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MEETING REPORT

Meeting report – Cell dynamics: host–pathogen interface

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ABSTRACT

Two years into the most significant infectious disease event of our generation, infections have populated every conversation and in-depth understanding of host–pathogen interactions has, perhaps, never been more important. In a successful return to in-person conferences, the host–pathogen interface was the focus of the third Cell Dynamics meeting, which took place at the glorious Wotton House in Surrey, UK. The meeting organised by Michaela Gack, Maximiliano Gutierrez, Dominique Soldati-Favre and Michael Way gathered an international group of scientists who shared their recent discoveries and views on numerous aspects, including cell-autonomous defence mechanisms, pathogen interactions with host cytoskeletal or membrane dynamics, and cellular immune regulation. More than 30 years into the beginning of cellular microbiology as a field, the meeting exhibited the unique aspect of the host–pathogen interface in uncovering the fundamentals of both pathogens and their hosts.

Cellular immune signalling

How eukaryotic cells sense and respond to pathogens and their molecules is central to understanding host–pathogen interactions. The meeting showcased the complexity of sensing mechanisms, which produce variable cellular outcomes.

Interferons (IFNs) are a major family of cytokines produced in response to microbial challenges and induce an anti-microbial state driven by hundreds of interferon-stimulated genes (ISGs). However, how individual ISGs contribute to protection against specific pathogens is mostly unknown. Joe McKellar (CNRS-University of Montpellier, France) presented how one ISG, MX1, inhibits both early and later stages of influenza A virus (IAV) infection. MX1 affects several different cellular pathways that are highjacked by IAV, including nuclear export and intracytoplasmic trafficking. This alters the localisation of different viral and cellular proteins and prevents viral replication (McKellar et al., 2021).

ISGs target all classes of pathogens. Eva Frickel (University of Birmingham, UK) presented an overview of her impressive work regarding the protective role of the interferon-inducible GTPases, guanylate-binding proteins (GBPs), during bacterial (*Salmonella* Typhimurium) or parasite (*Toxoplasma gondii*; *Tg*) infection in IFNγ-stimulated macrophages. GBP1 tackles *Tg* by promoting the disruption of parasite-containing vacuoles, which exposes the parasite DNA to the cytosol, leading to caspase-8-driven apoptosis. In case of *Salmonella*, GBP1 directly binds to the bacterial surface when they reach the cytosol, which in turn recruits and activates caspase-4, the non-canonical inflammasome, leading to pyroptosis, an inflammatory form of programmed cell death (PGD). The canonical inflammasome, via caspase-1, negatively regulates the amount of cell death through cleavage of GBP1. This work highlighted how GBP1 functions to distinctly recognise different pathogens and drive host cells into separate forms of PGD (Fisch et al., 2019, 2020).

Given the importance of IFN in controlling microbial infection, it is not surprising that pathogens have evolved a variety of mechanisms that inhibit IFN expression and signalling. Michaela Gack (Cleveland Clinic Florida, Port St. Lucie, USA) has contributed significantly to the understanding of the post-translational regulation of RIG-I-like receptors (RLRs). Activation of these ubiquitously expressed cytosolic RNA sensors induces IFNs and other cytokines. Michaela presented an elegant new mechanism that regulates the activation of MDA5 via conjugation of the ubiquitin-like IFN-inducible protein ISG15 (ISGylation). ISGylation can be targeted by pathogens, in particular SARS-CoV-2 utilizes a papain-like protease to directly promote de-ISGylation (Liu et al., 2021).

The other member of the RLR family, RIG-I, is not activated by ISGylation but rather by ubiquitylation, a mechanism that is also targeted by several viruses. Craig Roy (Yale University, USA) described what is, to our knowledge, the first example of a bacterial virulence factor that targets RLR signalling. Indeed, Roy and colleagues found that the causative agent of Q fever, *Coxiella burnetii*, encodes two type-IV secretion system effectors that are required for deubiquitylation and inhibition of RIG-I. The resulting inhibition of IFN induction is predicted to prevent host clearance because treatment of infected cells with IFN resulted in decreased bacterial replication. Finally, Charlotte Odendall (King's College London, UK) identified how *Shigella* blocks signalling downstream of IFN receptors, inhibiting ISG expression. This is mediated via inhibition of Ca^{2+} signalling by the conserved OspC family of virulence factors. Importantly, inhibition of IFN by OspCs or genetic deletion of IFN receptors promotes bacterial replication within host cells and colonisation of the murine gut (Alphonse et al., 2022).

A theme that became apparent from this discussion is the targeting of Ca^{2+} signalling pathways by virulence factors. Joana Sa Pessoa (Queen's University Belfast, UK) showed that a type-VI secretion system (T6SS) effector from *Klebsiella pneumoniae* triggers an increase in Ca^{2+} in the mitochondria from endoplasmic reticulum (ER) resources. This promotes mitochondrial fragmentation and production of reactive oxygen species (ROS) through the activation of the mitochondrial immune receptor NLRX1, impairing immune responses to the pathogen. This work illustrated unexpected important roles of T6SSs beyond their antimicrobial functions.

Cell death pathways are central defence mechanisms that contribute to the eradication of many intracellular pathogens. Jae U. Jung (Cleveland Clinic, USA) presented unpublished work describing a critical role for OASL, an IFN-induced protein of the OAS family. OAS-proteins promote antiviral responses upon sensing of foreign nucleic acids, and OASL was shown to promote non-canonical necroptosis by recruiting key necroptotic

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components, the innate sensor ZBP1 (also known as DAI), the RIPK3 kinase, and the effector MLKL, into liquid droplets, pointing to the importance of IFN in activating non-canonical necroptosis. Mitchell Pallett (Imperial College London, UK) described an atypical form of cell death, triggered upon actin depolymerisation by the *Salmonella* virulence factor SpvB. This caspase-independent pathway requires the activation of MAP4 kinase-dependent signalling cascades, culminating in lysosomal membrane permeabilization and cathepsin-dependent killing of *Salmonella*-infected cells.

One more beautiful *Salmonella* story was presented by Teresa Thurston (Imperial College London, UK). Its effector SteE induces phosphorylation of the transcription factor STAT3, altering the function of macrophages to skew them towards an anti-inflammatory 'M2' polarization. The underlying mechanism of action is fascinating and unique, with SteE binding the pleiotropic host serine/threonine kinase GSK3, and 'forcing it' to switch its specificity towards tyrosine 705 of STAT3 (Panagi et al., 2020). Similarly, by harnessing the power of pooled CRISPR knockout (Young et al., 2019) screens, Simon Butterworth (The Crick Institute, London, UK) showed that *T. gondii* also alters macrophage polarization by targeting STAT6 phosphorylation (Butterworth et al., 2022 preprint).

The environmental fungus *Cryptococcus neoformans* is another master manipulator of immunity that perturbs phagocyte functions, by not only evading phagocyte-mediated killing but also blocking antigen presentation by dendritic cells and T-cell proliferation. Although the specific mechanism of action has not yet been identified, Robin May (University of Birmingham, UK) shared that the capsule probably plays an important role in this process.

Whereas most of the presented studies were carried out in traditional cellular or animal models, Vivek Thacker (EPFL, Lausanne, Switzerland) described organ-on-chip methods, in which the co-culture of different cell types more accurately mimics tissue environments. Leveraging this model, this approach uncovered key mechanisms in endothelial cells that are responsible for the exacerbated immune responses associated with severe SARS-CoV-2 illness (Thacker et al., 2021).

An in-depth understanding of immune responses to infection is crucial in our fight against pathogens, as this knowledge can translate into developing therapies against infectious diseases. Indeed, as Sara Cherry (University of Pennsylvania, Philadelphia, USA) insisted, we need more and better antivirals. Because broad-spectrum antivirals are difficult to engineer owing to the vast genetic diversity among viruses, one avenue of choice is to boost the antiviral immune response. Sara described a high-throughput, imaging-based antiviral screening pipeline used to screen for compounds or agonists that block viral infection. This led to the identification of endogenous inducers of the IFN response, which protected animals from SARS-CoV-2 infection (Li et al., 2021), as well as a long noncoding RNA that blocked Chikungunya virus infection.

Selective autophagy in cell-autonomous defences

Macro-autophagy (hereafter autophagy) is essential for eukaryotic homoeostasis, and enables recycling and degradation of cytoplasmic content. Since the identification of its conserved core proteins (Atgs), different pathways have been shown to drive formation of membrane phagophores, promote cargo sequestration and enable subsequent fusion of autophagosomes with lysosomes for degradation (Mizushima, 2018; Ohsumi, 2014). Answering 'How cells defend themselves against invasion by cytosolic pathogens', a question recalled by Felix Randow (MRC Laboratory of Molecular Biology, UK), was critical to our understanding of selective autophagy, the selective engulfment of cargo via specific receptors. Numerous autophagy receptors were shown to recognise damaged vacuoles and exposed bacteria (Randow and Youle, 2014); this remarkably evergrowing list includes ubiquitin, galectin 8, optineurin, NBR1, GBPs, NDP52, p62, septins and, more recently, the enormous (584 kDa) E3 ligase RFN123, which displays unusual ubiquitylation activity towards lipopolysaccharide (LPS). As highlighted by Felix, RNF213 controls cytosolic targeting of *Salmonella* by promoting the direct ubiquitylation of LPS and favouring the recruitment of another E3 ligase, LUBAC, which increases pre-existing ubiquitin platforms. This work identified yet another element of host cytosolic defence mechanisms and points to the importance of non-protein ubiquitylation (Otten et al., 2021).

The ability of so-called professional cytosolic pathogens to promote infection within the cytosol inspired cellular microbiologists to search for host defences. Serge Mostowy (London School of Hygiene & Tropical Medicine, UK) shared with us his enthusiasm in following up those studies, which led him to the discovery that septins, considered the fourth component of the cytoskeleton, entrap *Shigella* within cage-like structures, limiting their replication and promoting their autophagic destruction (Lobato-Márquez et al., 2021). Serge described his novel *in vitro* approach to elucidate mechanisms underlying septin cage entrapment of *Shigella*, highlighting molecular details that will guide future findings, including the septin amphipathic helix domain, which plays a key role in recognising micron-scale bacterial curvature. Finally, analysis by cryo-electron tomography (cryo-ET) showed that septins assemble with a non-random architecture, with barbwire-like entanglements around the bacterial surface that are inhibited by the presence of LPS. Bacterial escape to the cytosol often occurs through the activity of type III secretion systems (T3SS), as for *Shigella* and *Salmonella*. Léa Swistak (Institut Pasteur, Paris, France) aims to characterize such vacuolar disruption events at the molecular resolution in a near-native state by using an automated workflow that also combines cryo-ET but with cryo-focused ion-beam sample milling (Swistak et al., 2021). Both presentations further highlighted the value of cutting-edge imaging techniques in illustrating the heterogeneity of lifestyles displayed by intracellular bacteria.

This heterogeneity has been investigated in depth by the group of Maximiliano Gutierrez (The Francis Crick Institute, London, UK), which follows the life cycle of *Mycobacterium tuberculosis* (*Mtb*), the causative agent of Tuberculosis or 'the other pandemic', still one the world's most significant infections, and one that disproportionally affects poor countries, further driving inequality (Moutinho, 2022). *Mtb* has been one of the paramount pathogens in the quest to understand host cytosolic defences and the heterogeneity of lifestyles displayed by intracellular bacteria (Gutierrez and Enninga, 2022). *Mtb* is found within enclosed or damaged vacuoles, surrounded by membrane remnants, or displaying autophagosomal targeting. Max presented a combination of high-resolution live microscopy and genetic studies, using infection of human induced pluripotent stem cell-derived macrophages as a model to identify the pathways that govern intracellular *Mtb* phenotypes (Pellegrino and Gutierrez, 2021). We learned that Atg7 and Atg14 restrict *Mtb* replication with distinct dynamics and effectiveness. Naomi Okugbeni (Stellenbosch University, South Africa) complemented these findings by extensively tracking autophagy progression during macrophage infection with *Mtb*. Different dynamics of p62 and LC3

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recruitment to bacteria correlate with variations in both bacterial replication and intracellular phenotypes (cytosol versus vacuole).

Damage to bacteria-containing vacuoles is an established feature of the cellular pathogenesis of various *Mycobacterium* species that also occurs through the activity of secretion systems, specifically the type VII secretion system ESX1 and the abundantly secreted pore-forming protein ESAT6. ESX1 and ESAT6 are required for virulence of different *Mycobacterium* species, including *Mycobacterium marinum* during infection of animal macrophages and of *Dictyostelium discoideum* (Dicty for short), the professional phagocyte amoeba model. Damages to endolysosomal membranes of Dicty caused by *M. marinum* led to the identification of a conserved ubiquitin E3-ligase (TrafE) as a relevant factor for sensing and repair of damaged compartments. Thierry Soldati (University of Geneva, Switzerland) presented detailed *in vivo* live-imaging experiments of membrane damage events, which revealed the role of TrafE in promoting K63-ubiquitylation within sites of endosomal damage (sterile or through the action of ESAT6). TrafE promotes both endosomal ESCRT-mediated membrane repair and selective autophagy of cytosolic bacteria, protecting Dicty during *M. marinum* infection (Raykov et al., 2021 preprint).

Pathogens have evolved multiple sophisticated mechanisms to curb autophagy, revealing important insights into the regulation of host autophagy processes. Maria Mota (University of Lisbon, Portugal) reminded us of the significant challenges with malaria, underscoring the importance of characterising the life-style transitions of the parasite *in vivo,* in order to uncover potential anti-malaria therapies. *Plasmodium falciparum* is recognised by selective autophagy; however, its recognition is diminished by the virulence protein UIS3. This observation prompted the identification of the compound-C4 (through high-throughput screening), which blocks the LC3–UIS3 interaction, disrupting the parasite ability to block autophagy and thus reducing infection (Setua et al., 2020).

Belinda Hall (Setua et al., 2020; University of Surrey, Guildford, UK) described how Mycolactone, an exotoxin secreted by *Mycobacterium ulcerans* helped uncover a ULK1-independent autophagy pathway (Hall et al., 2022). This pathway is activated by ER stress signals upon blockage of protein translocation to the ER by Mycolactone. Beyond the cytotoxicity of Mycolactone, this pathway might also be relevant to other physiological processes that involve the integrative stress response.

The interplay between viruses and host cytosolic defences was also extensively discussed during the meeting. Damaged mitochondria can accumulate during viral infections, releasing damage-associated molecular patterns (DAMPs), such as mitochondrial RNA, which trigger innate responses. These can be anti-infectious or exploited by pathogens. Sonja Best (Rocky Mountain Laboratories of the National Institute of Allergy and Infectious Diseases, Hamilton, USA) demonstrated how Zika virus (ZIKV) subverts an uncharacterised element of mitophagy in order to promote inflammatory responses that contribute to dissemination *in vivo* (Ponia et al., 2021). The viral non-structural protein NS5, which is also a potent IFN antagonist, targets the host protein Ajuba, preventing its binding to the mitophagy regulator kinase PINK1. This reduces Ajuba–PINK1–Parkin-mediated autophagy of damaged mitochondria close to viral replicative niches, which amplifies specific host immune signalling that is exploited by ZIKV for dissemination.

Other viral proteins, called viroporins, can induce non-canonical autophagy. These small proteins form transmembrane channels that perturb intracellular ion gradients and are crucial for viral assembly and entry. Rachel Ulferts (The Francis Crick Institute, London, UK) described a genome-wide CRISPR-Cas9 screen that identified modulators of LC3 lipidation during non-canonical autophagy responses to viral porins (M2 from influenza, E from coronaviruses) or ionophores (Ulferts et al., 2021). Among other factors, Rachel identified the vacuolar ATPase as general promoter of LC3 lipidation in response to altered pH compartments. The targeting of this pathway by various pathogens highlighted its significance, and was also discussed.

Vesicular trafficking and endomembrane organisation

Pathogenic control of host vesicular trafficking is crucial for infection. This enables phagolysosomal escape and formation of replicative niches, as well as exploitation of cellular membranes for pathogen structure and/or release from infected cells. The identification of these processes helps us to better understand pathogenesis and uncover fundamental features of eukaryotic membrane biology.

Nihal Altan-Bonnet [National Heart, Lung, and Blood Institute (NHLBI), Bethesda, USA] discussed different strategies and outcomes of non-lytic viral egress mechanisms. Initially focusing on RNA-enveloped viruses, Nihal summarised recent findings that describe the unexpected secretion of β-coronaviruses (CoVs) within secretory lysosomes, showing that virions do not require an intact biosynthetic pathway and instead depend on small GTPases that control transport and biogenesis of lysosomes (Arl8b and Rab7). Through a mechanism yet to be fully characterised, CoVs manipulate the function of terminal compartments, which enables their egress via lysosome secretion following budding within the ER and Golgi intermediate compartment (ERGIC) (Ghosh et al., 2020). During budding, CoVs also exploit host membrane organisation and lipid modification pathways. Francisco S. Mesquita (EPFL, Lausanne, Switzerland) presented details on the formation and infectivity of SARS-CoV-2 virions, which require massive host-mediated S-acylation, the reversible post-translational attachment of fatty acids to proteins. This understudied modification is increasingly being recognised as a fundamental ubiquitous eukaryotic process, and is used by CoVs for the modification of the viral fusion Spike glycoprotein within budding sites. This involves specific host acyltransferases and regulates the lipid composition, organization and infectivity of SARS-CoV-2 (Mesquita et al., 2021).

The trafficking mechanisms used by toxins and viruses have long guided cell biologists to discover vesicular transport routes, and conversely, uncovering regulators of host membrane sorting has contributed to finding determinants of pathogenesis. In this context, the final presentation on SARS-CoV-2 at the conference was given by James Daly (University of Bristol, UK), who described a proteomic screen to identify cargoes and regulators of retrograde transport from endosomes to the trans-Golgi network (TGN), which led to identification of the endosomal SNX-BAR sorting complex promoting exit 1 (ESCPE-1) as a regulator of SARS-CoV-2 co-receptor neuropilin-1 (NRP1). This pathway regulates intracellular transport of NRP1 ligands and is required for SARS-CoV-2 cellular infection (Simonetti et al., 2022).

Traditional models of viral budding, egress and dissemination generally assume release and transmission of individual viral particles. However, this view is incomplete and does not fully account for the heterogeneity of egress processes. Nihal also highlighted that non-enveloped viruses benefit from host-derived extracellular vesicles that are released within vesicle-cloaked clusters. Such vesicles consist of host membranes that contain

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fully infectious viral particles, as well as non-encapsulated genomes. Although the formation, release and uptake of such vesicles is poorly defined, Nihal demonstrated that membrane-enclosed viral clusters are released in stool, enable enhanced transmission and cell-to-cell spreading, and also provide protection against environmental damages (Santiana et al., 2018; Zhang et al., 2021).

Florence Niedergang (Institut Cochin, Paris, France) presented their work using human rhinovirus 16 (HRV16) to identify regulatory mechanisms of phagosome formation. Infections with HRV16 lead to defects in phagocytosis and bacterial clearance, causing macrophages to accumulate stalled phagocytic cups that fail to internalise bacteria. This phenotype revealed a role for arpin, a negative regulator of Arp2/3, during phagocytosis. Arpin localizes to membrane extensions within phagocytic cups and is required for efficient internalisation. HRV16 downregulates arpin, reducing phagocytic uptake during chronic obstructive pulmonary disease (Jubrail et al., 2020). Additional mechanisms implicated in phagosome maturation and manipulation of pathogen intracellular vacuoles were also discussed. Aby Anand (University of Osnabrück, Germany) described the hijacking of oxysterol-binding proteins (OSBPs), typically located at membrane contact sites, by pathogenic mycobacteria such as *M. marinum* in Dicty infections. OSPBs are lipid transfer proteins (LTPs) that shuttle sterols and phosphatidylinositol phosphates between membranes. OSBP8 accumulates at the *Mycobacterium* vacuoles and its depletion promotes mycobacteria replication through mechanisms that remain to be defined. Arthur Bienvenu (CNRS and University of Montpellier, France) identified a new *Coxiella* effector, Vice, that recruits lysobisphosphatidic acids (LBPAs) to bacterial vacuoles. LBPA is a major component of multivesicular endosomes and induces formation of luminal vesicles (Matsuo et al., 2004). Vice manipulates the biogenesis of multivesicular bodies and the protein profile of secreted extracellular vesicles, which might contribute to maturation of bacterial vacuoles. Similarly, *Plasmodium* parasites also modulate their intracellular niche. Maria Mota showed that *P. berghei* secretes a homologue of the host Rab5 GTPase, PbRab5b, which competes with its host counterpart to recruit the endocytic regulator APPL1. This competitive recruitment is important for the development of parasite exoerythrocytic forms within the parasitophorous vacuole (Lahree et al., 2022).

Cytoskeletal dynamics

The host cell cytoskeleton is a common target of all pathogens in order to promote or block intracellular access, subvert immunity, or enable pathogen spread. The study of cytoskeletal manipulation by pathogens has prompted the discovery of key cellular processes central to actin, microtubule, intermediate filaments and septin dynamics.

Host-pathogen interactions are characterized by a mutual manipulation of hosts and pathogens in complex biomechanical environments. Using traction force microscopy, Effie Bastounis (University of Tübigen, Germany) described interactions of *Borrelia burgdorferi* with endothelial cells and the activation of the immune system (Yuste et al., 2022). Daria Bonnazi (Institut Pasteur, Paris, France) focused on another model, meningococcal infection, and addressed the biophysics of vascular colonization by looking at changes in actomyosin and cell contractility. Daria showed that bacterial adhesion rapidly triggers a massive enrichment of Myosin-2 at the infection site and that *Neisseria meningitidis*-mediated remodelling of the endothelial cell surface induces changes in traction stresses exerted onto the extracellular matrix, impacting cell focal adhesions dynamics and cell shape. This points to a mechanism whereby meningococcal infection leads to the generation of contractile pulses through local actomyosin accumulation affecting cellular forces and tissue integrity. This work will pave the way to better understand the mechanical sensing and adaptation of the host cell to bacterial infection, a potential means for microbes to determine disease progression.

Matt Welch's (University of California, Berkeley, USA) pioneering work identified actin subversion by pathogens through a role of the Arp2/3 complex in *Listeria monocytogenes* actin-based motility. This time focusing on baculoviruses, he described how these viruses use Arp2/3-mediated actin-based motility to enter into and egress from the nucleus. Baculoviruses invade the nucleus through nuclear pore complexes, and following replication they recruit and engage nuclear actin to disrupt the nuclear envelope and escape from the nucleus. The molecular mechanism underlying nuclear envelope disruption remains uncharacterized but will be an interesting question for the future.

Using a pipeline of deep-learning analysis of automated microscopy, Derek Walsh (Northwestern University, Chicago, USA) described an interesting mechanism through which human cytomegalovirus (HCMV) provokes nuclear rotation (Procter et al., 2020). The cytoplasmic HCMV-assembly compartments function as microtubule-organizing centers that promote microtubule acetylation. In turn, acetylated microtubules enable dynein motors to pull and rotate the nucleus by binding to the linker of nucleoskeleton and cytoskeleton (LINC) complexes. This mechanism revealed a role for acetylated microtubules in controlling nuclear polarity which re-organizes nuclear actin to separate viral from host DNA facilitating viral replication (Furey et al., 2021; Procter et al., 2020). Microtubules are also targeted by HIV. Mojgan Naghavi (Northwestern University, Chicago, USA) illustrated that the microtubule end-binding protein (EB1) contributes to HIV infection by stabilizing microtubules; this not only promotes motility, but also delivers microtubule plus-end tracking proteins (+TIPs), such as CLIP170, which then bind to the viral particle and promote both its trafficking and uncoating. This may occur through structural mimicry of protein complexes with the HIV capsid that appear to have +-TIP-binding motifs (Santos da Silva et al., 2020).

Yue Zhang (Institute Pasteur of Shangai, China) presented the important role of the third cellular cytoskeletal polymer, the intermediate filaments, by demonstrating that vimentin coordinates the structural organisation of viral replication complexes. Vimentin is required for infection and, in addition to its structural role, also appears to bind ER-localized RNA-binding proteins and act as an RNA-binding-regulating hub in ZIKV infection (Zhang et al., 2022).

Finally, closing the chapter on virus-mediated targeting of cytoskeletal dynamics, Michael Way (The Francis Crick Institute, London, UK) summarized key findings regarding the interplay between vaccinia virus and all components of the cytoskeleton. These studies on the role of vaccinia actin tail motility in promoting cell-to-cell transmission were crucial to determining the various components of the signalling network that induce actin polymerization, as well as their dynamics. Similarly, vaccinia has been a paradigm in our understanding of clathrin-mediated sorting mechanisms and targeting of cytosolic pathogens by septins, which results in reduced viral release. A more recent focus for the Way lab has been the *in vitro* transport of vaccinia on purified microtubules. Reconstitution of microtubule motility of intracellular mature and

enveloped virions (IMVs and IEVs) allowed them to quantitatively dissect the role of kinesin-1 motors by describing the number of motors involved, virion velocities and other quantitative parameters. This model is an invaluable tool to study microtubular motors in a quantitative manner (Xu et al., 2022 preprint).

The Apicomplexa phylum includes obligate intracellular parasites, such as *Plasmodium* spp., the causative agent of malaria, and *T. gondii*, an opportunistic pathogen. These protozoa have their own cytoskeleton, but also interact with host cytoskeletal dynamics. First, Maria Mota presented work showing that formation of host actin rings around the plasmodium vacuole precedes parasite clearance. In the liver stages of *P. berghei*, the parasite vacuole membrane protein UIS4 interacts with actin and suppresses such still mysterious killing mechanism, which increases parasite survival and development (M'Bana et al., 2022). Friedrich Frischknecht (Heidelberg University, Germany) showed that *Plasmodium* sporozoites are fast moving in skin, the first barrier of infection for the parasite. For their motility, parasites require highly stable microtubules and unstable actin filaments linked to different surface proteins, the TRAP family adhesins, which have different roles during motility and infection (Frischknecht and Matuschewski, 2017).

Protozoans from the Apicomplexa phylum have evolved motile developmental stages unified under the term zoite. The polarized tachyzoite stage of *T. gondii* serves as a prototype to study the molecular machines that direct a specific type of adhesion-dependent and high-speed motility known as helical gliding. Using a combinatorial approach of force microscopy, expansion microscopy and high-speed live imaging, Isabelle Tardieux (Université Grenoble Alpes, France) and her team identified a timely interplay between the actomyosin motor and the peculiar helical microtubule cytoskeleton of the parasite that drives gliding. Using quantitative interference reflection contrast microscopy (qRICM), they demonstrated that the dynamics of surface–tachyzoite body contact could be mapped with high resolution and, by using traction force microscopy, the forces underlying gliding mobility could be monitored in real time (Pavlou et al., 2020). Dominique Soldati-Favre (University of Geneva, Switzerland) dissected in depth the structure of the conoid in *T. gondii*, a dynamic and enigmatic organelle. The conoid is formed by a cone of spiralling tubulin fibres that are associated with preconoidal rings (PCRs). Using ultrastructure expansion microscopy (U-ExM) and reverse genetics, they mapped five components of the PCRs and elucidate that the conoid extrusion is actomyosin driven and controls the F-actin flux during motility and invasion (Dos Santos Pacheco et al., 2022 preprint).

Concluding remarks

In summary, this latest edition of the Cell Dynamics meeting brought together scientists working at the forefront of host–pathogen interactions and was further proof that pathogens and their molecules guide researchers through the complexity of the mechanisms that coordinate disease and cell homeostasis. The field of cellular microbiology will not only continue to uncover fascinating fundamental cellular biology processes, but should also play a critical role in the quest of better understanding host–pathogen interactions, a key aspect to our preparedness for global infection challenges. Emerging questions will benefit from multidisciplinary approaches, cutting edge technologies, and creative and more-accurate model systems, which will certainly contribute to conceptual advances. Beyond this, a conclusion remains clear – cellular microbiology, encompassing viruses, bacteria, fungi and parasites, will continue to equip scientists with the most versatile and ingenious tools to understand all aspects of fundamental eukaryotic cell biology.

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Competing interests

The authors declare no competing or financial interests.

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