26. Guan, K. L. & Dixon, J. E. *Analyt. Biochem.* **192,** 262–267 (1991).
27. Smith, D. B. & Johnson, K. S. *Gene* **67,** 31–40 (1988).
28. Hata, Y., Davletov, B., Petrenko, A. G., Jahn, R. & Südhof, T. C. *Neuron* **10,** 307–315 (1993).
29. Robinson, P. J. *et al. Nature* **365,** 163–166 (1993).
30. Martin, R. G. & Ames, B. N. *J. biol. Chem.* **236,** 1372–1379 (1961).

ACKNOWLEDGEMENTS. We thank I. Leznicki, S. Afendis, C. Moomaw, E. Borowicz and A. Roth for technical assistance, and R. Jahn (New Haven, Connecticut) and M. Wilson (La Jolla, California) for antibodies. This work was supported by a postdoctoral fellowship to Y.H. from the Human Frontier Science Program.

GTP binding and hydrolysis by the signal recognition particle during initiation of protein translocation

Joshua D. Miller, Heike Wilhelm, Lila Gierasch*, Reid Gilmore† & Peter Walter

Department of Biochemistry and Biophysics,
University of California Medical School, San Francisco,
California 94143-0448, USA
* Department of Pharmacology,
University of Texas Southwestern Medical Center, Dallas,
Texas 75235-9041, USA
† Department of Biochemistry, University of Massachusetts,
Worchester, Massachusetts 01655-0001, USA

THE signal recognition particle (SRP) consists of one RNA and six protein subunits[1,2]. The N-terminal domain of the 54K subunit contains a putative GTP-binding site, whereas the C-terminal domain binds signal sequences and SRP RNA[3–7]. Binding of SRP to the signal sequence as it emerges from the ribosome creates a cytosolic targeting complex containing the nascent polypeptide chain, the translating ribosome, and SRP[8]. This complex is directed to the endoplasmic reticulum membrane as a result of its interaction with the SRP receptor[9–11], a membrane protein composed of two subunits, SRα and SRβ, each of which also contains a GTP-binding domain[12,13]. In the presence of GTP, SRP receptor binding to SRP causes the latter to dissociate from both the signal sequence and the ribosome[13,14]. GTP is then hydrolysed so that SRP can be released from the SRP receptor and returned to the cytosol[15]. Here we show that the 54K subunit (M_r 54,000) of SRP (SRP54) is a GTP-binding protein stabilized in a nucleotide-free state by signal sequences, and that the SRP receptor both increases the affinity of SRP54 for GTP and activates its GTPase. We propose that nucleotide-mediated conformational changes in SRP54 regulate the release of signal sequences and the docking of ribosomes at the endoplasmic reticulum.

To analyse the role of GTP in protein targeting to the endoplasmic reticulum, (ER), we pursued the observation that the interaction of SRP with its receptor induces GTP hydrolysis[16]. Purified SRP had no detectable GTPase activity and the purified SRP receptor hydrolysed GTP only poorly, but SRP and its receptor together hydrolysed GTP about ten times faster than the receptor alone (Fig. 1).

To determine which components of SRP interact with the SRP receptor to increase GTP hydrolysis, SRP was dissociated into its subunits under non-denaturing conditions. The dissociated proteins can be purified and reconstituted with SRP RNA to regenerate fully functional SRP[17]. Surprisingly, a partially reconstituted SRP containing only the 19K and 54K subunits and SRP RNA was almost as active as native SRP in the presence of SRP receptor (Fig. 1), but was inactive without it (not shown). Moreover, omission of SRP19, which stabilizes the binding of SRP54 to SRP RNA[18], reduced activity only slightly (Fig. 1). SRP RNA and SRP54, however, were both essential (Fig. 1), indicating that the complex of SRP54 and SRP RNA is both necessary and sufficient to elicit GTP hydrolysis in conjunction with the SRP receptor. All subsequent analysis was carried out with this 'minimal' SRP [SRP(54/RNA)].

To determine whether SRP54, SRα or SRβ catalyses the GTP hydrolysis, we monitored nucleotide binding to the proteins by ultraviolet crosslinking[19,20]. This approach allowed us to detect the relatively low-affinity binding of these proteins to GTP and to measure GTP binding to each of the three GTP-binding proteins in the reaction independently. When SRP receptor was incubated with [α-^{32}P]GTP and crosslinked using ultraviolet radiation, both SRα and SRβ were labelled (Fig. 2*a*, lane 1). Similarly, when SRP(54/RNA) was used, SRP54 was labelled (Fig. 2*a*, lane 2). SRP54 was crosslinked to the same extent when SRP RNA was omitted (not shown), and was the only SRP protein labelled when intact SRP was crosslinked, indicating that the labelling reaction was specific for GTP-binding proteins (not shown). SRP54, SRα and SRβ must therefore be GTP-binding proteins, as predicted from their amino-acid sequences.

When SRP(54/RNA) and SRP receptor were mixed to stimulate GTP hydrolysis (Fig. 1, reaction 7), GTP crosslinking to SRP54 was dramatically stimulated (Fig. 2*a*, lane 3), but there was no significant change in crosslinking to either SRα or SRβ.

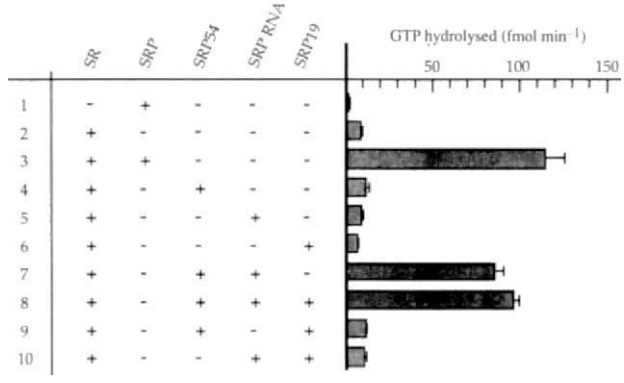

FIG. 1 Stimulated GTPase activity of SRP and partially reconstituted SRPs. GTP hydrolysis rates are the average of three independent experiments; the standard deviation of the measurements is indicated. The reaction was linear with time over the period analysed. tRNA could not replace SRP RNA in this reaction. In the presence of the SRP receptor (SR), all partially reconstituted SRPs that contained both the SRP RNA and SRP54 were about equally active; that is, the additional presence of SRP68/72 and/or SRP9/14 had no effect on the reaction. In the absence of SR, purified SRP proteins, SRP RNA and all partially reconstituted SRPs were inactive.
METHODS. SRP and SRP receptor were purified as described[28,29] as were the individual SRP components[18]. Partially reconstituted SRPs were formed by mixing components at a concentration of 500 nM each in 300 mM potassium acetate, 5 mM $Mg(OOCCH_3)_2$, 25 mM HEPES pH 7.5, 0.01% Nikkol detergent, (octaethyleneglycol mono-*n*-dodecyl ether; Nikko Chemical, Tokyo), 1 mM dithiothreitol (DTT). After mixing, reactions were incubated for 10 min on ice, 10 min at 37 °C and then kept on ice until the GTPase reaction. GTPase reactions (20 µl) contained 20 nM SR and/or either 20 nM SRP or 20 nM partially reconstituted SRPs in GTP hydrolysis buffer containing 50 mM $KOOCCH_3$, 50 nM triethanolamine, pH 7.5, 2.5 mM $Mg(OOCCH_3)_2$, 0.5% Nikkol detergent, 1 mM DTT. GTP 1 µM included 0.5 mCi ml^{-1} [γ-^{32}P]GTP (ICN). Reactions were incubated at 25 °C for 20 min and assayed by charcoal adsorption followed by Cerenkov counting.

There was no stimulation of GTP crosslinking to SRP54 when SRP RNA was omitted (not shown). Thus, GTP crosslinking to SRP54 (Fig. 2) and GTP hydrolysis (Fig. 1) are both stimulated by a functional interaction between SRP(54/RNA) and SRP receptor.

Most guanine nucleotide-binding proteins bind GDP tightly and are stimulated to bind GTP by specific guanine-nucleotide-releasing proteins (GNRPs), which decrease their affinity for GDP, creating a transiently empty nucleotide-binding site for GTP to enter[21]. To test whether SRP receptor was acting as a GNRP, we tested its effect on the ability of SRP54 to bind GDP. SRP(54/RNA) was crosslinked to [α-^{32}P]GDP in the presence of different concentrations of unlabelled competitor nucleotide. The concentration of nucleotide required to inhibit the crosslinking of SRP54 is a measure of its apparent affinity for the protein. As expected, crosslinking of SRP54 to [α-^{32}P]GDP could be competed with unlabelled GDP (Fig. 2*c*, open diamonds). Surprisingly, addition of receptor to these reactions did not alter the concentration of GDP required to inhibit the crosslinking (Fig. 2*c*, filled diamonds), indicating that SRP does not function as a GNRP to decrease the apparent affinity of SRP54 for GDP.

When unlabelled GTP was added to compete with [α-^{32}P]-GDP for crosslinking, (Fig. 2*d*, triangles), roughly tenfold higher concentration of unlabelled nucleotide was required for half-maximal inhibition (IC_{50}). In the absence of SRP receptor, the apparent affinity of SRP54 for GTP is thus ~10-fold lower than that for GDP. As SRP receptor stimulates GTP hydrolysis, a similar experiment with the receptor present would be difficult to interpret. We therefore used a non-hydrolysable GTP analogue, GMP-PNP, which has an apparent affinity for SRP54 which is threefold lower than GTP in the absence of the receptor (Fig. 2*d*, open squares). When SRP receptor was added to these reactions, the IC_{50} for GMP-PNP decreased ~90-fold (Fig. 2*d*, filled squares), indicating that the receptor substantially increased the apparent affinity of SRP54 for GTP. SRP receptor, therefore, does not act as a GNRP to release GDP, but instead appears to be a 'guanine-nucleotide-loading protein' which promotes GTP binding by increasing the affinity of SRP54 for GTP.

As SRP receptor stimulates the binding of GMP-PNP to SRP54 under conditions that lead to GTP hydrolysis, SRP54 may be responsible for nucleotide hydrolysis. If so, the labelled nucleotide crosslinked to SRP54 (Fig. 2*a*, lane 3) may be hydrolysed to GDP. The experiment shown in Fig. 2*a* was therefore repeated with [γ-^{32}P]GTP. The ^{32}P-labelled γ-phosphate would be released upon hydrolysis, resulting in the loss of the radiolabel from the crosslinked protein. SRα, SRβ and SRP54 were labelled with [γ-^{32}P]GTP to the same extent as they were with [α-^{32}P]GTP when reactions were done with either SRP receptor

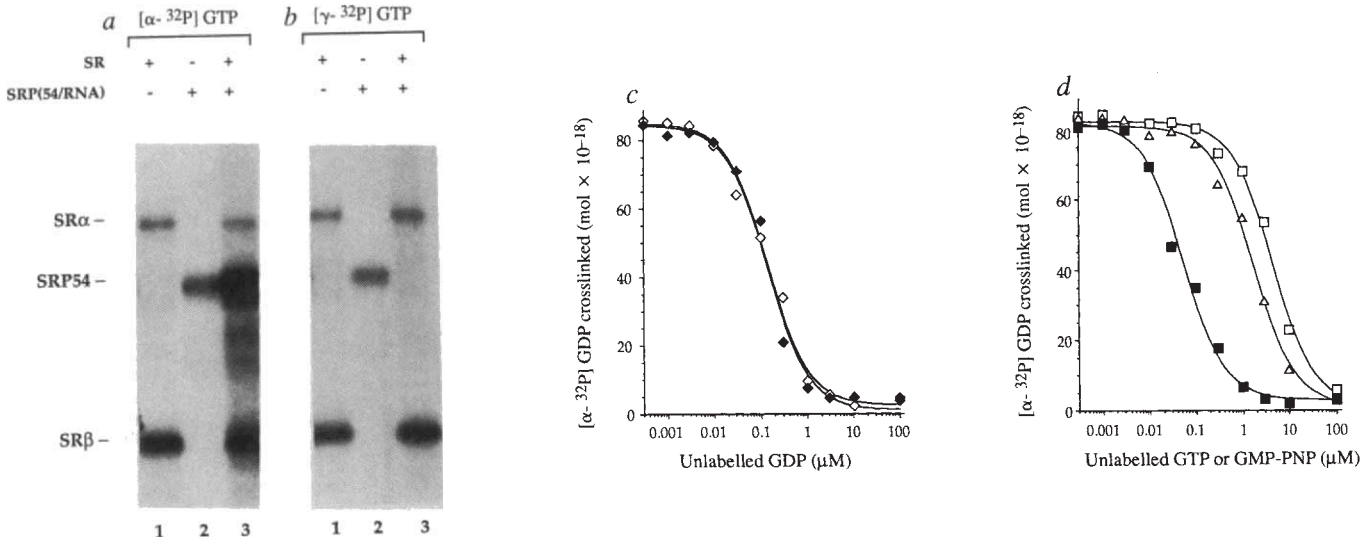

FIG. 2 SRP receptor both increases the affinity of SRP54 for GTP and functions as a GTPase-activating protein. *a*, SR (lane 1), SRP(54/RNA) (lane 2) or both complexes combined (lane 3) were ultraviolet-crosslinked to [α-^{32}P]GTP. *b*, Reactions were identical to those in *a*, except that [γ-^{32}P]labelled GTP was used. *c*, Crosslinking of radiolabelled GDP is competed with increasing amounts of unlabelled GDP in either the presence (◆) or absence (◇) of SR. The apparent K_i for GDP in the presence or absence of SR was 0.14 μM. *d*, Crosslinking of radiolabelled GDP is competed for by increasing amounts of unlabelled GTP in the absence of SR (△) or GMP-PNP in the presence (■) or absence (□) of SR. The apparent K_i for GTP in the absence of SR was 1.7 μM. This is consistent with the apparent K_d of SRP54 for GTP (2 μM) as determined by crosslinking directly to labelled GTP. The apparent K_is for GMP-PNP in the absence and in the presence of SR were 4.5 and 0.05 μM, respectively.

METHODS. *a* and *b*, Reactions (20 μl) containing 20 nM SRP(54/RNA) and/or SR were incubated for 20 min in GTP hydrolysis buffer at 25 °C. The GTP concentration was 0.3 μM, including 0.5 mCi ml^{-1} [α-^{32}P]GTP (Amersham) (*a* and *b*) or 0.5 mCi ml^{-1} [γ-^{32}P]GTP (*c*). After 20 min, reactions were pipetted into plastic weigh boats and irradiated with ultraviolet light at 6,000 W per cm^2 (using eight Model G15T8 15W Germicidal lamps (General Electric) 6 cm from the sample) for 5 min on ice to crosslink covalently the bound radiolabelled nucleotide to the protein[19]. Reactions were then precipitated with trichloroacetic acid to remove uncrosslinked label and analysed by SDS–PAGE followed by autoradiography. Ultraviolet crosslinking of labelled GTP to SRP54, SRα and SRβ was saturable and specific for GTP, as it could be inhibited by an excess of unlabelled GTP but not by ATP or CTP. *c* and *d*, Reactions (20 μl) contained 20 nM SRP(54/RNA) and/or SR in GTP hydrolysis buffer. All reactions also contained 0.1 μM GDP including 0.5 mCi ml^{-1} [α-^{32}P]GDP. Individual reactions were supplemented with unlabelled nucleotides to the concentrations indicated. Reactions were incubated for 4 h at 25 °C to reach equilibrium and then UV-crosslinked. Under saturating conditions, the level of crosslinking of [α-^{32}P]GDP to SRP54 was quantitatively identical to that of [α-^{32}P]GTP (not shown), indicating that crosslinking efficiencies are invariant for different nucleotides. At saturation, 4×10^{-4} mol nucleotide crosslinked to one mol SRP54. Crosslinked product was quantified using a PhosphorImager (Molecular Dynamics). Data points are experimental and the line is generated as a best fit to the equation: $B = B_{max}[1-[I]/([I]+K_i(1+([S]/K_d)))]$ (a modification of equation III-5 described in ref. 30) using the program Kaleidagraph (Abelbeck Software) on a Macintosh II computer; B, is the amount of [α-^{32}P]GDP crosslinked to SRP54; B_{max}, amount of [α-^{32}P]GDP crosslinked to SRP54 in the absence of competitor; $[I]$, concentration of competitor; K_i, dissociation constant of competitor; K_d, dissociation constant of [α-^{32}P]GDP; [S], concentration of [α-^{32}P] GDP.

or SRP(54/RNA) alone (compare Fig. 2*a* and *b*, lanes 1 and 2), confirming that neither component alone hydrolyses GTP significantly. In the reaction containing both receptor and SRP(54/RNA), however, the labelling of SRP54 was almost completely abolished (Fig. 2*b*, lane 3), whereas labelling of SRα and SRβ was unchanged, indicating that the nucleotide crosslinked to SRP54 was GDP. SRP54 must thus hydrolyse GTP upon interaction with SRP receptor, and the receptor therefore functions not only as a guanine-nucleotide-loading protein, but also as a GTPase-activating protein.

As SRP54 is bound to a signal sequence during the targeting reaction, we investigated whether a signal sequence could influence the receptor-dependent GTP hydrolysis reaction. Four synthetic peptides, derived from the signal sequence of the bacterial outer membrane protein LamB were tested (Fig. 3*a*): the wild-type signal sequence (wt), a deletion mutant (dm), which renders the signal sequence inactive *in vivo*, and two different second-site, single-amino-acid reversions that restore activity *in vivo* (r1 and r2)[22,23]. The three peptides corresponding to functional signal sequences all strongly inhibited receptor-dependent GTP hydrolysis by SRP54, with IC_{50}s of 2, 2 and 0.4 μM, respectively (Fig. 3*b*). The deletion mutant control peptide, however, did not affect the reaction even at 30 μM, the highest concentration tested (Fig. 3*b*).

To determine whether the peptides were inhibiting GTP hydrolysis by blocking GTP binding to SRP54 or by blocking the hydrolysis step, an ultraviolet crosslinking experiment was done in the presence of the peptides. Crosslinking of [α-^{32}P]GTP to SRP54 was drastically reduced in the presence of a functional signal peptide, with or without SRP receptor (compare Figs 2*a* and 3*c*). GTP crosslinking to SRα and SRβ was unaffected by the peptide (Fig. 3*c*), and the deletion mutant peptide did not inhibit GTP crosslinking to SRP54, SRα or SRβ (not shown). Binding of a functional signal peptide to SRP54 must thus prevent GTP binding and so inhibit GTP hydrolysis.

The inhibition of GTP binding to SRP54 mediated by the signal peptides might reflect either stabilization of a nucleotide-free state or stabilization of a bound nucleotide (co-purified with SRP54), preventing labelled GTP from entering the occupied binding site. To discriminate between these mechanisms, [α-^{32}P]GDP was prebound to SRP54 as shown in Fig. 3*d*, and then an excess of unlabelled GDP was added (Fig. 3*e*).

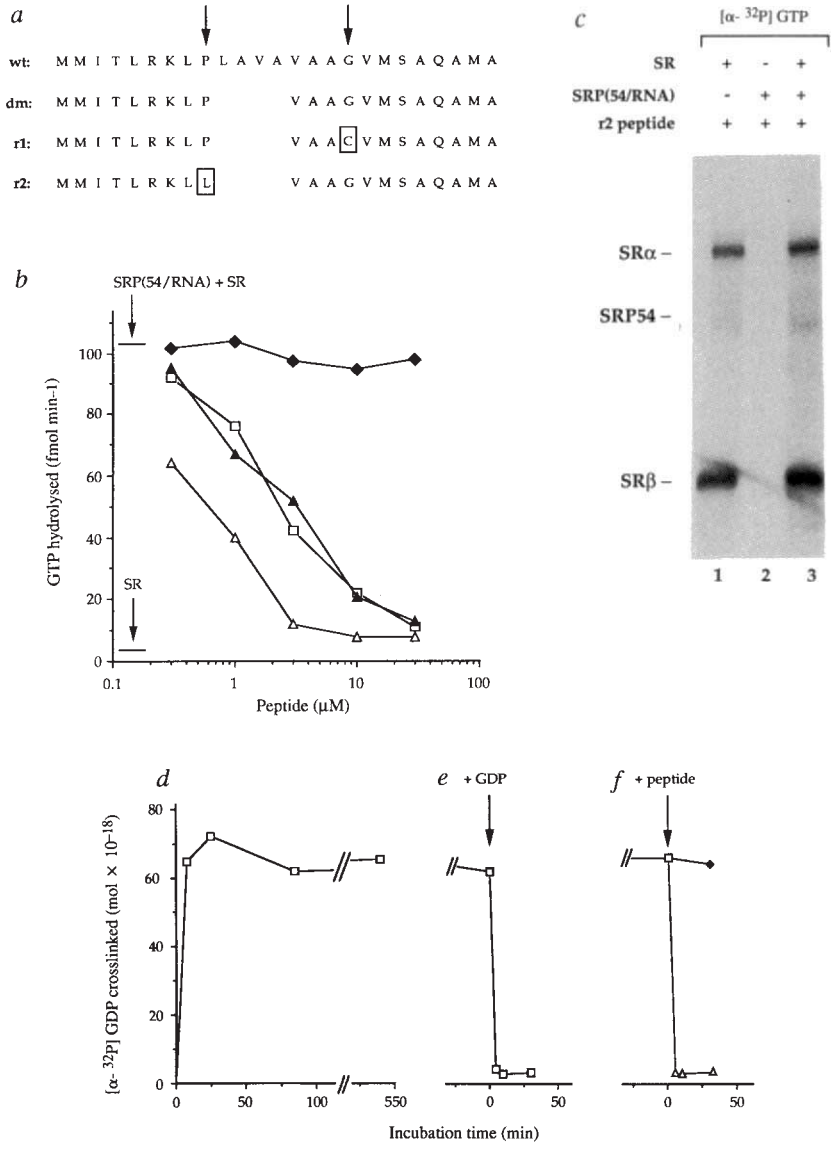

FIG. 3 Functional signal peptides inhibit GTP binding to and hydrolysis by SRP54. *a*, Synthetic peptides used in the GTPase assay. The wild-type (wt) peptide corresponds to the signal sequence of the *E. coli* LamB protein. The LamB signal sequence functions efficiently in a mammalian *in vitro* translocation system[31], and the synthetic peptides used here inhibit *in vitro* protein translocation across the membrane of *E. coli* inverted vesicles[32]. The synthetic wild-type peptide can readily adopt an α-helical conformation when analysed by circular dichroism spectroscopy[23]. The deletion mutant peptide (dm) removes four amino acids, thus bringing a proline and a glycine residue (arrows) closer together, such that these two residues function as helix breakers. The synthetic deletion mutant peptide does not form an α-helix[23], and the peptide does not function as a signal peptide *in vivo*[22]. In the second site revertants r1 and r2, either the glycine or the proline residue are changed to a different amino acid. Both the r1 and r2 peptides regain the ability to form an α-helix and function as signal sequences *in vivo*[22,23]. *b*, GTP hydrolysis reactions (as in Fig. 1) containing SRP(54/RNA) were supplemented with increasing concentrations of the synthetic wt (□), dm (◆), r1 (▲) or r2 (△) peptides. The degree of GTP hydrolysis in the absence of signal peptide [SRP(54/RNA) + SR] and the basal level of hydrolysis by SR are indicated (SR). The peptides were added to the reactions from a 150 μM stock solution in water, the concentration of which was determined by amino-acid analysis. *c*, Reactions were identical to those for Fig. 2*a*, except that the r2 signal peptide was added to 4 μM. *d*, [α-^{32}P]GDP was mixed with SRP(54/RNA) in GTP hydrolysis buffer. Aliquots were removed for UV crosslinking at the times indicated. The amount of GDP crosslinked to SRP54 was determined after SDS–PAGE by quantitation with a PhosphorImager. *e*, SRP(54/RNA) was incubated with GDP as in *d*. At time zero, unlabelled GDP is added to a final concentration of 10 μM. Aliquots were removed for UV crosslinking at the indicated times to monitor the dissociation of the prebound [α-^{32}P]GDP. *f*, SRP(54/RNA) was incubated with GDP as in *d*. At time zero, the r2 (△) or dm (◆) peptide were added to final concentrations of 4 μM. All reactions contained 20 nM SRP(54/RNA) in GTP hydrolysis buffer and 0.1 μM GDP including 0.5 mCi^{-1} ml [α-^{32}P]GDP. UV crosslinking and analysis were done as for Fig. 2.

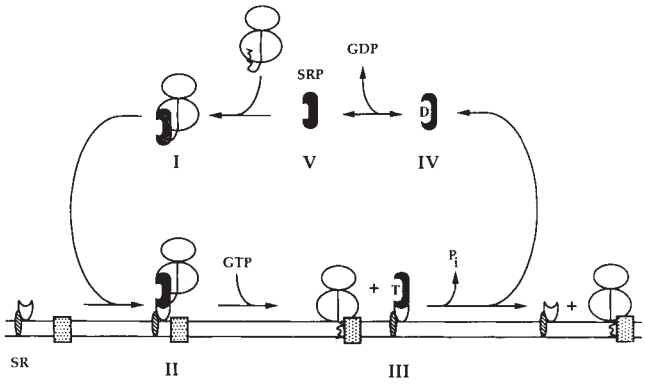

FIG. 4 Model depicting the proposed role of GTP binding to and hydrolysis by SRP54 during the initiation of protein translocation. As discussed in the text, SRP54 is proposed to cycle between three forms with respect to bound nucleotide: a nucleotide-free or 'empty' state, a GTP-liganded (T), and GDP-liganded (D) state. The translocation apparatus is indicated by the stippled box.

Crosslinking of labelled GDP to SRP54 was radically diminished even at the earliest time point after addition of unlabelled GDP, indicating that the radiolabelled GDP dissociated rapidly. Results were identical when the r2 signal peptide was added to SRP54 containing prebound [α-^{32}P]GDP (Fig. 3*f*, triangles), indicating that the signal peptide does not stabilize a GDP-bound state. Addition of the control deletion mutant peptide (Fig. 3*f*, diamond) did not reduce GDP crosslinking. A functional signal sequence thus appears to stabilize the nucleotide-free form of SRP54, although it remains a formal possibility that a signal sequence bound to SRP54 still allows nucleotide binding but causes a conformational change in the protein that completely disrupts ultraviolet crosslinking to the bound nucleotide.

Taken together, our data indicate that the guanine-nucleotide-bound state of SRP54 can be influenced by at least two ligands, the signal peptide and the SRP receptor, suggesting that the GTPase domain of SRP54 integrates information from the nascent polypeptide and the ER. We propose a model in which the occupancy of the SRP54 guanine-nucleotide-binding site defines discrete steps in a cycle of GTP binding and hydrolysis which operates during protein targeting to the ER (Fig. 4).

In the first step, signal sequence binding to SRP54 initiates formation of the targeting complex (Fig. 4, steps V→I). Previous studies have shown that guanine nucleotide and signal sequence bind to structurally separate domains on SRP54 (refs 5, 6). Moreover, chemical modification of the GTP-binding domain prevents signal sequence binding[24]. Our data suggest that binding of a signal sequence to one domain stabilizes an empty nucleotide binding site in the other. Thus, the two domains can communicate bidirectionally and the binding of a signal peptide and guanine nucleotide (GTP or GDP) to SRP54 may be mutually exclusive. We therefore propose that the targeting complex arrives at the ER membrane with SRP54 held in a nucleotide-free state by the signal sequence (Fig. 4, step I→II), when an SRP receptor-catalysed conformational change in SRP54 (Fig. 4, II) stimulates GTP binding and concomitantly reduces its affinity for the bound signal sequence (Fig. 4, III). As purified receptor is not sufficient to stimulate GTP binding to SRP54 in the presence of a signal peptide (Fig. 3*c*), this step may require some part of the targeting complex not included in the reconstituted assays, or a constituent of the membrane. This additional factor may be necessary to ensure that the signal sequence is not released from SRP unless essential translocation site components are available.

After GTP binding to SRP54 and release of the signal sequence, SRP and its receptor dissociate from the translocation apparatus as a stable complex[14,15](Fig. 4, III). SRP receptor-stimulated hydrolysis of GTP by SRP54 then ensues, allowing SRP to dissociate from its receptor and return to the cytosol (Fig. 4, IV). Given the rapid dissociation of GDP from SRP54 (Figs 2*c* and 3*e*), nucleotide-bound SRP54 probably exists in equilibrium with empty SRP54 (Fig. 4, step IV→V), which can bind a signal sequence to initiate another round of targeting (Fig. 4, step V→I).

According to this model, SRP54 closely resembles other GTPases (such as the trimeric G proteins or EF-Tu), in that interconversion between the different nucleotide-bound states causes the GTPase to interact successively with its effectors[25,26]. Whereas the crucial conformational switch for other well characterized GTPases is the interconversion between the GTP- and GDP-bound states, interconversion between the empty state and the GTP-bound state seems to be the important switch for SRP54.

This model attributes no function to the other GTPase domains in SRα and SRβ. It is not yet known whether the GTPase domain of SRβ is required for receptor function, but the GTP-bound form of SRα is necessary for the targeting reaction to progress through the cycle shown in Fig. 4 (ref. 27), suggesting that SRα may undergo GTP binding and hydrolysis like SRP54. It is possible that, just as SRP recruits ribosomes carrying nascent chains from the cytosol to the translocation site, so the SRP receptor may recruit essential components of the translocation apparatus from the plane of the membrane. □

Received 17 May; accepted 14 September 1993.

1. Walter, P. & Blobel, G. *Proc. natn. Acad. Sci. U.S.A.* **77,** 7112–7116 (1980).
2. Walter, P. & Blobel, G. *Nature* **99,** 691–698 (1982).
3. Bernstein, H. D. *et al. Nature* **340,** 482–486 (1989).
4. Römisch, K. *et al. Nature* **340,** 478–482 (1989).
5. Zopf, D., Bernstein, H. D., Johnson, A. E. & Walter, P. *EMBO J.* **9,** 4511–4517 (1990).
6. Römisch, K., Webb, J., Lingelbach, K., Gausepohl, H. & Dobberstein, B. *J. Cell Biol.* **111,** 1793–1802 (1990).
7. High, S. & Dobberstein, B. *J. Cell Biol.* **113,** 229–233 (1991).
8. Nunnari, J. & Walter, P. *Curr. Opin. Cell Biol.* **4,** 573–580 (1992).
9. Gilmore, R., Blobel, G. & Walter, P. *J. Cell Biol.* **95,** 463–469 (1982).
10. Gilmore, R., Walter, P. & Blobel, G. *J. Cell Biol.* **95,** 470–477 (1982).
11. Meyer, D. I., Krause, E. & Dobberstein, B. *Nature* **297,** 647–650 (1982).
12. Tajima, S., Lauffer, L., Rath, V. L. & Walter, P. *J. Cell Biol.* **103,** 1167–1178 (1986).
13. Connolly, T. & Gilmore, R. *Cell* **57,** 599–610 (1989).
14. Gilmore, R. & Blobel, G. *Cell* **35,** 677–685 (1983).
15. Connolly, T., Rapiejko, P. J. & Gilmore, R. *Science* **252,** 1171–1173 (1991).
16. Connolly, T. & Gilmore, R. *J. Cell. Biol.* (in the press).
17. Walter, P. & Blobel, G. *Cell* **34,** 525–533 (1983).
18. Siegel, V. & Walter, P. *J. Cell Biol.* **100,** 1913–1921 (1985).
19. Nath, J. P., Eagle, G. R. & Himes, R. H. *Biochemistry* **24,** 1555–1560 (1985).
20. Pashev, I. G., Dimitrov, S. I. & Angelov, D. *Trends Biochem. Sci.* **16,** 323–326 (1991).
21. Bourne, H. R., Sanders, D. A. & McCormick, F. *Nature* **349,** 117–127 (1991).
22. Emr, S. & Silhavy, T. J. *Proc. natn. Acad. Sci. U.S.A.* **80,** 4599–4603 (1983).
23. McKnight, C. J., Briggs, M. S. & Gierasch, L. M. *J. biol. Chem.* **264,** 17293–17297 (1989).
24. Lütcke, H., High, S., Römisch, K., Ashford, A. & Dobberstein, B. *EMBO J.* **11,** 1543–1551 (1992).
25. Bourne, H. R., Sanders, D. A. & McCormick, F. *Nature* **348,** 125–132 (1990).
26. Gilman, A. G. *A. Rev. Biochem.* **56,** 615–649 (1987).
27. Rapiejko, P. J. & Gilmore, R. *J. Cell Biol.* **117,** 493–503 (1992).
28. Walter, P. & Blobel, G. *Meth. Enzym.* **96,** 682–691 (1983).
29. Migliaccio, G., Nicchitta, C. V. & Blobel, G. *J. Cell Biol.* **117,** 15–25 (1992).
30. Segel, I. H. *Enzyme Kinetics: Behavior and Analysis of Steady State and Rapid Equilibrium Enzyme Systems* 105 (Wiley-Interscience, New York, 1975).
31. Watanabe, M., Hunt, J. F. & Blobel, G. *Nature* **323,** 71–73 (1986).
32. Chen, L., Tai, P. C., Briggs, M. S. & Gierasch, L. M. *J. biol. Chem.* **262,** 1427–1429 (1987).

ACKNOWLEDGEMENTS. We thank H. Bourne and J. Nunnari for discussion, members of P.W.'s laboratory for critically reading the manuscript, and C. Nicchitta for technical assistance. This work was supported by grants from the NIH and the Human Frontiers Science Foundation (to P.W.).