



Lincoln's Inn Fields

Cell Motility

www.london-research-institute.org.uk/michaelway

Group Leader **Michael Way**

Postdoctoral Scientists

Mark Dodding
Naoko Kogata
Morag Martin
Amy Ramsden

Graduate Students

Xing Chen
Joao Cordeiro
Sara Donnelly
Charlotte Durkin
Ina Weisswange

Scientific Officers

Theresa Higgins
Antonio Postigo
Sibylle Schleich

The correct spatial and temporal regulation of cell migration by signalling networks is essential during the development and throughout the lifetime of multi-cellular organisms. Consequently, deregulation of signalling networks and the induction of inappropriate cell migration can be devastating when it stimulates tumour cells to undergo metastasis and establish new tumours. Our research uses a variety of biochemical and imaging approaches, in a number of systems, including pathogen infected cells to understand the molecular basis of signalling networks and their role in regulating cell migration.

Signalling dynamics and regulation of actin based motility

Understanding how signalling networks regulate cell motility and adhesion will require a complete molecular understanding of the protein interactions occurring within each signalling network. It also requires detailed knowledge of the cellular localisation and dynamics of individual components and/or protein complexes within each network. Unfortunately, many signalling networks are often not amenable to such analysis, as they are frequently transient and dispersed. In contrast, many of the signalling pathways that are hijacked and manipulated by pathogens undergoing actin-based motility, are highly localised and sustained.

During vaccinia virus infection the extra-cellular enveloped virus attached to the plasma membrane induces an outside-in signalling cascade that locally activates Src and Abl family kinases. This activation results in phosphorylation of tyrosine 112 and 132 of A36, an integral viral membrane protein that is localised beneath the extra-cellular virus. Phosphorylation of tyrosine 112 creates a binding site for the SH2 domain of the adapter Nck, which is recruited beneath the extra-cellular virus as part of a tripartite complex that contains WIP and N-WASP. Phosphorylation of tyrosine 132 generates a binding site for the SH2 domain of the adapter Grb2. The recruitment of Grb2 however, requires the prior phosphorylation of tyrosine 112, as its association also depends on the presence of the polyproline rich region of N-WASP. Ultimately the recruitment of N-WASP locally stimulates the actin-nucleating activity of the Arp2/3 complex, resulting in the formation of an actin tail beneath the virus, which acts to enhance viral spread.

The signalling network vaccinia hijacks to stimulate actin polymerisation is also involved in a number of cellular processes such as the formation and regulation of invadopodia during tumour cell-induced matrix degradation and invasion. The highly localised and robust nature of the vaccinia signalling cascade make the virus a power model system to understand the molecular details of how a signalling network induces and regulates actin-based motility. Over the last few years Ina Weisswange, a graduate student in the laboratory has been analyzing the dynamics of GFP-tagged Grb2, Nck, WIP and N-WASP during Arp2/3 complex-dependent actin based motility of vaccinia. Using Fluorescence Recovery After Photobleaching (FRAP), Ina found that all four GFP-tagged proteins associated with the tips of vaccinia induced actin tails undergo rapid exchange. Ina found that Grb2 has an important role to help stabilise the vaccinia-signalling complex. This explains why the virus induces more actin tails in the presence of Grb2 than in its absence. Interestingly, the turnover of N-WASP beneath the

virus was found to be dependent on actin polymerisation nucleated by the Arp2/3 complex. This suggests there is a feedback mechanism, in which active actin polymerisation regulates the exchange of the vaccinia-signalling complex. Consistent with this hypothesis, the stability of N-WASP beneath the virus depends not only on its interaction with Grb2 but also the growing barbed ends of actin filaments. Disruption of either of these interactions leads to an increase in the rate of N-WASP exchange, which in turn results in a faster rate of virus movement. Our observations are consistent with a model in which the stability of N-WASP association regulates the rate of Arp2/3 complex-dependent actin-based motility by antagonising actin filament capping. Our future studies will be aimed at understanding the relationship between the stability of N-WASP, barbed end capping and Arp2/3 complex-dependent actin filament nucleation.

An E2-F12 complex is required for IEV morphogenesis during vaccinia infection

Vaccinia actin tail formation only occurs after intra-cellular enveloped virus (IEV) particles have fused with the plasma membrane. In order to reach the plasma membrane from their peri-nuclear site of assembly, IEV particles recruit kinesin-I and undergo microtubule dependent movements towards the cell periphery. Currently, only two viral proteins, A36 and F12, have been suggested to play a direct role in microtubule mediated movement of IEV. Loss of either A36 or F12 results in the accumulation of IEV at their peri-nuclear site of assembly. A36 appears to be responsible for recruiting the microtubule motor as it can interact directly with the kinesin-I light chain. In contrast, the role of F12 in the movement of IEV towards the plasma membrane remains obscure.

Over the last year Mark Dodding, a postdoc in the laboratory, has been imaging live cells infected with a recombinant virus expressing GFP-F12 to obtain additional insights into role of F12 during IEV transport. Mark found that GFP-F12 is recruited to IEV moving on microtubules and is released from virus particles when they fuse with the plasma membrane and switch to actin-based motility. Unexpectedly, Mark found that although the majority of IEV remain close to their peri-nuclear site of assembly in the absence of F12, a

small number of IEV are still capable of undergoing microtubule mediated transport. Using a recombinant virus expressing GST-F12 in conjunction with mass spectrometry analysis of glutathione resin pull downs, Mark was able to demonstrate that the viral protein E2 interacts directly with F12. In infected cells, GFP-E2 is observed on IEV moving on microtubules as well as in the Golgi region, but is not associated with actin tails. In the absence of the gene encoding E2, IEV accumulate in the peri-nuclear region and F12 is not recruited to virus particles. Conversely, GFP-E2 is not observed on IEV in the absence of F12. Ultra-structural analysis of cells infected with viruses lacking the genes encoding E2 or F12 by Lucy Collinson in the Electron Microscopy Unit revealed that loss of either protein results in defects in membrane wrapping during IEV formation. Taken together, our observations suggest that the primary role of the E2-F12 complex is in the morphogenesis rather than the microtubule-based transport of IEV.

Publications listed on page 130

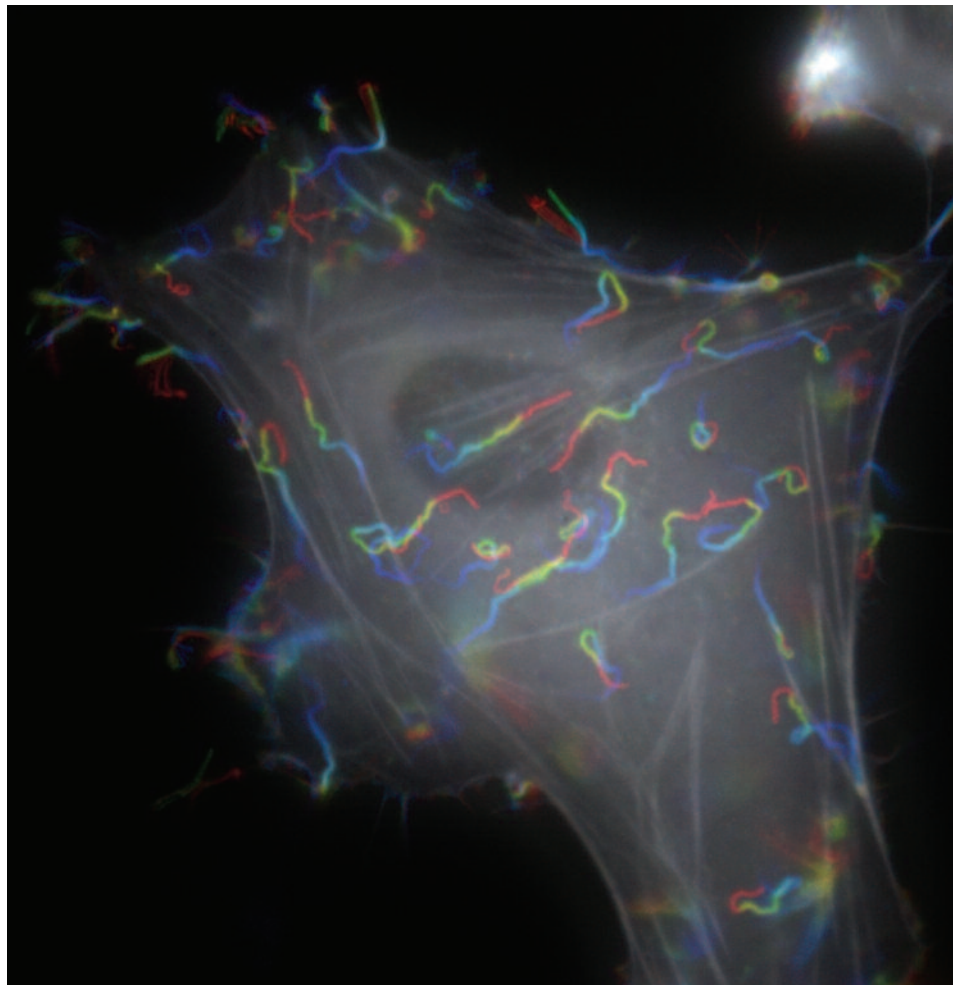


Figure 1. Fluorescence image of the paths taken by vaccinia induced actin tails over a 5 minute period.