clathrate structure Na_8Si_{46} is taken as the starting point, and used to generate a space-filling array of 14-sided cells of unequal sizes. The sizes were then equalized by using the 'Surface Evolver' computer package of K. Brakke published some 12 years ago. It equalizes the volumes while minimizing the surface area of each cell. A particular feature of this package, which runs on a work station rather than a supercomputer, is that it enables the original flat cell walls to be gradually curved. The area of each curved wall is estimated by taking it to be composed of steadily larger numbers of small flat pieces (the planes can just be seen on the polyhedra in the figure). In this way, Weaire and Phelan were able to design a new cell shape and show that it improved upon Kelvin's long-standing record holder.

The problem is still not wrapped up. The authors themselves have other candidates still to investigate and, as they point out, candidates are still coming forward on an *ad hoc* basis. Filling space economically is one of those mathematically formulated problems for which there is still no mathematical theory capable of providing a systematic approach. □

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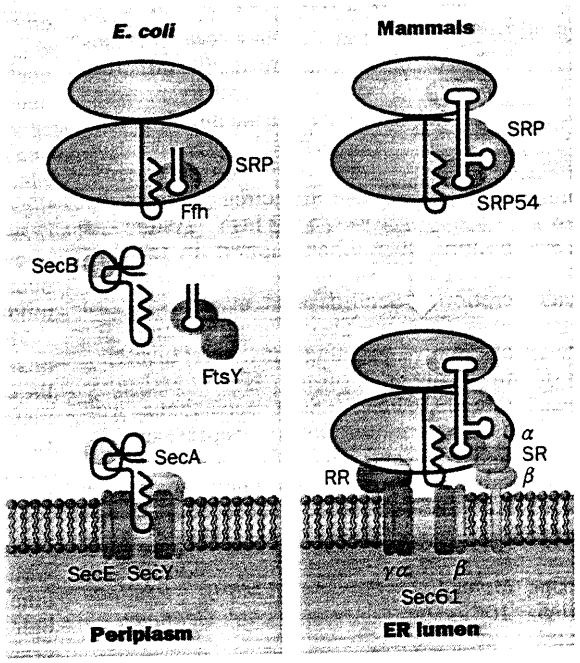
PROTEIN TRANSPORT

On the beaten pathway

Bernhard Dobberstein

TRANSPORT of proteins across bacterial plasma membranes is evolutionarily related to transport of proteins across the endoplasmic reticulum (ER) membrane in eukaryotic cells. In both systems, proteins destined for secretion are usually synthesized as precursors with a hydrophobic core signal sequence. At first, however, it looked like the respective targeting and translocation machinery would turn out to be very different — although the so-called signal recognition particle (SRP) and its receptor mediate targeting of nascent secretory proteins to the ER membrane, two structurally unrelated proteins, SecB and SecA, guide secretory proteins to the plasma membrane in bacteria[1]. But as more detail emerges, so that view is having to change. For example, papers by Miller *et al.*[2] and Hartmann *et al.*[3] (pages 657 and 654 of this issue) indicate that there is not only a striking similarity between the pro- and eukaryotic signal-recognition and protein-targeting apparatus, but also between the translocation sites. The implication, of course, is that the mechanisms involved must have much in common.

In eukaryotes, a ribosome synthesizing a secretory protein is targeted to the ER membrane by the sequential interactions of the signal sequence on a nascent polypeptide with SRP and the SRP receptor (SR) in the membrane (see figure). The SRP is a ribonucleoprotein composed of the 7S RNA and six polypeptides with relative molecular masses of 9,000–72,000 (see table). The signal sequence of the nascent secretory protein is recognized by the 54K protein subunit of SRP (SRP54). Contact between SRP and the signal sequence reduces the rate of polypeptide-chain elongation. Upon contact with the SR, the signal sequence is released from SRP, elongation proceeds and the nascent chain inserts into the ER membrane through a proteinaceous pore[4,5].



Factors involved in signal-sequence binding, targeting and membrane translocation in *E. coli* and mammalian cells. The signal sequence is shown as a zig-zag. Proteins that are similar in all organisms are in red, and those for which no counterpart is known yet in brown. RNA (4.5S RNA and 7S RNA) is shown as a thick purple line separated by a space. RR, ribosome receptor.

The process of targeting to the ER membrane is regulated by GTPases in SRP54, the SRα and probably also the SRβ subunits. *In vitro*, targeting and translocation requires the presence of GTP[6]. GTPases in a wide variety of molecules function as molecular switches which confer unidirectionality and specificity on biochemical processes. In the case of SRP-mediated protein translocation, cycles of GTP binding and hydrolysis appear to regulate the interactions of nascent secretory proteins with the targeting and translocation machinery. How exactly the GTPases work in the context of the ribosome nascent chain complex is not yet known. But from analysis of complexes formed between purified SRP or SRP subparticles and SR, some principles have emerged. For instance, GTP binding to SRP54 increases upon contact with SR (ref. 7), and if this holds true for the complete system then SRα acts as a 'guanine nucleotide loading' protein which promotes GTP binding to SRP54. SR itself probably needs to bind GTP in order to stimulate GTP binding to SRP54 (ref. 8).

Somewhat surprisingly, Miller and colleagues previously found that the presence of a signal sequence prevents SR from stimulating GTP binding and hydrolysis. This probably indicates that one or more additional components are needed for promoting GTP binding when a signal sequence interacts with SRP54. As GTP is required for signal-sequence release, it is likely that the additional factor ensures that the signal sequence is released from SRP only when appropriate components of the translocation site are available[5,7].

Molecules structurally similar to SRP 7S RNA, SRP54 and SRα occur in *Escherichia coli*, namely 4.5S RNA, Ffh (also known as P48) and FtsY respectively[9] (see table). As 4.5S RNA and Ffh form a ribonucleoprotein complex that specifically recognizes nascent polypeptides containing a signal sequence, the complex has been termed an SRP[10]. Miller *et al.*[2] now show that this SRP and FtsY communicate in like manner to their mammalian counterparts. In the presence of non-hydrolysable guanine nucleotides, *E. coli* SRP and an FtsY derivative form a stable complex, and GTP hydrolysis is significantly stimulated when they are combined. Miller *et al.*[2] find that, as with the mammalian proteins, FtsY is not able to stimulate GTP hydrolysis in the presence of a functional signal sequence. This suggests that bacteria have a signal-recognition and protein-targeting system that functionally resembles the SRP and SR system of eukaryotic cells. Furthermore, it seems

that in *E. coli*, as in mammalian cells, factors besides SRP and FtsY are required for the release of the signal sequence from SRP and successful targeting. These could be an as yet unknown SRβ homologue, a chaperone or components of the translocation site.

Protein components of the translocation site were first identified by genetic screens in *E. coli*[11], then in the yeast *Saccharomyces cerevisiae*[12], and most recently by a biochemical approach in mammalian cells[3]. Strikingly, the central part of the translocation complex in all three systems seems to consist of related proteins. Using crosslinking approaches, Sec61-α of mammalian cells, SEC61p of yeast and SecY of *E. coli* have been shown to line the postulated translocation pore[3] (see figure). Structurally, these proteins are quite similar.

Factors involved in protein secretion in mammalian cells, yeast and *E. coli*

	Mammals	Yeast	*E. coli*
Cytosol (signal recognition, chaperoning)			
SRP-RNA	7S RNA	SCR1	4.5S RNA
SRP proteins	9, 14, 19	SEC65	
	SRP54	SRP54	Ffh (P48)
	68, 72	?	
			SecB
Membrane (docking)			
SRP receptor	SRα (DP)	SRα	FtsY
(SR)	SRβ (DP)	?	?
	?	?	SecA
Membrane (translocation)			
Translocation	Sec61-α	SEC61p	SecY
machinery	Sec61-β	?	?
	Sec61-γ	SSS1p	SecE
			band 1
			SecD
			SecF

In all systems, other proteins associate with Sec61/SecY. These are Sec61-β and Sec61-γ in mammalian cells, SecE and band 1 in *E. coli*, and SEC62p, SEC63p and SEC66p in yeast. Mammalian Sec61-β and Sec61-γ have now been isolated and sequenced by Hartmann *et al.*[3]. Both proteins are predicted to span the membrane once. Of particular interest is that mammalian Sec61-γ bears significant homology to SSS1p of *S. cerevisiae* and can functionally replace it; SSS1p protein was discovered last year as a suppressor of *sec61* temperature-sensitive mutants[13]. Sec61-γ also has a small, but probably significant, resemblance to the SecE protein of bacteria.

Reconstitution studies have shown that, in *E. coli*, SecA and SecY/E are the only membrane proteins required for translocation of a pre-protein across membranes. Similarly, mammalian SR and the Sec61 complex reconstituted into lipid vesicles promote protein translocation[14,15]. It therefore seems that the core components of the translocation site have been characterized and consist of the pore protein, Sec61/Y, and associated proteins. Two small proteins associate with mammalian Sec61 and *E. coli* SecY, but until now only unrelated proteins have been found to associate with yeast SEC61p and it has been suggested that two of them (SEC62p and SEC63p) are involved in signal recognition and targeting[12]. So we can expect further growth in the number of proteins that are found to be in, or associated with, the translocation site. For instance, little is known about the release of the nascent chains from the translocation site. In *E. coli*, such a function could conceivably be performed by SecD and SecF.

No eukaryotic homologues for the *E. coli* SecB and SecA have yet been found. These two proteins interact during the targeting of some secretory proteins to the plasma membrane. It is perfectly possible that they constitute an alternative targeting pathway to the SRP/FtsY system, a notion which finds support in the fact that deletion of Ffh does not affect the secretion of SecB-dependent proteins[16]. In yeast there are also strong indications for alternative targeting pathway(s) involving the heat-shock protein HSP70 and possibly SEC62/63p[12]. So it seems that as well as the common pathway represented by SRP/SR and Sec61/Y, species- and substrate-specific secretion pathways have also evolved. □

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DAEDALUS

Deep insight

THE ocean floor is less well known than the surface of the Moon. At present, a few isolated patches can occasionally be glimpsed by robot submersibles. Daedalus now suggests a new approach. He is devising an undersea vehicle to crawl along the existing submarine cables.

This neat trick has many advantages. Each cable has a known diameter and surface texture, so a matching drive mechanism can be designed to grip and traverse it. The cable could easily transmit signals to the crawler, and pick up its results, by induction (spy submarines used to read cable traffic in this way). Even coaxial and fibre-optic cables could communicate with the crawler inductively via the power leads for their repeater–amplifiers. A signal sent along the cable to the crawler would take time to reach it, and time to return. This delay would give its exact position on the ocean floor.

For some of its length, a cable may be buried in the mud of the ocean floor, either by the impact of laying or subsequently by slow deposition of detritus from above. The crawler's drive would have to be powerful enough to push this overburden aside. It would also need a certain flexibility to negotiate splices and amplifier pods in the cable, and marine organisms such as shellfish which might be growing on it (though these should be rare in the depths). The crawler would project some sort of buoyant mast upwards to survey the ocean bottom from above the opaque muddy clouds raised by its passage.

The power supply for the crawler poses problems. Its inductive coupling to the cable could only provide it with milliwatts. It might burn carbonaceous fuel in the ocean's dissolved oxygen, as a fish does. But Daedalus prefers a metallic fuel which slowly dissolves in the surrounding water and generates electricity directly, in battery fashion. With certain chemical precautions, lithium seems the metal of choice. Being lighter than water, it adds no weight to the crawler. A fairly modest supply should power it across the widest ocean.

Cheap and effective, cable crawlers will transform oceanography. Along every cable, a succession of crawlers will map the ocean floor and log its changing fauna. Not only will they transmit images and data back to shore; on command they will gather specimens for later study. But careful traffic control will be needed. Two crawlers which met on the same cable could never get past each other. At least one would have to let go, develop buoyancy, and rise to the surface to await rescue. David Jones