Nucleic Acids Research, 1996, Vol. 24, No. 21 **4165–4170**

Monomeric scAlu and nascent dimeric Alu RNAs induced by adenovirus are assembled into SRP9/14-containing RNPs in HeLa cells

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Received August 1, 1996; Revised and Accepted September 6, 1996

ABSTRACT

Nearly 1 000 000 copies of Alu interspersed elements comprise ~5% of human DNA. Alu elements cause gene disruptions by a process known as retrotransposition, in which dimeric Alu RNA is a presumed intermediate. Dimeric Alu transcripts are labile, giving rise to stable left monomeric scAlu RNAs whose levels are tightly regulated. Induction of Alu RNA by viral infection or cell stress leads to a dramatic increase in dimeric Alu transcripts, while scAlu RNA increases modestly. Each monomer of the dimeric Alu element shares sequence homology with the 7SL RNA component of the signal recognition particle (SRP). The SRP protein known as SRP9/14 is also found in a discrete complex with scAlu RNA, although whether dimeric Alu RNA is associated with SRP9/14 had been unknown. Here we show that antiserum to human SRP9 immunoprecipitates both scAlu RNA and dimeric Alu RNAs and that these RNPs accumulate after adenovirus infection, while levels of SRP9, SRP14, SRP54 and 7SL SRP RNA are unaffected. Dimeric Alu RNAs are also associated with the La protein, indicating that these are indeed nascent RNA polymerase III transcripts. This report documents that induced Alu transcripts are assembled into SRP9/14-containing RNPs *in vivo* while SRP levels are unchanged. Implications for Alu RNA metabolism and evolution are discussed.

INTRODUCTION

Alu sequences comprise the most abundant class of short interspersed elements in primate DNA (1–3). *De novo* insertion of Alu elements causes human gene disruptions and is believed to occur via Alu RNA that is synthesized by RNA polymerase (pol) III (4; reviewed in 3). Alu repeats are ~285 nt long, composed of two non-identical monomers that are connected by a 20 nt spacer and followed by an A-rich or poly(A) tract. Primary Alu transcripts are derived from multiple loci and vary in sequence beyond their A-rich tracts (5–7). This variability is due to the fact that Alu elements must rely on fortuitous downstream transcription terminators for nascent RNA 3′-end formation. As a result, Alu primary transcripts are of heterogeneous length, ranging from 300 to 450 nt (6–11). Some of these full-length transcripts are shortened to a set of ~120 nt RNAs, representing Alu left monomer transcripts that accumulate as stable small cytoplasmic (sc)Alu RNAs of unknown function, while the rest appear to be degraded with rapid kinetics (7–9,12). Although by reducing the amount of dimeric Alu RNA available for retroposition, production of scAlu RNA represents one way to decrease Alu transposition, preferential stabilization and cytoplasmic compartmentation suggest an independent function for scAlu RNA.

Alu repeats are ancestrally related to the 7SL RNA component of the signal recognition particle (SRP), a ribonucleoprotein (RNP) that recognizes signal sequences on nascent polypeptides that are destined for secretion (13,14). Each of three SRP activities reside in a distinct domain of the particle (15). The first ~100 nt and last ~50 nt of 7SL RNA share nearly 90% homology with each monomer of the Alu sequence. Two SRP polypeptides, SRP9 and SRP14, form a stable heterodimer known as SRP9/14 that associates with the Alu-homologous region of 7SL RNA to form the translation arrest domain of SRP, while SRP19, SRP54, SRP68 and SRP72 associate with the internal ~150 nt of 7SL RNA, referred to as the S domain, which shares no homology with Alu (13,14,16–18).

Several lines of evidence suggest that interaction between the human SRP9/14 protein and Alu RNA influences Alu transcript metabolism and retrotransposition. SRP9/14 accumulates to levels 10- to 20-fold higher than other SRP subunits, including 7SL RNA, specifically in primate cells (19,20). Deregulation of SRP9/14 occurred during the evolutionary period that encompassed a dramatic change in the rate of Alu retrotransposition in primates and was associated with a substantial structural expansion of the C-terminus of SRP9/14 (19). A transgene-mediated increase in the level of human SRP14 in cells is associated with a corresponding increase in the level of scAlu RNA and this appears to occur at the expense of full-length Alu transcripts (7,21). This observation, in conjunction with the relative lability of full-length Alu RNA, suggests that although SRP9/14 may stabilize scAlu RNA (and/or facilitate its production), this protein may not associate efficiently with full-length Alu RNAs *in vivo* (6,12,20). Although full-length Alu transcripts were found to

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sediment in sucrose gradients with a mobility consistent with association with protein, no protein component was identified (6). Therefore, in order to better understand the metabolism of Alu RNA and the potential for an SRP-related function, it is important to determine the RNP nature of cellular Alu RNAs. Yet, whether SRP9/14 associates with full-length Alu RNA *in vivo* had remained unknown (20).

Viral infection as well as heat shock and other forms of cell stress stimulate Alu RNA expression (10,22,23). Under these conditions, as well as in other cases where Alu RNA is induced, full-length Alu transcripts increase dramatically while scAlu RNAs increase less than 5-fold (6,11,12,24). Some of the multiple adenoviral proteins required for Alu induction were previously known to act as RNA processing and transport factors (22; reviewed in 25). Thus, although Alu RNA induction was demonstrated at the transcriptional level, RNA accumulation may also involve alterations in Alu RNA-associated proteins, however, this aspect of Alu RNA induction had not been investigated (11,22–24).

Certain fundamental issues regarding an SRP9/14-like protein in human cells had also remained unresolved. Biochemical assays revealed an scAlu RNA binding activity that co-purified with two polypeptides of ~18 and ~10 kDa from HeLa cells (21). The ~18 kDa polypeptide was identified as human-specific SRP14 polypeptide, which contains an extended C-terminus accounting for its increased size relative to rodent SRP14, while evidence that the ~10 kDa polypeptide is SRP9 was indirect (19,21,26). Thus, in light of the variability of SRP9/14 in primates, its regulation independent of other SRP subunits and propensity to undergo degradation to smaller proteins, it is important to identify the ~10 kDa protein that co-purified with human SRP14 and scAlu RNA binding protein (9,19–21).

We also wanted to examine whether full-length Alu RNAs are associated with this Alu RNA binding protein *in vivo*. Further, because mechanisms that induce Alu RNA expression could conceivably lead to changes in the association of 7SL RNA and SRP9/14, we also examined whether adenoviral infection leads to an altered form of SRP. Our results demonstrate that both full-length Alu and scAlu RNAs are assembled into SRP9/14-containing RNPs in uninfected cells and that these are substantially increased after infection with adenovirus. The amount of 7SL RNA that remains associated with SRP9/14 as well as the amount of total SRP9/14 appears to be unchanged by infection.

MATERIALS AND METHODS

Human SRP9 cDNA was overexpressed as a glutathione S-transferase (GST)–SRP9 fusion protein from a pGEX-4T-2 plasmid (Pharmacia, Piscataway) designated pGST-hSRP9 (26). After purification by glutathione affinity chromatography, SRP9 was released from immobilized GST fusion protein by cleavage with thrombin (Pharmacia) and was used to immunize rabbits. Anti-SRP serum was provided by F. Miller (Federal Drug Administration, Bethesda, MD); this and other anti-SRP autoimmune sera recognize SRP54 specifically and do not recognize SRP9/14 (27,28; data not shown). Anti-La and anti-Sm autoimmune sera were provided as standards from the Centers for Disease Control (CDC, Atlanta, GA). Affinity-purified antibodies raised against an N-terminal peptide of human SRP14 and their use in chemiluminescent Western blotting were as described previously (19).

RNA electrophoretic mobility shift assays (EMSA) were performed as described (9). Briefly, scAlu and $scB1_{d40}$ [^{32}P]RNAs were synthesized from T7 promoter-containing scAlu and $scB1_{d40}$ DNA templates in the presence of [α-^{32}P]GTP and gel purified prior to use (29). A mixture of scAlu and $scB1_{d40}$ [^{32}P]RNAs and protein were incubated in 15 µl reactions containing 10 mM Tris–HCl, pH 7.5, 80 mM KCl, 5 mM $MgCl_2$, 0.1% Triton X-100, 1 mM DTT, 1 mM EDTA, 4 U RNasin, 5% glycerol (EMSA buffer) and 100 ng poly(rG). After a 40 min incubation at room temperature, samples were analyzed on non-denaturing 6% polyacrylamide gels as previously described for EMSA (9) or diluted to 300 µl with NET-2 (150 mM NaCl, 50 mM Tris–HCl, 0.05% Nonidet-P40) for immunoprecipitation (30). The source of SRP9/14 used for *in vitro* RNP reconstitutions was the heparin–agarose fraction purified from HeLa cells (21).

For immunoprecipitations, antibodies were first adsorbed onto protein A–Sepharose beads, washed with NET-2 and then incubated with cell-derived extracts or RNP reconstitution reactions for 90 min at 4°C, washed four times with NET-2 and RNA purified by phenol/chloroform extraction and ethanol precipitation (30). Carrier tRNA (1 µg) was included just prior to ethanol precipitation of RNP reconstitutions. The precipitated RNA was analyzed directly by polyacrylamide gel electrophoresis and autoradiography or by Northern blot analyses after hybridization to oligo [^{32}P]DNA probes complementary to Alu and 7SL RNAs as described previously (21).

Adenovirus type 2 was derived from high titer stocks provided by B. Howard's laboratory (11). Cytoplasmic extracts were produced from adenovirus-infected and control HeLa cells by a standard hypotonic lysis procedure followed by removal of nuclei by low speed centrifugation (30,31). Extracts from infected and uninfected cells were quantitated for protein content by a BioRad colorimetric assay and visually compared by SDS–PAGE with Coomassie blue staining.

RESULTS

Anti-SRP9 immunoprecipitates scAlu [^{32}P]RNPs reconstituted *in vitro*

Previous attempts to identify the ~10 kDa polypeptide that co-purified with scAlu RNA binding activity by direct amino acid sequencing were unsuccessful (21). We raised antisera against purified recombinant ~10 kDa protein that was expressed in bacteria from human SRP9 cDNA. This antiserum was used to probe samples representative of various stages of purification of Alu RNA binding activity by Western blotting (21,26). Both anti-SRP9 and a previously characterized anti-SRP14 serum (19) recognized polypeptides of the appropriate size (Fig. 1A). By comparing the relative amounts of SRP9 and SRP14 in the ammonium sulfate (AS) lane with the heparin–agarose (Hp) lane it appears that the amount of SRP9 antigen increased relative to the amount of SRP14 during the multiple chromatographic steps employed. This suggests that SRP14 is in excess over SRP9 in the crude extract represented by the AS fraction. In any case, since 48 µg protein in the AS fraction contained less SRP9 (Fig. 1A, lower panel, lane AS) than 10 ng protein in the final Hp fraction (lane Hp), the increase in antigenic specific activity observed here is somewhat higher than the increase in RNA binding activity that was observed previously, although the significance of this is unknown (21).

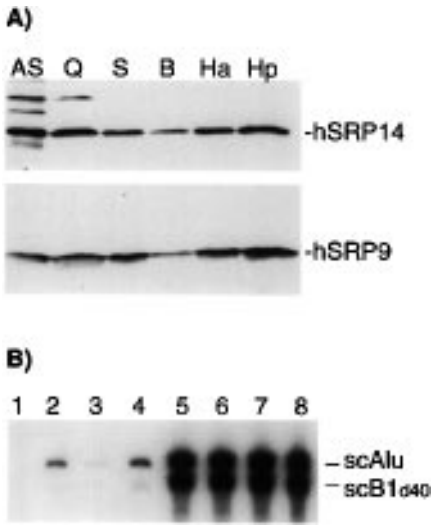

Figure 1. Antibodies that recognize SRP9 immunoprecipitate scAlu–SRP9/14 RNP complexes. (**A**) Samples representing various stages of purification of HeLa cell Alu RNA binding protein were analyzed by Western blot using anti-SRP9 (upper panel) and anti-SRP14 (lower panel) antisera as indicated. Lane 1, 48 µg ammonium sulfate precipitate of HeLa cytoplasm; lane 2, 18.6 µg Q Sepharose eluate; lane 3, 0.3 µg S Sepharose eluate; lane 4, 75 ng blue Sepharose eluate; lane 5, 20 ng hydroxyl apatite eluate; lane 6, 10 ng heparin agarose eluate (see 21). (**B**) Immunoprecipitation of scAlu RNP; immunoprecipitates (lanes 1–4) and supernatants (lanes 5–8) are shown. After incubation of *in vitro* reconstitution reactions that contained either 2 µg BSA (lanes 1 and 5), 50 ng purified SRP9/14 (lanes 2, 3, 6 and 7) or 2.5 µg crude HeLa cell extract (lanes 4 and 8) and a mixture of scAlu and $scB1_{d40}$ [^{32}P]RNAs, samples were subjected to anti-SRP9 antiserum (lanes 1, 2, 4–6 and 8) or preimmune serum (lanes 3 and 7), purified and electrophoresed in denaturing polyacrylamide gel and exposed to X-ray film. scAlu RNA is 115 nt in length, while $scB1_{d40}$ is 95 nt, lacking most of the Alu domain, rendering it unable to efficiently form an RNP (9).

Because some anti-SRP9 sera have been reported to interfere with RNP complex formation (32), we examined our anti-SRP9 serum for its ability to precipitate native RNP complexes reconstituted *in vitro* from scAlu RNA and SRP9/14. Previously, we demonstrated association of scAlu RNA and SRP9/14 by EMSA (9,19,21,26). However, immunoprecipitation as performed in Figure 1B allows a more direct assessment of the specificity of SRP9/14 for scAlu RNA while also qualifying the anti-SRP9 antiserum for immunoprecipitation. ScAlu and $scB1_{d40}$ [^{32}P]RNAs were mixed together and incubated with either purified SRP9/14, HeLa cell extract or BSA and then subjected to immunoprecipitation with anti-SRP9 IgG immobilized on protein A–Sepharose beads. $ScB1_{d40}$ [^{32}P]RNA served as a negative control; in this RNA most of the Alu domain is deleted, rendering it unable to bind (9). After stringent washing, RNA was extracted from the precipitate, purified and subjected to denaturing polyacrylamide gel electrophoresis and autoradiography. This showed that scAlu RNA, but not $scB1_{d40}$ RNA, was precipitated by anti-SRP9 (lanes 2 and 4). Preimmune serum did not immunoprecipitate either RNA (lane 3). Anti-SRP14 sera also specifically precipitated scAlu RNA after reconstitution with SRP9/14 (not shown). These data, in conjunction with previous results which showed that SRP9 and SRP14 bind RNA as a stable heterodimeric protein, indicate that scAlu–SRP9/14 RNP reconstituted *in vitro* can be specifically precipitated by this anti-SRP9 serum. We conclude that the highly purified activity previously referred to as Alu RNA binding protein (9,19,21,26) is indeed SRP9/14 and that anti-SRP9 sera can immunoprecipitate scAlu RNP.

Full-length Alu and scAlu RNAs form SRP9/14 RNPs in HeLa cells

Preliminary experiments revealed that detection of Alu RNA from anti-SRP9 precipitates of uninduced HeLa cell cytoplasm would require substantial amounts of extract as well as antiserum. This was expected since Alu and scAlu RNAs accumulate only to 100–1000 copies/cell (6,9) and theoretically should occupy no more than 0.01% of the total cellular SRP9/14 antigen. After immunoprecipitation, RNA was purified and analyzed by Northern blot using an oligonucleotide probe specific for Alu RNA (Fig. 2). This reproducibly revealed that both the 300–450 nt Alu transcripts and scAlu RNA were immunoprecipitated by anti-SRP9 (lane 2) but not by preimmune serum (lane 3). As alluded to in the Introduction, full-length Alu primary (1°) transcripts appear diffuse because of their expected size heterogeneity (7). The broad band of Alu RNA precipitated by anti-SRP9 suggested that nascent unprocessed Alu transcripts are associated with SRP9/14. To confirm this we used antiserum to the human La protein, a pol III transcription termination factor that transiently associates with all pol III nascent transcripts by binding to their common oligo(U) 3′-termini (33–35). Alu RNAs precipitated by anti-La also exhibited a broad size distribution (lane 5), but markedly less scAlu species as compared with anti-SRP9. We also noted an Alu-homologous RNA slightly larger than scAlu that is barely detectable in HeLa input RNA (lane 1) which was reproducibly enriched by anti-La serum (Fig. 2, lane 5, scAlu*) (see Discussion). Another control serum, anti-Sm, precipitated little if any Alu-homologous RNA (lane 4). These experiments showed for the first time that uninduced HeLa cells accumulate full-length Alu transcripts in the form of SRP9/14-containing RNPs as well as scAlu RNPs.

Adenovirus-induced Alu transcripts are associated with SRP9/14

Cytoplasmic extract isolated from adenovirus-infected HeLa cells was also subjected to immunoprecipitation. It was previously shown that 7SL RNA levels do not change after infection with adenovirus, although other subunits of SRP have not been examined (11,22). Extracts from infected and uninfected cells that were equalized for their protein and 7SL RNA content (not shown) were immunoprecipitated separately with anti-SRP54 and anti-SRP9 and subjected to Northern blot analysis (Fig. 3). The results indicate for the first time that the 300–450 nt Alu transcripts and the scAlu RNAs that are induced by adenovirus infection were associated with SRP9/14 (Fig. 3A, lane 1). Because Alu RNA is easily detectable in infected cells, we chose to conserve infected extract by using less than required to detect Alu RNA from immunoprecipitates of uninfected extract. Thus, although this amount was clearly sufficient for detection of the Alu RNA that was immunoprecipitated by anti-SRP9 from infected cells, Alu RNA was barely detectable in the uninfected cells in this experiment, as expected (lane 2). The high degree of Alu RNA induction may be seen by comparing lanes 1 and 2 of Figure 3A and B, which represent different probings of the same blot. The association of full-length Alu RNA and scAlu RNA with SRP9/14 was specific, since antisera directed to the SRP54 subunit did not precipitate these RNAs

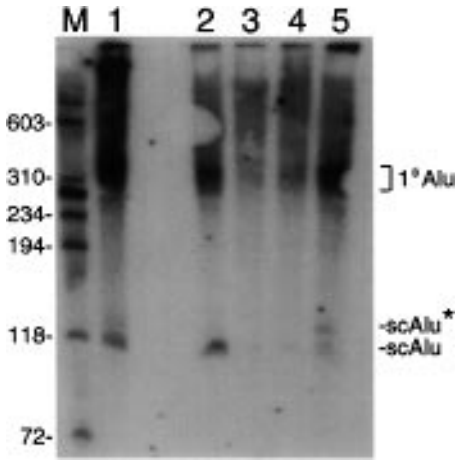

Figure 2. Cellular Alu RNAs are immunoprecipitated by anti-SRP9 serum. After incubation of HeLa cell cytoplasmic extract with antibody immobilized on protein A–Sepharose beads and washing, RNA was purified and examined by Northern blot using an oligoDNA probe specific for the Alu left monomer (lanes 2–5) (9). Immunoprecipitating antisera: lane 2, anti-SRP9; lane 3, preimmune serum; lane 4, anti-Sm; lane 5, anti-La. Lane 1 shows RNA purified from 15% of the input extract that had not been subjected to immunoprecipitation. Lane M shows ^{32}P-end-labeled, denatured, *Hae*III/ΦX174 DNA fragments as mobility markers; sizes are indicated to the left in nucleotides.

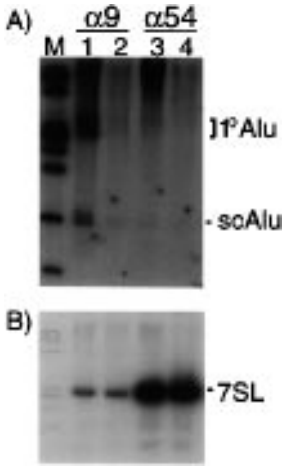

Figure 3. Northern blot analysis of RNA purified from immunoprecipitated extract derived from uninfected (lanes 1 and 3) and adenovirus-infected (lanes 2 and 4) HeLa cells. Immunoprecipitating antisera: lanes 1 and 2, anti-SRP9; lanes 3 and 4, anti-SRP54. (**A**) The probe was specific for Alu RNA; exposure time 4 days. (**B**) The probe was specific for 7SL RNA; exposure time 16 h.

(lanes 3 and 4). The full-length Alu transcripts that accumulated in infected cells were also efficiently precipitated by anti-La (not shown).

SRP integrity in adenovirus-infected HeLa cells

It was previously shown that SRP can be assembled *in vitro* in the absence of SRP9/14 and that these RNPs recognize signal sequences and promote nascent polypeptide translocation but exhibit no translation arrest activity (18). This raises the possibility that SRP9/14-deficient SRPs might exist in cells under

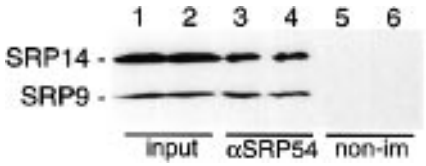

Figure 4. Western blot analysis of anti-SRP54 immunoprecipitates of infected (lanes 3 and 5) and uninfected (lanes 4 and 6) cell extracts. The immunoprecipitating antisera were anti-SRP54 (lanes 3 and 4) and non-immune (lanes 5 and 6). Total extract equivalent to 10% of the input used for immunoprecipitation from infected and uninfected cells was loaded in lanes 1 and 2 respectively. The blot was probed first with anti-SRP14 and then with anti-SRP9. The composite results after the second probing are shown with the reactive bands indicated to the left.

certain conditions even though the level of 7SL RNA may appear unchanged (11,22). Therefore, we wanted to examine whether Alu RNA induction might lead to a significant amount of SRPs that lack SRP9/14. As one approach to evaluate this, we reprobed the blot shown in Figure 3 for 7SL RNA (Fig. 3). In addition to providing a control for Figure 3, these results revealed that anti-SRP9 and anti-SRP54 each immunoprecipitated similar amounts of 7SL RNA from uninfected (Fig. 3, lanes 1 and 3) and infected (lanes 2 and 4) cell extracts, although anti-SRP54 immunoprecipitated 7SL RNA more efficiently than did anti-SRP9 from both extracts. Since anti-SRP9 immunoprecipitated comparable amounts of 7SL RNA from infected and uninfected cell extracts, these results suggested that infection did not lead to a significant amount of SRP9/14-deficient SRPs.

As another approach to examine whether viral infection leads to SRP9/14-deficient SRP complexes, we examined SRP isolated from infected and uninfected cell extracts for their SRP9 and SRP14 content. For this we immunoprecipitated SRPs from the two extracts with antiserum specific for SRP54 and compared their SRP9 and SRP14 content by Western blot analysis. As shown in Figure 4, both SRP9 and SRP14 polypeptides were co-immunoprecipitated in comparable amounts from infected and uninfected cell extracts by anti-SRP54 (lanes 3 and 4) but not by non-immune serum (lanes 5 and 6). Since the anti-SRP54 serum does not recognize either SRP9 or SRP14 directly (28; unpublished observation), these data indicate that SRP9/14 was co-immunoprecipitated with SRP54 by virtue of its association with 7SL RNA as a subunit of SRP. These results, together with those shown in Figure 3 provide the first evidence to indicate that SRP9/14 remains associated with 7SL RNA in extracts from adenovirus-infected cells.

Levels of SRP proteins are unaltered in adenovirus-infected HeLa cells

As alluded to above, SRP protein levels had not previously been examined in adenovirus-infected cells. The levels of total SRP9, SRP14 and La antigen were determined by Western blot analyses of equal amounts of protein isolated from infected and uninfected cell extracts. The levels of these three Alu RNA binding proteins remained virtually unchanged after infection (Fig. 5A), as did SRP54 (not shown).

Finally, we examined the levels of SRP9/14-mediated Alu RNA binding activity in infected and uninfected cell extracts by an EMSA that has been characterized previously in our laboratory (9,19,21,26). The results in Figure 5B show that the amount of

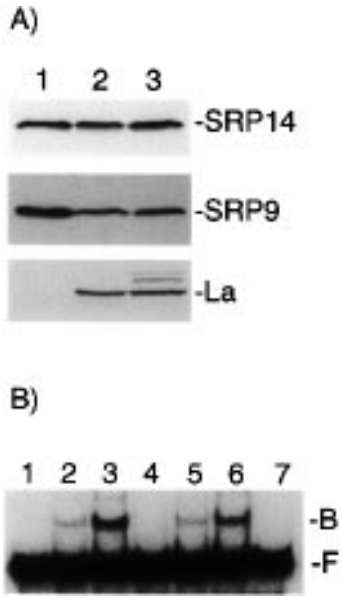

Figure 5. (**A**) Western blot analysis of equal amounts of extract from adenovirus-infected (lane 2) and uninfected (lane 3) HeLa cells; lane 1 contains purified human SRP9/14 to serve as a marker (21). The same blot was probed sequentially with three antisera, rabbit anti-SRP14 (upper panel), rabbit anti-SRP9 (middle panel) and human autoimmune anti-La (lower panel; see text). (**B**) Comparison of Alu RNA binding activity from extracts derived from adenovirus-infected (lanes 1–3) and uninfected (lanes 4–6) cells. Equal amounts of protein from each extract, 0.5 (lanes 1 and 4), 1.0 (lanes 2 and 5) or 1.5 µg (lanes 3 and 6), were used in a quantitative EMSA. The reaction shown in lane 7 contained no extract. The free RNA (F) and bound (B) probe are indicated.

extractable SRP9/14 RNA binding activity was comparable before and after infection by adenovirus.

DISCUSSION

The major conclusion from this work is that the Alu primary transcripts as well as the scAlu RNAs that are induced by adenovirus are assembled into SRP9/14-containing RNPs. In addition, we observed that HeLa cells contain a sufficient capacity of accessible SRP9/14 to accommodate a large increase in Alu RNA without affecting SRP integrity as it has been assayed here. Moreover, the fact that SRP9/14 RNA binding activity (Fig. 5B) was essentially unchanged after Alu induction supports the idea that SRP9/14 is in substantial excess over its RNA ligands, 7SL RNA and Alu RNA, even in viral-infected HeLa cells. Thus, after induction, full-length Alu transcripts are organized into SRP9/14-specific RNPs in the context of a fixed amount of SRP.

The findings reported here provide an opportunity to consider mechanisms of Alu RNA metabolism. First, the fact that anti-La serum immunoprecipitates a small amount of scAlu RNA but a relatively large amount of full-length Alu transcripts as compared with anti-SRP9/14 (Fig. 2) supports the proposal that most scAlu RNA is not the nascent product of pol III, but is derived by RNA processing from Alu primary transcripts (7,8). Since SRP9/14 is primarily cytoplasmic, it was previously unclear if it would be able to associate with nascent Alu transcripts (20). The existence of nascent Alu–SRP9/14 RNPs suggests that scAlu–SRP9/14 RNPs are derived from these. After adenovirus infection Alu sequences become derepressed to yield an ~50-fold increase in full-length Alu transcripts, while scAlu RNA levels increase <5-fold (11,22). Increases in Alu expression induced by transfection, heat shock and protein synthesis inhibitors also lead to preferential accumulation of full-length Alu transcripts, indicating that scAlu RNA levels are more tightly regulated than nascent Alu transcripts (6,10,12). An increase in the level of SRP9/14 in cells is associated with an ~5-fold increase in scAlu RNA levels, suggesting that SRP9/14 may be able to exert a modest regulatory influence over scAlu RNA (21). These cumulative results are consistent with a pathway for scAlu RNA expression that becomes overwhelmed by the large amount of nascent Alu transcripts that are induced during viral infection and cell stress and that SRP9/14 levels alone do not determine Alu RNA levels. Presumably, limiting amount of an as yet unidentified factor governs the levels to which scAlu RNA can accumulate. Identification of this putative factor(s) and its relationship to SRP9/14 and the Alu RNA expression pathway may shed light on Alu retrotransposition as well as provide clues to the function of Alu RNPs.

An unexpected finding was that anti-La serum immunoprecipitated an Alu left monomer transcript that is slightly larger than scAlu (Fig. 2, lane 5, scAlu*). A similar left monomer transcript was previously detected as a prominent RNA, for which the coding DNA was localized to human chromosome 15 (see 7, fig. 3A, lane 15, and 21, fig. 7B). This La-associated small Alu RNA most likely represents a nascent pol III synthesized transcript, whose biogenesis may be due to termination in the Alu intermonomeric A+T-rich spacer. Nucleotide substitutions in this linker region of Alu elements may create a $(dT)_4$ pol III termination signal (36). Alternatively, scAlu* RNA may represent a unique locus that harbors an Alu free-left monomer (37,38). Since La is found associated with nuclear precursors of small cytoplasmic RNAs, it may be of interest to determine whether La-associated scAlu* RNA is efficiently compartmentalized to the cytoplasm, as is genuine scAlu RNA, or if it is primarily nuclear (7,9,39).

Finally, we would like to understand why changes in the structure and deregulation of the SRP9/14 protein occurred during primate evolution. Specifically, whether selection for these traits was related to Alu retrotransposon activity (19). Amplification of the majority of Alus in the human genome ceased ~30 000 000 years ago and a substantial proportion of these have since accumulated mutations, including in their transcriptional control elements. Therefore, ancestral primates may have been able to produce higher levels of Alu RNA as compared with human cells. In addition, non-mutated Alu RNA sequences might have been higher affinity ligands for SRP9/14 as compared with the Alu sequences induced in human cells. In the ancestral primate that presumably existed prior to the deregulation of SRP9/14, a massive induction of Alu RNA might indeed have interfered with SRP function. In this scenario, the genetic deregulation that accompanied the 10- to 20-fold increase in SRP9/14 could plausibly have been an adaptive response that allowed induction of Alu RNAs while protecting the integrity of the SRP. This reasoning implies that the ability to induce Alu RNA was beneficial to the species. We wish to emphasize that although Alu RNA induction is not accompanied by disruption of SRP in human cells, this does not exclude the possibility that Alu–SRP9/14 RNPs may play a role in translation (40). It has been reported that cellular mRNAs are blocked at translational elongation after infection by adenovirus (41,42). Thus, it is tempting to speculate that Alu–SRP9/14 RNPs may be involved in the modulation of translational elongation that occurs upon

infection of HeLa cells. The results reported here suggest that it is reasonable to examine this possibility in the future.

ACKNOWLEDGEMENTS

We are grateful to G. Humphrey and members of the Howard laboratory for generously providing adenovirus and extracts, to F. Miller for anti-SRP sera, to the CDC for standard sera, to E. Englander for critical reading and to A. Sakulich, LMGR members and anonymous reviewers for discussion and suggestions. K.H. was supported by the NIH Research Scholars Program of the Howard Hughes Medical Institute. D.Y.C. was supported by an Interpersonnel Act between NICHHD and the Department of Biochemistry, University of Maryland Medical School.

REFERENCES

1 Jelinek,W.R. and Schmid,C.W. (1982) *Annu. Rev. Biochem.*, **51**, 813–844.
2 Singer,M.F. (1982) *Cell*, **28**, 433–434.
3 Schmid,C. and Maraia,R. (1992) *Curr. Opin. Genet. Dev.*, **2**, 874–882.
4 Wallace,M.R., Andersen,L.B., Saulino,A.M., Gregory,P.E., Glover,T.W. and Collins,F.S. (1991) *Nature*, **353**, 864–866.
5 Sinnett,D., Richer,C., Deragon,J.-M. and Labuda,D. (1992) *J. Mol. Biol.*, **226**, 689–706.
6 Liu,W.-M., Maraia,R.J., Rubin,C.M. and Schmid,C.W. (1994) *Nucleic Acids Res.*, **22**, 1087–1095.
7 Maraia,R.J., Driscoll,C., Bilyeu,T., Hsu,K. and Darlington,G.J. (1993) *Mol. Cell. Biol.*, **13**, 4233–4241.
8 Matera,A.G., Hellmann,U. and Schmid,C.W. (1990) *Mol. Cell. Biol.*, **10**, 5424–5432.
9 Chang,D.-Y. and Maraia,R.J. (1993) *J. Biol. Chem.*, **268**, 6423–6428.
10 Liu,W.-M., Chu,W.-M., Choudary,P.V. and Schmid,C.W. (1995) *Nucleic Acids Res.*, **23**, 1758–65.
11 Russanova,V.R., Driscoll,C.T. and Howard,B.H. (1995) *Mol. Cell. Biol.*, **15**, 4282–4290.
12 Chu,W.M., Liu,W.M. and Schmid,C.W. (1995) *Nucleic Acids Res*, **23**, 1750–1757.
13 Weiner,A.M. (1980) *Cell*, **22**, 209–218.
14 Walter,P. and Blobel,G. (1982) *Nature*, **299**, 691–698.
15 Siegel,V. and Walter,P. (1988) *Cell*, **52**, 39–49.
16 Gundelfinger,E.D., Di Carlo,M., Zopf,D. and Melli,M. (1984) *EMBO J.*, **3**, 2325–2332.
17 Ullu,E., Murphy,S. and Melli,M. (1982) *Cell*, **29**, 195–202.
18 Siegel,V. and Walter,P. (1986) *Nature*, **320**, 81–84.
19 Chang,D.Y., Sasaki-Tozawa,N., Green,L.K. and Maraia,R.J. (1995) *Mol. Cell. Biol.*, **15**, 2109–2116.
20 Bovia,F., Fornallaz,M., Leffers,H. and Strub,K. (1995) *Mol. Biol. Cell*, **6**, 471–484.
21 Chang,D.Y., Nelson,B., Bilyeu,T., Hsu,K., Darlington,G.J. and Maraia,R.J. (1994) *Mol. Cell. Biol.*, **14**, 3949–3959.
22 Panning,B. and Smiley,J.R. (1993) *Mol. Cell. Biol.*, **13**, 3231–3244.
23 Panning,B. and Smiley,J.R. (1994) *Virology*, **202**, 408–417.
24 Panning,B. and Smiley,J.R. (1995) *J. Mol. Biol.*, **248**, 513–524.
25 Panning,B. and Smiley,J.R. (1995) In Maraia,R.J. (ed.), *The Impact of Short Interspersed Elements (SINEs) on the Host Genome.* R.G.Landes Co., Austin, TX, pp. 143–161.
26 Hsu,K., Chang,D.Y. and Maraia,R.J. (1995) *J. Biol. Chem.*, **270**, 10179–10186.
27 Reeves,W.H., Nigam,S.K. and Blobel,G. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 9507–9511.
28 Targoff,I.N., Johnson,A.E. and Miller,F.W. (1990) *Arthritis Rheumatism*, **33**, 1361–1370.
29 Maraia,R. (1991) *Nucleic Acids Res.*, **19**, 5695–5702.
30 Steitz,J.A. (1989) *Methods Enzymol.*, **180**, 468–481.
31 Dignam,J.D., Lebovitz,R.M. and Roeder,R.G. (1983) *Nucleic Acids Res.*, **11**, 1475–1489.
32 Strub,K. and Walter,P. (1990) *Mol. Cell. Biol.*, **10**, 777–784.
33 Stefano,J.E. (1984) *Cell*, **36**, 145–154.
34 Gottlieb,E. and Steitz,J.A. (1989) *EMBO J.*, **8**, 851–861.
35 Maraia,R.J., Kenan,D.J. and Keene,J.D. (1994) *Mol. Cell. Biol.*, **14**, 2147–2158.
36 Liu,W.-M., Leeflang,E.P. and Schmid,C.W. (1992) *Biochim. Biophys. Acta*, **1132**, 306–308.
37 Jurka,J. and Zuckerkandl,E. (1991) *J. Mol. Evol.*, **33**, 49–56.
38 Quentin,Y. (1992) *Nucleic Acids Res.*, **20**, 3397–3401.
39 Rinke,J. and Steitz,J.A. (1982) *Cell*, **29**, 149–159.
40 Andreazzoli,M. and Gerbi,S.A. (1991) *EMBO J.*, **10**, 767–777.
41 Katze,M.G., DeCorato,D. and Krug,R.M. (1986) *J. Virol.*, **60**, 1027–1039.
42 Katze,M.G., Lara,J. and Wambach,M. (1989) *Virology*, **169**, 312–322.