History of the Signal Hypothesis
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The signal hypothesis, which describes how secretory and membrane proteins are targeted to the endoplasmic reticulum, was proposed in 1971 by Günter Blobel and David Sabatini and demonstrated by Blobel and Bernhard Dobberstein in 1975. Subsequent research identified the key components of the membrane insertion and translocation machinery, including the signal recognition particle (SRP), the SRP receptor and the protein-conducting channel. Ultimately, the signal hypothesis was shown to be true for not only eukaryotes but also prokaryotes. Most importantly, the concept of signal-mediated targeting was expanded into a general hypothesis of cellular topogenesis that helps to explain how proteins are distributed to their correct locations within the cell following their synthesis in the cytoplasm.

Introduction: Historical Background of the Signal Hypothesis

Although all advancements in science build on earlier results, one of the striking aspects of research on the signal hypothesis is its continuity with previous studies in the Laboratory of Cell Biology at the Rockefeller Institute (later University) (Table 1). The signal hypothesis, as will be elaborated in later sections, essentially addressed the first step in the process of protein secretion from the cell. In the 1930s and early 1940s, very little was known about secretion or even about protein synthesis. Light microscopic observations of highly secretory tissues, such as the exocrine pancreas, revealed the presence of large granules in the cytoplasm. Histochemical staining of tissue sections by Brachet in Belgium in the 1920s and 1930s, combined with ribonuclease treatment of parallel sections suggested that cellular components that stained intensely red with the dye pyronine were ribonucleic acids (RNAs) (Bechtel, 2006). In the 1930s and 1940s, Robert Bensley at the University of Chicago and Albert Claude at the Rockefeller Institute in New York developed techniques of differential centrifugation that permitted them to separate subcellular components from broken and homogenised cells (Bechtel, 2006). Claude called one of the cellular fractions that he isolated microsomes. Biochemical analysis of microsomes indicated that they contained significant amounts of ribonucleic acids. However, as late as 1948 Claude considered that the function of microsomes in the cell was unknown (Claude, 1948). See also: History of Molecular Biology; Rockefeller Foundation: Biomedical and Life Sciences Offshoots

In 1939, Keith Porter joined Claude’s laboratory at Rockefeller and soon thereafter began to develop electron microscopy for use on biological specimens. Electron microscopy, which had been invented by engineers, had a much higher resolution than light microscopy due to the short wavelengths achievable with an electron beam. The promise of improved resolution was tempered, however, by the threat of damage to biological specimens caused by the electron beam and the requirement that samples be viewed in a vacuum. Furthermore, although the electron beam was powerful, it was unable to penetrate the thickness of most cells and typical tissue sections (Bechtel, 2006). Porter initially circumvented these problems by growing cells in tissue culture directly on a thin plastic film that was then transferred to the fine screen or ‘grid’ used to suspend specimens in the electron microscope, and later developed techniques for cutting tissue sections sufficiently thin for the electron beam to penetrate. Among the structures newly visible in the first electron micrographs, published in 1945, was a ‘lace-like reticulum’ extending through the cytoplasm (Porter et al., 1945).

In the next few years, Porter continued his studies of the reticulum, now named the endoplasmic reticulum (ER), in collaboration with George Palade, who had joined the laboratory in 1946. Gradually, as Porter concentrated on
## Table 1: Key events in the history of the signal hypothesis

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
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<tr>
<td>1971</td>
<td>Günter Blobel and David Sabatini propose the first version of the signal hypothesis, suggesting that N-terminal information helps direct nascent secretory polypeptides to the endoplasmic reticulum.</td>
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<td>1972</td>
<td>Tim Harrison and Cesar Milstein hypothesise that a larger form of immunoglobulin light chain has an N-terminal signal related to segregation of the protein in the endoplasmic reticulum.</td>
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<td>1975</td>
<td>Blobel and Bernhard Dobberstein propose a new version of the signal hypothesis and provide strong supporting evidence using a completely reconstituted assay system.</td>
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<td>1977</td>
<td>Dobberstein and Nam-Hai Chua discover a possible precursor of the plant carboxylase small subunit that they propose is important for its transport from the cytoplasm into the chloroplast. Peter Highfield and John Ellis prove this to be the case the following year and demonstrate that transport into the chloroplast occurs post-translationally.</td>
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<td>1978</td>
<td>Hiroshi Inouye and Jon Beckwith discover a precursor of <em>Escherichia coli</em> alkaline phosphatase and link it to bacterial protein secretion.</td>
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<td>1979</td>
<td>Maria-Luisa Maccecchini, Gottfried Schatz and Blobel show that precursors of mitochondrial proteins are transported into the mitochondria from the cytoplasm and processed post-translationally.</td>
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<td>1980</td>
<td>Peter Walter and Blobel identify the salt-extractable factor required for translocation as a complex of six polypeptides, later calling it the signal recognition protein (SRP). When a small ribonucleic acid (RNA) is found to be part of the complex, SRP is renamed signal recognition particle.</td>
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<td>1980</td>
<td>David Meyer and Dobberstein discover a microsomal membrane protein required for protein translocation that they later call ‘docking protein’. Reid Gilmore in Blobel’s laboratory later confirms and extends their results, renaming the protein the SRP receptor.</td>
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<tr>
<td>1980</td>
<td>Blobel postulates the existence of protein ‘topogenic sequences’ responsible for the targeting and disposition of proteins in locations throughout the cell.</td>
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<td>1987</td>
<td>Ray Deshaies and Randy Schekman discover the sec61 mutant in yeast that is defective in an early stage of secretion. Sec61 is later shown to be part of the protein-conducting channel originally hypothesised by Blobel in 1975.</td>
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<tr>
<td>1991</td>
<td>Sandy Simon and Blobel provide biophysical data consistent with the existence of a protein-conducting channel in the endoplasmic reticulum.</td>
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<tr>
<td>1993</td>
<td>Dirk Görlich and Tom Rapoport report protein translocation into proteoliposomes reconstituted only with the sec61 complex and the SRP receptor.</td>
</tr>
<tr>
<td>1999</td>
<td>Günter Blobel receives the Nobel Prize for his work on the signal hypothesis.</td>
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The development of electron microscopy and its associated technologies, and extended his morphological studies to other types of tissues and cells, Palade took the lead in determining the function of the ER. Palade (1955) described a ‘particulate component of the cytoplasm’ visible by electron microscopy. These particles, which were distributed throughout the cytoplasm, were, in some cases, observed to be associated with the membranes of the ER, forming linear and spiral patterns. ER membranes with these attached particles were referred to as a ‘rough surfaced variety’ or later simply rough ER. Palade (1955) also noted that microsomes isolated from liver cells were closely associated with the particulate component. In the following year, Palade and Philip Siekevitz, a biochemist and expert on protein synthesis recently recruited to Rockefeller, published a study that combined biochemical analysis, cell fractionation and electron microscopy to establish that microsomes were derived by cell homogenisation from the endoplasmic reticulum (Palade and Siekevitz, 1956). When the cell was disrupted, rough ER was converted into rough microsomes, that is, microsomes with bound particles. Within a short time the particles were renamed ribosomes. See also: Palade, George Emil

Over the next 10 years, as Palade’s focus turned more and more to the study of the overall secretory pathway in cells, Siekevitz and his collaborators established that the ER, with its attached ribosomes, were involved in the synthesis of secretory proteins. Siekevitz and Palade (1966) showed that amylase, radioactively-labelled during its synthesis *in vivo* in the guinea pig pancreas, rapidly appeared in microsomes isolated from the labelled tissue. Much of the newly synthesised or nascent amylase was seemed to be contained within the lumen of microsomal vesicles that resealed after disruption of the ER membrane network during homogenisation of the tissue because it could only be released by permeabilising the microsomal membrane with detergent. Furthermore, Siekevitz observed that “ribosomes with nascent... protein still bound to them... more firmly attached to the membrane than... ribosomes devoid of nascent protein”. Redman *et al.* (1966) described a cell-free or *in vitro* system containing pigeon microsomes that was capable of
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When Redman left Rockefeller, his work on the nature of the ribosome–membrane interaction was taken over by David Sabatini, who had collaborated with Redman in his studies of the effects of puromycin (Redman and Sabatini, 1966). Sabatini, an Argentinian MD and an expert electron microscopist, had come to Rockefeller to learn biochemistry. He was soon joined by Günter Blobel, who came to Rockefeller to learn biochemistry. Both were known to prematurely terminate protein synthesis leading to the release of the unfinished polypeptides from the ribosome. When added to microsomes, the released polypeptides could be found within the closed microsomal vesicles, again defined as the proportion of radioactivity that could be solubilised only by detergent disruption of the microsomal membranes (Redman and Sabatini, 1966). Overall, the results of both the in-vivo and in-vitro experiments suggested that association of ribosomes with the ER membrane was mediated at least in part by the polypeptides that they were synthesising and discharging into the lumen. See also: History of Cell Biology

- Proteins being synthesised by free ribosomes contained ‘a common sequence of amino acids’ at their N-terminus, designated by an X in the model, that would direct the entire synthetic apparatus to the membrane, facilitating ribosome binding that somehow led to translocation of the nascent chain across the membrane. Given its brevity and speculative character, along with, no doubt, the lag between the volume’s publication and distribution, the model did not appear to attract any attention. Indeed, in a subsequent joint publication on ribosome–membrane interaction from their laboratory submitted in mid-1972, the 1971 model was not mentioned (Adelman et al., 1973).

- Unbeknownst to Blobel and Sabatini, experiments were being conducted at the Medical Research Council...
laboratories at Cambridge University that would profoundly influence the next phase of their work. Cesar Milstein, an immunologist at Cambridge, was investigating the generation of antibody diversity by characterising messenger RNAs (mRNAs) for immunoglobulin light and heavy chains in collaboration with George Brownlee, an RNA expert. Tim Harrison, a graduate student of Brownlee, carried out a key aspect of this project. To isolate light-chain mRNA, Harrison purified microsomes from a mouse myeloma tumour capable of producing only a single variant light chain. The light-chain mRNA was extracted from the microsomes and separated from other RNAs by sucrose density sedimentation. Because no means existed to specifically identify the light-chain message, Harrison incubated each of the gradient fractions in an in-vitro protein synthesis system derived from another mouse tumour, and then separated the synthesised proteins by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. With this assay, fractions enriched in light-chain mRNA would be expected to produce a polypeptide that migrated on the gel with a mobility identical to that of authentic light chain isolated from myeloma cell cultures. In his first experiment, Harrison observed authentic light chain in some fractions, but also observed another polypeptide in the same fractions that migrated slightly more slowly with an estimated molecular mass approximately 1.5 kDa larger than the authentic light chain. In subsequent experiments, Harrison discovered that his protein synthesis system was sometimes contaminated with microsomal membranes, and that authentic light chain was only evident when contaminating membranes were present. To determine if the larger polypeptide made in the absence of membranes was related to light chain, Harrison and Milstein peptide-mapped the two polypeptides and determined that they were indeed almost identical. The one exception was a peptide known to be located at the N-terminus of the protein. On the basis of Harrison’s knowledge of the cell biology of secretion, he, Milstein, and their collaborators proposed, in a paper published in Nature in 1972, that the larger polypeptide was a precursor of authentic light chain and that the extra sequence at the N-terminus might act as a ‘signal’ to direct ribosomes synthesising light chain to the ER membrane (Milstein et al., 1972). At the time, nobody in the group was aware of the Blobel and Sabatini, 1971 model, but soon learned of it when Mike Matthews, a coauthor on the paper, encountered Blobel at a meeting in the United States that summer.

By this time, Sabatini had moved on, becoming Chairman of the new Department of Cell Biology at New York University. Blobel continued the work on his own, stimulated by the Cambridge results that suggested that their 1971 model might be correct. To prove the model rigorously, Blobel decided to construct a completely reconstructed in-vitro system that was capable of synthesising a secretory protein from beginning to end, converting the putative precursor to its authentic size and, in the process, translocating the polypeptide into the interior of microsomal vesicles. After carefully purifying components and optimising reactions, Blobel finally succeeded at the end of 1974. By the end of the following year, Blobel and his collaborators published their results in the Journal of Cell Biology (Blobel and Dobberstein, 1975a, 1975b).

In the two papers published together in December 1975, Blobel and his postdoctoral fellow Bernhard Dobberstein described two in-vitro systems that they called a ‘read-out’ system and an ‘initiation’ system (Blobel and Dobberstein, 1975a, 1975b). The readout system resembled the one of Redman a decade earlier in that ribosomes and mRNA already bound to microsomes were used for the synthesis of proteins. These existed on the membrane as polysomes, that is, multiple ribosomes associated with a single light-chain mRNA, with each ribosome corresponding to a partially completed polypeptide. When these were extracted from the rough microsomes with detergent under mild conditions, and then incubated in an in-vitro system in the absence of membranes, nascent light-chain polypeptides associated with the polysomes were completed or ‘read-out’. In samples taken at different times during the incubation, they observed that the first chains completed had been ‘processed’ to the molecular weight of authentic light chain, whereas those completed later were the size of the putative precursor. Their interpretation of this experiment was that polypeptides mostly complete when the polysomes were extracted from the microsomes had had sufficient time to pass through the membrane N-terminus first and be processed proteolytically to the correct size. In contrast, polypeptides that had only begun to be synthesised before extraction of the polysomes had had insufficient time to pass through the membrane and therefore appeared as the larger form when their synthesis was completed in vitro. Thus, this experiment established that the larger form was a precursor of the authentic form (Blobel and Dobberstein, 1975a).

In the second paper, Blobel and Dobberstein combined purified ribosomal subunits with isolated immunoglobulin light-chain mRNA and microsomal membranes from which any attached ribosomes had been removed, or ‘stripped’ (Blobel and Dobberstein, 1975b). When an enzyme mixture consisting of the required initiation and elongation factors was incubated with the ribosomes, membranes and mRNA, authentic light chain was synthesised and was resistant to degradation by proteases added at the end of the incubation (Figure 2). This suggested that the synthesised light chain had been translocated into the interior of the microsomal vesicles, where it was protected from proteolytic degradation. If the microsomes were omitted, then only precursor light chain was synthesised, and it was fully susceptible to proteolysis (Figure 2). In contrast, when mRNA for the cytoplasmic protein globin (part of the haemoglobin molecule) was added to the system instead of light-chain mRNA, only authentically sized globin was synthesised with or without membranes, and, even in the presence of membranes, none of it was protected from proteolysis, indicating that it was not translocated (Figure 2).

See also: Cotranslational Translocation of Proteins Into Microsomes: Methods
Blobel and Dobberstein (1975a) called their overall model for targeting and translocation of the ‘signal hypothesis’ and illustrated it in a diagram in their first paper (Figure 1b). What had changed from the 1971 version was that now the N-terminus, or, as it was now called, the signal sequence of the secretory protein, was proteolytically removed following the protein’s translocation to the microsome interior. The new model also speculated on how the secretory protein was able to cross the membrane. Interaction of the ribosome and the signal sequence with the membrane was proposed to initiate the assembly of a ‘tunnel’ in the plane of the membrane through which the nascent polypeptide could pass on its way to the interior (Figure 3) (Blobel and Dobberstein, 1975a).

In addition to these two papers, a third appeared at the same time from the Blobel lab, which reported the N-terminal sequences of a variety of pancreatic secretory proteins synthesised in vitro in the absence of microsomal membranes. All had previously unrecognised homologous peptide extensions of approximately 20 amino acids that were proposed to be signal sequences (Devillers-Thiery et al., 1975). This time, the signal hypothesis was not ignored by the scientific community, earning a News and Views report in Nature in early 1976 (Amar-Costescu, 1976).

See also: Protein: Cotranslational and Posttranslational Modification in Organelles

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Extension of the Signal Hypothesis

All of Blobel’s initial results dealt with secretory proteins such as immunoglobulins and pancreatic enzymes. However, a variety of other proteins were known to utilise the ER and secretory pathway for their transport through the cell. Among these were integral membrane proteins that were destined for the plasma membrane. Integral membrane proteins cross the lipid bilayer, embedded in the membrane by a stretch of largely hydrophobic amino acids known as the transmembrane segment. Even so, some mechanism was necessary to target the ribosomes that
synthesised them to the ER membrane where, it was presumed, they would be inserted. Blobel and his colleagues hypothesised that signal sequences were involved in this process as well.

The reconstituted assay system was dependent on exogenous mRNAs purified from specific cells or tissues. In the mid-1970s, obtaining such mRNAs was a challenge, as recombinant DNA techniques to manufacture such molecules from cloned DNA sequences did not exist. Immunoglobulin-secreting tumours that produced large amounts of light chain, or the exocrine pancreas, mainly making digestive enzymes, yielded mRNAs predominantly coding for these proteins. In the case of membrane proteins, however, most cells and tissues produced a wide diversity of species, with no single one being particularly abundant. To circumvent this problem, Blobel collaborated with Harvey Lodish at the Massachusetts Institute of Technology. Lodish was studying an animal virus called vesicular stomatitis virus or VSV. Particles of VSV consist of a lipid-bilayer membrane wrapped around a complex of nucleic acids and proteins that form the viral genome. The viral membrane contains an integral membrane protein called G-protein that binds the virus to cells, facilitating infection. After infection, VSV overwhelms the host cell, producing enormous amounts of mRNAs for viral proteins. Thus, a large proportion of the mRNAs extracted from cells during infection code for the G-protein.

Results of the first experiments in which G-protein mRNA was translated in vitro were confusing. Secretory proteins translated in vitro in the presence of microsomal membranes migrate faster by gel electrophoresis than the precursor forms synthesised in the absence of membranes because the signal sequence is proteolytically cleaved during translocation (Figure 2). However, when G-protein mRNA was translated in vitro in the presence of membranes, the protein produced was larger than its putative precursor. This mystery was solved when it was realised that G-protein, like many other integral membrane proteins, was a glycoprotein, and that a core set of sugars or oligosaccharides were likely added as translocation into the interior of the microsomal vesicles occurred (Katz et al., 1977b). Thus, it was possible that G-protein did indeed utilise a signal sequence to target it to the ER membrane during its synthesis, and that this sequence was proteolytically removed. However, the reduction in molecular mass resulting from cleavage of the signal sequence was more than compensated for by oligosaccharide addition, rendering the final product larger rather than smaller. Treatment of the protein synthesised in the presence of microsomes with enzymes that removed the sugars, as well as sequencing of the N-terminal amino acids of the forms made both in the presence and absence of microsomal membranes confirmed this, and the results were published in 1977 and 1978 (Katz et al., 1977a; Lingappa et al., 1978). Just like secretory proteins, integral membrane proteins, as exemplified at least by G-protein, relied on signal sequence mediated targeting. See also: Membrane Insertion of Tail-anchored Proteins; Protein Glycosylation, an Overview

By this time, the size of Blobel’s lab had expanded tremendously due to the interest generated by his work on the signal hypothesis. Each new person was assigned a different protein to investigate and methods were developed to extract and purify mRNAs from a variety of tissues. The technique of immunoprecipitation was also incorporated in the studies, which permitted the synthesis of less abundant proteins to be examined. In short order, the general applicability of the signal hypothesis to the targeting and translocation, or integration, in the case of membrane proteins, began to be clear. Among the proteins investigated were lysosomal hydrolases that began life in the ER before being sorted to the lysosome (Erickson and Blobel, 1979). In addition, other, nonviral integral membrane proteins, some of which spanned the membrane multiple times, were also studied (Friedlander and Blobel, 1985). Furthermore, because experiments were conducted with a completely reconstituted assay system consisting of components from a variety of species, the evolutionary conservation of the mechanism became evident. Demonstration that the hormone insulin, for example, utilised a signal sequence for targeting to and translocation across the ER, was accomplished with mRNA isolated from fish islets of Langerhans, ribosomes and factors from wheat germ, and microsomal vesicles purified from dog pancreas (Shields and Blobel, 1977). Many laboratories also began to accumulate evidence indicating that precursors to bacterial secretory proteins and components of bacterial viruses might also exist, suggesting that a mechanism related to the signal hypothesis might also operate in prokaryotes (Inouye and Beckwith, 1977; Randall et al., 1978; Ito et al., 1979).

The Mechanism of Targeting and Translocation

Although it was evident from these studies that signal sequences were involved in targeting nascent polypeptides to the ER membrane, nothing was known about how the process of targeting and translocation actually worked. Presumably, there was some kind of signal sequence receptor on the membrane. After the signal sequence bound, the revised signal hypothesis postulated that a tunnel assembled to permit the nascent polypeptide to penetrate the ER membrane (Figure 1a, Figure 3). However, the existence of this tunnel was pure speculation motivated by arguments based on the thermodynamics that hydrophilic amino acids in the nascent chain could not easily cross the hydrophobic core of the ER membrane.

In Blobel’s laboratory, investigation of these mechanisms was initiated by Bernhard Dobberstein. He began this work by focusing on the microsomal membrane component of the assay system. The in-vitro system used for protein synthesis in the original papers was complex, containing ribosomes and enzymes purified from a variety of sources including mouse tumours, rat liver, and rabbit
reliculocytes and microsomal membranes derived from dog pancreas. To simplify preparation of the system, Dobberstein continued to use dog pancreatic microsomes, but substituted an extract of wheat germ as a source for ribosomes and other essential enzymes. In this new system, however, he was unable to achieve protein translocation, even though precursor proteins were synthesised. He finally found an explanation in the method used to prepare the microsomes. Before their use in the assay, microsomes were typically treated with puromycin and washed with high concentrations of salt to eliminate any ribosomes or mRNA bound to the membranes. Reasoning that this harsh ‘stripping’ procedure might have removed an essential translocation factor, Dobberstein added back the fraction from the high salt wash to the stripped microsomes and was able to reconstitute translocation activity.

By this time, however, Dobberstein had obtained a position at the European Molecular Biology Laboratory in Heidelberg, and decided to take the reconstitution project with him. Once settled in Germany, Dobberstein was able to replicate and publish his basic results in *Nature* in 1978, although no biochemical characterisation of the key extracted components was presented (Warren and Dobberstein, 1978). Shortly thereafter, he presented a paper at a meeting of the German Biochemical Society and reported that he had fractionated the translocation activity by centrifugation on a sucrose density gradient and then analysed the fractions by SDS gel electrophoresis. The activity appeared to correspond to fractions enriched in six specific polypeptides.

In the Blobel laboratory, investigation of the translocation mechanism continued after Dobberstein’s departure. Peter Walter, a graduate student who had inherited the project, had briefly overlapped with Dobberstein and was very much aware of his results. However, he was unable to inactivate the translocation activity of the microsomal membranes using salt extractions alone, and was only successful in doing so by using a combination of mild proteolysis with trypsin and salt extraction. With this approach, he was able to reconstitute the translocation activity by adding back the trypsinised material, concluding that proteolysis had cleaved a protein from the microsomes, but that it could still function when permitted to rebind to the membrane. Walter, Blobel and their collaborators quickly published their findings, with the paper appearing in early 1979 (Walter et al., 1979).

Back in Heidelberg, the expansion of Dobberstein’s lab had permitted him to hand off the reconstitution project to David Meyer, a new postdoctoral fellow. Meyer began by trying to replicate the original results obtained with salt extractions, but was unable to do so. Once Walter’s paper appeared, he decided, like Walter, to exploit proteolysis, settling on the more specific protease elastase instead of trypsin. As with trypsin, elastase inactivated the microsomes, and translocation could be reconstituted by adding back the proteins cleaved by elastase to the inactive membranes. However, when fractionated on a sucrose gradient, the elastase-released activity migrated completely differently than the original activity discovered by Dobberstein. Using conventional protein purification techniques, Meyer was able to partially purify the activity, identifying it as a single polypeptide with a molecular mass of approximately 60 kDa in a paper appearing in 1980 (Meyer and Dobberstein, 1980).

In New York, Walter was frustrated by his slow progress and began modifying his assay system. In his original experiments on the trypsin-releasable factor, he had used a rabbit reticulocyte lysate to provide ribosomes and enzymes needed for protein synthesis *in vitro*. Although this was a well-established system, its production was labour intensive and unpleasant because it involved bleeding of anaemic rabbits. As an alternative, Walter switched to an optimised wheat germ extract similar to the one that Dobberstein had previously used in the Blobel laboratory. Now he was able to inactivate the microsomal membranes by salt washing them without protease treatment, and reconstitute translocation activity by adding back a factor from the salt extract. When the factor was purified it turned out to be an 11S complex of six polypeptides, the same ones seen earlier by Dobberstein (Walter and Blobel, 1980). Further characterisation revealed that a small 7S RNA molecule was also part of the complex (Walter and Blobel, 1982). As its function appeared to be in part binding of the signal sequence as it emerged from the ribosome during translation, Walter named it the signal recognition particle or SRP (Figure 1c).

As the work in New York and Heidelberg proceeded, the mystery of the appearing and disappearing salt extractable factor was gradually solved. In Dobberstein’s original experiments in New York and initially in Heidelberg, he had used wheat germ extract to provide protein synthesis components. Later, when Meyer picked up the project, he had switched to rabbit reticulocyte lysate and was unable to detect the factor. Conversely, Walter had begun his studies using the reticulocyte lysate, and was only able to inactivate the microsomal membranes with trypsin and salt extraction. When he then switched to wheat germ extract, he was able to detect and purify SRP. The key was that, unexpectedly, reticulocyte lysate contained significant amounts of SRP. Thus, when salt-washed microsomes were assayed in a system containing reticulocyte lysate, the SRP in the lysate replaced that washed from the microsomal membranes, making it appear as if inactivation had not occurred. Wheat germ extract, however, contained no SRP.

But what was the 60 kDa protein that Meyer had discovered? After further experiments, Meyer found out that it was a proteolytic fragment of a larger, integral membrane protein of the microsomal membrane that acted as a receptor for the nascent chain–ribosome complex. To emphasise that it was where the complex docked on the membrane after floating through the cytoplasm, he named it ‘docking protein’ (Meyer et al., 1982). Back in New York, Reid Gilmore, one of Blobel’s postdoctoral fellows, succeeded in purifying the same protein using SRP as an affinity probe, calling it the ‘SRP receptor’ (Gilmore et al.,
1982a; Gilmore et al., 1982b). With this, the first parts of the mechanism for targeting proteins to the ER membrane were clear: SRP bound the signal sequence of the nascent chain as it emerged from the ribosome, and then transferred the synthetic machinery to the ER membrane through its interaction with the SRP receptor (Figure 1c).

Although the 1975 version of the signal hypothesis had not predicted anything like SRP, it did postulate a membrane tunnel (Figure 1b, Figure 3). The actual existence of this tunnel proved to be the most controversial part of the mechanism. Indeed, work on prokaryotic cells hinted that such a tunnel might not be necessary (Wickner, 1979). Although prokaryotes did not have an ER, they both secreted proteins across and inserted them into their outer membranes. One model for the latter was a small, hydrophobic coat protein of the bacterial virus M3. Though the coat protein was made as a precursor that was processed during insertion, insertion occurred after synthesis was complete and appeared from modelling studies with artificial membranes to be spontaneous, that is, without the need for a tunnel or other complex of proteins in the membrane (Wickner, 1979; Ito et al., 1980).

Evidence began to accumulate, however, that a protein-conducting channel, as it gradually came to be called, did, in fact, exist. Clever cross-linking experiments identified specific proteins that contacted the signal sequence during its translocation (Wiedmann et al., 1987). In Blobel’s laboratory, an ion-conducting channel linked to protein translocation was detected in planar lipid bilayers containing microsomal membrane proteins (Simon and Blobel, 1991). A crucial finding came from studies of yeast, a eukaryotic microorganism. Randy Schekman’s laboratory at Berkeley had adopted yeast to study the secretory pathway because mutants defective in various steps of secretion could be generated and the mutant genes readily identified. In 1987, they discovered a mutant defective in an early stage of the secretory process that they called Sec61S (Deshaies and Schekman, 1987). In 1993, Dirk Görlich and Tom Rapoport incorporated the Sec61 complex and SRP receptor in lipid vesicles and were able to reconstitute protein translocation. Ultimately, structural characterisation of Sec61 homologues and its associated subunits revealed that it formed the long sought after channel in the membrane that bound ribosomes and provided a pathway for the signal sequence and nascent chain (Beckmann et al., 1997). See also: Protein Export from Endoplasmic Reticulum to the Cytosol: In Vitro Methods; Ribosome Structure and Shape.

### Topogenesis and Generalisation of the Signal Hypothesis

The original signal hypothesis focussed on the mechanism by which proteins were targeted to the ER membrane and translocated across it. However, it was known that other proteins synthesised in the cytoplasm of eukaryotic cells were also targeted to locations distinct from the ER. These included the mitochondria and, in plants, chloroplasts. It was possible, then, that signal sequences analogous to those specific for the ER might also function in these organelles. The first indication that this was the case came from a collaboration between Dobberstein while he was still in Blobel’s laboratory, and Nam-Hai Chua, a colleague of Blobel’s in the Laboratory of Cell Biology at Rockefeller. Chua worked on the green algae *Chlamydomonas reinhardtii* and was interested in the structure and biogenesis of chloroplasts. The most abundant protein in chloroplasts was the enzyme ribulose bis-phosphate carboxylase. Carboxylase consisted of two subunits, a small one made in the cytoplasm that had to somehow be transported into the chloroplast, and a large one coded by the chloroplast DNA and synthesised within the chloroplast itself. When Dobberstein extracted mRNA from the algae and translated it in vitro, the resulting polypeptide was larger than the form isolated from the chloroplast, but could be proteolytically processed by cell extracts to the correct size (Dobberstein et al., 1977). Hence, it was postulated to be a precursor of the mature form. Shortly thereafter, Peter Highfield and John Ellis in Britain demonstrated that a small subunit precursor from peas was taken up by intact chloroplasts after it was synthesised and proteolytically processed to the correct size during or after transport (Highfield and Ellis, 1978), a finding later corroborated by Chua who also provided a complete sequence of the precursor segment (Schmidt et al., 1979). While the chloroplast studies were occurring, Blobel collaborated with Gottfried Schatz in Switzerland to determine if there were also precursors of cytoplasmically synthesised mitochondrial proteins that facilitated their targeting and transport into the mitochondria. In 1979 they reported that, indeed, three subunits of the mitochondrial F1-adenosine triphosphatase (ATPase) were synthesised as larger precursors and transported after translation into the mitochondria, where they were proteolytically processed to the correct size (Macececcchini et al., 1979). See also: Mitochondrial Disorders: Nuclear Gene Mutations; Mitochondrial Dynamics: Mechanisms and Pathologies; Mitochondria Protein Import: Methods; Plant Chloroplasts and Other Plastids.

Blobel (1980) published a paper that generalised the signal hypothesis into a scheme for intracellular protein ‘topogenesis’. In addition to signal sequences he proposed ‘sorting sequences’ and other ‘topogenic sequences’ that were responsible for correctly targeting proteins to different locations within the cell and ensuring that they were placed in the correct orientation in those locations. Thus, according to this proposal, topogenic sequences helped to determine not only to which organelle a particular protein was targeted, but also the topographical disposition of that protein. Secretory proteins, for example, were targeted to the ER by signal sequences, and also to the interior of the ER. Similarly, signal sequences were also responsible for the insertion of membrane proteins into their target membrane with their N- and C-termini on the correct side.
of the membrane. Topogenic sequences functioned not only cotranslationally, as proposed in the original signal hypothesis, but also post-translationally as observed in the mitochondria and chloroplasts, and later, in the ER itself. Different topogenic sequences might also function sequentially; a signal sequence, for example, might cause initial insertion of a membrane protein into the ER membrane, with a second sorting sequence making sure that the final destination of that protein was the plasma membrane.

Blobel’s idea was transformational because it linked the information content of a linear deoxyribonucleic acid (DNA) sequence, ultimately translated into a polypeptide, to the three-dimensional spatial location of the protein in the cell. Critically, decoding of this information relied on the preexisting cellular form, suggesting that the organised cell and its membranes, which are also inherited, were the repositories of intergenerational spatial information as critical to life as the genetic code itself. Indeed, as noted by Lenny Moss:

The biological meaning of a protein is not realised simply at the level of its amino acid sequence but is dependent on its localization in a particular cellular or extracellular compartment or milieu.

(Moss, 2003, p. 85).

This form of ‘structural inheritance’ is part of the epigenetic system of living organisms, as important to evolution as the DNA code (Jablonka and Lamb, 2005).

References


Further Reading


