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Abstract

The signal recognition particle (SRP) is a conserved ribonucleoprotein particle that targets membrane and secreted proteins to translocation channels in membranes. In eukaryotes, the Alu domain, which comprises the 5' and 3' extremities of the SRP RNA bound to the SRP9/14 heterodimer, is thought to interact with the ribosome to pause translation elongation during membrane docking. We present the 3.2 Å resolution crystal structure of a chimeric Alu domain, comprising Alu RNA from the archaeon Pyrococcus horikoshii bound to the human Alu binding proteins SRP9/14. The structure reveals how intricate tertiary interactions stabilize the RNA 5' domain structure and how an extra, archaeal-specific, terminal stem helps constrain the Alu RNA into the active closed conformation. In this conformation, highly conserved noncanonical base pairs allow unusually tight side-by-side packing of 5' and 3' RNA stems within the SRP9/14 RNA binding surface. The biological relevance of this structure is confirmed by showing that a reconstituted full-length chimeric archaeal-human SRP is competent to elicit elongation arrest in vitro. The [...]
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Crystal structure of a signal recognition particle Alu domain in the elongation arrest conformation

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ABSTRACT

The signal recognition particle (SRP) is a conserved ribonucleoprotein particle that targets membrane and secreted proteins to translocation channels in membranes. In eukaryotes, the Alu domain, which comprises the 5′ and 3′ extremities of the SRP RNA bound to the SRP9/14 heterodimer, is thought to interact with the ribosome to pause translation elongation during membrane docking. We present the 3.2 Å resolution crystal structure of a chimeric Alu domain, comprising Alu RNA from the archaean Pyrococcus horikoshii bound to the human Alu binding proteins SRP9/14. The structure reveals how intricate tertiary interactions stabilize the RNA 5′ domain structure and how an extra, archaeal-specific, terminal stem helps constrain the Alu RNA into the active closed conformation. In this conformation, highly conserved noncanonical base pairs allow unusually tight side-by-side packing of 5′ and 3′ RNA stems within the SRP9/14 RNA binding surface. The biological relevance of this structure is confirmed by showing that a reconstituted full-length chimeric archaeal-human SRP is competent to elicit elongation arrest in vitro. The structure will be useful in refining our understanding of how the SRP Alu domain interacts with the ribosome.

Keywords: signal recognition particle; translation; X-ray crystallography; RNA; RNA folding

INTRODUCTION

In eukaryotic cells, secretory and membrane proteins are targeted to the endoplasmic reticulum (ER) by the universally conserved ribonucleoprotein particle SRP and its membrane-associated receptor (SRP receptor [SR] or docking protein) (for review, see Saraogi and Shan 2011; Nyathi et al. 2013). A nascent polypeptide chain emerging from a translating ribosome with an N-terminal hydrophobic signal sequence, the hallmark of ER-targeted proteins, is recognized by the SRP54 component of SRP and targeted to the ER via the interaction of SRP and SR. This process is controlled by GTP-binding and hydrolysis by SRP54 and SR leading to the transfer of the nascent chain to the translocation channel. The protein then integrates into or transfers across the membrane in a cotranslational manner. During targeting, the mammalian SRP delays nascent chain elongation, which ensures the efficient delivery of the nascent chain to the ER in human cells (Lakkaraju et al. 2008).

Eukaryotic SRPs possess a composite structure, comprising the universally conserved S-domain and the Alu domain that is found in eukaryotes, archaea, and some bacteria (Walter and Blobel 1980). Human SRP exemplifies a typical eukaryotic particle and consists of six proteins (Walter and Blobel 1980), which bind to the functionally essential 7SL RNA (human SRP RNA) of 300 nt. The signal recognition and targeting functions are assigned to the S-domain; whereas, the Alu domain harvests the elongation arrest function (Siegel and Walter 1988). The Alu domain includes the 5′ and 3′ extremities of 7SL RNA, forming the Alu RNA, bound to the SRP9/14 heterodimer (Fig. 1A). Our previous structural studies revealed that human SRP9 and SRP14 are structurally homologous (Birse et al. 1997) and together form a six-stranded β-sheet with a concave surface which is the major binding site for Alu RNA (Weichenrieder et al. 2000).

In most Alu domains, the 5′ and 3′ portions are linked by a single-stranded hinge region (Fig. 1A,B), but are otherwise mobile with respect to each other (Weichenrieder et al. 2000).
In previous work, we only succeeded in crystallizing this structure by restricting its flexibility, which was done by producing an artificial, circularly permuted \textit{Alu} RNA with a rigid linkage (Weichenrieder et al. 2000, 2001). The resulting \textit{Alu} domain structure was a domain-swapped dimer with each RNA in the extended, “open” conformation and each SRP9/14 heterodimer bound to two sites, consistent with those mapped by hydroxyl radical cleavage experiments (Strub et al. 1991), but on different adjacent RNA molecules (Fig. 1C; Weichenrieder et al. 2000). The likely monomeric physiological structure was inferred from the crystallographic model by proposing that the \textit{Alu} RNA must fold back on to itself such that one SRP9/14 heterodimer interacts with a single RNA molecule, in a “closed” conformation (Fig. 1D; Weichenrieder et al. 2000, 2001). This model was later confirmed by modeling into the low-resolution cryo-electron microscopy map of the entire mammalian SRP–ribosome complex (Halic et al. 2004). However, a high-resolution structure of the physiological, closed form the \textit{Alu} domain is still lacking.

The elongation arrest activity of the \textit{Alu} domain primarily involves SRP9/14. Cell-free translation and translocation assays and in vivo studies in human cells revealed a stretch of basic amino acid residues in SRP9 and at the C-terminus of SRP14 to be essential for elongation arrest activity (Thomas et al. 1997; Lakkaraju et al. 2008; Mary et al. 2010). To better understand how the SRP \textit{Alu} domain interacts with the ribosome and to elucidate the mechanism of elongation arrest, a more precise model of the closed conformation \textit{Alu} domain structure is required. As the human \textit{Alu} domain complex was resistant to crystallization, we switched to work with the \textit{Alu} domain of the archaeon \textit{Pyrococcus horikoshii}. Archaeal SRP RNAs possess both \textit{Alu} and \textit{S}-domains, but protein homologues of only SRP19 and SRP54 have been identified, and very little is known about protein translocation in archaeal species (Fig. 1E; Zwieb and Bhuiyan 2010). Archaeal SRP RNAs have extra sequences at their 5′ and 3′ ends, which are predicted to form an additional helix (H1) in the \textit{Alu} domain (Fig. 1E; Larsen and Zwieb 1991). This would create a three-way junction comprising H1, H2, and H5 (Fig. 1F), which we hypothesized might stabilize the closed conformation of the archaeal \textit{Alu} domain. A similar structure is also predicted to exist in certain eubacteria which possess an \textit{Alu} domain, such as \textit{Bacillus subtilis} (Poritz et al. 1988).

Here, we describe the crystal structure of a chimeric \textit{Alu} domain comprising the \textit{P. horikoshii} \textit{Alu} RNA (\textit{PhAlu}) in complex with human SRP9/14. In this structure, the overall fold of the archaeal \textit{Alu} domain RNA and in particular the conserved tertiary contacts between the 5′ and 3′ domains are revealed in detail. Furthermore, we demonstrate that a reconstituted full-length chimeric SRP is active in elongation arrest assays suggesting that the new structure will be a robust model for the human \textit{Alu} domain.

**RESULTS**

Structure determination of the \textit{P. horikoshii} \textit{Alu} domain with human SRP9/14

For structure determination, two \textit{PhAlu} domain RNAs, \textit{PhAlu110} (110 nt) and \textit{PhAlu134} (134 nt) which include...
different lengths of helix 5 closed by an artificial tetraloop were designed (Fig. 1F). These were synthesised by in vitro transcription and shown to form stable 1:1 complexes with human SRP9/14 (h9/14) by gel filtration (Supplemental Fig. 1). Crystallization trials of both RNAs alone and in complex with h9/14 were performed. However, differing crystals were only obtained of the RNP in each case. The structure of PhAlu134-h9/14 was solved by the MAD method using seleno-methionine labeled protein and refined to 3.20 Å resolution, with one complex per asymmetric unit. The structure of PhAlu110-h9/14 structure was subsequently solved by molecular replacement at 3.35 Å resolution and contains two complexes in the asymmetric unit. Most of the RNA and protein is well defined in the electron density (Supplemental Fig. 2) apart from the extra, distal section of helix 5 in PhAlu134, the C-terminal extremities of h9 (beyond Met83) and h14 (beyond Lys96) and the internal loop of h14 (43–49 have poor electron density). All three independent examples of the core of the PhAlu-h9/14 RNP are very similar (Supplemental Fig. 3) and so only the higher resolution PhAlu134-h9/14 structure is described in detail. Crystallographic details are given in Supplemental Tables 1 and 2.

**Structural features of the* P. horikoshii Alu**

**RNA 5′ domain**

The PhAlu domain RNAs are of approximate dimensions 112 × 38 × 30 Å (PhAlu134) and 87 × 38 × 30 Å (PhAlu110). The overall RNA fold resembles a shepherd’s crook, with the 3′ domain (helix H5) representing the staff and the 5′ domain its hook (Fig. 2A,B). As in the human Alu RNA, the PhAlu 5′ domain (stems 2, 3, and 4) forms a three-way τ-junction in which stems 2 and 4 are coaxially stacked and connected by a U-turn to stem 3 (Weichenrieder et al. 2000). The corresponding hairpin loops L3 and L4 interact through extensive base-pairing (Fig. 2A). Both τ-junction and helix 5 are accommodated within the concave β-sheet surface of h9/14 as predicted in the model for the active conformation of the human Alu domain.

The PhAlu RNA structure is crowned by a three-way junction formed by helices H1, H2, and H5 (hereafter referred to as J1/2/5), which has the form of an inverted tuning fork (Fig. 2A, C). Helix 1 comprises five base pairs from the 5′ and 3′ extremities of the PhAlu RNA and forms the fork handle. The junction orients the two arms of the tuning fork, helix H2, and helix H5 into a quasiparallel orientation as required for binding to SRP9/14. A buckled base-triple, formed by A68 interacting with the minor groove of the G13·C65 base pair, plays a central role in the three-way junction. The inflexible J1/2/5 junction constrains the RNA to be in the active conformation, rigidifies the junction and likely prevents rotation of helix 5 relative to the 5′ domain, a movement that was postulated to occur during assembly of the human Alu RNA in which the 5′ and 3′ ends are not base-paired (Weichenrieder et al. 2000, 2001).

Loops L3 and L4, which in PhAlu are, respectively, 7 and 11 nt long, are stabilised by interloop base pairs and additional buttressing interactions. Five G:C Watson–Crick base pairs between nucleotides 25–29 (CGGCCG) and 52–48 (GCCGCG) create a robust double helix that runs perpendicular to stems 3 and 4 (Fig. 2A,B). The G29·C48 end of this helix is extended by an additional noncanonical base pair between U30 and A46, C47 being flipped out. Sharp kinks in the RNA backbone at C48 and A53 of one strand and C25 of the other, bring neighboring nucleotides back behind the interacting loops such that stabilizing base triples and additional hydrogen bonds are formed. The flanking bases 22-GUU of loop 3 stack onto each other but are not base-paired. G22 makes a single hydrogen bond with U30 and U24 makes a U-turn interaction (hydrogen bond via N3) with the phosphate of G27, buttressing this region of the structure. Several unpaired nucleotides in L4 are involved in base-triples (Fig. 2D). A45 stacks on the noncanonical G57:A44 that forms the last base pair of stem 4 and makes a buckled triplet with the interloop Watson–Crick G29–C48 base pair. A55 binds to the minor groove of the loop–loop base pair G26·C51 and represents an archetypal A-minor motif (Nissen et al. 2001). A53, which stacks on A55, forms hydrogen bonds with the base pair C25·G52 via its Hoogsteen edge in a similar manner as seen in the A platform (Cate et al. 1996) and in the A–A sidestep (Conn et al. 1999) motifs. The intervening base U54 is flipped out, like U38 in the human Alu RNA domain structure and is completely exposed to solvent. The multiple canonical and noncanonical interactions between L3 and L4 create a particularly intricate and stable structure. Compared with the human Alu 5′ domain structure, where there are only three interloop base pairs and no base triplets, the extra structural buttressing of the PhAlu 5′ domain probably reflects the need for higher thermal stability in thermophilic organisms.

**Stem 5 structure and tertiary interactions between the 5′ and 3′ domains**

The PhAlu134 RNA comprises 30 base pairs (roughly half) of helix H5 and is capped by an artificial tetraloop. H5 is ∼90 Å in length and slightly bent (Fig. 2A,B). Superposition of the PhAlu110 and PhAlu134 RNAs shows that despite the high degree of similarity of the two RNAs, the direction of helix 5 diverges slightly after the τ tertiary interaction (Supplemental Fig. 3). This may be due to crystal packing but likely reflects flexibility in the structure at this point, which is approximately where bending of SRP RNA (hinge 2) was observed in a cryo-EM structure of mammalian SRP bound to the ribosome (Halic et al. 2004). The majority of helix H5 base pairs are Watson–Crick, but there are also three regions with noncanonical base pairs (boxed in Fig. 2A). Of these, the most significant are G78·A298 and C79·C297, which are at a position analogous to the first asymmetric internal loop found in helix H5 of human SRP RNA (Fig. 1B). The PhAlu RNA structure
confirms that this is the major site of contact between the 5′ and 3′ domains, as previously deduced from the low-resolution human Alu domain structure (Weichenrieder et al. 2000). Now the significantly improved resolution permits the precise nature of the interdomain contacts to be determined. Stem 3 of PhAlu RNA contains a conserved G17-U35 wobble base pair that is strictly conserved in most Alu RNAs (e.g., the human counterpart is G4·U23) (Fig. 1B). This base pair mediates the interaction of the 5′ domain with the noncanonical G78-A298 and C79-C297 base pairs resulting in a local distortion of the helix 5 structure (Fig. 2E).

The nitrogen N2 of G17 is exposed at the surface of the minor groove of helix 3 and forms a network of hydrogen bonds with the 2′ and 3′ ribose oxygen atoms as well as N3 of the base of A298 of helix 5 as another A-minor motif (Doherty et al. 2001). Both O2 and O2′ of U35 make additional hydrogen bonds with O2′ of A298. The consequence of this motif is to distort helix 5, flattening the curvature of the double helix and allowing close contact of the two stems (Fig. 2E). Interstem contacts are also made from residues G17–G19 and U34 of the 5′ domain, to atoms of C297 and C299 of the 3′ domain. We presume that a very similar stem–stem interaction occurs at this site in other SRPs.
contact can be made by the human \textit{Alu} RNA with the equivalent G4-U23 wobble base pair interacting with noncanonical base pairs of the first asymmetric loop (notably G57:A289), as suggested by the low resolution human \textit{Alu} domain structure (Weichenrieder et al. 2000). Therefore, we propose to extend the nomenclature of SRP RNAs (Zwieb et al. 2005) to take into account this additional conserved contact and suggest to refer to this tertiary interaction as “t3” (Fig. 2A).

\textbf{hSRP9/14 binding to PhAlu RNA}

Despite the evolutionary distance between the two organisms, it is remarkable that the hSRP9/14 proteins bind to the PhAlu RNA in an essentially identical manner to observed previously in the human \textit{Alu} domain structures (Fig. 3A; Weichenrieder et al. 2000). Indeed, superposition of the PhAlu RNA structure with the derived model of the active, closed form of the human \textit{Alu} domain RNA (Fig. 3B) shows that the conformation of the RNA core of the \textit{Alu} domain (both 5’ and 3’ domains) is well-predicted by our previous model (Fig. 1D) and strikingly well-conserved between very distant related species. Fifty-three bases can be structurally superposed in this way, of which 26 are identical, with an RMSD of the phosphate-ribose backbone atoms of 1.77 Å. The similarity of the RNA structure explains why the detailed protein–RNA interactions are also highly conserved. The β-sheet of hSRP9/14 contacts both the U-turn and the neighboring helices H3 and H4, with basic residues from hSRP14 providing the majority of these contacts, as previously described (Weichenrieder et al. 2000). Interestingly, the internal loop of hSRP14 (residues 34–54), which was disordered in previous human \textit{Alu} domain structures (i.e., in the absence of stem 5), is partially visible when bound to PhAlu RNA. It extends in the direction of the J1/2/5 junction with contacts to the U-turn nucleotide A38 and C65 of stem 2 (Fig. 3C). Specifically, Arg36 interacts with the phosphate of A38, the main chain of residues 37–39 stack on the base of A38 and Lys38 interacts with the phosphate of C65. These observations are consistent with previous work showing that the N-terminal part of the loop was the most critical for RNA binding (Bui et al. 1997).

Jointly, SRP9/14 bury \sim 1280 Å² of the surface area of the RNA, of which about two-thirds is by SRP14. Whereas SRP14 contacts only the 5’ domain, the contiguous β-sheet of SRP9 interacts equally with the 5’ and 3’ domains, clasping them together (Fig. 3C). While SRP9 strand β1 interacts with residues of the U-turn in the 5’ domain, strands β2 and β3 contact the minor groove of stem 5, as do residues Asp45 to Cys48 of the β2–β3 loop (Fig. 3D). A network of hydrogen bonds is central to this interaction, including an interaction of the carbonyl group of Leu46 with the 2’ hydroxyl group.

\begin{figure}[ht]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{SRP9/14 binding to PhAlu SRP RNA. (A) Overall structure of the chimeric \textit{Alu} domain RNP. The backbone of PhAlu134 RNA is drawn as a ribbon and colored purple and cyan for the 5’ and 3’ domains, respectively. The hSRP9 and hSRP14 are drawn as, respectively, red and green cartoons. SRP14 contacts exclusively the 5’ and 3’ domain whereas SRP9 contacts both RNA domains. The long internal loop, which extends toward the J1/2/5 junction, is shown dotted where poorly visible. The basic C-terminal extension of SRP14 extends toward the S-domain. (B) Comparison of PhAlu134 RNA with the human \textit{Alu} RNA structure model. A structural superposition of the human \textit{Alu} domain model, derived from the structure of a circularly permuted RNA (Weichenrieder et al. 2000) (yellow backbone with bases as sticks) onto PhAlu134 RNA (red) highlights the highly conserved tertiary fold of the \textit{Alu} RNA core from divergent organisms. Superposing just the 5’ domain (nucleotides 15–65 in PhAlu and 2–47 in human \textit{Alu} RNA) allows matching of 31 nt (19 identities) with an RMSD of 1.47 Å. For the 5’ and 3’ domain taken together, 53 bases can be matched (26 identities) with an RMSD of 1.77 Å. (C) Diagram showing how both the 5’ domain and 3’ stem of PhAlu134 RNA are accommodated side by side within the concave β-sheet surface of SRP9/14. (D) Protein–RNA interactions mediated by SRP9 at the t3 tertiary interaction. SRP9 (red with yellow side chains) interacts with nucleotides from both the \textit{PhAlu} 5’ (purple) and 3’ (cyan) domains thus reinforcing the t3 tertiary RNA interaction.}
\end{figure}
of G77. The side-chain of Lys52 is also engaged in hydrogen bonds to the phosphate G80 of helix 5. Interestingly, these SRP9–RNA contacts colocalize with the t3 tertiary RNA interaction, adding further stabilization.

**Functional replacement of the human Alu domain by the P. horikoshii Alu domain**

We next investigated whether the PhAlu domain could function in a similar manner to 7SL Alu domain. For this purpose, we constructed the PAHS RNA comprising the PhAlu 5’ domain (including a short stretch of nucleotides from the 3’ end) fused to the S-domain of human 7SL RNA (Supplemental Fig. 4A). We first examined whether such a chimeric SRP RNA was functionally active. Denaturing and native PAGE was used to compare the integrity and heterogeneity of human full-length synthetic 7SL (7SLC) (Mary et al. 2010) and PAHS RNAs, respectively (Fig. 4A). In addition to some heterogeneity observed for both RNAs in native PAGE, PAHS RNA migrated more slowly than 7SLC RNA in a distinct major band consistent with its larger size. 7SLC and PAHS RNAs were reconstituted into SRP using recombinant and native SRP proteins (Huck et al. 2004).

Reconstitution reactions (RCs) were directly added to wheat-germ lysate programmed for translation with preprolactin and cyclin mRNAs. The elongation arrest and translocation activities were assayed by determining the relative inhibition of preprolactin synthesis as compared with cyclin synthesis and by quantifying the processing of preprolactin to prolactin in the presence of microsomes. This heterologous system has been extensively used for the characterization of elongation arrest activity (Walter and Blobel 1981; Halic et al. 2004; Mary et al. 2010). The use of wheat-germ lysate is necessary because of the presence of endogenous SRP in the equivalent mammalian translation system. In this system, the elongation arrest activity of reconstituted particles is dependent of the presence of hSRP9/14 (Siegel and Walter 1985). More particularly, a conserved basic pentapeptide located in the C-terminal portion of SRP14 was shown to be required for SRP elongation arrest activity (Fig. 4B; Lakkaraju et al. 2008). PAHS RCs (gray) exhibited similar levels of activity as compared with 7SLC RCs (black) (Fig. 4C,D; Supplemental Table 3). Particularly, we observed that similarly to 7SLC RCs, the presence of h9/14 was required for PAHS-RCs to perform elongation arrest activity.

To further confirm that the mechanism of elongation arrest is the same, we tested both RNAs with previously characterized mutated SRP9/14s (Fig. 4D; Lakkaraju et al. 2008; Mary et al. 2010). The h14–A12 protein lacks the residues comprised between the lysine 95 and the alanine tail including the basic pentapeptide.

H14-100 is truncated after the basic pentapeptide. Again, PAHS-RCs behaved similarly to 7SLC-RCs showing that same amino acids were critical for elongation activity (Fig. 4C). The defects in elongation arrest were confirmed in translocation experiments. Particles reconstituted with both RNAs behaved similarly and the translocation efficiencies were reduced in the absence of elongation arrest activity (Fig. 4D).

**DISCUSSION**

Using an archaean Alu RNA, in which the presence of the J1/2/5 junction prevents the 180° rotation of the 5’ relative to the 3’ domain, we have succeeded in determining the structure of the closed, active conformation of the complex. This state was predicted from the crystallographic work on the human Alu domain (Weichenrieder et al. 2000) and confirmed by modeling into the low-resolution cryo-electron microscopy map of the entire mammalian SRP–ribosome complex (Halic et al. 2004). Thus nature may have evolved two ways to stabilize the active closed conformation of the Alu domain RNA. Higher eukaryotes utilize the SRP9/14 heterodimer to clamp together the 5’ and 3’ domains, while archaea (and probably euubacteria with an Alu domain), use an extra helix (H1) which forms a rigid three-way junction, combined with an intricate network of tertiary base-pairing to stabilize the required RNA conformation, possibly also in the absence of protein.

Since the more rigid P. horikoshii Alu RNA is able to functionally replace the equivalent human domain in elongation
arrest, it is likely that the conformational interchange between open and closed states described for the human Alu domain (Weichenrieder et al. 2001), is not required for this function, but may have another role in eukaryotic systems, where SRP9/14 interacts not only with SRP but also with Alu RNA derived from highly repetitive Alu repeats in the genome (Bovia et al. 1995; Hsu et al. 1995). So far, SRP9 or SRP14 homologs have not been identified in archaea (http://rnp.uthscsa.edu/rnp/SRPDB/SRPDB.html). This could imply either that they are unrecognizable due to sequence divergence or that there are no Alu binding proteins in archaea and the Alu RNA functions as naked Alu RNA. In the absence of a crystal structure of the naked PhAlu RNA it is not known whether it could fold into the same structure without protein. A third possibility is that paralogous Alu RNA binding protein(s) might exist since otherwise it is not easy to understand why the evolutionarily distant archaeal RNA is still able to bind hSRP9/14 in essentially the same manner as human Alu RNA. The only other explanation for this observation is that the RNA structure needs to be conserved in order to be able to interact with highly conserved features of the ribosome.

As observed in the current and previous structures, the SRP14 C-terminal tail that is required for elongation arrest activity extends toward the S-domain when bound to SRP RNA (Fig. 3A). Cross-linking studies in ongoing translation demonstrated that SRP14, and thus the Alu domain, is already in close proximity of the large ribosomal subunit in the absence of a signal sequence in the nascent chain. Upon signal sequence recognition, changes occur at the SRP–ribosome interface and SRP14 is now cross-linked to proteins of the large and the small subunit (Huck et al. 2004). Cryo-electron microscopic (cryo-EM) studies of SRP bound to elongation-arrested ribosomes (Halic et al. 2004, 2006) shows that the Alu domain binds in a cleft between the subunits, which is the site of interaction of the eukaryotic elongation factor II (eEF2) (Gomez-Lorenzo et al. 2000; Halic et al. 2004) and SRP9/14 contacts three helices of the 18S rRNA (Halic et al. 2004). Specifically, residues 57–75 including the functionally essential residues 60, 61, and 64 of SRP9 (Mary et al. 2010) are in close proximity to helices h5 and h15 of the small subunit. However, these EM structures have a rather low resolution (~12 Å) and fail to explain satisfactorily the observed cross-links with ribosomal proteins of the large subunit and how the essential basic amino acid residues in SRP14 mediate elongation arrest. The new high-resolution structure of the active Alu domain conformation will be valuable in conjunction with a future, improved resolution EM structure of the SRP–ribosome complex in answering these outstanding questions.

DATA DEPOSITION

Structure factors and coordinates for the complexes of PhAlu134 and PhAlu110 with hSRP9/14 are available in the PDB with codes 4UYK and 4UYJ, respectively.

SUPPLEMENTAL MATERIAL

Supplemental material is available for this article.

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Crystal structure of a Signal Recognition Particle Alu domain in the elongation arrest conformation

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Supplementary Material
Supplementary (Online) Methods

Reconstitution of \textit{PhAlu} RNA complexes with hSRP9/14

Human SRP9 residues 1 to 85 and SRP14 residues 1 to 107 proteins were expressed in \textit{Escherichia coli} BL21(DE3) using pET vectors, as described previously (Bovia et al. 1997). Selenomethionine (Se-Met) labeled SRP9 and SRP14 were produced in the same strain, under modified growth conditions (Van Duyne et al. 1993). SRP9 and SRP14 were purified separately on Heparin Sepharose (GE Healthcare) and Bio-S ion exchange columns, respectively (Bio-Rad), followed by reconstitution of the heterodimer in solution at an equi-molar ratio. The SRP9/14 complex further purified using a Bio-S column followed by size exclusion chromatography (Superdex 200, GE Healthcare). \textit{P. horikoshii} SRP Alu DNA coding sequences were obtained by gene synthesis (Hoover and Lubkowski 2002). GUAA tetraloops were inserted to replace the SRP RNA S-domain, together with a 3’ hammerhead ribozyme. PCR fragments were cloned into pSP64 vector and the DNAs of each of the plasmids were fully sequenced. Amplified vectors were linearised with \textit{Hind} III. Alu RNAs were transcribed in vitro and gel purified as described previously (Price et al. 1995) and refolded prior to complex formation by annealing at 65°C followed by a slow cooling step. Complex formation with SRP9/14 proceeded at 37°C for 10 minutes with a 30% excess of protein. RNPs were then purified on a Superdex 200 gel filtration column, concentrated up to 5 mg/ml and then stored at 4°C.

Crystallization and data collection

Crystals of selenomethionine-labeled and native SRP9/14–RNA complex were grown in hanging drops formed by a 1:1 mixture of the complex at 5 mg/ml and
crystallization buffer. *PhAlu*134 complex crystals grew in a crystallization solution containing 17.5% PEG 400, 5% glycerol buffered in 100 mM sodium acetate pH 5.0. Crystals grew at 4°C in three days and belong to the space group *P*43212 (*a*=*b*=100.45 Å, *c*=196.71 Å, α=β=γ=90°). Crystals of the *PhAlu*110 complex were obtained with a crystallization solution containing 17.5% PEG 3350, 2.5% glycerol, 0.3 M ammonium sulphate and 0.1 M sodium acetate at pH 4.6. Crystals grew at 4°C in 24 hours and belong to the orthorhombic space group *P*212121 (*a*=104.13 Å, *b*=108.8 Å, *c*=128.26 Å, α=β=γ=90°).

Cryoprotection was achieved by adding 20% glycerol before freezing at 100K. A *PhAlu*134 crystal diffracting to 3.6 Å resolution on ESRF beamline BM30A allowed collection of a three-wavelength MAD dataset. A higher resolution dataset to 3.2 Å was subsequently collected on the ESRF beamline ID14-1. *PhAlu*110 crystals diffracted X-rays to 3.35 Å on ESRF beamline ID23-1. Diffraction data were processed using XDS(Kabsch 1993). Data collection statistics are summarized in Supplementary Table 1.

**Phasing and refinement**

Six of the seven selenium atoms of the *PhAlu*134 complex were located from the MAD data using reflections to 4.5 Å resolution with SOLVE(Terwilliger and Berendzen 1999) and the final figure of merit of the experimental phases was 0.53. Phases were improved by solvent flattening using SOLOMON and DM(Cowtan 1993) as implemented in SHARP with a 60% solvent content. The electron density map was of sufficient quality (Supplementary Figure 2) for a modeling and refinement to proceed using COOT(Emsley and Cowtan 2004) and REFMAC, resulting in an atomic model with an *R*<sub>work</sub>/*R*<sub>free</sub> of 0.193/0.228. The RNA geometry was significantly improved with
PHENIX-ERRASER  (https://www.phenix-online.org/documentation/erraser.htm). The structure of the PhAlu110 complex was solved by molecular replacement using Phaser(McCoy et al. 2007) with the PhAlu134 structure as model and refined to an $R_{\text{work}}/R_{\text{free}}$ of 0.192/0.237. For both structures, map quality was considerably enhanced using map sharpening as implemented in REFMAC. The structure of SRP9/14 is relatively unchanged compared to that found in previous complexes lacking RNA stem 5 (RMSD of 0.9 Å over 147 SRP9/14 Cα positions, compared to the model with PDB accession code 1E8O(Weichenrieder et al. 2000)). Nevertheless, certain residues that were disordered in previous structures of SRP9/14 were found to be traceable in the electron density, including half of the large internal loop of SRP14. Figures were prepared using PYMOL(DeLano 2002) and MOLSCRIPT(Kraulis 1991) and BOBSCRIPT(Esnouf 1997).

**Elongation arrest and translocation activities**

7SLC reconstitutes more efficiently into SRP than the previously used construct and exhibited similar activity in elongation arrest activity (Lakkaraju et al. 2008; Mary et al. 2010). 7SLC RNAs were expressed as described previously (Huck et al. 2004). After purification on G50 columns, the RNAs were extracted 2x with phenol/dichloromethane and 2x with dichloromethane and lyophilized. The RNA concentrations were determined by OD$_{260}$ and compared by denaturing gel electrophoresis. Native gels were performed as described (Huck et al. 2004). Particles were assembled at 0.5 µM of recombinant human SRP19, recombinant canine SRP54 and canine SRP68/72 and at 2 µM synthetic RNAs (7SLC or PAHS). Concentration of SRP9/14 was 4uM. The excess of PAHS RNA, and as a control also of 7SLC RNA, is required for maximal yield of functional particles,
since not all of the synthetic RNA is in its native structure. For protein purifications and elongation arrest and translocation assays see Huck et al., 2004. Briefly, the final concentration of SRP in the wheat germ translation reactions was 100 nM based on the SRP54 concentration. For the processing assay EDTA and salt-washed membranes (EKRM) were added to translation reactions at final concentrations of 0.02 eq/μL, respectively, as described (Thomas et al. 1997). For elongation arrest and translocation assays, the reactions were stopped at 20 min. and 35 min., respectively. The in vitro synthesized proteins were quantified by phosphorescence imaging. The relative inhibition of preprolactin accumulation was calculated as follows:

\[
\text{Inhibition} [%] = \left[ 1 - \left( \frac{P_s}{C_s} / \frac{P_o}{C_o} \right) \right] \times 100
\]

\(P_s\) and \(C_s\) are the amounts of preprolactin and cyclin quantified in the sample and \(P_o\) and \(C_o\) the amounts present in the negative control (buffer). The translocation efficiency was calculated as follows: 

\[
T = 100 \times \frac{\text{PL}}{\text{PL} + \text{pPL}},
\]

where PL is prolactin and pPL preprolactin.

**RNA transcript sequences**

The ‘PAHS’ RNA used in the translocation assays consisted of stems 1, 2, 3 & 4 of archaeal SRP RNA fused to the complementary stems of human SRP RNA, and was of the following sequence:

```
GGGGCTAGCCGGGGGTTCGCGTCCCCTGTAAACCGGAAACCGCCGATATGCCGGGGCCGAAGAGTGTTAGGAGATCGCTTTGAGCCGAGTTCTGGGCTGACTGCGCTATGCGAGTCCGC
ACGTATAGTTGCTTCTCAATATGTTGACCTCCTCGGAGGACCCAGGTTCGTCTCTAGTGGATCGCTGTACCTATACCGGACCCAGTGCACTGC
```

```
TT
```
The ‘7SLC’ RNA represents a circular permutation of 7SL RNA (5’ and 3’ ends at positions 280 and 277 in 7SL RNA, respectively). The sequence of 7SLC transcribed in the translocation assays was as follows:

GATAGCGAGACCCCGCCTCTTTTGGCGGCGCGGGATCGCTGAGTCCAGCTACTCGGG
AGGCTGAGGCCGCAGTCCCTTTGGCGGCGAGTTCTGGGCTGCAGTGCGCTATGCCGAGGG
TGTCGCCGACTAAAGTTCCGAGCATCAATATGGTGACCTCCGGGCAGGGGGACCCAGGTTGCTTA
AGGAGGGGTGAACCAGGGCCAGGTTGCCGAAAAACGGAGCAGGTCCTCCGCTGATCAGTACGG
GGATCGCGCCTGTGATAGCACTGCACTCCAGGCTTT
## Supplementary Tables

### Supplementary Table 1. Data collection statistics.

§ $R_{\text{sym}} = \frac{\sum_{hkl} \sum_i |I_{hkl}| - I_{hkl,i}}{\sum_{hkl} \sum_i I_{hkl,i}}$, where $\langle I \rangle_{hkl}$ is the mean intensity and $I_{hkl,i}$ is the intensity of the $i$th measurement of reflection $I_{hkl}$. †Values in parentheses refer to the highest resolution shells. *Friedel pairs were treated as separate reflections.

<table>
<thead>
<tr>
<th>hSRP9/14 with</th>
<th>PhAlu134</th>
<th>PhAlu110</th>
</tr>
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<tbody>
<tr>
<td>Identifier</td>
<td>Peak*</td>
<td>Inflection*</td>
</tr>
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<td></td>
<td>BM30A</td>
<td>BM30A</td>
</tr>
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<td>0.9808</td>
<td>0.9809</td>
<td>0.9763</td>
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<td>$P4_32_12$</td>
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<td>Unit cell parameters (Å)</td>
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<tr>
<td>$a=100.5, b=100.5$, $c=196.9$</td>
<td>$a=100.9, b=100.9$, $c=197.5$</td>
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</tr>
<tr>
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<td>$a=\beta=\gamma=90^\circ$</td>
<td>$a=\beta=\gamma=90^\circ$</td>
</tr>
<tr>
<td>Resolution (Å)</td>
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<tr>
<td>(outer shell limits)</td>
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</tr>
<tr>
<td>50 - 3.6 (3.7-3.6)</td>
<td>50 - 3.6 (3.7-3.6)</td>
<td>50 - 3.6 (3.7-3.6)</td>
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<tr>
<td>$R_{\text{sym}}$ (%)§</td>
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</tr>
<tr>
<td>11.1 (54.3)</td>
<td>10.1 (49.7)</td>
<td>13.0 (65.8)</td>
</tr>
<tr>
<td>$(I)/\langle\sigma(I)\rangle$†</td>
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<tr>
<td>11.7 (3.22)</td>
<td>11.8 (3.10)</td>
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<td>Reflections measured†</td>
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<td>94090 (6548)</td>
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<td>85617 (5866)</td>
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<td>Unique reflections measured†</td>
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<tr>
<td>21989 (1587)</td>
<td>22024 (1601)</td>
<td>22059 (1575)</td>
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<tr>
<td>Completeness (%)</td>
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<tr>
<td>99.5 (97.2)</td>
<td>99.4 (98.8)</td>
<td>99.5 (96.4)</td>
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**Supplementary Table 2.** Refinement statistics.

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<tr>
<td>$R_{\text{work}}$ (last shell)</td>
<td>0.193 (0.342)</td>
<td>0.192 (0.363)</td>
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<tr>
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<td>0.228 (0.424)</td>
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<td>1379</td>
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<td>4722</td>
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<td>RNA</td>
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<td>116.0</td>
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<td>R.M.S. deviations</td>
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<td>Ramachandran plot</td>
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<tr>
<td>Favoured (%)</td>
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<td>Outliers (%)</td>
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**Supplementary Table 3**: Quantitation of elongation arrest and translocation assays

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<tr>
<td>Mean</td>
<td>SEM</td>
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<td>A12</td>
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<tr>
<td>100</td>
<td>85</td>
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<tr>
<td>(-9/14)</td>
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<table>
<thead>
<tr>
<th>Inhibition [%]</th>
<th>Translocation [%]</th>
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</thead>
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<tr>
<td>RC PAHS</td>
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<tr>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>WT</td>
<td>76</td>
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<tr>
<td>A12</td>
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<tr>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>(-9/14)</td>
<td>28</td>
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Supplementary References


Supplementary Figure Legends

Supplementary Figure 1

Characterization of PhAlu RNA : SRP9/14 complexes by size exclusion chromatography using a Superdex200-16/60 column (GE Healthcare) equilibrated in a buffer containing 20 mM Heps pH 7.5, 150 mM NaCl and 5 mM MgCl₂.

(A) Elution profile of PhAlu134 RNA alone (dashed line) and PhAlu134 RNA:SRP9/14 complex (solid line).

(B) Elution profile of PhAlu110 RNA alone (dashed line) and PhAlu110 RNA:SRP9/14 complex (solid line).

(C) SDS-PAGE analysis of purified PhAlu134:SRP9/14 complex revealed by silver staining.

(D) SDS-PAGE analysis of purified PhAlu110 RNA:SRP9/14 complex revealed by Coomassie blue (left panel) and urea-PAGE gel stained with methylene blue.

Supplementary Figure 2

Stereo view of the experimental electron density map of PhAlu134 RNA superposed with the final model (yellow backbone, red bases), contoured at 0.8 sigma calculated from MAD data after heavy atom substructure refinement, phase calculation and solvent flattening with SHARP (Bricogne et al. 2003).

Supplementary Figure 3

(A) Superposition of PhAlu134 (red) and PhAlu110 (green) RNAs showing very high structural similarity except in the distal 5’ stem region. PhAlu134 shows a bend in the region assigned to hinge 2 in the cryo EM map of the complete mammalian SRP bound to the ribosome (Halic et al. 2004).
(B) Schematic depiction of the secondary structure of the PhAlu134 (green and red nucleotides) and PhAlu110 (green only) RNAs.

Supplementary Figure 4

(A) Secondary structure of PAHS RNA.

(B) and (C) Elongation arrest and translocation activities of SRPs. \[^{35}\text{S}\]-labeled translation products displayed by SDS-PAGE. SRP was reconstituted with either 7SLC or PAHS RNA together with recombinant (SRP19, SRP54) and canine (SRP68/72) proteins as well as the SRP9/14 proteins indicated. WT: SRP9/14; -9/14: without SRP9/14; A12: SRP9/14A12; -100: SRP9/14-100. Cyc: Truncated version of cyclin; pPL: Preprolactin; PL: Prolactin. Final SRP concentration in the translation reaction: 100 nM, EKRM: 0.02 eq./µl.
Supplementary Figure 2