Bacterial protein secretion and trafficking down under

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In July 2008, Australian microbiologists braved wintry Melbourne weather to attend the Annual Scientific Meeting of the Australian Society for Microbiology. The meeting attracted more than 1100 delegates from around the Asia-Pacific region and offered over 400 scientific presentations covering the latest research findings from leading local and international research and clinical laboratories. The theme of the meeting, 'Changing Times', highlighted the immense challenges ahead for the microbiology community in responding quickly and appropriately to often unpredictable environmental, political, public health and regulatory changes in the face of microbes that are changing just as rapidly. Keynote speakers addressed many of these issues within their presentations including Nobel Laureate, Peter Doherty (Department of Microbiology & Immunology, University of Melbourne, Australia) who delivered the opening address. Mike Levine (Center for Vaccine Development, Baltimore, USA) gave the Commonwealth Serum Laboratory-sponsored Bazeley Oration and used his invaluable clinical insight to discuss the history and future development of typhoid vaccines as well as the use of *Salmonella* as a vaccine carrier, and the construction and testing of new-generation *Salmonella* vaccines. Alan Cowman (Walter and Eliza Hall Institute, Australia) gave the prestigious Rubbo Oration and presented a stunning overview of malaria biology as well

as recent progress on mechanisms to control this devastating infectious disease. The meeting also hosted a special 'Infection Control Day', which comprised a series of workshops and presentations designed to draw attention to the problems of antibiotic resistance and the measures needed to reduce hospital-acquired infections and their transmission.

In the area of microbial and molecular pathogenesis, a major topic of the meeting was the secretion of bacterial proteins and their trafficking within infected cells. There are now at least six recognized bacterial systems (Type I–VI) dedicated to the export of bacterial proteins that often play a direct role in the interaction of pathogens with their hosts. The study of these secretion systems and their substrates is therefore an essential part of understanding how a pathogen causes disease. In some cases, the export of a protein leads to the construction of zones within the bacterial membranes that serve as an adhesive organelle to contact host surfaces. In other cases, the secreted factors are translocated directly into a eukaryotic cell, where they interfere with host cell function. In this instance, a bacterial protein may need to cross two bacterial membranes and one or more mammalian cell membranes to reach a target site. The substrate specificity of secretion systems is remarkable and a single secretion system may be dedicated to a single substrate, only a few substrates or,

in the case of *Legionella*, more than 150 proteins. Current work on the function of bacterial secretion systems and their substrates at the molecular and structural level is driven by the expectation of greater insight into how pathogens evolve and cause disease.

Getting out & about: protein secretion & bacterial membranes

Tony Pugsley (Institut Pasteur, France) presented the latest advances in understanding how Type II secretion systems are assembled. In *Klebsiella oxytoca*, the function of the secretory apparatus depends on the assembly of a dodecamer of the protein PulD in the bacterial outer membrane. A specific chaperone, PulS, carries targeting information that directs the PulD protein subunits to assemble only in the outer bacterial membrane; in the absence of the PulS chaperone, PulD assembles into dodecamers equally well in the outer or inner membrane. Multimerization and membrane insertion of PulD appear independent of the membrane environment or any membrane-associated factors. This apparently spontaneous multimerization and insertion is most likely restricted to outer membrane proteins closely related to PulD. Given that PulD-related structures are at the heart of both Type II secretion systems and capsule translocation machinery, ongoing work to understand, in structural detail, the assembly of these ring complexes is highly exciting and will be biomedically important.

While PulD-related proteins are assembled in outer membranes without assistance, a protein called Omp85 is required to drive the assembly of β -barrel proteins – the most common structural class of proteins in bacterial outer membranes. Trevor Lithgow (University of Melbourne, Australia) presented work identifying Omp85 proteins from

all classes of bacteria with outer membranes. In *Escherichia coli* and *Neisseria meningitidis*, Omp85 sits as a core subunit of the so-called 'bacterial membrane assembly machinery (BAM) complex', which also has several lipoprotein subunits. It is now clear that this is also true in α -proteobacteria. Given that bacteria of the α -proteobacterial lineage are the ancestors of mitochondria, it should perhaps not have been a surprise that our own mitochondria have a 'sorting and assembly machinery (SAM) complex' with homology to the bacterial BAM complex. Some effector proteins, such as PorB from *N. meningitidis*, are able to be assembled in bacterial outer membranes by the BAM complex to form a nutrient pore, and in mitochondrial outer membranes by the SAM complex to regulate host cell apoptosis.

Getting to the right place: targeting Golgi function

In a symposium on the cell biology of translocated virulence effector proteins, the Golgi emerged as an unexpected target of several bacterial pathogens. Nat Brown (Griffith University, Australia) presented evidence that a new Type III secretion system effector of *Salmonella* (SseK3) was targeted to the Golgi. A key question under study now is how SseK3 and several other Golgi-associated virulence factors are targeted within host cells. Elizabeth Hartland (University of Melbourne) showed that EspI, another Type III secretion system effector from attaching and effacing *E. coli*, has a C-terminal sequence that docks to PDZ domain proteins. If this seven-amino acid motif is removed, EspI no longer localizes to the Golgi of host cells. Whether Golgi localization is necessary and sufficient for EspI function is less clear, given that the deletion of this motif has only a modest effect on virulence and intestinal colonization in a mouse model.

Craig Roy (Yale University, USA) presented work on a family of effector proteins secreted by Type IV secretion systems of *Legionella pneumophila* and *Coxiella burnetii*. This family of virulence factors contain a domain of

ankyrin repeats, a protein interaction domain characteristic of eukaryotes. Ectopic expression of one of these 'Ank proteins', AnkX, results in fragmentation of the Golgi: the protein acts on microtubule-dependent vesicle transport in the cell and, ultimately, contributes to the control *L. pneumophila* exerts on membrane trafficking to avoid fusion of its intracellular compartment with lysosomes. An astonishing feature of the Type IV secretion system of *L. pneumophila* is the number of substrates it can recognize and secrete – at least 150 are known but some estimates predict approximately 250 proteins may be translocated from the pathogen into infected cells by the Type IV secretion system. This virulence 'pipe' allows the bacteria to regulate a range of processes, including bacterial entry and egress, membrane trafficking and host gene expression.

A pocketful of proteases: proteolysis & pathogenesis

Bacterial pathogens often secrete proteases to modify host tissues during infection and colonization. *Dichelobacter nodosus* secretes several subtilisin serine proteases to generate the extensive tissue destruction characteristic of footrot in sheep. Julian Rood (Monash University, Australia) explained that while distinct protease activities have been correlated with virulent and benign strains of *D. nodosus*, sheep trials using chimeric strains in which proteases from benign and virulent strains were exchanged did not support the simple virulent/benign distinction. The detailed mechanism by which these proteases enhance disease progression requires a re-evaluation. The dental pathogen *Porphyromonas gingivalis* is also an abundant protease producer. Eric Reynolds (University of Melbourne) presented work to show that the trypsin-like proteases of *P. gingivalis* target inflammatory cytokines for destruction. This sets up a gradient of cytokine concentration around the site of infection in the dental pocket between the tooth root and the mucosa that prevents effective recruitment of immune cells, resulting in chronic inflammation and infection.

Getting around: bacterial motility
Exciting new work was presented on organelles that facilitate bacterial motility. Cynthia Whitchurch (University of Technology, Sydney, Australia) has captured video footage of *Pseudomonas aeruginosa* crawling on surfaces through twitching motility that depends on Type IV pili. By tracking the movements of individual bacteria, an invisible network of paths became evident along which the pathogens move and retreat. Accumulating evidence suggests that these paths consist of DNA that has been secreted by the bacteria; live imaging showed 'explosions' of DNA release as the bacterial population moves out across a surface. The use of extracellular DNA to colonize a surface is likely to have a complex genetic basis.

Another spectacular mode of bacterial movement is seen with pathogens such as *Shigella flexneri* after entry into host cell cytoplasm. Renato Morona (University of Adelaide, Australia) explained that *S. flexneri* secretes the protein IcsA at one pole of the bacterium and this surface protein binds to the host protein N-WASP. This, in turn, nucleates actin polymerization to generate an 'actin tail' to propel the bacterium through the cytoplasm of an infected cell. Work presented at the meeting showed that specific characteristics of the lipopolysaccharide surface of *S. flexneri* strains affects binding to N-WASP. This suggests that there is more to the IcsA–N-WASP molecular interaction than previously thought. Ongoing research further suggests a novel N-WASP binding domain as well as a domain needed for polar targeting of IcsA.

Future perspective

Protein targeting is a central theme in understanding diverse host–pathogen interactions. The translocation of proteins into or across membranes provides surface-located proteins for attachment to host cell epithelia and allows for a number of pathogenic processes, including: the cloaking of pathogens from immune surveillance; toxin secretion to disrupt homeostatic

processes; and pathogen survival in otherwise hostile environments within host cells. Recent and current work using both prokaryotic and eukaryotic model systems has already provided a glimpse of:

- How specific substrate proteins are selected for secretion
- The mechanism by which each type of bacterial secretion system operates to translocate protein substrates
- How secreted 'effector' proteins enter into host cells
- How bacterial proteins subvert host cell biology to enhance persistence, replication and transmission of the pathogens

Given the rapid progress over the past few years, a full understanding of this area of host–pathogen interplay is on the cards.

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