When cells and microbes meet in Krakow

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The sixth edition of the biannual 'At the Joint Edge of Cellular Microbiology & Cell Biology' meeting took place in October 2010 in Krakow with the support of EMBO. The meeting highlighted that research at the interface between cell biology and microbiology continues to bloom, and that cell biologists still have a lot to learn from bugs, as do microbiologists from cell biology.

In the mid-to-late 1990s a new scientific discipline, 'cellular microbiology', emerged at the interface of microbiology and cell biology to address fundamental questions in both fields. The creation in 2000 of a meeting series that brought together the two communities was important in the fostering of productive interactions between them. The sixth edition of this meeting was organized by Ivan Dikic (Goethe U., Germany), Dominique Soldati (U. Geneva, Switzerland), Marta Miacynska (IICMB, Poland) and Ryszard Przewlocki (Institute of Pharmacology PAS, Poland), and showed that after a decade of working together, members of both fields are as active, engaged and inventive as ever.

How bugs use the 'doors' into cells

By using genome-wide RNA-interference (RNAi) screening combined with high-throughput microscopy to look at the endocytosis of different ligands—both physiological and infectious—Prisca Liberali (ETH Zürich, Switzerland) found that when ligands are clustered according to the genes required for their cellular uptake, the vesicular stomatitis virus clustered with transferrin and EGF, whereas the SV40 virus clustered with cholera toxin B and the glycosyl phosphatidyl inositol-entry pathway GEEC. This study indicates that classes of ligand have different requirements, probably at least partly reflecting the pathway that they use to enter cells. However, it is increasingly apparent that a cargo—be it a physiological ligand–receptor complex or a microbe—has a specific requirement for a subset of endocytic or endocytosis-related proteins.

Scott Emr (Cornell U., USA) reported that retrieval of unfolded proteins from the cell surface in yeast requires the arrestin-related

protein Art1, which in turn recruits the E3 ubiquitin-ligase *rsp5* to the unfolded cargo protein, leading to its ubiquitination and clathrin-dependent uptake. Interestingly, Art1 seems to cycle between an active dephosphorylated state at the plasma membrane—under stress conditions—and a phosphorylated inactive state on the Golgi—under resting conditions—a cycle that is controlled by the kinase *npr1*. Interestingly, Gisou van der Goot (EPF Lausanne, Switzerland) reported that the anthrax toxin also requires β -arrestins to enter mammalian cells. Similar to findings in yeast, arrestins recruit the E3 ligase that modifies the cytosolic tail of the anthrax toxin receptors, leading to clathrin-mediated uptake. This endocytosis was also dependent on the adaptor complex AP1, rather than the classical endocytic adaptor AP2, further

highlighting the specific requirements of a given cargo (Abrami *et al*, 2010). Similarly, Kirsten Sandvig (Oslo University Hospital, Norway) reported that rather than entering cells only by exciting clathrin coated pits, Shiga toxin also triggers *de novo* formation of pits in a syk-dependent manner.

Phagosomes

Bacteria themselves are also able to use cellular entry routes to get into cells. To enter macrophages, pathogens generally exploit phagocytosis. After their initial formation, phagosomes undergo a maturation process through several interactions with successive compartments of the endocytic pathway, by this they become an aggressive compartment—in terms of pH and the presence of damaging enzymes—that ultimately leads to the death of the invading bacterium. Maximiliano Gutierrez (Helmholtz Centre for Infection Research, Germany) showed that the delivery of acid sphingomyelinase and prosaposin to the phagosome follows an unusual route directly from the Golgi to the phagosome, bypassing the endosomes (Wahe *et al*, 2010). The group found that the transmembrane protein, Sortilin, has a role in this pathway; it is not only a receptor for neuromediators and growth factors at the plasma membrane, but it has also been implicated in the transport of lysosomal proteins (Wahe *et al*, 2010). Killing of bacteria in phagosomes also depends on the HOPS (homotypic fusion and vacuole protein sorting) complex. More specifically, Helmut Krämer (U. Texas Southwestern Medical Center, Texas, USA) showed that when the HOSP component *vps16B* was knocked out in *Drosophila*, phagosomes failed to acidify and kill the bug, leading to the nomenclature of fly *vsp16b*: Fob for 'full of bacteria'.

A successful pathogen must interfere with the normal process of phagosome maturation, and one of the strategies for this is to break the phagosomal membrane. The group of Thierry Soldati (U. Geneva, Switzerland) found that, 24 h after infection of *Dictyostelium* with *Mycobacterium marinum*, the vacuole ruptures and the bacterial cell-wall components, as well as vacuole remnants undergo ubiquitination, subsequently recruiting the autophagic machinery. *M. marinum* nevertheless continues to proliferate in the cytosol. Surprisingly, infection of autophagy-deficient *Dictyostelium* mutants resulted in premature escape to the cytosol and massive ubiquitination, but did not, counterintuitively, lead to improved bacterial replication. These data raise the possibility that autophagy plays a more complicated and ambiguous role in controlling mycobacteria infection than has been previously suggested.

Molecular machines

As for all vesicular transport steps, endocytosis and phagocytosis require membrane budding. Harvey McMahon (U. Cambridge, UK) reported a role for F-BAR-containing protein family FCHo1/2 (Fer/Cip4 homology domain-only proteins 1 and 2)—membrane-sculpting proteins—during early plasma membrane invagination, before AP2 and clathrin are recruited. The group showed that a signal present in FCHo defines where a clathrin-coated pit forms, and that changes in FCHo1/2-expression levels correlate with the number of clathrin-coated vesicle-budding events. This activity depends on the F-BAR domain, suggesting that FCHo sculpts the initial bud site, leading to the subsequent recruitment of the clathrin machinery (Henne *et al*, 2010).

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After endocytic or other vesicles bud, the next step is fusion with the target compartment. In this context, Reinhard Jahn (MPI for Biophysical Chemistry, Germany) reminded us of the importance of numbers. SNARE proteins, which mediate membrane fusion, are highly abundant on synaptic vesicles—as opposed to endosomes—to

ensure that they do not become limiting. Despite this abundance, Jahn's group used proteoliposomes and Förster resonance energy transfer (FRET) analyses to show that one SNARE complex is sufficient to induce fusion between liposomes or between liposomes and synaptic vesicles (van den Bogaart *et al*, 2010). Their work supports the notion that zippering of a SNARE complex is a process that cannot be stopped once it is initiated, and that it requires high levels of energy to disassemble.

Autophagy

Autophagy, is an evolutionarily conserved cellular 'self-eating' programme that mediates the clearance of damaged organelles, large protein aggregates and pathogens in a highly coordinated manner, by targeting them to lysosomes. Defects in autophagy lead to several diseases and the molecular mechanisms driving this important phenomenon have been intensively studied. Sharon Tooze (Cancer Research UK, London Research Institute) gave an in-depth insight into the molecular machinery driving autophagy and highlighted the role of WIPI2, a mammalian orthologue of ATG18, in the formation of LC3-positive autophagosomes. WIPI2 is required for the formation of LC3-positive autophagosomes and is recruited early. One of the important questions in the field pertains to autophagy receptors, and evidence was presented that monoubiquitination of long-lived proteins is sufficient for the degradation of cytosolic proteins and peroxisomes. The clearance process is dependent on adaptor molecules such as p62/SQSTM1 and/or NBR1 (neighbour of BRCA1 gene 1), which can bind to the targeted proteins and members of the autophagic machinery to elicit degradation of the selected cargo. Selective adaptors for clearance of damaged organelles were also discussed by David McEwan (Goethe U., Germany)—for example, a mitochondrial protein, NIX, interacts directly with LC3/GABARAP to elicit mitophagy during erythrocyte differentiation (Novak *et al*, 2010).

Apart from recognition of the cargo, the origin of the double membrane of autophagosomes is also a topic of great interest and debate. Jennifer Lippincott-Schwartz (National Institute of Child Health and Human Development, USA) used live-cell imaging and transmission electron microscopy to reveal that autophagosomes are formed from mitochondrial outer

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membranes in serum-starved cells (Hailey *et al*, 2010). By expressing fluorescently tagged markers that had localized to various subcellular compartments, a robust transfer of the outer-mitochondrial membrane protein cytochrome b5 (YFP-cb5-Mito) to the newly forming autophagosomes was detected, without an apparent involvement of mitophagy. Endoplasmic reticulum (ER) has been thought to be the source of autophagosomes for a long time, but these results add a new twist to our understanding of autophagosome biogenesis. These observations are supported by the fact that during starvation, mitochondria rather than ER could act as the source of phosphoethanolamine, which is required for the conjugation of proteins such as LC3 to autophagosomal membranes. Phosphoethanolamine can be synthesized in the mitochondria by decarboxylation of phosphatidylserine, which normally localizes to the ER. The transport of phosphatidylserine from the ER to mitochondria is dependent on physical tethering of mitochondria to the ER by Mitofusin 2. Whether mitochondria act as a cradle of autophagosomes during other autophagy-inducing stress conditions will be an exciting question to answer in the future.

Ubiquitin signalling

Conjugation of ubiquitin to cellular proteins has profound effects on their stability, activity and localization, which in turn influence almost every cellular process. Recent studies have revealed that proteins can either be conjugated to one or several ubiquitin molecules, or to ubiquitin chains of various kinds. The specificity of the chain alters the affinity of the protein to its interacting partners, which in turn modulates the assembly, dynamics, stability and functionality of various macromolecular signalling platforms. Ivan Dikic introduced the world of ubiquitin to the conference and highlighted the role of various ubiquitin chains synthesized during the activation of the crucial transcriptional factor NF- κ B by TNF α , a key inflammatory cytokine. The role of Lys48 and Lys63 linked ubiquitin chains in this pathway is well documented, whereas emerging evidence supports a crucial role

for linear ubiquitin chains (head-to-tail) in the activation of NF- κ B.

In particular, the recruitment of LUBAC (linear ubiquitin chain assembly complex)—a novel E3 ligase complex composed of the two RING-finger proteins HOIL-1L and HOIP—is required for the stabilization of the TNFR1-receptor signalling complex, thereby controlling TNF α -mediated gene induction and apoptosis resistance. Furthermore, LUBAC has been shown to conjugate linear polyubiquitin chains to NEMO, which is required for the activation of the canonical NF- κ B pathway. By using biochemical methods, a new member of this complex was found to regulate TNF-dependent NF- κ B activation and apoptosis resistance *in vivo*, reaffirming the crucial role of linear ubiquitin chains in this pathway.

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By inventively using mouse genetics, Manolis Pasparakis (U. Köln, Germany) unravelled the *in vivo* role of NF- κ B in mediating epithelial and immune homeostasis. Although NF- κ B inhibition has been shown to reduce inflammation in various settings, recent studies have shown, surprisingly, that inhibition of NF- κ B actually triggers severe chronic inflammation in the skin and gut. In most cases, the observed inflammation of the epithelia is dependent on TNFR1 signalling, highlighting the crucial role of this cytokine in regulating skin inflammation. As anti-TNF targeted therapies are proving to be effective in treating inflammatory skin diseases such as psoriasis, the exact mechanisms by which inhibition of NF- κ B in the epidermis triggers inflammation in the skin remain to be elucidated.

Considering its importance, the NF- κ B pathway is a privileged target of bacterial pathogens. Pascale Cossart (Institut Pasteur, France) added a new protein to the list of bacterial virulence factors that affect NF- κ B signalling. Her lab revealed that when

Listeria monocytogenes is in the host-cell cytosol it secretes InlC, a member of the internalin family, which directly interacts with IKK α , an important component of the kinase complex that phosphorylates I κ B proteins. This leads to proteasomal degradation and activation of NF- κ B. Infection with InlC-deficient strains leads to increased production of proinflammatory cytokines, supporting a crucial role of this protein in dampening innate immune responses.

Equally important to the generation of ubiquitin chains is their disassembly by deubiquitinating enzymes (DUBs). Alain Israel (Institut Pasteur, France) revealed the role of a deubiquitinating enzyme in positively regulating the activation of the transmembrane receptor Notch. Notch signalling is highly conserved and has a crucial role during development, and deregulation of this pathway is associated with various diseases. Using an RNAi-mediated loss-of-function approach, Christel Brou (Institut Pasteur, France) and colleagues found that eIF3f—a known member of the multiprotein translation initiation factor eIF3 complex—functions as a Notch-deubiquitinating enzyme (Moretti *et al*, 2010). Silencing of eIF3f led to the accumulation of mono-ubiquitinated forms of activated Notch and inhibited signalling. Interestingly, the activated form of Notch is only able to interact with eIF3f in the presence of the E3 ubiquitin ligase Deltex, and Notch deubiquitination is required for processing by γ -secretase, a step that is essential for nuclear translocation and regulation of gene expression.

Subversion of host cell signalling

Pathogens are experts at subverting host functions for their own benefit. Thus, studies of their modes of infection seem to provide a never-ending source of new mechanisms that shed light on normal cellular functions. By exploiting a chlamydia infection model, Krishna Rajalingam (Goethe U., Germany) reported the non-redundant role of inhibitors of apoptosis (IAPs) in regulating cell death. IAPs were found to be organized in multi-meric protein complexes (IAPosomes) and further studies revealed the non-apoptotic role of these molecules in regulating C-Raf stability, modulating Rho GTPases and

cell migration (Dogan *et al*, 2008). Thomas Meyer (MPI for Infections Biology, Germany) used genome-wide RNAi screens to identify host proteins involved in regulating chlamydia and influenza virus infections. Chiara Zurzolo (Institut Pasteur, Paris, France) reported the way in which prions exploit tunnelling nanotubes for intercellular spreading and Maria Mota (Institute of Molecular Medicine, U. Lisbon, Portugal) revealed the world of *Plasmodium*–host interactions. Finally, Brett Finley (U. British Columbia, Canada) discussed the role of microbiota and metabolite balance in regulating enteric diseases caused by pathogenic *Escherichia coli* and *Salmonella* in murine models.

Conclusion

This meeting illustrated the marvel of cellular microbiology—revealing the repertoire of mechanisms that have evolved in pathogens to subvert and undermine every cellular process. Just as co-evolution of pathogens with their hosts has sculpted sophisticated host–pathogen interactions, bringing together cell biologists and microbiologists will continue to shed light on these fascinating wars that have shaped us. We look forward to the next meeting, in which, no doubt, new tricks in the eternal attack and counter-attack cycle will be revealed.

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