Interaction of E. coli Ffh/4.5S ribonucleoprotein and FtsY mimics that of mammalian signal recognition particle and its receptor

Joshua D. Miller, Harris D. Bernstein* & Peter Walter†

Department of Biochemistry and Biophysics, University of California Medical School, San Francisco, California 94143-0448, USA

The mechanism of protein translocation across the endoplasmic reticulum membrane of eukaryotic cells and the plasma membrane of prokaryotic cells are thought to be evolutionarily related 1. Protein targeting to the eukaryotic translocation apparatus is mediated by the signal recognition particle (SRP), a cytosolic ribonucleoprotein, and the SRP receptor, an endoplasmic reticulum membrane protein 2. During targeting, the 54K SRP subunit (Mr, 54,000; SRP54), a GTP-binding protein 3, binds to signal sequences 4, and then interacts with the alpha unit of the SRP receptor (SRα), another GTP-binding protein 5. Two proteins from Escherichia coli, Ffh and FtsY, structurally resemble SRP54 and SRα 6,7,11,12. Like SRP54, Ffh is a subunit of a cytosolic ribonucleoprotein that also contains the E. coli 4.5S RNA 3,11,13. Although there is genetic and biochemical evidence that the E. coli Ffh/4.5S ribonucleoprotein has an SRα-like function 14,19, there is no evidence for an SRα-like role for FtsY. Here we show that the Ffh/4.5S ribonucleoprotein binds tightly to FtsY in a GTP-dependent manner. This interaction results in the stimulation of GTP hydrolysis which can be inhibited by synthetic signal peptides. These properties mimic those of mammalian SRP and its receptor, suggesting that the E. coli Ffh/4.5S ribonucleoprotein and FtsY have functions in protein targeting that are similar to those of their mammalian counterparts.

To test for an interaction between the Ffh/4.5S ribonucleoprotein (RNp) and FtsY, Ffh and 4.5S RNA were purified from overproducing strains and reconstituted into an RNp. FtsY was purified as a fusion protein with glutathione S-transferase (FtsY-GST) and immobilized on a glutathione-affinity resin, which was then incubated with Ffh/4.5S RNp in the presence of either GDP or the non-hydrolysable GTP analogue GMP-PPN. ATP was included in these reactions because both Ffh and FtsY contain GTP-binding domains which might regulate their interaction. More than 90% of the Ffh co-fractionated with the FtsY-GST resin in the presence of GMP-PPN; most of the Ffh was recovered in the supernatant fraction in the presence of GDP (Fig. 1). Similarly, most of the Ffh was recovered in the supernatant fraction in the presence of the non-hydrolysable ATP analogue AMP-PPN (not shown), indicating that the interaction requires guanosine triphosphate. Free Ffh protein failed to bind to FtsY-GST resin even in the presence of GMP-PPN (Fig. 1), indicating that FtsY binding requires both Ffh and 4.5S RNA. Neither Ffh/4.5S RNp nor free Ffh bound to a control resin containing immobilized GST, indicating that binding is specific for FtsY (Fig. 1).

Interestingly, no binding was observed when the reaction shown in Fig. 1 was performed with GTP instead of GMP-PPN (not shown), indicating that GTP might be hydrolysed during the reaction. We therefore analysed the ability of these components to hydrolyse GTP. As shown in Fig. 2, purified Ffh hydro-

* Present address: Genetech and Biochemistry Branch/NIDDK, National Institutes of Health, Building 10, Room 90.15, Bethesda, Maryland 20892, USA.
† To whom correspondence should be addressed.
FIG. 1 Ffh/4.5S binds to immobilized FtsY–GST in the presence of GDP-PNP. Purified Ffh protein and Ffh/4.5S RNP were assayed for their ability to bind to FtsY–GST or GST immobilized on glutathione–agarose beads in the presence of either GDP or GDP-PNP. After binding, the beads were collected by centrifugation, and the proteins recovered in the pellet (p) and supernatant (s) fractions were analysed on SDS–polyacrylamide gels and visualized by staining with Coomassie blue. The amount of Ffh protein in each lane is expressed as a percentage of the total Ffh protein recovered in pellet and supernatant fractions.

METHODS. Ffh protein and 4.5S RNA were prepared as described. The FtsY–GST fusion protein was produced by fusing amino acids 486–494 of FtsY in-frame to the C terminus of GST in the pGEX1 vector. The region of strong homology between FtsY and SrfA encompasses amino acids 197–494 and includes the GTP-binding domain. Purified FtsY–GST fusion protein (10 pmol) or GST protein was incubated under constant mixing with 50 μl of a 50% suspension of glutathione–agarose beads (Sigma) in phosphate-buffered saline for 1 h at room temperature. The resin was washed with buffer A containing 50 mM triethanolamine/acetic acid (TEA), pH 7.5, 25 mM potassium acetate, 2.5 mM Mg(OOC·CH₂)₂, 0.1 mM EGTA, 0.1 mM EDTA, 1 mM dithiothreitol, 0.1% Nikkol detergent (Nikko Chemicals, Tokyo) and then added to 100 μl of the same buffer containing 5 pmol of Ffh or Ffh/4.5S RNP. GDP or GDP-PNP was added to a final concentration of 1 mM where indicated. Reactions were incubated with constant mixing at room temperature for 30 min. The resin was pelleted by centrifugation in a microfuge for 30 s and the supernatant removed. Proteins in the supernatant were precipitated with an equal volume of 30% trichloroacetic acid and solubilized in SDS sample buffer. The resin was washed with buffer A containing either GDP or GDP-PNP at 1 mM, and bound proteins were then solubilized by boiling the beads in SDS sample buffer. Quantification was by laser scanning densitometry of the stained gel. The Ffh/4.5S RNP was prepared by incubating a solution containing 10 μM Ffh and 20 μM 4.5S RNA in buffer B containing 50 mM TEA, 500 mM potassium acetate, 5 mM Mg(OOC·CH₂)₂, 1 mM DTT, 0.01% Nikkol and 10% glycerol for 10 min on ice followed by 10 min at 37°C.

FIG. 2 Enhanced GTP hydrolysis results from interaction of Ffh/4.5S RNP and FtsY. Purified Ffh/4.5S RNP and purified Ffh protein were assayed for the ability to hydrolyse GTP in the presence or absence of FtsY–GST or GST.

METHODS. Reactions (100 μl) were incubated in buffer A containing 10% glycerol. Ffh/4.5S RNP complexes were formed as described in Fig. 1 legend. Ffh was present at 5 nM, 4.5S RNA at 10 nM, FtsY–GST and GST at 150 nM. [γ-32P]GTP (0.5 μCi ICN) was added at 0.5 μM. After a 20-min incubation at 25°C an 8 μl aliquot of the reaction was mixed with 200 μl of a 5% suspension of activated charcoal (Sigma) in 20 mM phosphoric acid, incubated on ice for 10 min and then centrifuged for 10 min at maximum speed in a microfuge to pellet the charcoal and bound nucleotide. A 100 μl aliquot of the supernatant fraction containing the phosphate liberated in the reaction was analysed by Cerenkov counting in a liquid scintillation counter and corrected for background counts from a reaction performed with buffer only. The Michaelis constant of the Ffh/4.5S RNP for GTP hydrolysis was 0.4 μM; the rate of hydrolysis was linear during the 20-min incubation.
FIG. 3 Synthetic signal peptides inhibit GTP hydrolysis.
a. Effect of titrating synthetic signal peptides into GTP hydrolysis reactions containing Ffh/4.5S RNP and FtsY. b. Effect of titrating synthetic signal peptide into GTP hydrolysis reactions containing Ffh/4.5S RNP in the absence of FtsY. Reactions are described in Fig. 2 legend, except that addition of synthetic signal peptide was preincubated for 30 min at 25 °C with the appropriate synthetic signal peptide before addition of GTP. Four different peptides were used: wild-type peptide (E); sequence MMILRLPLA-VAAVAGVMSQAAM, corresponding to the signal sequence of LamB, a bacterial outer membrane protein; deletion mutant peptide (A), corresponding to the LamB signal sequence with residues L10–A13 deleted; r1 (A) (corresponding to the deletion mutant peptide with a G17C change) and r2 (Δ) (corresponding to the deletion mutant peptide with a P9L change) peptides, which correspond to second-site, single-amino-acid revertants of the deletion mutant. The synthetic wild-type peptide can readily adopt an α-helical conformation as analysed by circular dichroism spectroscopy. The missing four amino acids in the deletion mutant peptide cause a proline and a glycine residue to be brought closer so that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.

that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix, and does not function as a signal peptide in vivo. Both r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences in vivo.