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Functional Divergence of the Plastid and Cytosolic Forms of the 54-kDa Subunit of Signal Recognition Particle

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Chloroplast and cytoplasmic signal recognition particles (cpSRP and cySRP) each contain a similar subunit, SRP54. The chloroplast homologue binds to cpSRP43, which is absent from cytosolic SRP, and cySRP54 binds to SRP-RNA, which appears to be absent from cpSRP. In the presence of cpSRP43, cpSRP54 posttranslationally forms a soluble targeting intermediate, transit complex, with the major light harvesting protein of the thylakoid membrane. In contrast, cySRP54 functions cotranslationally. In this study we investigated whether cytosolic and chloroplast forms of SRP54 were interchangeable in three types of functional assays: complementation of an *Escherichia coli* SRP54 mutant, formation of the transit complex, and heterologous binding between the SRP54 subunits, cpSRP43, and SRP-RNA. In no cases were the 54-kDa subunits able to substitute for each other suggesting that the two proteins are fundamentally different.

Key Words: **protein-targeting; membrane-biogenesis; thylakoid membrane; Ffh; cpSRP54.**

Chloroplasts contain numerous pathways for targeting proteins to the thylakoid membrane [1]. Based on sequence similarities among the components of the targeting machinery in chloroplast and prokaryotes, one might predict that the targeting pathways operate by fundamentally similar mechanisms. For example, one pathway is reminiscent of the Sec pathway described in *Escherichia coli*. Proteins that use this pathway require ATP and a chloroplast homologue of the protein translocation ATPase SecA [2, 3]. Chloroplast homologues of two translocon proteins SecE (D. Schuenemann, E. Hartmann, and N. E. Hoffman, unpublished results) and SecY [4] have also been identified. A second pathway distinguished by a trans thylakoid pH gradient was originally thought to be unique to the thylakoids but was recently shown to involve a protein, Hcf 106 [5], that also has prokaryotic homologues [6–8]. A third pathway utilizes a chloroplast homologue of the 54-kDa subunit of signal recognition particle (cpSRP54) [9]. However initial studies suggest that cpSRP is not a functional homologue of the cytoplasmic form.

Cytoplasmic SRP (cySRP) is a ribonucleoprotein that cotranslationally interacts with ribosome nascent chains and facilitates targeting to the endoplasmic reticulum and cytoplasmic membrane in eukaryotes and prokaryotes, respectively [10]. cpSRP differs from cySRP in two major ways. First, a functional cpSRP lacks an RNA and contains a 43-kDa protein unrelated to any protein subunits of SRP [11]. Second, cpSRP interacts with substrates posttranslationally [12]. Thus, cpSRP appears to be structurally and functionally different than cySRP.

Previous studies have indicated that *E. coli* and mammalian cySRP54 are interchangeable in some respects. The *E. coli* cySRP54, termed Ffh (fifty-four homologue), binds to mammalian SRP RNA and vice versa [13, 14]. Heterologous particles containing Ffh instead of the mammalian cySRP54 are functional in signal sequence binding, GTP hydrolysis, and translation arrest but not receptor binding [15]. To address whether cpSRP54 is interchangeable with Ffh, we have expressed the chloroplast protein alone or in the presence of cpSRP43 in a strain of *E. coli* conditionally deficient in Ffh. We have examined whether Ffh alone or in the presence of cpSRP43 can bind to Lhcb1. Lastly, we have tested whether cpSRP54 is able to bind to *E. coli* SRP-RNA and whether Ffh is able to bind to cpSRP43. Under the conditions of the three assays, neither protein was able to substitute for the other

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Abbreviations used: cpSRP, chloroplastic signal recognition particle; cySRP, cytoplasmic signal recognition particle; EST, expressed sequence tag; Ffh, *E. coli* cySRP54 (fifty four homologue); GS, glutathione-Sepharose; GST, glutathione-*S*-transferase; GST-43, glutathione-*S*-transferase fused to the 43-kDa subunit of chloroplast signal recognition particle; LHCP, light harvesting chlorophyll a/b binding protein; SRP, signal recognition particle; UTL, untranslated leader.

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suggesting that cpSRP54 has specialized into a fundamentally different protein from cySRP54.

MATERIALS AND METHODS

DNA constructs. A chloramphenicol selectable plasmid expressing mature cpSRP54his in the vector pACYC184 has been described [11]. To make a mature construct of cpSRP43, the full length cDNA [16] was used as a template for PCR with the following forward and reverse primers, respectively: AAGGATCCATGGCCGCCGTACAAAGAAAC, CCCCCAAGCTTTCATTCATTCATTGGTTG. The resulting PCR fragment was digested with *Bam*HI and *Hin*dIII and cloned into the same sites in Bluescript SK+ to form BSSK + chaos(m). A spectinomycin selectable version of the plasmid, BSSK + chaos(m)Sp^R, was constructed by subcloning the *Bam*HI–*Xho*I fragment from BSSK + chaos(m) into the *Bam*HI–*Xho*I sites of BSSK + spec (provided by Devaki Bhaya). BSSK + spec was made by replacing the *Dra*I fragment of Bluescript containing the Bla gene with a *Dra*I fragment from pHP45Ω [17] containing the gene for spectinomycin resistance. *E. coli* Ffh was subcloned as a *Sal*I–*Nhe*I fragment from pBYO3 [18] (provided by Greg Phillips) into the same sites of pBR322. The resulting clone, pFfh, contains the entire Ffh coding region flanked by 161 and 214 nt of sequence at the 5′ and 3′ ends respectively. A *Hin*dIII fragment from pHP45Ω containing a spectinomycin resistance gene was subcloned into the *Hin*dIII site of pFfh and the clone was designated pFfhSp^R. To make constructs for wheat germ translations of Ffh and mature cpSRP54, the coding regions were subcloned behind the untranslated leader (UTL) from a pea *rbcS* clone SS8.0 [19] where an *Nco*I site was introduced at the initiation Met codon [9]. To facilitate cloning, an *Nco*I site was introduced at the initiating met codon of pFfh to form pAF15 and a *Bsp*HI site was introduced at the processing site of cpSRP54. The Ffh gene was subcloned as an *Nco*I–*Hin*dIII fragment into the same sites of a pGEM4 vector containing the mutated *rbcS* UTL to form the plasmid pSSFfh. Mature cpSRP54 was subcloned as a *Bsp*HI–*Xba*I fragment into the *Nco*I and *Xba* sites of a pSP73 vector containing the mutated *rbcS* UTL to form the plasmid pAF1. The gene encoding SRP-RNA was amplified from pSN1 [20] (obtained from Harris Bernstein), using the forward and reverse oligonucleotides: GGAATTCTAATACGACTCACTATAGGGGGCTCTGTTGGT and CGGGATCCCGGGGTGGGGGCCCTG. The forward oligonucleotide includes an *Eco*RI restriction site followed by the T7 promoter. The reverse oligonucleotide includes restriction sites for *Bam*HI and *Sma*I. The PCR product was digested with *Bam*HI and *Eco*RI and subcloned into the same sites of Puc19 to form pFfs.

Strains. The *E. coli* strain WAM113 [21] was a gift from Dr. Greg Phillips. WAM113 contains a kanamycin resistance gene inserted into the chromosomal copy of *ffh* and an arabinose inducible copy of *ffh* linked to an ampicillin resistance gene integrated into the chromosome at a second site. Competent WAM113 cells were made as described [22] and individually transformed with either pACYC54his, pBsSK + chaos(m)Sp^R, or pFfhSp^R to yield strains WAM113-54, WAM113-43, or WAM113-Ffh respectively. Competent WAM113-54 cells were prepared and transformed with pBsSK + chaos(m)Sp^R to yield WAM113cpSRP. WAM113 strains were plated on LB-agar containing 100 μg/ml ampicillin, 50 μg/ml kanamycin ± 0.2% (L-)arabinose. For the WAM113 strains transformed with additional plasmids, the following antibiotics were added as noted: WAM113-54, 35 μg/ml chloramphenicol; WAM113-43 or WAM113-Ffh, 20 μg/ml spectinomycin; WAM113 cpSRP, 35 μg/ml chloramphenicol and 20 μg/ml spectinomycin.

Complementation assays. WAM113 strains were grown overnight in LB + 0.2% arabinose + the appropriate antibiotics as listed above. In the morning, the overnight culture was diluted 100-fold into fresh media and grown until the OD_{600} was approximately 1. The resulting culture was diluted 10^6-fold, 200 μl was plated onto LB-agar containing the appropriate antibiotics either in the presence or the absence of 0.2% arabinose, and colonies that grew after overnight incubation at 37°C were counted. To test whether cpSRP54 and cpSRP43 were expressed in WAM113, strains were grown overnight in LB, 0.2% arabinose, and the appropriate antibiotic containing either 10 or 100 μM IPTG, and cpSRP43 and cpSRP54 were extracted as previously described [11]. The antisera raised against cpSRP54 [9] and cpSRP43 [16] were used in immunoblots as previously described [23].

In vitro transcription and translation. A construct encoding *E. coli* SRP-RNA was engineered as described above to enable synthesis of the unmodified RNA by *in vitro* transcription. The placement of the T7 and *Sma*I sites specified correct initiation and termination of the transcript derived from *Sma*I linearized DNA template. SRP-RNA synthesized by *in vitro* transcription (Promega, Ribomax system) was quantitated by UV absorption and used in binding studies without any further modification. pAF1 and pSSFfh were used to synthesize [^{35}S]-met radiolabeled cpSRP54 and Ffh in wheat germ extracts as described [24]. Neither template was linearized prior to transcription/translation.

Binding of SRP54 to SRP-RNA. To ensure translation products were completely dissociated from ribosomes, translation products were incubated in 5 mM EDTA for fifteen minutes at 25°C. Each binding reaction contained, in 10 μl, wash buffer (5 mM MgOAc, 500 mM KOAc, 50 mM Tris-OAc pH 7.5) and 0.35 μCi translation product (approx. 50 fmol). Where noted, 1 μg of tRNA or SRP-RNA was added (approx. 3 pmol). Reactants were incubated for 30 min at 25°C and transferred to tubes containing 500 μl of wash buffer and 30 μl of Q-Sepharose Fast Flow. Tubes were rotated end over end for 15 min at 4°C. The tube contents were transferred to syringes attached to minicolumns (Wizard, Promega) and the beads and flow through were separated and saved. After washing the beads with an additional 3 ml of wash buffer, the columns were spun dry in a microcentrifuge, and incubated with 100 μl of elution buffer (2 M KCl, 5 mM MgOAc, 50 mM Tris-OAc, pH 7.5) for 1 min at room temperature. The eluate was recovered upon centrifuging the columns for 2 minutes. Proteins in the eluate were precipitated by adding sodium deoxycholate (final 200 μg/ml), trichloroacetic acid (final 10%), incubating for 30 minutes at 0 °C, and centrifuging the samples for 15 minutes at 4 °C. The pellets were washed with 100% acetone, briefly dried, resuspended in 20 μl of 4× sample buffer, and analyzed by SDS–PAGE on 13% acrylamide gels.

Binding of cpSRP54 and ffh to cpSRP43. Recombinant GST-43 (100 μg protein) [11] was bound to glutathione-Sepharose beads (25 μl bed-volume) in B buffer (20 mM Hepes–KOH pH 8.0, 150 mM NaCl) by rotating the mixture end over end for 2 h at 4 °C. The beads were washed two times with B buffer and resuspended in the same. The binding efficiency (90%) was determined by protein measurement [25] of the GST-43 solution before and after the binding reaction. The indicated amounts of cpSRP54 or Ffh translation products were incubated with 13 μg GST-43 beads in B buffer containing 1 mM PMSF and 8 μl wheat germ extract in a total volume of 50 μl. Control samples contained GS-beads lacking GST-43. After 20 min at 25 °C, the slurry was transferred to Wizard Mini-columns (Promega) and washed with 5 ml of B buffer. Protein was eluted with 50 μl of 2× SDS–PAGE sample buffer. The radioactivity in the samples was determined by liquid scintillation counting.

Sequence alignments. A partial pea cpSRP54 cDNA was isolated by screening a pea expression library with antibodies against arabidopsis cpSRP54 and both strands of the insert were sequenced. The pea cDNA has the GenBank Accession No. AF089724. The maize sequence was provided by Robert Meeley and was derived from Pioneer Hi-Bred's EST collection. Sequences were aligned using the program Clustal W and shaded using the program Gene.doc.

TABLE 1

cpSRP Does Not Complement the Growth Defect in the WAM113 Mutant

Strain	IPTG (μM)	Colonies + 0.2% arabinose	Colonies − arabinose
WAM113	10	367	0
	100	483	0
WAM113-ffh	10	107	123
	100	135	143
WAM113-54	10	272	0
	100	307	0
WAM113-43	10	156	0
	100	193	0
WAM113-cpSRP	10	225	0
	100	220	0

Note. Cells were grown in the presence of 0.2% arabinose to an OD_{600} of 1.0 and then diluted and plated onto media containing or lacking arabinose, IPTG, and the appropriate antibiotics (see Materials and Methods).

RESULTS

An intact cpSRP does not accumulate in WAM113. The *E. coli* gene *ffh* is essential. Phillips and Silhavey [21] created a conditional mutant by introducing an arabinose inducible copy of *ffh* into a second site and inactivating the endogenous copy. In the absence of arabinose, the protein Ffh is no longer synthesized, the pre-existing protein becomes depleted by dilution and turnover, and the cells die. WAM113 requires arabinose for survival and the requirement can be alleviated by transforming the strain with a plasmid expressing Ffh (Table 1). However neither cpSRP54, cpSRP43, nor the combination of the two could complement the mutant (Table 1). Although mature cpSRP43 accumulates in both WAM113-43 and WAM113-cpSRP (Fig. 1, lanes 3 and 4), cpSRP54 was not detected in WAM113-54 and only a 30-kDa fragment derived from cpSRP54 is observed in WAM113-cpSRP (Fig. 1, lanes 10 and 12). This 30-kDa fragment binds to Ni^{2+}–NTA (Fig. 1, lane 12) and therefore represents the C-terminus of cpSRP54, which was expressed with a C-terminal His_6 tag. cpSRP43 also bound the Ni^{2+}–NTA column when co-expressed with cpSRP54 (Fig. 1, lane 8) indicating that cpSRP54 interacts with cpSRP43 through its C-terminus. Given the difficulty of expressing intact cpSRP54 in WAM113, it was not possible to ascertain whether cpSRP54 could substitute for the *E. coli* homologue in the experiments described above. However the instability of the chloroplast protein in *E. coli* suggested that it is substantially different from the *E. coli* protein.

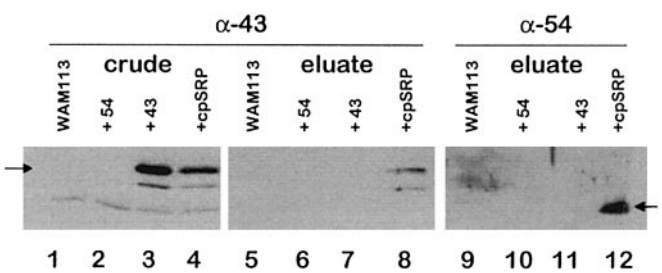

FIG. 1. Expression of cpSRP43 and cpSRP54 in WAM113 lines. An overnight culture was diluted 1:100 in LB + 0.2% arabinose, appropriate antibiotics, and 100 μM IPTG and grown to an OD_{600} = 1. Protein extracts derived from the cells were analyzed by SDS–PAGE on 10% acrylamide gels and assayed for cpSRP43 and cpSRP54 by immunoblotting with ECL detection. Extracts were also applied to Ni^{2+}–NTA–agarose and the eluates prepared by eluting with 250 mM imidazole. The left arrow indicates the position of mature cpSRP43 and the right arrow indicates the position of the cpSRP54 degradation product.

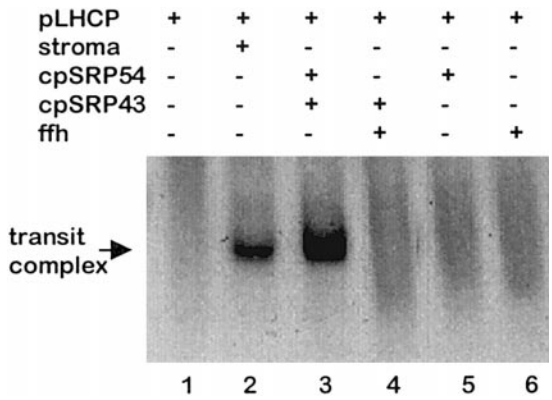

FIG. 2. Ffh does not form transit complex with cpSRP43 and Lhcb1. The indicated components: Lhcb1, 8×10^4 cpm synthesized in wheat germ extracts; arabidopsis stroma (equivalent to 36 μg of chlorophyll); cpSRP54 or Ffh (8 μl, approximately 160 ng) synthesized in wheat germ extracts; purified recombinant cpSRP43 (50 ng) were mixed and assayed for the formation of transit complex on nondenaturing gels by radioimaging exactly as described [11].

Ffh does not form the Lhcb1 transit complex. One of the documented functions of cpSRP is to maintain the solubility of Lhcb1, an abundant and hydrophobic thylakoid membrane protein, as it is transported through the soluble phase of the chloroplast [26, 27]. A characteristic complex, named transit complex, is detectable on nondenaturing gels when radiolabeled Lhcb1 synthesized in a wheat germ translation extract is incubated with stroma (Fig. 2, lane 2) [12, 27]. Transit complex can be reconstituted in the absence of stroma with purified recombinant cpSRP43 and cpSRP54 synthesized in a wheat germ translation extract (Fig. 2, lane 3, [11]). To test whether Ffh either alone or in combination with cpSRP43 could interact with Lhcb1, we replaced cpSRP54 with a wheat germ translation extract of Ffh in the transit complex assays. Neither cpSRP54 nor Ffh bind Lhcb1 in the absence of cpSRP43 (Fig. 2, lanes 5 and 6). However Ffh clearly differs from cpSRP54 in being unable to form transit complex in the presence of cpSRP43 (Fig. 2, lane 4).

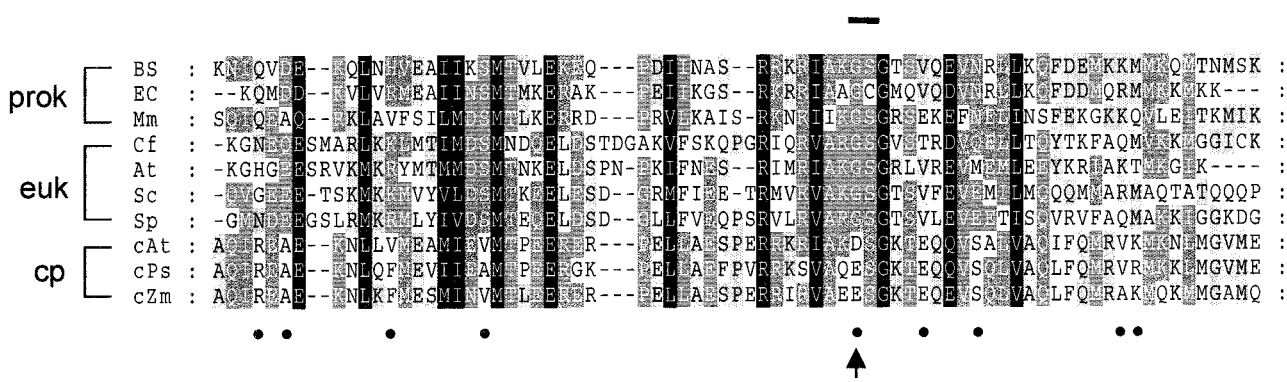

FIG. 3. Alignment of the RNA binding region of cySRP54 from prokaryotes and eukaryotes to the corresponding sequences in cpSRP54. Bs, *B. subtilis* [32]; Ec, *E. coli* [18]; Mm, *M. mycoides* [33]; Cf, *C. familiaris* [34]; At, *A. thaliana* [35]; Sc, *S. cerevisiae* [36]; Sp, *S. pombe* [36]; Ps, *P. sativum;* Zm, *Z. mays.* Conserved residues found in all 10 sequences are shaded black, in 6–9 sequences are shaded dark gray, and in 3–5 sequences are shaded light gray. Conserved residues are based on the following assignments: D=E=N=Q; S=T; H=K=R; F=Y=W; L=I=V=M; G=P; A=C. Deviations in the plastid sequence from the cytosolic consensus are indicated by the closed circles. The GSG motif is overscored. The Bs sequence begins with K364. D404G in cpSRP54 is indicated by the arrow.

cpSRP54 does not bind SRP RNA. All cySRP54 proteins bind to SRP-RNA. This binding is essential for activity of the cySRP [14, 28]. Furthermore, binding occurs between proteins and RNA from evolutionarily distant organisms such as *E. coli* and humans where *in vivo* complementation fails [13, 14, 29]. Hence, if the RNA binding function of cpSRP54 is conserved, cpSRP54 should bind to *E. coli* SRP-RNA as does mammalian SRP54.

The RNA binding domain of *Bacillus subtilus* Ffh has been defined [30]. In Fig. 3, the corresponding regions of cytosolic SRP54 proteins from three prokaryotic species and four eukaryotic species are aligned with three chloroplast homologues. As has been previously noted, there is substantial conservation of amino acids among the three types of proteins [30]. However, at least 9 residues are more conserved among the cytosolic proteins than the chloroplast proteins (marked with ● in Fig. 3). One of the most drastic changes occurs in the conserved sequence GSG (overscored in Fig. 3) where the first glycine is replaced by an acidic residue in the chloroplast protein. Kurita *et al.* [30] observed that mutation of either glycine reduced binding to SRP-RNA by approximately 2 orders of magnitude. Using arabidopsis cpSRP54, we examined whether wild type cpSRP54 or the D404G mutant would bind to *E. coli* SRP-RNA.

To test for RNA binding of the two proteins, we employed a frequently used assay that measures the RNA dependent binding of SRP54 to a strong anion exchange resin [14]. Yeast tRNA was used to control for non-specific binding. Radiolabeled Ffh and cpSRP54 of comparable specific activity were synthesized in a wheat germ translation extract and the binding of these proteins to SRP-RNA was tested. A large excess of RNA was added relative to protein to maximize potential binding of the radiolabel. Neither protein significantly bound to the strong anion exchange column, Q-Sepharose, when RNA was absent nor when tRNA was present (Fig. 4). In the presence of SRP-RNA, however, a substantial fraction of the Ffh (60%) bound to the column whereas cpSRP54 did not. This result suggests that cpSRP54 has little if any affinity for *E. coli* SRP-RNA. Likewise, the D404G mutation did not significantly alter the binding properties of cpSRP54. Thus there are multiple differences between cpSRP54 and Ffh that contribute to their altered binding properties.

Ffh does not bind to cpSRP43. Approximately 50% of cpSRP54 in the chloroplast stroma are bound to the protein cpSRP43 [11]. To verify that the cpSRP54 that did not bind to RNA was capable of binding cpSRP43, we performed binding studies between cpSRP54 and cpSRP43 fused to GST. Keeping the level of GST-43

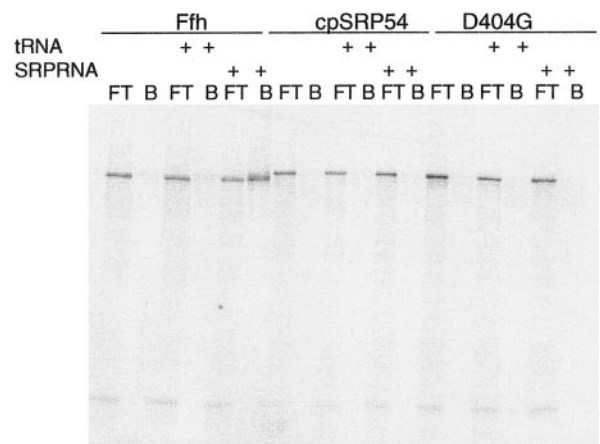

FIG. 4. CpSRP54 does not bind SRP-RNA. Ffh and cpSRP54 were radiolabeled by translation in wheat germ extracts. 0.35 μCi of product (50 fmol) was incubated with 1 μg of RNA (3 pmol) as indicated and then allowed to bind to Q-Sepharose as described under Materials and Methods.

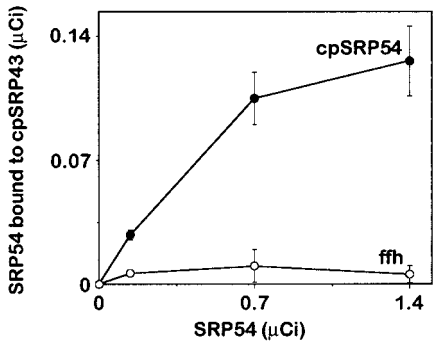

FIG. 5. Ffh does not bind to cpSRP43. Radiolabeled Ffh or cpSRP54 was synthesized in wheat germ extracts and incubated with GST-43 (13 μg) prebound to glutathione-Sepharose as described under Materials and Methods.

fixed, we varied the amount of radiolabeled cpSRP54 and measured the amount that bound to GS-Sepharose. Likewise, similar experiments were performed with Ffh. We observed that cpSRP54 indeed binds to GST43 while Ffh shows little if any affinity for the protein (Fig. 5). Thus, the binding properties of cpSRP54 and cytosolic SRP54 are fundamentally different. The inability of Ffh to form transit complex is probably due to its inability to bind to cpSRP43.

DISCUSSION

Despite the higher sequence similarity between prokaryotic cySRP54 and the chloroplast homologue, cySRP54 from eukaryotes and prokaryotes are functionally similar and differ from the chloroplast protein. With regard to RNA binding, cytosolic SRP54 from prokaryotes and eukaryotes are interchangeable. Under conditions where heterologous cySRP54 and SRP-RNA bind, cpSRP54 does not bind the RNA. Thus in this basic function, the chloroplast protein is clearly distinguishable from cySRP54. Just as SRP-RNA is required for the function of cySRP, so too is cpSRP43 required for cpSRP function [11]. We found that Ffh can neither bind to cpSRP43 nor form transit complex with cpSRP43 and Lhcb1. The binding data support the notion that cpSRP54 is fundamentally different from the cytosolic homologues.

SRP54 contains two principal domains, the NG domain at the N-terminus containing the GTP binding and hydrolysis site and the M domain at the C-terminus, which binds signal sequences and SRP RNA [10]. The NG domain of cpSRP54 is 70% identical to the prokaryotic protein and presumably has an analogous function [9]. While protein interacting domains of cpSRP54 remain to be identified, preliminary evidence (Fig. 1) indicates that the C-terminus of cpSRP54 binds to cpSRP43. Thus, it is formally possible that the RNA binding region of SRP54 corresponds to the cpSRP43-binding region of cpSRP54.

Why should plants have evolved a new type of SRP? The substrates for cpSRP include many of the light harvesting chlorophyll proteins (LHCP) [12] (P. Amin and N. Hoffman, unpublished results). These proteins are extremely abundant membrane proteins that are posttranslationally targeted to the chloroplast through the aqueous phase [26]. It would appear that cpSRP has evolved as a means to facilitate this process exploiting the ability of cySRP54 to interact with hydrophobic proteins. cpSRP43 does not resemble any subunit of cySRP but is essential for the post-translational interaction between cpSRP54 and LHCP [11, 16]. It is likely that cpSRP54 took on a specialized role as it evolved from a cytoplasmic prokaryotic progenitor which lost its ability to bind cytoplasmic SRP-RNA and gained the ability to bind cpSRP43.

Recently, it was observed that null mutants in cpSRP43 [16] have a distinct phenotype from mutants lacking cpSRP54 (P. Amin, L. Nussaume, and N. Hoffman, unpublished results). A mutation in cpSRP43 specifically affects proteins in the LHCP family while mutation of cpSRP54 affects both LHCP proteins and chloroplast encoded proteins targeted by a co-translational mechanism [16, 23] (P. Amin and N. Hoffman, unpublished results). A possible explanation for the non-overlapping functions of the two proteins is offered by the finding that there are two pools of cpSRP54 but just one pool of cpSRP43. All of the cpSRP43 is complexed with cpSRP54 while a second pool of cpSRP54 is associated with the 70S ribosomes free of cpSRP43 [11]. Based on these observations we have speculated that cpSRP, consisting of cpSRP43 and cpSRP54 facilitates the post-translational targeting of LHCP type proteins while cpSRP54 bound to the ribosome is engaged in co-translational targeting reactions [16]. We can imagine that the association of cpSRP54 with the ribosome is required either for its function or for proximity of cpSRP54 to its putative substrate, the nascent chains. In the former case, the activity of cpSRP54 may depend on binding to an RNA or protein component of the ribosome. It was recently reported that sequences related to SRP-RNA are found in the chloroplast genome of red algae and diatoms [31] and conceivably such an RNA may be present in higher plant chloroplasts and bound to the ribosome. The results presented here suggest that if such an RNA exists in plant chloroplasts, it must be modified from cytosolic SRP-RNA to be able to bind cpSRP54.

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