MicroMeeting

Getting out: protein traffic in prokaryotes

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Summary

Protein secretion systems in prokaryotes are increasingly shifting from being considered as experimental models for 'more complex' processes (i.e. eukaryotes) to being a major source of key biological questions in their own right. The pathways by which proteins move between compartments or insert into membranes in prokaryotic cells are certainly less numerous than in eukaryotes (though not dramatically so). However, the quality and complexity of bacterial protein targeting systems indicate that virtually all mechanistic problems associated with protein traffic were solved very efficiently well before eukaryotes appeared on the Earth crust. Indeed, recent studies have both increased the number of known prokaryotic protein traffic systems and indicated new layers of complexity for those that were already well characterized. This report describes some recent developments in bacterial protein traffic that were presented at two meetings in the autumn of 2003.

Introduction

This report highlights some of the more recent developments that were presented at two recent meetings: the European Science Foundation EURESCO meeting 'Protein Targeting' organized by Arnold Driessen in Spa (Belgium), and the Juan March Foundation Workshop 'Finding

the Way Out: Protein Traffic in Bacteria' organized by Victor de Lorenzo and Anthony Pugsley at the Juan March Foundation in Madrid (Spain). The EURESCO meeting covered protein traffic systems in eukaryotes as well as in bacteria, whereas the Juan March meeting was devoted exclusively to bacterial export and secretion systems, mostly in Gram-negative bacteria. The wealth of information on *Escherichia coli* and its close relatives reflects mainly the availability of more genetic tools for this bacterium and its long-standing use as a model organism. However, given the limited number of niches that *E. coli* inhabits, it is certain that much of the whole prokaryotic secretion landscape is still to be explored.

SecYEG

Components of the bacterial Sec translocase share many features with their counterparts in the archaeal and eukaryotic Sec translocases, although they differ in some mechanistic aspects. Overwhelming evidence shows that the core element of the Sec translocase, SecY protein (Sec61α in eukaryotes and archaea), together with two other integral membrane proteins, the bacterial proteins SecE (Sec61β) and SecG (a third subunit, Sec61γ, is also present in eukaryotes and archaea but it is not homologous to SecG), probably forms the channel through which proteins are translocated through the plasma (inner) membrane. Although studies of mutations in the *E. coli sec* genes and cross-linking experiments have provided much information on the relative positions of transmembrane α-helices and possible contacts with presecretory proteins, definitive information on the organization of the translocase requires structural information. The three-dimensional X-ray crystallographic analysis (at 3.2 Å resolution) of the Sec complex isolated from the archeaon *Methanococcus jannaschii* [presented at the Juan March Workshop by Ian Collinson (Max Planck Institute, Frankfurt, Germany) and at the EURESCO meeting by Bert van den Berg (Harvard Medical School, Boston, USA)] (Fig. 1) therefore represents a major step forward in understanding secretory protein translocation in prokaryotes and in eukaryotes.

The crystal structure of SecY reveals 10 transmembrane segments, as predicted previously from biochemical analyses and as observed in electron diffraction patterns of two-dimensional crystals of *E. coli* SecYEG (Breyton *et al.*,
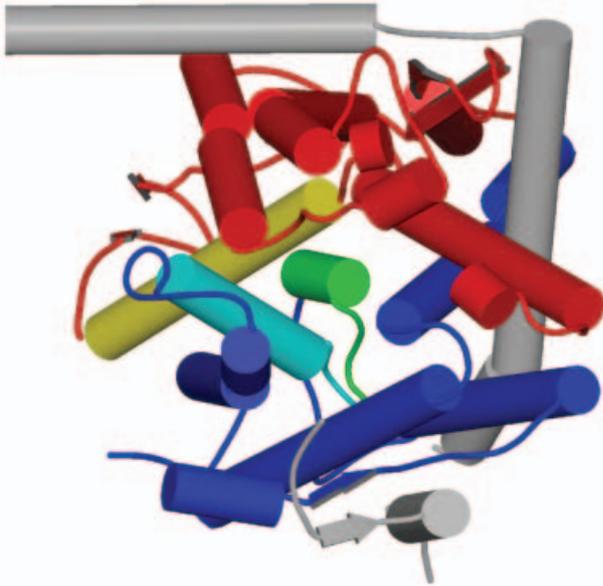
Fig. 1. Crystal structure of the Sec complex from *M. jannaschii*, looking through the channel in the centre of the Sec61α component from the upper (extracytoplasmic) side. The crossed transmembrane helices 2 and 7 of Sec 61α are indicated in cyan and yellow, respectively, the *N*- and *C*-terminal domains of the rest of this protein are coloured blue and red, respectively, and the plug loop is coloured green. Sec 61β and Sec61γ are both in grey. The authors are grateful to William Clemons for preparing this figure and to Tom Rapoport for permission to use it.

2002). The steeply angled Sec61β (SecE) transmembrane helix that forms a girdle around part of the Sec61α (SecY) barrel explains the stabilizing effect of SecE on SecY (Matsuyama *et al.*, 1990), and the position of the peripherally located Sec61γ (SecG) is also conserved in bacteria and archaea. The juxtaposition of Sec61β from adjacent complexes in the 2D crystal is also consistent with previous studies showing that adjacent SecE proteins can be cross-linked (Veenendaal *et al.*, 2001). The crossed organization of Sec61α TM segments 2 and 7, on the opposite side of the barrel from Sec61β could form a lateral opening, described by Ian Collinson as rather like a crab's claw, through which signal peptides insert laterally into the translocase and transmembrane segments of integral membrane proteins escape from the lumen of the channel into the lipids of the membrane. This observation is consistent with cross-linking studies showing that the signal sequence is sandwiched between TM2 and TM7.

Of particular interest is the presence of a continuous, albeit rather narrow, channel running through the centre of the Sec61α barrel. This channel is occluded by a 'plug' formed by the loop region between two transmembrane (TM) segments and has a 'seal' near its centre where several hydrophobic amino acids come together. The plug loop mentioned above could form a gate that controls access to the channel on the cytoplasmic side of the

membrane, and the 'sphincter' could fit snugly around the polypeptide chain of the presecretory protein to prevent ion movement though the channel during translocation. Some of the *prl* mutations (suppressors of signal peptide defects) map in the pore region around the 'plug' in SecY and, based on the evidence that *prl* suppression correlates with tighter SecA-translocase binding, these could be the residues that define the SecA biding site, although other interpretations are also possible. Dilation of the sphincter would create a channel large enough to accommodate a single polypeptide sequence with secondary structure, and possibly even slightly more bulky structures such as a small disulphide-bonded loop that can be translocated by *E. coli* Sec translocase (Tani *et al.*, 1990).

The proposal that this channel represents the pathway by which secretory proteins cross the membrane is very attractive but must be reconciled with previous data. Earlier electron microscopy studies of single particles of the *E. coli* translocase revealed what appeared to be stages in multimerization that were induced by recruitment of the SecA ATPase (see below) and presecretory protein (Manting *et al.*, 2000). The largest structure observed (probably containing four SecY monomers) was proposed to be the active complex, with a 5 nm central cavity between the four subunits being the channel for presecretory protein translocation. This model is attractive because the dimensions of such a channel would be similar to the experimentally determined internal diameter of the Sec61 channel in eukaryotes (Hamman *et al.*, 1997; Wirth *et al.*, 2003) and would be sufficient to accommodate segments of SecA protein that are proposed to insert into the channel (Economou *et al.*, 1995). However, it is also possible that SecA does not penetrate through the centre of the SecY channel but, instead, penetrates into the lipid bilayer (van Voorst *et al.*, 1998) to contact the translocating polypeptide chain (in the SecY channel) on both sides of the membrane. Finally, the size of largest SecYEG complex visualized by negative staining is similar to the presumed Sec61 complex observed underneath the ribosome in electron micrographs of the two complexes (Beckmann *et al.*, 2001).

On the other hand, electron diffraction analysis of *E. coli* SecYEG suggested that the complex forms a dimer, with a putative translocation channel located at the interface between the two monomers. This would be consistent with biochemical studies indicating that the dimeric form is the minimal functioning *E. coli* SecYEG complex (Bessonneau *et al.*, 2002). However, the crystal structure reveals that this 'channel' is really a depression in the surface contour of adjacent complexes and probably does not form a continuous conduit. Moreover, the outer surface of the SecY monomers appears to be uniformly hydrophobic, making it unlikely that two or four of them could come together to form a hydrophylic channel.

The successful resolution of this crystal structure is clearly a major event in the long-running history of studies on the Sec translocase. The data provide a huge amount of 'food for thought' and cause us to re-evaluate many of our previous ideas on how translocase works. Clearly the SecYEG the story is nowhere near the end but the structure provides a superb basis for further exploration, notably the creation of mutations affecting key residues involved in the positioning of the plug loop and the amino acids in the sphincter. Further crystallographic studies already under way, most notably those on the translocase in co-complex with SecA and stabilized by monoclonal antibodies, could provide a valuable snapshot of the translocase in action.

SecA

Related to the structure of the SecYEG complex is that of SecA, the ATPase that drives presecretory proteins though the translocation channel in batches of approximately 25 residues at a time (van der Wolk *et al.*, 1997). The structural organization of SecA was presented and discussed by John Hunt (Columbia University, New York, USA) at the EURESCO meeting and by Tassos Economou (FoRTH, Iraklion, Greece) at the Juan March Workshop. The crystal structures of several SecA proteins are now known at high resolution. These structures show that SecA is divided into several interacting domains and reveal several possibilities for dynamic interactions and substantial movements of different segments in the polypeptide. John Hunt emphasised the analogy to the well-characterized F_1F_OATPase, whereas Tassos Economou emphasised the analogy to 'DEAD' family RNA and DNA helicases. Overall sequence similarities with DEAD helicases are actually not very compelling, but key motifs are well-conserved, whereas the overall topology and the positioning of the nucleotide interaction site between two domains of the protein are also quite like those of the F_1F_OATPase. Indeed, all of these proteins belong to the same super family, along with proteins such as RecA that display a similar ATP binding fold, and mechanistic differences are unlikely to be particularly dramatic. Indeed, both John Hunt and Tassos Economou underscored the potential for considerable lateral movement upon nucleotide binding and, especially, hydrolysis that would lead to changes in relative positions or conformations of specific domains.

Molecular dissection of the different domains of SecA in the Economou lab [achieved by expressing, purifying and analysing distinct domains revealed by the crystal structure or by proteolysis (Sianidis *et al.*, 2001)] has revealed intricate details of internal controls over activation, ATP turnover and recognition of presecretory proteins. Surprisingly, regions of the protein that become

accessible to the periplasmic side of the membrane in the cycles of insertion and withdrawal that are proposed to occur during translocation are dispersed throughout the protein in the crystal structure, meaning that a vast surface of the large SecA monomer (>100 kDa) is exposed to agents on the *trans* side of the membrane.

YidC

Another interesting aspect of translocase function was presented at the two meetings by Martin van der Laan and Arnold Driessen (both from the University of Groningen, the Netherlands). Although not initially considered as a core component of the bacterial translocase, YidC, an essential protein in *E. coli* (Samuelson *et al.*, 2000), can be co-purified with it and can be cross-linked to transmembrane segments of Sec-dependent plasma membrane proteins. A YidC homologue, Oxa1, is involved in protein insertion into the inner membrane of mitochondria, which do not have a Sec-type apparatus. YidC seems to play a similar role to Oxa1, because it is required for the SecYEG-independent insertion of phage coat proteins, like the bacteriophage M13 procoat, into the *E. coli* plasma membrane. Is this the only function of YidC? Surely not, because YidC is an essential protein and must therefore play a role in at least one essential membrane-related function in *E. coli*.

According to one attractive hypothesis, YidC acts in conjunction with the SecYEG translocase to facilitate the lateral release of hydrophobic, transmembrane segments of plasma membrane proteins during translocation (see the SecYEG crab's claw model discussed above). In this model, YidC would be essential because, in its absence, clearance of the SecYEG channel would be slowed down and precursors of presecretory proteins (presumably using the same translocation machinery as plasma membrane proteins) would accumulate. If this model is correct, then depletion of YidC *in vivo* and *in vitro* should decrease the rate of plasma membrane protein integration, whereas increasing YidC levels should have the opposite effect. As reported by van der Laan and Driessen, their studies with the model plasma membrane protein FtsQ revealed that both increasing **and** decreasing YidC levels impede the insertion of FtsQ into the membrane.

What, then, is the physiological function of YidC and why is it essential? van der Laan and co-workers observed that a ∼ 25 kDa plasma membrane protein identified as the phage shock response protein PspA is drastically overproduced when YidC levels are depleted (van der Laan *et al.*, 2003). PspA levels are known to increase when the proton motive force is reduced [for example, as a result of proton leakage across the plasma membrane (Kleerebezem *et al.*, 1996)]. van der Laan and Driessen ruled out a role for YidC in sealing the translocation chan-

nel against proton leakage and showed, instead, that YidC is actually required for the insertion of certain subunits of the F_1F_OATPase and cytochrome o oxidase into the plasma membrane. In the absence of YidC, the levels of these protein complexes in the membrane are insufficient to maintain the normal proton motive force (pmf) and PspA production is increased. Hence the cells lose viability when YidC levels are reduced because a full pmf cannot be established, with all other effects of YidC depletion probably being an indirect consequence of this effect on the pmf. The role of YidC was clearly demonstrated by showing that purified YidC reconstituted into proteoliposomes in the absence of translocase is sufficient to promote cotranslational insertion of F_Oc subunit into the membrane. These observations do not rule out a role for YidC in translocation channel clearance but they do indicate that such a role is unlikely to explain the effects of its depletion on viability.

Where are SecYEG translocase complexes located?

Before moving on to other topics discussed at these two meetings, we would like to mention another intriguing aspect of Sec translocase function. Recent studies are beginning to show that certain proteins are located at specific sites in bacterial cell envelope. For example, at the Juan March Workshop, Pascale Cossart (Institut Pasteur, Paris, France) reported that the pathogenic bacillus *Listeria monocytogenes* has several cell-surface, infection-related proteins that seem to locate specifically to one of its poles. However, the mechanisms by which such selective localization is achieved remain mysterious. One possibility is that these proteins are selectively targeted to specific Sec translocases that are positioned to ensure correct localization of the proteins they translocate. Recent studies in *E. coli* suggest that the translocase in this bacterium is evenly distributed throughout the plasma membrane (Brandon *et al.*, 2003). However, Jan Jongbloed (University of Groningen, the Netherlands) revealed a rather different story at the EURESCO meeting. He showed that fluorescent protein-tagged, fully functional derivatives of Sec components form discrete clusters throughout the plasma membrane of exponentially growing *Bacillus subtilis* (Fig. 2). These clusters, which presumably represent several translocases linked together, became more diffuse as the culture aged or when protein synthesis was arrested by adding chloramphenicol. Furthermore, antibodies to amylase Q, a highly overproduced protein, also exhibited a patchy labelling pattern similar to that obtained with SecA and SecY, presumably because it accumulates near sites of protein export. Possible explanations for this phenomenon include translocase interactions with murein (Young, 2003) or actin cytoskeletons (Carballido-Lopez and Errington, 2003) or with specific
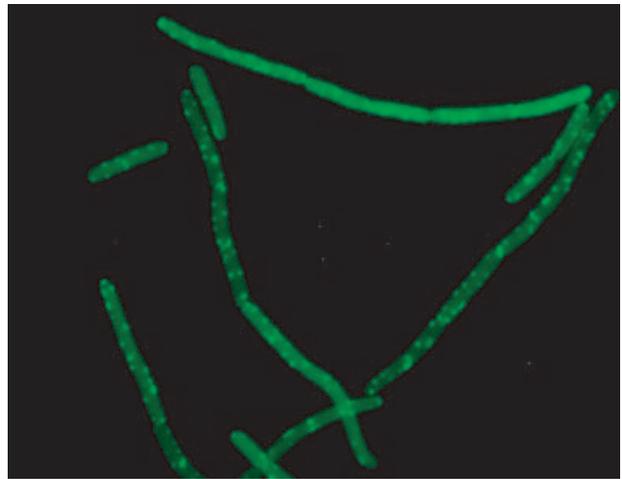

Fig. 2. Subcellular localization of SecA-GFP in *B. subtilis*. Fluorescence microscopy of *B. subtilis* cells expressing SecA-GFP, grown in rich medium until mid-exponential growth phase. (N. Campo, H. Tjalsma, G. Buist, D. Stepniak, *et al*. in prep.). The authors are grateful to Jan Jongbloed for supplying this illustration.

lipid domains (Vanounou *et al.*, 2003). Each translocase cluster might have specific associated factors that allow it to select particular secretory proteins and target them to unique subcellular sites, such as the pole(s) or the mid cell.

Autotransporters

Among the different pathways by which extracellular proteins can cross the outer membrane in Gram-negative bacteria, the so-called autotransporter or autosecretion pathway is apparently the simplest. Initially discovered as the IgA protein secretion pathway in *Neisseria gonorrhoeae*, many Gram-negative bacteria are now known to use this pathway to secrete a variety of cell-surface and extracellular enzymes and adhesins. Autotransporters are composed of essentially three domains: a signal peptide, the extracellular domain and a β-barrel domain with features similar to those found in most outer membrane proteins (i.e. predicted antiparallel amphipathic β-strands linked by short periplasmic loops and longer extracellular loops).

In the original model for IgA protease secretion, the extracellular domain (passenger) did not play an active role in translocation (it can be replaced by other, unrelated protein sequences) and was presumed to be threaded through the centre of the barrel formed by the β-domain. Translocation was proposed to be an energy-favourable reaction caused by the folding of the passenger protein on the outer surface of the cell, consistent with the observation that folded polypeptides were inefficient passengers. Protein (antifolding) chaperones prevent the folding of the passenger domain to permit its translocation through the narrow channel of the β-translocator (upper
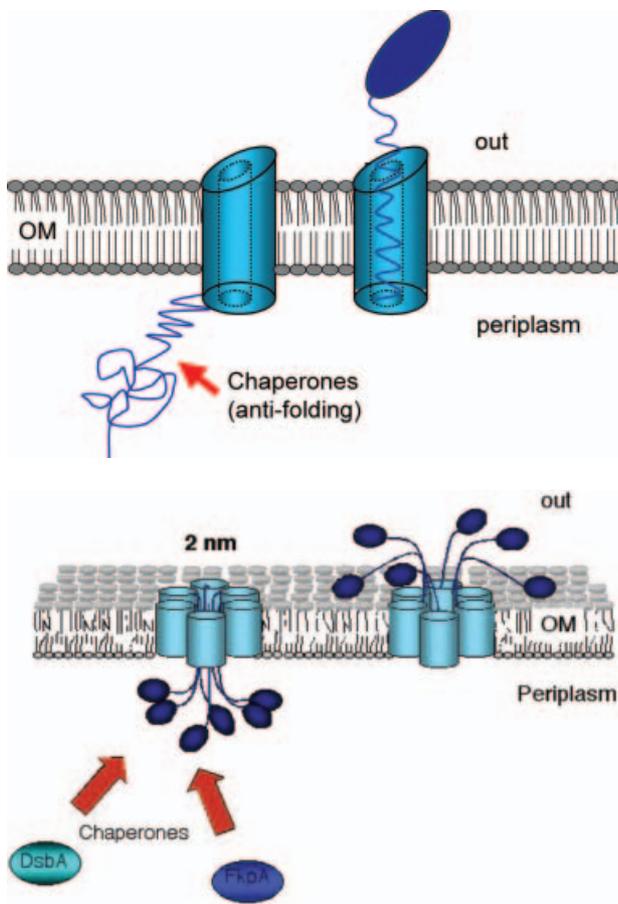
Fig. 3. Model for translocation of passenger proteins via a channel formed by a single β-barrel (upper panel) of multimers thereof (lower panel) from an 'autotransporter' protein such as IgA protease. In these models, periplasmic chaperons either prevent (upper panel) or assist (lower panel) the folding of passenger proteins before they are translocated across the outer membrane. The authors thank Luis Angel Fernández for permission to use his drawing in this figure.

panel of Fig. 3). As discussed at the Juan March Workshop by Luis Angel Fernández (CSIC, Madrid, Spain) this view was recently challenged when it was demonstrated that the IgA protease β-barrel could translocate globular proteins with considerable tertiary structure and an estimated diameter of about 2 nm (lower panel of Fig. 3). In this model, passengers are translocated irrespective of whether they have acquired tertiary structure through the action of periplasmic (folding) chaperones, provided the folded structure is not too large. Indeed, measurements of saccharide movement through the β-domain channel are consistent with this idea. Furthermore, electron microscopy of single particles of the IgA protease β-barrel revealed an oligomeric structure with an apparent central cavity that could correspond to this translocation channel (Veiga *et al.*, 2002). Moreover, Fernández presented evidence suggesting that passengers that cannot be translocated (because they are too bulky) actually interfere with

the secretion of passengers that can be translocated, which seems to confirm the multimeric nature of the translocation channel. However, it is difficult to see how translocation of folded proteins could be energetically favourable.

Jan Tommassen (University of Utrecht, the Netherlands) presented the eagerly awaited structure of an autotransporter β-barrel domain at the Juan March Workshop. He showed the crystal structure of the barrel domain of NalP, a *Neisseria meningitidis* cell surface protease (Van Ulsen *et al.*, 2003), crystallized from *E. coli* inclusion bodies upon urea denaturation and refolding. The barrel has 12 antiparallel β strands. The entire exterior surface of the barrel is hydrophobic, which seems to exclude the possibility that a water-filled channel could be formed by β-barrels in a ring-like structure such as that depicted in the lower panel of Fig. 3. Instead, the barrel has a central channel that, in the crystal structure, is occupied by an α–helix corresponding to the C-terminal end of the passenger domain. Does translocation occur via this channel, as initially proposed? The crystal structure can only give us a snapshot of autotransporter function but it is tempting to speculate that the fact that the helix is in the centre of the barrel indicates that this is its normal position. However, as the polypeptide that was crystallized was not derived from the native outer membrane protein, other possibilities cannot be excluded. Nevertheless, the internal diameter of the barrel (without the helix) is too small to accommodate a 2 nm diameter folded polypeptide.

How can we resolve these apparently conflicting data? Perhaps the word translocation is inappropriate in this particular case, because the evidence that passengers actually move through any form of channel is only circumstantial and presentation of passenger proteins on the cell surface might occur during insertion of the β-domain into the outer membrane. However, this idea does not explain all of the data obtained by Fernández and colleagues and other explanations must be sought. A provocative alternative model, proposed by Jan Tommassen, is that passenger domain translocation could require the Omp85 machinery that is involved in insertion of outer membrane proteins (Voulhoux *et al.*, 2003). This mechanism would then be analogous to the two-partner secretion, exemplified by the filamentous haemagglutinin secretion of *Bordetella pertussis*, presented at the Juan March Workshop by Françoise Jacob-Dubuisson (Institut Pasteur, Lille, France). The outer membrane component of this secretion machine shares homology with Omp85 (Jacob-Dubuisson *et al.*, 2001). In fact, the term 'autotransporters' for this type of secretion could quite easily be inappropriate.

TAT translocation

A similar logistics problem is faced by the TAT translocase,

the major alternative to the Sec pathway that is used mainly, though not exclusively, for exporting metalloproteins that acquire their prosthetic groups in the cytoplasm. These proteins, which are of different sizes, probably achieve their final conformation before they are translocated, which means that the channel though which they are transported must have a large and flexible internal diameter and must be tightly regulated to prevent simultaneous proton influx. Tracy Palmer (John Innes Institute, Norwich, UK) and Kirstin Model (Max Planck Institute, Frankfurt, Germany) at the EURESCO meeting and Ben Berks (University of Oxford, UK) at the Juan March Workshop presented evidence that the TAT complex (composed of TatA, TatB and TatC subunits) undergoes complex subunit recruitment and exchange reactions when it becomes activated for translocation. Unfortunately, the analysis by electron microscopy of negatively stained particles corresponding to the different states of the TAT translocase does not yet reveal sufficient information on which to speculate about how these changes induce the formation of the translocation channel. However, biochemical analyses in bacteria (Matthias Müller, University of Freiburg, Germany) and in the homologous chloroplast system (Ken Cline, University of Florida, Gainesville, USA), presented at the EURESCO meeting, are beginning to reveal information on which TAT components recognize substrate precursors and what triggers the different transitions.

Tracy Palmer presented another of the many other interesting aspects of the TAT system at the Juan March Workshop. She reported fascinating evidence, obtained by her colleague Frank Sargent, that chaperones exert a 'quality control' function on proteins that are exported by the TAT pathway in order to avoid export of incorrectly folded or unassembled substrates. The specific example she cited was that of an *E. coli* TAT substrate composed of one protein with a TAT signal peptide (HybO) and one that lacks its own targeting signal (HybC) and must therefore ride 'piggy back' with its partner protein. Palmer presented evidence obtained using a two-hybrid system and chimeras that a specific chaperone (HybE) probably recognizes the *N*-terminal region of the HybO signal peptide. A TorA-HybO chimera (in which the signal peptide is derived from the TAT substrate TorA) would still co-export HybC but only when the TorA-specific chaperone (TorD) was present in large amounts. In one of the models that Palmer presented, the signal peptide of HybO is masked by the chaperone until such time as the full tripartite complex has been formed. Once this happens, the signal peptide is unmasked and interacts with its receptor in the TAT complex.

Needles, rulers and earmarks

The type III secretion system (T3S) of Gram-negative bacteria is closely related to and probably evolved from the flagellum biogenesis machinery present in both Gram-negative and Gram-positive bacteria. One of the most fascinating features of the T3S is its ability to inject proteins into target cells, from which this particular nanomachine derives the name, injectisome. The business end of the injectisome is a needle-like structure of very uniform length that, in some cases (like the T3S nanomachines of plant pathogens or enteropathogenic *E. coli*), is extended by a much longer pilus. Both the needle and the pilus have a central channel through which secreted proteins are presumed to travel in an unfolded configuration. Indeed Sheng Yang He (Michigan State University, East Lansing, USA) presented evidence at the Juan March Workshop that proteins actually do transit via the inside of the pilus of the T3S of plant pathogens (Jin and He, 2001). Interestingly, this had been largely assumed in view of the structure of the needle/pilus and the close similarities between the T3S and flagellum biogenesis and yet it had never been shown before.

Also at this meeting, Guy Cornelis (Biozentrum Basel, Switzerland) addressed the question of how needle length is controlled in the TTS of *Yersinia enterocolitica*. The needles of this TTS are difficult to measure on the cell surface by electron microscopy but they break off very easily and broken needles are easy to measure. Surprisingly, they are of uniform length (approx 60 nm), suggesting that they always break at the same site (Fig. 4). Because the cell-associated needles are also about 60 nm long, the fracture point must be close to the cell surface. These needles are composed almost exclusively of a single protein, YscF, but another protein, the 515 residue-long, proline-rich YscP protein, was found in clus-
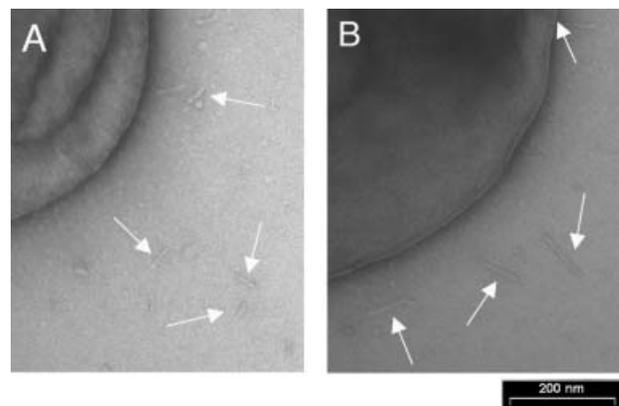

Fig. 4. The molecular ruler YspP controls needle length in *Y. enterocolitica*.
A. The effect of replacing wild-type YscP (needle length 58 ± 10 nm) by $YscP_{\Delta222-306}$ (439 residues), causing the needle length to fall to 43 ± 8 nm.
B. The opposite effect (lengthening of needle to 88 ± 12 nm) following introduction of $YscP_{\Omega222-381}$ (680 residues). The authors thank Laure Journet and Guy Cornlis for permission to use this figure.

ters at discrete sites on the cell surface that could be the sites where needles are produced. Deletion of this protein (or its homologue) from the T3S systems of *Y. enterocolitica* and other bacteria (Tamano *et al.*, 2002) abolishes needle length control. YscP of *Y. enterocolitica* has an internal repeat region that is not present in YscP from two related bacteria, *Y. pestis* and *Y. pseudotuberculosis.* When the shorter *Y. pestis* YscP is used to replace the endogenous protein, the *Y. enterocolitica* needles that were produced were correspondingly shorter, suggesting that the length of YscP determines the length of the needle. Cornelis proposed that the species-specific needle length might correlate with the depth of the cell surface lipopolysaccharide layer, which the needles must bridge in order to contact their target cells.

The concept of YscP as a molecular ruler was confirmed by artificially shortening or lengthening YscP, which caused a corresponding decrease or increase in the length of the needle (an example is shown in Fig. 4) (Journet *et al.*, 2003). How does this molecular ruler mechanism work? As discussed by Cornelis, YscP could then insert inside the needle as it grows, while remaining anchored at its base. Needle elongation (which probably occurs as subunits are translocated through the needle and assembled at the tip) would be arrested when the YscP ruler is fully extended. At this stage, the domain of YscP that is anchored at the base would signal the secretion apparatus to switch substrate, from needle subunits to effectors (the YOPS). YscP is actually secreted along with the YOPS when secretion is activated by calcium-depletion. In non-activated cells, however, the protein copurifies with needles, lending support to this novel idea. Its release into the medium when secretion is activated could be explained by the need to increase the space within the needle to allow secreted proteins to move more freely.

Despite all these new details on the structure of the T3S needle of *Yersinia enterocolitica* (which might apply to many other pathogens), a pivotal aspect of this type of mechanism (i.e. how otherwise cytoplasmic proteins are selectively loaded into this particular injection device) remains fuzzy. At the Juan March Meeting, Alan Collmer presented an exhaustive computational study on structural motifs present in a large number of T3S-secreted proteins of *Pseudomonas syringae pv. tomato* (a pathogen of tomato and *Arabidopsis*). The outcome of this analysis is quite perplexing, as sequences that earmark proteins for secretion by this T3S are not as clear-cut as, for example typical Sec or TAT signal peptides or C-terminal secretion signals of polypeptides secreted by the type 1 secretion system. Perhaps the signal recognized by the T3S is a constellation of protein folds rather than one or more specific sequences. One might even entertain the hypothesis that some structural motifs of the mRNAs

encoding T3S-secreted proteins cause the preferential location of the translating ribosome at the base of the needle (Ramamurthi and Schneewind, 2003). All of these possibilities deserve further analysis, as they open the way to enticing applications, e.g. injecting proteins 'à la carte' into selected target cells.

Pili and adhesins

Probably the best characterized pilus biogenesis pathway is the chaperone/usher pathway for type 1 and P pili in *E. coli*. Scott Hultgren (Washington University, St Louis, USA) presented the recent crystallographic and biochemical data supporting the donor strand exchange model of pilus assembly (Sauer *et al.*, 2002) and demonstrating that FimH, the tip adhesin of type 1 pili, initiates pilus assembly as the first subunit to bind the outer membrane usher FimD.

Scott Hultgren also described an interesting mouse model of *E. coli* bladder infection, where the FimH adhesin is both necessary and sufficient to promote invasion of the bladder epithelium. Interestingly, once inside the epithelial umbrella cells, bacteria multiply and form intracellular communities that are embedded in an extracellular fibrous matrix and are highly resistant to antimicrobial agents and neutrophils. While the invasion-induced inflamatory cascade causes rapid exfoliation and destruction of bladder epithelium, the bacteria undergo cycles of multiplication inside these biofilm-like structures, termed pods, followed by rapid dispersal to invade new cells. The second generation of bacteria emerge from a pod differentiated into a filamentous form that also appears to be resistant to neutrophils. This first example of an intracellular biofilm could account for the recurrence of UPEC caused urinary tract infections and their resistance to treatment (Anderson *et al.*, 2003). In addition, it represents and unusual and hitherto unreported aspect of the ability of *E. coli* to change shape according to prevailing circumstances, although the underlying molecular mechanisms of this unique developmental switch remain to be explored.

Concluding remarks

Unfortunately, space limitations do not allow us to discuss more than a few of the excellent presentations at the two meetings covered in this MicroMeeting Report. However, a developing trend that was obvious in many of the presentations is the increasing impact of structural information. As much as 30% of the genome codes for proteins that associate with, integrate into or are translocated across membranes. Very few such proteins, and particularly those in the membrane, have been successfully analysed at the structural level. More and more high-resolution structures of membrane proteins are being solved, how-

ever, revealing new insights into how they might function and, at the same time, providing a firm basis for future biochemical and molecular biology experiments. Nevertheless, one must be cautious not to rely too heavily only on structures for understanding secretion mechanisms. Structures require the mass production of protein, which might alter their native properties, and proteins in crystals are highly organized, static entities that might not be good representations of their structure during the highly dynamic processes that they carry out. It is thus essential to continue to combine structural information with both genetic approaches and novel non-disruptive procedures to monitor protein–protein interactions *in vivo.*

The growing interest in understanding how secretion works is not altogether devoid of practical implications, which go beyond the use of protein export systems in cell factories for secreting high-added value polypeptides into the external culture medium. They can also be instrumental for delivering active proteins to the niches where such microorganisms naturally reside (the animal gut, the skin, polluted soil, the rhizosphere), for protection against pathogens, for remediation of chemical contamination or for biological control of pests. Finally, some secreted proteins are essential to the virulence of many bacterial pathogens. These features open up new possibilities of screening novel drugs and compounds specifically able to inhibit given secretory mechanisms. Whether basic or applicable, all of these challenges in protein secretion will keep the secretion community in full activity for many years to come.

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