

PROTEIN TARGETING

Nobel Lecture, December 8, 1999

by

GÜNTER BLOBEL

Laboratory of Cell Biology, Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA.

PROLOGUE

I began research in the 60ties, first as a graduate student in the laboratory of Van R. Potter (Fig.1) at the McArdle Institute for Cancer Research of the University of Wisconsin in Madison. I continued as a postdoctoral fellow in the laboratory of George E. Palade (Fig. 2) at The Rockefeller University in New York City. At that time, the intracellular pathway of secretory proteins, from their synthesis to their extrusion from the cell, the so-called 'secretory pathway', had already been worked out by George Palade and his coworkers, primarily Philip Siekevitz, Jim Jamieson and Lucien Caro (for review see 1974 Nobel lecture by George Palade [1]). Using pulse-chase labeling with radioactive amino acids in tissue slices in conjunction with cell fractionation and autoradiography, Palade and coworkers established that, during or shortly



Figure 1. Van R. Potter, 1964. Mc Ardle Laboratory, University of Wisconsin, Madison, Wisconsin.



Figure 2. George E. Palade, 1970. The Rockefeller University, New York.

after their synthesis, secretory proteins cross the rough (ribosome-studded) endoplasmic reticulum (ER) membrane. It was proposed that translocation across the ER yields segregation of secretory proteins from cytosolic proteins. From the ER, secretory proteins are transported via vesicular carriers through the cisternae of the Golgi apparatus. Finally, vesicular carriers budding from the trans-cisternae of the Golgi complex were observed to fuse with the plasma membrane, yielding externalization or exocytosis of the secretory proteins (Fig. 3). The biochemical mechanisms underlying this pathway were unknown at that time.

Several reports in the mid-sixties suggested that mRNAs for secretory proteins are sequestered in rough microsomes that represent the vesicular remnants of the fractured rough ER. However, it was not clear how the sequestration of these mRNAs was accomplished. One possibility was that an *untranslated* region that might be common to all mRNAs coding for secretory proteins mediated their attachment to the ER membrane. Other such distinct *untranslated* regions of other mRNAs might mediate attachment to other organelles. Once attached to their 'cognate' organelles, the translation products might then be vectorially transported into these organelles (2). Another idea was that free and organelle-bound ribosomes might differ in their composition. The distinct organelle-bound ribosomes might function to select 'cognate' mRNAs to the organelles, again via cognate *untranslated* regions. These and other ideas were frequently discussed in the Palade laboratory in the late 60ties.

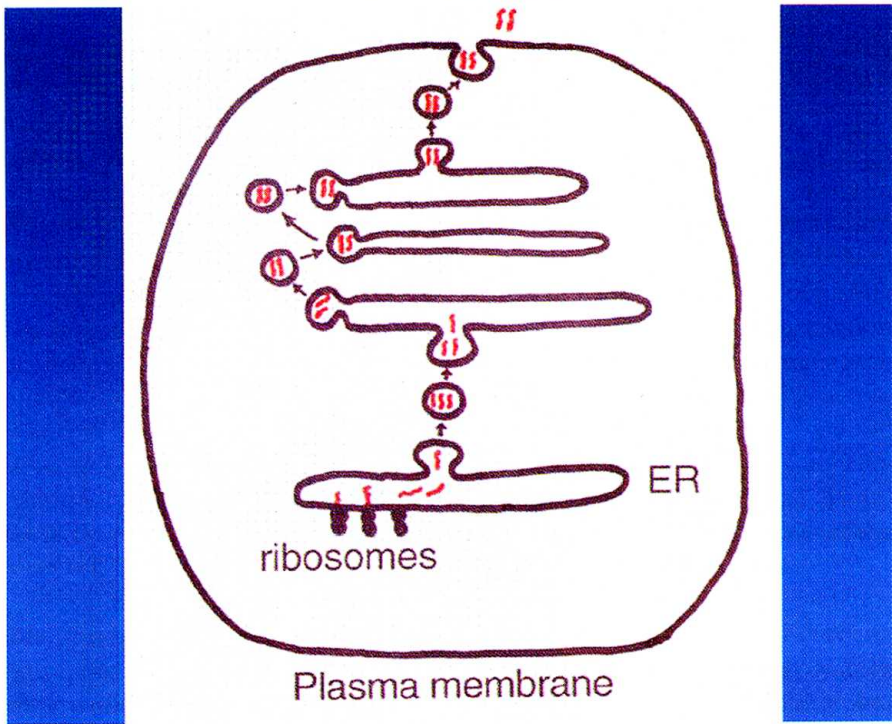


Figure 3. The secretory pathway. Secretory proteins (indicated in red) are synthesized on ribosomes bound to the endoplasmic reticulum (ER). They are then transported via vesicular carriers through the Golgi complex and are finally exocytosed.

This simple cartoon does not show important branches:

1. The branch for lysosomal proteins from the distal Golgi cisternae and the branch for peroxisomal membrane proteins from the ER.
2. The retrograde recycling branches of this pathway, including endocytosis.
3. Most importantly, essentially all integral membrane proteins (except for those of mitochondria and chloroplasts share this pathway).

THE FIRST VERSION OF THE SIGNAL HYPOTHESIS

David Sabatini (Fig. 4) and I tested one of these ideas, namely whether free and ER-bound ribosomes differ in their protein composition. We used one-dimensional sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE). Although this method did not resolve all ribosomal proteins, we could not detect significant differences in the protein pattern between these two ribosome populations. Therefore, we disfavored the idea that sequestration to the ER of mRNAs coding for secretory proteins occurs via distinct features of ER-bound ribosomes.

Instead, we hypothesized in 1971, that selection of mRNA to the ER membrane is not via direct binding of the mRNA itself, but rather via binding of its nascent translation product (3). We postulated that all mRNAs for secretory proteins code for a signatory amino-terminal sequence element that is recognized by a soluble factor that, in turn, binds the nascent chain-ribosome complex to the ER membrane (Fig. 5). We also postulated that ribosomes,



Figure 4. David D. Sabatini, 1970. The Rockefeller University, New York.

free or ER-bound, are indistinguishable and that they cycle between an ER-bound and a cytosolic pool. At this point, there were only a few sequences of secretory proteins established and there was no evidence that these proteins share a common amino-terminal sequence element. However, it occurred to us that such an amino-terminal sequence tag might be a transient and not a permanent feature of nascent secretory proteins. Therefore, we had no hesitation to publish this idea (3). Nevertheless, our proposals at this point were pure speculation without any supporting evidence.

To experimentally test the predictions of this hypothesis, we sought to develop a cell-free system, in which protein translation and protein translocation across microsomal membrane vesicles was faithfully recapitulated. We hoped to reconstitute such an *in vitro* system from defined components. To accomplish this, we began with a series of deconstruction experiments. We started with polysomes. Using puromycin and high concentrations of salt, we isolated functional ribosomal subunits (4) and mRNA still attached to its binding proteins (5, 6, 7). Using the same methods, we succeeded in disassembling rough microsomes. This yielded a virtually mRNA- and ribosome-free membrane fraction (8). We also isolated 'native' small ribosomal subunits that contained translation initiation factors. These initiation factors could be dissociated as multi-subunit complexes (9, 10). With these components in hand, we then attempted to reconstruct a functional protein translation/translocation system from defined components.

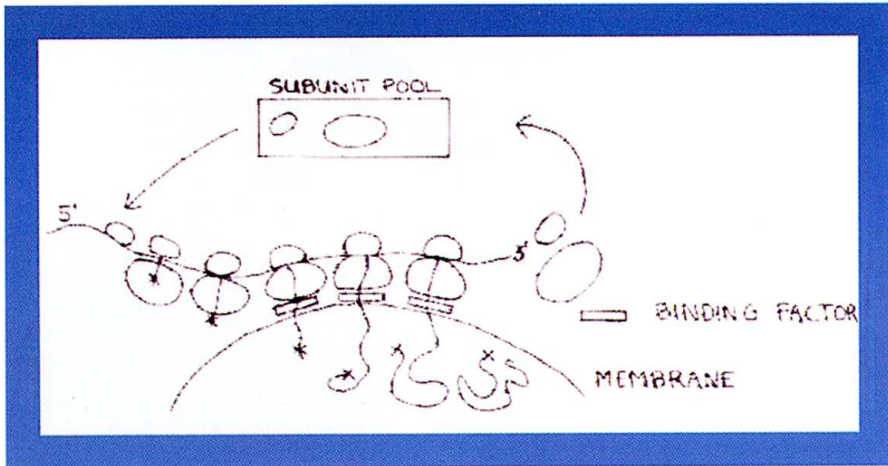


Figure 5. The signal hypothesis in its first version (1971). We announced this idea in a symposium on Biomembranes in 1971 (3) and published it in the proceedings of this symposium. To us the idea was very appealing, even though there was essentially no evidence for it. The signal sequence is indicated by an x and was predicted to be recognized by a "binding factor" that mediates binding to the ER membrane. The signal sequence was not indicated to be a transient feature of the nascent protein, although it occurred to us that it may no longer be present in the mature secreted protein. After completion of translocation the ribosome was predicted to be dissociated into subunits that would join a cytoplasmic pool. No specific proposals were yet made as to how the chain crosses the membrane. From Blobel and Sabatini (3), with permission.

While these deconstruction experiments were going on, two important papers appeared in 1972 from the laboratories of Philip Leder (11) and of Cesar Milstein (12). These investigators studied translation of poly(A) containing mRNAs from myeloma cells (containing primarily mRNA for the light chain of IgG) in a cell-free system that lacked microsomal vesicles. The translation yielded one major product that was larger by about 2–3 kDalton than the mature light chain of IgG which was secreted from the myeloma cells. By peptide mapping of the radioactively labeled translation product they could show that it contained an extension located at the amino terminus. A partial sequence of this amino-terminal extension was subsequently established by Schechter and colleagues (13) and revealed a preponderance of hydrophobic residues.

Could this additional peptide function as the determinant for attachment to the ER and subsequent translocation across the ER, as we had postulated in 1971? Or was the larger form the result of an *in vitro* translation artifact, e.g., perhaps the consequence of an erroneous upstream initiation? Interestingly, *in vitro* completion of nascent chains in isolated rough microsomes of myeloma cells yielded the mature light chain and not the larger form (12). Therefore, it was suggested that the microsomal membranes might contain a protease that converts the larger form into the mature form of the light chain of IgG by removing its amino-terminal extension (12).

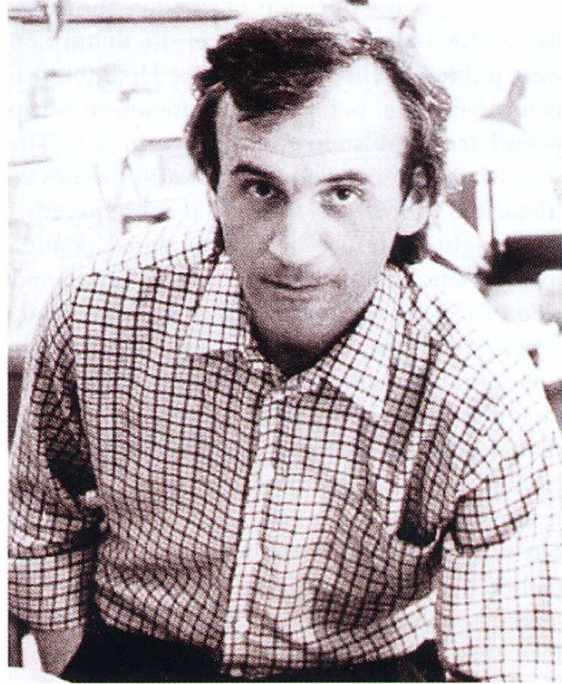


Figure 6. Bernhard Dobberstein, 1975. The Rockefeller University, New York.

SIGNAL PEPTIDASE

Together with Bernhard Dobberstein (Fig. 6), we succeeded in reproducing (14) the results of Leder's and Milstein's laboratories. We translated poly(A) containing mRNA from myeloma cells in a cell-free translation system that was reconstructed from heterologous components. We obtained a major polypeptide (Fig. 7, lane 1) that was larger by about three kDalton than the mature form of the light chain that was secreted from these myeloma cells (Fig. 7, lane 2).



Figure 7. Synthesis of the large (pLi) and mature (mLi) form of the light chain of IgG. ^{35}S -methionine-labeled proteins were separated by SDS-PAGE and visualized by autoradiography. Lane 1, translation products of poly(A) containing mRNAs isolated from myeloma cells; lane 2, mature light chains secreted from myeloma cells; lane 3, *in vitro* readout of polysomes ("detached polysomes") prepared by detergent from isolated rough microsomes of myeloma cells. From Blobel and Dobberstein (14) with permission.

To address the question of whether the larger form of the IgG light chain was a physiological precursor of the mature chain or an *in vitro* artifact, we were guided by three conjectures. First, the processing enzyme (subsequently termed signal peptidase) is a membrane-associated protease, as had been postulated by Milstein and co-workers (12). Therefore, this enzyme might be solubilized when isolated rough microsomes are treated with detergent; the 'detached' polysomes that could subsequently be sedimented by centrifugation might therefore be free of signal peptidase. Second, as the signal sequence was presumed to mediate the attachment of the ribosome to the cis-side of the membrane (see Fig. 5), the signal peptidase activity is likely associated with the trans-side of the microsomal membrane. This would assure that signal sequence removal occurs only after signal sequence-mediated attachment on the cis-side of the membrane. Third, after signal sequence-mediated attachment to the membrane the signal sequence would be translocated across the membrane and thereby gain access to its trans-side. There it would be cleaved 'co-translationally' and 'co-translocationally', i.e., during but not after translation and translocation were completed. Hence, in 'detached polysomes', the ribosomes near the 5' end of the mRNA might contain nascent chains with a signal sequence. In contrast, ribosomes located near the 3' end of the mRNA should contain nascent chains that already had their signal peptide removed (see model in Fig. 8).

In agreement with these assumptions, we observed (14) that nascent chains present in detached polysomes can be completed in a cell-free translation system (in the presence of an initiation inhibitor to prevent re-initiation) to yield both the larger and the mature forms of the light chain (Fig. 7, lane 3). Moreover, in a time course experiment, we observed that nascent chains lack-

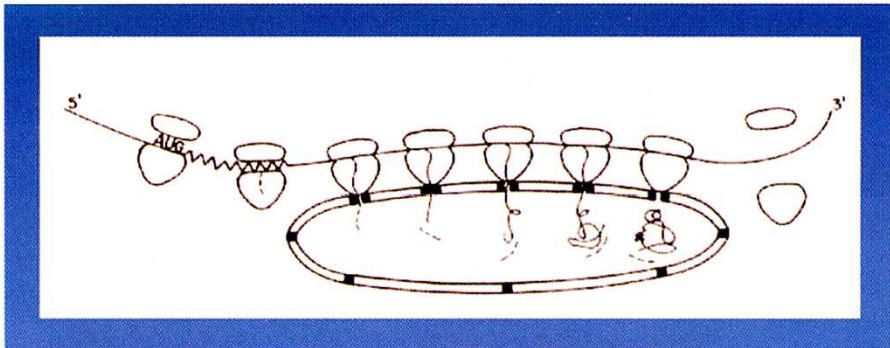


Figure 8. The 1975 version of the signal hypothesis. Signal peptidase, not indicated here was postulated to be associated with the trans side of the microsomal membrane. When the membrane is solubilized by detergent, signal peptidase would be absent in isolated detached polysomes. The detached polysomes should contain two kinds of chains: those still containing their signal peptide at the N-terminus and those with their signal peptide cleaved off by signal peptidase. If signal peptide cleavage occurs during translocation then those ribosomes near the 3' end of mRNA should contain chains without signal peptide and those near the 5' end of mRNA should still contain their signal peptide. In a time course of translation one should therefore first see the completion of chains that have lost their signal peptide and then the completion of chains that still contain their signal peptide.

ing a signal peptide were completed first, followed by the completion of chains still containing their signal peptide (Fig. 9). These results supported the notion that microsomal ribosomes near the 3' end of mRNA contained nascent chains from which the signal sequence had already been removed, co-translationally and co-translocationally. In contrast, ribosomes near the 5' end still contained their signal sequence. This signal sequence could no longer be cleaved as the signal peptidase had been removed during the preparation of the 'detached polysomes'. These 'readout' data with 'detached polysomes' suggested that a *completed* larger form of the light chain of IgG is indeed an *in vitro* artifact. *In vivo*, the signal peptide is cleaved off the *nascent* chain but cannot be cleaved off the *completed* chain. Hence, the *completed* larger form is not a physiological precursor of the mature form of the light chain.

RECONSTITUTION OF CO-TRANSLATIONAL TRANSLOCATION

The data on signal sequence removal provided us with important clues for our subsequent attempts to reconstitute translation and translocation of the light chain of IgG. In view of the signal hypothesis, it appeared unlikely that the larger form would be translocation-competent. That is, cell free synthesis of the completed larger form in the absence of membranes followed by post-translational incubation of the reaction with microsomal membrane vesicles was unlikely to yield translocation and conversion of the larger form to the mature form. Indeed, we found that the post-translational addition of microsomal vesicles to the *in vitro* translation reaction containing the larger form of the light chain of IgG did not yield conversion to the mature form.

At this point, we experienced an extremely frustrating period in our efforts to reconstitute co-translational translocation. All microsomal vesicle preparations that we prepared from several tissues of the usual laboratory animals (mice, rats, chicken, guinea pigs, pigeons and rabbits) severely inhibited the synthesis of the light chain of IgG in the translation reaction. As translation

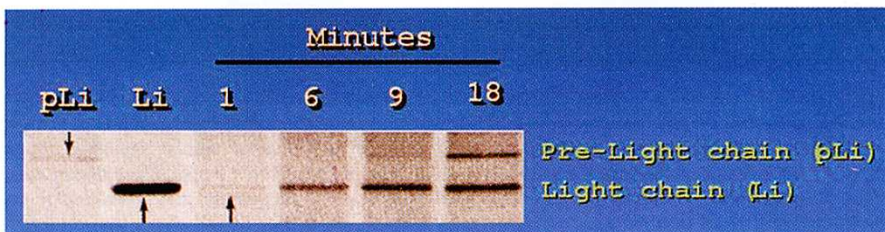


Figure 9. Time course of readout of "detached polysomes" (see Figs 7 and 8) from myeloma cells in a cell-free translation system. ^{35}S -methionine-labeled proteins were separated by SDS-PAGE and visualized by radioautography. Lane 1, major *in vitro* translation products of poly(A) containing mRNAs from myeloma cells; lane 2, light chains secreted from myeloma cells; lanes 1, 6, 9, 18, readout products from detached polysomes after 1, 6, 9 and 18 min of incubation in a translation system in the presence of an inhibitor of initiation. From Blobel and Dobberstein (14), with permission.

was completely inhibited by the added microsomal membranes, we were not able to detect translation-coupled translocation across these membranes.

A way out of this dilemma came in December of 1974. The chance arose to prepare and to test yet another rough microsome preparation from yet another source: canine pancreas. Surprisingly, addition of canine pancreas microsomal membranes to the translation reaction did not inhibit translation!! And, indeed, analysis of the translation products by SDS-PAGE showed that in the co-translational presence of microsomal vesicles most of the light chain was synthesized in its mature form (Fig.10, lane 3) (15). This strongly suggested that the signal sequence of the nascent chain engaged the translocation machinery of the membrane and gained access to signal peptidase on the trans-side of the membrane. The mature chain co-sedimented with the vesicles, suggesting that it was segregated within the vesicle lumen.

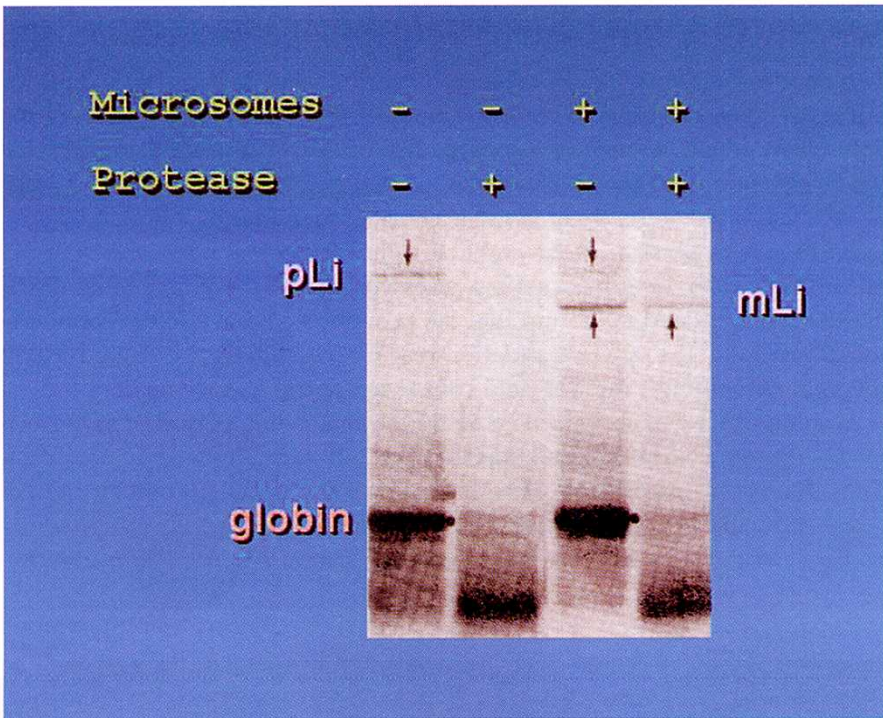


Figure 10. Reconstitution of translocation. Poly(A) containing mRNA from rabbit reticulocytes (coding primarily for the two globin chains) and from myeloma cells (coding primarily for the pre-light chain of IgG) were translated in the absence or presence of canine pancreas rough microsomes. Aliquots were post-translationally incubated in the absence or presence of protease. ^{35}S -methionine-labeled translation products were separated by SDS-PAGE and visualized by radioautography. Note that the mature light chain is synthesized only in the co-translational presence of microsomes and is protected from protease. From Blobel and Dobberstein (15), with permission.

As a control, we also translated rabbit reticulocyte mRNA (15). Translation of this mRNA yielded primarily the two globin chains (Fig. 10, lane 1). The two globin chains are cytosolic proteins and therefore should not contain a

signal sequence for translocation into the ER lumen. Moreover, there should not be any shortening of the two globin chains as a result of signal peptidase cleavage in the co-translational presence of pancreas microsomal vesicles. Indeed, we did not observe conversion to smaller forms of the two globin chains in the co-translational presence of microsomal membranes (Fig. 10, lane 3). Unexpectedly, however, the two globin chains co-sedimented with the microsomal vesicles. Could co-sedimentation result from co-translational segregation of the two globin chains into the lumen of the ER? Or was co-sedimentation of the two globin chains with the microsomal vesicles the consequence of non-specific binding of the newly synthesized globin chains to the cis-side of the vesicle membrane rather than of translocation to the trans-side of the vesicle membrane, i.e., into the vesicle lumen. To distinguish between these possibilities, we carried out post-translational incubation with proteolytic enzymes. We had shown earlier (16) that membranes of microsomal vesicles are sealed in such a way that they protect the content proteins from attack by externally added proteolytic enzymes while allowing proteolysis of surface-bound proteins and even detachment of ribosomes. Hence, post-translational incubation with proteolytic enzymes should distinguish between proteins bound to the surface of microsomal vesicles and those segregated in the vesicle lumen. We found that the two globin chains were largely degraded by post-translationally added proteases, regardless of whether translation was carried out in the absence of vesicles (Fig. 10, lane 2) or in the presence of vesicles (Fig. 10, lane 4).

Most importantly, post-translational proteolysis of the translation reaction that was carried out in the presence of microsomal vesicles yielded degradation of the larger form of the light chain but not the mature form of the light chain (Fig. 10, lane 4). These results suggested that even in the presence of microsomal membranes some of the nascent chains failed to engage the translocation machinery of the membrane, never gained access to signal peptidase on the trans-side of the vesicles and therefore were completed in the translation reaction as larger forms. Having not been translocated, the larger forms were degraded by added proteolytic enzymes.

Together, these data strongly suggested that we had succeeded in recapitulating the initial events in the secretory pathway in an *in vitro* system. This opened the way for biochemical analysis of protein translocation across the ER. These experiments marked the beginning of molecular cell biology. Successful *in vitro* reconstitution was subsequently achieved for many other membrane-coupled reactions.

AN EXPANDED SIGNAL HYPOTHESIS

The results of the reconstitution experiments encouraged us to further elaborate the simple model of 1971 (Fig. 5). The most significant additional postulate was that protein transport across the microsomal membrane proceeds through a protein-conducting channel (Fig. 11) (14). The protein-conducting channel was envisaged to consist of intergral membrane protein sub-

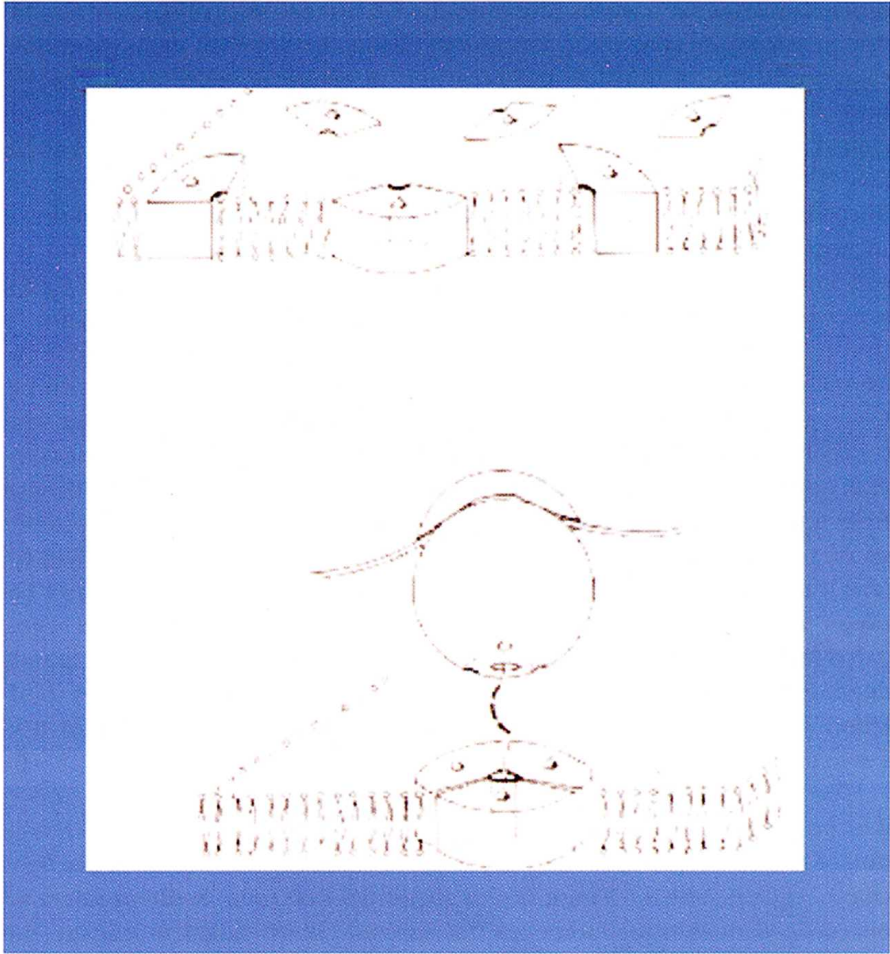


Figure 11. Signal hypothesis 1975. Proposal of a protein-conducting channel (PCC). The PCC was envisaged to be assembled from integral membrane proteins. The signal sequence, cooperatively with the ribosome, was proposed to gate open the channel, with the ribosome attaching to the channel. This arrangement provided for an alignment of a protein-conducting tunnel in the large ribosomal subunit with the protein-conducting channel in the membrane, allowing translation-coupled (co-translational) translocation of the chain across the membrane (see below [Fig. 46] showing relevant data obtained twenty-two years later). This proposal became one of the most controversial aspects of the signal hypothesis. Other proposals suggested a direct transfer of the nascent protein across the lipid bilayer. From Blobel and Dobberstein (14), with permission.

units. The signal sequence and the ribosome were proposed to bind to these subunits and to assemble them into an aqueous channel (Fig. 11) allowing transport of the chain in an aqueous conduit across the membrane. The concept of a protein-conducting channel remained controversial for many years until definitive evidence for it was provided by electrophysiological experiments in 1991 (17). In the intervening 15 years, alternative hypotheses that proposed transfer of secretory proteins across the membrane directly through the lipid bilayer, unassisted by proteins, flourished and became widely accepted.

We also suggested that integral membrane proteins that experience a partial translocation of a segment of the nascent chain to the trans-side of the membrane might use a signal sequence to initiate translocation.

Finally, we predicted that the translocation of proteins across other cellular membranes, e.g., import of cytoplasmically synthesized proteins into mitochondria, is mediated by a signal sequence that would be distinct from that of a secretory protein.

MEMBRANE PROTEIN ASSEMBLY

To test whether an integral membrane protein does, in fact, use a signal sequence to translocate a domain to the trans-side of the ER, we studied the glycoprotein of the vesicular stomatitis virus (G protein of VSV) (18, 19). This protein is part of the viral membrane and contains an amino-terminal domain exposed on the surface of the viral membrane, a single transmembrane segment and a carboxy-terminal domain that interacts with the viral capsid. To achieve this topology in the viral membrane, the amino-terminal domain of the G protein must be translocated to the trans-side of the ER, whereas the carboxy-terminal domain needs to remain untranslocated on the cis-side of the ER.

To accomplish this asymmetric integration into the ER membrane, we reasoned that a signal sequence, indistinguishable from those of secretory proteins, might initiate the translocation process in the ER. An additional sequence, that we termed 'stop-transfer' sequence, would then terminate the translocation process. We speculated that the stop-transfer sequence would open the channel laterally, thereby allowing displacement of the stop-transfer sequence from the aqueous protein-conducting channel to the lipid bilayer. The stop-transfer sequence would then become the trans-membrane segment that is embedded in the lipid bilayer. The carboxy-terminal remainder of the protein would not be translocated, and would remain on the cis-side of the membrane (see Fig. 12).

It should be remembered that in those days there were still no pure mRNAs available that could be readily obtained by *in vitro* transcription from recombinant DNA. All we could do was to isolate total mRNA from cells or tissues that contained major species of mRNA whose translation would give rise to major and readily detectable translation products. The VSV-infected cell was a good model system as most of the total mRNA from these infected cells coded for VSV proteins. Of these, the G protein is the largest and is readily identifiable on the basis of its migration in SDS-PAGE. These experiments were done in collaboration with Harvey Lodish's laboratory at MIT, particularly his graduate student Flora Katz, and by a graduate student in our laboratory, Vishwanath ('Vishu') Lingappa (Fig. 13).

As expected, translation of mRNA from VSV-infected cells yielded the G protein and other VSV proteins (Fig. 14, lane 1, arrow pointing to G_0). However, contrary to our expectation, in the co-translational presence of the

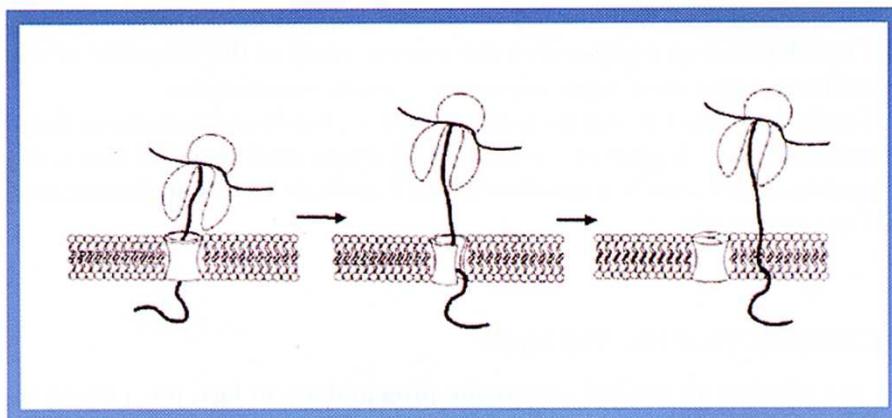


Figure 12. Model for catalyzed integration into the lipid bilayer of integral membrane proteins. The proposal that the initial steps in the integration of membrane proteins are the same as those for the translocation of secretory proteins was made already in the 1975 version of the signal hypothesis. Translocation across the protein-conducting channel (PCC) was envisaged to be initiated by a signal sequence, as in the case of secretory proteins. A stop-transfer sequence, corresponding to the trans-membrane segment of the translocating chain would be recognized by the PCC and open it laterally. The trans-membrane segment would then be displaced from the aqueous PCC, to the lipid bilayer, resulting in the closure of the PCC and the integration of the nascent chain into the lipid bilayer.

microsomal vesicles, at least half of the G protein molecules appeared to be synthesized as a *larger* form (Fig. 14, lane 3, arrow pointing to G_1). Rather than having cleaved off a putative signal sequence, the membranes appeared to have added something to the G protein. We were very puzzled by this result until it occurred to us that the canine microsomal vesicles might contain ac-

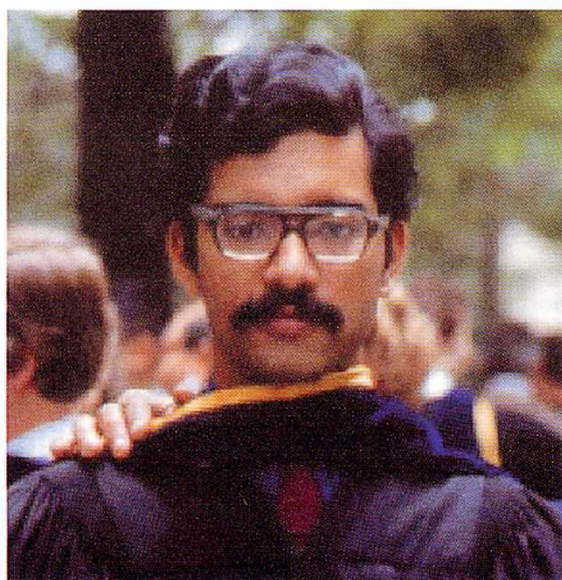


Figure 13. Vishwanath (Vishu) Lingappa, 1979. The Rockefeller University, New York.

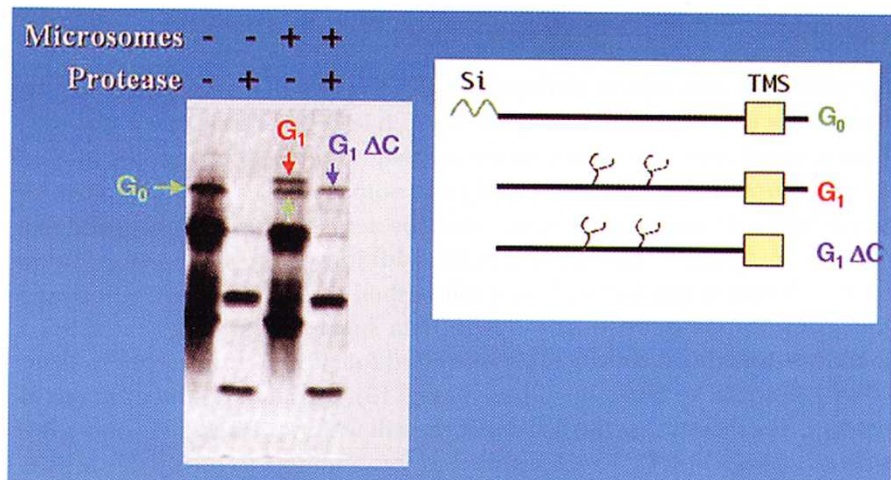


Figure 14. Membrane assembly *in vitro*. Poly(A) containing mRNA from VSV-infected cells was translated in the absence or presence of canine pancreas microsomal membranes. Equal aliquots were post-translationally incubated in the absence or presence of protease. The ^{35}S -methionine-labeled translation products were separated by SDS-PAGE and visualized by radioautography. G_0 , translation product in the absence of membranes; it still contains the amino-terminal signal sequence; G_1 , translation product in the presence of microsomes; it is core-glycosylated and has lost its signal sequence; $G_1\Delta C$, a form of G_1 that has lost its vesicle-exposed carboxy-terminal tail during post-translational proteolysis. From Katz, *et al.* (18) and Lingappa, *et al.* (19), with permission.

tivated oligosaccharides and oligosaccharidyl transferase to carry out co-translocational core-glycosylation of the G protein. The added core sugars might more than compensate in mass for the loss of a signal sequence and therefore result in a slower migration in SDS-PAGE. Biochemical characterization of both the G_0 and the G_1 species indeed confirmed that G_0 contained an amino-terminal signal sequence that was structurally and functionally equivalent to those of secretory proteins (18, 19). The G_1 species had lost its signal sequence and had acquired core sugars. Moreover, post-translational proteolysis experiments showed complete digestion of G_0 whereas G_1 was converted to a slightly smaller form (Fig. 14, lane 4). This smaller form represented G_1 that had lost its carboxy-terminal tail. These post-translational proteolysis data suggested that the G protein had been correctly integrated into the microsomal vesicles!! As *in vivo*, the bulky amino-terminal domain was translocated to the trans-side of the membrane, whereas the smaller carboxy-terminal domain of about 40 amino acid residues remained untranslocated on the cis-side of the membrane.

Microsomal vesicles were not only able to translocate secretory proteins. They were also competent to asymmetrically integrate membrane proteins and to core glycosylate them! These results demonstrated that the asymmetric integration of membrane proteins is not a spontaneous event but is catalyzed in the ER, presumably by the same machinery that mediates the translocation of secretory proteins.

THE SIGNAL RECOGNITION PARTICLE

Our next goal now was to deconstruct the translocation machinery of the microsomes into functional components. It had been shown that a salt wash of microsomal vesicles removes an activity that is required for translocation of secretory proteins. Such salt washed microsomal vesicles were incapable of co-translational translocation of secretory proteins that were synthesized in a wheat germ cell-free translation system. Addition of the salt wash to the *in vitro* translocation system containing salt-washed microsomal vesicles restored their translocation activity (20). These data suggested that salt extracted a membrane-associated activity that is required for protein translocation. Peter Walter (shown in an Einsteinian pose in Fig. 15), a graduate student in the laboratory, discovered that the activity of the salt wash vanished after only a few hours of storage at 0°C. This frustrated all his attempts to purify this activity until he made the important discovery that minute concentrations of the non-ionic detergent Nikkol stabilized the activity. We reasoned that Nikkol protected a hydrophobic site of the activity, perhaps the binding site for the hydrophobic signal sequence. Incorporating the hydrophobic-site-idea into his purification protocol, he was able to rapidly purify the activity. The activity sedimented at about 11S and by SDS-PAGE consisted of six proteins (21). Based on an extensive analysis of its function (22, 23, 24) we termed the protein complex "signal recognition protein" (SRP).

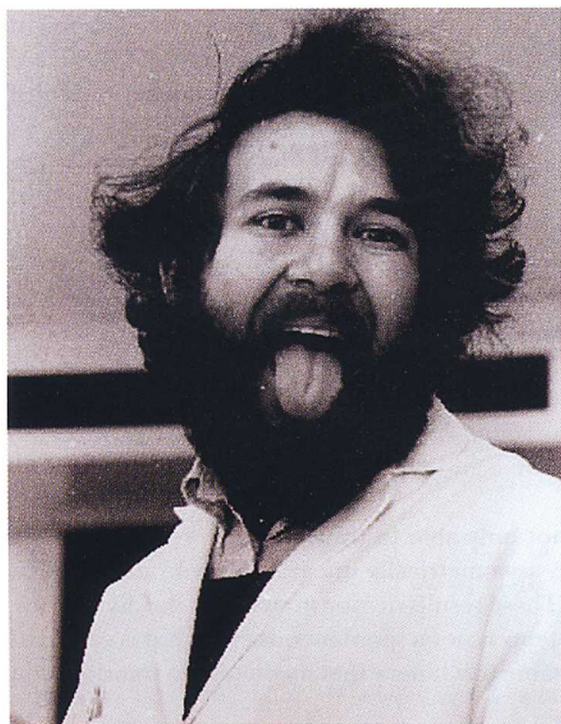


Figure 15. Peter Walter, 1980. The Rockefeller University, New York. Obviously inspired here by the famous photo of Einstein.

It took us another two years before we realized that the purified activity also contained a 7S RNA (25)! We then changed the name to signal recognition particle, leaving the acronym SRP intact. A considerable amount of work in both Peter Walter's and Bernhard Dobberstein's laboratory has led to a functional and structural model for the SRP and to the discovery of an interesting homolog in bacteria (Fig. 16) (26).

Functional analyses (22, 23, 24) revealed that canine pancreas SRP bound with low affinity to wheat germ ribosomes. However, SRP bound with high affinity, when wheat germ ribosomes were programmed with a mRNA coding for a secretory protein (bovine preprolactin), not when programmed with a mRNA coding for cytosolic proteins, such as the globin chains. Also, if translation was carried out in the presence of hydroxy-leucine instead of leucine, there was greatly reduced binding of SRP. As the signal sequence for preprolactin is rich in leucine, this result suggested that SRP interacted directly with the signal sequence. Moreover, the interaction of SRP caused a translation ar-

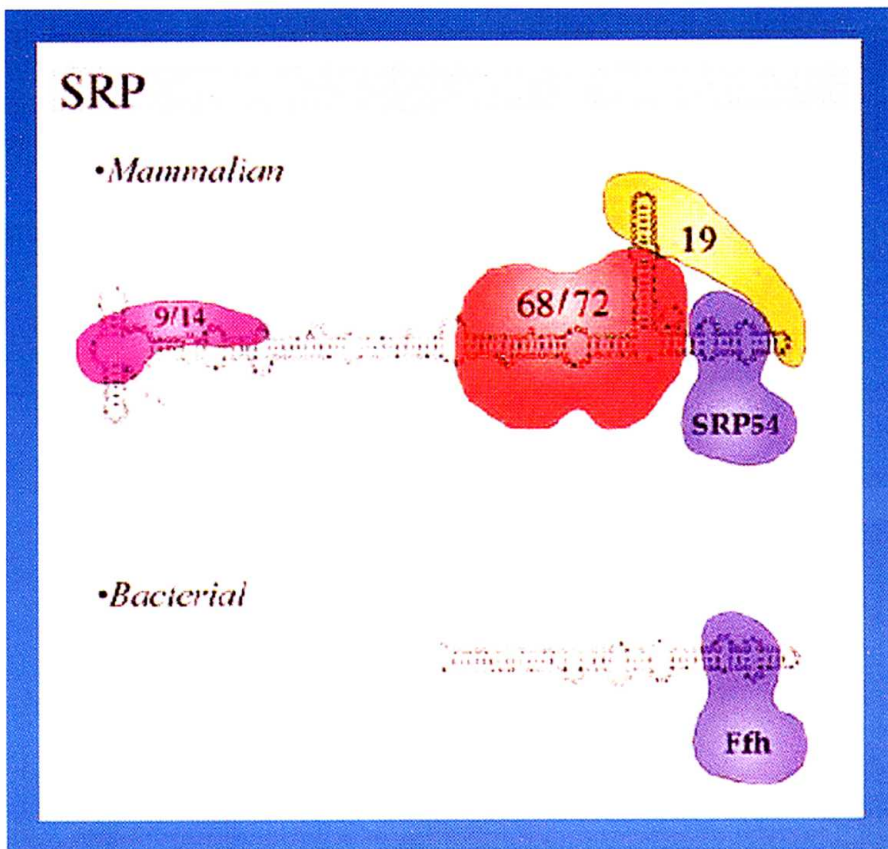


Figure 16. Signal Recognition Particle (SRP) in mammalian cells and in bacteria. Both SRPs contain an RNA and proteins. The mammalian SRP contains six proteins of indicated mol.wt. in kDaltons. The bacterial SRP contain a shorter RNA and a single protein (ffh, fifty-four-homolog). From Walter and Johnson (26), with permission.

rest when ribosomes were programmed with a mRNA for a secretory protein, but not when programmed with mRNA for cytosolic proteins. The size of the arrested nascent secretory protein corresponded to about 70 amino acid residues, suggesting that binding of the signal sequence occurred after the signal sequence was fully exposed following exit from the tunnel in the large ribosomal subunit (Fig. 17). When microsomal membranes were added, there was release of the translation arrest, suggesting that the ER membrane contains an SRP receptor that is capable of releasing SRP-mediated translation arrest.

We found that the *in vitro* assembled polysomes synthesizing secretory proteins, but not those synthesizing cytosolic proteins, bound to SRP-depleted (salt-washed) microsomes, but only in the presence of SRP. Binding was abolished when SRP was pretreated with N-ethyl maleimide.

These data strongly supported the notion that protein translocation across the ER is a receptor-mediated event and ruled out proposals that chain translocation occurs spontaneously and unassisted by proteins.

The discovery of the signal recognition particle was an important milestone in the molecular analysis of protein translocation across the ER membrane. Its function corresponded to that of the "binding factor" whose existence had been predicted in 1971 (see Fig. 5). It was the first component of any of the cellular membrane translocation systems that was isolated and characterized. It provided strong support for the predictions made in the signal hypothesis.

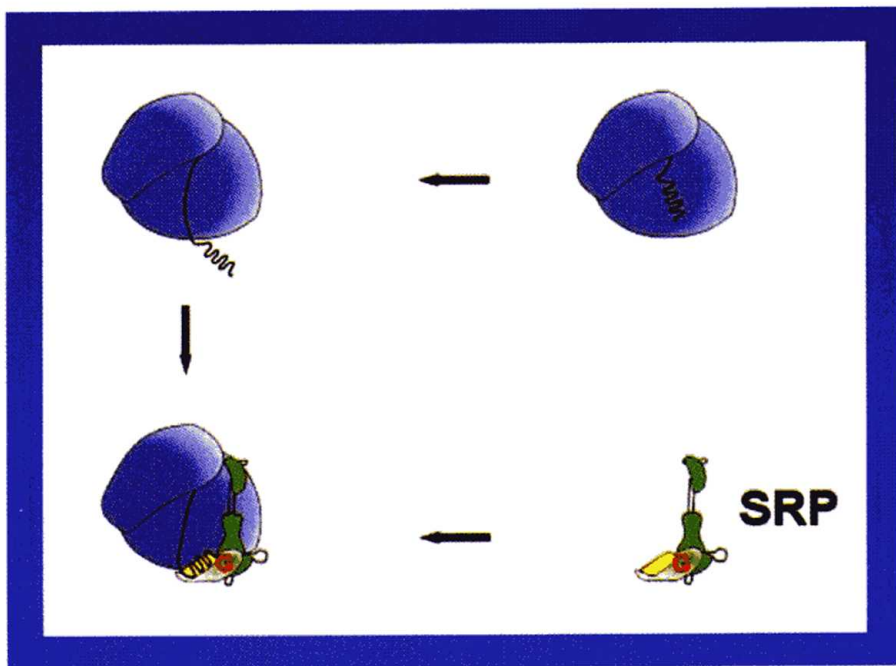


Figure 17. Recognition of the signal sequence by the Signal Recognition Particle (SRP). SRP 54 is a GTPase and is therefore marked G. As soon as the signal sequence has emerged from the large ribosomal subunit, SRP can bind to it.



Figure 18. Reid Gilmore, 1985.
The Rockefeller University,
New York.

SRP RECEPTOR

Reid Gilmore, a postdoctoral fellow in our laboratory (Fig. 18) used the ability of salt-washed microsomal membranes to release the SRP-mediated elongation arrest of the synthesis of secretory proteins as an assay to follow

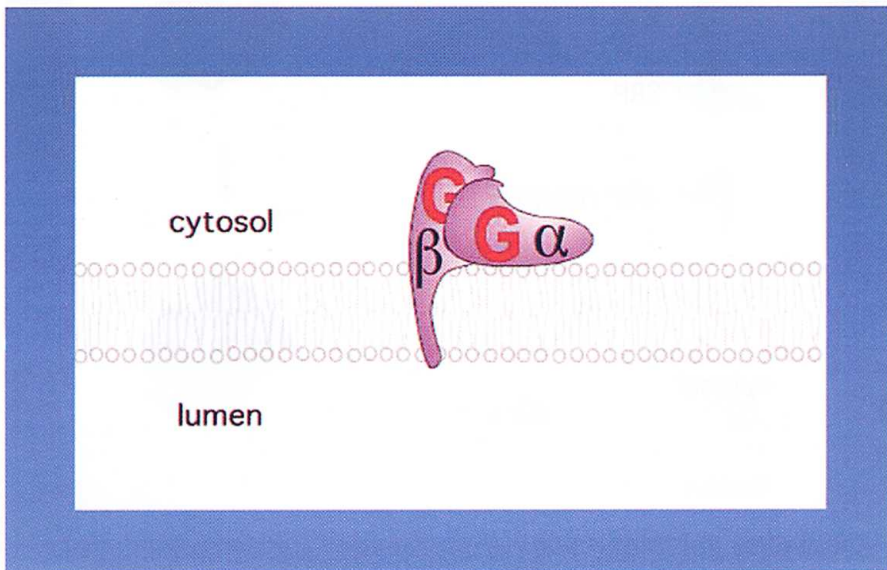


Figure 19. The SRP receptor is a heterodimer of two GTPases; the beta subunit is an integral membrane protein and the alpha subunit a peripheral membrane protein.

the purification of this activity. Together with the membrane's signal peptidase (see below), the arrest-releasing activity could be solubilized by treatment of the salt-washed microsomal membranes with non-ionic detergent and moderate salt concentrations. The purified SRP receptor consisted of two subunits, a larger subunit and a smaller subunit (27–31). The larger subunit is a peripheral membrane protein whereas the smaller subunit is an integral membrane protein with one transmembrane segment. Both proteins were shown to be GTP binding proteins (30, 31) (Fig. 19). SRP and SRP receptor interact with each other (32) (Fig. 20). The SRP receptor is located exclusively to the ER (including the outer nuclear envelope) and is present in the ER in sub-stoichiometric quantities relative to membrane-bound ribosomes (27). This suggested that the SRP-SRP receptor interaction is transient in nature. It serves in the targeting but not in the subsequent translocation of the chain.

With the discovery of the SRP and its cognate SRP receptor, the components involved in signal sequence recognition and targeting to the ER had been isolated.

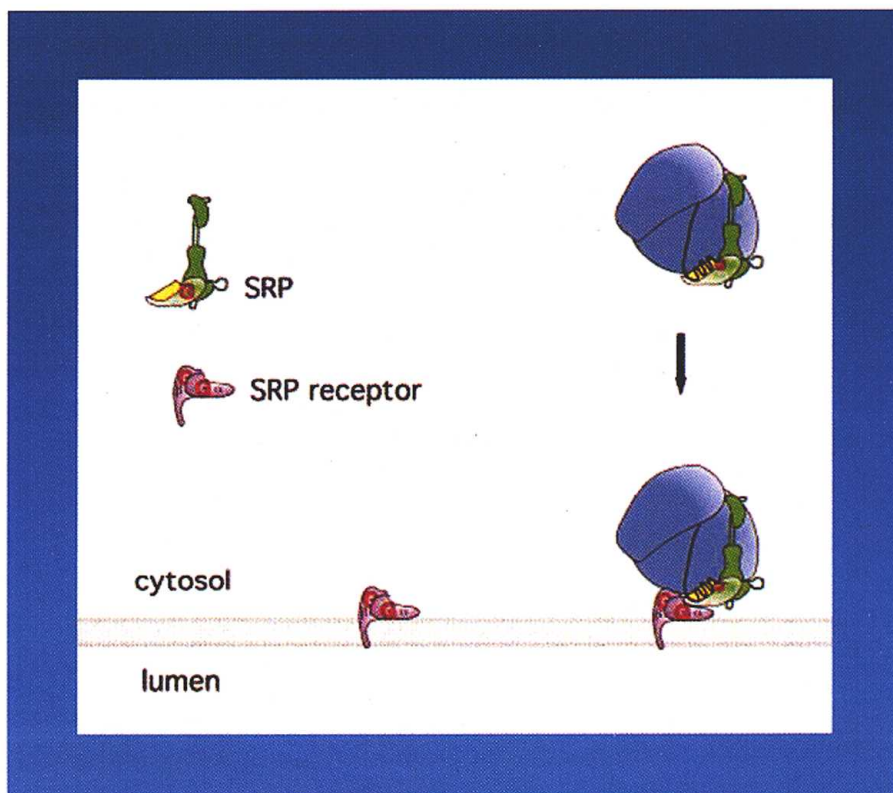


Figure 20. The ribosome/nascent chain/SRP complex binds to the SRP receptor in the ER.

SIGNAL PEPTIDASE

The signal peptidase cleavage site of nascent secretory proteins is accessible to signal peptidase only on the trans-side of the membrane (see above). Therefore, signal sequence cleavage is dependent on translocation. Robert Jackson in our laboratory developed a translocation-independent assay for signal peptide cleavage. He solubilized the microsomal signal peptidase by detergent. Using an *in vitro* synthesized presecretory protein as a substrate he demonstrated correct endoproteolytic removal of the signal sequence by the detergent form of the enzyme (33).

The solubilized microsomal signal peptidase was purified by Emily Evans, a graduate student in our laboratory (Fig. 21). The purification of this enzyme was a major challenge as its activity required the presence of lipids and detergents throughout all purification procedures. Omission of lipids led to rapid inactivation of the enzyme. Surprisingly, the purified microsomal signal peptidase turned out to be a complex of five non-identical subunits (34) (Fig. 22), all of which are integral membrane proteins (35, 36, 37).

With the SRP receptor and signal peptidase, two components of the microsomal membrane that could be solubilized by detergents and assayed independently of intact vesicles and of translocation, had been purified. Our next goal was to purify the putative protein-conducting channel. The proteins making up such a channel were likely to be integral membrane proteins. To purify them from other membrane proteins required detergent solubilization. However, unlike SRP receptor and signal peptidase, channel proteins require reconstitution into proteoliposomes to unambiguously assay their activity.



Figure 21. Emily Evans, 1986.
The Rockefeller University,
New York.

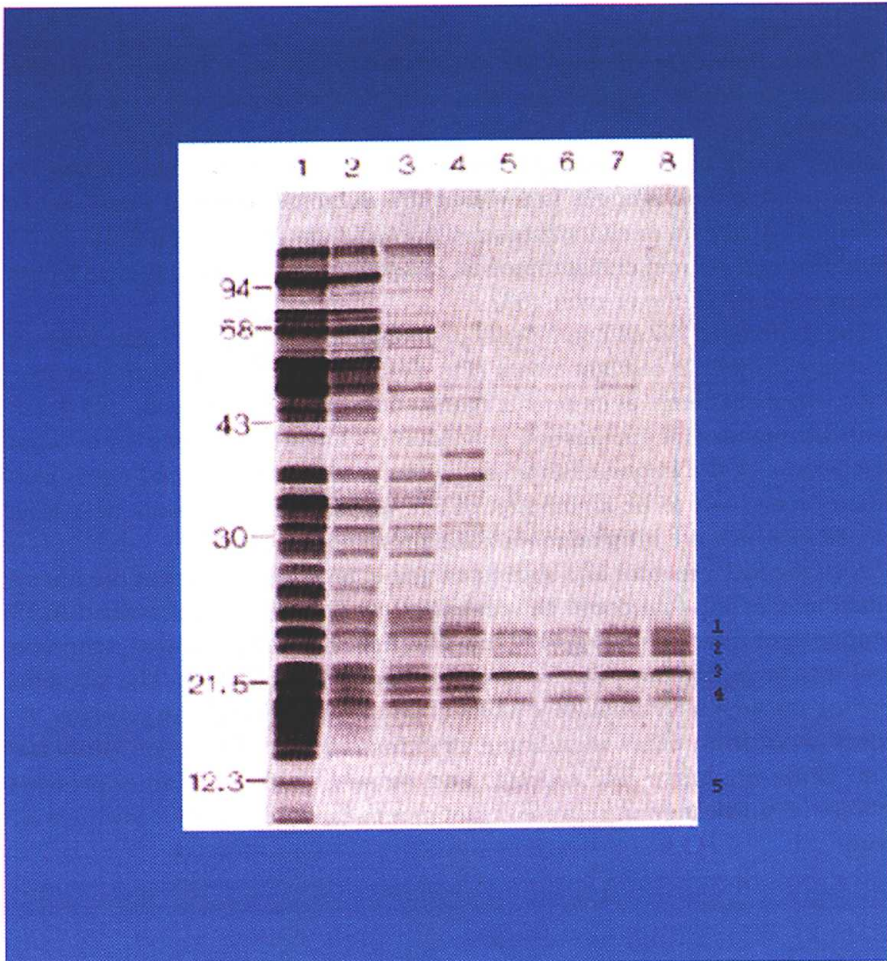


Figure 22. Purification of mammalian signal peptidase, a pentameric complex of integral membrane proteins. Band 2 represents the glycosylated and non-glycosylated form of the same polypeptide. From Evans, *et al.* (34), with permission.

RECONSTITUTION OF TRANSLOCATION-ACTIVE PROTEOLIPOSOMES

The reconstitution of translocation-active proteoliposomes had been a high priority on our agenda for quite some time before Christopher Nicchitta (Fig. 23) joined our laboratory as a postdoctoral fellow. However, many attempts by the most talented people in our laboratory had failed to achieve this goal. We had arrived at the conclusion that reconstitution, if at all possible, would probably occur with very low efficiency. Presumably, after detergent solubilization and removal of the detergent to form proteoliposomes, the components that are required for co-translational translocation are not necessarily reconstituted in the asymmetric orientation and in the stoichiometry in which they exist in the microsomal vesicles. Instead, reconstituted proteoli-



Figure 23. Christopher Nicchitta, 1989. The Rockefeller University, New York.

posomes may contain these proteins in a scrambled fashion and in non-stoichiometric amounts rendering them inefficient or inactive in translocation. The SRP receptor, the signal peptidase complex, or the constituents of the protein-conducting channel and perhaps other components that participate in protein translocation may not regain their asymmetric orientation in the reconstituted proteoliposome vesicles. Signal peptidase and the SRP receptor, for example, exhibit their active sites in opposite orientations, on the trans-side and on the cis-side of the vesicles, respectively.

Through careful choice of detergents and of other conditions (pH as well as salt- and detergent concentrations) we were able to obtain translocation-competent proteoliposomes (38) (Fig. 24).

These experiments were seminal. They showed that after complete detergent solubilization of microsomal membranes, the detergent forms of integral membrane proteins required for protein translocation, including a putative protein-conducting channel, could be reconstituted in an active form. This paved the way for the isolation of the protein-conducting channel. It also allowed the subsequent reconstitution of translocation-active proteoliposomes from purified components.

ELECTROPHYSIOLOGICAL DETECTION OF THE PROTEIN-CONDUCTING CHANNEL

We first proposed the concept of a protein-conducting channel (PCC) made up of integral membrane proteins in 1975 (14). However, it remained a highly controversial idea, particularly as there was no direct evidence for it. Alternative models of protein translocation directly through the lipid bilayer

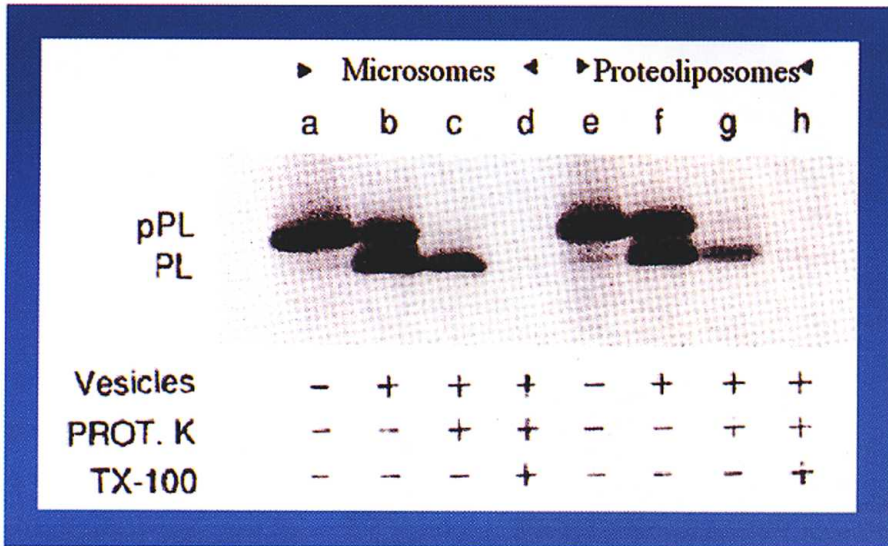


Figure 24. Reconstitution of protein translocation activity into proteoliposomes. Comparison of protein translocation activity of microsomes and reconstituted proteoliposomes. pPL, preprolactin; PL, prolactin; Prot. K, proteinase K. TX-100, the non-ionic detergent Triton X-100. From Nicchitta and Blobel (38), with permission.

flourished (39, 40). In 1985, we showed (41) that nascent chains in the process of translocation are accessible to aqueous perturbants. Although these data were consistent with an aqueous PCC, they could not rule out that an aqueous environment was created transiently by the hydrophilic head groups of the lipids rather than proteins forming such an aqueous channel.

Although electrophysiology was a standard approach for identifying and characterizing ion-conducting channels, it had never been applied to the detection and characterization of any protein-conducting channels. It was difficult to predict how such channels would behave electrophysiologically. We had postulated that they would be opened and closed for each protein translocation event (14). Moreover, we reasoned that the conductance of a protein across the channel is unlikely to be accompanied by a significant co-conductance of ions or small molecules. If this were the case, it would be difficult to maintain the distinct composition of ions (particularly calcium) and small molecules in the ER lumen, the 'reticuloplasm', as compared to that in the cytoplasm. PCCs are likely to be closed when they are not conducting a polypeptide chain. Moreover, they are most likely designed to prevent the co-conduction of ions when they are opened for and during translocation of a protein. Therefore, it was not clear whether PCCs could be revealed at all by electrophysiological measurements. With these caveats in mind, Sanford Simon (Fig. 25), a post-doctoral fellow in our laboratory, set out on a most remarkable and exciting journey into completely uncharted territory: to explore PCCs by electrophysiological approaches. These experiments were begun in collaboration with Joshua Zimmerberg at NIH (42).

Because rough microsomes were too small for patch-clamp experiments.

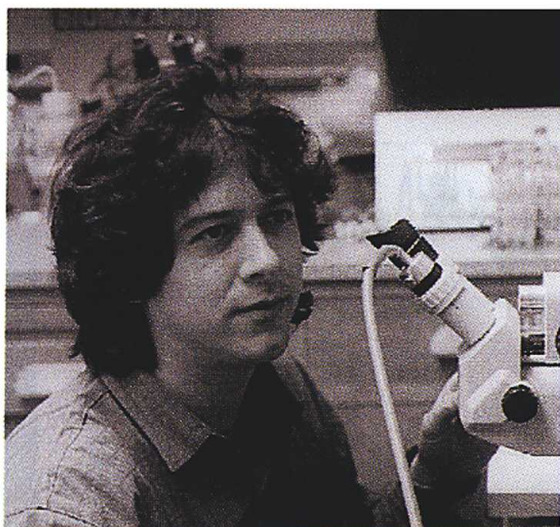


Figure 25. Sanford M. Simon, 1991. The Rockefeller University, New York.

we decided to use the planar lipid bilayer system developed by Mueller *et al.* in 1962 (43). In this system, vesicles are fused to a planar bilayer that is formed in a hole of a partition separating two chambers (Fig. 26). Addition of microsomes to the cis-chamber yielded occasionally fusion of a single rough microsomal vesicle as evidenced by an increase in conductance. Some preparations yielded unitary conductances of 20, 55, 80 and 115 pico Siemens (pS) in 45 mM potassium glutamate (42) (Fig. 27). By electron microscopy it can be estimated that each of the rough microsomal vesicles contains about a hundred membrane-bound ribosomes (Fig. 28). Many of these membrane-bound ribosomes are potentially in the process of conducting a chain across the membrane. Therefore, it was unlikely that the few unitary conductances that we observed were related to PCCs. Nevertheless, the fact that we could

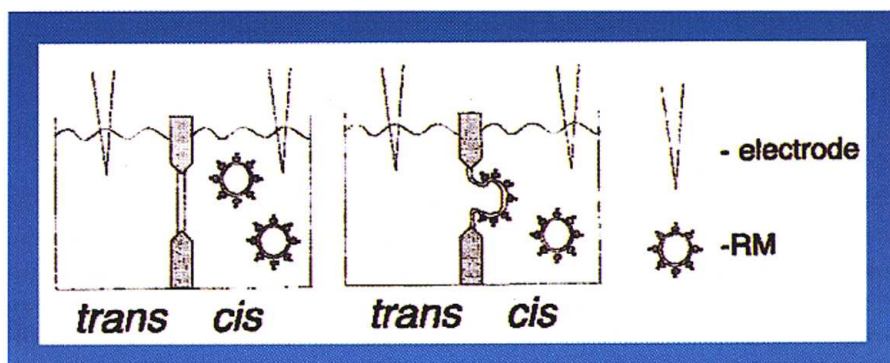


Figure 26. Schematic representation of the bilayer system. The cis chamber is separated from the trans chamber by a plastic division (thick line) with a small circular hole that contains a planar lipid bilayer. Left, rough microsomes (RM) are added to the 'cis' chamber. Right, a single rough microsomal vesicle has fused with the planar bilayer. From Simon and Blobel (17), with permission.

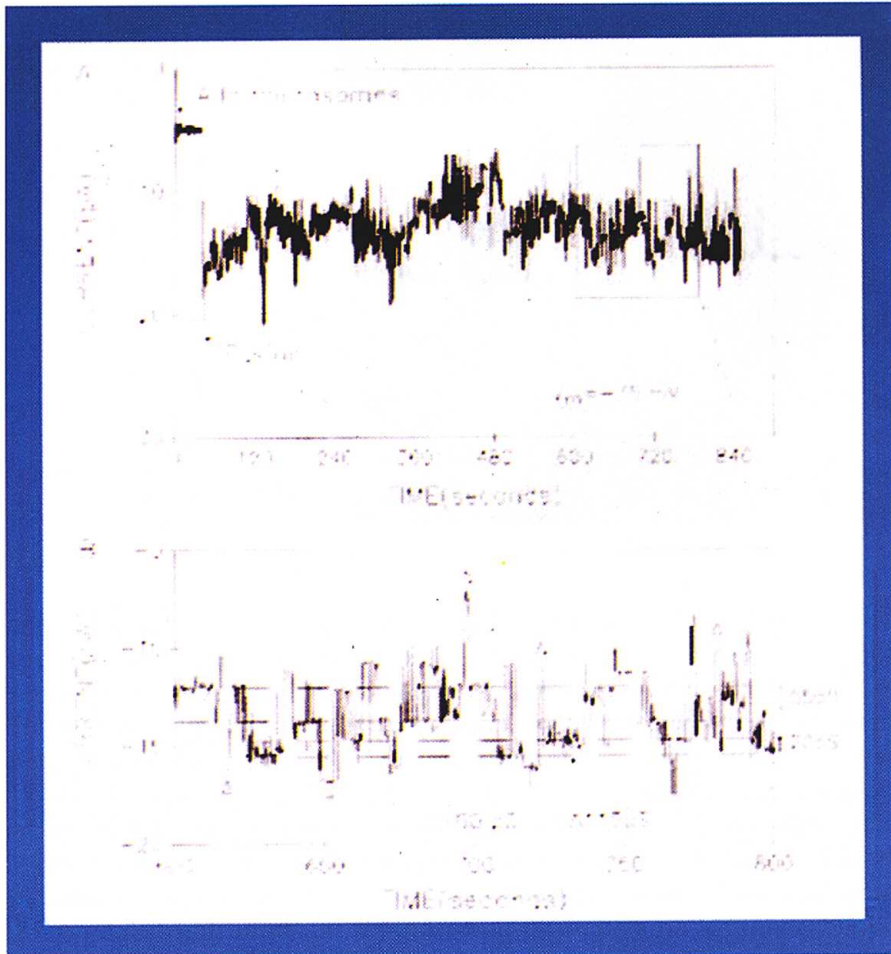


Figure 27. Channels in rough microsomes. (A) Fusion of a single rough microsomal vesicle to the planar bilayer. After 45 sec there was a substantial increase in the fluctuations of the current (B). A segment of the record of A from 600 to 800 sec is shown in an expanded time scale. Four distinct channel sizes can be distinguished: 20 pS, 50 pS, 80 pS and 115 pS. The function of these channels remains to be determined. From Simon, *et al.* (42), with permission.

detect conductances at all suggested that fusion of a rough microsomal vesicle with the planar bilayer had taken place.

If the PCCs are indeed either closed or electrically silent when occupied by nascent chains, would it be possible to reveal these channels by releasing the nascent chain without closing them? Could puromycin, an analog of amino acyl tRNA (Fig. 29) achieve this? The peptidyl transferase activity of ribosomes couples puromycin to the carboxy-terminal end of the nascent chain. This causes chain release from the ribosome. In the case of rough microsomes, it had been established some time ago that the chain is vectorially discharged into the lumen of the vesicle (2). When the puromycin reaction is carried out at low salt concentrations the chains are vectorially discharged,

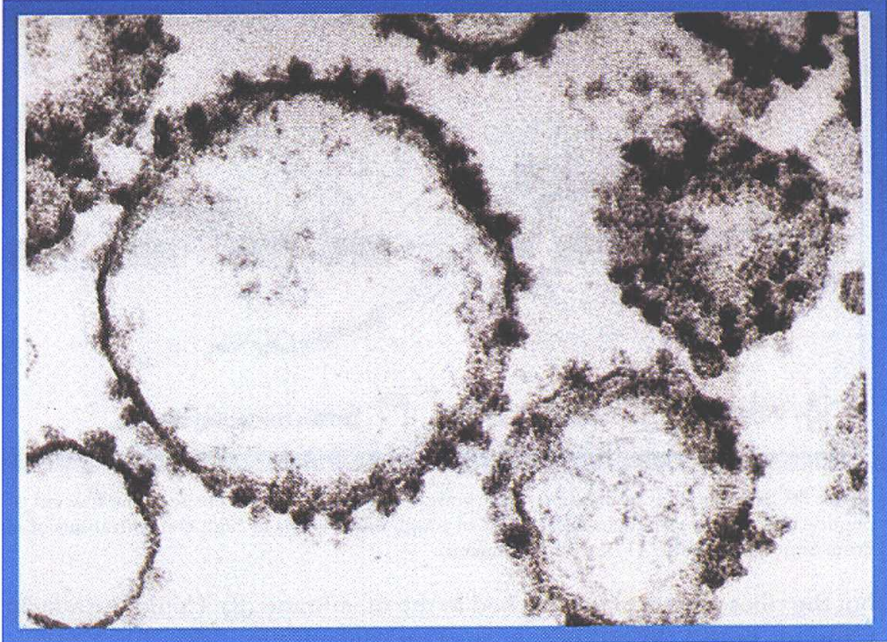


Figure 28. Electron micrograph of isolated canine pancreas rough microsomes.

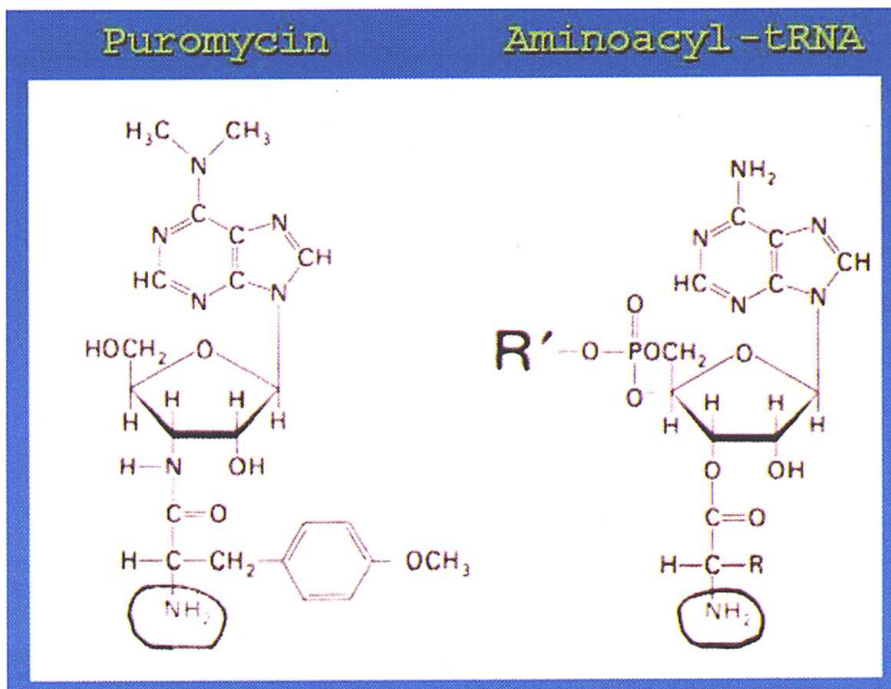


Figure 29. Puromycin is an analog of aminoacyl-tRNA.

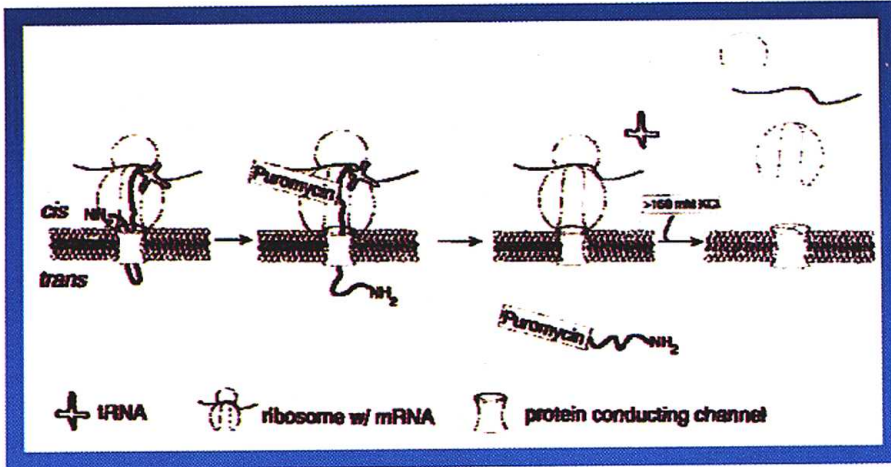


Figure 30. Schematic representation of puromycin-induced vectorial release of the nascent polypeptide chain and subsequent disassembly of rough microsomes by high concentrations of salt. From Simon and Blobel (17), with permission.

but the ribosomes remain attached to the membrane (8). Could the attached ribosome keep a cleared PCC in an open configuration (Fig. 30)? And could subsequent treatment with high salt release the ribosome and close the PCC (Fig. 30) (8)?

Based on these considerations we added puromycin to the cis-chamber of the bilayer that contained a fused rough microsome vesicle (Fig. 26). We observed a huge increase in conductance (17) (Fig. 31). This effect was specific

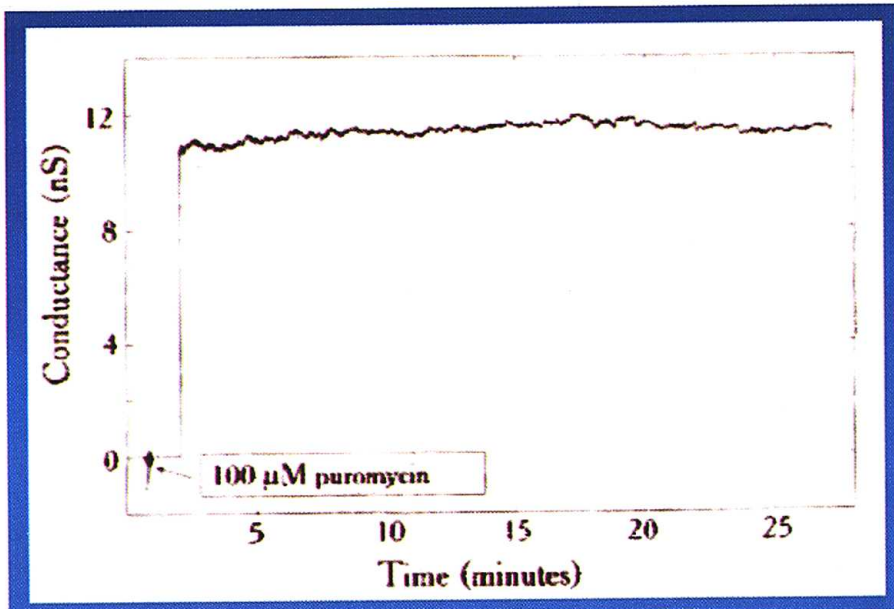


Figure 31. Puromycin-induced clearance of protein-conducting channels in rough microsomes. A sharp increase in conductance occurred within 45 sec of addition of puromycin (100 μ M) to the cis side of the microsomal membrane. From Simon and Blobel (17), with permission.

as it was observed only when puromycin was added to the cis-chamber, not when it was added to the trans-chamber (17). The cis-chamber, but not the trans chamber, contained the exposed ribosomes of the fused rough microsomal membrane (see Fig. 26). Puromycin, when added to the trans-chamber, could not access the ribosomes and be coupled to the nascent chain. But when subsequently added to the cis-chamber, it again caused a huge increase in conductance (Fig. 32). These side-specific data strongly suggested that puromycin indeed cleared the nascent chain and that at low salt the PCCs stayed in an open configuration and were able to conduct ions.

How many PCCs were cleared by the addition of puromycin? Could very low concentrations of puromycin make it possible to demonstrate a time-resolved clearance of individual PCCs? Indeed, when much lower concentrations of puromycin were added to the cis-chamber, we observed clearance, one at a time, of individual PCCs (17) of 220 pS (Fig. 33). Hence, the PCC contains a very large aqueous pore that conducts about ten times more ions than an ordinary ion-conducting channel (17).

Could one close the puromycin-revealed PCC by dissociating the ribosome by high salt concentrations? Indeed, when a single PCC (Fig. 34) was revealed at low concentrations of puromycin and at low salt, it was closed by a subsequent increase in salt concentration (Fig. 35). This result was consistent with

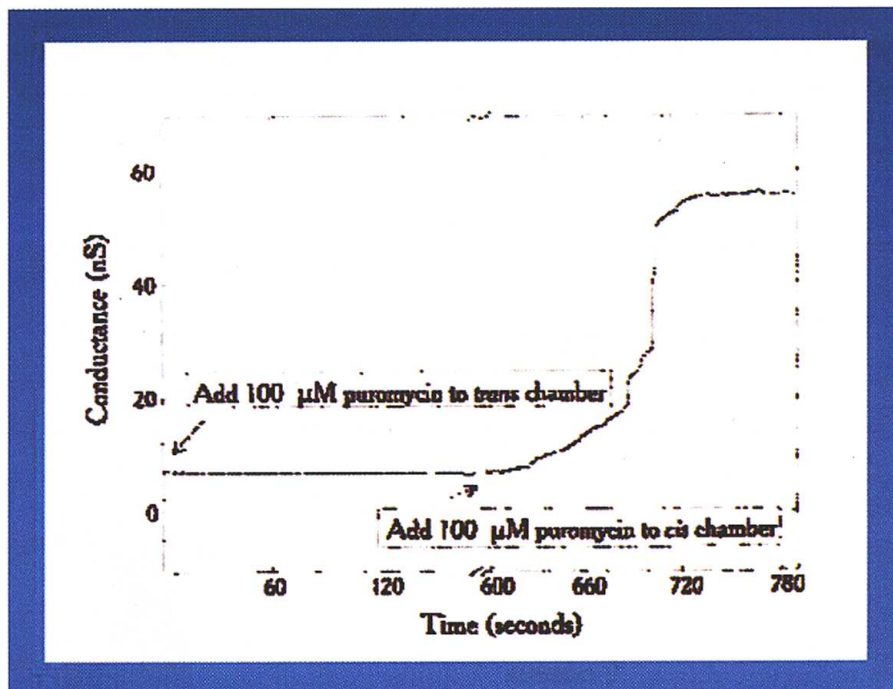


Figure 32. Specificity of puromycin action. At the first arrow puromycin was added to the trans chamber (luminal side of the microsomal membrane). After 10 min, puromycin was added to the cis chamber (ribosomal side of the microsomal membrane). A substantial increase in conductance was observed. From Simon and Blobel (17), with permission.

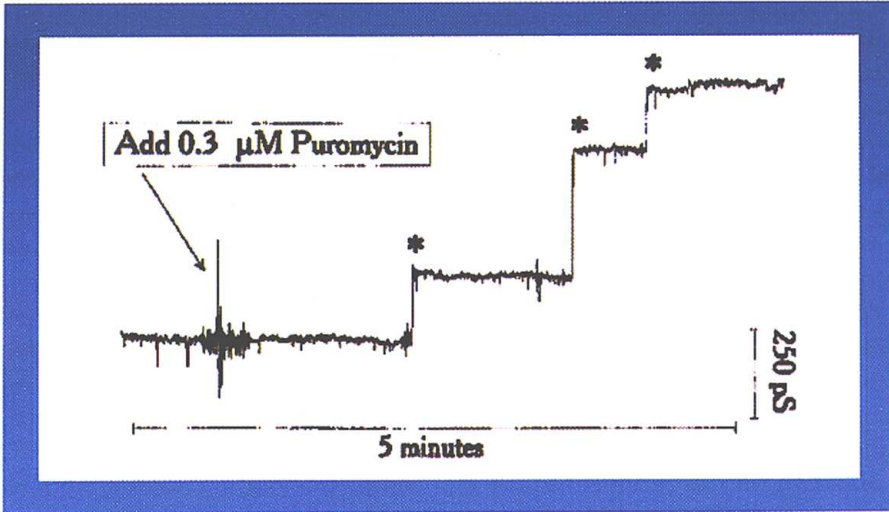


Figure 33. Single puromycin-revealed channels. Puromycin ($0.3 \mu\text{M}$) was added to the ribosomal side of the microsomal membrane. Discrete consecutive jumps in the conductance of 220 pS, 440 pS and 220 pS were observed (at asterisks). At faster time resolution, the 440 pS jump resolved into two discrete 220 pS steps. From Simon and Blobel (17), with permission.

the idea that the puromycin-revealed PCCs are kept open by attached ribosomes but are closed when ribosomes are dissociated (see model Fig. 30).

Can PCCs be demonstrated also in other membranes and by methods other than puromycin-induced clearance of translocating chains? Does the signal sequence serve as a ligand to open (or assemble) the PCC as was postulated in the signal hypothesis (14)? To answer these questions we turned to the prokaryotic plasma membrane. It had been suggested that in evolution

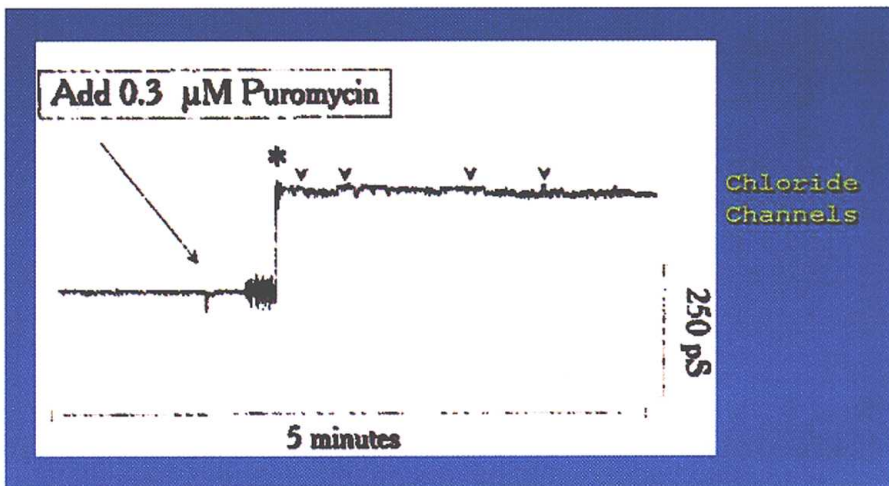


Figure 34. A single puromycin-revealed channel. Puromycin was added to the ribosomal side of the microsomal membrane and then removed. Small chloride channels are marked by asterisks.

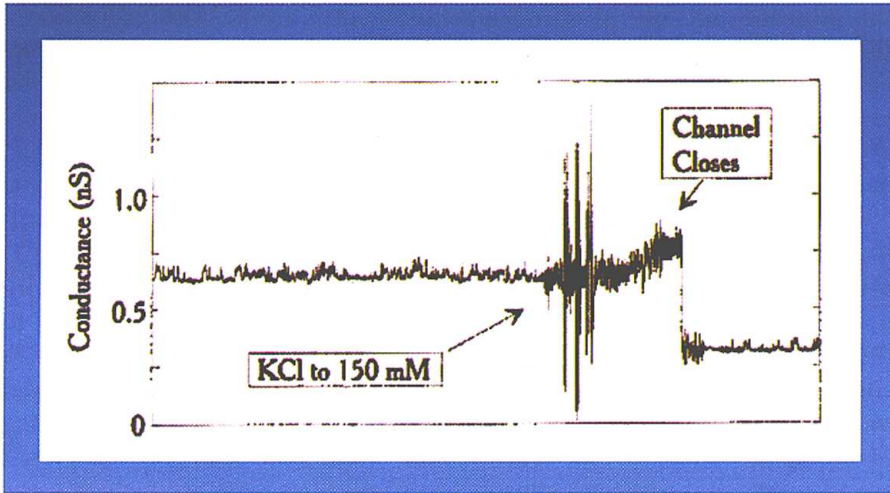


Figure 35. Closure of puromycin-revealed channel. Addition of 150 mM KCl closed a single puromycin-revealed channel. From Simon and Blobel (17), with permission.

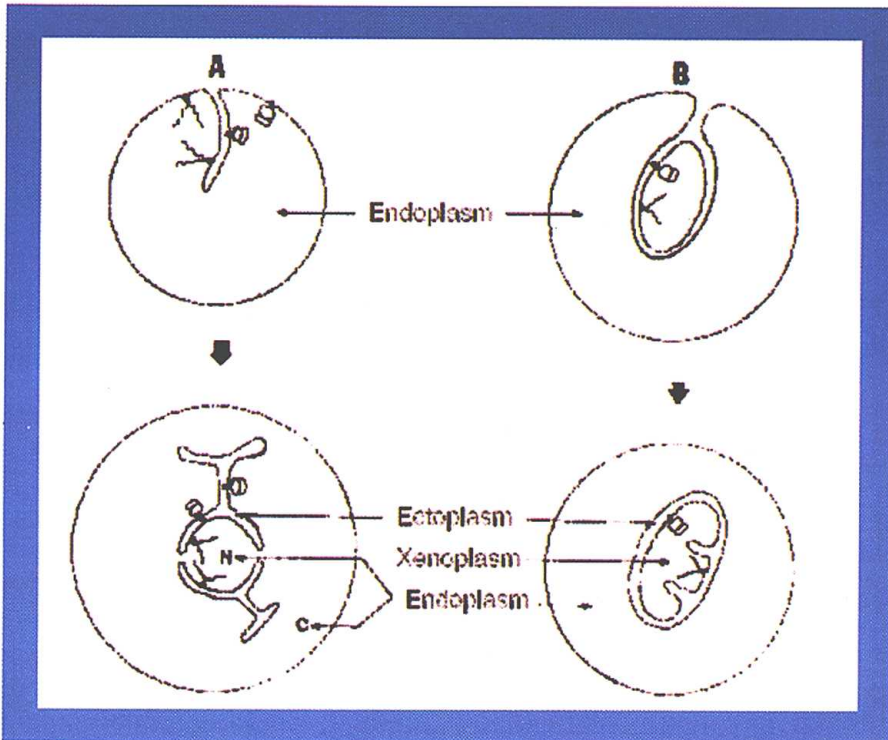


Figure 36. Evolution of eukaryotic intracellular membranes. Left, invagination of the prokaryotic plasma membrane with indicated ribosome and DNA binding sites yields the endoplasmic reticulum and the nuclear envelope. Right, uptake of a prokaryotic progenitor cell yields mitochondria and chloroplasts. From Blobel (44), with permission.

the eukaryotic ER arose by invagination of the prokaryotic plasma membrane (Fig. 36) (44). In fact, signal sequences for bacterial secretory proteins function in translocation across the ER of eukaryotic cells and, vice versa, signal sequences addressed to the eukaryotic ER function in translocation across the prokaryotic plasma membrane. It was therefore conceivable that the PCC of the prokaryotic plasma membrane resembles that of the eukaryotic ER.

When protoplasts of *Escherichia coli* (Fig. 37) are fused from the cis-chamber to a planar bilayer, the putative signal sequence-binding site of the PCC will be exposed on the trans-chamber (Fig. 38). The addition of low concentrations of synthetic signal peptide of the bacterial LamB protein (Fig. 39) to the trans-chamber, but not to the cis-chamber, caused a stepwise increase in conductance (Fig. 40). The stepwise increase in conductance was observed only in the presence of high salt. Each step is likely to represent the binding of one signal peptide to one signal peptide binding site of the PCC. High salt would reinforce the largely hydrophobic interactions between the hydrophobic signal peptide and the PCC and thus keep the PCC in an open configuration for long periods of time (45). The conductance properties of the prokaryotic plasma membrane PCC were found to be similar to those of the PCC

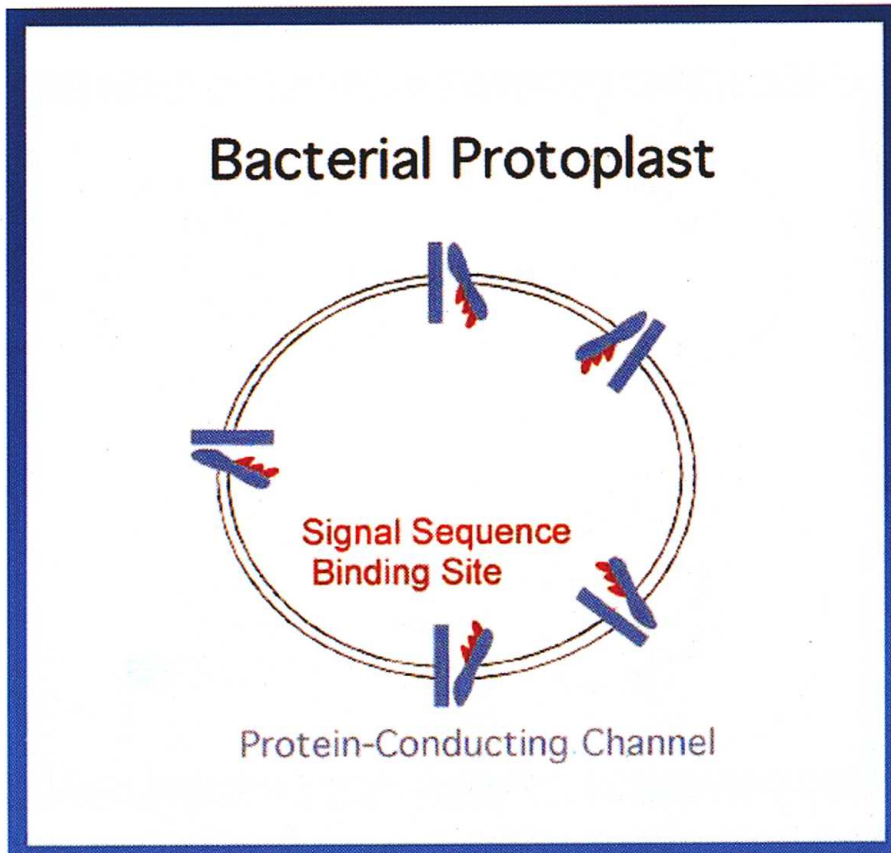


Figure 37. Schematic drawing of a bacterial protoplast with protein-conducting channels and their signal sequence binding sites.

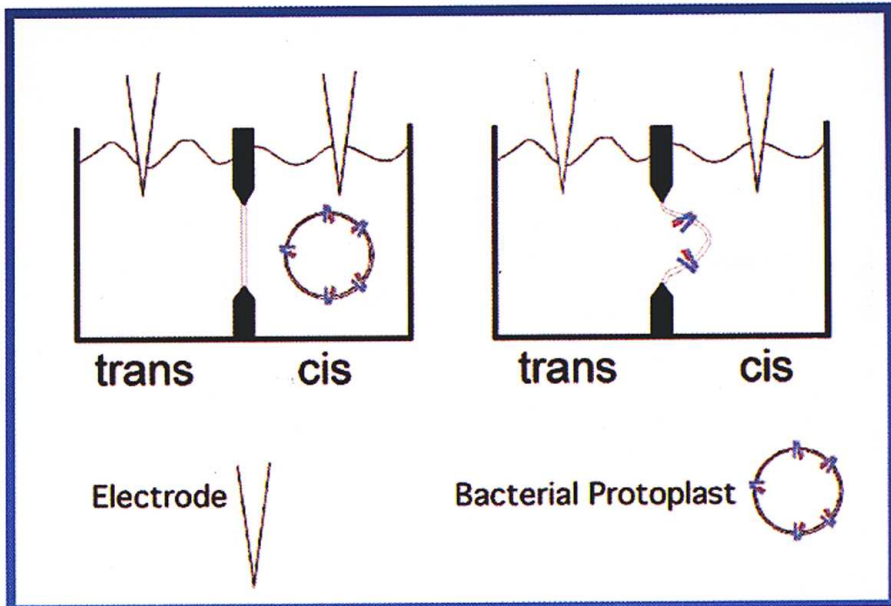


Figure 38. Schematic drawing of a bacterial protoplast added to the cis chamber and fused to the planar bilayer. Note that the cis side of the bacterial plasma membrane with the signal sequence binding sites of the protein-conducting channels is exposed to the trans chamber.

in the ER, in support of the notion that the two channels share a common history and are conserved.

Taken together, these data strongly supported the existence of aqueous protein-conducting channels that, like ion-conducting channels, were composed of proteins and that the signal sequence indeed served as one of the ligands to open the channel. These results led to a paradigm shift in cell biology and strongly argued against models that had reigned supreme for many years and that had proposed transfer of proteins directly through the hydrophobic core of the lipid bilayer.

VISUALIZATION OF THE PROTEIN-CONDUCTING CHANNEL

By genetic approaches, Schekman and co-workers (46) had identified Sec61 as a candidate for the protein-conducting channel in yeast. Rapoport and co-workers, used subfractionation and reconstitution into proteoliposomes (see

Met-Met-Ile-Thr-Leu-Arg-Lys-Leu-
Pro-Leu-Ala-Val-Ala-Val-Ala-Ala-
Gly-Val-Met-Ser-Ala-Gln-Ala-Met-Ala

Figure 39. Synthetic signal peptide of the preLamB protein of *E. coli*.

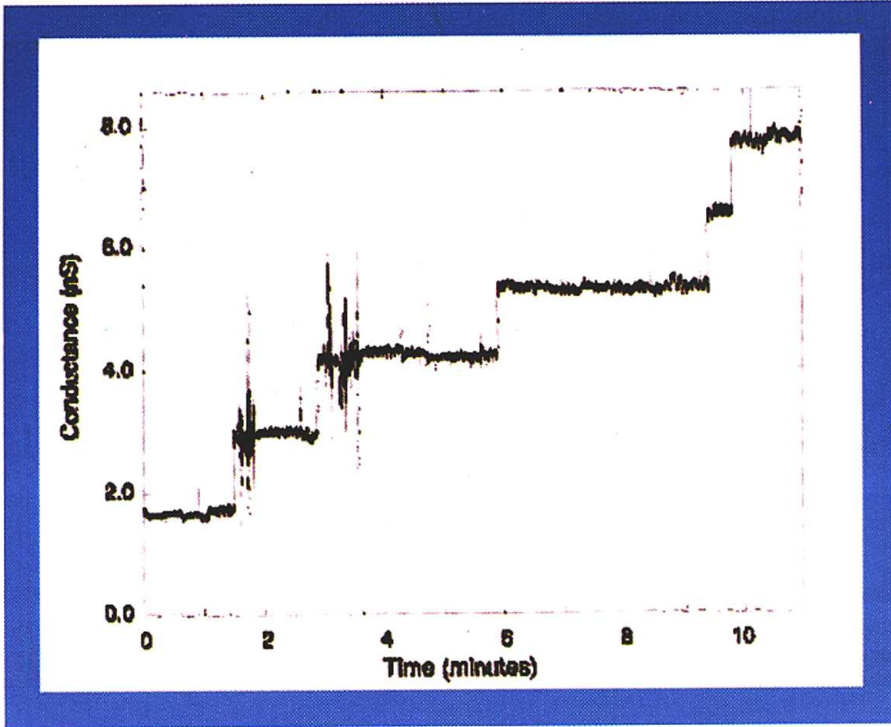


Figure 40. Signal peptide-gated channel remains open in high salt. *E. coli* protoplasts were fused to the planar bilayer. Synthetic preLamB signal peptide was added to the trans chamber (see Figure 38) in a final concentration of 0.2 nM and in the presence of 700 mM KCl. Single channels of 220 pS remained open in high salt. Presumably, the high concentration of salt reinforced the hydrophobic interactions of the signal peptide with the signal peptide binding site of the protein-conducting channels, leaving them in an open configuration. From Simon and Blobel (45), with permission.

above) to isolate the mammalian counterparts of the yeast PCC (47, 48). Their work established that the PCC consisted of a heterotrimer, termed the alpha, beta and gamma subunits of Sec61 (Fig. 41).

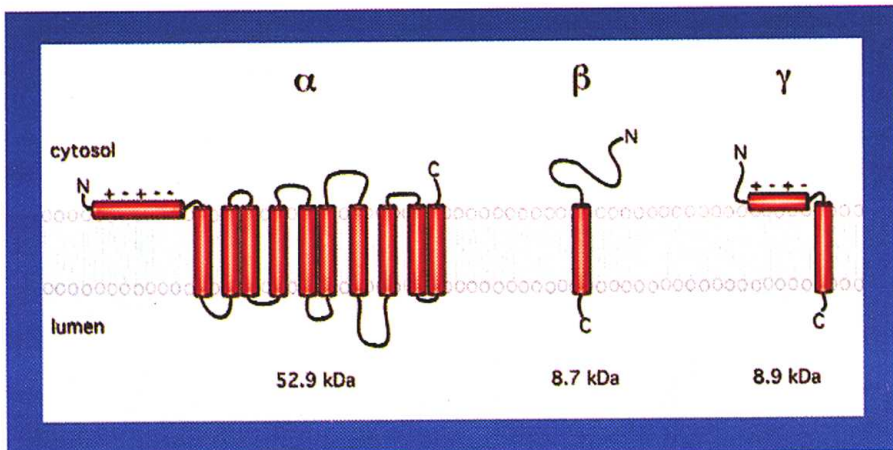


Figure 41. Topology of the Sec61 heterotrimer in the endoplasmic reticulum membrane.



Figure 42. Roland Beckmann, 1999. The Rockefeller University, New York.

To visualize the PCC attached to the ribosome, Roland Beckmann, a post-doctoral fellow in our laboratory (Fig. 42), was able to isolate the Sec61 complex by detergent solubilization of yeast microsomes (Fig. 43) and to bind it to isolated yeast monomeric ribosomes (48). Binding was saturable and sug-

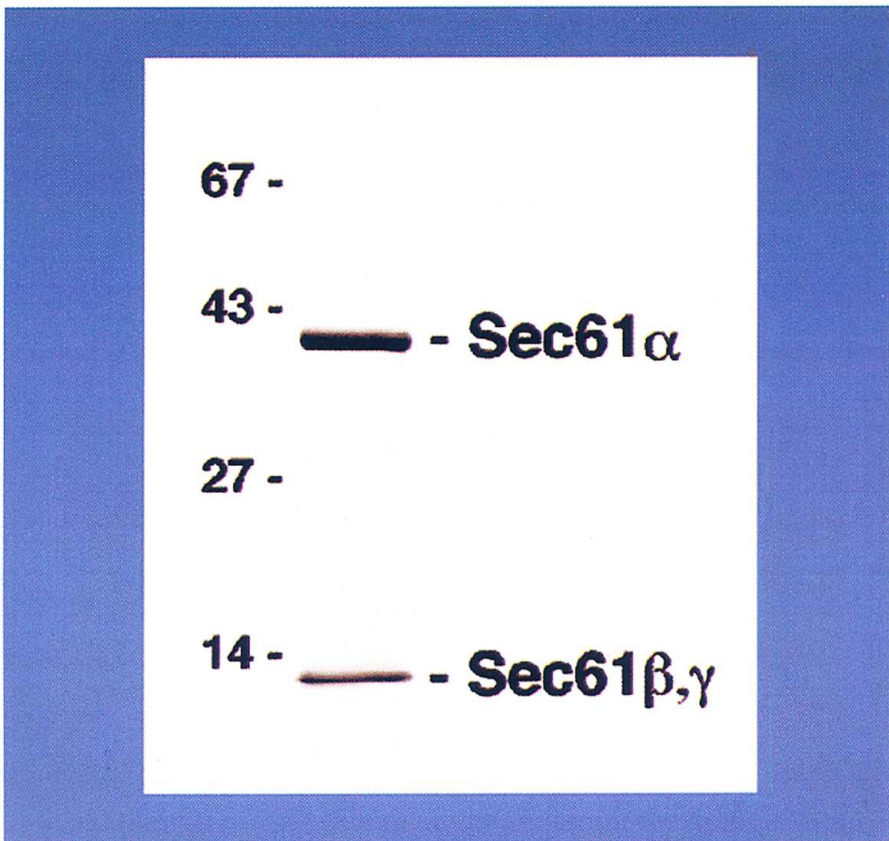


Figure 43. The purified Sec61 heterotrimer. The proteins of the purified yeast Sec61 heterotrimer were separated by SDS-PAGE and visualized by Coomassie Blue staining. From Beckmann, *et al.* (49), with permission.

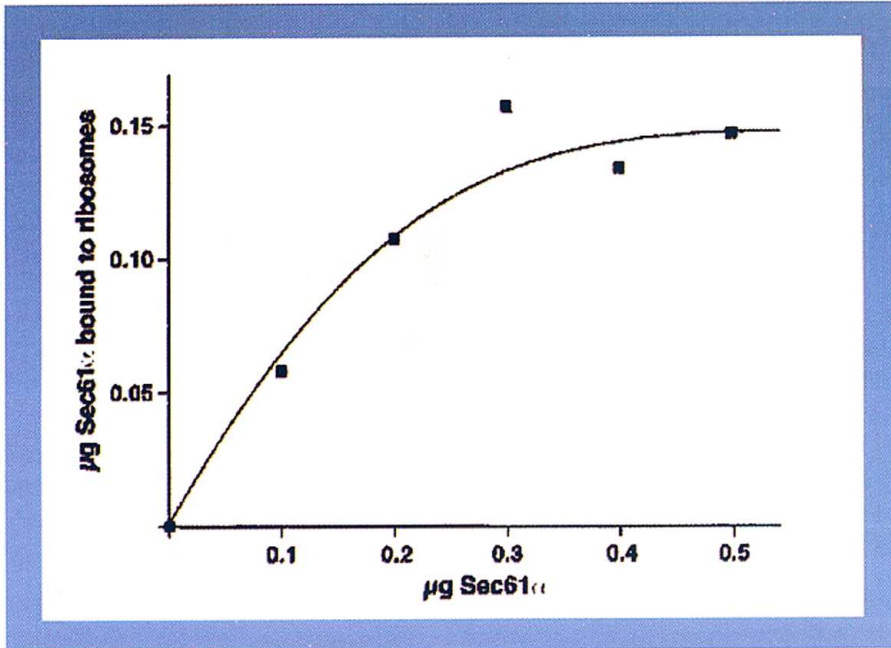


Figure 44. Binding of purified Sec61 to purified yeast ribosomes. Binding is saturable at an estimated binding of two Sec61 heterotrimers per ribosome. From Beckmann, *et al.* (49), with permission.

gested that two Sec61 trimers bound per ribosome (Fig. 44). Cryo-electron microscopy of these complexes (Fig. 45) and three-dimensional image reconstruction (Fig. 46, left panel), done in collaboration with Joachim Frank's laboratory, revealed that the Sec61 trimer bound to a region of the large

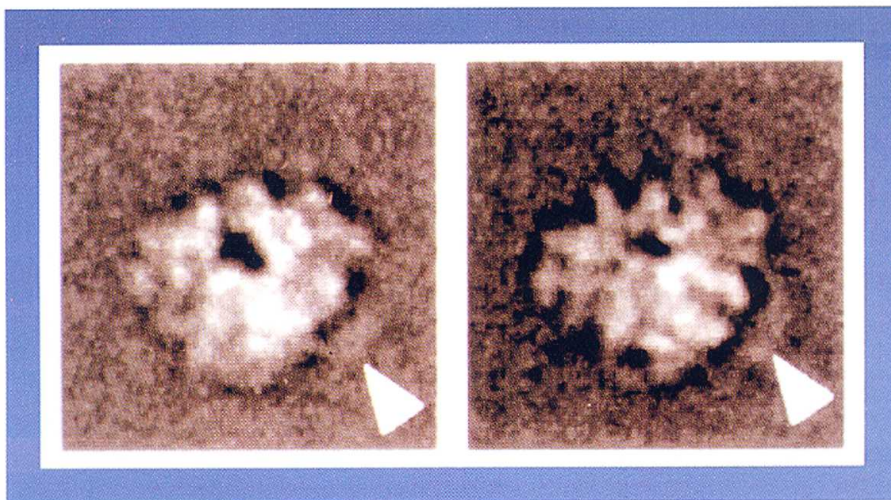


Figure 45. Cryo-electron micrograph of ribosome/Sec61 complexes. Arrowhead points to the Sec61 complex visible as a mass lying parallel to the ribosome surface. From Beckmann, *et al.* (49), with permission.

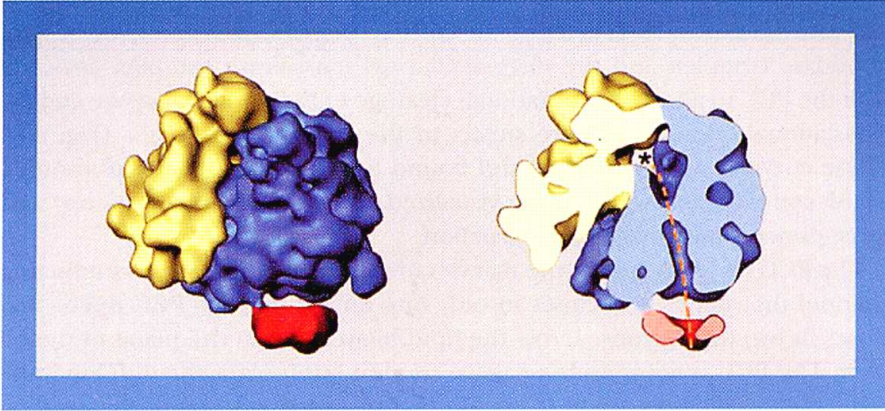


Figure 46. Three dimensional reconstruction of the ribosome/Sec61 complex. Small ribosomal subunit in yellow; large ribosomal subunit in blue; and Sec 61 complex in red. Left, note single attachment site of Sec61 to the large ribosomal subunit. Right, same orientation as left, but cut along a plane that sections the PCC of the Sec61 and the ribosome tunnel. The space between the two ribosomal subunits is indicated by an asterisk. The ribosomal tunnel and its alignment with the Sec61 PCC is indicated by a broken red line. Hence the conduit of the nascent polypeptide chain in the large ribosomal subunit tunnel and the Sec61 PCC is aligned. From Beckmann, *et al.* (49), with permission.

ribosomal subunit that was near to the previously identified exit site of the nascent chain. The Sec61 complex formed a funnel-shaped structure with a diameter of about 35 Ångström at the side facing the ribosome and a diameter of 15-20 Ångström at the side facing the ER lumen. The Sec61 complex was attached to the large ribosomal subunit at one site. Most interestingly, a section across the center of the ribosome (Fig. 46, right panel) revealed that a tunnel in the large ribosomal subunit aligned with the Sec61 channel. This arrangement suggests a likely conduit of the nascent chain within the large ribosomal subunit and across the Sec61 channel into the lumen of the ER.

The visualization of the PCC attached to the large ribosome and the alignment of the large ribosomal subunit tunnel with the PCC was an extremely gratifying confirmation of the predictions that had been made in 1975 (see Fig. 11) (14).

We are presently repeating these experiments with ribosomes that contain nascent secretory proteins in order to visualize the PCC in its active (open) form. For these experiments the ribosomes are programmed with truncated mRNA whose translation yields nascent chains long enough for the signal sequence to be exposed on the surface of the large ribosomal subunit. These nascent chain-ribosome complexes can then be used to engage SRP, SRP receptor and Sec61. These experiments should sublocalize SRP and SRP receptor on the active ribosome and should reveal the PCC in an active (open) configuration.

Likewise, truncated mRNAs coding for integral membrane proteins can be translated on ribosomes to investigate the PCC morphology that is accompanied by the proposed lateral opening of the PCC to the lipid bilayer. Attempts

will also be made to crystallize the Sec61 complex. Moreover, the signal peptidase complex and the oligosaccharidyl transferase complex associate with the PCC to allow co-translational cleavage of the signal sequence and co-translational addition of core sugars to the translocating chain (Fig. 47). These enzymes will be isolated and bound to the nascent chain/ribosome/Sec61 complex and will then be visualized by cryo-electron microscopy and three-dimensional image reconstruction.

The PCC is clearly one of the marvels of nature. Unlike an ion-conducting channel that opens and closes in only one dimension, the PCC opens and closes in two dimensions, across the lipid bilayer and in the plane of the bilayer. The PCC is not merely a passive conduit but it scans the unfolded nascent chain as it passes across it, responding to a passing stop-transfer sequence by lateral opening. Moreover, the PCC is constructed in such a way that it does not leak significant amounts of small molecules. More recently, evidence has accumulated for reverse translocation and disintegration of membrane proteins. In order to allow degradation of secretory and integral membrane proteins by the cytoplasmic proteasomes, proteins can be reverse-translocated from the lumen of the ER or disintegrated (membrane proteins) from the ER membrane. Reverse translocation and disintegration are likely to occur by opening the channel from the trans-side of the membrane (for translocated proteins) and by additional lateral opening of the PCC from the bilayer (for integral membrane proteins).

The demonstration that the PCC consists of integral membrane proteins confirmed a prediction made in 1980 (44). Virchow's dictum on cells: 'omnis cellula e cellula' (each cell comes from a pre-existing cell) was extended to membranes: 'omnis membrana e membrana' (each membrane comes from a pre-existing membrane). Membranes and compartments are not created *de*

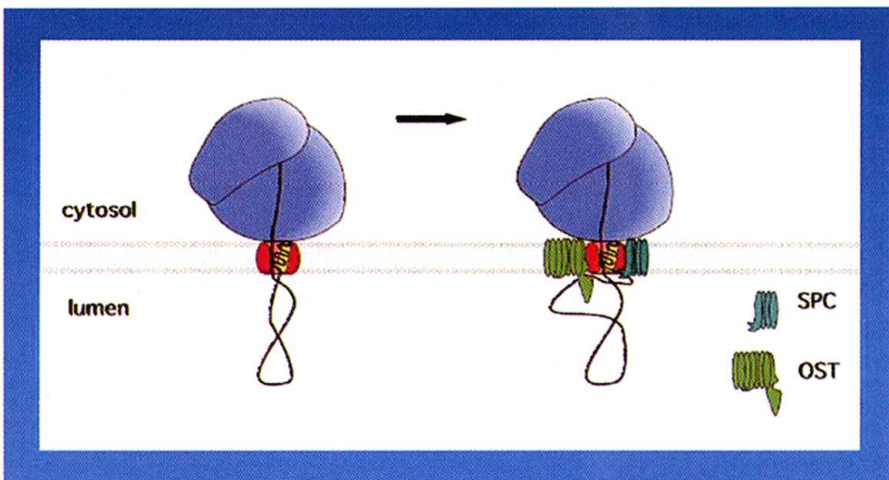


Figure 47. Recruitment of the signal peptidase complex (SPC) and the oligosaccharidyl transferase complex (OST) to the ribosome/nascent chain/Sec61 complex in the endoplasmic reticulum membrane. SPC endoproteolytically removes the signal peptide. OST attaches core oligosaccharides to the translocating nascent chain.

novoo, but recreate themselves. The asymmetric integration of the integral membrane proteins of the Sec61 complex requires the pre-existence of asymmetrically integrated Sec61 complex. The asymmetrically oriented Sec61 complex catalyzes asymmetric integration of all other integral membrane proteins of the exocytotic and endocytotic membranes as specified by their intrinsic sequence elements (*signal*- and *stop transfer* sequences) (50). This assures that the asymmetric integration of a membrane protein proceeds with high fidelity which is absolutely crucial for the membrane protein's function. In contrast, reconstitution of proteoliposomes by mixing lipids, detergents and proteins usually does not result in a high fidelity asymmetric topology of membrane proteins. It should be noted, however, that some proteins are designed to insert "spontaneously" into lipid bilayers (e.g., certain toxins). The asymmetric integration of most membrane proteins, however, is catalyzed by PCCs of the ER or by PCCs of mitochondrial or chloroplast membranes (see below).

POST-TRANSLATIONAL TRANSLOCATION ACROSS THE ER

Protein translocation across the ER can also occur post-translationally. This was first discovered in yeast (51, 52). Instead of SRP and SRP receptor this system uses other factors, primarily heat shock proteins, to keep the protein in an unfolded and translocation-competent configuration (53, 54). Moreover, additional membrane proteins interacting with the Sec61 complex are required to facilitate presentation of the signal sequence for opening the Sec61 PCC.

OTHER PROTEIN TRANSLOCATION SYSTEMS

Work on the protein translocation system of the ER established the basic principles. The other systems for protein translocation across distinct cellular membranes (Fig. 48) were subsequently shown to work on similar principles. Entry of a protein into any of these other "public" translocation systems requires a cognate signal sequence. As in the case of a signal sequence addressed to the ER, each signal sequence is recognized by a cognate signal recognition factor that, in turn, targets the complex to a cognate receptor. These receptors are restricted in their localization to distinct cellular membranes (Fig. 48). Translocation then occurs through channels to the other side of the membrane. There are fascinating similarities and differences in the construction of these various translocation systems.

The translocation system of the ER has evolved from the major protein translocation system in the plasma membrane of prokaryotes. Most of the eukaryotic cellular membranes arose by invagination of the prokaryotic plasma membrane and by relegation of specific membrane protein functions of the prokaryotic plasma membrane to these intracellular membranes (Fig. 36) (44). The signal sequence of the ER is related to the signal sequence addressed to the major prokaryotic plasma membrane translocation system.

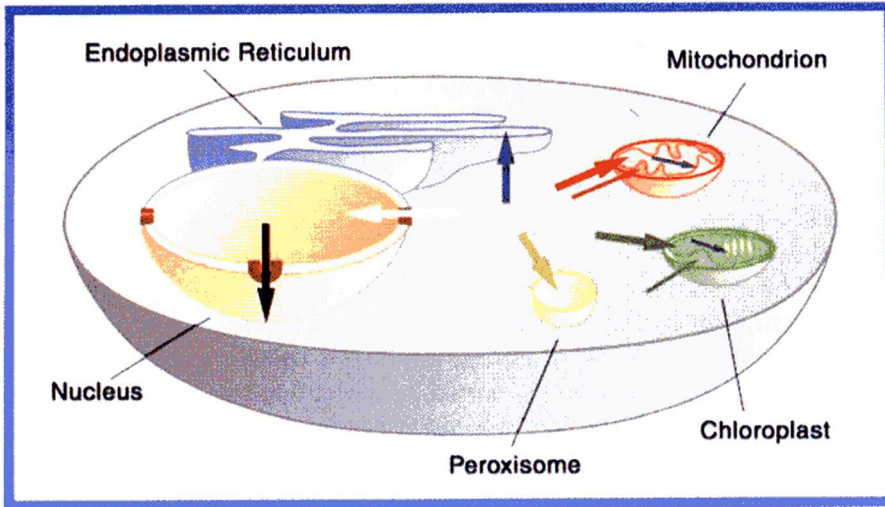


Figure 48. "Public" protein translocation systems in the cell. Bold blue arrow indicates protein translocation system in the ER; slim blue arrows indicate the related systems within the mitochondrial matrix and the chloroplast stroma. Bold and slim red and green arrows indicate protein translocation systems for import across the outer and inner mitochondrial or chloroplast membrane, respectively. White and black arrows indicate transport into and out of the nucleus across the nuclear pore complex.

Moreover, homologs of SRP (see above) and SRP receptor exist in prokaryotes and the SecY complex of bacteria is the homolog of the Sec61 complex of the ER. Hence, protein translocation across membranes and protein integration into membranes are very ancient and highly conserved membrane functions. Related systems exist for protein translocation from the mitochondrial matrix or the chloroplast stroma across the inner mitochondrial membrane or the thylakoid membranes of chloroplasts, respectively (Fig. 48).

Although the protein import system into mitochondria and chloroplasts are not related to those of the ER, the principles by which they operate are similar: distinct signal sequences, cognate signal recognition factors, cognate receptors and protein conducting channels. Like the PCCs in the ER, the PCCs in the outer and inner membrane of these organelles can also open laterally to achieve integration of membrane proteins. Proteins that are targeted to the matrix contain two signal sequences, one for translocation across the outer membrane and one for translocation across the inner membrane. The two PCCs in the outer and inner membrane can transiently interact with each other forming a continuous conduit for the unfolded nascent chain from the cytosol into the matrix. Protein translocation can occur post-translationally. Hence, in addition to signal recognition factors there are additional proteins required that keep the completed chain in an unfolded and translocation-competent configuration.

The protein import system into peroxisomes operates with at least two distinct signal sequences and cognate signal recognition factors and receptors. However, it appears that *integral membrane proteins* of the peroxisomal mem-

brane are integrated using the ER translocation system and are then sorted to the peroxisomal membrane, as was first suggested in 1978 (55). It appears that peroxisomal *content proteins* can be transported across the peroxisomal membrane post-translationally and in a folded and even oligomeric form. A putative peroxisomal membrane pore may not be able to open laterally to the lipid bilayer and, therefore, may not be competent for the integration of membrane proteins. This would explain why integral membrane proteins of the peroxisomal membrane need to be integrated by another translocation system, namely, that of the ER.

Import and export of macromolecules into and from the nucleus is not restricted to proteins but includes ribonucleoproteins and deoxyribonucleoproteins (viruses). The nuclear pore complex (NPC) is the common conduit for both import and export of all these molecules and molecular complexes. It is a huge organelle, at least 20 times the mass of the ribosome with a central pore of about 25nm diameter. Again, protein import and export are mediated by distinct signal sequences. These signal sequences are recognized by cognate signal recognition factors (karyopherins or kaps) that ferry between the nucleus and the cytoplasm. The kaps target the import and export substrates to receptors that are represented by a subset of nuclear pore complex proteins (collectively termed nucleoporins or nups). The NPC is connected on the nuclear side to tubes that extend all the way to the nucleolus. These tubes form a chromatin-free zone and are likely to facilitate intranuclear diffusion of macromolecules. Nuclear import and export, the nuclear pore complex and intranuclear traffic in these tubes are at present intensely investigated by many laboratories. The next years will bring considerable progress in the detailed understanding of these important cellular activities.

OTHER TOPOGENIC SEQUENCES

It is clear that *signal* sequences for protein translocation across distinct cellular membranes and a combination of *signal-* and *stop-transfer* sequences for the asymmetric integration of proteins into membranes are not the only "topogenic" sequences that serve as determinants for protein localization (44). Following translocation across or asymmetric integration into membranes, many proteins undergo further traffic. For example, lysosomal proteins contain signal sequences indistinguishable from those of secretory proteins (56, 57) and like secretory proteins, they are first segregated within the lumen of the ER. Subsequently, they have to be sorted to the lysosomes. *Sorting* sequence elements are required to achieve sorting to the lysosome.

Another example is the polyIg receptor. It is asymmetrically integrated into the ER using a signal sequence and a stop-transfer sequence (58, 59). It is then directed to the basolateral plasma membrane where it binds IgA or IgM. The ligand receptor complex is then transcytosed to the apical plasma membrane where the ligand-binding domain of the polyIg receptor is cleaved and shed as the so called 'secretory component' of secreted IgA or IgM. *Sorting*

sequences direct this transcytotic pathway. The existence of *sorting* sequences has been postulated in 1980 (44), long before such sequences were actually identified. Like signal and stop-transfer sequences, sorting sequences need to be recognized and various effectors are required to decode them and to direct them into the various pathways they specify.

EPILOGUE

What began as an inquiry about how secretory proteins are translocated across the ER proceeded into an exciting voyage that revealed the principles by which cells organize themselves into distinct membranes and compartments. Most cellular proteins, including integral membrane proteins, contain intrinsic sequence elements. These sequence elements are decoded by cognate recognition factors. Cognate receptors and effectors localize the protein to their proper location.

ACKNOWLEDGEMENTS

Foremost, I want to thank my two mentors, Van R. Potter and George E. Palade. David Sabatini was a particularly inspiring and forming influence, especially in the early stages of my career. David Luck's critical input was extremely valuable, steering me away from the ancillary and keeping me focused on the major questions.

I was fortunate to have extremely talented graduate students and postdoctoral fellows working with me. Those who made seminal contributions on the ER translocation system were mentioned in the text. Other major contributors in this area were: Mark Adelman, Nica Borgese, Christoph Freienstein, Anne Devillers-Thiery, Dennis Shields, Stefano Bonatti, Ann Erickson, Mike McCune, Keith Mostov, David Anderson, Ibrahim Ibrahimi, Gregory Conner, Martin Friedlander, Gerald Waters, Yves Audigier, William Chirico, Sanjay Nigam, Gregory Shelness, Yashpal Kanwar, Jacques YaDeau, Gary Greenburg, and Giovanni Migliaccio. Our work on the bacterial translocation system was carried out by: Chung Nan Chang, Matthias Müller, Irene Fecycz and Makoto Watanabe. Major contributors to the work on mitochondrial protein import were: Maria-Luisa Macchecchini, Katsuyoshi Mihara, Sherry Goltz, Jim Kaput, Debkumar Pain, Norbert Schuelke, and Hiroshi Murakami. Our work on protein import into chloroplasts was carried out by: Debkumar Pain, Danny Schnell and Felix Kessler. Work on peroxisomal protein import was done by: Barbara Goldman and Ralf Erdmann. The work on nuclear structure and nuclear import was carried out by: Ariane Monneron, Robert Aaronson, Nancy Dwyer, Larry Gerace, Paul Fisher, Miguel Berrios, David Fisher, Daniel Fisher, Nilabh Chaudhary, Laura Davis, Spyros Georgatos, Richard Wozniak, John Aris, Howard Worman, Jeffrey Yuan, Eckart Bartnik, Ruben Henriquez, Jerome Appelbaum, Jean-Claude Courvalin, Thomas Meier, Karima Djabali,

Mary Shannon Moore, Susan Wente, Michael Rout, Jun Sukegawa, Susan Smith, Aurelian Radu, Einar Hallberg, Doris Kraemer, Lucy Pemberton, Junona Moroianu, Jian Wu, Michael Matunis, Elias Coutavas, Makoto Hijikata, Cordula Enenkel, Michael Rexach, Caterina Strambio-de Castillia, Michael Hurwitz, John Aitchison, Monique Floer, Ulf Nehrbass, Nabeel Yaseen, Neris Bonifaci, Erica Johnson, Jonathan Rosenblum, Markus Albertini, Beatriz Fontoura, Yuh Min Chook, Anton Titov and Kimihasa Yoshida.

Absolutely essential were the office and technical support. I would like to mention Peggy Beaudet-Fisher, Gisele Nimic, Nancy Dwyer, Parkash Cashmore, Evette Ellison and Sean Campbell. Special thanks are due to Lourdes R. Quiroigico, who has managed the laboratory and office for over 14 years in a flawless and highly efficient manner.

Our research was supported by fellowships and grants from the National Institutes of Health, the American Cancer Society, The Damon Runyon-Walter Winchell Cancer Fund, The Helen Hay Whitney Foundation, The Jane Coffins Child Foundation, The Leukemia Society of America, The Life Sciences Research Foundation, Boehringer Fond, Deutsche Forschungsgemeinschaft, EMBO Fund, Humboldt Stiftung, Thyssen Stiftung, Human Frontier Science Program, NATO, and Canadian, French, Japanese, Swedish and Swiss research organizations.

Most importantly, however, was the **steady** support from the Howard Hughes Medical Institute. Without the encouragement and support of Purnell Choppin, Max Cowan, Claire Winestock, Dinah Singer and Carolyn McGinnis and their staff we would not have been able to initiate or execute many of the risky and exciting experiments of the past 14 years.

I thank the Geschwister Scholl Gymnasium in Freiberg/Saxony where I received a very gratifying education; the Universities of Frankfurt/Main, München, Kiel and Tübingen, where I trained for my M.D.; the hospitals in Neuenbürg, Obergünzburg and Wolfratshausen, where I did my internship; and the University of Wisconsin in Madison, where I was generously supported by a fellowship and where I received a Ph.D.

Special thanks go to Rockefeller University where I spent the past 33 years of my life. It is an extraordinarily beautiful, congenial and inspiring place to work. The administrations of Detlev Bronk, Frederick Seitz, Joshua Lederberg, David Baltimore, Torsten Wiesel and Arnold Levine have been supportive and encouraging at every point in my career. David Rockefeller, in particular, has been an inspiring presence throughout my entire career here at Rockefeller University.

My parents, Margarete and Dr. Bruno Blobel; my sisters Ruth, Siegrid and Ingeborg; my brothers Hans, Reiner, Karl, and Albrecht and their families; and especially my wife, Laura Maioglio, were the pillars of my life. I thank them from the bottom of my heart.

This lecture is dedicated to the memory of my beloved oldest sister, Ruth Blobel, who died at age 19, on April 10, 1945, during a bomb attack on a train near Schwandorf, Bavaria.

ABBREVIATIONS

EMBO	European Molecular Biology Organization
ER	endoplasmic reticulum
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
Kap	karyopherin
mRNA	messenger ribonucleic acid
NATO	North Atlantic Treaty Organization
NIH	National Institutes of Health
NPC	nuclear pore complex
Nup	nucleoporin
PCC	Protein conducting channel
pS	pico Siemens
RM	rough microsome
SDS-PAGE	sodium dodecylsulfate polyacrylamide gel electrophoresis
SRP	signal recognition particle
VSV	vesicular stomatitis virus

REFERENCES

1. Palade, G.E. *Science* **189**:347–358, 1975.
2. Redman, C.M. and Sabatini, D.D. *Proc. Natl. Acad. Sci. USA*. **56**:608–615, 1966.
3. Blobel, G. and Sabatini, D.D. In *Biomembranes*, ed. by L.A. Manson, pp. 193–195, Plenum Publishing Corporation, New York, USA, 1971.
4. Blobel, G. and Sabatini, D.D. *Proc. Natl. Acad. Sci. USA*. **68**: 390–394, 1971.
5. Blobel, G. *Proc. Natl. Acad. Sci. USA*. **68**:832–835, 1971.
6. Blobel, G. *Biochem. Biophys. Res. Comm.* **47**:88–95, 1972.
7. Blobel, G. *Proc. Natl. Acad. Sci. USA*. **70**:924–928, 1973.
8. Adelman, M., Sabatini, D.D. and Blobel, G. *J. Cell Biol.* **56**:206–229, 1973.
9. Freienstein, C. and Blobel, G. *Proc. Natl. Acad. Sci. USA*. **71**: 3435–3449, 1974.
10. Freienstein, C. and Blobel, G. *Proc. Natl. Acad. Sci. USA*. **72**:3392–3396, 1975.
11. Swan, D., Aviv, H., and Leder, P. *Proc. Natl. Acad. Sci. USA*. **69**:1967–1971, 1972.
12. Milstein, C., Brownlee, G.G., Harrison, T.M., and Mathews, M.B. *Nature New Biology* **239**:117–120, 1972.
13. Schechter, I., McKean, D.J., Guyer, R., and Terry, W. *Science* **188**:160–162, 1975.
14. Blobel, G. and Dobberstein, B. *J. Cell Biol.* **67**:835–851, 1975.
15. Blobel, G. and Dobberstein, B. *J. Cell Biol.* **67**:852–862, 1975.
16. Sabatini, D.D. and Blobel, G. *J. Cell Biol.* **45**: 146–157, 1970.
17. Simon, S.M. and Blobel, G. *Cell* **65**: 371–380, 1991.
18. Katz, F.N., Rothman, J. E., Lingappa, V.R., Blobel, G. and Lodish, H.F. *Proc. Natl. Acad. Sci. USA*. **74**: 3278–3282, 1977.
19. Lingappa, V.R., Katz, F.N., Lodish, H.F. and Blobel, G. *J. Biol. Chem.* **253**: 8667–8670, 1978.
20. Warren, G. and Dobberstein, B. *Nature* **273**:569–571, 1978.
21. Walter, P. and Blobel, G. *Proc. Natl. Acad. Sci. USA*. **77**:7112–7116, 1980.
22. Walter, P., Ibrahimi, I. and Blobel, G. *J. Cell Biol.* **91**:545–550, 1981.
23. Walter, P. and Blobel, G. *J. Cell Biol.* **91**:551–556, 1981.
24. Walter, P. and Blobel, G. *J. Cell Biol.* **91**:557–561, 1981.
25. Walter, P. and Blobel, G. *Nature (London)* **299**: 691–698, 1982.

26. Walter, P. and Johnson, A.E. *Annu. Rev. Cell Biol.* **10**:87–119, 1994.
27. Gilmore, R., Walter, P. and Blobel, G. *J. Cell Biol.* **95**:470–477, 1982.
28. Meyer, D.I., Krause, E., and Dobberstein, B. *Nature* **297**:503–508, 1982.
29. Tajima, S., Lauffer, L., Rath, V.L., and Walter, P. *J. Cell Biol.* **103**:1167–1178, 1986.
30. Connolly, T. and Gilmore, R. *Cell* **57**:599–610, 1989.
31. Miller, J.D., Tajima, S., Lauffer, L., and Walter, P. *J. Cell Biol.* **128**:273–282, 1995.
32. Gilmore, R. and Blobel, G. *Cell* **35**:677–685, 1983.
33. Jackson, R.C. and Blobel, G. *Proc. Natl. Acad. Sci. USA.* **74**:5598–5602, 1977.
34. Evans, E., Gilmore, R. and Blobel, G. *Proc. Natl. Acad. Sci. USA.* **83**:581–585, 1986.
35. Shelness, G.S., Kanwar, Y.S. and Blobel, G. *J. Biol. Chem.* **263**:17063–17070, 1988.
36. Greenburg, G., Shelness, G. and Blobel, G. *J. Biol. Chem.* **264**:15762–15765, 1989.
37. Shelness, G.S. and Blobel, G. *J. Biol. Chem.* **265**:9512–9519, 1990.
38. Nicchitta, C. V. and Blobel, G. *Cell* **60**:259–269, 1990.
39. von Heijne, G. and Blomberg, C. *Eur. J. Biochem.* **97**:175–181, 1979.
40. Engelman, D.M., and Steitz, T.A. *Cell* **23**:411–422, 1981.
41. Gilmore, R. and Blobel, G. *Cell* **42**: 497–505, 1985.
42. Simon, S., Blobel, G. and Zimmerberg, J. *Proc. Natl. Acad. Sci. USA.* **86**:6176–6180, 1989.
43. Mueller, P., Rudin, D.O., Ti Tien, H. and Wescott, W.C. *Nature* **194**:979–980, 1962.
44. Blobel, G. *Proc. Natl. Acad. Sci. USA.* **77**:1496–1500, 1980.
45. Simon, S.M. and Blobel, G. *Cell* **69**:677–684, 1992.
46. Stirling, C.J., Rothblatt, J., Hosobuchi, M., Deshaies, R., and Schekman, R. *Mol. Biol. Cell* **3**:129–142, 1992.
47. Goerlich, D., Prehn, S., Hartmann, E., Kalies, K.U., and Rapoport, T.A. *Cell* **71**:489–503, 1992.
48. Goerlich, D., and Rapoport, T.A. *Cell* **75**: 615–630, 1993.
49. Beckmann, R., Bubeck, D., Grassucci, R., Penczek, P., Verschoor, A., Blobel, G. and Frank, J. *Science* **278**:2123–2126, 1997.
50. Friedlander, M. and Blobel, G. *Nature (London)* **318**:338–344, 1985.
51. Waters, M.G. and Blobel, G. *J. Cell Biol.* **102**:1543–1550, 1986.
52. Hansen, W., Garcia, P.D., and Walter, P. *Cell* **45**: 397–406, 1986.
53. Chirico, W.J., Waters, M.G. and Blobel, G. *Nature (London)* **332**:805–810, 1988.
54. Deshaies, R., Koch, B.D., Werner-Washburne, M., Craig, E., and Schekman, R. *Nature* **332**: 800–805, 1988.
55. Goldman, B. and Blobel, G. *Proc. Natl. Acad. Sci. USA.* **75**:5066–5070, 1978.
56. Erickson, A.H. and Blobel, G. *J. Biol. Chem.* **254**:11771–11774, 1979.
57. Erickson, A.H., Conner, G. and Blobel, G. *J. Biol. Chem.* **256**:11224–11231, 1981.
58. Mostov, K.E. and Blobel, G. *J. Biol. Chem.* **257**:11816–11821, 1982.
59. Mostov, K.E., Friedlander, M., and Blobel, G. *Nature (London)* **308**:37–43, 1984