Microbial strategies to exploit host cells

Meeting on Spatial and Temporal Dynamics of the Endomembrane System

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The European Research Conference (EURESCO) on Spatial and Temporal Dynamics of the Endomembrane System was held in sunny San Feliu de Guixols, Spain, between 16 and 21 October 2004. The conference was organized by D. Holden and H. Stenmark

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process of rapid equilibration rather than using vectorial transport or the maturation of cisternae. The role of actin polymerization in the *cis*-Golgi to coordinate retrograde transport through cell-division cycle protein 42 (CDC42), neural Wiskott-Aldrich syndrome protein (N-WASP) and the actin-related protein complex ARP2/3 was elegantly shown by G. Egea (Barcelona, Spain).

At the plasma membrane (PM), successful pathogenic invaders choose the endocytic pathway often, although not exclusively, through the use of specific receptors (Fig 1). Pathogenic bacteria using type III and IV secretion systems inject effectors into the cytoplasm to manipulate host cell functions, such as triggering uptake and phagosome deviation, as exemplified by *Salmonella* and *Legionella*. Owing, in part, to motifs in the carboxy-terminal domains of the receptors, the chosen pathway might have opposite consequences in terms of signalling or degradation. Signalling through the transforming growth factor- β receptor (TGF- β R) is enhanced after uptake through clathrin-mediated endocytosis, whereas TGF- β R is downregulated on engulfment by the caveolae (Di Guglielmo *et al*, 2003).

For vesicles to internalize receptors, the main thermodynamic limitation of membrane invagination must be overcome. H. McMahon (Cambridge, UK) detailed different ways of promoting membrane bending. This phenomenon might be caused by partial protein insertion into one leaflet of the bilayer (as in the case of epsin), protein oligomerization (as seen in dynamin), or restricting or changing the lipid composition of the membrane. Several proteins that are involved in vesicle budding have Bin/amphiphysin/Rvs (BAR) domains (Peter *et al*, 2004) that confer dimerization, membrane binding and, more importantly, membrane curvature-sensing abilities to the protein. Amphiphysins, endophilins, nadrins and other proteins that are important in the entry of clathrin-coated vesicles have N-BAR domains with a further amino-terminal amphipathic helix, which inserts into the membrane and promotes curvature.

The internalization of clathrin-coated vesicles and caveolae is controlled by the GTPase dynamin, which forms a collar for the vesicle and helps the pinching-off process. A. Luini (Santa Mario Imbaro, Italy) suggested that C-terminal-binding protein/brefeldin A-ADP-ribosylated substrate (CtBP/BARS), which was recently found to promote Golgi membrane fission (Carcedo *et al*, 2004), might exert similar actions during clathrin-independent macropinocytosis. This pathway is also regulated by the small GTP-binding protein ADP-ribosylation factor 6 (Arf6). A. Dautry-

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Introduction

By bringing together scientists from the fields of microbiology and cell biology, the European Research Conference (EURESCO) on 'Spatial and Temporal Dynamics of the Endomembrane System' combined the best of both worlds at the intersection where intracellular pathogens and host cells meet. The mixture of studies, which focused on the molecular mechanisms behind endocytic and secretory transport, and the pathogenic microbes that exploit these pathways, was extremely fruitful for both sides. Excellent talks by young scientists and lively poster sessions contributed to the excitement and quality of this meeting.

Mechanisms of sorting to vesicular pathways

J. Lippincott-Schwartz (Bethesda, MD, USA) started the meeting by providing evidence that proteins move through the Golgi by a

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Fig 1 | Pathways for pathogen and toxin entry and their transport in cells. Bacteria tend to choose either phagocytosis or macropinocytosis for their entry. In both pathways, ADP-ribosylation factor 6 (Arf6) has been proposed to regulate the entry step. The carboxy-terminal-binding protein/brefeldin A-ADP ribosylated substrate (CtBP/BARS) might also regulate the entry of the fluid-phase tracer dextran. Many viruses, such as vesicular stomatitis virus (VSV) and adenovirus type 2 (AD2), and toxins, such as diphtheria toxin (DT) and anthrax toxin (AT), enter the cell through clathrin-coated vesicles, although other pathways are also used in parallel (for example, AD2 and shiga toxin). From the early endosome, some of the cargo (such as AT and VSV) probably reaches the intraluminal vesicles with the help of the sorting machinery, including hepatocyte growth factor-regulated tyrosine kinase substrate (HRS), phosphatidylinositol triphosphate (PI3P), clathrin and endosomal sorting complexes required for transport (ESCRT). DT is released to the cytoplasm from the early endosomes, whereas VSV and AT are probably released by the back fusion of intraluminal vesicles with the limiting membrane of late endosomes. In macrophages, human immunodeficiency virus (HIV) uses a similar sorting machinery to bud into late endosomes. Shiga toxin might enter the Golgi directly from early endosomes (K. Sandvig, Oslo, Norway). *Brucella* and *Legionella* escape the degradation pathway by acquiring endoplasmic reticulum (ER) membranes after entry. *Mycobacteria* block maturation of their phagosomes at an early endosomal stage, whereas *Salmonella* enters the endosomal pathway further towards the late endosomal stage and *Leishmania* survives in late endosomal compartments.

Varsat (Paris, France) reported that Arf6 might also regulate the entry of *Chlamydia* into host cells.

To invade host cells efficiently, pathogens or their toxic products interact with specialized domains of the host PM (Fig 1). Some pathogenic bacteria, such as *Brucella*, *Salmonella* and *Shigella*, use areas of lipid rafts for host-cell entry. Anthrax toxin (AT) uses both lipid rafts and clathrin-coated regions, whereas shiga toxin uses only the latter. For adenovirus type 2, clathrin- and dynamin-dependent uptake is crucial for infection. As reported by U. Greber (Zurich, Switzerland), this pathway activates dynamin-independent macropinocytosis of extracellular fluids and lysis of macropinosomes. The entry of viruses through both macropinocytosis and the clathrin-mediated endocytosis of virus-antibody complexes by the immunoglobulin G (IgG)-binding Fc- γ receptor leads to enhanced infection. In a

provocative poster talk, B. Nichols (Cambridge, UK) used fluorescence resonance-energy transfer (FRET) experiments to question the existence of rafts. He showed that raft markers failed to cluster after T-cell receptor ligation.

Several talks focused on the molecular mechanisms of multi-vesicular endosome formation and sorting. As H. Stenmark (Oslo, Norway) pointed out, receptor proteins that are destined for lysosomal degradation are ubiquitinated at the cytoplasmic tail and sorted into the lumen of early endosomes by various protein-protein interactions at the membrane. Hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) recognizes ubiquitinated cargo and sorts it to the degradative pathway by forming intraluminal vesicles. This process involves budding events from the vesicle membranes into the lumen. HRS colocalizes with unconventional clathrin, which is a muscle

isoform of the clathrin heavy chain, in the early endosomes, but does so poorly with early endosome antigen 1 (EEA1). This indicates that microdomains for protein sorting are separate from those for other early endosome actions.

The silencing of HRS leads to decreased degradation of ligands and reduced amounts of multivesicular endosomes, which points to a role for HRS in the formation of intraluminal endosomal vesicles. In addition to HRS, endosomal sorting complexes required for transport (ESCRT)-I, -II and -III are recruited to the microdomains and are probably also used for the intraluminal sorting of cargo. J. Gruenberg (Geneva, Switzerland) reported that the lipid lysobisphosphatidic acid (LBPA) is restricted to the internal membranes of late endosomes (Fig 1). Alix, which is the mammalian homologue of the yeast class E vacuolar protein-sorting 31 (vps31) protein, interacts with ESCRT proteins. Alix seems to bind LBPA on the limiting membrane of late endosomes and might even be incorporated into endosomes, as implied by its presence on exosomes (Matsuo *et al*, 2004). Interestingly, H. Göttlinger (Boston, MA, USA) showed that human immunodeficiency virus (HIV) exploits the cellular ESCRT machinery through a sequence (PT/SAP) in the Gag protein that allows viral budding from the PM.

How to exploit the endosomal pathway

Interesting new data were presented on the fate of pathogens and toxins in the endosomal pathway (Fig 1). Disturbing LBPA function using specific antibodies or by silencing Alix inhibits vesicular stomatitis virus (VSV) infection and prevents the release of the AT lethal factor (LF) from intraluminal vesicles (Gruenberg). Intact LBPA microdomains seem to be required to recycle transit proteins. However, according to Stenmark, Alix depletion has no effect on epidermal growth-factor receptor (EGFR) downregulation, which indicates that LBPA function is not essential for lysosomal sorting. It will be interesting to learn how the complex multivesicular structure facilitates the release of viral genomes and toxins to the cytoplasm. What are the molecular mechanisms for the 'back fusion' of intraluminal vesicles with the limiting membrane and how is it inhibited under physiological conditions? As pointed out by Göttlinger, the conserved PT/SAP sequence in the HIV Gag protein allows binding to Alix and thereby promotes viral budding. In his talk, M. Marsh (London, UK) showed that immature HIV particles bud into the late endosomal compartment in a manner that is topologically similar to intraluminal vesicles in multivesicular endosomes (Pelchen-Matthews *et al*, 2003). Preliminary experiments imply that these viral particles are more infective than those derived from T cells. In an excellent poster that was awarded the first Blackwell Science-sponsored poster prize, J. Blume (Cologne, Germany) characterized the intracellular localization, tissue distribution and function of members of a new protein family, EPS15 homology domain (EHD) 1–4, which are implicated in vesicular transport.

F. Rey (Gif-sur-Yvette, France) showed that some viruses produce class II fusion proteins that seem to form trimers to facilitate fusion. Similarly, central coils in class I fusion proteins, such as N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), might mediate membrane fusion at the PM or within endosomes and/or multivesicular bodies (MVBs). Rey also pointed out that low pH conditions in endosomes lead to a rearrangement of viral glycoproteins on fusion, as seen in the Semliki forest and dengue fever viruses, or cause them to refold, as in the case of

influenza haemagglutinin. In some cases, fusion might occur early during internalization, but the virus might still travel to the late endosome before the release of its genome to the cytoplasm, as seems to be the case for VSV (Gruenberg).

G. van der Goot (Geneva, Switzerland) presented exciting data on the mechanism of entry into cells of AT, which consists of a protective antigen (PA), oedema factor and LF (Fig 1). Whereas diphtheria toxin enters the cytoplasm directly from early endosomes, it seems that AT must be targeted to late endosomes before it can escape into the cytoplasm. Distinct delivery sites might be important for AT activity at the perinuclear region. One toxic consequence of AT cell entry is the cleavage of mitogen-activated protein kinase kinases (MEKs), such as MEK1, by LF. PA and LF enter the cell through clathrin-coated vesicles and, in early endosomes, LF is translocated by PA into the lumen of intraluminal vesicles and ends up in the MVBs. This might protect LF from being degraded. The downstream targets of LF—MEK1 and its scaffolding complex—were both shown to associate with late endosomes (Abrami *et al*, 2004).

Entering the late endosomes might pose a threat to pathogens owing to the low pH conditions and hydrolytic activity. One way to keep the pathogens safe would be to block phagosome maturation (Fig 1). G. Griffiths (Heidelberg, Germany) suggested that *Mycobacterium tuberculosis* inhibits the p38 kinase pathway, which participates in phagosome maturation. D. Russell (Ithaca, NY, USA) advocated taking into account the kinetics of the intraphagosomal lumen when studying phagosome maturation rather than using static approaches. By measuring the intraphagosomal pH, protease activity, and the proximity of labelled molecules on the bacterial surface and endocytosed fluorescent tracers using FRET, Russell challenged the model that Toll-like receptors influence phagosome maturation (Blander & Medzhitov, 2004).

Brucella makes an interesting escape from late endosomes and lysosomes by fusing with the endoplasmic reticulum shortly after traversing the early endosomal stage, as shown by J.-P. Gorvel (Marseille, France). The VirB type IV secretion system of *Brucella* is not only essential for entering the host cell but is also required for ER recruitment to the bacterial phagosome at a later stage of infection (Fig 1).

Learning to survive in host cells

Microbiologists have now uncovered the fascinating abilities of microbes to exploit cellular systems to establish infection. T. Meyer (Berlin, Germany) showed how pili-expressing *Neisseria* exploit cholesterol-rich domains on epithelial cells to form microcolonies. Caveolin is recruited to the area of bacterial adhesion. However, in caveolin-negative cells, or those with depleted cholesterol or knocked-down caveolin-1, *Neisseria* invade host cells instead of forming microcolonies at the PM. Caveolin-1 might therefore prevent cell entry but foster colonization of the epithelial surface.

The fascinating strategies that *Salmonella* use to establish themselves in cells were discussed in several talks (Fig 1). V. Koronakis (Cambridge, UK) reported that six essential *Salmonella* virulence effectors, including *Salmonella*-invasion protein A (SipA) and SipC, which are delivered by the type III secretion system to the host cell, associate with the PM (Cain *et al*, 2004). In his poster, R. Hayward from the same group showed the direct interaction of type III translocated effectors with cholesterol. Two such effectors, SipA and SipC, bind actin to

induce bundles for *Salmonella* entry. S. Meresse (Marseille, France) used the yeast two-hybrid system to show that another *Salmonella* effector protein, SifA, which is also translocated into the host cell, interacts with a 150-kDa pleckstrin-like molecule that is known as SKIP. This seems to affect kinesin activity on the cytoskeleton, which might be important for the movement, localization and stability of *Salmonella* vacuoles. As kinesin is negatively regulated by SifA, vacuoles of a SifA⁻ mutant interact more strongly with kinesin, which leads to aberrant phagosome localization in the cell, their destabilization and, ultimately, the release of *Salmonella* into the cytoplasm. As reported by M. Marsman (Amsterdam, the Netherlands) in her poster talk, the fusion of *Salmonella*-containing vacuoles with lysosomes through the GTP-binding protein rab7 effector, rab-interacting lysosomal protein (RILP), is controlled by the motor protein dynein.

G. Cornelis (New Haven, CT, USA) pointed out the structural similarity and sequence homology between the type III secretion apparatus of *Yersinia* and the flagellum hook, through which bacterial flagellae are inserted into the membrane and cell wall of Gram-negative bacteria. The type III secretion apparatus resembles a needle that protrudes from the bacterial surface and perforates the host-cell membrane to transfer bacterial effector proteins. By physically detaching these needles from the *Yersinia* surface, their length was determined to be 58 and 48 nm for *Y. pseudotuberculosis* and *Y. pestis*, respectively. In elegant experiments in which the needles (encoded by the *ycsP* gene) were exchanged between these two species or needles of different lengths were expressed, it was shown that the number of *ycsP* residues dictates needle length and, therefore, acts as a molecular ruler. The optimal length of the needles is determined by the *yadA* protein, which is an adhesin-forming microfilament that extends beyond the bacterial outer membrane. Increasing the *yadA* length increases the distance between the bacteria and the host cell, and so reduces the efficiency of effector transfer by shorter needles (Mota *et al*, 2005).

With respect to bacterial type III secretion systems, an interesting hypothesis was put forward by N. Andrews (New Haven, CT, USA). The calcium-sensing cellular protein synaptotagmin VII (SytVII) participates in the resealing of wounded cell membranes. SytVII-knockout mice are highly susceptible to *Salmonella* infection, which implies that this repair system has evolved to counteract membrane injury that is caused by the bacterial type III secretion apparatus (Roy *et al*, 2004). Calcium influx after cell wounding triggers the exocytosis of lysosomes, which prompts the appearance of lysosome-associated membrane protein 1 (LAMP1) at the PM for a short period, before it is resealed. SytVII-knockout cells showed a decreased release of lysosomal material on wounding and SytVII contributed to resealing the wounded PM.

Salmonella can also enter host cells independently of the type III secretion system. F. García-del Portillo (Madrid, Spain) described one such alternative entry route: a mutant that lacked the *Salmonella* pathogenicity island 1 (SPI-1) and, therefore, the type III secretion system, was engulfed by a zipper-like mechanism, and was sensitive to inhibitors of phosphatidylinositol triphosphate (PI3P) kinase and MEK1. This mode of uptake is different from the trigger-mediated host-cell entry that is used by wild-type *Salmonella*, which requires the type III secretion system-mediated transfer of effector proteins, as described above. Following this mode of uptake, there was a delay in the acquisition

of late endosomal markers to the *Salmonella* vacuole and intracellular colonization was not accompanied by the cytotoxicity that is observed in cells that are infected with wild-type *Salmonella*. Unlike the wild-type strain, which remains in vesicles and has attenuated growth in fibroblasts, the mutant shows no such activity. In mice, these mutants were found in non-phagocytic CD18-negative cells of the ileal lamina propria, whereas wild-type *Salmonella* were found in phagocytes.

J. Leong (Worcester, MA, USA) described the mechanism by which enterohaemorrhagic *Escherichia coli* manipulate the cytoskeleton to induce actin pedestals on epithelial cells by transporting the translocated intimin receptor (Tir) and at least one other effector, through the type III secretion system. Using a red-assisted pathogenicity island deletion (RAPID) screen, the *Escherichia*-secreted protein (Esp) F_U was shown to interact, probably indirectly, with Tir and directly with N-WASP, to promote actin assembly into pedestals. This work, by K. Campellone from the group of Leong, was awarded the second poster prize (Campellone *et al*, 2004). Another gastrointestinal pathogen, *Helicobacter pylori*, uses a type IV secretion apparatus to deliver effector proteins into the host cell. As P. Sansonetti (Paris, France) reported, it also transports peptidoglycan, which is recognized by the intracytoplasmic pathogen-recognition molecule NOD1, and this process leads to a proinflammatory host response. Consequently, *Nod1*-knockout mice are more susceptible to *H. pylori* infection than wild-type mice (Viala *et al*, 2004).

The group of M. Desjardin (Montreal, Canada) has studied phagosomes using a global proteomic approach, and has shown that phagosomal proteins and lipids are not equally distributed throughout the phagosome, but are organized in microdomains. These microdomains can be targets for pathogen-derived lipids, such as *Leishmania* lipophosphoglycan, which influence phagosome maturation. The observation that endoplasmic reticulum membranes participate in phagocytosis and bring the major histocompatibility complex (MHC) class I processing and presentation machinery to phagosomes has now been applied to vaccine development. By showing that herpes simplex virus (HSV) particles that are coupled to beads are more efficiently processed and presented by MHC class I molecules than HSV particles alone, a vaccine that uses virus-coated beads has been developed.

F. Niedergang (Paris, France) showed in her poster that late endosomal membranes also participate in phagocytosis, which indicates that membranes from various sources can be recruited for this process (Braun *et al*, 2004). When discussing antigen presentation, J. Neefjes (Amsterdam, the Netherlands) reported that connexin 43 (Cx43) translocates antigenic peptides through tight junctions between cells, thereby allowing cross-presentation to T cells (Neijssen *et al*, 2005). To interfere with a proper immune response, some viruses have highly sophisticated mechanisms to modulate the MHC class I pathway. P. Lehner (Cambridge, UK) showed that the K3 protein from Kaposi's sarcoma herpesvirus uses its really interesting new gene (RING) variant domain to promote the ubiquitylation of MHC class I molecules for their internalization and degradation.

Pathogens, parasites and the cytoskeleton

According to M. Way (London, UK), the vaccinia virus uses both actin filaments and microtubules to move out of the host cell. In the cell, newly formed viruses interact with kinesin through the envelope protein A36R and are moved by microtubules to the PM

to exit the host cell. There, the virus is not detached from the PM but uses another envelope protein, B5R, to activate the tyrosine kinase Src through an unknown host cell receptor. Src in turn phosphorylates A36R to facilitate its dissociation from kinesin. The actin-polymerization machinery of the host cell, which includes N-WASP and ARP2/3, is thereby recruited to the PM to induce actin filaments that protrude from its surface. These finger-like extensions contain the attached virus at the tip and spread the infection to a new host cell (Newsome *et al*, 2004).

Cytoskeleton-based cell motility was discussed by M.-F. Carlier (Gif-sur-Yvette, France), who provided the molecular basis for the rapid actin processes during movement that are induced by the processive motor protein formin (Romero *et al*, 2004). The FH1–FH2 domains of formin accelerate ATP hydrolysis coupled to profilin–actin polymerization. The free energy is then used to enhance the profilin–actin association 15-fold with the barbed ends of actin filaments.

Movement in the world of parasites was the topic of D. Soldati (Geneva, Switzerland), who described class XIV myosin motors, which are restricted to apicomplexan parasites, such as *Toxoplasma*. Because the deletion of one such motor protein gene, *MyoA*, produces a non-motile attenuated strain, it is believed to be responsible for the gliding motility of the parasite. Finally, D. Dobbelaere (Bern, Switzerland) introduced the strategy that the cattle parasite *Theileria* uses to immortalize T cells through nuclear factor- κ B (NF- κ B) activation, which promotes expansion of its host-cell population and, thereby, the propagation of the parasite.

In this collection of excellent talks, the remarkable interactions between microbes and their host cells provided new insights into eukaryotic cell biology. The innovative approach of combining the complementary disciplines of microbiology and cell biology proved fruitful for both sides, and will hopefully foster similar meetings in the future.

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