interactions beyond 8.5 Å smoothed to zero by use of a shifting function for electrostatic terms and a switching function for van der Waals terms. A boundary potential was used to keep the dimethylphosphates within the sphere. Langevin molecular dynamics simulated annealing was performed on this potential surface for 4.75 ps, starting at 1000 K and cooling slowly to 10 K. The final configuration was further optimized by performing 100 steps of ABNR (adopted-basis Newton-Raphson) minimization. The entire procedure was repeated five times starting from different random configurations and the interaction energy of each dimethylphosphate pair with the arginine was calculated. The configuration with the most favorable interaction energy is shown (Fig. 6B).

14. Coordinates of two tRNA crystal structures (yeast Phe and Asp tRNAs) in the Brookhaven Protein Data Bank [F. C. Bernstein *et al., J. Mol. Biol.* **112**, 535 (1977)] were searched to determine the location of phosphate pairs oriented in a manner similar to the phosphates in Fig. 6B. We required the P-P distance to be between 6.5 and 7.5 Å and the free oxygens (O1P and O2P) to point toward each other. Although more stringent criteria are possible (for example, requiring oxygen-oxygen distances to match the modeling results more closely), a broader range of phosphate orientations was accepted to accommodate other possible conformations indicated by the modeling (see legend to Fig. 6B) or conformational changes that might occur upon arginine binding. Three types of phosphate pairs were found: phosphates adjacent in the sequence (i, $i+1$), those one away from each other (i, $i+2$), and those distant in primary sequence but near each other in the tertiary structure. In no case did phosphate pairs in double-stranded RNA match the template. The (i, $i+1$) pattern frequently appeared surrounding the first or last unpaired base in a bulge or loop, and the (i, $i+2$) pattern frequently appeared in a bulge or loop that bound a hydrated magnesium ion. In fact, water molecules coordinated to Mg^{2+} produce an array of hydrogen-bond donors similar to that of an arginine side chain (Fig. 6A) and bind to a phosphate pair in a manner similar to Fig. 6B.
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Requirement of GTP Hydrolysis for Dissociation of the Signal Recognition Particle from Its Receptor

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The signal recognition particle (SRP) directs signal sequence specific targeting of ribosomes to the rough endoplasmic reticulum. Displacement of the SRP from the signal sequence of a nascent polypeptide is a guanosine triphosphate (GTP)–dependent reaction mediated by the membrane-bound SRP receptor. A nonhydrolyzable GTP analog can replace GTP in the signal sequence displacement reaction, but the SRP then fails to dissociate from the membrane. Complexes of the SRP with its receptor containing the nonhydrolyzable analog are incompetent for subsequent rounds of protein translocation. Thus, vectorial targeting of ribosomes to the endoplasmic reticulum is controlled by a GTP hydrolysis cycle that regulates the affinity between the SRP, signal sequences, and the SRP receptor.

RIBOSOMES SYNTHESIZING PROTEINS with rough endoplasmic reticulum (RER)–specific signal sequences are cotranslationally recognized by SRPs and then delivered to the RER membrane via interaction between the SRP and the SRP receptor or docking protein (*1–4*). The SRP receptor–mediated displacement of the SRP from the signal sequence of the nascent polypeptide is a GTP-dependent reaction (*5–7*). One protein subunit from both the SRP receptor (SRα) (7) and the SRP (SRP54) (*8, 9*) contains protein sequence motifs that are similar to those in GTP binding proteins (*10*). We examined the role of GTP hydrolysis in SRP receptor function by replacing GTP with the nonhydrolyzable analog β-γ-imidoguanosine 5′-triphosphate [Gpp(NH)p] during the targeting and insertion steps of a protein translocation reaction.

A truncated mRNA encoding the NH_2-terminal 90 residues of the G protein of vesicular stomatitis virus was translated in vitro in the presence of ^{125}I-labeled SRP to prepare complexes containing SRP, ribosomes, and a nascent polypeptide. After translation, ribonucleotides were removed by gel filtration chromatography, and the SRP-ribosome complexes were incubated in the absence or presence of Gpp(NH)p and microsomal membranes that were depleted of SRP (K-RM) (Fig. 1, A and B). The SRP-ribosome complexes were then separated from free SRPs by sedimentation on sucrose density gradients that were underlayered with a 2 M sucrose cushion. Under these conditions, membrane vesicles sediment at the interface between the sucrose layers. Addition of K-RM and GTP to the complexes increased the amount of unbound SRP recovered after centrifugation while the amount of SRP bound to ribosomes decreased (Fig. 1, A and C), indicating that the SRP enters a soluble pool. In

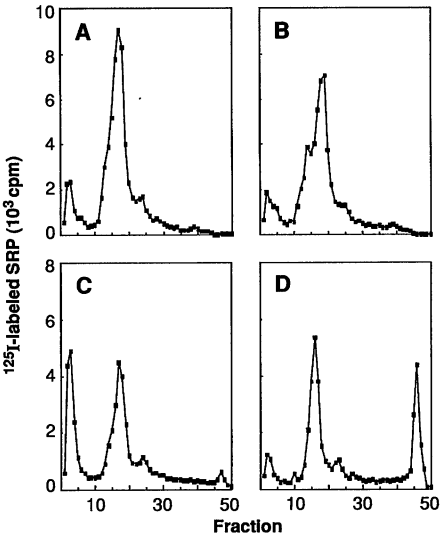

Fig. 1. Recycling of SRP after GTP hydrolysis. A truncated mRNA transcript (*7, 16*) was incubated for 20 min in a wheat germ system containing 6.5 nM SRP (including ^{125}I-labeled SRP) (*3, 7, 19*). SRP-ribosome complexes were separated from ribonucleotides (*5*) and incubated in 50 mM triethanolamine-acetate, pH 7.5, 150 mM potassium acetate, 2.5 mM magnesium acetate, and 1 mM dithiothreitol for 5 min at 25°C as follows. (**A**) No additions, (**B**) K-RM [5 equivalents, as defined (*3*)], (**C**) K-RM (5 equivalents) and 100 μM GTP, and (**D**) K-RM (5 equivalents) and 100 μM Gpp(NH)p. The abbreviation K-RM refers to rough microsomal membranes depleted of SRPs by extraction with 0.5 M potassium acetate (*3*). Samples were applied to sucrose density gradients (10 to 30%) underlain with 0.5 ml of 2 M sucrose. The gradients contained 50 mM triethanolamine-acetate, pH 7.5, 150 mM potassium acetate, 5 mM magnesium acetate, and 1 mM dithiothreitol. Centrifugation, fractionation, and quantitation of gradients were as described (*3, 7*). The top and bottom of the gradient were in fractions 1 and 50, respectively. The interface between the sucrose layers was in fraction 45. The sedimentation position of 80*S* ribosomes (fractions 14 to 20) was determined from the ultraviolet-absorbence profile as recorded with a continuous flow cell. Free SRPs sedimented in fractions 1 to 5 in gradients lacking ribosomes. Similar results were obtained in three separate experiments.

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contrast, the SRP cosedimented with the membranes when GTP was replaced by Gpp(NH)p (Fig. 1D). Complexes of SRP, a ribosome and a nascent polypeptide that are targeted to the membrane in the absence of GTP or Gpp(NH)p, do not remain stably associated with the membrane under the ionic strength conditions used for the sucrose gradient analysis (Fig. 1B). These results indicate that GTP hydrolysis precedes dissociation of SRP from the membrane.

We next considered the possibility that the SRP–SRP receptor complexes undergo a translocation-independent guanine nucleotide exchange reaction. SRP–SRP receptor complexes form at reduced ionic strength (50 mM potassium acetate) and dissociate when the ionic strength is raised (*1, 2, 11*). ^{125}I-labeled SRP was incubated with K-RM and Gpp(NH)p in buffers of various ionic strength. Unbound SRP was then separated from K-RM by gel filtration chromatography in a high ionic strength buffer (250 mM potassium acetate). Incubation at low ionic strength allowed the formation of complexes that were resistant to dissociation at high ionic strength (Fig. 2A). The stability of SRP–SRP receptor complexes formed at 50 mM potassium acetate in the presence or absence of GTP or Gpp(NH)p was evaluated by fractionation in buffers containing 50, 150, or 500 mM potassium acetate (Fig. 2B). Approximately 20% of the added SRP remained bound to K-RM at 50 mM potassium acetate, but little SRP remained bound at concentrations of potassium acetate approximating physiological ionic strength, when either GTP or no ribonucleotide was included. SRP–SRP receptor complexes were more stable when formed by initial incubation in the presence of Gpp(NH)p. Membranes rendered deficient of SRP receptors by digestion with trypsin (*2, 12, 13*) were used to evaluate the nonspecific binding of ^{125}I-labeled SRP to K-RM. SRP binding to trypsin-digested membranes was not enhanced by Gpp(NH)p (*14*). Hydrolysis of GTP catalyzed by the SRP–SRP receptor complex probably accounts for the failure of GTP to enhance binding of SRPs to membranes (*14*).

The above experiments suggest that the SRP–SRP receptor complex is stabilized by bound Gpp(NH)p. To confirm that the receptor was a component of these complexes, purified preparations of the two proteins were incubated together at low ionic strength with Gpp(NH)p. The formation of complexes between the SRP and its receptor was verified by analysis of sucrose density gradients, with ^{125}I-labeled SRP serving as an internal standard for the sedimentation position of SRP (Fig. 3A). The location of the SRP receptor was determined by protein immunoblot analysis of fractions from the sucrose density gradient with monoclonal antibodies to the α and β subunits of the receptor (*15*). Both the α and β subunits of the SRP receptor sediment at the top of the sucrose gradient after incubation with SRP in the absence of ribonucleotides (Fig. 3A). In contrast, we observed that 50 to 65% of both SRP receptor subunits cosedimented with SRP after incubation in the presence of Gpp(NH)p (Fig. 3B).

Gpp(NH)p can replace GTP in a translocation assay designed to monitor a single round of SRP-dependent targeting of ribo-

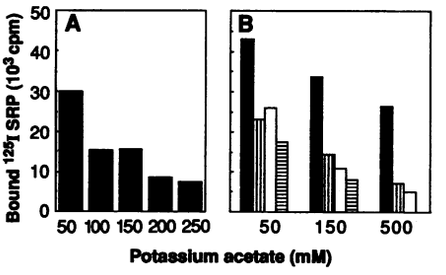

Fig. 2. Binding of SRP to K-RM is enhanced by Gpp(NH)p. (**A**) Thirty fentomoles of ^{125}I-labeled SRP (80,000 cpm) was incubated for 10 min at 25°C with K-RM (2.5 equivalents) and Gpp(NH)p (100 μM) in 50 μl of buffer A [50 mM triethanolamine-acetate, pH 7.5, 2.5 mM magnesium acetate, bovine serum albumin (1 mg/ml), gelatin (100 μg/ml), 0.002% Nikkol, 1 mM dithiothreitol] adjusted to final potassium acetate concentrations between 50 and 250 mM. Nikkol is the nonionic detergent octaethyleneglycol-mono-*N*-dodecyl ether. The samples were then adjusted to 250 mM potassium acetate and applied to Sepharose CL-2B columns (1 ml) equilibrated in buffer A containing 250 mM potassium acetate. Membrane-bound ^{125}I-labeled SRP eluting in the void volume was quantitated by counting. (**B**) Thirty fentomoles of ^{125}I-labeled SRP (80,000 cpm) was incubated as above with 2.5 equivalents of K-RM (solid, open, and vertically striped bars) or 2.5 equivalents of trypsin digested K-RM (horizontally striped bars) in buffer A containing 50 mM potassium acetate. Trypsin-digested K-RM samples were prepared and assayed for SRP receptor activity as described (*12*). Individual 50-μl samples also contained no ribonucleotide (vertically and horizontally striped bars); 100 μM GTP (open bars); or 100 μM Gpp(NH)p (solid bars). The samples were fractionated as described above at final potassium acetate concentrations of 50, 150, and 500 mM (see abscissa). Protein immunoblots of trypsin- digested membranes revealed no intact SRα. (A) is the result of a single experiment. Values in (B) are averages of between two and six separate determinations.

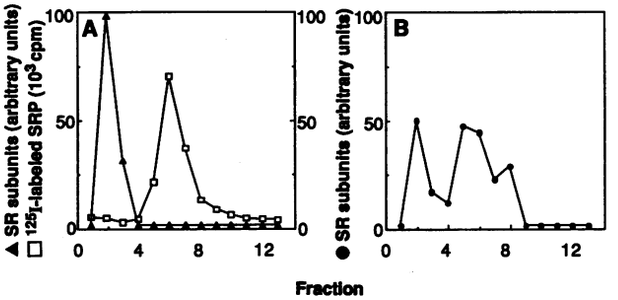

Fig. 3. Stabilization of SRP-SRP receptor complexes by Gpp(NH)p. Complexes of SRP [12 pmol, ^{125}I-labeled SRP (150,000 cpm)] and SRP receptor [4 pmol, purified as described (*28*)] were formed in buffer A adjusted to 50 mM potassium acetate, 0.1% Nikkol, and 0 μM (▲) (**A**) or 100 μM (●) (**B**) Gpp(NH)p. The SRP and the SRP receptor preparations were comparable in purity to previously described preparations (*28*) and are estimated to be greater than 95% homogeneous on the basis of staining polyacrylamide gels with Coomassie blue. The proteins were resolved on sucrose density gradients (5 to 20%) containing 50 mM triethanolamine-acetate, 200 mM potassium acetate, 5 mM magnesium acetate, 1 mM dithiothreitol, gelatin (100 μg/ml), and 0.1% Nikkol. Centrifugation was for 6 hours at 50,000 rpm in a Beckman SW 50.1 rotor. The top and bottom of the sucrose gradients were fractions 1 and 14, respectively. ^{125}I-labeled SRP (□) was detected by gamma counting (A). SRP receptor (▲ and ●) was quantitated by densitometric scanning of protein immunoblots that had been simultaneously probed with monoclonal antibodies to the α and β subunits of the SRP receptor (*15, 29*). Similar results were obtained in four separate experiments.

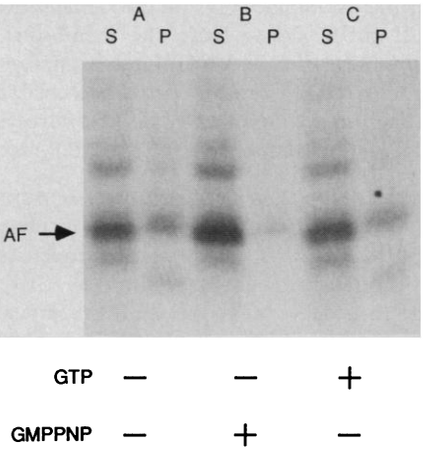

Fig. 4. Inhibition of binding of SRP-ribosome complexes to microsomal membranes treated with SRP and Gpp(NH)p. K-RM (1 equivalent) and SRP (2 pmol) were incubated for 10 min at 25°C in buffer A adjusted to 50 mM potassium acetate either without any ribonucleotides (**A**), or with 100 μM Gpp(NH)p (**B**), or 100 μM GTP (**C**). SRP-ribosome complexes bearing [^{35}S]methionine-labeled nascent preprolactin polypeptides were assembled by in vitro translation of bovine pituitary mRNA (*4, 5*). SRP-ribosome complexes were incubated for 10 min at 25°C with the treated K-RM in the presence of 1 mM cycloheximide. Differential centrifugation in an Airfuge after treatment with 25 mM EDTA (*5*) yielded supernatant fractions (S) or membrane-bound pellet fractions (P). The nascent preprolactin chain (AF) was resolved by polyacrylamide gel electrophoresis in SDS (*4, 5*). Similar results were obtained in three separate experiments.

somes to the membrane (*5, 6, 16*). To determine whether prevention of the SRP recycling reaction would interfere with subsequent translocation cycles, we first incubated K-RM with the guanine ribonucleotides and saturating quantities of the SRP relative to the membrane content of the SRP receptor. The treated membranes were then tested for activity in a nascent polypeptide insertion assay (*5*). Cosedimentation of a portion of the nascent preprolactin polypeptide with the membranes indicated that the treated membranes remained competent for ribosome targeting and nascent chain insertion (Fig. 4, A and C). Prior incubation of K-RM with Gpp(NH)p and the SRP reduced the amount of nascent preprolactin associated with membranes (Fig. 4B). Prior incubation of K-RM with Gpp(NH)p in the absence of the SRP did not inhibit subsequent insertion of nascent preprolactin in the presence of GTP (*14*).

The GTP hydrolysis cycle of the SRP–SRP receptor complex may function in regulating protein translocation across the endoplasmic reticulum. The 54-kD subunit of the SRP (SRP54) can be cross-linked to nascent signal sequences (*17, 18*) and has been shown to contain the signal sequence recognition domain of the SRP (*19*). Upon binding ribonucleotide, GTP binding proteins display an increased affinity for a downstream effector protein (*20, 21*). We propose that a GTP-induced increase in association between SRP and its receptor is directly coupled to release of the nascent polypeptide from the signal sequence binding site of SRP54. Displacement of SRP54 allows insertion of the signal sequence into a translocation competent site in the RER membrane (*22–26*). The translocation site contains a 35- to 39-kD glycoprotein that has been termed the signal sequence receptor or mp39 (*24–26*). Vectorial insertion of the nascent chain into the RER would be ensured by the inherent delay in GTP hydrolysis which is a characteristic of GTP binding proteins, so that SRP54 is unable to rebind the signal sequence prior to membrane insertion of the polypeptide. The subsequent GTP hydrolysis reaction would convert the receptor to a GDP-bound form with a reduced affinity for SRP, thereby allowing the return of SRP to the cytoplasm for participation in subsequent cycles of ribosome targeting (*27*).

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Effect of Wnt-1 and Related Proteins on Gap Junctional Communication in *Xenopus* Embryos

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The proto-oncogene *wnt*-1 (previously referred to as *int*-1) is thought to be important in embryonic pattern formation although its mechanisms of action are unknown. Premature and increased expression of the Wnt-1 protein, achieved by injection of synthetic *wnt*-1 RNA into fertilized *Xenopus* eggs, enhanced gap junctional communication between ventral cells of the developing embryo. This result is consistent with the hypothesis that Wnt proteins activate a receptor-mediated signal transduction pathway and that gap junctional communication can be a target of this pathway. The effects of two Wnt-1–related proteins on gap junctional communication were also investigated: overexpression of Xwnt-8 increased gap junctional coupling in a manner similar to Wnt-1, whereas Xwnt-5A did not. These findings are consistent with the existence of multiple receptors for Wnt proteins.

THE PROTO-ONCOGENE *INT*-1 encodes a 44-kD product that associates with the cell surface or extracellular matrix (*1*) after its secretion. Isolation of genes related to *int*-1 led to the reclassification of these genes as members of the *wnt* family (*2*). On the basis of its transient and spatially restricted expression in the neural tube of mouse embryos (*3*) and its homology with the *Drosophila* segment polarity gene *wingless* (*4*), it has been proposed that *wnt*-1 (*int*-1) is important in pattern formation in vertebrate embryos (*2, 5, 6*). This view is supported by the demonstration that microinjection of synthetic *wnt*-1 RNA into fertilized *Xenopus* eggs leads to overexpression of Wnt-1 and a bifurcation of the embryonic axis (*6*) and by the observation that deletion of this gene in mouse by homologous recombination results in embryos that lack the midbrain and some parts of the rostral metencephalon (*7*).

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We have attempted to elucidate potential cellular mechanisms by which the products of the *wnt* gene family may affect embryonic development. Microinjection into fertilized *Xenopus* eggs of synthetic *wnt*-1 and *Xwnt*-8 RNA, but not *Xwnt*-5A RNA, resulted in increased gap junctional coupling between blastomeres in the ventral region of 32-cell embryos. Gap junctional communication has been implicated in pattern formation (*8*) and is thought to be modulated by receptor-mediated signaling pathways (*9*). Therefore, our data are consistent with the hypothesis that premature and increased expression of Wnt-1 and Xwnt-8 leads to activation of receptor-mediated signal transduction pathways, which have the potential for modulating gap junctional communication and thus influencing pattern formation. The inability of Xwnt-5A to affect gap junctional communication suggests that it may act at a distinct receptor.

Gap junctional communication in *Xenopus* embryos has been measured by microinjection of Lucifer yellow (*10*). With the use of this dye, and fluorescein isothiocyanate (FITC)–conjugated dextran as a negative