RNA Editing Associated with the Generation of Two Distinct Conformations of the Trypanosomatid Leptomonas collosoma 7SL RNA*

(Received for publication, April 8, 1999)

Herzel Ben-Shlomo, Alexander Levitan, Naomi Editha Shay, Igor Goncharov, and Shulamit Michaeli‡

From the Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel

Analysis of the trypanosomatid Leptomonas collosoma 7SL RNA revealed the existence of two distinct stable 7SL RNA conformers (7SL I and II). Sequence analysis of the RNAs indicated a single base difference between the conformers at position 133 (C in 7SL II and U in 7SL I) located in domain III. This change appears to be the result of a post-transcriptional editing event, since the single-copy 7SL RNA gene codes exclusively for a C at this position. The edited form (7SL I) was found preferentially in the cytoplasm, and the pre-edited form in the nucleus. 7SL I is mainly bound to ribosomes, whereas 7SL II is more abundant in ribosome-free particles. Mutations introduced in regions outside the editing site were found to occur in a single conformation, suggesting that the editing event is not the only factor that determines the conformation of the molecule. This study is the first description of an editing event on a small RNA other than tRNA and is the first report of C → U editing in trypanosomes. We propose a novel role for RNA editing in controlling the conformation of the 7SL RNA in vivo.

The signal recognition particle (SRP)† functions as an adaptor between the protein synthesis machinery and the protein translocation apparatus (1). SRP was shown in vitro to bind the signal sequence emerging from translating ribosomes and to trigger a transient pause in elongation. The arrest in translation was documented by canine SRP in a wheat-germ cell-free system (2) but not when canine SRP was added to either reticulocyte lysate or HeLa cell extracts (3). However, SRP was shown to cause translational pausing at multiple sites in the nascent polypeptide in reticulocyte lysates (4). In the second step of the SRP cycle, elongation arrest is relieved when SRP interacts with the SRP receptor. The SRP is released and can recycle, whereas the ribosome remains attached to the membrane and the nascent chain is then translocated co-translationally into the lumen of the endoplasmic reticulum (ER).

The best studied eukaryotic SRP is the canine particle that is composed of one RNA molecule, the 7SL RNA, and six proteins: SRPs 9, 14, 19, 54, 68, and 72 (5). In vitro studies with canine SRP indicated that SRP54 binds the signal peptide as it emerges from the ribosome, SRP9/14 bind to domain I and function in elongation arrest, SRP68/72 promote translocation into the ER, whereas SRP19 facilitates the binding of SRP54 to the RNA (6). SRP exists in the cell in different states. About 15% of the SRPs are found as free (not bound to polysomes or microsomal membranes). The association of SRPs to monosomes is weak compared with its tight binding to polysomes (7).

7SL RNA was cloned and sequenced from a variety of eukaryotes and these RNAs appear to fit a canonical secondary structure model (8). Despite extensive phylogenetic studies on the 7SL RNA, the only experimental data supporting the secondary structure model are the nuclease digestion (9) and a-sarcin cleavage data (10). The trypanosome 7SL RNA also fits the canonical secondary structure model, except that domain I appear to deviate in length and structure from the human RNA (11–13). Only 70% identity exists between the 7SL RNAs of the monogenetic trypanosomatid Leptomonas collosoma and Trypanosoma brucei (13). Whereas domains I and IV of these trypanosomatid 7SL RNAs are highly conserved, domain III is divergent (13). The presence of a co-migrating tRNA-like molecule, which co-purifies with the T. brucei 7SL RNA (12), led us to hypothesize that the trypanosomatid SRP may differ from other SRPs and is composed of two small RNAs. Using affinity selection with antisense biotinylated oligonucleotide, we have recently demonstrated that L. collosoma SRP complex is indeed composed of two RNA molecules, the 7SL RNA and a tRNA-like molecule (sRNA-85).2

The exact function of the 7SL RNA within the SRP complex is unknown. 7SL RNA most probably does not function simply as a passive scaffold for the SRP proteins but rather has an active role in the translocation process. SRP may therefore undergo structural rearrangements during the functional cycle of the SRP. Indeed, one study, performed on soluble polysome-bound and membrane-bound SRPs, revealed that the secondary structure of the 7SL RNA in these particles is different, suggesting that the 7SL RNA may play an active role in the SRP cycle (14). The potential for the 7SL RNA to exist in more than one conformation was observed for human 7SL RNA (14–16) and the sequences that are essential for adopting the different conformations were determined (15, 16).

RNA editing is a process that co or post-transcriptionally modifies RNA primary sequence from that encoded by the gene by either deletion, insertion, or base modification (17). The phenomenon was first described in trypanosomatid mitochondrial mRNA and was shown to occur through a guide RNA-
mediated cleavage and ligation (18). RNA editing has since been found in diverse lower and higher eukaryotes. The process can occur in the nucleus, chloroplast, and mitochondria, and it involves base modification such as C → U, U → C, and A → I transitions on mRNAs (17). RNA editing was also shown to alter the sequence of tRNA and rRNA (17).

In this study, we demonstrate that the L. collosoma 7SL RNA is present in the cell in two stable conformational states. The conformational change is associated with an RNA editing event of C → U conversion at position 133. This event, however, is not the only factor that determines whether 7SL RNA will undergo the structural changes, since 7SL RNA mutants, altered in regions outside the editing site, were found in a single conformation. The conversion between the two conformations is a dynamic process that takes place in the cytoplasm; 7SL I was also found preferentially attached to ribosomes. This is the first report that correlates conformational change of a small RNA molecule with RNA editing and is the first to report the existence of C → U editing in trypanosomes.

EXPERIMENTAL PROCEDURES

Primers to 7SL RNA—5′-GCTTCACAGGATCGC-3′ is complementary to nt 265–278; 17208 (5′-GCTTCACAGGATCGC-3′) is antisense to domain IV, complementary to nt 180–197; 8721 (5′-GCTTCACAGGATCGC-3′) is sense to position 1–16 including a BamHI site; 15137 (5′-GCTTCACAGGATCGC-3′) is complementary to nt 304–323 including BamHI site; 16865 (5′-GCTTCACAGGATCGC-3′) is complementary to nt 72–86 of snoRNA-2 (19).

Growth and Extract Preparation—L. collosoma cells were grown as described previously (20). Cells (5 × 10^6) were harvested, washed with phosphate-buffered saline, and resuspended in buffer A containing 35 mM Hepes-KOH (pH 7.9), 10 mM MgCl2, 50 mM KCl, 5 mM NaCl, and 5 mM β-mercaptoethanol. The cell suspension was equilibrated in a nitrogen cavitation bomb (Parr Instruments Co.) at 1000 psi for 10 min, and disrupted by release from the bomb (20). Post-ribosomal supernatant (PRS) was prepared as described previously (21).

Plasmid Construction and DNA Transformation—The construction of mutants in domains II and IV was as described previously (13). The 7SL RNA mutant carrying the double domain I was constructed by ligating to the construct (pDI) carrying the 7SL RNA domain I and 1.3 upstream sequences (13) a (335-base pair) polymerase chain reaction product generated with oligonucleotide 8721 and 15137 and carrying the 7SL RNA coding region and its transcription termination signals. The fragments carrying the mutations were cloned into the pX expression vector (22). L. collosoma cells were transfected with 50–100 µg of plasmid DNA, and stable cell lines were generated as described previously (22).

7SL RNA Preparation and Modification with Diethyl Pyrocarbonate (DEPC)—Fractions enriched with 7SL RNA obtained from PRS prepared from 10^11 cells was fractionated on a DEAE-Sephacel column (13), and the flow-through fraction was concentrated on 0.2-mL DEAE column by binding the RNPs at 50 mM KCl and eluting at 0.4 mM KCl. The RNA was deproteinized and separated on preparative 10% denaturing gel (13). 7SL RNA molecules were eluted from the gel in 0.4 M sodium acetate, 0.1% SDS, 0.1 mM EDTA; 2–5 µg of pure 7SL RNA were obtained from each preparative gel. Modification with DEPC was performed using 0.1 µg of pure 7SL RNA as described previously (23). RNA was suspended in 300 µL of buffer II containing 200 mM Hepe (pH 8.0), 1 mM EDTA, 10 µL of DEPC was added, and incubation was for 45 min at 30 °C and 1 min at 90 °C. The reaction was terminated by chilling on ice, 5 µg of tRNA was added, and the RNA was precipitated and analyzed by primer extension.

Primer Extension—Total RNA (20 µg) was mixed with 50,000 cpm of gel-purified [γ-32P]ATP-labeled oligonucleotide (−1 pmol). After annealing at 60 °C for 15 min, the sample was kept on ice for 1 min, 1 unit of AMV reverse transcriptase was added, and the extension was performed at 42 °C for 90 min. The reaction was analyzed on a 6% polyacrylamide gel next to DNA sequencing reaction performed with the same primer. Primer extension sequencing was performed as described previously (24).

Preparation of Nuclei—L. collosoma cells (2 × 10^8) were pelleted, washed with phosphate-buffered saline, and suspended in buffer A. Nonidet P-40 was added to the swollen cells to 0.3% (% v/v), and the cells were lysed by douching 20–30 times with a type “A” pestle. After microscopic examination the nuclei were pelleted at 10,000 × g. The pellet and cytoplasmic fractions were deproteinized, and the RNA was separated on a denaturing gel and subjected to Northern analysis.

RESULTS

The L. collosoma 7SL RNA Is Present in Cells in Two Stable RNA Conformations—We have previously demonstrated that 7SL RNA is a single-copy gene in L. collosoma and that no allelic differences exist in this gene (13). However, two stable 7SL RNA molecules were observed when RNA from L. collosoma was fractionated on a 6% polyacrylamide denaturing gel (13). To examine whether the two transcripts represent two distinct RNA structures, the migration of the RNA was examined in urea gels that vary in the percentage of polyacrylamide. In addition, the migration was examined in gels containing both urea and formamide and in native gels. The results, presented in Fig. 1, indicate that the apparent mobility of 7SL I and II relative to the DNA marker varied in gels containing different acrylamide concentrations, suggesting that both molecules migrate abnormally not as linear RNA molecules. This is surprising since the 7 m concentration of urea used in these gels is assumed to provide totally denaturing conditions. Such partial denaturation phenomenon was previously reported for the Escherichia coli 5 S rRNA (26). Our data, as well as the 5 S rRNA study, suggest that in the presence of 7 m urea, under standard electrophoresis conditions, most, but not all, secondary structures are melted. The RNA migrated as a single molecule or form, approximately at the expected size, only in the presence of 75% formamide and 7 m urea. In native gels, however, four 7SL RNA bands were observed, since each of the single conformations, i.e. 7SL I (the fast migrating form) and 7 SL II (the slow migrating from), can be further separated into two bands. The ability to denature the RNA under very stringent denaturation conditions suggests that some of the secondary or tertiary RNA structures are stabilized by the formation of covalent bonds.

The Level of 7SL I and II Changes during Growth and Is Affected When Protein Synthesis Is Inhibited—To explore whether the two 7SL conformers represent two functional forms of the molecule, the level of the two conformers was first examined during growth. The results (Fig. 2A) indicate that the level of 7SL I to II changes during growth, suggesting that in actively growing and metabolizing cells the dominant form is 7SL I. To control for the amount of RNA in each lane, the level of another stable RNA, U3, was examined. The results indicate that the amount of RNA was comparable in each lane and that the level of U3 did not change during growth.

To correlate the conformational change with the translation process, the level of the two conformers was examined when protein synthesis was inhibited by cycloheximide. The results presented in Fig. 2B indicate that, upon inhibition of protein synthesis with cycloheximide, the high ratio of 7SL I to 7SL II remains constant (≈1.7), whereas in the control the ratio decreased from 1.7 to 0.9. These results suggest that inhibition of translation, and consequently protein translocation, arrests the conversion process and may therefore suggest that the conformational change takes place only during ongoing protein synthesis.

Studies of the mammalian 7SL RNA indicate that the 7SL
RNA undergoes conformational changes during the translocation cycle (14). This conclusion is based on differential chemical sensitivity of the 7SL RNA found in free particles versus polysome-bound or membrane-bound SRPs (14). To explore whether 7SL I and II are associated with different subpopulations of the SRP, the level of 7SL RNA was examined on free and ribosome-bound SRPs. Low salt, whole cell extract was fractionated on sucrose gradients. RNA was extracted from the

**Fig. 1.** Separation of the 7SL RNA in polyacrylamide gels. Fractions enriched in 7SL RNP obtained from a DEAE-Sephacel column were deproteinized, and the RNA was separated in different gel systems next to a DNA marker (M; 1-kilobase pair ladder, Life Technologies, Inc.). The RNA was visualized by EtBr staining. RNA was separated in 6% polyacrylamide gel containing 7 M urea (A), 10% polyacrylamide gel containing 7 M urea (B), 10% polyacrylamide containing 75% formamide and 7 M urea (C), and 10% native polyacrylamide gel (D).

**Fig. 2.** The ratio between 7 SL I to 7 SL II during growth and upon treatment with cycloheximide. A, *L. collosoma* cells were grown for 60 h. Every 6 h cells were counted. RNA was prepared from cells every 12 h and subjected to Northern analysis with anti-7SL and anti-U3 oligonucleotide probes. The growth curve and the Northern analysis with the probes are presented. Densitometric analysis was performed, and the ratio between 7SL I and II was determined and is schematically presented. B, *L. collosoma* cells (5 x 10⁶/ml) were grown for 50 h in the presence of 200 µg ml⁻¹ cycloheximide. RNA was prepared from control and treated cells and was subjected to Northern analysis with anti-7SL and U3 oligonucleotide probes. The data were subjected to densitometric analysis, and the ratio between 7SL I and II was determined and is schematically presented. The squares represent the ratio between 7SL I and II in control cells, and the circles indicate the ratio in cells treated with cycloheximide.
Fig. 3. A, fractionation of 7SL RNP on sucrose gradients. Low salt, whole cell extracts were layered on a continuous 10–30% (w/v) sucrose gradient in buffer A containing 100 mM KCl. Gradients were centrifuged at 4 °C for 3 h at 35,000 rpm in a Beckman SW41 rotor. S values were determined using standards, i.e. 30, 50, and 70 S ribosomes from E. coli and the enzyme catalase (10 S). RNA was extracted and separated on a 10% denaturing gel. The RNA was stained with EtBr and was subjected to Northern analysis with antisense oligonucleotide probes to the 7SL and 5 S RNA. The level of 7SL I to II across the gradient was determined by densitometric analysis and the ratio between 7SL I to 7SL II is presented. B, the distribution of 7SL I and II on ribosomes and PRS. Low salt, whole cell extracts was subjected to high speed spin (150,000 × g). RNA was extracted from the ribosomal pellet and PRS and subjected to Northern analysis with 7SL and 5S RNA probes. Densitometric analysis was performed on the level of 7SL I and II, and the ratio between the two molecules is presented. The results are expressed as mean ± S.E. (n = 5) *, p < 0.05.


differences between 7SL I and 7SL II Revealed by Partial Enzymatic Cleavage with Base Specific Nucleases—The sequence of four independent 7SL RNA genes cloned in our laboratory was identical (13). Therefore, changes in sequence of 7SL I and 7SL II may take place post-transcriptionally. To directly analyze the RNA sequence and to obtain information on the RNA structure, the susceptibility to nucleases was determined. For this purpose, the purified 7SL RNA species were separately end-labeled and subjected to partial hydrolysis with base-specific nucleases. The results presented in Fig. 4 indicate that the two 7SL molecules are very stable and that there is no conversion in vitro between these species, i.e. 7SL I was not converted to II or vice versa. The sequences seem to be identical except for position C138 in loop III. Ribonuclease CL3 digested the C in 7SL I but not in 7SL II. The failure to digest this C may result from base modification, since it has been reported that modified bases are less susceptible to digestion with nucleases (27). It should be noted that 7SL RNA is among the few small RNAs that were shown not to carry modified bases. Indeed, this is the case for the L. collosoma 7SL RNA. 7SL I and II were analyzed for the presence of modified nt using high performance liquid chromatography and mass spectrometry and no modified nt were detected. The reason for the peculiar lack of digestion of C138 in 7SL II may result from its inaccessibility to the nucleases due to local secondary structure of the RNA. It is known that secondary structure interferes with the cleavage of site-specific nucleases (28). Additional evidence for the existence of stable structures in 7SL RNA is the presence of compression in the sequence. The compressed regions are marked with brackets in Fig. 4, and are located between loop III and the loop around nt 160. These compressed regions are, however, common to both molecules.

Analysis of the 7SL I and II Structures Using AMV Reverse Transcriptase and Differential Sensitivity of 7SL I and II to Modification with DEPC—It is well documented that the presence of highly structured regions in an RNA molecule induces the reverse transcriptase to pause. We have used this property

3 James A. McCloskey, personal communication.
of reverse transcriptase to monitor differences in the structures of 7SL I and II. Primer extension was performed on the two separated 7SL RNA molecules. The results are presented in Fig. 5 (A and B) and indicate that stops common to both molecules are located in the 3′ part of domain II and IV. However, there are also stops that are unique to both 7SL I or 7SL II and these are indicated in Fig. 5C. In all the experiments that we have performed, the number of stops on 7SL II was higher compared with 7SL I. Because of the numerous strong stops on 7SL II, the level of the extension product from 7SL II that reached the 111 position was only 1/10 of that observed for 7SL I. Most of the unique stops on 7SL II were mapped to domain II in the region adjacent to domain III and in domain III itself.

To further elucidate the structure of the two molecules, each 7SL RNA conformer was exposed to chemical modifications and the locations of the modified sites were mapped by primer extension. To differentiate natural reverse transcriptase stops from stops elicited by chemical modification, untreated RNA sample was analyzed next to the chemically modified RNA. The experiments were performed with both dimethyl sulfate and DEPC. Because dimethyl sulfate modifies both guanosine and cytosine (23) and since the G+C content of 7SL RNA is 65%, the modification created many obstacles for the reverse transcriptase. Only very short extension products were obtained and therefore the data could not be used to deduce structural information on the two molecules. However, treating the RNA with DEPC, which modifies adenines (23), revealed that 7SL I is more susceptible to the chemical compared with 7SL II, as presented in Fig. 6. The location of the modified bases is indicated in Fig. 5C. The reverse transcriptase stops were located one nucleotide before the modified base as was previously reported (29). This data suggests that 7SL I have a more open structure and therefore is more accessible to chemical modifications. Most of the modified bases are located in domains I and II of the RNA, suggesting differences in the structures of these domains between the two conformers. However, the differential pause sites on domain III (observed in Figs. 5 and 6) suggests differences also in the structure of this domain. These differences may have not been revealed by the DEPC treatment because few adenines exist in this domain.

An Editing Event Is Associated with the Two 7SL RNA Conformers—To examine whether the differences between the 7SL molecules could also be attributed to minor sequence differences that are introduced post-transcriptionally, the separated RNA molecules were subjected to primer extension sequencing. The results, presented in Fig. 7, demonstrate a single change. This change was mapped to position 138 where a U was found in 7SL I versus a C in 7SL II. The DNA sequence of all four independently cloned 7SL RNA genes indicate the presence of C at this position, suggesting that the C→U conversion takes place post-transcriptionally. To examine in which cell compartment (nucleus or cytoplasm) this post-transcriptional modification may take place, the cellular distribution of 7SL I and II was examined. To assess the quality of the nuclei, the distribution of the small nucleolar RNA snoRNA-2 RNA was assayed. RNA was prepared from the nuclear and cytoplasmic fractions, and was subjected to Northern analysis with 7SL and...
snoRNA-2 probes (Fig. 8). The distributions of snoRNA-2 indicate that the nuclei were intact and no snoRNA-2 leaked to the cytoplasm. 7SL I was preferentially found in the cytoplasm; minor contamination of the nuclei by the cytoplasmic fraction can explain the small amount of 7SL I found in the nuclear fraction. The 7SL II was found exclusively in the nucleus, suggesting that this conformer undergoes the conformational change and the editing in the cytoplasm. However, we cannot rule out the possibility that editing does take place in the nucleus and that the edited RNA is rapidly translocated to the cytoplasm.

7SL RNA Mutated in Strategic Domains of the Molecule, but Located outside the Editing Site, Are Found in a Single Conformation—To relate the functionality of the 7SL RNP with the

**Fig. 5.** A, mapping the AMV reverse transcriptase pause sites on 7SL I and 7SL II. 7SL I and 7SL II were eluted from a preparative gel, and ~100 ng were subjected to primer extension with: 1) oligonucleotide 5277, complementary to the 3' end of the molecule, and 2) oligonucleotide 17208, complementary to loop IV. 7SL DNA sequencing with the same oligonucleotides used for primer extension was used as reference. Primer extension stops on 7SL I are indicated with white arrows and those on 7SL II with black arrows. B, primer extension sequencing of 7SL I and II. RNA was prepared as in A. The primer extension was performed with oligonucleotide 17208 in the presence of dideoxynucleotides. The stops on 7SL I and II are indicated with arrows as in A. C, localization of the major AMV reverse transcriptase pause sites on the secondary structure of the 7SL RNA. The structural stops on 7SL I and II are indicated with gray and black arrows, respectively. Stops elicited from the DEPC modification are indicated with arrows and circles.
conformational change of the 7SL RNA undergoes, the conformation of three 7SL RNA mutants was examined in vivo. Cells lines expressing 7SL RNA genes mutated in strategic domains of the 7SL RNA were prepared (Fig. 9A). One mutant carries an insertion in the most conserved loop IV, which should affect the binding of the SRP54 protein; the second mutant carries an insertion in domain II in the region shown in the mammalian 7SL RNA to interact with ribosomes (14); and the third mutant carries a double domain I. RNA was prepared from cell lines expressing the mutated 7SL RNA genes and was subjected to Northern analysis with 7SL RNA probe. The results, presented in Fig. 9B, indicate that mutations in either domain II or IV expressed form the multicopy plasmid repressed the synthesis of the wild type 7SL RNA as shown by the disappearance of the wild type 7SL I and II transcripts (13). These mutants failed to undergo the conformational change, and the mutated 7SL RNA appeared as a single transcript, since the same hybridization pattern was observed when the RNA was hybridized with a specific probe that detects the mutant but not the wild type RNA (results not shown). However, the 7SL RNA carrying the double domain I failed to repress the synthesis of the wild type RNA, but did not undergo the conformational change. Hybridization with a probe designed to specifically identify the mutated RNA (antisense to the boundary between the two domains I) was used to confirm that the mutated 7SL RNA is present in a single conformation (results not shown). These results suggest that mutated 7SL RNA that engage inactive particles fail to undergo the conformational change.

DISCUSSION

This study demonstrates that the trypanosomatid 7SL RNA undergoes a conformational change in vivo that is associated with an RNA editing event. This is the first description of a C → U editing on a small RNA apart from tRNA, and is also the first case of C → U editing in trypanosomes.

The L. collosoma 7SL RNA, unlike all 7SL RNA described so far, is not fully denatured in 7 M urea. Such a property was previously observed for E. coli 5 S rRNA (26). In the latter case, the fast migrating molecule carried a U in position 92, whereas the slow migrating variant contained a C in the same position (26). This is exactly the case in this study, since the fast migrating 7SL I carries a U in position 133 and the slow migrating molecule (7SL II) carries a C in the same position. In the case of the 5 S rRNA, it was suggested that the only difference observed between the two variants is responsible for the drastic reduction in the stability of the two 5 S rRNA molecules (26). It is currently unknown whether this is also the case in the L. collosoma 7SL RNA. However, the findings that mutated 7SL RNAs located outside the editing site were found in a single conformation may suggest that the editing is not the only factor that controls the structural change. The mechanism that elicits the conformational change of 7SL RNA is unknown. Since the editing site is situated close to loop III and to the region that was shown to bind SRP19 in the canine SRP (30), it may alter SRP19 binding. Alternatively, the editing event may affect long range tertiary interactions.

The differences in pause sites observed between 7SL I and 7SL II may represent regions on the molecule that present obstacles for reverse transcriptase due to RNA structures that are not melted because of strong secondary or tertiary interactions. The differential stops between 7SL I and 7SL II reflect differences in the structures of the two molecules. These different structures are mostly located in domains II and III. Previous studies performed on naked human 7SL RNA indicated that in vitro transcribed human 7SL RNA can exist in two different conformations that can be separated on native gels (15, 16). By site-directed mutagenesis, different sites were shown to be important for the formation of these two conformations. Most of the mutations affecting the conformation of the RNA were located in domains II and III of the RNA (16). These are also the domains where major differences were seen in stop sites between the trypanosomatid 7SL I and II. A small region located between positions 129 to 134 of the mammalian 7SL RNA, which is part of the SRP 19 binding site, was shown to be most critical for the conformational change that the mammalian 7SL RNA undergoes (16). The editing site detected in this study is located in this same domain.

Studies performed on ribosome-bound, membrane-bound, and free canine SRPs have indicated that the 7SL RNAs in these SRPs are found in different conformations. This conclusion was based on variation in sensitivity of the different SRPs.
to chemical modifications (14). Initially, it was expected that polysome-bound SRP should be less sensitive to chemical modification than free SRPs because of potential protection of the particles by ribosomes. Instead, the converse was found, and chemical accessibility of polysome-bound SRP was actually higher than that of soluble SRPs, suggesting that the polysome-bound SRP has a more open conformation than that of the 7SL RNA in free particles (14). The results presented in this study support this notion, since 7SL I, which is preferentially associated with ribosomes, is the molecule that was found to be more accessible to chemical modification and therefore has a more open structure, whereas the 7SL II, which is found mostly associated with free SRPs possesses a more closed structure, as revealed by the many strong pause sites and inaccessibility to modification by DEPC. The location of the pause sites observed on the 7SL RNA agrees well with the regions that were shown to be involved in binding to ribosomes or to the rough ER membrane (14). In particular, attention should be drawn to the region around nt 120 in domain III, since homologous regions on the mammalian 7SL RNA were shown to bind to ribosomes or the ER membrane. In addition, the regions in domain II around nt 220 were also shown in the mammalian system to be involved in binding to ribosomes or ER membrane (14). Interestingly, only domain I of the 7SL I but not of 7SL II was accessible to interaction with DEPC, suggesting major differences in the structure of this Alu-like domain between the conformers. In the mammalian SRP, however, the Alu domain was less accessible to chemical modification compared with S domains II and III (14).

It is currently unknown why the 7SL RNA undergoes a conformational change during the translocation cycle. Two steps in the translocation cycle may require changes in the 7SL RNA: first when SRP interacts with the ribosome and induces an arrest or a pause in translation, and again when SRP is released from the ribosome after its interaction with the SRP receptor (1). Conformational changes of rRNA have been shown to take place in the transition from inactive to active 30 S ribosomal subunits (31), in the assembly of subunits to monosomes (32) and during tRNA translocation (33). In this context, the 7SL RNA may cause translation arrest by interfering with the rRNA, thus interfering with the conformational changes rRNA undergoes during protein synthesis. However, the arrest function of SRP was shown to be mediated by domain I (6). It has been suggested that domain I functions in protein arrest by mimicking the shape of a tRNA and thereby blocking the entry of incoming tRNAs. Our finding that a tRNA-like molecule is present in the SRP complex in a 1:1 ratio with the 7SL RNA
supports the notion that the tRNA-like domain may play a role in the arrest (2). The data presented in this paper, as well as the studies on the canine SRP (14) demonstrating that the structure of domains II and III are different in ribosome-free and ribosome-bound SRP, suggest that additional interactions apart from the domain I but with domains II and III of the molecule are also essential for the interaction of the SRP with the ribosome. Further studies are needed to accurately map the site of interaction of 7SL RNA with rRNA, e.g. by in vivo UV-induced psoralen cross-linking.

C → U conversion is among the best documented RNA editing events and has been shown to take place in mitochondria, chloroplast, and the nucleus (17). The first reported C → U editing by base deamination was for the apolipoprotein B mRNA that takes place in the nucleus post-transcriptionally (17). C → U editing was found also in the mitochondria and chloroplast of land plants (34). This type of editing is, however, not restricted to mRNA and was found to modify the anticodon loop and acceptor stem of tRNAs (35). Whereas the editing of apoB mRNA is directed by a mooring sequence located adjacent to the editing site, tRNA editing, especially editing sites in the acceptor stem, may be guided by the base-paired region opposite the edited nucleotide (34, 35). This could also be the case for the 7SL RNA editing, since the site is present in an 8-base pair stem-loop structure. It is currently unknown what type of activity could mediate the editing event revealed in this study. However, the conversion of C → U may "freeze" the 7SL I. Since the results presented in this study suggest that the conversion of 7SL I to 7SL II is a dynamic process, it may imply that an activity that mediates U → C conversion should also exist to convert 7SL I to 7SL II. U → C editing was reported mainly in the mitochondria and chloroplast of land plants and mammalian tRNAs (34). This conversion could be achieved by transamination.

The finding of C → U editing in trypanosomes is especially interesting because it suggests that U insertion and deletion present in the kinetoplast (18) is not the only editing pathway in trypanosomes and that two different mechanisms of editing can co-exist in the same organism. Because of the early divergence of trypanosomes from the eukaryotic lineage, the finding of C → U editing in trypanosomes suggests that this process evolved early in eukaryotic evolution.

The localization of 7SL I in the cytoplasmic fraction suggests that this editing takes place in the cytoplasm. We could not, however, rule out the possibility that editing takes place in the nucleus and that the edited RNA is rapidly translocated to the cytoplasm. The finding that 7SL RNA mutants examined in this study exist in a single conformation may suggest that only 7SL RNA molecules that engage active particles undergo the conformational change. We therefore favor the hypothesis that the editing function may be associated with the ribosome. This hypothesis is not unprecedented, since deaminases involved in editing were found in the cytoplasm of plants (34).

The combination of the unique properties of the L. colosoma 7SL RNA with the ability to express in vivo mutated 7SL RNA will be further used to identify sequences that are involved in: (a) the editing event, (b) the ability to undergo the conformational change, and (c) binding of ribosomes. The establishment of an in vitro system that is amenable to convert 7SL I is essential for better understanding the mechanism and machinery that carries out this novel editing event. We anticipate that other editing events of C → U on small RNAs, such as tRNAs and other cellular and kinetoplast mRNAs, may be found in trypanosomes.

REFERENCES

1. Walter, P., and Johnson, A. E. (1994) Annu. Rev. Cell Biol. 10, 87–119
2. Walter, P., and Blobel, G. (1981) J. Cell Biol. 91, 557–561
3. Meyer, D. I. (1985) EMBO J. 4, 2301–2303
4. Wolin, S. L., and Walter, P. (1993) J. Cell Biol. 121, 1211–1219
5. Walter, P., and Blobel, G. (1982) Nature 299, 691–698
6. Siegel, V., and Walter, P. (1988) Trends Biochem. Sci. 3, 314–316
7. Walter, P., and Blobel, G. (1983) J. Cell Biol. 97, 1693–1699
8. Althoff, S., Selinger, D., and Wise, J. A. (1994) Nucleic Acids Res. 22, 1933–1947
9. Gundelfinger, E. D., Carlo, M. D., Zepf, D., and Melli, M. (1984) EMBO J. 3, 2325–2332
10. Siegel, V., and Walter, P. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 1801–1805
11. Michaeli, S., Podell, D., Agabian, N., and Ullu, E. (1992) Mol. Biochem. Parasitol. 1, 55–65
12. Beja, O., Ullu, E., and Michaeli, S. (1993) Mol. Biochem. Parasitol. 57, 223–230
13. Ben-Shlomo, H., Levitan, A., Beja, O., and Michaeli, S. (1997) Nucleic Acids Res. 25, 4977–4984
14. Andreazoli, M., Gerbi, S. A. (1991) EMBO J. 10, 767–777
15. Zwieb, C., and Ullu, E. (1986) Nucleic Acids Res. 4, 4639–4658
16. Goward, K., and Zwieb, C. (1997) Nucleic Acids Res. 25, 2835–2840
17. Smith, H. C., Gott, J. A., and Hanson, M. R. (1997) RNA 3, 1105–1123
18. Stuart, K., Allen, T. E., Kable, M. L., Lawson, S. (1997) Curr. Opin. Chem. Biol. 1, 340–346
19. Levitan, A., Xu, X. Y., Ben-Dov, C., Ben-Shlomo, H., Zhang, Y., and Michaeli, S. (1998) Nucleic Acids Res. 26, 1775–1783
20. Goldring, A., Karchi, M., and Michaeli, S. (1995) Exp. Parasitol. 80, 333–338
21. Michaeli, S., Roberts, T. G., Watkins, K. P., and Agabian, N. (1999) J. Biol. Chem. 265, 10582–10588
22. Goldring, A., Zimmer, Y., Ben-Yehuda, E., Goncharov, I., and Michaeli, S. (1996) Exp. Parasitol. 84, 28–41
23. Krol, A., and Carbon, P. (1989) Methods Enzymol. 180, 212–227
24. Patzelt, E., Perry, K. L., and Agabian, N. (1989) Mol. Cell. Biol. 9, 4291–4297
25. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
26. Digweed, M., Kumagai, I., Pieler, T., and Erdmann, V. A. (1982) Eur. J. Biochem. 127, 531–537
27. Dennis-Keller, H., Muzam, A. M., and Gilbert, W. (1997) Nucleic Acids Res. 4, 2527–2538
28. Lockard, R. E., Alzner-Deweerd, B., Heckman, J. E., MacGee, J., Tabor, M. V., and RajBhandary, U. L. (1978) Nucleic Acids Res. 5, 37–56
29. Youvan, D. C., and Hearst, J. E. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 3751–3754
30. Siegel, V., and Walter, P. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 1801–1805
31. Moazed, D., and Noller, H. F. (1986) Cell 47, 895–904
32. Stebbins-Boaz, B., and Gerbi, S. A. (1990) J. Mol. Biol. 228, 93–112
33. Moazed, D., and Noller, H. F. (1989) Nature 342, 142–148
34. Marchfelder, A., Binder, S., Brennack, A., and Knoop, V. (1998) in Modification and Editing of RNA (Grosjean, H., and Benne, R., eds) pp. 307–323, ASM Press, Washington, D. C.
35. Price, D., and Gray M. W. (1996) in Modification and Editing of RNA (Grosjean, H., and Benne, R., eds) pp. 289–305, ASM Press, Washington, D. C.