

Meeting preview

Delivering the goods

Bruce Ward

A preview of the topics to be discussed at the SGM Main Symposium *How Do Molecules Cross Microbial Membranes?* at the University of Leeds, 7–8 September 1999

● Transport is an essential component of our lives. Yet cells display a dexterity in speed of conveyance, complexity of the variety of molecules carried and specificity of carriers that makes the traffic problems of the M25 look trivial. A major problem in both cases is getting to the right place at the right time.

In eukaryotes, some proteins are destined for particular organelles (mitochondria, chloroplasts, peroxisomes, lysosomes or the nucleus), some of which have multiple compartments. Others are retained within the general secretory pathway (endoplasmic reticulum and Golgi), exported to the plasma membrane or secreted from the cell. Even for the relatively simple prokaryotic cells, the problem of delivering molecules to the right place at the right time is not trivial. In a Gram-negative bacterium, macromolecules may be destined for the inner membrane, periplasm, outer membrane or the external medium.

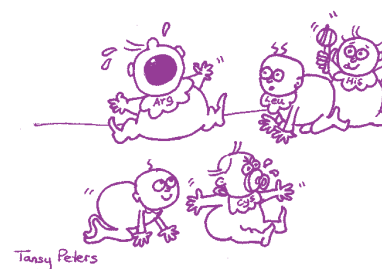
No previous SGM symposium has focused entirely on microbial transport, although individual papers on transport of small molecules (late 1970s), protein secretion mechanisms (1989–1991) and, more recently, protein transport in relation to host infection (1993–1997) have been written for SGM symposia. The wealth of molecular information on transport systems has led to an improved understanding of the shared features of transport in prokaryotic and eukaryotic cells and the complexity of machinery required to ensure the safe delivery of molecules to the correct location in an active state. Some of the many current interests in microbial transport are: the nature of transmembrane channels and the regulation of their gating; the structure and mechanisms of protective chaperone proteins; and the mechanisms for ensuring correct targeting, modulating protein–protein interactions and controlling ligand specificity. Hence a symposium on transport of molecules across biological membranes seems timely.

● Prokaryotic protein secretion

Bacterial membranes provide essential barriers between the inside of the cell and the external medium. Yet protein secretion is essential for secretion of virulence factors and extracellular enzymes, and also for the assembly of cell appendages like pili and flagella. Other proteins have to be integrated into the inner and outer membranes or exported to the periplasm. Gram-negative bacteria have evolved a number of systems for coping with the problem of traversing two rather different lipid membranes (the inner proton-impermeable membrane is composed primarily of phospholipid and proteins while the outer membrane contains lipopolysaccharide as a major component but relatively few proteins and has channels, called porins, that facilitate entry or exit of small molecules). The SecYEG translocase of *Escherichia coli* plays a vital role in transporting proteins into and across the inner membrane. Its eukaryotic counterpart is the Sec61 complex for protein translocation

into and across the endoplasmic reticulum (ER) membrane to the ER lumen. The hydrophobic N-terminal signal peptide on the exoprotein is recognized by the Sec system. In many Gram-negative bacteria, Sec-dependent translocation serves to present a subset of proteins to the Type II secretion machinery, which is then responsible for the second (terminal) transport step across the outer bacterial membrane. Type II secretion requires some 12–16 proteins. Filloux will discuss the composition of the proteins in the Type II secretion system and speculates on how the ‘patch’ secretion signals of exoproteins are recognized by the machinery. Proteins can be delivered to the SecYEG translocase either via chaperones, most notably via the export-dedicated chaperone, SecB, or through their interaction with a signal recognition particle (SRP). In eukaryotes a cytosolic SRP binds the hydrophobic signal peptides of presecretory proteins as they emerge from the ribosome and delivers the nascent polypeptides to the translocase at the ER membrane. In their review High and colleagues will discuss the evidence for a bona fide SRP-dependent targeting pathway in *E. coli*.

By contrast Type I and Type III systems involve one-step mechanisms for protein translocation. Type III systems are found in *Shigella*, *Salmonella*, certain *E. coli* strains, *Yersinia* and plant-pathogenic *Pseudomonas*, *Xanthomonas* and *Erwinia* spp. These systems comprise a sizeable set of proteins capable of delivering a number of virulence factors into mammalian or plant host cells. Schneewind will review the Type III secretion machinery of the pathogenic yersiniae. Some of their proteins are directly injected into the cytoplasm of the eukaryotic host cell (so have to cross three membranes), whereas others are secreted into the surrounding medium, or remain bound to the surface of the bacterium. At least three of them require dedicated chaperones for successful targeting. Type I systems utilize a periplasmic ABC transporter (ATP-binding cassette) of which the α -haemolysin of *E. coli* is the best known example. Type I systems generally secrete only one or a few exoproteins, and these are recognized as substrates by the ABC protein exporter, largely by virtue of the secondary structure of their C-termini. The dynamics of the assembly of this relatively simple machinery and of the Type I secretion process are now beginning to be understood. Bacterial diversity is shown in Type II systems with alternative second steps and in the use of other transport systems, e.g. Type IV systems for export of T-DNA by *Agrobacterium tumefaciens*.



● Eukaryotic protein transport

Proteins, which are destined for secretion, are firstly delivered to and transported across the ER membrane, then transported to the Golgi apparatus and thence to secretory vesicles. These vesicles move to and fuse with the plasma membrane from whence they are secreted. This process has to be tightly regulated all the way to control *inter alia* protein conformation and, in the case of hydrolytic enzymes, premature activation. Preproteins can either be co-translationally targeted to the ER membrane by SRP, or post-translationally by the Sec63-dependent route. In both cases the preprotein crosses the ER membrane via a transmembrane channel, which in both yeast and mammalian cells is composed primarily of three proteins (the Sec61 complex). The Sec61p of yeast shows homology to the SecY component of the *E. coli* SecYEG translocase. Recent work has revealed that the transmembrane channel is much wider than that required to accommodate a linearly extruded polypeptide, and thus to the realization that substantial folding of the protein may occur whilst it is still being translocated across the membrane. This and other recent advances, such as the revelation that gating of the translocase may be accomplished by transduction of conformational changes, that occur in the ribosome as it translates, will be discussed by Stirling.

Whilst a branch of this general secretory pathway is used for the delivery of proteins into lysosomes, most proteins of the mitochondria, peroxisomes, chloroplasts and the nucleus arrive at their destination as the result of post-translational import from the cytoplasm. Proteins transported from the plant cytosol to the thylakoid lumen of chloroplasts have to traverse three membranes: the outer and inner chloroplast envelope membranes and the thylakoid membrane. Chloroplast protein transport is more complicated than originally envisaged. Once proteins containing chloroplast transit sequences have been imported into the stroma they may undergo SRP-dependent targeting and Sec-dependent or Δ pH-

dependent translocation across the thylakoid membrane. These pathways all reflect the prokaryotic origins of chloroplasts. Preproteins destined for the Δ pH-dependent pathway have N-terminal signal peptides that differ subtly from classical signal peptides by the inclusion of a twin-arginine motif bordering the hydrophobic region. The Sec- and Δ pH-dependent pathways seem to be mutually exclusive and, remarkably, the latter pathway acts on fully folded precursors. The discovery of the second Δ pH-dependent pathway has led to the recognition of the same type of pathway in bacteria for the export of folded periplasmic proteins requiring cofactors. A fourth pathway, that may also have a bacterial counterpart, appears to involve the spontaneous insertion of proteins into the thylakoid membrane.

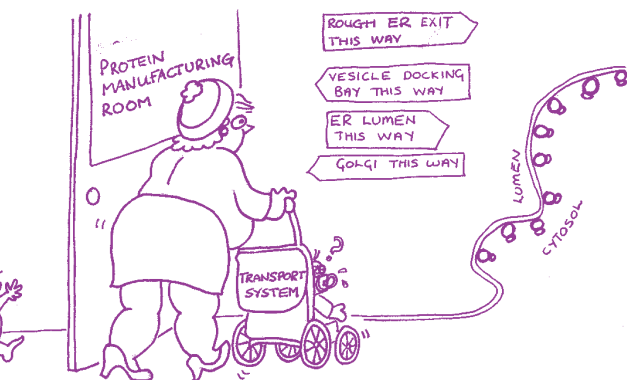
Protein transport from the cytosol to peroxisomes is conceptually simpler than chloroplast transport as there is only a single membrane to cross. Whilst the vast majority of peroxisomal proteins are post-translationally imported from the cytosol (by virtue of short C-terminal targeting signals), it is becoming increasingly clear that peroxisomal lipids and some important peroxisomal membrane proteins derive from the ER. In her contribution Baker will focus on the biogenesis of peroxisomes.

● Influx and efflux systems for small molecules

For uptake of nutrients the problems are slightly different. Often the major one is that the concentration of a metabolite outside the cell is much lower than that in the cell and uptake has to be against a concentration gradient. One solution is to convert the transported solute into a modified form as in the phosphorylation of sugars by the phosphotransferase system (PTS). Regulation of sugar transport at the protein level involves inducer exclusion for Gram-negative bacteria but inducer expulsion in Gram-positive bacteria. Primary and secondary transport systems for solutes respectively use energy from ATP hydrolysis and from ion gradients; they can catalyse both uptake and efflux. Poolman will review these systems with particular emphasis on transport systems used in adaptation to osmotic stresses.

Efflux systems are used to pump out antibiotics, heavy metals and toxic metabolites (including waste products from intermediary metabolism) from cells and thus help regulate the environment within the cell. Rosen will describe the ubiquitous arsenite efflux pumps and the ways in which accessory factors improve the efficacy of the resistance mechanism. Specific binding of a peripheral membrane ATPase to bacterial pmf-dependent arsenite carriers convert them into primary arsenite pumps, and possession of an arsenate reductase effectively extends the range of substrates that arsenite transporters can extrude.

Lewis will describe the multidrug resistance pumps (MDRs) that occur in bacterial, animal and plant cells. By contrast with solute influx transporters, MDRs have remarkably broad substrate specificity; amphipathic cations are generally the preferred substrates, even though MDRs



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occupy four different membrane protein superfamilies. A variety of mechanisms and pathways for the ligand are used; these include a pathway whereby the ligand is flipped from the inner leaflet to the outer leaflet of the membrane and another where transport is from the outer leaflet to the external medium using a porin to traverse the outer membrane. The polarity of the ligand and its partitioning in the lipid bilayer may

determine the route of efflux. Lewis will also discuss the possibility that naturally occurring plant alkaloids would be potent antimicrobials if not for the evolution of MDRs. As overexpression of MDRs causes clinically significant multidrug resistance, the possibility of combating this resistance by using the natural MDR inhibitors produced by plants (alongside the alkaloids) in combination with antimicrobials will also be discussed.

Sequence comparison studies have been applied to transport proteins and are particularly valuable when applied to membrane proteins, since so little high-resolution structural data exist. Such studies have proved useful in grouping proteins into evolutionarily related families, identifying conserved motifs of functional significance, etc. Proteins with six transmembrane segments are found in many transport families and seem to have evolved independently in several families; the preference for this configuration is still unknown. Saier will discuss repeat elements within permeases, the recognition of fused domains and variations in permeases with multidomains.

● Role of lipids

Lipids play crucial and often undervalued roles in transport of molecules across membranes. The phospholipid bilayer stabilizes the hydrophobic membrane-spanning α -helices from which most membrane proteins are constructed. Phospholipids may interact directly with the hydrophobic signal peptides of presecretory proteins. The internal and external layers of membrane bilayers may be asymmetric in their lipid composition and this may play a role in the recognition of sequences responsible for correct orientation of integral membrane proteins in membranes. For at least one MDR the membrane influences the substrate specificity of the MDR, by selection only of ligands that partition in the membrane. For example the mammalian P-glycoprotein works by a flippase mechanism (transporting molecules from the inner to the outer leaflet of the membrane by flipping them over). Lipids also play vital roles in vesicular transport of proteins between organelles.

● Recurrent themes

Cells use different signal peptides as sorting codes for delivery via different systems. Bacterial, chloroplast and yeast proteins using the Sec-dependent route have the 'classical' signal peptide, whereas signal peptides for the Δ pH-dependent route in chloroplasts and bacteria contain twin-Arg regions next to the hydrophobic core. These two systems appear to have evolved to transport unfolded and folded proteins, respectively. In some cases bipartite signal sequences are used, each to cross one of two successive membranes. The old concept of proteins always being translocated in the unfolded state is obviously not true. Sequence comparisons have proven useful in identifying motifs characteristic of particular proteins, e.g. MDR efflux translocases can be identified by sequence analysis. Chaperones are important in controlling the folding and unfolding of proteins. Proteins like SecB and Bip bind unfolded proteins to prevent unwanted hydrophobic interactions. Some chaperones, as in the Yop system, are specific to particular proteins to ensure delivery in good shape. *You'll never walk alone* could well be the theme tune of the transport world.

Space regrettably precludes further discussion of recurrent themes or of the many applications like antibiotic resistance, inherited disease, inhibitor design, etc., that stem from the fundamental science.

However, the organizers of the Symposium on *How Do Molecules Cross Microbial Membranes?* hope that this preview of the programme is sufficient to whet your appetite to attend the session at Leeds in September.

See you there and may you arrive safely!

● *Dr Bruce Ward helped to organize this symposium and can be contacted at Institute for Cell and Molecular Biology, University of Edinburgh Tel. 0131 650 5370; e-mail bward@srvo.bio.ed.ac.uk*

Other symposium organizers

● *Professor S. Baumberg
Department of Biology, University of Leeds*

● *Professor C.J. Stirling
School of Biological Sciences, University of Manchester*

● *Dr J.K. Broome-Smith
School of Biological Sciences, University of Sussex*

● *Dr P.M. Goodwin
The Wellcome Trust, London*

Further details of the meeting appear on p. 84 and a booking form is on p. 101. The symposium will be published as a book. A review and order form will appear in a future issue of Microbiology Today.