Stochasticity and organisation in microbes: challenges from imaging

Wednesday 7th July

Titles & Abstracts:

Marek Cyrklaff (Heidelberg) - Cryo-electron tomography of whole cells

Electron tomography is a method for three-dimensional structure characterization and is highly valued in studies on molecular assemblies that lack evident symmetry, are pleiomorphic or too large for standard averaging approaches. For that we preserve biological samples in an intact state by rapid freezing and the resulting tomographic maps closely reproduce the life conformation of the object under study. We applied the cryo-electron tomography (cryo-ET) to gain 3D views into whole, intact cells that were grown (or deposited) on the EM grids. Using the whole cell approach we’ve recently focused on studying different pathogens, often in the stages of dynamic interactions with host cells.

I will focus my presentation on the morphology of Plasmodium, the causative agents of malaria. In the sporozoites, Plasmodium extracellular forms, we were able to characterize novel morphological features of cell organelles and cytoskeleton. However, although our study originally aimed on revealing the cytoskeletal structures involved in sporozoite movement, the actin and myosin, we are not able to detect them in the crowded tomograms of intact cytoplasm. In silico modelling allowed us to determine the detection limit of small molecules and short actin fibres. We investigate the ways toward improving resolution and feature detection in the tomograms. Nevertheless, in another approach we scoped into the cytoplasm of the erythrocytes invaded with Plasmodium (thus on the intracellular stages of malaria parasites) and detected extended actin networks in the cytoplasm of host cells. A more detailed characterization allowed us to conclude that the actin networks are regulated by the parasite within the host cell, and are largely responsible for export of the parasite encoded proteins onto the surface of invaded cell. This causes erythrocytes aggregation in blood capillaries often with lethal outcomes.

Judith Armitage (Oxford) - Bacterial swimming behaviour and insights into protein dynamics

The bacterial flagella motor is composed of multiple copies of about 20 different proteins arranged in rings spanning from the cytoplasm through the cytoplasmic membrane to the outer membrane. Synthesis is highly regulated with the transmembrane rotor attached to an extracellular semi-rigid flagellar helix and rotated at about 100Hz by the movement of ions through a ring of peptidogylcan associated stator proteins. Cryo-EM studies gave the impression of a stable structure, very similar to an electric motor. However, using genomic expression of fluorescent fusions to the stator proteins we could not count the number of stator proteins using TIRF microscopy, we showed they are dynamic, exchanging with a pool of proteins in the cytoplasmic membrane. Each stator unit only remains in the motor for about 30s. We have now shown that not only do the stator proteins exchange, but the rotor proteins also exchange, even while rotating. Interestingly the rotor proteins only exchange if the chemosensory protein, CheY, is present. These data will be discussed, along with the implications for sensory signalling and future approaches and developments in imaging required to understand the mechanisms and causes of exchange.

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Delalez, N, Leake, MJ, Wadhams, GH, Berry, RM and Armitage, JP (2010) Dynamics of protein turnover in the functioning rotor of the bacterial flagellar motor *PNAS doi:10.1073/pnas.1000284107*

Kenn Gerdes (Newcastle) - ***Dynamic cytoskeletal proteins, ParM and ParA, segregate DNA by different mechanisms***

It has been difficult to unravel the underlying molecular mechanisms of chromsosome segregation in bacteria. By contrast, the mechanisms of plasmid segregation is better understood (Gerdes et al., 2010). The *parMRC* locus of plasmid R1 encodes actin homologue ParM, centromere-binding protein ParR and centromere *parC* to which ParR binds. ParM forms actin-like filaments in vivo and in vitro (Moller-Jensen et al., 2002; Garner et al., 2004). ParM filaments are dynamically unstable – that is – their bidirectional polymerization switches stochastically to rapid unidirectional decay. ParR and *parC* forms a complex that caps the ParM filament ends (Moeller-Jensen et al., 2003; Garner et al., 2007) and thereby prevent dynamic instability of the filaments. Since the capped filaments continue to polymerise, the plasmids carrying the ParR/*parC* complex tethered to the filament ends are pushed apart in a simple mitotic-like process. The ParM-mediated DNA segregation process has been fully reconstituted in vitro and an almost full understanding of the process may be within reach (Garner et al., 2007). Interestingly, ParM and microtubules, which segregate eukaryotic chromosomes, are the only filament-forming proteins known to exhibit dynamic instability.

The more common type of *par* loci from plasmids and chromosomes that encode Walker Box ATPases has been more difficult to understand. We use the Type Ib *par2* locus of *Escherichia coli* plasmid pB171 as a model system. *par2* encodes ATPase ParA and centromere-binding protein ParB in an operon (Ebersbach et al., 2001). ParB dimers bind cooperatively to two centromere sites, *parC1* and *parC2* that flank the *parAB* operon and thereby form the “partition” complex that is recognized by ParA (Ringgaard et al., 2007). ParA also has non-specific DNA binding activity and therefore associates with the nucleoid (Ringgaard et al., 2009; Ebersbach 2001). Strikingly, ParA forms dynamic cytoskeletal-like structures that oscillate over the nucleoid. Simultaneously, the *par2* locus distributes plasmids regularly, on average, over the nucleoid (Ebersbach et al., 2006). We showed recently that the dynamic ParA patterns are not simple oscillations (Ringgaard et al., 2009). Rather, ParA nucleate in-between plasmids and polymerize bidirectionally. When a ParA assembly reaches a plasmid, the assembly-reaction reverses into disassembly. Strikingly, plasmids consistently migrate behind disassembling ParA cytoskeletal structures, suggesting that ParA filaments pull plasmids by depolymerization. The perpetual cycles of ParA assembly and disassembly result in continuous relocation of plasmids which, at the cell population level results in a “positional averaging” of the plasmids. Mathematical modeling of ParA and plasmid dynamics supports these interpretations. Mutational analysis of the N-terminus of ParB supports a molecular mechanism in which ParB (bound to *parC*) controls ParA filament depolymerization. The proposed molecular model that explains plasmid movement by ParA is reminiscent of how MinE of *E. coli* controls the dynamics of MinD, a ParA homologue. We also succeeded, for the first time, in constructing Z-stacks of time lapses showing that *par2* not only separates but also fuses plasmid foci, consistent with the proposal that plasmid pairing is an intermediate step in plasmid segregation mediated by *par2*.

Gerdes K, Howard M, Szardenings F. (2010) Pushing and pulling in prokaryotic DNA segregation. ***Cell*** 141, 927- 942.

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Møller-Jensen, J., Borch, J., Dam, M., Jensen, RB., Roepstorff, P. and Gerdes, K. (2003) Bacterial Mitosis: ParM of plasmid R1 moves plasmid DNA by an actin-like insertional polymerization mechanism. *Mol. Cell* 12, 1477–1487

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