

Shedding light on the translocation pore

Fluorescence techniques show that signal sequences are in an aqueous environment during protein translocation, which provides strong evidence for the existence of the long sought-after translocation pore.

The secretion of proteins from cells presents a fundamental biochemical problem: as all protein synthesis occurs in the cytoplasm, for a protein to be secreted it must cross a membrane. Secreted proteins, like all water-soluble proteins, have extensive hydrophilic regions. Membranes, however, have evolved as permeability barriers to such polar molecules. How is this apparent paradox resolved? Secreted proteins do not seem to be special in any way that would imply they can transiently enter the hydrophobic core of the membrane, leading to the suggestion that an aqueous environment is created across the membrane to allow their passage [1]. Although this idea was first put forward almost two decades ago, it has never been demonstrated experimentally.

Early in the study of the molecular mechanisms of secretion, it was discovered, however, that proteins destined for secretion are distinguished from those that will remain in the cytosol by an amino-terminal extension of some 20 to 25 amino acids [1]. In recognition of its role as the feature that identifies proteins to be secreted, this extension was termed the 'signal sequence'. The signal sequence is cleaved from the remainder of the protein chain early in its translocation across the membrane of the endoplasmic reticulum (ER) by a protease, known as a signal peptidase, that acts on the luminal side of the membrane. The signal sequence consequently plays no part in the function of the mature protein, but has a pivotal role in its translocation across the membrane.

Close examination of the signal sequences from a large number of different secreted proteins has revealed a number of puzzling features. Signal sequences seem to be sequences of hydrophobic amino acids with no apparent primary-structure conservation and which vary in length from one secreted protein to another. While puzzling in an era that emphasizes recognition of specific sequences, these properties suggested a solution to the problem of getting a hydrophilic protein across the hydrophobic barrier of the membrane: the hydrophobic nature of the signal sequence would allow it to partition directly into a lipid bilayer, bringing the attached protein chain to be translocated along with it [2,3]. The lack of sequence similarity among signal sequences would not be a problem: it would be the tendency of the signal sequences to partition into the lipid bilayer that would allow them to play their part in protein translocation.

Is this idea feasible? Theoretical thermodynamic analysis suggested that it is possible for a signal sequence both to insert spontaneously into a lipid bilayer, and to act subsequently as the component that makes possible the entry

of hydrophilic sequences into what would otherwise be the energetically unfavorable environment of the lipid bilayer [3]. Experimental approaches have concentrated on examining the properties of model signal sequence peptides and have verified that they do, indeed, insert spontaneously into lipid bilayers, and that mutations preventing them from acting as signal sequences *in vivo* also prevent this insertion *in vitro* [4].

What has been missing from these studies is the ability to examine the environment of the signal sequence when it is actually part of a nascent chain and in the act of translocation across a membrane. A recent paper by Crowley, Reinhart and Johnson [5] provides this context. To membrane vesicles derived from the ER, the authors delivered ribosomes programmed in an *in vitro* translation system to make specific nascent chains. The ribosomes attach to the membranes and translocation ensues as translation continues. The authors used a clever trick to monitor the environment of the nascent protein chain: by specifically modifying the ϵ -amino group of a lysyl-tRNA and then using the tRNA charged with the modified amino acid in the *in vitro* translation reaction, they were able to incorporate modified lysine residues at specific points within the nascent chain [6]. The authors used a nascent secretory protein with two lysine residues in its signal sequence and, as a modifying group, a fluorophore with fluorescence properties that are sensitive to the polarity of the environment (in other words, whether it is aqueous or hydrocarbon). By using messenger RNAs truncated at specific points within their coding sequences, the authors produced a set of stable translocation intermediates with nascent chains of defined length and fluorophores incorporated into their signal sequences. In their paper, Crowley *et al.* focused on short nascent chains that are not long enough to have had their signal sequences removed on the luminal side of the membrane by the signal peptidase. For these nascent chains, they examined whether the fluorophores are in an aqueous or a hydrocarbon environment.

The results of these experiments are clear: in all of the translocation intermediates examined, the fluorophores are in an aqueous environment. In no case is one in a hydrophobic environment. This is not what would be predicted by a model in which the initiating event of translocation is the spontaneous insertion of the signal sequence into the lipid bilayer. Crowley *et al.* conclude that, at least during the initial stages of translocation, the signal sequence is in an aqueous environment and is prevented from coming into contact with the hydrocarbon core of the membrane. The authors also conclude that

the aqueous environment in which the signal sequence is found is not contiguous with the cytoplasm. This conclusion is drawn from the authors' inability to quench the fluorophore with small, water-soluble quenching agents, such as iodide ions, delivered from the exterior of the membrane vesicles. Thus, the ribosome must sit down on the membrane in such a way that it forms a tight seal (Fig. 1). This is a satisfying finding as it implies that a potential translocation tunnel that opens up underneath the ribosome would only conduct passage of the nascent protein chain and not generally perturb the permeability barrier of the membrane. This is consistent with results from electrophysiological experiments showing that translating ribosomes prevent passage of ions across the membrane [7].

Taken together, these results suggest that, rather than being in contact with the hydrophobic core of the lipid bilayer, the signal sequence is surrounded by membrane components that sequester it in an aqueous environment. This suggests that the role of the signal sequence in the initiation of translocation is that of a ligand recruiting a competent translocation apparatus [8]. These results argue against the idea that signal sequences provide the thermodynamic drive to make possible the entry of the remainder of the nascent chain into the membrane. Such a role for the signal sequence as a ligand for a membrane component is reminiscent of its role in the cytoplasm. The initial identification of a secretory protein as it is being synthesized is made by a soluble cytoplasmic component, the

signal recognition particle (SRP), that binds to the signal sequence as it emerges from the ribosome. In doing so, the SRP selectively marks those ribosomes making secretory proteins for interaction with the ER membrane [9]. Association with the membrane is made through the interaction of SRP with its receptor, an ER membrane protein, which induces SRP to relinquish the signal sequence into the environment examined by Crowley *et al.* Thus, it is possible that during the course of translocation the role of the signal sequence is to participate as a ligand in a series of interactions, each one organizing components required for a particular stage of the process.

These results demonstrating that the signal sequence resides in an aqueous compartment during translocation come tantalizingly close to proving the existence of a translocation pore, the long sought-after aqueous tunnel through which the nascent chain would cross the membrane. Recently, Simon and Blobel [7], using electrophysiological techniques, demonstrate that large aqueous pores can indeed exist in the ER membrane, and could be the avenue through which nascent chains cross it. Crowley *et al.* are in a position to test directly if the compartment in which they find the signal sequence is indeed such a membrane-spanning channel. They can now ask whether that compartment extends all the way to the luminal side of the membrane, and whether probes incorporated throughout the remainder of the nascent chain are also in an aqueous environment while crossing the plane of the membrane. If the answer to each of these questions is

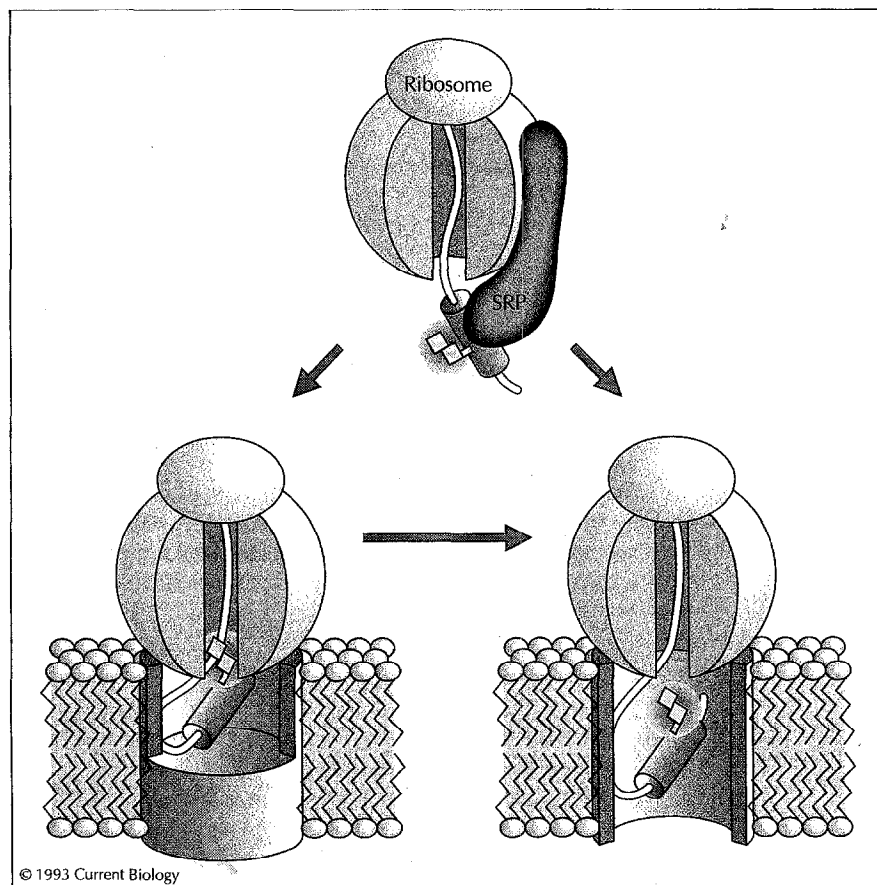


Fig. 1. The top shows a free ribosome synthesizing a short nascent chain, as in the recent experiments of Crowley *et al.* [5]. The signal sequence is indicated by the cylinder and the fluorescent group by the two yellow squares projecting from it. The signal recognition particle (SRP) is bound to both the signal sequence and the ribosome. Below are two possible configurations for a ribosome-channel complex consistent with the results of Crowley *et al.* On the left, the ribosome is engaged with an incomplete channel, either an intermediate in channel assembly or a mature channel that is gated; on the right, it is engaged with a membrane-spanning channel. The arrows indicate that the state shown on the left could be an intermediate or, alternatively, the free ribosome could associate directly with a membrane-spanning channel.

positive, it would constitute long-awaited, direct proof of an aqueous channel for protein translocation, and would suggest that the role of the signal sequence is to trigger assembly or activation of that channel.

As Crowley *et al.* point out, it remains possible that an all-or-nothing view of membrane spanning is an oversimplification. Perhaps there are early translocation intermediates in which the signal sequence is harbored in an aqueous environment that does not extend all the way across the membrane. Such might be the case at an initial stage in which the signal sequence is responsible for recruiting the components required for assembly of a complete membrane-spanning channel. Alternatively, it is possible that the compartment Crowley *et al.* have detected is the fully assembled channel but that it does not extend all the way to the lumen, being gated on the luminal side to maintain the permeability barrier of the membrane until protein translocation is fully under way. The experimental means to test these possibilities are now in hand.

In addition to its use in examining the environment in which water soluble, secreted proteins pass through the membrane during translocation, the technique used by Crowley *et al.* should help reveal the mechanism of the other essential function of any translocation channel, that of recognizing integral membrane proteins and integrating them into the membrane. To date, all experimental evidence suggests that the same membrane components are required for both the translocation of soluble proteins and the integration of membrane proteins. How, then, can a membrane protein escape an aqueous channel laterally to achieve its proper, asymmetric orientation in the membrane? This process must involve the recognition of the nascent transmembrane region while it is still in an aqueous environment, followed by its transfer into the hydrophobic interior of the membrane. For some multispinning integral membrane proteins, such recognition and transfer would have to occur as many as ten times. Any aqueous translocation channel, then, cannot

be simply a static pipe surrounding the nascent chain. It must instead be a much more dynamic structure, capable of monitoring the nascent chain as it passes, and transiently and selectively abdicating the role of a barrier between aqueous and hydrophobic environments. Thus, continued use of these techniques may not only provide light at the end of the tunnel but may also shed light on its sides as well.

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