Meeting report

A hitchhiker's guide to cell biology: exploitation of host-cell functions by intracellular pathogens

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A report on the 'Pathogen-host cell interactions' minisymposium at the 41st Annual Meeting of the American Society for Cell Biology, Washington DC, USA, 8-12 December 2001.

It is becoming a widely accepted principle that studying the mechanisms by which pathogens subvert processes in a host cell can teach us about interesting cell biology. Accordingly, the Annual Meeting of the American Society for Cell Biology (ASCB) has featured minisymposia and poster sessions on host-pathogen interactions for the past several years. One example of this subject is the motility of several intracellular pathogens that use the host cell's actin cytoskeleton, including the Gram-positive bacterium *Listeria monocytogenes*. This topic has become so mainstream that talks and posters on it (more than a dozen from eight different laboratories) are now found among almost all sessions devoted to actin polymerization and cell motility (abstracts are freely available at the ASCB meeting website [http://www.ascb.org/]). The poster subjects included the determination of the force-velocity curve for this form of movement, which showed that contrary to other known molecular motors, the bacterium generates more mechanical power under increased load (James McGrath and co-workers, Johns Hopkins University, Baltimore, USA). Other posters addressed cell biological or biochemical issues, for example, Fred Southwick and colleagues (University of Florida, Gainesville, USA) presented a poster that showed that a decrease in the intracellular calcium concentration causes a decrease in the actin depolymerization rate while not affecting the actin polymerization rate. Two talks in different minisymposia discussed the role of the cellular protein VASP in *Listeria* motility - both its function in actin nucleation at the bacterial surface (Matthew Welch, University of California, Berkeley, USA) and its role in enhancing the speed of intracellular movement (Joseph Loureiro, Massachusetts Institute of Technology, Cambridge, USA).

Listeria is not the only pathogen capable of intracellular movement using actin polymerization. Like *Listeria*, vaccinia virus (related to the causative agent of smallpox) also forms actin 'comet tails' by recruiting actin filaments behind a moving pathogen particle, typical of this form of motility. Michael Way (Imperial Cancer Research Fund, London, UK) reported that, unlike *Listeria*, vaccinia forms actin comet tails only at the periphery of infected cells, where the virus can interact with the plasma membrane. Actin nucleation requires the viral membrane protein A36R as well as host factors. But the viral particles are too large to move from their replication site near the center of the cell to the cell periphery by passive diffusion. Direct visualization of viral particles immediately after replication showed that they moved outward in a processive, and at times saltatory, manner consistent with microtubule-based transport. This movement is also dependent on the A36R protein and involves interactions between viral particles and the host motor protein kinesin. Here, a single viral protein is capable of subverting both the microtubule and actin cytoskeleton systems of the host cell and, apparently, of choosing which cytoskeletal system to use according to intracellular location.

Another great example of a pathogen carefully regulating its exploitation of the host-cell cytoskeleton is the bidirectional movement of herpes simplex virus 1 in neuronal axons in the peripheral nervous system. Herpes simplex virus 1 infects through the mucosal membranes and invades tissue until it encounters nerve terminals. It then enters the neurons and moves in a 'retrograde' manner, along the axon toward the cell body, where it replicates. Subsequently, anterograde transport of viral particles back down the axon towards peripheral tissues can cause recurrent symptoms of infection.

Greg Smith (Northwestern University, Chicago, USA) showed direct visualization of virus particles labeled with green fluorescent protein (GFP) in the axons of cultured neurons. The rates of viral movement are in the same range as the fast axonal transport of synapse components and are therefore most likely due to attachment of the viral particles to microtubule motors. Early in infection the predominant movement is retrograde, towards the cell body, although shorter, slower spurts of anterograde movement are also seen. Later time points favor movement in the anterograde direction, also with spurts of reversal in direction. Thus, herpes simplex virus 1 is capable of regulating its association with directional microtubule motors depending on the stage of infection and the target location to which the virus is heading.

In addition to exploitation of the host cytoskeleton for intracellular transport, many intracellular pathogens alter membrane traffic in their host cells. For example, the protozoan parasite *Leishmania donovani* is phagocytosed by host cells and then alters the endosomal compartment to avoid further endosome maturation and fusion with lysosomes where the parasite would be degraded. Jean-François Dermine (University of Montreal, Canada) provided evidence that, although normal maturing phagosomes accumulate flotillin-1-enriched lipid rafts (cholesterol-rich membrane microdomains where signaling molecules, such as flotillin-1, are concentrated), phagosomes containing the infective form of *Leishmania* do not. The lipophosphoglycan (LPG) on the surface of *Leishmania* is responsible for preventing lipid-raft accumulation as well as for inhibiting phagosome-lysosome fusion. The fact that this parasite can survive in the phagosomes and prevent lipid-raft accumulation through production of a surface molecule that inhibits phagosome-lysosome fusion strengthens the hypothesis that lipid rafts are normally required for phagosome maturation.

The creation of specialized internal membrane-bound replication compartments is a common theme among intracellular pathogens that manipulate the host cell's environment to their benefit. The bacterial pathogen *Legionella pneumophila*, which causes legionnaire's disease (a community-acquired pneumonia), modifies the endocytic vacuole it occupies to create a compartment that contains markers typical of the endoplasmic reticulum (ER). As reported by Craig Roy (Yale University, New Haven, USA), *Legionella* does this by delivering effector molecules required for infection into the host cytoplasm through a type IV secretion apparatus that is encoded by 24 *dot/icm* genes. This type of secretion mechanism is found in other pathogens, including extracellular pathogens such as the tumor-producing *Agrobacterium tumefaciens*, which transfers its viral T-DNA into plant cells. *Helicobacter pylori* also uses this secretion mechanism to deliver the CagA protein, a major virulence factor, into gastric epithelial cells where it becomes phosphorylated and subsequently causes dramatic spreading and elongation of these host cells. No effector protein secreted through the type IV apparatus had previously been identified in any intracellular pathogen. Roy and colleagues succeeded by using a creative *in silico* genome-based method. The host cell's small Ras-like GTPase ARF1, implicated in traffic between the ER and the Golgi, had been recently shown to be required for *Legionella*'s modification of the endocytic vacuole. By searching the *Legionella* genome for homologs to known ARF1-interacting proteins, a gene with similarity to the yeast protein Sec7 (containing the Sec7 domain, the common sequence between the known highly divergent ARF guanadine-nucleotide exchange factors) was identified. The product of this gene, RalF, was further shown to be injected into macrophages specifically by the Dot/Icm secretion apparatus and to act as a guanine-nucleotide exchange factor for ARF1. Furthermore, RalF was found not to be essential for intracellular replication of *Legionella*, on the basis of past genetic screens aimed at identifying an effector protein, explaining why it had not been previously discovered by traditional mutagenesis techniques.

In addition to the use of genome informatics to identify new bacterial virulence factors, genome-level analysis of the host transcriptional response to infection by a variety of viral, bacterial, and protozoan pathogens has recently helped to define the early signaling pathways involved in innate immunity. A few very 'clever' pathogens have apparently developed mechanisms to subvert the innate immune responses, which are usually effective and robust. Barbara Burleigh (Harvard School of Public Health, Boston, USA) presented the two distinct mechanisms by which the long (25 μm) protozoan parasite *Trypanosoma cruzi* manages to enter host cells within ten minutes. The first, a well-established mechanism, involves local lysosome exocytosis and subsequent formation of an intracellular vacuole constructed primarily of lysosomal membrane. The parasite then enters the host cell inside the vacuole in a microtubule-dependent but actin-independent manner. The second entry mechanism requires actin-dependent plasma membrane ruffling. Burleigh concluded with a briefly described, but potentially extremely interesting, result. Microarray experiments performed to characterize gene regulation upon *Trypanosoma* infection of fibroblasts showed that no host genes seem to be upregulated within the first few hours after entry. This is strikingly different from the innate immune response characteristic of infection by almost all other microbial pathogens examined to date, all of which activate the transcription factor NF-κB and various downstream genes early during infection. In contrast, a substantial number of genes were induced after 24 hours of *Trypanosoma* infection. Thus, Burleigh suggested that there might be a mechanism by which *Trypanosoma* represses the normal innate immune response that is triggered by other pathogens. Further investigation has the potential to identify a mechanism by which an intracellular pathogen can manipulate not only the host-cell environment but also the host's response to infection in such a way that detection of the pathogen in the cell is prevented. Understanding such mechanisms will provide further insights into basic cellular processes and is likely to lead to new approaches for tackling the diseases caused by intracellular pathogens.