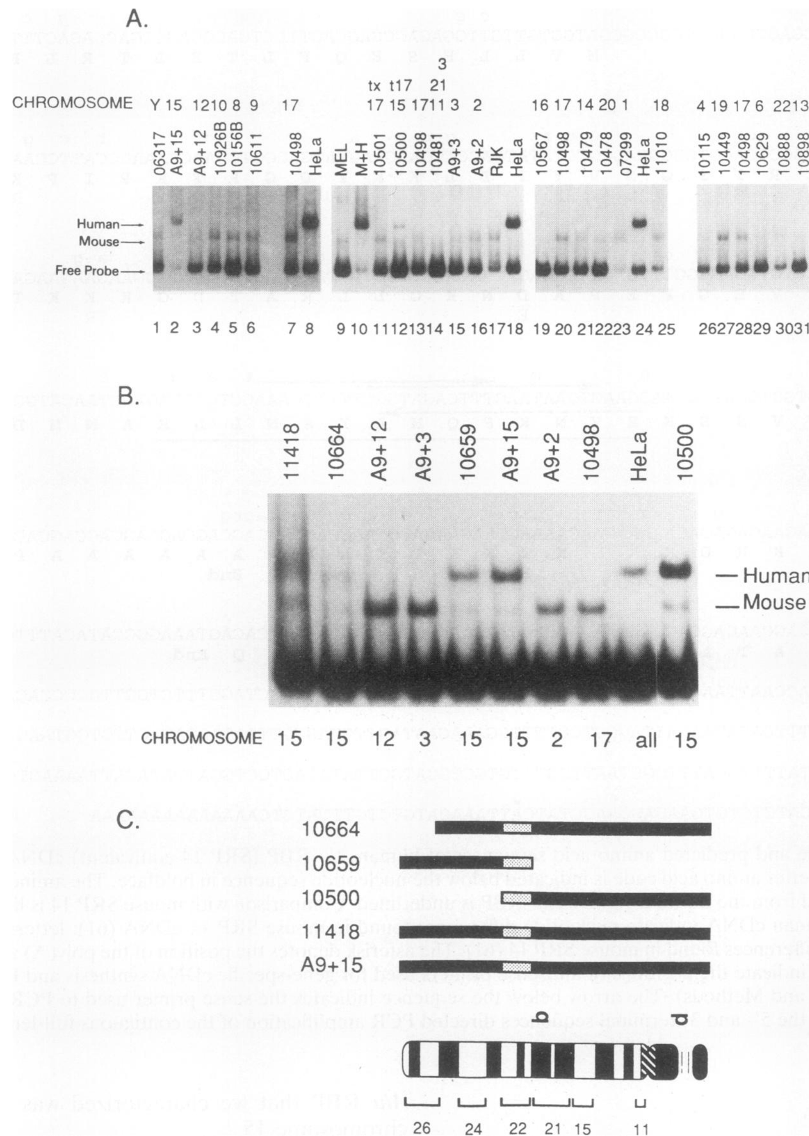


FIG. 5. An active gene for *Alu* RBP is expressed from human chromosome 15q22. Whole cell extracts were made from a well-characterized panel of rodent × human SCHs which retain single human chromosomes or parts thereof (21, 37, 43) and used for RNA mobility shift analyses. (A) Hybrids representing the full complement of human chromosomes. The positions of the human- and rodent-specific RNA-protein complexes are indicated on the left. HeLa extract produces a human-specific complex which is shown in lanes 8, 10, 18, and 24. A9+15 (lane 2) and GM10500 (lane 12) hybrid extracts reveal the human-specific complex. Identities of the cell lines and their human chromosomal contents are listed above the lanes. MEL, murine erythroleukemia cells (mouse); RJK, Chinese hamster ovary cells; M+H, mixture of MEL and HeLa extracts. Although chromosomes 5 and 7 are not represented here, our results revealed the rodent-specific complex only. (B) Additional hybrids containing chromosome 15 or a part thereof were used for regional assignment of the *Alu* RBP gene. (C) Schematic diagram of human chromosome 15 and its representatives in SCHs GM11418, GM10500, GM10659, A9+15, and GM10664, which expressed *Alu* RBP. The rectangles indicate the regions of chromosome 15 present in each cell line; hatched areas indicate overlap in the q22 region.

GM10659, and GM10664, which each retain human chromosome 15 or a part thereof (21). GM11418 contains a full-length chromosome 15 as its only human chromosome (21). GM10659 contains the terminal half of the q arm of chromosome 15 (q22 > qter) translocated onto chromosome 17 but lacks the p arm and proximal q arm of chromosome 15. GM10664 contains a chromosome 15 which lacks the terminal portion of the q arm (q25 > qter) (21). Each of these cell lines expressed human *Alu* RBP (Fig. 5B). We have not encountered a cell line which contains chromosome 15 and does not express human *Alu* RBP. Thus, the results are completely concordant with expression of *Alu* RBP from chromosome 15. Since GM10500 (15pter > 15q22) and GM10659 (15q22 > 15qter) both unambiguously express human *Alu* RBP (Fig. 5B) and share human chromosomal overlap only in 15q22, we can assign an active *Alu* RBP gene to 15q22 (Fig. 5C).

To confirm that the gene encoding *Alu* RBP physically resides on human chromosome 15, we used Southern blots of somatic cell hybrid DNAs digested with *Pst*I for probing with *Alu* RBP cDNA. Figure 6A shows a blot from hybrid cells which each retain multiple human chromosomes. *Pst*I generated three bands of 5.5, 3.3, and 2.3 kbp from human cell DNA

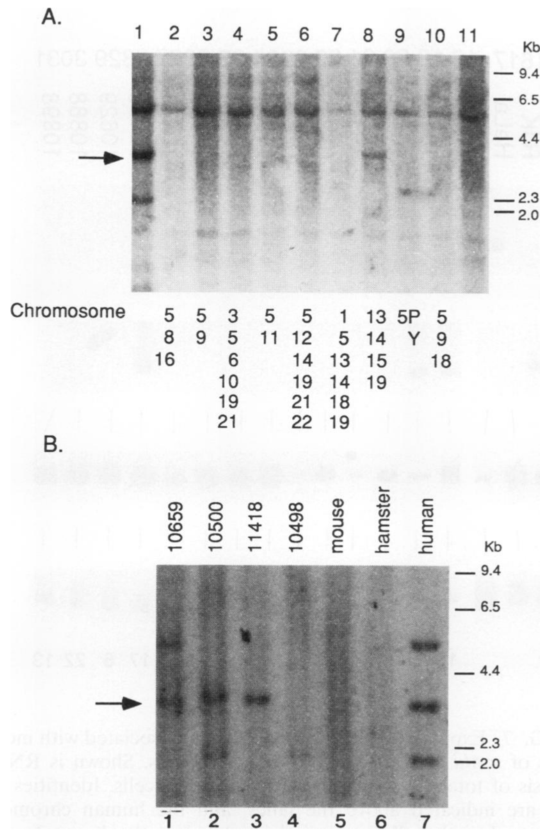


FIG. 6. Physical mapping of *Alu* RBP (SRP 14-equivalent) DNA to chromosome 15q22. Shown is Southern analysis of *Pst*I-digested SCH DNA for chromosomal assignment of the *Alu* RBP gene. The probe was derived from *Alu* RBP cDNA. (A) Hybrid cell blot obtained from Bios Laboratories Inc. Each hybrid sample (lanes 2 to 10) contained a complex mixture of cells which collectively contained multiple human chromosomes as indicated below the lanes. The arrow on the left indicates 3.3-kbp human-specific band with most homology to the sc*Alu* cDNA probe (lane 1; see text). Lane 11 contains rodent DNA. (B) Lanes contained *Pst*I-digested DNA prepared from well-characterized hybrid cell lines which harbor human chromosome 15 as their only human chromosome (GM10500 and GM11418) or with other human chromosomes (GM10659) in a high percentage of the cells (21). GM10498 carries chromosome 17 as its only human chromosome and does not reveal the 3.3-kbp band. Mouse, hamster, and human DNAs are also shown. Size markers are indicated on the right.

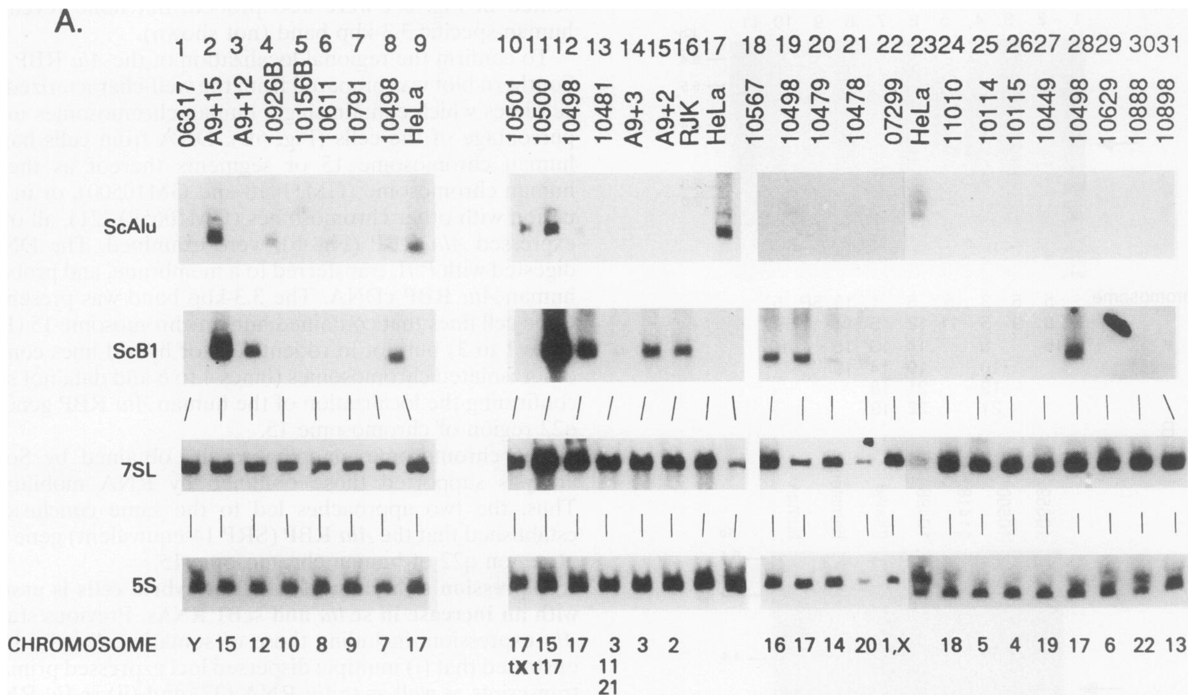
(lane 1). The 5.5-kbp fragment was detected in all hybrid cell lines as well as rodent DNA and was therefore not informative. Signal from the 2.3-kbp band was preferentially lost as stringency increased, while the 3.3-kbp band remained after high-stringency washes (not shown). Moreover, the 3.3-kbp band was not present in rodent DNA (lane 11). This finding indicated that the 3.3-kbp band best represented the human-specific fragment with greatest homology to *Alu* RBP cDNA (arrow in Fig. 6A). This 3.3-kbp fragment was detectable in only one of the multiple chromosome-containing SCHs, which contained human chromosomes 13, 14, 15, and 19 (Fig. 6A, lane 8). The 3.3-kbp band could not be detected in any of the other hybrid lines, including the one in lane 7, which contained all of the chromosomes in lane 8 except chromosome 15. The Southern analysis confirmed the analysis of expressed protein in assigning the human *Alu* RBP gene to chromosome 15. Blots of hybrids representing all human chromosomes not represented in Fig. 6A were also probed, but none revealed the human-specific 3.3-kbp band (not shown).

To confirm the regional localization of the *Alu* RBP gene, a Southern blot was prepared from the well-characterized hybrid cell lines which contain single human chromosomes in a high percentage of the cells (Fig. 6B). DNA from cells harboring human chromosome 15 or segments thereof as their only human chromosome (GM11418 and GM10500), or in combination with other chromosomes (GM10659) (21), all of which expressed *Alu* RBP (Fig. 5), were examined. The DNA was digested with *Pst*I, transferred to a membrane, and probed with human *Alu* RBP cDNA. The 3.3-kbp band was present in all three cell lines that contained human chromosome 15 (Fig. 6B, lanes 1 to 3) but not in rodent cells or hybrid lines containing other isolated chromosomes (lanes 4 to 6 and data not shown), confirming the localization of the human *Alu* RBP gene to the q22 region of chromosome 15.

The chromosome mapping results obtained by Southern analysis supported those obtained by RNA mobility shift. Thus, the two approaches led to the same conclusion and established that the *Alu* RBP (SRP 14-equivalent) gene resides at region q22 of human chromosome 15.

Expression of human *Alu* RBP in hybrid cells is associated with an increase in sc*Alu* and scB1 RNAs. Previous studies of *Alu* expression, including those of somatic cell hybrids, demonstrated that (i) multiple dispersed loci expressed primary *Alu* transcripts as well as sc*Alu* RNA (37) and (ii) sc*Alu* RNA was derived from the left monomers of larger (dimeric) *Alu* transcripts (37, 40). However, it was also noted that a hybrid line which contained human chromosome 15 expressed substantially more sc*Alu* RNA than other hybrid lines did, presumably at the expense of *Alu* primary transcript intermediates (37). Expression of *Alu* RBP in human chromosome 15-containing cells suggested that this protein might be involved in the increase in sc*Alu* RNA observed in the same cells. Thus, it became imperative to explore a correlation between expression of *Alu* RBP and sc*Alu* RNA. This was done by examining RNA levels in the expanded panel of SCHs, which includes several independent hybrid cell lines that retain chromosome 15 and express human *Alu* RBP. The fact that the human *Alu* RBP exhibited higher apparent affinity for the RNA probe than did the rodent protein when the two proteins were coexpressed in hybrid cells (regardless of whether the probe was sc*Alu* or scB1 RNA) (Fig. 5B) suggested a direct role for *Alu* RBP in effecting high levels of sc*Alu* RNA. This observation also suggested that expression of human *Alu* RBP in chromosome 15-containing SCHs might lead to an increase in the level of endogenous scB1 RNA.

To test this hypothesis, sc*Alu*, scB1, and other RNAs were independently probed on Northern blots of hybrid cells (Fig. 7). The results showed that a greater amount of sc*Alu* RNA was present in each of the five independently derived chromosome 15-containing hybrids, GM10500, A9+15, GM10659, GM10664, and GM11418, than in other hybrids (Fig. 7). This amount of sc*Alu* RNA was comparable to that of HeLa cells (Fig. 7A, lanes 9, 17, and 23) but substantially more than the little or no sc*Alu* RNA expressed in the numerous hybrids which contained other human chromosomes (37) (Fig. 7). It is noteworthy that hybrids containing human chromosomes other than 15 do contain genes for sc*Alu* RNA but accumulate only low levels of sc*Alu* RNA (37) (Fig. 7A, lanes 4, 6, 8, and 10). Although this analysis demonstrates a strong correlation between expression of human *Alu* RBP and accumulation of sc*Alu* RNA, our conclusions were cautioned by the facts that sc*Alu* RNA genes expressed in these cells also reside on chromosome 15 and that these genes may be atypical com-



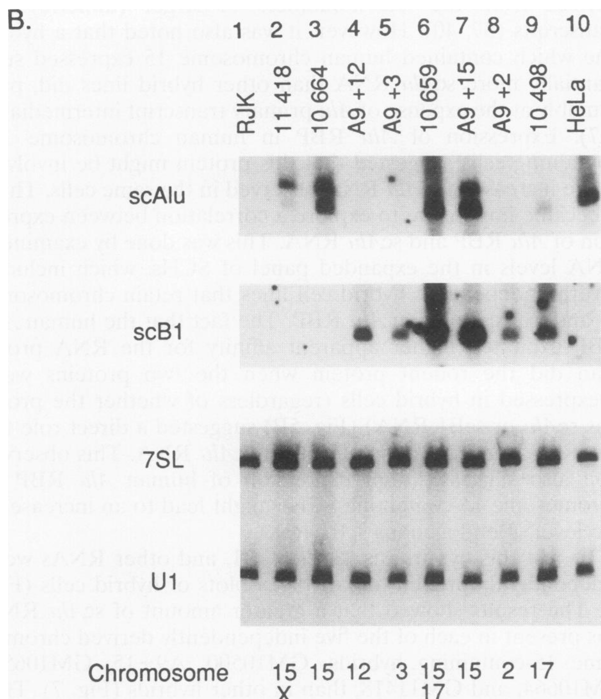


FIG. 7. Expression of human *Alu* RBP is associated with increased levels of sc*Alu* and scB1 RNAs in hybrid cells. Shown is RNA blot analysis of total RNAs extracted from hybrid cells. Identities of cell lines are indicated above the lanes, and the human chromosomal content of each cell line is indicated below the lanes. Lanes are numbered across the top. (A) Hybrids representing full complement of human chromosomes, using sc*Alu*-, scB1-, 7SL-, and 5S-specific oligonucleotide probes. The same filters were hybridized with each probe separately. (B) Cell lines RJK (Chinese hamster), GM11418, GM10664, A9+12, A9+3, GM10659, A9+15, A9+2, GM10498, and HeLa, using sc*Alu*-, scB1-, 7SL-, and U1-specific oligonucleotide probes. The B1 probe does not detect scRNA in hamster-derived cells (see Materials and Methods for identification).

pared with sc*Alu* RNA genes on other chromosomes. We reasoned that if the increase in sc*Alu* RNA was due to overexpression of human *Alu* RBP, this protein should also elevate scB1 RNA which is expressed from the fixed complement of mouse chromosomes in these cells. Thus, we examined scB1 RNA to control for sc*Alu* template variability. Mouse-derived SCHs GM10498, GM10479, GM10478, GM10567, A9+2, A9+3, and A9+12, which harbored human chromosomes other than 15, contained basal levels of scB1 RNA (Fig. 7A and data not shown). In contrast to these cell lines, scB1 RNA was elevated in each of the chromosome 15-containing mouse-derived SCHs A9+15 (Fig. 7A, lane 2), GM10500 (Fig. 7A, lane 11), and GM10659 (Fig. 7B, lane 6). In particular, the four A9-derived hybrids represent a fixed mouse genetic background in which the effects of human chromosome 15 on scB1 RNA accumulation can be appreciated (Fig. 7A; compare lanes 2, 3, 14, and 15). Increased levels of scB1 RNA were detectable in each of the three mouse-derived SCHs that contained chromosome 15 (Fig. 7A, lanes 2 and 10; Fig. 7B, lanes 6 and 7), whereas significantly lower levels were detectable in SCHs containing other human chromosomes. Unfortunately, we were unable to detect scB1 RNA in the Chinese hamster cell line RJK (Fig. 7B, lane 1) or its SCH derivatives (Fig. 7A), including the chromosome 15-containing lines GM11418 and GM10664 (Fig. 7B, lanes 2 and 3), using our probe, and therefore were unable to evaluate *Alu*-equivalent RNA in these SCHs. Nonetheless, in each of the five chromosome 15-containing SCHs tested, we detected a substantial elevation in the levels of sc*Alu* RNA, scB1 RNA, or both. Phosphor storage densitometry revealed a four- to fivefold increase in each of these scRNA species after correction for sample loading. Thus, the combined amount of sc*Alu* and scB1 RNAs in chromosome 15-containing cells clearly exceeded the amount of these RNAs in other hybrids. Levels of 7SL RNA,

5S rRNA, and U1 small nuclear RNA (snRNA) were not affected by the presence of chromosome 15 (Fig. 7). We conclude that a significant increase in sc*Alu* and scB1 RNAs was specific and limited to human chromosome 15-containing cells that expressed human *Alu* RBP. These results indicate that *Alu* RBP encoded by chromosome 15q22 acts as a *trans*-acting factor which positively affects scAlu and scB1 RNA levels in vivo.

DISCUSSION

Human *Alu* RBP was purified, and its cDNA was isolated. The high degree of identity between human *Alu* RBP and mouse SRP 14, coupled with their RNA binding characteristics, leaves little doubt that these proteins are equivalent gene products. Thus, the human SRP 14-equivalent polypeptide is 18 kDa.

Previous studies identified a single-strand region within the scB1 RNA secondary structure which is required for competition in scB1 RNA mobility shift assays (11, 34, 48). However, this single-strand sequence in 7SL RNA was not apparently protected by SRP 9/14 (60). Moreover, compounded mutations in the highly conserved SRP 9/14-protected site of 7SL RNA (60) did not diminish its ability to compete with scB1 RNA for *Alu* RBP in mobility shift assays (12). We initially interpreted these results and results with anti-9-k Da antisera as indications that the *Alu* RBP was distinct from SRP 9/14 (11). However, in light of the present results, we must reconsider the possibility that *Alu* RBP exhibits a different mode of binding to its ligand than the canine SRP 9/14 used to establish the footprint on 7SL RNA (60). Nevertheless, the SRP 9/14 footprint on 7SL RNA and mobility shift results with scB1 and sc*Alu* RNAs are in general agreement, since they localized multiple regions within the ~50-nucleotide tRNA-like *Alu* secondary structure as important for binding (11, 60).

The transient transfection experiment demonstrated that *Alu* RBP cDNA is required and sufficient to produce human-specific sc*Alu* RNA binding activity when expressed in rodent cells. However, SRP 14 does not bind RNA independently in vitro; it must do so in association with its heterodimeric partner SRP 9 (62). According to this model, the 18-kDa *Alu* RBP expressed from transfected human cDNA would have to associate with endogenous rodent SRP 9 to produce a human-specific mobility shift. This expectation is reasonable since (i) canine SRP 9 can heterodimerize with mouse SRP 14 to form a functional RBP (62) and (ii) sequence comparison of human and canine SRP 9 cDNAs and their predicted polypeptides reveals a high degree of identity (20). This model is further supported by preliminary results which indicate that in vitro-translated 18-kDa *Alu* RBP does not form the human-specific sc*Alu* RNA mobility shift unless SRP 9 is also provided (20). Although we were unable to obtain amino acid sequence from the ~10-kDa polypeptide which copurified with the 18-kDa RBP (Fig. 1), its size, elution profile, and the requirement of the 18-kDa *Alu* RBP for SRP 9 in in vitro RNA binding assays strongly suggest that it is the human SRP 9 polypeptide (62).

Surprisingly, we found that the *Alu* RBP cDNA contains a trinucleotide repeat region. Trinucleotide repeats have been found in several genes responsible for human genetic disorders. These repeat regions are unstable; they are hypervariable in the normal population and expanded in affected individuals (reviewed in reference 10). The fragile X-mental retardation syndrome is caused by expansion of the trinucleotide repeat CCG which occurs in the gene encoding the *FMR1* RBP (2, 58, 66). It is within the *FMR1* locus that the chromosomal fragile site is also encountered. The presence of a trinucleotide repeat region in the *Alu* RBP gene suggests that this might be a region of variability or instability. In this regard, it is noteworthy that characteristics previously mapped to human chromosome 15q22 include a fragile site (3), a breakpoint found in most cases of acute promyelocytic leukemia (30), and a restriction fragment length polymorphism (7). Examination of variability in the trinucleotide repeat region of the *Alu* RBP gene at chromosome 15q22 may be informative. After the sequence of *Alu* RBP was determined, we discovered an essentially identical cDNA (HSSRP 14A) contributed to the sequence data base by workers at the Danish Centre for Human Genome Research.

The amounts of sc*Alu* and scB1 RNAs are about equal to those of some low-abundance snRNAs at about 10^3 copies per cell (34, 41). *Alu* primary transcripts also accumulate to comparable levels (32). The amounts of sc*Alu* and scB1 RNAs detectable in vivo each represent a small fraction of the more abundant 7SL RNA (11, 32, 34, 57). The sparsity of *Alu* scRNA relative to 7SL scRNA is analogous to U12 snRNA, which represents less than 1% of the amount of U1 snRNA, yet these snRNAs form independent complexes with the abundant Sm antigen (4, 41). We propose that the SRP 9/14 protein is distributed between 7SL RNA as a component of SRP and sc*Alu* RNA as a discrete non-SRP RNP. We propose that *Alu* RBP stabilizes sc*Alu* and scB1 RNAs in vivo. Levels of mRNAs containing *Alu* sequences in their untranslated regions may also be positively affected by this protein. The amount of *Alu* RNA contained in pol II-generated transcripts appears to be more than in small *Alu* RNAs. Thus, the possibility exists that the amount of non-SRP associated *Alu* RBP in rodent and primate cells is substantial.

The SRP 9/14 heterodimeric protein together with the *Alu*-homologous region of 7SL RNA comprises the translation arrest domain of SRP (52, 53, 62, 73). After emerging from the ribosome, the signal sequence of a growing polypeptide which is destined for secretion interacts with the S region of SRP (52, 53, 54, 62, 73). This brings the *Alu* domain of SRP into close proximity to the ribosome, arresting translation until contact with the endoplasmic reticulum and disengagement of SRP occurs (54, 68, 73). Attempts to detect translation arrest activity from the isolated *Alu* domain subparticle of SRP have not been successful (52). It is therefore unlikely that scB1 or sc*Alu* RNA complexed with the 9/14 heterodimeric protein would exhibit this activity on its own. Yet the association of *Alu* RNA with an SRP protein compels a role for *Alu* RNA in an SRP-like function. Thus, we consider the possibility that an sc*Alu* RNA-9/14 scRNP might have translational modulatory activity if brought to the ribosome via an SRP-independent mechanism. By analogy to the S region of 7SL RNA, the non-*Alu* region of scB1 RNA (34) and sc*Alu* RNA may target certain mRNAs for translational control. Tiedge and coworkers also proposed that the *Alu*-related transcript BC200 might bind SRP proteins and mediate translational control (63). *Alu* sequences located in untranslated regions of ribosome-associated mRNAs might assemble the SRP 9/14 heterodimer into an active subparticle with *cis* access to the ribosome. According to this scenario, mRNAs which acquired *Alu* insertions in their untranslated regions might have become subjected to a novel mode of translational control as a result.

As discussed in the introduction, the posttranscriptional metabolism of *Alu* RNA is likely to be a critical determinant in the *Alu* transposition pathway (49, 51). The cumulative data suggest that accumulation of sc*Alu* RNA and scB1 RNA is due to association with human *Alu* RBP. Although it was previously noted that the ratio of primary to processed *Alu* RNA was greatly reduced in hybrid cells which contained chromosome

15, the mechanism for this was unclear (37). The compelling possibility that human *Alu* RBP somehow shifts the equilibrium between *Alu* primary transcripts, which represent transposition intermediaries, and processed sc*Alu* RNA toward the latter awaits further experiments designed to dissect the mechanism by which *Alu* RBP leads to preferential accumulation of sc*Alu* RNA. Regardless of the mechanism, however, the present data suggest that genetic variability in *Alu* RBP in the form of a potentially variable trinucleotide repeat might have multifarious effects on human *Alu* mobility and thereby have important implications for human genetics.

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