CBCB Symposium 2016: Abstracts

Heath Murray

A new bacterial replication origin element specifies single-strand initiator binding

DNA replication is tightly controlled to ensure accurate inheritance of genetic information. In all organisms initiator proteins possessing AAA+ (ATPases associated with various cellular activities) domains bind replication origins to license new rounds of DNA synthesis1. In bacteria the master initiator protein, DnaA, is highly conserved and has two crucial DNA binding activities2. DnaA monomers recognise the replication origin (oriC) by binding double-stranded DNA sequences (DnaA-boxes); subsequently, DnaA filaments assemble and promote duplex unwinding by engaging and stretching a single DNA strand3-5. While the specificity for duplex DnaA-boxes by DnaA has been appreciated for over thirty years, the sequence specificity for single-strand DNA binding remained unknown. Here we identify a new indispensable bacterial replication origin element composed of a repeating 3mer motif that we term the DnaA-trio. We show that the function of the DnaA-trio is to stabilise DnaA filaments on a single DNA strand, thus providing essential precision to this binding mechanism. Bioinformatic analysis detects DnaA-trios in replication origins throughout the bacterial kingdom, indicating that this element comprises part of the core oriC structure. The discovery and characterisation of the novel DnaA-trio extends our fundamental understanding of bacterial DNA replication initiation, and because of the conserved structure of AAA+ initiator proteins these findings raise the possibility of specific recognition motifs within replication origins of higher organisms.

Katharina Peters

The redundancy of peptidoglycan carboxypeptidases ensures robust cell shape main-tenance in Escherichia coli

Peptidoglycan (PG) is an essential structural component of the bacterial cell wall and important to maintain the integrity and shape of the cell by forming a continuous layer around the cytoplasmic membrane. The thin PG layer of Escherichia coli resides in the periplasm, a unique compartment whose composition and pH can vary depending on the cell's environment. Hence, the growth of the PG layer must be sufficiently robust to allow cell growth at different conditions. We have analysed the PG composition of 28 mutants lacking multiple PG enzymes (Penicillin-binding proteins, PBPs) after growth in acidic or standard (near neutral) medium. Statistical analysis of the obtained muropeptide profiles identified DD-carboxypeptidases (DD-CPases, i.e. PG hydrolases trimming pentapeptides to tetrapeptides) that were more active in cells growing at acidic pH. In particular, the absence of a previously considered minor DD-CPase, PBP6b, caused a significant increase in the pentapeptide content in PG and morphological defects when cells grew at acidic pH. Other DD-CPases (PBP4, PBP4b, PBP5, PBP6, PBP7, AmpH) and the PG synthase PBP1B had a smaller or no contribution to the pentapeptide-trimming activity at acidic pH. We solved the crystal structure of PBP6b and show that the enzyme is more stable and binds its substrate stronger at acidic pH, explaining why PBP6b is more active. Hence, PBP6b is a specialist DD-CPase that contributes to cell shape maintenance at low pH. E. coli may utilize seemingly redundant DD-CPases to be able to grow with normal shape at a range of different growth conditions.

Richard Daniel

Crossing the wall

The bacterial cell wall provides an important structural component of the cell that protects the cell from its environment, but its presence restricts aspects of cell growth. Thus determining the properties of the the wall and how it can be "breached" without resulting in osmotic instability should provide new insights into cell wall metabolism and other essential processes.

Paula Salgado

Clostridium difficile S-layer: insights at molecular level

Clostridium difficile expresses a surface layer (S-layer) which coats the surface of the bacterium and is proposed to facilitate interaction of the bacterium with host enteric cells, as well as mediate the host's immune response. In C.difficile, the S-layer is composed of two SLPs derived from post-translational cleavage of a single precursor, SlpA. The mature S-layer proteins (SLPs: HMW, ~40kDa; LMW, ~35kDa) form a tightly associated non-covalent complex that, together with other cell wall proteins, assemble to form the S-layer. Recently, we have obtained diffracting crystals of the whole LMW/HMW complex from different C. difficile strains and determined the first S-layer envelope by electron diffraction. We have also made considerable progress in understanding S-layer biogenesis by determining the structure of SecA2, the accessory secretion system responsible of secretion of SlpA and other cell wall proteins. Together, the results of our structural efforts contribute to the understanding of the assembly and structure of the mature S-layer in C. difficile.

Lauren Drage

Integrating Clinical and Basic Research to understand Asymptomatic Bacteruria host-pathogen interactions

Urinary tract infections (UTIs) account for up to 3% of GP visits in UK. In people of 60 years of age and over it is estimated that up to 20% are susceptible to asymptomatic bacteriuria (ABU). ABU is defined as bacterial colonisation of the bladder without any UTI symptoms. ABU patients, however, frequently present episodic symptoms of acute UTI, complicating their management. The mechanisms of the transition from asymptomatic to symptomatic are poorly understood. Integrating clinical and basic research approaches underpins our objective to further our appreciation of ABU, develop better diagnostic tools and consider improved point-of-care management for ABU patients. We have conducted a clinical study of 30 ABU patients followed for 6 months providing us with urine every two weeks. Our primary aim was to ask: how do patients react to ABU? The analysis of clinical data suggests the urinary tract of patients that carry uropathogenic Escherichia coli (UPEC) asymptomatically react more dynamically to this state than was expected. It is clear that despite strong activation of immune biomarkers, UPEC is able to evade host-defences and thrive in the bladder long-term. The innate defence system of the host uroepithelium is a front line defence strategy against pathogens like UPEC. Using clinical UPEC isolates we find that not all UPEC strains induced the innate immune system. This has led to the question: how does E. coli modulate innate recognition? We find that UPEC exploits the population heterogeneity of innate immune recognition factors to evade innate immune recognition. Our data suggests UPEC in the bladder can maintain a selective advantage by expressing specific colonisation factors in a heterogeneous manner; at a level below a threshold that is recognised by the innate immune response.

Ling Juan Wu

Microfluidic microscopy of cell wall-less Bacillus subtilis – an update

Bacterial cell wall is an essential component of most bacteria. It requires the action of a large number of proteins to synthesis and to maintain its integrity. Mutants that are able to propagate in a wall-less state, called L-forms, grow and divide in a manner that is very different from the walled cells, making the genes involved in cell wall synthesis and cell division, many of them essential for walled cell viability, dispensable. L-form cells are amorphic but are able to adapt to a given shape. We have used Bacillus subtilis L-forms and microfluidic microscopy to study the roles of cell wall and cell geometry on chromosome segregation, and on the localisation of proteins important for cell division and shape determination. We found that wall-less B. subtilis can efficiently grow, segregate their chromosomes, and properly assemble important machineries important for walled cells.

Jeremy Lakey

Why most diagrams of the Gram negative bacterial outer membrane are wrong

Most textbook representations of the gram-negative bacterial outer membrane show a modified Singer-Nicholson fluid mosaic model with outer membrane proteins floating in a bilayer. The better ones show the outer membrane lipids to be asymmetric with lipopolysaccharide on the outside and phospholipids making up the inner leaflet. Just as we no longer accept the fluid mosaic as a complete picture for cytoplasmic membranes, we should apply the discoveries of the last 30 years to update our image of the OM. The proteins are often arranged in highly ordered areas and this results from an interaction with LPS that is much more intimate than previously thought. Furthermore LPS binding is calcium dependent and essential for OmpF folding in the outer membrane. To further investigate the dynamics of the OM we have developed a fully asymmetric model which can be studied using neutron scattering and initial results from this work will be presented. Furthermore Bert Van Den Berg's group at Newcastle have recently solved the structure of trimeric OmpE36, from E. cloacae, with bound LPS which reveals how the tight barrier of the OM is maintained when proteins are inserted.