MOLECULAR AND CELLULAR BIOLOGY, Apr. 1995, p. 2109–2116 Vol. 15, No. 4
0270-7306/95/$04.00+0
Copyright © 1995, American Society for Microbiology

A Trinucleotide Repeat-Associated Increase in the Level of *Alu* RNA-Binding Protein Occurred during the Same Period as the Major *Alu* Amplification That Accompanied Anthropoid Evolution

DAU-YIN CHANG, NORIKO SASAKI-TOZAWA, LAURA K. GREEN, AND RICHARD J. MARAIA*

Laboratory of Molecular Growth Regulation, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892-2753

Received 27 October 1994/Returned for modification 7 December 1994/Accepted 9 January 1995

Nearly 1 million *Alu* elements in human DNA were inserted by an RNA-mediated retroposition-amplification process that clearly decelerated about 30 million years ago. Since then, *Alu* sequences have proliferated at a lower rate, including within the human genome, in which *Alu* mobility continues to generate genetic variability. Initially derived from 7SL RNA of the signal recognition particle (SRP), *Alu* became a dominant retroposon while retaining secondary structures found in 7SL RNA. We previously identified a human *Alu* RNA-binding protein as a homolog of the 14-kDa *Alu*-specific protein of SRP and have shown that its expression is associated with accumulation of 3′-processed *Alu* RNA. Here, we show that in early anthropoids, the gene encoding SRP14 *Alu* RNA-binding protein was duplicated and that SRP14-homologous sequences currently reside on different human chromosomes. In anthropoids, the active SRP14 gene acquired a GCA trinucleotide repeat in its 3′-coding region that produces SRP14 polypeptides with extended C-terminal tails. A C→G substitution in this region converted the mouse sequence CCA GCA to GCA GCA in prosimians, which presumably predisposed this locus to GCA expansion in anthropoids and provides a model for other triplet expansions. Moreover, the presence of the trinucleotide repeat in SRP14 DNA and the corresponding C-terminal tail in SRP14 are associated with a significant increase in SRP14 polypeptide and *Alu* RNA-binding activity. These genetic events occurred during the period in which an acceleration in *Alu* retroposition was followed by a sharp deceleration, suggesting that *Alu* repeats coevolved with C-terminal variants of SRP14 in higher primates.

Ample evidence indicates that amplification of short interspersed elements (SINEs) occurred via reverse transcription of small RNA intermediaries in a process termed retroposition (52). The SINEs of many organisms are related to one or another tRNA, and these have been found widely distributed in nature, including in plants, fish, and mammals (see references 13, 43, and 44 and references therein). The *Alu* family of SINEs was derived from the terminal portions of the 7SL RNA component of the signal recognition particle (SRP), a small ribonucleoprotein involved in intracellular protein trafficking (6, 24, 27, 70, 71, 74, 77). In contrast to tRNA-like SINEs, and despite the ubiquity of 7SL SRP RNAs, *Alu*-related SINEs are for unknown reasons mostly limited to rodents and primates (for a recent review, see reference 13).

Evolution of *Alu*-related SINEs apparently occurred in two major phases: an ancient period during which *Alu* sequences emerged as monomeric elements followed by a period of remodelling and proliferation (see reference 50 and references therein). The discovery of fossil *Alu* sequences provided evidence that an *Alu* monomer originated in an ancestor common to rodents and primates (50). The monomeric *Alu*-equivalent SINE referred to as B1 has since undergone substantial divergence from 7SL during its amplification to ~50,000 to 80,000 copies per haploid genome in rodents (3, 29). Trace levels of ancient *Alu* monomers have also been identified in primate DNA; however, these are no longer active (26, 28, 48, 49). In early primates, *Alu* monomers underwent modest divergence from 7SL and were remodeled into a dimeric element of great transpositional potential while preserving 7SL RNA secondary structures in each monomer (26, 31, 56, 62). However, although the dimeric *Alu* structure appeared in the earliest primates, its amplification rate has been significantly lower in prosimians than in simians (anthropoids) (11, 12). Furthermore, while rodent and prosimian genomes amplified 7SL-related SINEs in addition to tRNA-related SINEs, simian genomes expanded only one dominant SINE, i.e., *Alu* (3, 11, 13).

Classification of *Alu* repeats into subfamilies of distinctive evolutionary ages in conjunction with examination of *Alu* sequences in several primate genomes has revealed a more detailed temporal image of the *Alu* amplification pattern in primates (6, 12, 27, 47, 57, 64, 78). *Alu* proliferation in prosimian primates appears to have occurred in that isolated lineage since (i) galago (prosimian) *Alu* sequences are diagnostically different from anthropoid *Alu*s (12) and (ii) individual *Alu* repeats common to anthropoid genomes cannot be found at the orthologous loci in prosimians (55, 57, 59). These studies indicate that a dimeric founder *Alu* existed in the earliest primate genome but that it subsequently amplified to different extents and evolved along different pathways in prosimian and simian genomes. In the simian lineage, *Alu* elements underwent a concentrated amplification that was specific to early anthropoids. Thus, by 30 to 50 million years ago, the great majority of *Alu* repeats to be ultimately inherited by the human genome were amplified de novo in early anthropoid DNA, and the rate of amplification slowed dramatically thereafter (5–7, 12; reviewed in references 57 and 59).

While relatively few *Alu* sequences continue to transpose in humans, a substantial fraction of these have caused genetic variability and/or gene disruptions that have come to the at-

* Corresponding author. Phone: (301) 402-3567. Fax: (301) 480-9354. Electronic mail address: maraia@ncbi.nlm.nih.gov.

tention of geneticists (1, 14, 22, 25, 39, 42, 54, 73). Further characterization of human-specific *Alu* sequences has allowed the calculation of an *Alu* de novo transposition rate of 1 in 100 human births (13). Yet despite their effects on the human genome and the existence of an insightful model of retroposition (23, 72), much remains unknown about factors which facilitate *Alu* mobility (58).

Conservation of 7SL RNA-like secondary structures in transcripts of active *Alu* and B1 sequences suggests a function related to SRP (6, 33, 34, 40, 62, 63). However, such conservation also suggests that these RNA structures may simply stabilize *Alu* RNA or provide recognition by the *Alu* retroposition machinery (9, 30, 31, 33, 62, 63). Recent advances in detecting cellular *Alu* and B1 transcripts has provided new impetus to their study and a better understanding of their regulation in human cells (9, 10, 15, 32, 33, 35–37, 40, 45, 53, 63). Under basal conditions, a small fraction of *Alu* elements are transcribed by RNA polymerase III to produce poly(A)$^+$-containing nascent transcripts, whereas *Alu* transcription increases dramatically in response to viral infection (10, 36, 40, 45, 46, 53, 63). A subset of these *Alu* nascent transcripts are thought to represent transposition intermediaries.

According to the current model of *Alu* retroposition, the 3′ terminal oligo(U) residues produced as a result of termination by polymerase III intramolecularly base pair with the internal poly(A) tract to provide a self-primed template for reverse transcriptase (23, 41; for reviews, see references 13 and 56). The discovery of an *Alu* left monomer RNA (40) led to the suggestion that some nascent *Alu* transcripts can be diverted from the transposition pathway by conversion to poly(A)$^-$ transcripts known as small cytoplasmic *Alu* (sc*Alu*) RNA (9, 10, 36, 40). Such 3′ processing would reduce the potential of *Alu* primary transcripts for retroposition by decreasing the likelihood of generating a full-length reverse cDNA. Thus, on the basis of 7SL-like RNA structure conservation and several lines of experimental evidence, researchers have proposed that post-transcriptional mechanisms of *Alu* RNA expression may constitute important determinants of *Alu* mobility and evolution (13, 26, 31, 33, 35–37, 56, 63).

The *Alu*-homologous region of 7SL RNA is associated with two SRP polypeptides with molecular masses of 9 and 14 kDa which together form a stable heterodimer known as SRP9/14 (4, 18, 60, 65, 67, 68, 75). The human SRP14 gene produces a protein of ~18 kDa because of a trinucleotide repeat-containing region (TCR) in the 3′-coding sequence of its mRNA (10). This ~18-kDa SRP14 protein interacts with *Alu* sequence RNA efficiently in vitro (10). The ~18-kDa human SRP14 protein is overexpressed relative to the corresponding endogenous rodent protein in somatic cell hybrids (10).

Overexpression of human SRP14 in these cells is reflected by increased *Alu* RNA-binding activity, and this leads to accumulation of sc*Alu* RNA at the apparent expense of *Alu* nascent transcripts, suggesting a direct role in *Alu* RNA metabolism (10). The evolutionary conservation of 7SL RNA secondary structure by active *Alu* sequences in conjunction with these observations indicates that primate SRP14 is a candidate factor involved in *Alu* expression and evolution (10). Here, we examine the relationship between the origin of the TCR in primate SRP14 genes and the expression of SRP14-mediated *Alu* RNA-binding activity in primate cells for which *Alu* evolution has been previously characterized.

MATERIALS AND METHODS

Cell lines. The following cell lines were obtained from the American Type Tissue Culture (Rockville, Md.): green monkey (CRL 1650), baboon lymphoblast 26CB-1 (CRL 1495), chimpanzee WES (CRL 1609), and NIH 3T3 (CRL 1658). HeLa (S3), Sumatran orangutan GM04272, and gorilla skin fibroblast AG05251A were from the Corriell Institute for Medical Research (Camden, N.J.). Simian virus 40 T antigen-transformed *Galago senegalensis* fibroblasts (GSe-TAg) and owl monkey kidney (OMK [American Type Culture Collection catalog no. 637-69]) cells were kindly provided by P. Deininger (Louisiana State University Medical School, New Orleans).

Western blot (immunoblot) analysis. Whole-cell extracts were prepared as previously described (10). Protein contents were determined by the Bio-Rad colorimetric protein assay and confirmed by side-by-side comparison of Coomassie blue-stained sodium dodecyl sulfate (SDS)-polyacrylamide gels (not shown). Rabbits were immunized with a synthetic peptide consisting of amino acids 5 to 19 of SRP14; the amino acid sequence of this peptide is 100% identical in mouse and human SRP14 (10, 66). Antibodies were affinity purified on SRP14–glutathione-*S*-transferase (GST)–glutathione-Sepharose by using recombinant human GST-SRP14 as follows. A 200-μl volume of antiserum was incubated with 50 μl of GST-SRP14–Sepharose for 2 h at room temperature and washed with 1 ml of 50 mM Tris-Cl (pH 8.0)–0.15 M NaCl–1 mM EDTA–0.05% Nonidet P-40 six times. Antibodies were then eluted with 0.2 M glycine (pH 2.8), neutralized with Tris base, and stabilized with albumin. Whole-cell extracts were fractionated by SDS-polyacrylamide gel electrophoresis (PAGE) (18%) and were transferred to nitrocellulose. After blocking, blots were incubated with affinity-purified anti-SRP14-peptide antibody, washed, and developed with the enhanced chemiluminescence kit from Amersham according to the manufacturer's instructions.

***Alu* RNA-binding activity.** Electrophoretic mobility shift analysis (EMSA) using whole-cell extracts was as previously described (9, 10). EMSA results were quantitated with a PhosphorImager (Molecular Dynamics). For reliable detection and quantitation, it was necessary to use more total protein from galago and NIH 3T3 whole-cell extract than from other cell lines; however, EMSAs performed at various protein concentrations and poly(rG) (nonspecific inhibitor) concentrations yielded similar results.

PCR analysis of the 3′ TCR of the SRP14 gene. DNA was purified from cultured cells by overnight treatment with SDS-proteinase K and then by phenol-chloroform extraction, ethanol precipitation, and solubilization in Tris-EDTA buffer. After boiling, DNA was added to PCR components, which were preheated to 80°C, and cycling was begun immediately thereafter. PCR parameters were 94°C for 40 s, 55°C for 30 s, and 72°C for 30 s, for a total of 40 cycles (see the legend to Fig. 3A for positions of primers). Aliquots were analyzed by 3% agarose gel electrophoresis-ethidium bromide staining-UV visualization. PCR products were gel purified and used as templates for direct sequencing by using the PCR-based *fmol* sequencing kit from Promega. For PCR from somatic cell hybrids, purified DNA samples were provided by the Corriell Institute for Medical Research (21). PCR was as described above, except that the cycling parameters were 94°C for 40 s, 60°C for 30 s, and 72°C for 30 s, for a total of 40 cycles (see the legend to Fig. 3B for positions of primers).

Southern blot analysis. Purified DNA (10 μg) was incubated with restriction enzymes (50 Units; New England Biolabs) overnight and fractionated through 0.7% agarose, briefly acid depurinated, and transferred to a nylon membrane (GeneScreen Plus; Dupont), all 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 16 h. Probe was generated by random priming in the presence of [α-^{32}P]dCTP of the first 305 bp of the SRP14 *Alu* RBP cDNA by standard procedures (10). The blots were washed nonstringently at 60°C with 1× SSPE–10% SDS for 2 h and were then washed with 1× SSPE–0.1% SDS for 1 h and were visualized by autoradiography.

RESULTS

Anthropoid-specific expression of SRP14 polypeptide correlates with elevated levels of *Alu* RNA-binding activity. Figure 1 shows a Western blot of the SRP14-homologous proteins isolated from cultured cells derived from various species. The antibody used was raised against a synthetic peptide located close to the N terminus whose amino acid sequence is 100% identical in mice and humans (10, 66). As expected, the antibodies detected an ~18-kDa polypeptide from HeLa whole-cell extract (Fig. 1A, lane 1) which comigrated with highly purified ~18-kDa SRP14 (previously described as a component of *Alu* RNA-binding protein [RBP]) (10) (Fig. 1A, lane 2). Also as expected, these antibodies detected polypeptides of ~14 kDa in mice (NIH 3T3) and galagos (prosimian) (Fig. 1B, lanes 4 and 5), whereas all subsequently evolved primates examined exhibited proteins with larger sizes (lanes 1 to 3). The owl (New World) monkey protein, with an apparent mass of

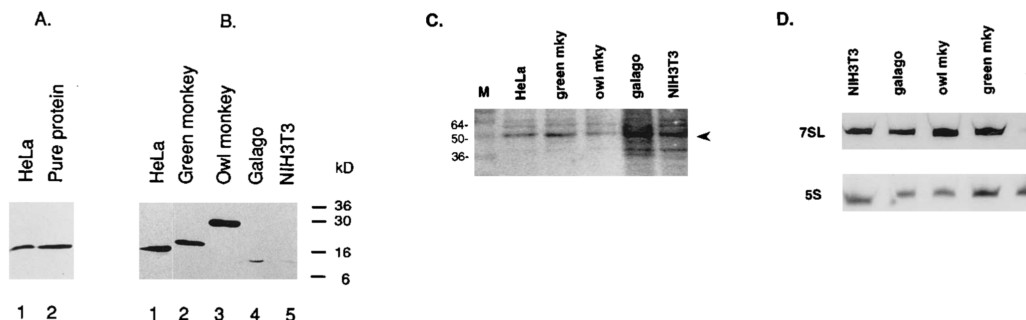

FIG. 1. SRP14 proteins vary in size and accumulate in anthropoid cell extracts. Western analysis of SRP14 was done with affinity-purified, anti-peptide-SRP14 antibody. (A) Lanes: 1, HeLa whole-cell extract (15 μg); 2, highly purified *Alu* RBP (20 ng) (10). (B) Lanes: 1 to 5, HeLa, green monkey (Cos1), owl monkey, galago, and mouse NIH 3T3 whole-cell extracts, respectively. Lanes 1 to 3 contained 15 μg of extract, while lanes 4 and 5 contained 70 μg of extract. The blots in panels A and B were visualized by chemiluminescense (Materials and Methods). Size markers are indicated on the right in kilodaltons. (C) Western blot prepared as in panel A probed with anti-La serum and visualized with ^{125}I-labeled protein A. As expected on the basis of intentional overloading of galago and NIH 3T3 samples (see panel B), PhosphorImager quantitative analysis revealed a two- to fourfold-greater amount of La antigen in galago and NIH 3T3 samples than in HeLa and monkey samples (not shown). (D) Northern (RNA) blot analysis. Total RNA from whole-cell extracts equalized for total protein content (212 μg) was purified, fractionated by 8 M urea–6% PAGE, and hybridized with oligo-DNA probes as described elsewhere (10). Upper panel shows results of probe complementary to positions 106 to 134 of 7SL RNA, which is 100% identical in humans and rodents (51). Direct PhosphorImager analysis indicated a difference of less than 2-fold in 7SL RNA levels in all samples except HeLa cells, which demonstrated 5- to 10-fold less 7SL RNA; similar results for 7SL RNA are demonstrable by ethidium staining (not shown). The lower panel shows the same blot reprobed with oligo-DNA complementary to 5S rRNA. The specific activities of probes and exposure times were significantly different for 7SL and 5S RNA probes. Both autoradiographs were intentionally underexposed. Mky, monkey.

~28 kDa, is the largest of these (Fig. 1B, lane 3), while the green (Old World) monkey protein migrates at ~20 kDa (lane 2). Orangutan, gorilla, and chimpanzee cells also expressed an ~18-kDa protein, while baboon cells expressed a protein whose mobility was indistinguishable from that of the green monkey protein (data not shown). Thus, all anthropoid cells examined expressed a larger SRP14 than did other mammals. As will be shown below, this is due to variation in the 3′-coding TCR. Similar results were obtained from multiple extract preparations; in all cases, the amounts of SRP14 polypeptide from galago and NIH 3T3 cells were significantly less than those from anthropoid extracts. More protein was loaded in Fig. 1B, lanes 4 and 5, than in lanes 1 to 3 (see Fig. 1 legend), as determined by a colorimetric assay and SDS-PAGE–Coomassie staining (data not shown [but see Fig. 1C]). The antibodies used were directed to a peptide sequence that was 100% conserved from mice to humans. These antibodies detected comparable amounts of galago, mouse, and human SRP14 proteins when SRP was first enriched by immunoprecipitation with a constant amount of anti-SRP54 serum (data not shown), further indicating that they can recognize SRP14 comparably from these species. Thus, in addition to the size differences, the affinity-purified anti-SRP14-peptide antibodies consistently detected more SRP14 polypeptide in whole-cell extracts of anthropoid primate cells than they did in rodent and prosimian cell extracts. As will be shown below, the increased amounts of SRP14 polypeptide in anthropoid cells are reflected by increased amounts of *Alu* RNA binding activity in the same extracts.

As indicated above and in the legend to Fig. 1B, we analyzed more protein from mouse and galago extracts than from simians because we reproducibly detected less SRP14 per microgram of protein in rodent and prosimian cell extracts (Materials and Methods). Figure 1C shows the results of Western analysis with antiserum that recognizes a ubiquitous ~50-kDa RBP, La, which is involved in transcription termination by RNA polymerase III (17, 37). These results demonstrated that as expected, the lanes containing NIH 3T3 and galago samples contained more La antigen, which reflected the greater amount of protein loaded. Thus, on the basis of these results in conjunction with those in Fig. 1B, we conclude that simian cells contain more SRP14 polypeptide than do other mammals, including prosimians.

In order to evaluate the relative amounts of SRP in these extracts, we examined 7SL RNA by Northern (RNA) blot analysis. The 7SL RNA subunit of SRP is efficiently contained in SRP and therefore should reflect SRP levels (76). Total RNA was prepared from aliquots of the whole-cell extracts used above; each aliquot contained an equal amount of total protein. The RNA was fractionated by denaturing PAGE, transferred to a nylon membrane, and hybridized with an oligonucleotide probe complementary to a region of 7SL RNA that is 100% identical in rodents and humans (51) (Fig. 1D, upper panel). This procedure revealed comparable amounts of 7SL RNA in NIH 3T3, galago, owl monkey, and green monkey cell extracts and smaller amounts of this RNA in HeLa extracts (see the legend to Fig. 1). The relatively small amount of 7SL RNA in HeLa extracts was significant, as was seen by reprobing this blot with an oligonucleotide complementary to 5S rRNA (Fig. 1D, lower panel). These results suggest among other things that the ratio of SRP to ribosomes is lower in HeLa cells than in the other cells examined. Cumulatively, the results shown in Fig. 1 suggest that 7SL RNA and SRP14 *Alu* RBP subunits are not coordinately regulated to the same extent in these cells and that the ratio of SRP14 to its ligand, 7SL RNA, is specifically increased in anthropoids relative to other mammals, including prosimians.

The size difference between rodent and human SRP14 *Alu* RBPs is apparent in the *Alu* RNA-mediated EMSA (9, 10). Figure 2A shows that the mobilities of *Alu* RNA-protein complexes formed from the various extracts used above reflected the mobility profile expected on the basis of Western analysis. Furthermore, as was anticipated from the results of Fig. 1, anthropoid primate cell extracts (Fig. 2A, lanes 1 to 3) consistently exhibited more *Alu* RNA binding activity per microgram of total cell protein than did the other species (lanes 4 and 5), as quantitated for Fig. 2B. Human fetal fibroblast, human hepatoma, chimpanzee, gorilla, orangutan, and baboon cell extracts all produced an RNA-protein complex that was indistinguishable in mobility from that of HeLa cells, and all of these contained high levels of *Alu* RNA-binding activity that were similar to that of HeLa cells, whereas lemur cell (pro-

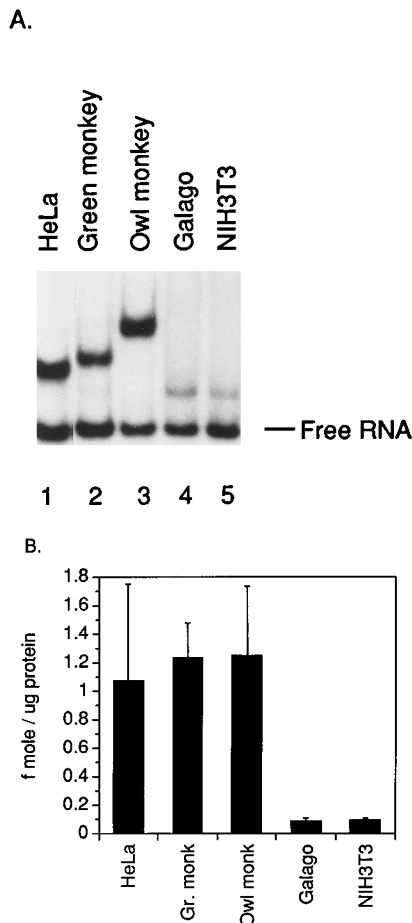

FIG. 2. SRP14-mediated *Alu* RNA-binding activity is increased in anthropoids. (A) EMSA for ^{32}P-labeled *Alu* RNA binding using whole-cell extracts. Mobility of free RNA probe is indicated. For the purpose of reliable detection and quantitation, reaction mixtures in lanes 1 to 3 contained 1 μg of whole-cell extract, while lanes 4 and 5 contained 5 μg of whole-cell extract (see Materials and Methods). (B) Specific activity of *Alu* RBP expressed as femtomoles of RNA specifically shifted per microgram of protein as previously described (10). Means and standard deviations were calculated and plotted on the basis of three independent experiments; however, for any given experiment, HeLa, green monkey (Gr. monk), and owl monkey (Owl monk) extracts each exhibited at least 8.5-fold higher RNA-binding activity than galago and NIH 3T3 extracts.

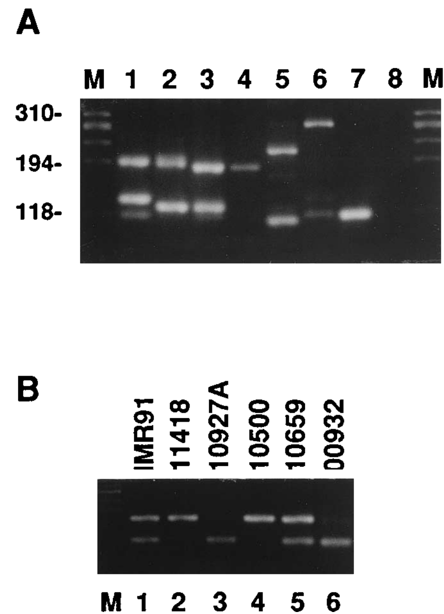

FIG. 3. (A) SRP14 3′ trinucleotide repeat length variations in primates. PCR analysis of various species' genomic DNAs was done with primers which flank the human SRP14 3′ TCR. The sense oligonucleotide primer spanned nucleotides 276 to 298; the antisense primer was complementary to nucleotides 469 to 446 (as numbered in reference 10). The expected size of the PCR product on the basis of the sequence of the cDNA clone of the human SRP14 *Alu* RBP is 194 bp (10). Lanes 1 to 8, human, chimpanzee, gorilla, orangutan, green monkey, owl monkey, galago, and mouse DNA, respectively; M, size markers with *Hae*III restricted ϕX174; sizes in base pairs are indicated on the left. (B) Two SRP14 sequences reside on different human chromosomes. Somatic cell hybrid DNAs were amplified by PCR. The sense oligonucleotide primer spanned nucleotides 299 to 330; the antisense primer was complementary to nucleotides 461 to 432 (as numbered in reference 10). The expected size of the PCR product on the basis of the sequence of human SRP14 *Alu* RBP cDNA is 163 bp (10). The identities of cell lines are indicated above the lanes. IMR91 is male DNA representing the entire human genome, GM11418 contains chromosome 15 as its only human chromosome, GM10927A contains chromosome 11 as its only human chromosome, GM10500 contains a single rearranged human chromosome in which a portion of the q arm of chromosome 17 has been translocated to a fragment of chromosome 15, GM10659 contains chromosomes 15 and 11 in addition to other human chromosomes, and GM00932 contains human chromosome 11 in addition to other chromosomes but does not contain human chromosome 15 (10, 21).

simian) extract as well as several mouse and hamster cell extracts produced the rodent-specific mobility and low levels of this *Alu* RNA-binding activity (data not shown). Cumulatively, the results indicate that anthropoid cells accumulate more SRP14 *Alu* RBP than do other mammalian species and that this appears to be the basis of the increased *Alu* RBP activity in the same cells. This interpretation is consistent with results which demonstrate that recombinant human SRP14 polypeptides that either contain or lack the C-terminal tail bind *Alu* RNAs equally well (20) (see Discussion).

Variable-length GCA repeat-containing region in anthropoid SRP14 genes. Human (~18-kDa) SRP14 *Alu* RBP is larger than those reported for mouse and dog cells because it contains a 28-residue, alanine-rich, C-terminal tail which is translated from a 3′ GCA-rich TCR (10, 66). In order to determine whether variability in the 3′ TCR lengths accounted for the size difference between the SRP14-homologous proteins, we used primers which flank the human TCR to amplify genomic DNAs from various primate species by PCR. As predicted from the mouse SRP14 cDNA sequence, this PCR assay would not be expected to amplify mouse DNA (10, 66). The expected band of 194 bp was readily amplified from human DNA (Fig. 3A, lane 1), in addition to an unexpected band of ~120 bp (see below). Direct sequencing of the larger PCR band from human DNA revealed that it was the ~18-kDa SRP14 3′-coding region containing the TCR (10) (Table 1). Using these primers, we could amplify products with variable sizes from the genomic DNAs from owl monkey, green monkey, orangutan, gorilla, and chimpanzee cells (Fig. 3A). In each case, the size of the larger PCR fragment was consistent with the size of the SRP14 *Alu* RBPs detected by Western blots and EMSAs. Sequence analyses of these PCR products confirmed, in all cases examined (owl monkey, green monkey, chimpanzee, and human cells), the presence of an expanded 3′ TCR flanked by SRP14 sequences, as summarized in Table 1. Trinucleotide repeats are known to be genetically unstable, leading to repeat length hypervariability in genes which have been identified by their association with specific diseases (8, 16). Therefore, we examined genomic DNAs from many different humans, five additional chimps, two gorillas, and three additional orangutans. Surprisingly, we could not detect intraspe-

TABLE 1. Comparison of sequence encoding the carboxy terminus of galago SRP14 with those of other species[a]

Species	Lys codon	No. of codons in the TCR[b]	Substitution codon[c]	Ala codon	Gln codon	Stop codon
Mouse	. . .	0	C. .	. . .	. . .	.G.
Galago	AAA	0	GCA	GCA	CAG	TAA
Owl monkey	. . .	52	. . .	. . .	. . .	. . .
Green monkey	. . .	33	. . .	. . .	. . .	. . .
Human	. . .	26	. . .	. . .	. . .	. . .

[a] Dots indicate identity with the galago sequence; differences are indicated directly above by the base.

[b] In human SRP14 cDNA, this TCR region contains mostly (nineteen) GCA triplets, but is also rich in ACA repeats (10). Owl monkey and green monkey TCRs contain GCA triplets in the same relative proportions as that in the human sequence (not shown).

[c] The nucleotide position which, substituted in galagos, constitutes two adjacent GCA codons which are immediately 3′ to the expanded GCA-rich TCR in anthropoids; in mice, this CCA codon encodes proline, whereas the corresponding GCA codon in humans encodes alanine (10, 66).

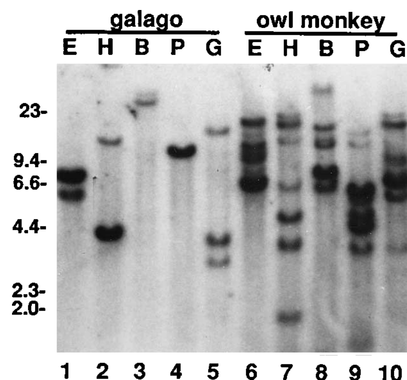

FIG. 4. Southern blot analysis of SRP14 sequences from galago (prosimian) and owl monkey (anthropoid) DNAs. The restriction enzymes used are indicated above the lanes. E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; P, *Pst*I; G, *Bgl*II. The probe was derived from the first 305 bp of SRP14, which does not include the GCA TCR (10).

cies size variability by our PCR assay, although slight size variability might be detectable by a more sensitive assay (not shown). In any case, the size differences of different primate species' SRP14 proteins are due to corresponding size differences in the translated 3′ TCR encoded by their genomic DNAs.

As evidenced in Fig. 1 and 2, the size of galago SRP14 is similar to those reported for rodent and dog SRPs (10, 66), which therefore suggests that it is expressed from a gene which does not contain a TCR. As such, the PCR primers used would be expected to amplify a galago-derived fragment of ~120 bp, precisely the size of the single PCR product seen after amplification of galago DNA (Fig. 3A, lane 7). A single PCR product identical in size to the single galago product was also readily produced from lemur DNA (data not shown). Direct sequencing of the galago PCR product confirmed that it encoded the SRP14 C-terminal region and that it lacked an expanded TCR (Table 1). However, it is noteworthy here that while the relevant sequence of mouse SRP14 cDNA in this region is CCA GCA (66) (Table 1), the galago sequence and those of all primates examined harbor the sequence GCA GCA in this region (substitution codon in Table 1), suggesting that the C→G base substitution which fortuitously generated two consecutive GCA codons in the prosimian galago facilitated GCA expansion in later primates.

The SRP14 gene is duplicated in anthropoids. A second, smaller PCR product amplified from human and other primate DNAs is similar to the galago band of ~120 bp (Fig. 3A). These results suggested that anthropoids contain two SRP14-homologous sequences, one with an expanded TCR and the other more similar to those of galagos and lemurs. Each of four different primer pairs which flank the TCR amplified two different products from human and other primate DNAs, a larger one containing a TCR and a smaller one more similar to those of galagos and lemurs, while none of these primer pairs produced a larger band in galago or lemur DNA (not shown). The smaller PCR bands exhibited slight size variations in the different primates tested (Fig. 3A). Direct sequencing of the smaller band from human DNA revealed it to be identical to the larger SRP14 sequence but containing eight GCA trinucleotide repeats in the region occupied by 28 codons in the ~18-kDa SRP14-encoding DNA (10) (not shown). Likewise, the sequences of the smaller bands from chimpanzees and green monkeys revealed SRP14 homologs with five and two GCA triplets, respectively (not shown). Thus, TCR length variability accounts for the size variation of the larger and smaller PCR products in primate genomes. These data suggest that two SRP14-homologous sequences reside in the genomes of anthropoids; this was corroborated by Southern blotting and by demonstrating that each of the two PCR-amplified human SRP14-homologous sequences resides on a distinct chromosome.

We previously demonstrated using rodent × human somatic cell hybrids (SCH) that an active gene encoding the human ~18-kDa SRP14 *Alu* RBP resides on human chromosome 15 at 15q22 (10). We assumed that this gene served as a template for the larger of the two PCR products amplified from human DNA (Fig. 3A) on the basis of the sequence of the PCR product and because a product of the same size was amplified when human ~18-kDa SRP14 cDNA was used as a template (not shown). Figure 3B demonstrates the chromosomal origins of the two human SRP14-related PCR products using DNA from well-characterized SCH which contain different human chromosomes (21). As expected, the larger PCR product was amplified from GM11418, a SCH which contains chromosome 15 as its only human chromosome (Fig. 3B, lane 2) (10, 21). By contrast, the smaller PCR product was not amplified from this SCH (lane 2). Another monochromosomal hybrid, GM10500, which contains chromosome 15, also served as a template for the amplification of the larger PCR product but not for that of the smaller (Fig. 3B, lane 4). Pilot experiments using multiple SCH indicated that the smaller band was encoded by chromosome 11. This was confirmed by demonstrating that the smaller PCR product could be amplified from SCH GM10927A, which contains chromosome 11 as its only human chromosome (21), whereas the larger PCR product was not amplified from this SCH (Fig. 3B, lane 3). The smaller PCR product could be amplified from other SCH which contain human chromosome 11 (lanes 5 and 6). SCH GM10659, which contains both chromosomes 11 and 15, served as a template for both PCR products (lane 5). Therefore, at least two SRP14-homologous sequences exist in the human genome, and these currently reside on different chromosomes.

Previous Southern blot analysis identified a single band representing the SRP14 sequence in rodents, multiple bands from total human DNA, and a single band from human chromosome 15 (see Fig. 6 of reference 10). The results shown in Fig. 3A demonstrated a single SRP14 PCR product in galagos. To further investigate SRP14 gene copy number, we performed

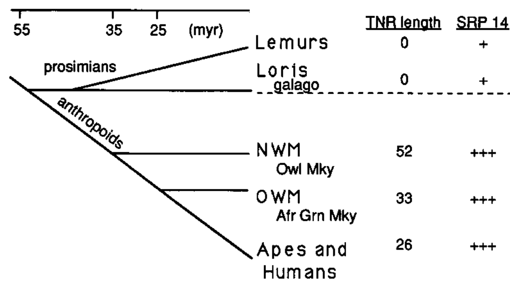

FIG. 5. Phylogenetic tree showing relationships among the primate species represented in this study, their SRP14 TCR lengths (see Table 1), and their levels of SRP14 expression. +, normal; +++, high. The broken horizontal line separates prosimians (above) and anthropoids (below). NWM, New World monkey; OWM, Old World monkey; Afr Grn Mky, African green monkey. The upper horizontal line indicates the time scale in millions of years (myr) before the present. Approximately 85% of the *Alu*s in human DNA were inserted by the time New World monkeys split from the anthropoid lineage about 35 million years ago; the majority of the remaining *Alu*s were inserted over the subsequent 10 million years, with only ~0.5% inserted in the last 8 million years. Thus, there exists a correlation between *Alu* insertion rates and trinucleotide repeat (TNR) lengths. The tree is a composite modified from references 38 and 59.

Southern blot analysis of primate DNAs (Fig. 4). Several restriction enzymes each yielded a multiplicity of bands in owl monkey (anthropoid [New World]) DNA compared with galago (prosimian) DNA (Fig. 4). Although galago DNA produced two or three bands with some enzymes (Fig. 4, lanes 1, 2, and 5), other enzymes produced a single band (lanes 3 and 4). By contrast, each enzyme produced at least twice as many bands from owl monkey DNA (Fig. 4, lanes 6 to 10) as from galago DNA (lanes 1 to 5). All other anthropoids examined revealed a multiplicity of bands with a complexity similar to that seen with owl monkey and human DNA (not shown). These results are consistent with the contention that the prosimian galago contains a single SRP14 gene, while later primates contain more than one SRP14-homologous sequence. It was previously shown that the human gene represented by a single ~3.3-kbp *Pst*I fragment from chromosome 15q22 actively produces the ~18-kDa SRP14 *Alu* RBP (10). The SRP14-homologous sequence which resides on chromosome 11 may not be active, since the expected protein of ~14 kDa was not observed, although low amounts might not be detectable by our assays (Fig. 1 and 2A).

DISCUSSION

The major conclusion that can be drawn from this study is that several genetic events affected the SRP14 gene during early primate evolution. These included gene duplication, origin and expansion of a TCR in the 3′-coding sequence of the active SRP14 gene, and increased expression. The net result was the creation of an overexpressed variant of the SRP14 *Alu* RBP in anthropoids. Cumulatively, the data presented here are consistent with previous results which demonstrated that rodent × human SCH expressed substantially more human-specific *Alu* RBP activity than the endogenous rodent-specific RBP activity expressed in the same cells (10). Western blots of such hybrid cells reveal correspondingly more human SRP14 than rodent SRP14, providing further support of the interpretation that greater accumulation of human SRP14 accounts for a higher level of RNA-binding activity (17a). An additional conclusion drawn from Fig. 1 and 2 is that the ratio of SRP14 (and its RNA-binding activity) to its ligand 7SL RNA is substantially increased in anthropoid cells.

The modifications of the SRP14 gene that are associated with increased levels of *Alu* RNA-binding activity occurred during the evolutionary period that encompassed the major amplification of *Alu* elements and therefore suggest that *Alu* RBP influenced *Alu* expansion in anthropoids (5) (Fig. 5). However, we wish to emphasize that although these results demonstrate a correlation among the origin of a TCR, increased expression of SRP14, and the major wave of *Alu* amplification in primates, they do not prove a cause and effect relationship. At the present time, we do not know if the putative effect of SRP14 on *Alu* expression was positive or negative in early anthropoids. For example, we do not know if the multiple genetic events that affected SRP14 occurred simultaneously or as discrete events; nor do we know the temporal order of these events in relation to the acceleration and deceleration in the amplification rates that shaped the wave of *Alu* expansion. In any case, we suspect that determinants such as evolution of the *Alu* nucleotide sequence itself and the effects of such changes on the ability of *Alu* sequences to interact with the transposition machinery contributed significantly to shaping the complex pattern of *Alu* amplification during primate evolution. It is hoped that future investigations will resolve some of these relationships and address more precisely the nature of the correlations presented here.

Human ~18-kDa SRP14 *Alu* RBP can interact with *Alu* RNA in vitro (9) and affect *Alu* RNA levels in vivo (10). It was previously shown that chromosome 15-containing, *Alu* RBP-expressing, SCH contain an abundance of processed sc*Alu* RNA but much less nascent *Alu* RNA, while this ratio was reversed in *Alu* RNA-expressing SCH which do not express *Alu* RBP (see Fig. 3A of reference 36). Thus, overexpression of human ~18-kDa SRP14 *Alu* RBP led to accumulation of processed sc*Alu* RNAs, presumably at the expense of nascent *Alu* intermediary transcripts (10). This suggests that increased levels of SRP14 may directly affect *Alu* RNA metabolism. As noted in the introduction, 3′ processing of nascent *Alu* transcripts would be expected to decrease the potential of an *Alu* transcript for retroposition. On the basis of these observations, we are compelled to consider the possibility that the genetic events which led to increased expression of SRP14 were selected for as a result of a negative effect on a high rate of *Alu* mobility. The *Alu* amplification rate was about 100-fold greater in early anthropoids than in humans (5, 13, 59). In this scenario, anthropoid-specific SRP14 would have evolved as a factor whose increased expression would limit *Alu* expansion.

We should also consider an alternative interpretation of the relationship between primate SRP14 and *Alu* expansion. Once *Alu* elements amplified, many would be transcribed as part of heterogeneous nuclear RNAs (hnRNAs) and mRNAs. Such interspersed *Alu* dimeric RNA elements, each representing two SRP9-SRP14-binding sites, might confer a formidable burden on the level of free SRP9/14 that was available to bind 7SL RNA. The level of SRP9/14 *Alu* RBP would have to increase to meet the demand. According to this model, the increase in *Alu* RBP levels in primates would have been due to pressure exerted by *Alu* amplification, and selection for increased expression of SRP14 would facilitate rather than limit *Alu* amplification. In this model, increases in SRP14 *Alu* RBP would be a response to *Alu* amplification but would not have been involved in the amplification process per se. Recent results are consistent with either model. The *Alu*-homologous region of 7SL RNA facilitates its nucleocytoplasmic transport, possibly via interaction with SRP9/14, suggesting that the latter is a nuclear protein (19). Thus, SRP9/14 *Alu* RBP may have access to large amounts of hnRNA and mRNA. *Alu* RBP can indeed recognize and bind to *Alu* sequences embedded within larger transcripts such as mRNAs (20). In light of these observations,

it is difficult to imagine how the amount of *Alu* RNA contained in hnRNA and mRNA could have appeared in primate cells without having an impact on the integrity of SRP structure and perhaps function unless compensatory elevations in *Alu* RBP also occurred.

With the above considerations in mind, it is reasonable to suggest that the *Alu* sequences in human DNA and the genetic alterations in SRP14 *Alu* RBP coevolved in early anthropoids. The idea that this may have been attributable to a C→G substitution that predisposed the SRP14 locus to GCA triplet expansion and aberrant expression is intriguing and may provide a model not only for triplet expansions in other genes but for coevolution of genetic elements in complex biological systems as well.

The data reported here suggest that the evolutionary modifications of SRP14 have implications for human cell biology. The results represented by Fig. 1 suggest that individual subunits of SRP need not be tightly regulated in cultured cells. It is possible that the extended structure of primate-specific SRP14 facilitates dissociation of SRP9/14 from SRP; what effects, if any, the primate-specific C-terminal tail may have on SRP assembly or function have yet to be examined. Through such investigations, we may learn, among other things, whether the recently developed C-terminal tail of SRP14 enhanced, impaired, or remained neutral with respect to SRP function in primate cells and may provide insight into the idea that primate SRP14 evolution might have occurred for reasons other than those directly related to SRP (i.e., related to *Alu* amplification).

SRP9/14 together with the *Alu*-homologous region of 7SL RNA confers translational control on a subset of mRNAs (60, 61, 65, 67). SRP9/14 *Alu* RBP binding sites in the form of *Alu* sequences in pre-mRNAs as well as many mature mRNAs suggest the possibility that *Alu* RBP affects the metabolism or translatability of these RNAs in primates (10). Finally, we must also consider the possibility that primate-specific *Alu*-homologous transcripts such as sc*Alu* RNA, full-length *Alu* transcripts, and neuron-specific BC200 RNA (9, 36, 40, 45, 63, 69), in conjunction with SRP9/14 *Alu* RBP, mediate novel forms of translational control in a manner similar to that of the SRP9/14-mediated elongation arrest domain of SRP.

ACKNOWLEDGMENTS

We thank P. Deininger for helpful discussion and for sharing the galago transformed cell line before publication and K. Strub for discussion of results prior to publication. We also thank G. Darlington for gifts of cell lines and extracts and for cytologic analyses, M. Batzer and J. Marini for DNAs, and F. Miller and B. Dobberstein for antisera. We thank H. Bernstein, D. Labuda, T. Kokubo, E. Englander, B. Howard, and A. Furano for helpful discussions and criticisms; and A. Weiner, M. Singer, the members of the LMGR, and the NIH Friday seminar group for encouragement.

L.K.G. was supported by an Intramural Research Training award. D.Y.C. was supported by an Interpersonnel Act between the NICHHD and the Department of Biochemistry, University of Maryland Medical School.

REFERENCES

1. **Batzer, M. A., G. E. Kilroy, P. E. Richard, T. H. Shaikh, T. D. Desselle, C. L. Hoppens, and P. L. Deininger.** 1990. Structure and variability of recently inserted Alu family members. Nucleic Acids Res. **18:**6793–6798.
2. **Batzer, M. A., C. W. Schmid, and P. L. Deininger.** 1993. Evolutionary analyses of repetitive DNA sequences. Methods Enzymol. **224:**213–232.
3. **Bennett, K. L., R. E. Hill, D. F. Pietras, M. Woodworth-Gutai, C. Kane-Haas, J. M. Houston, J. K. Heath, and N. D. Hastie.** 1984. Most highly repeated dispersed DNA families in the mouse genome. Mol. Cell. Biol. **4:**1561–1571.
4. **Bovia, F., N. Bui, and K. Strub.** 1994. The heterodimeric subunit SRP9/14 of the signal recognition particle functions as permuted single polypeptide chain. Nucleic Acids Res. **22:**2028–2035.
5. **Britten, R. J.** 1994. Evidence that most human *Alu* sequences were inserted in a process that ceased about 30 million years ago. Proc. Natl. Acad. Sci. USA **91:**6148–6150.
6. **Britten, R. J., W. F. Baron, D. B. Stout, and E. H. Davidson.** 1988. Sources and evolution of human *Alu* repeated sequences. Proc. Natl. Acad. Sci. USA **85:**4770–4774.
7. **Britten, R. J., D. B. Stout, and E. H. Davidson.** 1989. The current source of human *Alu* retroposons is a conserved gene shared with Old World monkey. Proc. Natl. Acad. Sci. USA **86:**3718–3722.
8. **Caskey, C. T., S. Pizzuti, Y.-H. Fu, R. G. Fenwick, Jr., and D. L. Nelson.** 1992. Triplet repeat mutations in human disease. Science **256:**784–789.
9. **Chang, D.-Y., and R. J. Maraia.** 1993. A cellular protein binds B1 and Alu small cytoplasmic RNAs *in vitro*. J. Biol. Chem. **268:**6423–6428.
10. **Chang, D.-Y., B. Nelson, T. Bilyeu, K. Hsu, G. J. Darlington, and R. J. Maraia.** 1994. A human *Alu* RNA-binding protein whose expression is associated with accumulation of small cytoplasmic *Alu* RNA. Mol. Cell. Biol. **14:**3949–3959.
11. **Daniels, G. R., and P. L. Deininger.** 1991. Characterization of a third major SINE family of repetitive sequences in the galago genome. Nucleic Acids Res. **19:**1649–1656.
12. **Daniels, G. R., G. M. Fox, D. Loewensteiner, C. W. Schmid, and P. L. Deininger.** 1983. Species-specific homogeneity of the primate Alu family of repeated DNA sequences. Nucleic Acids Res. **11:**7579–7593.
13. **Deininger, P. L., and M. A. Batzer.** 1993. Evolution of retroposons. Evol. Biol. **27:**157–196.
14. **Economou-Pachnis, A., and P. N. Tsichlis.** 1985. Insertion of an Alu SINE in the human homologue of the *Mlvi*-2 locus. Nucleic Acids Res. **13:**8379–8387.
15. **Englander, E. W., A. P. Wolffe, and B. H. Howard.** 1993. Nucleosome interactions with a human Alu element: transcriptional repression and effects of template methylation. J. Biol. Chem. **268:**19565–19573.
16. **Gostout, B., Q. Liu, and S. S. Sommer.** 1993. "Cryptic" repeating triplets of purines and pyrimidines (cRRY(i)) are frequent and polymorphic: analysis of coding cRRY (i) in the proopiomelanocortin (POMC) and TATA-binding protein (TBP) genes. Am. J. Hum. Genet. **52:**1182–1190.
17. **Gottlieb, E., and J. A. Steitz.** 1989. Function of the mammalian La protein: evidence for its action in transcription termination by RNA polymerase III. EMBO J. **8:**851–861.

17a. **Green, L. K., D.-Y. Chang, and R. J. Maraia.** Unpublished observations.

18. **Gundelfinger, E. D., E. Krause, M. Melli, and B. Dobberstein.** 1983. The organization of the 7SL RNA in the signal recognition particle. Nucleic Acids Res. **11:**7363–7374.
19. **He, X.-P., N. Bataille, and H. M. Fried.** 1994. Nuclear export of signal recognition particle RNA is a facilitated process that involves the *Alu* sequence domain. J. Cell Sci. **107:**903–912.
20. **Hsu, K., D. Y. Chang, and R. J. Maraia.** Human signal recognition particle Alu-associated protein also binds Alu interspersed repeat sequence RNAs. Submitted for publication.
21. **Human Genetic Mutant Cell Repository.** 1992. 1992–1993 catalog of cell lines, p. 579–585. NIH publication no. 92-2011. National Institute of General Medical Sciences, Bethesda, Md.
22. **Hutchinson, G. B., S. E. Andrew, H. McDonald, Y. P. Goldberg, R. Graham, J. M. Rommens, and M. R. Hayden.** 1993. An *Alu* element retroposition in two families with Huntington disease defines a new active *Alu* subfamily. Nucleic Acids Res. **21:**3379–3383.
23. **Jagadeeswaran, P., B. G. Forget, and S. M. Weissman.** 1981. Short interspersed repetitive DNA elements in eucaryotes: transposable DNA elements generated by reverse transcription of RNA Pol III transcripts? Cell **26:**141–142.
24. **Jelinek, W. R., and C. W. Schmid.** 1982. Repetitive sequences in eukaryotic DNA and their expression. Annu. Rev. Biochem. **51:**813–844.
25. **Jurka, J.** 1993. A new subfamily of recently retroposed human Alu repeats. Nucleic Acids Res. **21:**2252.
26. **Jurka, J., and A. Milosavljevic.** 1991. Reconstruction and analysis of human Alu genes. J. Mol. Evol. **32:**105–121.
27. **Jurka, J., and T. Smith.** 1988. A fundamental division in the *Alu* family of repeated sequences. Proc. Natl. Acad. Sci. USA **85:**4775–4778.
28. **Jurka, J., and E. Zuckerkandl.** 1991. Free left arms as precursor molecules in the evolution of Alu sequences. J. Mol. Evol. **33:**49–56.
29. **Krayev, A. S., D. A. Kramerov, K. G. Skryabin, A. P. Ryskov, A. A. Bayev, and G. P. Georgiev.** 1980. The nucleotide sequence of the ubiquitous repetitive DNA sequence B1 complementary to the most abundant class of mouse fold-back RNA. Nucleic Acids Res. **8:**1201–1215.
30. **Labuda, D., D. Sinnett, C. Richer, J.-M. Deragon, and G. Striker.** 1991. Evolution of mouse B1 repeats: 7SL RNA folding pattern conserved. J. Mol. Evol. **32:**405–414.
31. **Labuda, D., and E. Zietkiewicz.** 1994. Evolution of secondary structure in the family of 7SL-like RNAs. J. Mol. Evol. **39:**506–518.
32. **Liu, W.-M., and C. W. Schmid.** 1993. Proposed roles for DNA methylation in Alu transcriptional repression and mutational inactivation. Nucleic Acids Res. **21:**1351–1359.
33. **Maraia, R.** 1991. The subset of mouse B1 (*Alu*-equivalent) sequences ex-

pressed as small processed cytoplasmic transcripts. Nucleic Acids Res. **19:** 5695–5702.
34. **Maraia, R., M. Zasloff, P. Plotz, and S. Adeniyi-Jones.** 1988. Pathway of B1-*Alu* expression in microinjected oocytes: *Xenopus laevis* proteins associated with nuclear precursor and processed cytoplasmic RNAs. Mol. Cell. Biol. **8:**4433–4440.
35. **Maraia, R. J., D.-Y. Chang, A. P. Wolffe, R. L. Vorce, and K. Hsu.** 1992. The RNA polymerase III terminator used by a B1-*Alu* element can modulate 3′ processing of the intermediate RNA product. Mol. Cell. Biol. **12:**1500–1506.
36. **Maraia, R. J., C. Driscoll, T. Bilyeu, K. Hsu, and G. J. Darlington.** 1993. Multiple dispersed loci produce small cytoplasmic *Alu*, RNA. Mol. Cell. Biol. **13:**4233–4241.
37. **Maraia, R. J., D. J. Kenan, and J. D. Keene.** 1994. Eukaryotic transcription termination factor La mediates transcript release and facilitates reinitiation by RNA polymerase III. Mol. Cell. Biol. **14:**2147–2158.
38. **Martin, R. D.** 1994. Primate palaeontology: bonanza at Shanghuang. Nature (London) **368:**586–587.
39. **Matera, A. G., U. Hellmann, M. F. Hintz, and C. W. Schmid.** 1990. Recently transposed Alu repeats result from multiple source genes. Nucleic Acids Res. **18:**6019–6023.
40. **Matera, A. G., U. Hellmann, and C. W. Schmid.** 1990. A transpositionally and transcriptionally competent *Alu* subfamily. Mol. Cell. Biol. **10:**5424–5432.
41. **Mitchell, G. A., D. Labuda, G. Fontaine, J. M. Saudubray, J. P. Bonnefont, S. Lyonnet, L. C. Brody, G. Steel, C. Obie, and D. Valle.** 1991. Splice-mediated insertion of an *Alu* sequence inactivates ornithine d-aminotransferase: a role for *Alu* elements in human mutation. Proc. Natl. Acad. Sci. USA **88:**815–819.
42. **Muratani, K., T. Hada, Y. Yamamoto, T. Kaneko, Y. Shigeto, T. Ohue, J. Furuyama, and K. Higashino.** 1991. Inactivation of the cholinesterase gene by Alu insertion: possible mechanism for human gene transposition. Proc. Natl. Acad. Sci. USA **88:**11315–11319.
43. **Ohshima, K., R. Koishi, M. Matsuo, and N. Okada.** 1993. Several short interspersed repetitive elements (SINEs) in distant species may have originated from a common ancestral retrovirus: characterization of a squid SINE and a possible mechanism for generation of tRNA-derived retroposons. Proc. Natl. Acad. Sci. USA **90:**6260–6264.
44. **Okada, N.** 1991. SINEs. Curr. Opin. Genet. Dev. **1:**498–504.
45. **Panning, B., and J. R. Smiley.** 1993. Activation of RNA polymerase III transcription of human *Alu* repetitive elements by adenovirus type 5: requirement for the E1b 58-kilodalton protein and the products of E4 open reading frames 3 and 6. Mol. Cell. Biol. **13:**3231–3244.
46. **Panning, B., and J. R. Smiley.** 1994. Activation of RNA polymerase III transcription of human Alu elements by herpes simplex virus. Virology **202:**408–417.
47. **Quentin, Y.** 1988. The Alu family developed through successive waves of fixation closely connected with primate lineage history. J. Mol. Evol. **27:**194–202.
48. **Quentin, Y.** 1992. Fusion of a free left Alu monomer and a free right Alu monomer at the origin of the Alu family in the primate genomes. Nucleic Acids Res. **20:**487–493.
49. **Quentin, Y.** 1992. Origin of the Alu family: a family of Alu-like monomers gave birth to the left and the right arms of the Alu elements. Nucleic Acids Res. **20:**3397–3401.
50. **Quentin, Y.** 1994. A master sequence related to a free left Alu monomer (FLAM) at the origin of the B1 family in rodent genomes. Nucleic Acids Res. **22:**2222–2227.
51. **Reddy, R.** 1988. Compilation of small RNA sequences. Nucleic Acids Res. **16:**71–85.
52. **Rogers, J. H.** 1985. The origin and evolution of retroposons. Int. Rev. Cytol. **93:**187–279.
53. **Russanova, V. R., C. T. Driscoll, and B. H. Howard.** Chromatin-mediated repression of Alu repetitive sequence expression. Submitted for publication.
54. **Ryan, S. C., and A. Dugaiczyk.** 1989. Newly arisen DNA repeats in primate phylogeny. Proc. Natl. Acad. Sci. USA **86:**9360–9364.
55. **Sawada, J., and C. W. Schmid.** 1986. Primate evolution of the alpha-globin gene cluster and its Alu-like repeats. J. Mol. Biol. **192:**693–709.
56. **Schmid, C., and R. Maraia.** 1992. Transcriptional regulation and transpositional selection of active SINE sequences. Curr. Opin. Genet. Dev. **2:**874–882.
57. **Schmid, C. W., and C. K. J. Shen.** 1985. The evolution of interspersed repetitive DNA sequences in mammals and other vertebrates, p. 323–358. *In* R. J. MacIntyre (ed.), Molecular evolutionary genetics. Plenum Press, New York.
58. **Sharp, P. A.** 1983. Conversion of RNA to DNA in mammals: *Alu*-like elements and pseudogenes. Nature (London) **301:**471–472.
59. **Shen, M. R., M. A. Batzer, and P. L. Deininger.** 1991. Evolution of the master Alu gene(s). J. Mol. Evol. **33:**311–320.
60. **Siegel, V., and P. Walter.** 1986. Removal of the *Alu* structural domain from signal recognition particle leaves its protein translocation activity intact. Nature (London) **320:**81–84.
61. **Siegel, V., and P. Walter.** 1988. Each of the activities of signal recognition particle (SRP) is contained within a distinct domain: analysis of biochemical mutants of SRP. Cell **52:**39–49.
62. **Sinnett, D., C. Richer, J.-M. Deragon, and D. Labuda.** 1991. *Alu* RNA secondary structure consists of two independent 7 SL RNA-like folding units. J. Biol. Chem. **266:**8675–8678.
63. **Sinnett, D., C. Richer, J.-M. Deragon, and D. Labuda.** 1992. Alu RNA transcripts in human embryonal carcinoma cells: model of post-transcriptional selection of master sequences. J. Mol. Biol. **226:**689–706.
64. **Slagel, V., E. Flemington, V. Traina-Dorge, H. Bradshaw, Jr., and P. L. Deininger.** 1987. Clustering and sub-family relationships of the Alu family in the human genome. Mol. Biol. Evol. **4:**19–29.
65. **Strub, K., J. B. Moss, and P. Walter.** 1991. Binding sites of the 9- and 14-kilodalton heterodimeric protein subunit of the signal recognition particle (SRP) are contained exclusively in the *Alu* domain of SRP RNA and contain a sequence motif that is conserved in evolution. Mol. Cell. Biol. **11:**3949–3959.
66. **Strub, K., and P. Walter.** 1989. Isolation of a cDNA clone of the 14-kDa subunit of the signal recognition particle by cross-hybridization of differently primed polymerase chain reactions. Proc. Natl. Acad. Sci. USA **86:**9747–9751.
67. **Strub, K., and P. Walter.** 1990. Assembly of the *Alu* domain of the signal recognition particle (SRP): dimerization of the two protein components is required for efficient binding to SRP RNA. Mol. Cell. Biol. **10:**777–784.
68. **Strub, K., N. Wolff, and S. Oertle.** 1993. The Alu-domain of the signal recognition particle, p. 635–645. *In* K. H. Nierhaus (ed.), The translational apparatus. Plenum Press, New York.
69. **Tiedge, H., W. Chen, and J. Brosius.** 1993. Primary structure, neural-specific expression, and dendritic location of human BC200 RNA. J. Neurosci. **13:** 2382–2390.
70. **Ullu, E., S. Murphy, and M. Melli.** 1982. Human 7SL RNA consists of a 140 nucleotide middle-repetitive sequence inserted in an Alu sequence. Cell **29:**195–202.
71. **Ullu, E., and C. Tschudi.** 1984. *Alu* sequences are processed 7SL RNA genes. Nature (London) **312:**171–172.
72. **Van Arsdell, S. W., R. A. Denison, L. B. Bernstein, and A. M. Weiner.** 1981. Direct repeats flank three small nuclear RNA pseudogenes in the human genome. Cell **26:**11–17.
73. **Wallace, M. R., L. B. Andersen, A. M. Saulino, P. E. Gregory, T. W. Glover, and F. S. Collins.** 1991. A *de novo Alu* insertion results in neurofibromatosis type 1. Nature (London) **353:**864–866.
74. **Walter, P., and G. Blobel.** 1982. Signal recognition particle contains a 7S RNA essential for protein translocation across the endoplasmic reticulum. Nature (London) **299:**691–698.
75. **Walter, P., and G. Blobel.** 1983. Disassembly and reconstitution of signal recognition particle. Cell **34:**525–533.
76. **Walter, P., and G. Blobel.** 1983. Subcellular distribution of signal recognition particle and 7SL RNA determined with polypeptide-specific antibodies and complementary DNA probe. J. Cell Biol. **97:**1693–1699.
77. **Weiner, A. M.** 1980. An abundant cytoplasmic 7S RNA is complementary to the dominant interspersed middle repetitive DNA sequence family in the human genome. Cell **22:**209–218.
78. **Willard, C., H. T. Nguyen, and C. W. Schmid.** 1987. Existence of at least three distinct Alu subfamilies. J. Mol. Evol. **26:**180–186.