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A highly conserved nucleotide in the Alu domain of SRP RNA mediates translation arrest through high affinity binding to SRP9/14

Dau-Yin Chang[1], John A. Newitt[2], Karl Hsu[1,3], Harris D. Bernstein[2] and Richard J. Maraia[1,*]

[1]Laboratory of Molecular Growth Regulation, National Institute of Child Health and Human Development, National Institutes of Health, [2]Genetics and Biochemistry Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health and [3]HHMI/NIH Research Scholars Program, Howard Hughes Medical Institute, National Institutes of Health, Bethesda, MD 20892, USA

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ABSTRACT

Binding of the signal recognition particle (SRP) to signal sequences during translation leads to an inhibition of polypeptide elongation known as translation arrest. The arrest activity is mediated by a discrete domain comprised of the Alu portion of SRP RNA and a 9 and 14 kDa polypeptide heterodimer (SRP9/14). Although very few nucleotides in SRP RNA are conserved throughout evolution, the remarkable conservation of G24, which resides in the region of SRP9/14 interaction, suggests that it is essential for translation arrest. To understand the functional significance of the G24 residue, we made single base substitutions in SRP RNA at this position and analyzed the ability of the mutants to bind SRP9/14 and to reconstitute functional SRPs. Mutation of G24 to C reduced binding to SRP9/14 by at least 50-fold, whereas mutation to A and U reduced binding ~2- and 5-fold respectively. The mutant RNAs could nevertheless assemble into SRPs at high subunit concentrations. SRPs reconstituted with mutant RNAs were not significantly defective in translation arrest assays, indicating that the conserved guanosine does not interact directly with the translational machinery. Taken together, these results demonstrate that G24 plays an important role in the translation arrest function of SRP by mediating high affinity binding of SRP9/14.

INTRODUCTION

The mammalian signal recognition particle (SRP) is a ribonucleoprotein composed of six polypeptides and a 300 nt RNA (7SL RNA) that recognizes signal sequences of nascent secreted and membrane proteins and targets them as ribosome-associated intermediates to the endoplasmic reticulum (ER) (reviewed in 1). The binding of SRP to signal sequences leads to a transient inhibition of further polypeptide elongation known as 'translation arrest'. This translational block is relieved after interaction between SRP and the SRP receptor anchored in the ER membrane. Interaction between SRP and its receptor also catalyzes release of the nascent chain from SRP and its insertion into a protein translocation complex or 'translocon' composed of the Sec61 complex and an additional protein called TRAM (2,3). Although some organisms contain a much smaller SRP (4,5), the basic elements of the SRP pathway as well as the translocon are highly conserved throughout evolution (1,6,7).

Extensive dissection of SRP has provided an integrated model of its structure and function. Signal sequence recognition, translation arrest and nascent polypeptide translocation activities reside in distinct domains of SRP (8). Signal sequences are recognized by a 54 kDa polypeptide (SRP54) as they emerge from translating ribosomes (9,10). Translation arrest is mediated by a domain located at the opposite end of the rod-like SRP. Release of translation arrest and translocation of the nascent chain across the ER membrane requires activity of the S domain of the particle, which consists of the SRP 19, 54, 68 and 72 kDa subunits plus the ~150 nt of 7SL RNA to which they are bound (11,12). The translation arrest domain consists of the Alu-homologous region of 7SL RNA, which folds into a tRNA-like cruciform structure (Fig. 1), and the associated 9 and 14 kDa protein heterodimer SRP9/14 (13). It has been proposed that the Alu domain tRNA-like structure of 7SL RNA might effect translation arrest by blocking the access of incoming aminoacylated tRNA molecules (14,15). This suggests that although SRP9/14 is required for translation arrest, the Alu portion of SRP RNA may play a direct role in this activity.

Despite efforts to dissect SRP function, the function of the RNA moiety of the particle remains obscure. Although the overall secondary structure of SRP RNA appears to be highly conserved (13), very few highly conserved nucleotides or sequence motifs have been identified. One sequence motif appears to have been conserved at least in part to allow binding of SRP54 (16). Another conserved sequence motif, referred to as SRP9/14 binding site IIB, is found in the Alu domain of SRP RNAs in bacteria, yeast, plants and animals (6,17). In mammalian SRP, the central part of

*To whom correspondence should be addressed at: Building 6, Room 416, 9000 Rockville Pike, Bethesda, MD 20892-2753, USA. Tel: +1 301 402 3567; Fax: +1 301 480 9354; Email: maraia@ncbi.nlm.nih.gov

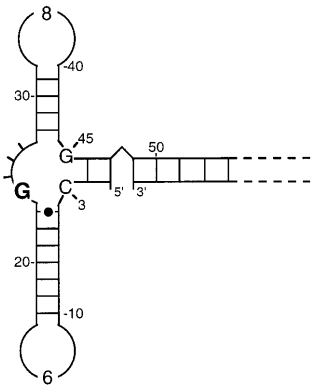

Figure 1. Secondary structure model of the region of the Alu-homologous domain of 7SL RNA studied in this report. This structure is supported by phylogenetic comparative analysis (13). The filled circle represents a G4–U23 base pair that is phylogenetically conserved. The guanosine at position 24 (G24) is indicated in large bold font. The positions of C3 and G45 are also indicated.

this site lies between two hairpins as a single-stranded region that is reminiscent of the anticodon loop of tRNA (Fig. 1; 11–13). The most highly conserved residue in this site is located at position 24 of human 7SL RNA. Guanosine is found at this position in ~95% of the sequences in the SRP RNA database, with adenosine found in most of the others (13).

The high degree of conservation of G24 suggests that it plays an important role in SRP structure or function. Consistent with this hypothesis, mutation of the G4 residue of *Schizosaccharomyces pombe* SRP RNA which is equivalent to mammalian G24 produces a conditional growth defect (18,19). There are two possible mechanisms by which G24 might participate in the translation arrest function. One possibility is that although SRP9/14 makes a large number of contacts with the cruciform structure of the Alu domain (17), G24 might be particularly important for binding. Alternatively, the G24 nucleotide of 7SL RNA may play a direct role in translation arrest by interacting with the ribosome. Previous studies have not distinguished clearly between these two possibilities. They have raised doubts, however, about the significance of G24 in SRP9/14 binding. Experiments with chemical probes indicate that U23–G24–U25 is not protected by SRP9/14 (20) and therefore suggest the possibility that G24 may be available in some stages of the SRP cycle for interaction with the ribosome.

Elucidation of the role of G24 in SRP function is also of interest in light of the fact that it is replaced by a U in the closely related cytoplasmic RNA scB1, found in rodent cells (the human homolog scAlu retains G). ScB1 and scAlu RNAs are ~125 nt and exhibit high affinity for SRP9/14 and secondary structures nearly indistinguishable from the Alu domain of 7SL RNA. The SRP9/14 heterodimer is normally produced at a level that far exceeds the levels of 7SL RNA and other SRP subunits in human cells (21,22) and an increase in the level of SRP9/14 leads to a corresponding increase in the levels of scB1 and scAlu RNAs (23,24), suggesting concentration-dependent interaction *in vivo*. Because scB1 and scAlu RNAs bind SRP9/14 but do not contain binding sites for the other SRP proteins, it has been proposed that they may play a role in translational control distinct from SRP-mediated elongation arrest. If the G24 residue of SRP RNA influences the translational machinery directly, however, then this hypothesis is probably incorrect.

In order to address these questions we produced mutant SRP RNAs that contain substitutions at position 24. The results show clearly that G24 is not directly involved in the translation arrest function of SRP. Rather, the results demonstrate that G24 is a strong determinant of the binding between SRP RNA and SRP9/14 and this accounts for its function in translation arrest. The results also demonstrate that SRP RNAs substituted with residues other than G at position 24 function as well as the wild-type RNA.

MATERIALS AND METHODS

Synthesis of 7SL RNA molecules with single base substitutions

Oligonucleotide primers containing the promoter for T7 RNA polymerase precisely juxtaposed to the first 31 nt of the human 7SL RNA gene *7L30.1* (30) or scB1 were used in standard PCR amplification reactions to produce templates for transcription of full-length 7SL RNA and scB1 RNA as described (25). Mutagenic primers contained an A, T or C instead of a G at position 24 of the 7SL RNA gene and created or destroyed a diagnostic restriction site. After purification by phenol/chloroform extraction and ethanol precipitation, the PCR products containing G24 or point mutations were used directly for T7 polymerase-directed RNA synthesis as described (25). Free NTPs were removed by gel filtration using High Capacity Quick Spin RNA Columns (Boehringer Mannheim) according to the manufacturer's instructions. RNA was further purified by phenol/chloroform extraction followed by ethanol precipitation and was stored at –20°C as a precipitate until just before use.

Measurement of SRP9/14 binding to Alu RNA

RNA electrophoretic mobility shift assays (EMSA) using the 145 nt Alu domain of 7SL RNA and highly purified human SRP9/14 have been described previously (28). Templates for the 145 nt Alu domain of 7SL RNA were constructed by PCR amplification of an S domain deletion mutant of the 7SL RNA gene previously used for SRP9/14 binding studies (28,30). All templates were transcribed by T7 RNA polymerase (Promega) in parallel reactions that contained a pre-mixed solution of NTPs including [α-^{32}P]GTP to ensure that each RNA was labeled to the same specific activity (25). All EMSA reactions contained 10 ng poly(G), which was used as a non-specific competitor (28). Quantitation was performed using a PhosphorImager and accompanying ImageQuant software (Molecular Dynamics).

Assembly and purification of SRPs

SRP protein subunits were purified from a high salt extract of canine pancreatic rough microsomes as described (31). To reconstitute SRPs, the proteins were added at a final concentration of 2 μM together with 6 μM synthetic human 7SL RNA into a buffer containing 50 mM HEPES, pH 7.5, 500 mM potassium acetate, 5.5 mM magnesium acetate, 0.5 mM EDTA, 1 mM DTT and 0.01% (w/v) Nikkol (octaethyleneglycol mono-*N*-dodecylether; Nikko Chemical Corp., Tokyo, Japan). Whereas all components are typically added at equimolar concentrations (32,33), we used an excess of synthetic RNA because it assembles into particles less efficiently than native RNA. Reconstitution reactions were

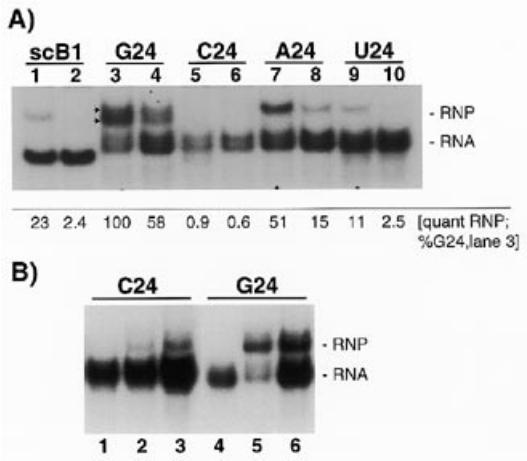

Figure 2. Analysis of SRP9/14 binding to 7SL-Alu RNAs by [^{32}P]RNA electrophoretic mobility shift assays. (**A**) 1 ng scB1 or 7SL-Alu [^{32}P]RNAs were incubated with 1.33 ng human SRP9/14 and 10 ng poly(rG) in a reaction volume of 13.5 µl and examined after electrophoresis on a native 6% polyacrylamide gel. Unlabelled scB1 RNA (10 ng) was added as specific competitor to the reactions in the even numbered lanes. The [^{32}P]RNAs used in each reaction are indicated above the lanes. The mobilities of the free RNAs and the RNA–protein (RNP) complexes are indicated on the right. The 135 nt scB1 RNA migrates faster than the 145 nt 7SL-Alu RNA. Two electrophoretic forms of 7SL-Alu G24 RNP complexes are indicated by arrows (lanes 3 and 4; see text). Numbers below the lanes indicate quantitation of the RNP bands in percentages relative to the G24 RNP in lane 3. (**B**) As in (A) except that 1 ng (lanes 1, 2, 4 and 5) or 3.3 ng (lanes 3 and 6) of the 7SL-Alu [^{32}P]RNAs indicated above the lanes were incubated with SRP9/14 and 10 ng poly(rG) in a reaction volume of 9.6 µl. Lanes 1 and 4 contained no added protein.

incubated on ice for 10 min and then at 37°C for an additional 10 min. Subsequent purification steps were performed at 4°C. Intact SRP particles were purified away from free subunits and incomplete particles by spin filter chromatography using Ultrafree-MC DEAE anion exchange membrane units (Millipore). Typical 50 µl reconstitution reactions were first diluted with 150 µl 50 mM HEPES, pH 7.5, 10% (v/v) glycerol, 0.5 mM EDTA, 1 mM DTT, 0.01% Nikkol (UDEAE buffer) containing 500 mM potassium acetate and 5.5 mM magnesium acetate and then with 200 µl 0.5 mM magnesium acetate in UDEAE buffer to yield final potassium acetate and magnesium acetate concentrations of 250 and 3 mM respectively. The samples were then centrifuged for 2 min at 16 000 g_{max} to remove any insoluble material. Supernatants were loaded onto DEAE filter units pre-equilibrated with 250 mM potassium acetate and 3 mM magnesium acetate in UDEAE buffer and centrifuged at 4500 g_{max} for ~5 min until all the solution had passed through the membranes. The membranes were then washed by repeating the centrifugation with 400 µl UDEAE buffer containing 350 mM potassium acetate and 4 mM magnesium acetate. Intact SRPs were eluted by centrifuging 30 µl UDEAE buffer containing 600 mM potassium acetate and 6.5 mM magnesium acetate through the membranes twice. Nearly all the SRP eluted in the first step. Incomplete SRPs remained bound to the membranes under these elution conditions. The concentration of SRPs was determined by comparison of SRP68 staining intensity with that of a bovine serum albumin standard curve on a Coomassie Brilliant Blue stained SDS–PAGE gel. The stoichiometry of SRP9 and SRP14 in reconstituted SRPs was verified by examination of silver stained SDS–PAGE gels (34).

SRP activity assays

Translation arrest and translocation assays were performed as described (31,35) with the following minor modifications. CyclinΔ90 and preprolactin capped mRNAs were used at final concentrations of 3 and 2 µg/ml respectively. Wheat germ translation reactions were precipitated with 10% (w/v) trichloroacetic acid, re-dissolved with SDS sample buffer (5% w/v SDS, 105 mM Tris–HCl, pH 8.2, 12.5% v/v glycerol, 1 mM EDTA, 100 mM DTT), heated to 65°C for 30 min and analyzed by electrophoresis on 14% acrylamide Tris–glycine SDS–PAGE gels. After the gels were fixed and dried, translation products were quantitated using a Fuji BAS2000 PhosphorImager. Translation arrest activity was calculated as described (36) except that cyclinΔ90 (37) was used instead of globin as the non-secretory control. Percent processing was calculated by a method similar to that described previously (36) except that any variations in sample loading were corrected by normalizing the radioactivity in the preprolactin and prolactin bands to that of the cyclinΔ90 band in the same lane.

RESULTS

Effects of point mutations at position 24 of 7SL RNA on SRP9/14 binding

The EMSA was previously shown to be useful for monitoring relative affinities of SRP9/14 for the Alu domain of 7SL RNA and Alu-related RNAs such as small cytoplasmic (sc) B1 RNA (28). The equilibrium K_d value of 2×10^{-10} M for the interaction between SRP9/14 and 7SL-Alu RNA determined using this assay agreed with the value determined in solution by others (38). The affinity of SRP9/14 for scB1 RNA was previously found to be between 5- and 10-fold lower than for 7SL-Alu RNA (28). To determine the relative affinities of SRP9/14 for the Alu domain of 7SL RNAs containing different nucleotides at position 24, we synthesized ^{32}P-labeled transcripts of the Alu region of 7SL (hereafter referred to as 7SL-Alu; see Materials and Methods) which varied only at this position.

In the experiment shown in Figure 2A, purified scB1 [^{32}P]RNA and 7SL-Alu [^{32}P]RNAs were incubated with SRP9/14 alone (odd numbered lanes) or with SRP9/14 plus non-radioactive scB1 RNA competitor (even numbered lanes) and binding was analyzed by EMSA. PhosphorImager quantitation of the radioactivity in the RNP bands is shown below the lanes of Figure 2A and is expressed as a percentage of the radioactivity contained in the wild-type 7SL G24 RNP band in lane 3. SRP9/14 exhibited the highest affinity for wild-type (G24) 7SL-Alu RNA (lane 3), followed by A24 (lane 7), U24 (lane 9) and C24 (lane 5). SRP9/14 exhibited 2- and 5-fold lower affinities for 7SL-Alu A24 and 7SL-Alu U24 RNAs respectively. 7SL-Alu U24 RNA and scB1 RNA exhibited similar binding affinity (compare lanes 1 and 9), probably because scB1 RNA contains a U at the analogous position (25,28). The order of relative affinities of SRP9/14 for 7SL RNAs containing substitutions at position 24 determined here correlate with the frequency with which the individual bases are found in the SRP RNA database (13; Zwieb at http://pegasus.uthct.edu/SRPDB/SRPDB.html).

The relative affinities reported above were supported by the results obtained with scB1 RNA, which was used as a reference to calibrate binding affinity. Comparison of lanes 1 and 3 revealed that ~5-fold fewer SRP9/14-containing RNP complexes formed

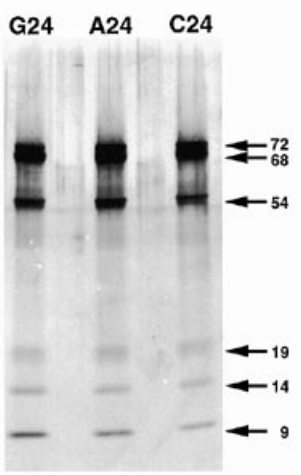

Figure 3. Reconstitution of SRP with 7SL RNAs that contain substitutions at position 24. SRPs were assembled using 7SL RNA that contained the nucleotide at position 24 indicated above each lane. After purification of particles, SRP polypeptide subunits were analyzed on a 10–20% acrylamide Tris–tricine SDS–PAGE gel and silver stained. Identities of the SRP polypeptide subunits are indicated on the right.

with scB1 [^{32}P]RNA than with 7SL G24 [^{32}P]RNA. This result demonstrates that this assay accurately reflects differences in binding affinity, since it was previously determined that SRP9/14 exhibits a 4- to 9-fold lower affinity for scB1 than for 7SL-Alu RNA (28). We also used unlabeled scB1 RNA as a competitor (even numbered lanes). Unlabeled scB1 RNA did not compete as well with 7SL G24 [^{32}P]RNA (lane 4) or 7SL A24 [^{32}P]RNA (lane 8) as it did with scB1 [^{32}P]RNA (lane 2), while it competed well with 7SL U24 [^{32}P]RNA (lane 10). These results agree with the relative affinities of wild-type 7SL-Alu RNA and scB1 RNA for SRP9/14 determined previously (28).

Although 7SL-Alu RNA substituted with C24 formed virtually no detectable SRP9/14 RNP complexes in the experiment shown in Figure 2A, several lines of evidence indicate that this RNA can associate with SRP9/14. In some experiments, 1–2% of the amount of the G24 RNP was observed using the same conditions (data not shown). On the basis of these results, which reflect equilibrium binding constants, the G24→C mutation must reduce the affinity of SRP9/14 for 7SL-Alu RNA at least 50-fold. In addition, the observation that in these experiments much of the radiolabeled 7SL-Alu C24 RNA reproducibly produced a smear on acrylamide gels (data not shown) suggests that weak complexes with SRP9/14 formed but then dissociated during electrophoresis (39). We could also demonstrate 7SL-Alu C24 RNA binding to SRP9/14 by increasing the concentration of 7SL-Alu [^{32}P]RNA, as shown in Figure 2B. Under these conditions, 7SL-Alu G24 RNA efficiently bound SRP9/14, while the C24 RNA bound <50% of the SRP9/14 (compare intensity of RNP bands in lanes 3 and 6). Thus, although this experiment does not allow determination of an equilibrium K_d, the results nonetheless demonstrate that 7SL RNA containing the C24 mutation can bind SRP9/14.

We often observed that 7SL-Alu G24 RNA when bound to SRP9/14 yielded two electrophoretically distinct complexes (Fig. 2A, lanes 3 and 4), whereas the other RNAs yielded only one RNP complex (lanes 7–10). With regard to this observation and the high degree of conservation of G24, the following considerations are noteworthy. Structure predictions derived from RNA minimal free energy calculations (40) indicated that: (i) 7SL-Alu G24 RNA exhibited the lowest free energy among the four RNAs differing at position 24; (ii) G24 was base paired with C3. This base pair could not form in any of the mutant RNAs; instead, C3 was base paired with G45 in each of the mutants, the latter of which remained unpaired in the wild-type RNA. Thus it is conceivable that the Alu domain of 7SL RNA may exist in two forms when bound to SRP9/14. In one form, G24 is base paired with C3; this leaves G45 unpaired, as in the predicted minimal free energy structure. In the other form, G24 is unpaired while C3 is base paired with G45, as in the phylogenetically determined structure represented in Figure 1. SRP RNAs exhibit a very high degree of conservation at each of these three positions (13; Zwieb at http://pegasus.uthct.edu/SRPDB/SRPDB.html; K.Hsu and R.Maraia, unpublished observation). This observation, in conjunction with the data in Figure 2A, suggests that the three residues may be coordinately conserved to preserve the ability of 7SL RNA to switch between the two isoforms.

Reconstitution of SRPs containing 7SL RNAs substituted at position 24

The observation that introduction of mutations at position 24 of 7SL RNA reduced, but did not abolish, binding of SRP9/14 implies that reconstitution of SRPs with mutant RNAs should be possible provided that subunit concentrations are sufficiently high. SRP reconstitutions are typically performed with subunit concentrations in the 1 µM range, which allows addition of reconstituted particles to biochemical reactions at physiological concentrations (~10 nM) (41). Because the K_d for the interaction between SRP9/14 and 7SL RNA is 5000-fold lower than the concentration of subunits in the reconstitution reactions, we reasoned that even the most severe mutation at position 24 (G→C), which raises the K_d to ~10^{-8} M, would still be compatible with SRP assembly.

To determine whether SRP RNAs that contain substitutions at position 24 could be used to assemble complete SRPs, we synthesized full-length 7SL RNAs containing G24, A24 and C24 and used them in reconstitution reactions (see Materials and Methods). Following incubation of SRP proteins with the 7SL RNAs, assembled SRPs were isolated by DEAE chromatography. Equal portions of the samples were analyzed by SDS–PAGE followed by silver staining (Fig. 3). The observation that similar amounts of each SRP polypeptide were present in each sample indicates that wild-type and mutant RNAs assembled equally efficiently into complete particles. Hence, the mutations in SRP RNA at position 24 did not prevent binding of SRP9/14 (or any other subunit) to the RNA under standard reconstitution conditions. It is particularly noteworthy that SRP reconstituted with 7SL C24 RNA contains nearly as much SRP9 and SRP14 as SRP reconstituted with wild-type RNA. Thus once the mutant SRPs were assembled, they were sufficiently stable to be recovered after purification with minimal loss of SRP9/14.

Analysis of activity of mutant SRPs

To test for activity, reconstituted SRPs were added to wheat germ *in vitro* translation reactions and translation arrest activity was measured by specific inhibition of preprolactin synthesis (36). The concentration of SRP required to cause 50% translation arrest can be used to define relative activity (31). By this criterion,

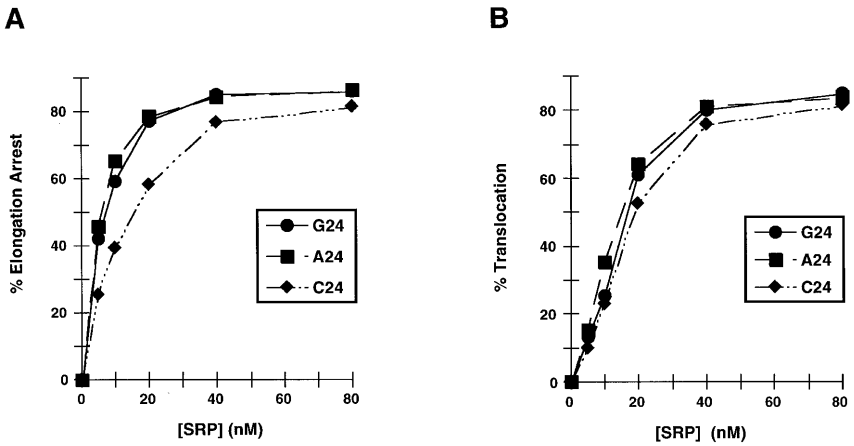

Figure 4. (**A**) Translation arrest and (**B**) translocation activity of SRPs reconstituted with G24 (●), A24 (■) or C24 (◆) 7SL RNAs. SRPs were added at varying concentrations to wheat germ translation reactions containing a mixture of preprolactin and cyclinΔ90 mRNAs. (A) Translation arrest activity at a given SRP concentration was measured as specific inhibition of preprolactin synthesis relative to that of a translation reaction containing no added SRP. (B) Translation reactions were supplemented with EDTA and salt-washed canine pancreatic rough microsomes. Translocation activity was determined by measuring the percentage of preprolactin that was processed to prolactin.

purified SRP reconstituted with wild-type synthetic 7SL RNA supports the same level of translation arrest activity (Fig. 4A) as SRP reconstituted with native RNA (31). Furthermore, an SRP containing 7SL A24 RNA exhibited translation arrest activity that was nearly indistinguishable from wild-type (Fig. 4A). SRP containing 7SL C24 RNA, however, was ~2-fold less active than the other two SRPs. This reduction in translation arrest activity may be explained by the fact that binding of SRP9/14 to the 7SL C24 RNA was slightly less efficient (compare Fig. 3, lanes G24 and C24) and that a small amount of SRP9/14 might have dissociated from the mutant RNA during the assay. In any case, the effect of the G24→C mutation on translation arrest activity was extremely small compared with the effect this mutation had on binding of SRP9/14.

Previous work has shown that the Alu domain does not play a role in the protein translocation activity of SRP (11). Hence, we would expect that mutations at nucleotide 24 of 7SL RNA would not affect protein translocation except in the unlikely case that they produce a global disruption of RNA structure that interferes with the function of the S domain of SRP. To rule out this possibility, we added pancreatic microsomes to *in vitro* translation reactions and monitored translocation by measuring the conversion of preprolactin to the mature prolactin form. As expected, SRPs containing either 7SL A24 or C24 RNA exhibited translocation activities that were nearly identical to that of SRP containing wild-type 7SL G24 RNA (Fig. 4B). This result demonstrates that G24A and G24C are not gain-of-function mutations that affect the protein translocation activity of SRP.

DISCUSSION

In this study we have explained the functional significance of one of the few nucleotides in SRP RNA that is highly conserved throughout evolution. Given that the Alu domain is known to interact with the translation machinery to produce an inhibition of polypeptide elongation, a reasonable hypothesis is that the conserved G24 residue participates in a key interaction between the ribosome and SRP. Our results demonstrate clearly, however, that whereas substitution of A or C in this position significantly reduces binding of SRP9/14, the mutations have little or no effect on the ability of SRP to mediate translation arrest. Introduction of a C at position 24 of 7SL-Alu RNA reduced the equilibrium binding affinity for SRP9/14 by at least 50-fold, as determined by our assay, but 7SL RNA with this mutation nonetheless assembled into a functional SRP provided that subunits were present at high concentrations. The relatively small (<2-fold) decrease in translation arrest observed in experiments with the C24 mutant can probably be attributed to a slight reduction in the amount of SRP9/14 successfully bound to the particle or to a slight loss of SRP9/14 during the experiment. Thus, the data are most consistent with the interpretation that the role of G24 in 7SL RNA is to promote efficient binding to SRP9/14.

One interesting question that arises from this work is whether G24 facilitates binding of SRP9/14 by directly interacting with the proteins or by promoting a folded structure of the RNA that the proteins recognize. The report that G24 was not observed to be a point of contact in chemical protection studies supports the latter hypothesis (20). The observation of a fast-migrating form of the 7SL RNA–SRP9/14 RNP complex when G was present at position 24 (Fig. 2A) suggests that this residue may play a role in the overall structure of the Alu domain. Although the G4 residue in *S.pombe* SRP RNA that is equivalent to G24 resides in a loop that is part of a single hairpin instead of an internal loop between two hairpins, genetic analysis is consistent with the notion that it contributes significantly to the structure of the Alu domain (18). In any case, it is noteworthy that this single nucleotide appears to exert a very significant influence over the binding of SRP9/14, despite evidence that a large number of contacts are made between the RNA and protein heterodimer (17).

The data presented here, together with results from studies on domain IV of SRP RNA (16), indicate that the highly conserved residues in SRP RNA that have been studied thus far are required for protein binding and not for interaction with ribosomes. Indeed, previous work suggests that the interaction between SRP and the ribosome is complex and may require the participation of all the subunits (42). Consistent with this notion, a 7SL-Alu-SRP9/14 RNP fragment of SRP is unable to compete with SRP in translation arrest assays (11). Given that 7SL RNA appears to

undergo conformational changes in different phases of the SRP cycle (20), one of its functions may be to allow the protein subunits to change their relative position with respect to one another. The proteins may have evolved as part of an RNP, so that in bacteria which have lost a significant portion of the primordial SRP, the remaining protein(s) must still bind to RNA to adopt a functional conformation.

Given that SRP9/14 binds to SRP RNA with a K_d of ~10^{-10} M and that the concentration of SRP subunits in the cell has been measured to be ~10^{-8} M (41), the mutation G24→C, which reduces equilibrium binding to $\leq 2 \times 10^{-8}$ M, is likely to impair function *in vivo*. However, an interesting puzzle is why G24 is so highly conserved if mutation to A or U reduces the affinity of the RNA for SRP9/14 by only a few-fold. The notion that U at position 24 is compatible with SRP9/14 binding is supported by the observation that scB1 and scAlu RNAs maintain stable association with SRP9/14 despite the fact that scB1 RNA harbors a U at the G24-homologous position (21,23,25,28). One explanation for the conservation of G24 is that 7SL RNA or SRP has an additional function *in vivo* that has not been measured in *in vitro* assays.

The observation that 7SL RNAs that contain substitutions at position 24 can assemble into functional particles despite a decreased affinity for SRP9/14 provides an explanation for previous findings that scB1 and scAlu RNAs increase in response to elevated intracellular SRP9/14 levels (23). Although these RNAs are present at only ~0.1% the level of 7SL RNA, the high concentration of SRP9/14 should drive the assembly of RNP complexes. Moreover, the observation that 7SL RNA containing U at position 24 is active in translation arrest is consistent with the possibility that scB1 and scAlu RNAs play a regulatory role in translation.

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REFERENCES

1 Walter,P. and Johnson,A.E. (1994) *Annu. Rev. Cell Biol.*, **10**, 87–119.
2 Görlich,D., Hartmann,E., Prehn,S. and Rapoport,T.A. (1992) *Nature*, **357**, 47–52.
3 Görlich,D. and Rapoport,T.A. (1993) *Cell*, **75**, 615–630.
4 Poritz,M.A., Bernstein,H.D., Strub,K., Zopf,D., Wilhelm,H. and Walter,P. (1989) *Science*, **250**, 1111–1117.
5 Ribes,V., Römisch,K., Giner,A., Dobberstein,B. and Tollervey,D. (1990) *Cell*, **63**, 591–600.
6 Althoff,S., Selinger,D. and Wise,J.A. (1994) *Nucleic Acids Res.*, **22**, 1933–1947.
7 Schatz,G. and Dobberstein,B. (1996) *Science*, **271**, 1519–1526.
8 Siegel,V. and Walter,P. (1988) *Cell*, **52**, 39–49.
9 Kurzchalia,T.W., Wiedmann,M., Girshovich,A.S., Bochkareva,E.S., Bielka,H. and Rapoport,T.A. (1986) *Nature*, **320**, 634–636.
10 Krieg,U.C., Walter,P. and Johnson,A.E. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 8604–8608.
11 Siegel,V. and Walter,P. (1986) *Nature*, **320**, 81–84.
12 Gundelfinger,E.D., Krause,E., Melli,M. and Dobberstein,B. (1983) *Nucleic Acids Res.*, **11**, 7363–7374.
13 Larsen,N. and Zwieb,C. (1991) *Nucleic Acids Res.*, **19**, 209–215.
14 Siegel,V. and Walter,P. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 1801–1805.
15 Walter,P. and Lingappa,V.R. (1986) *Annu. Rev. Cell Biol.*, **2**, 499–516.
16 Wood,H., Luirink,J. and Tollervey,D. (1992) *Nucleic Acids Res.*, **20**, 5919–5925.
17 Strub,K., Moss,J.B. and Walter,P. (1991) *Mol. Cell. Biol.*, **11**, 3949–3959.
18 Liao,X., Selinger,D., Althoff,S., Chiang,A., Hamilton,D., Ma,M. and Wise,J.A. (1992) *Nucleic Acids. Res.*, **20**, 1607–1615.
19 Selinger,D., Brenwald,P., Althoff,S., Reich,C., Hann,B., Walter,P. and Wise,J.A. (1994) *Nucleic Acids Res*, **22**, 2557–2567.
20 Andreazzoli,M. and Gerbi,S.A. (1991) *EMBO J.*, **10**, 767–777.
21 Bovia,F., Fornallaz,M., Leffers,H. and Strub,K. (1995) *Mol. Biol. Cell*, **6**, 471–484.
22 Chang,D.Y., Sasaki-Tozawa,N., Green,L.K. and Maraia,R.J. (1995) *Mol. Cell. Biol.*, **15**, 2109–2116.
23 Chang,D.Y., Nelson,B., Bilyeu,T., Hsu,K., Darlington,G.J. and Maraia,R.J. (1994) *Mol. Cell. Biol.*, **14**, 3949–3959.
24 Chang,D.Y., Hsu,K. and Maraia,R.J. (1996) *Nucleic Acids Res.*, **24**, 4165–4170.
25 Maraia,R. (1991) *Nucleic Acids Res.*, **19**, 5695–5702.
26 Maraia,R.J., Driscoll,C., Bilyeu,T., Hsu,K. and Darlington,G.J. (1993) *Mol. Cell. Biol.*, **13**, 4233–4241.
27 Sinnett,D., Richer,C., Deragon,J.-M. and Labuda,D. (1991) *J. Biol. Chem.*, **266**, 8675–8678.
28 Hsu,K., Chang,D.Y. and Maraia,R.J. (1995) *J. Biol. Chem.*, **270**, 10179–10186.
29 Chang,D.-Y. and Maraia,R.J. (1993) *J. Biol. Chem.*, **268**, 6423–6428.
30 Ullu,E. and Weiner,A.M. (1985) *Nature*, **318**, 371–374.
31 Zopf,D., Bernstein,H.D. and Walter,P. (1993) *J. Cell Biol.*, **120**, 1113–1121.
32 Walter,P. and Blobel,G. (1983) *Cell*, **34**, 525–533.
33 Walter,P. and Blobel,G. (1983) *J. Cell Biol.*, **97**, 1693–1699.
34 Morrisey,J.H. (1981) *Anal. Biochem*, **117**, 307–310.
35 Strub,K. and Walter,P. (1990) *Mol. Cell. Biol.*, **10**, 777–784.
36 Siegel,V. and Walter,P. (1985) *J. Cell Biol.*, **100**, 1913–1921.
37 Murray,A.W., Solomon,M.J. and Kirschner,M.W. (1989) *Nature*, **339**, 280–286.
38 Janiak,F., Walter,P. and Johnson,A.E. (1992) *Biochemistry*, **31**, 5830–5840.
39 Carey,J. (1991) In Sauer,T.R. (ed.), *Methods in Enzymology.* Academic Press, San Diego, Vol. 208, pp. 103–118.
40 Jaeger,J.A., Turner,D.H. and Zuker,M. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 7706–7710.
41 Siegel,V. and Walter,P. (1988) *EMBO J.*, **7**, 1769–75.
42 Powers,T. and Walter,P. (1996) *Curr. Biol.*, **6**, 331–338.