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# Functional substitution of the signal recognition particle 54-kDa subunit by its *Escherichia coli* homolog

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**ABSTRACT** **The 54-kDa subunit of the mammalian signal recognition particle (SRP54) binds to the signal sequences of nascent secretory and transmembrane proteins and facilitates their cotranslational targeting to the membrane translocation apparatus in the endoplasmic reticulum (ER). A 48-kDa *Escherichia coli* protein that shares extensive sequence similarity with SRP54 was identified in homology searches. Recent genetic experiments by Phillips and Silhavy [Phillips, G. J. & Silhavy, T. J. (1992) *Nature (London)* 359, 744–746] have shown that depletion of this protein, designated Ffh (*fifty-four homolog*), leads to a significant secretory defect *in vivo*. We demonstrate here that Ffh is structurally and functionally related to SRP54 by virtue of its ability to mimic closely its mammalian counterpart in several established biochemical assays, thereby suggesting that it plays a direct role in protein export. Ffh assembled efficiently with mammalian SRP components into a chimeric ribonucleoprotein ["SRP(Ffh)"] and bound at the site normally occupied by SRP54. Like SRP54, the Ffh moiety of the chimeric particle specifically recognized the signal sequence of preprolactin in a photocrosslinking assay. Moreover, Ffh could also act in concert with other SRP components to arrest elongation of preprolactin upon recognition of the signal sequence. In all of these assays, Ffh had approximately the same specific activity as SRP54. In contrast, SRP(Ffh) did not promote the translocation of preprolactin across the membrane of microsomal vesicles, suggesting that Ffh cannot mediate an interaction with a membrane component that is required for the translocation of nascent chains.**

The signal recognition particle (SRP) is a ribonucleoprotein (RNP) that plays an important role in eukaryotic protein secretion *in vitro* (1) and *in vivo* in the yeast *Saccharomyces cerevisiae* (2). In cell-free translation systems SRP recognizes signal sequences of secreted and transmembrane proteins as they emerge from ribosomes and then transiently inhibits polypeptide chain elongation ("elongation arrest") (3, 4). Subsequently, SRP binds to the heterodimeric SRP receptor ("docking protein") (5, 6) in the membrane of the endoplasmic reticulum (ER), which allows resumption of protein synthesis and delivery of ribosome–nascent chain complexes to the translocation apparatus (7, 8).

Studies involving UV crosslinking (9, 10) and selective alkylation (11) have shown that the 54-kDa subunit of SRP (SRP54) contains the signal sequence binding site. Signal sequence recognition as well as binding of the protein to SRP RNA are mediated by the unusually methionine-rich, positively charged C-terminal domain of SRP54 (M domain) (12, 13). The methionine residues have been proposed to play a key role in binding a broad spectrum of hydrophobic signal sequences (14). SRP54 also has an N-terminal domain (G domain) that contains a GTP binding site, and experiments in which this domain has been chemically modified (15) or removed from SRP (16) have suggested that it influences the signal recognition activity of the M domain. Recent studies have suggested that the G domain also plays an essential role in mediating the interaction between SRP and the SRP receptor. A mutant RNP that is missing the G domain of SRP54 is not able to form a salt-stable complex with the SRP receptor (16). Moreover, hydrolysis of GTP bound to the G domain of SRP54 is stimulated by interaction with the SRP receptor (J. Miller, H. Wilhelm, and P.W., unpublished data).

Evidence for a SRP-mediated protein export pathway in bacteria was obtained from the discovery of homologs of components of the mammalian SRP pathway by sequence analysis. These findings were unexpected because extensive genetic studies of bacterial secretion (reviewed in refs. 17 and 18) had neither identified any of the homologs nor suggested the existence of an SRP-dependent or obligatorily cotranslational export pathway. One of the homologs is highly related to SRP54 (14, 19) and was originally identified as an open reading frame (20). This protein, designated Ffh ("*fifty-four homolog*"), contains a homologous G domain as well as the hallmark methionine-rich, basic C-terminal domain and has been shown experimentally to hydrolyze GTP (L. S. Kahng and P.W., unpublished data). The observation that *in vivo* Ffh is complexed with 4.5S RNA (21, 22), which shares a conserved structural motif with mammalian SRP RNA (23, 24), provided evidence that Ffh and SRP54 have common features beyond sequence similarity. Finally, a protein designated FtsY (25) has been shown to share homology with the $\alpha$ subunit of the SRP receptor (14, 19). Intriguingly, purified FtsY stimulates the GTPase activity of Ffh *in vitro*, suggesting that it can interact with Ffh in a manner resembling the interaction of SRP54 with the SRP receptor (A. Farrell, J. Miller, and P.W., unpublished data).

Taken together, the discoveries that *Escherichia coli* Ffh, 4.5S RNA, and FtsY are structurally similar to mammalian SRP54, SRP RNA, and the $\alpha$ subunit of the SRP receptor and that Ffh and 4.5S RNA are assembled *in vivo* in a RNP complex that can interact with FtsY suggest that these components have analogous functions in the respective organisms. This notion is strongly supported by the recent observation that depletion of Ffh *in vivo* causes a significant accumulation of the untranslocated forms of several periplasmic and outer membrane proteins (26), indicating that, like mammalian SRP, the Ffh/4.5S particle plays a role in protein translocation. Moreover, it was recently shown that a signal sequence can be crosslinked to the Ffh/4.5S RNP in a crude *E. coli* cell extract (27). In this study we have characterized the structure and function of Ffh by taking advantage of quantitative biochemical assays that have been established

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Abbreviations: AF, arrested fragment; ER, endoplasmic reticulum; pPL, preprolactin; RNP, ribonucleoprotein; SRP, signal recognition particle; TCA, trichloroacetic acid; EKRMs, EDTA-stripped and salt-washed microsomal membranes.

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for examining the function of SRP. Given the striking homology between Ffh and SRP54 and the ability of SRP54 to bind specifically to *E. coli* 4.5S RNA (21), we reasoned that, conversely, Ffh might bind to mammalian SRP RNA and that a chimeric particle composed of Ffh in place of SRP54 might be functionally analogous to SRP. Our data indicate that a chimeric SRP that contains Ffh in place of SRP54 closely resembles mammalian SRP in many but not all functions.

## MATERIALS AND METHODS

**Rough Microsomal Membranes and RNP Components.** Canine rough microsomal membranes and EDTA-stripped and salt-washed microsomal membranes (EKRMs) were prepared using standard methods (28). SRP protein subunits and SRP RNA were purified from a high salt extract of canine rough microsomes as described (16). The gene encoding Ffh was placed under control of the T7 promoter and expressed at high levels in *E. coli*. An abundant 48-kDa soluble protein that accumulated upon expression was purified and shown to be Ffh on the basis of its ability to bind to 4.5S RNA and to react on Western blots with an antibody raised against a glutathione transferase–Ffh fusion protein (23).

**Reconstitution Reactions and Analysis by Sucrose Gradient Sedimentation.** To assemble RNPs, Ffh or SRP54 plus the desired SRP protein subunits and SRP RNA were mixed at a final concentration of 2 μM each in SRP buffer (50 mM Hepes, pH 7.5/500 mM potassium acetate/5 mM magnesium acetate/1 mM dithiothreitol/0.01% Nikkol detergent) and incubated at 0°C for 10 min and then at 37°C for 10 min (29). To monitor assembly or to purify assembled particles, as much as 25 μl of each reaction mixture was loaded onto 220 μl 5–20% sucrose gradients in SRP buffer. These gradients were formed in 7 × 20 mm tubes by allowing 5%, 10%, 15%, and 20% sucrose layers (55 μl each) to diffuse for 2 hr at 4°C. Samples were centrifuged at 55,000 rpm for 2.5 hr at 4°C in a Beckman TLS 55 rotor and then fractionated. Cold trichloroacetic acid (TCA) was added to each fraction to a final concentration of 10% (wt/vol) and the precipitated proteins were resolved by SDS/PAGE on 10–15% gradient gels.

**UV Crosslinking Assays.** RNPs were added at a 50 nM final concentration to 25 μl wheat germ translation reactions supplemented with $N^{\epsilon}$-(5-azido-2-nitrobenzoyl)lysine tRNA and programed with a truncated preprolactin (pPL) mRNA that encodes the first 86 amino acids of pPL (PPL86). Translation reactions were performed and processed as described (9, 12). After incubation at 26°C, reaction mixtures were UV-irradiated and nascent chains were released from ribosomes with puromycin and 500 mM potassium acetate. Reactions were analyzed by sedimentation on 220 μl 5–20% sucrose gradients in SRP buffer. After SDS/PAGE, the $^{35}$S-labeled translation products were visualized by fluorography.

**Elongation Arrest and Translocation Assays.** SP6 transcriptions and 10 μl wheat germ cell-free translations were performed essentially as described (30), except that each reaction was programed with 0.1 μl of pPL mRNA and a control mRNA encoding a nonsecretory protein. Reassembled RNPs, free SRP54, or free Ffh were diluted to the appropriate concentration in SRP buffer and added to the reaction mixtures. In elongation arrest assays, reactions were performed at 26°C for 20 min and then stopped by the addition of cold TCA. TCA-precipitated proteins were then resolved by SDS/PAGE. The $^{35}$S-labeled translation products were visualized by autoradiography and the level of synthesis of each protein was quantitated using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Percent elongation arrest was defined previously (31). The level of synthesis of the control nonsecretory protein, which was not measurably affected by the addition of any of the RNPs or free proteins, was used as an internal standard to normalize each reaction. Translocation reaction mixtures contained 1 equivalent of EKRMs (28). Reactions were performed at 26°C for 45 min, stopped by the addition of cold TCA, and then processed as described above. Percent translocation was defined as the percentage of pPL synthesized in the absence of any RNP that was converted to prolactin (31).

## RESULTS

**Assembly of Ffh into a Chimeric Particle.** To test the hypothesis that Ffh can bind to mammalian SRP RNA in place of SRP54, reconstitution reactions were performed in which Ffh or, as a control, SRP54 was mixed with SRP RNA and the five other SRP protein subunits under stringent particle assembly conditions (29). The products of these reactions were analyzed by sucrose gradient sedimentation (Fig. 1 *A* and *B*). In each case a particle of the size of native SRP was observed that contained roughly equimolar amounts of all of the added protein components (lanes 8 and 9). Ffh assembled as efficiently as SRP54 into an 11S particle, which is henceforth referred to as SRP(Ffh). Whereas most of each of the other protein subunits of SRP was found in particles (lanes 8 and 9), some of the Ffh and SRP54 remained in a free form and was found at the top of the gradients (lanes 3 and 4). The presence of the unbound protein suggests that either the concentration of Ffh and SRP54 was slightly underestimated or that the two protein homologs bind to SRP RNA slightly less efficiently than the other subunits.

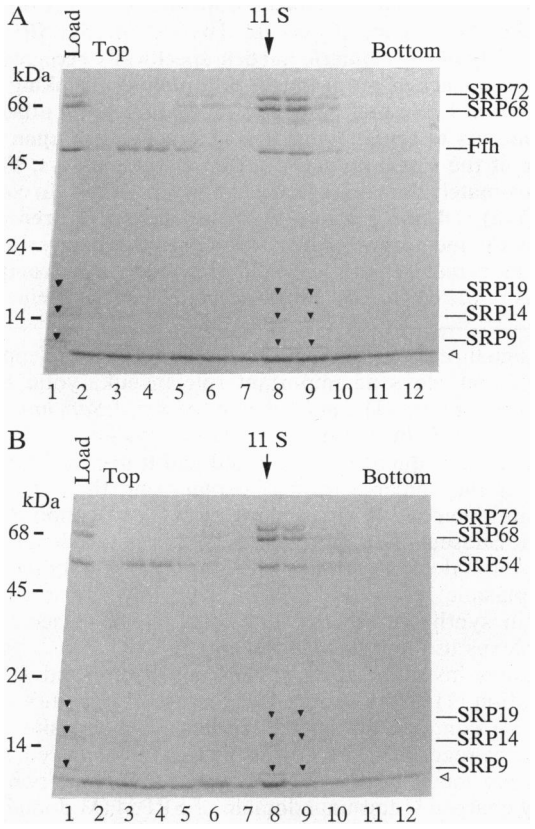


FIG. 1. Sucrose gradient fractionation of reconstitution reactions in which *E. coli* Ffh (*A*) or canine SRP54 (*B*) was incubated with SRP RNA plus the five other protein subunits of SRP. Twenty microliters of 25 μl reconstitution reaction mixtures was loaded onto each gradient. After centrifugation, 11 equal fractions were collected and TCA precipitated in the presence of aprotinin (band labeled ◁) as a carrier protein. TCA precipitates were subjected to SDS/PAGE (lanes 2–12), and proteins were visualized by Coomassie blue staining. Five microliters of each reaction mixture was saved and run separately on the gel (lane 1, Load).

The observation that Ffh did not interfere with the binding of the other subunits to SRP RNA suggested that it bound at a specific site, most likely where SRP54 normally binds. To test this idea directly, we performed reconstitution reactions in which increasing amounts of Ffh were added to compete with SRP54 for binding to SRP RNA. Reaction mixtures were sedimented through sucrose gradients that were subsequently divided into a top fraction containing free proteins (Fig. 2, lanes 1, 3, and 5; "F") and a bottom fraction containing assembled 11S particles (Fig. 2, lanes 2, 4, and 6; "B"). The appearance of virtually all of the 9-, 14-, 19-, 68-, and 72-kDa SRP subunits in the bottom fractions indicated that efficient particle assembly occurred in all reactions (SRP68 is shown; see Fig. 2, lanes 2, 4, and 6). In the absence of Ffh, most of the SRP54 was bound to 11S particles (Fig. 2, lanes 1 and 2). When an equimolar amount of Ffh was added but the concentration of all other components was kept the same, approximately half of the 11S particles contained SRP54 and half contained Ffh (Fig. 2, lanes 3 and 4). As the concentration of Ffh was increased to 10 times that of the other components, the proportion of particles containing SRP54 was greatly reduced, and the proportion containing Ffh exceeded 90% (Fig. 2, lanes 5 and 6). These results not only confirm the notion that Ffh binds to SRP RNA at the same location as SRP54 but also suggest that Ffh has approximately the same affinity for SRP RNA as its mammalian counterpart.

**Recognition of a Signal Sequence by SRP(Ffh).** We first used a crosslinking approach to assess the ability of the Ffh moiety of SRP(Ffh) to interact with the signal sequence of pPL and thereby functionally replace SRP54. This method provides direct physical evidence for signal sequence binding. Reconstituted SRP or SRP(Ffh) was added to a translation system supplemented with $N^{\epsilon}$-(5-azido-2-nitrobenzoyl)lysine tRNA to incorporate photoreactive lysine residues into the signal sequence of pPL at positions −27 and −22 (9, 12). A truncated synthetic pPL mRNA was translated in each reaction. The binding of SRP to the pPL signal sequence produces an ≈70-amino acid elongation-arrested fragment (AF). The only lysines in the AF that are exposed outside the ribosomes, and that are therefore accessible for crosslinking, are contained within the signal sequence. After termination of the translation reactions, samples were irradiated with UV light and the $^{35}$S-labeled nascent polypeptide chains were released from ribosomes with puromycin and high salt (9, 12). Free and crosslinked products were then separated by sucrose gradient sedimentation.

Analysis of the products of the crosslinking reactions clearly shows that SRP(Ffh) recognized the pPL signal sequence. When SRP(Ffh) was present in the reaction mixture, a crosslinked product of ≈56 kDa was observed that sedimented at the position of SRP (Fig. 3*A*, lanes 7–9). The size of the product suggests that it is comprised of the AF covalently linked to Ffh. This conclusion was supported by the observation that the product could be immunoprecipitated with an Ffh-specific antibody (data not shown). When SRP was present in the reaction mixture, an ≈62-kDa product identical in size to the previously characterized SRP54-AF crosslinked product (9, 12) was observed to sediment at 11 S (Fig. 3*B*, lanes 7–9). Densitometric analysis of the autoradiographs shown in Fig. 3 showed that the efficiency of the crosslinking was about equal for SRP and SRP(Ffh), suggesting that Ffh and SRP54 have similar affinities for the pPL signal sequence. As previously observed (9, 12), some uncrosslinked AF, which is highly hydrophobic, associated nonspecifically with molecules in the extract and consequently appeared in all gradient fractions. A small amount of full-length PPL86, which migrates slightly slower than the AF, could also be observed in some of the fractions.

To characterize the chimeric particle further, we next tested its activity in an elongation arrest assay. Elongation arrest is strictly dependent on the binding of SRP to the signal sequence and thus provides an independent and more quantitative means of measuring signal sequence recognition. Moreover, elongation arrest requires communication of the signal recognition event to the domain of SRP that contains the heterodimeric SRP9/14 subunit (31, 32) and presumably

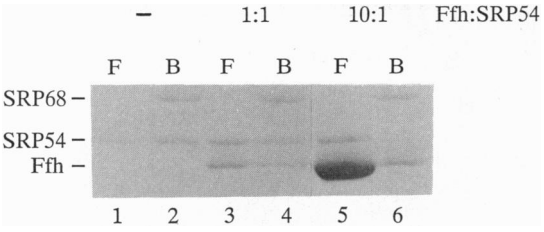


FIG. 2. Competition between Ffh and SRP54 for binding to SRP RNA. Reconstitution reactions that contained equimolar concentrations of all SRP components (2 μM) and varying amounts of Ffh were performed under standard conditions. The reaction mixtures were then subjected to sucrose gradient sedimentation and two 100-μl fractions, containing free proteins (F) and proteins bound to 11S particles (B), respectively, were collected. Proteins were TCA precipitated, resolved by SDS/PAGE, and visualized by Coomassie blue staining. Reaction mixtures contained no Ffh (lanes 1 and 2), equimolar Ffh (lanes 3 and 4), or a 10-fold molar excess of Ffh (lanes 5 and 6).

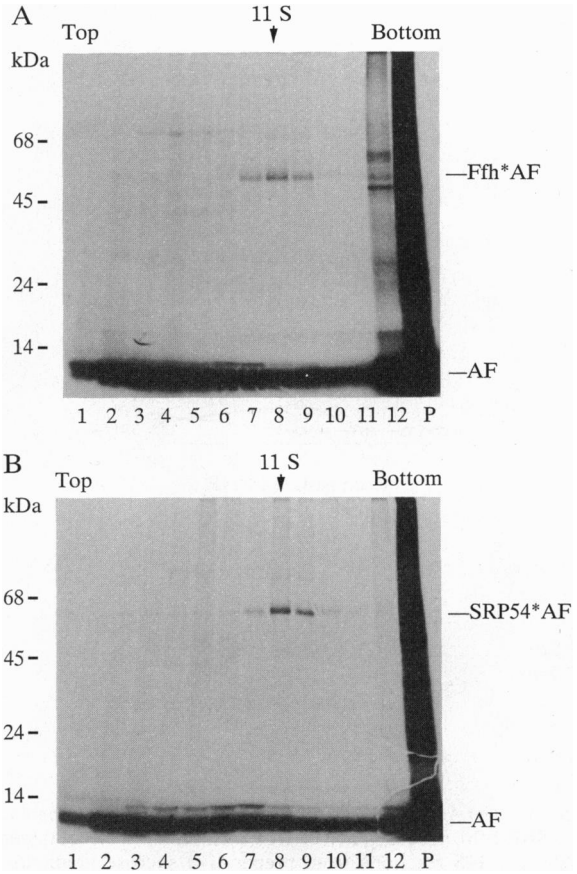


FIG. 3. Sucrose gradient sedimentation of UV crosslinking products. SRP(Ffh) (*A*) or SRP (*B*) was added to UV crosslinking reaction mixtures and the products were analyzed by sucrose gradient sedimentation. Twelve fractions (lanes 1–12) plus a pellet (lane P) were collected. TCA-precipitated proteins were resolved by SDS/PAGE and labeled bands were visualized by fluorography. The positions of the free AF and the AF crosslinked to Ffh (Ffh*AF) and SRP54 (SRP54*AF) are indicated. Most of the labeled products that were recovered in the pellet fractions have been shown to be nonspecific and independent of protein synthesis and UV irradiation (9, 12).

places additional constraints on SRP54 structure or function. Addition of gradient-purified SRP(Ffh) to wheat germ cell-free translation reactions programed with synthetic bovine pPL mRNA and α-globin mRNA to yield a control nonsecretory protein indeed resulted in a concentration-dependent, specific inhibition of pPL synthesis (Fig. 4*A*). SRP(Ffh) was only slightly less effective in the assay than a control SRP reconstituted from mammalian components. This observation supports the idea that SRP54 and Ffh have similar affinities for the pPL signal sequence. Furthermore, neither free SRP54 nor free Ffh affected the translation of pPL (Fig. 4*A*), indicating that the elongation arrest required participation of other SRP subunits. As expected, a partially reconstituted SRP lacking SRP54 was also completely inactive in this assay (data not shown) (31).

If the chimeric particle affects translation by the same mechanism as SRP, then the SRP(Ffh)-mediated elongation arrest should be dependent on the presence of the SRP9/14 heterodimer. To test this notion, reconstitution reactions were performed in which SRP54 or Ffh and the other SRP subunits except SRP9/14 were mixed together to yield partially reconstituted RNPs, termed SRP(−9/14) and SRP(Ffh, −9/14), respectively. The RNPs were added to translation reaction mixtures at a concentration of 20 nM and assayed for elongation arrest activity. Reconstituted SRP and SRP(Ffh) efficiently inhibited the synthesis of pPL (Fig. 4*B*, compare lane 1 with lanes 2 and 4), whereas SRP(−9/14) and SRP(Ffh, −9/14) did not (Fig. 4*B*, compare lane 1 with lanes 3 and 5). The fully reconstituted particles were approximately as active as those prepared for other experiments (e.g., see Fig. 4*A*). None of the RNPs affected the synthesis of a control nonsecretory protein, a truncated form of B cyclin. As expected from previous results (31, 32), SRP(−9/14) was fully active in a translocation assay, thereby demonstrating that it was a functional particle (data not shown).

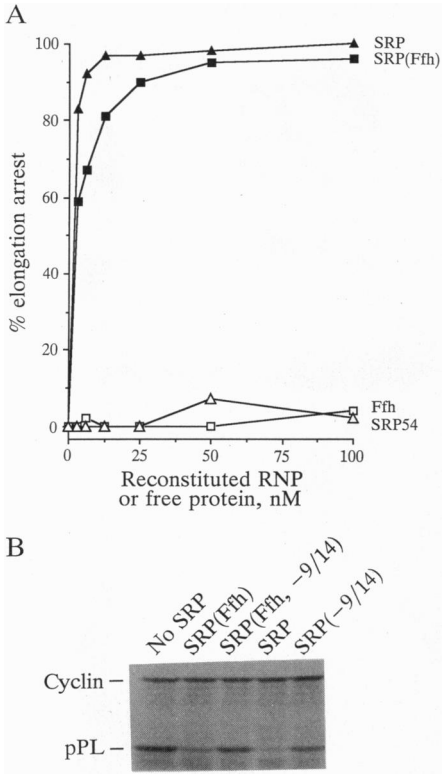


FIG. 4. (*A*) Assay of elongation arrest mediated by reconstituted SRP, SRP(Ffh), free SRP54, or free Ffh. RNPs were obtained by pooling the 11S fractions from preparative sucrose gradients. Increasing concentrations of each purified RNP or free protein were added to translation reactions programed with bovine pPL mRNA and control *Xenopus* α-globin mRNA. (*B*) Assay of elongation arrest mediated by SRP(Ffh) (lane 2), SRP(Ffh, −9/14) (lane 3), reconstituted SRP (lane 4), or SRP(−9/14) (lane 5). The level of protein synthesis in the absence of SRP is shown in lane 1. Translation reactions programed with bovine pPL mRNA and control sea urchin B cyclinΔ90 mRNA (4) were performed in the presence of an RNP added at a 20 nM final concentration. TCA-precipitated proteins from each reaction were resolved by SDS/PAGE and the translation products were visualized by autoradiography.

**Test of SRP(Ffh) Activity in a Translocation Assay.** Given the observation that Ffh could replace SRP54 in signal sequence recognition assays, it was of interest to determine whether SRP(Ffh) could also perform subsequent stages of SRP function and promote the translocation of pPL across microsomal membranes to yield processed, mature prolactin. Promotion of translocation would be dependent on the ability of Ffh to mimic the interaction between SRP54 and the SRP receptor. In contrast to SRP, however, SRP(Ffh) was inactive in translocation assays (Fig. 5). Moreover, whereas interaction between SRP and the ER-associated SRP receptor leads to release of elongation arrest, the SRP receptor present in the microsomal vesicles in this assay did not release the SRP(Ffh)-mediated arrest (Fig. 5*B*, lane 2). Thus the inactivity of the chimeric SRP(Ffh) in the translocation assay may be due to an inability of Ffh to interact with the SRP receptor. These observations suggest that although SRP54 and Ffh have closely related sequences, the specific contacts that allow SRP54 to interact with the SRP receptor are not evolutionarily conserved.

## DISCUSSION

Because Ffh can replace mammalian SRP54 in reconstitution, crosslinking, and elongation arrest assays, we conclude

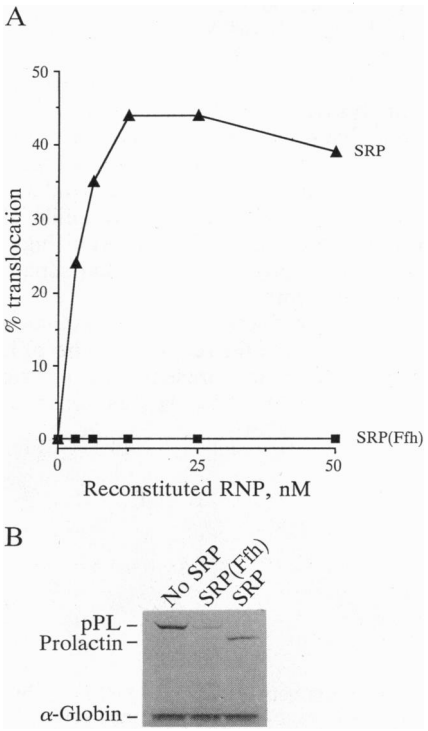


FIG. 5. (*A*) Translocation of pPL across microsomal vesicles facilitated by reconstituted SRP or SRP(Ffh). Increasing concentrations of each RNP were added to *in vitro* translation reaction mixtures containing 1 equivalent of EKRMs. Reactions were programed with bovine pPL mRNA and control *Xenopus* α-globin mRNA. (*B*) Autoradiograph of labeled products from a translocation assay in which no RNP (lane 1), 50 nM SRP(Ffh) (lane 2), or 50 nM reconstituted SRP (lane 3) was added to the reaction mixture.

that in addition to amino acid sequence homology, Ffh and SRP54 share significant common structural features. Moreover, the observation that SRP(Ffh) can promote elongation arrest nearly as well as SRP suggests that Ffh binds signal sequences sufficiently tightly to transmit the information to the remainder of SRP(Ffh) and the ribosome and that Ffh and SRP54 bind signal sequences via similar mechanisms.

Given that the M domain of SRP54 has been shown to bind to RNA and signal sequences, it is likely that the homologous domain of Ffh was responsible for much of the activity observed in our assays. Intriguingly, the M domains of the two proteins are somewhat different in size and share only 38% amino acid identity. Secondary structure predictions, however, suggest that both M domains contain similar structural elements (14). In both proteins several positively charged amphipathic $\alpha$-helices may be brought together to form an RNA-binding site on one surface and a signal sequence binding pocket composed of conformationally flexible methionines and other large hydrophobic amino acids on the other. Recently, it has been shown that the G domain may also influence the efficiency of signal sequence recognition. An SRP mutant that is missing the G domain of SRP54 binds signal sequences but is approximately 5- to 10-fold less active than wild-type SRP in crosslinking and elongation arrest activities (16). One explanation of these results is that the G domain promotes tighter binding of the signal sequence to the M domain. Our observation that SRP and SRP(Ffh) have very similar levels of activity in signal sequence recognition assays thus suggests that the region of the G domain that influences signal sequence binding is evolutionarily conserved.

Although the Ffh G domain can perform the role of its mammalian homolog in signal recognition, our data imply that in one respect that domain is too divergent from the SRP54 G domain to replace its function completely. Previous work indicates that the G domain of SRP54 is required for proper SRP–SRP receptor interaction (16). A simple interpretation of our results is that Ffh cannot bind to the mammalian SRP receptor, although an inability to interact with another ER membrane component cannot be excluded. As Ffh can interact with FtsY *in vitro*, it seems likely that the SRP54–SRP receptor interaction is conserved in each species and that it may not be possible to reconstitute this interaction from heterologous components.

The results presented here provide a link between sequence homology data and genetic studies by showing directly that *E. coli* Ffh can interact with a signal sequence within a nascent polypeptide substrate as efficiently and as selectively as its mammalian counterpart. Consistent with our results, complementary methods have been used to suggest that Ffh binds to a wild-type but not a mutant pPL signal sequence (27). Because signal sequences constitute a large diverse family of sequences whose principal features have been conserved throughout evolution (33), the pPL signal sequence is structurally indistinguishable from bacterial signal sequences that Ffh would encounter *in vivo*. Indeed, a recent study has suggested that, conversely, Ffh and SRP54 can bind with micromolar affinity to the *E. coli* lamB signal sequence *in vitro* (J. Miller, H. Wilhelm, and P.W., unpublished data), reinforcing the notion that in this system signal sequences are highly interchangeable.

Our results add to the increasing body of evidence that not only is Ffh related to SRP54 by sequence but that it, as part of an RNP complex, also plays an analogous role in protein targeting. A likely explanation as to why Ffh was not identified in genetic screens for components of the export machinery is that Ffh/4.5S RNP-dependent and -independent targeting pathways may exist in *E. coli* (23). A clear precedent for the existence of redundant protein targeting pathways has been established in the yeast *S. cerevisiae* (2). The observation that depletion of Ffh or yeast SRP *in vivo* affects the translocation of different proteins to variable degrees (2, 26) strongly supports the model that many proteins can use an SRP-independent targeting pathway, albeit often with much reduced efficiencies. Intriguingly, as in *E. coli*, genetic studies in yeast initially failed to identify SRP but were successful when a presumably more SRP-dependent protein substrate was used (34). A role for yeast SRP in secretion was established upon identification of an SRP54 homolog and subsequent disruption of the gene encoding it (2). These studies demonstrate the usefulness of phylogenetically comparative approaches that were deemed inappropriate when hints of the existence of an SRP-dependent protein targeting pathway in *E. coli* first emerged from primary structure similarities (35, 36).

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