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A Meeting of Good Friends: When the Cell Biology of Prokaryotes and Eukaryotes Meet

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Since the time of Louis Pasteur, microorganisms and especially bacteria, served as a handy experimental system for deciphering the basic molecular mechanisms of life. Bacterial models played a pivotal role in deciphering the metabolic pathways, the mechanisms of synthesis, the role of proteins and nucleic acids and the principles of gene regulation and expression. Moreover, bacterial models served in the development of new genetic techniques and studies on bacteriophage restriction in the late sixties, yielded the *in vitro* recombinant DNA genetic technology of the seventies, which generated new biotechnologies of the eighties and literally revolutionized biomedical sciences. Fundamental research on bacteria, hence, provided the basis for an explosive progress in the understanding of molecular principles of life over the last two decades. In spite of that, in the early eighties, many biologists tended to think that bacterial cell biology is coming to the limits of what it can contribute as new concepts and discoveries. The impressive advance of eukaryotic cell biology and immunology in the eighties redirected a significant part of biological research activities and support from prokaryotic to eukaryotic models. In parallel, an almost complete control of life-threatening bacterial infections in the developed world, due to improved hygiene, antibiotics and vaccination, was achieved. This promoted the spread of the naive belief that the problem of bacterial infections had been solved. Some basic bacteriology projects even had to be proposed as biotechnological research to get funded. However, new pathogens continued to be discovered and many almost forgotten pathogens, such as *Mycobacterium tuberculosis*, are becoming a serious public health problem again, even in the developed countries. Evolution of virulence traits and acquisition of multiple antibiotic resistance, bring pathogenic bacteria back to the forefront of the biological research scene as one of the prominent players in the postantibiotic era of the upcoming 21st century. Simply, new knowledge is needed to fight old diseases.

The good news is that once again public health concern provides a new cutting edge in biological research, *viz.* the cellular microbiology (Cossart *et al.* 1996). Here two good old friends, molecular cell biology of prokaryotes and that of eukaryotes, meet again in a productive and mutually enriching way. Bacterial pathogens and the mammalian hosts have a long history of co-evolution, over which the ones devised sophisticated mechanisms to subvert the evolving and finely tuned defense of the others. Logically, a detailed understanding of the molecular mechanisms of action of bacterial virulence factors, *e.g.*, the mode of action of toxins, adhesins and other molecules and analysis of the process of pathogen-induced bacterial invasion into nonphagocytotic cells, teaches us how our own cell signals, cytoskeleton and organelles function (Finlay and Cossart 1997). In turn, analysis of bacterial interactions with host cells and mechanisms of adaptation to various environments within the host leads to the discovery of new bacterial genes and functions that could hardly be found otherwise.

The last decade was a period of unparalleled accumulation of knowledge and understanding of the mechanisms underlying this fascinating host–pathogen cross-talk.

In order to summarize and to promote another cross-talk, namely communication between good friends, specialists in molecular and cellular microbiology of bacterial pathogens, with the general microbiological audience, the *Czechoslovak Society for Microbiology* organized the 1st Minisymposium on Cellular Microbiology. It was held at the *Institute of Microbiology, Academy of Sciences of the Czech Republic*, in Prague, on October 6th 1997 and focused on cell biology of host–pathogen interactions and signaling. Ten of the invited lectures given by outstanding researchers in the field, are collected in this special issue of *Folia Microbiologica.* A digest of the enclosed reviews is given below, highlighting the major achievements presented at the meeting.

The common theme of the lectures (reviews) was bacterial signaling and subversion of host cell functions by virulence mechanisms inducing cytoskeleton rearrangements, organelle dysfunction and impairment of bactericidal functions. The meeting was launched by P. SANSONETTI, reporting on the molecular and cellular aspects of bacillary dysentery, a *Shigella* infection accounting annually for about 600 000 deaths. The cell biology of *Shigella* infection is now being studied in fascinating molecular detail (Sansonetti 1998). The bacteria pass from the colorectal mucosa into the subepithelial area *via* M-cells of Peyer's patches and escape the

awaiting macrophages by inducing their apoptosis. To generate this complex weapon, the type III secretion pathway is used (see two other reviews of this issue) to inject Ipa protein effectors into cells. One of them, IpaB, appears to activate the macrophage interleukin 1b (IL-1b)-converting enzyme ICE (caspase 1), thereby routing the macrophage towards programmed cell death and IL-1b release starting an inflammatory process. Inflammation eventually leads to permeabilization of the epithelial barrier and passage of more bacteria into the subepithelial area, where *Shigella* invade polarized epithelial cells from the basolateral side by the "trigger" mechanism of macropinocytosis. Ipa-mediated signaling induces recruitment of the cortactin-phosphorylating protooncogene $pp60^{c\text{-}src}$ kinase and of the stress cable formation-controlling small GTPases of the Rho family, at the site on the host cells where an entry focus forms. This resembles in its composition the cell adhesion plaques and massive rearrangements of the cell cytoskeleton, dominated by actin polymerization, are initiated. Filopodia then extend from the entry focus, forming membrane ruffles in which the bacterium is trapped and engulfed by the normally nonphagocytotic epithelial cells. Invasion is followed by the IpaBC-mediated escape of bacteria from the membrane-bound vacuole, their proliferation within cells and bacterial cell-to-cell spread within the epithelial layer. For this, *Shigella* subverts once again the cell functions to move within them. At one pole of the bacterial cell, decorated by the IcsA protein, the actin polymerization apparatus is recruited and the bacteria are propelled from cell to cell within the epithelial layer on the tip of polymerizing F-actin comet tails inside membrane bound protrusions. Upon their endocytosis by the neighboring cells, *Shigella* escapes from the organelle surrounded by three membrane bilayers for a new cycle of proliferation in a new cell, bypassing the antibody response.

The other paradigm of an intracellular pathogen, the Gram-positive *Listeria monocytogenes*, exploiting induced host cytoskeleton rearrangements and actin tail propulsion for cell-to-cell spread in its life cycle, was presented by yet another pioneer of cell microbiology, P. COSSART (Cossart 1998). Invasion of *Listeria* into cells differs from the spectacular membrane ruffle-mediated macropinocytosis of *Shigella* and uses a more subtle "zipper" mechanism, where *Listeria* push the cell membrane until it surrounds the bacterium. The first mechanistic features of this process are now being unraveled. Two surface-located bacterial proteins are involved in inducing this invasion, the InlB protein interacting with an unknown receptor, and an internalin, interacting with host-cell E-cadherin, which normally mediates calcium-dependent cell-to-cell adhesion. On the host cell side, intact cytoskeleton and receptor-mediated tyrosine phosphorylation of membrane proteins are required for the invasion. Upon an uncharacterized receptor stimulation event, the P85/P110 PI-3 kinase is recruited by the phosphotyrosine-containing membrane proteins. This kinase appears as a key player in *Listeria* entry as it can interact with pp125FAK and Rho GTPases controlling actin cytoskeleton and possibly recruiting the cell components of the endocytotic pathway. In addition, the kinase generates the PI-3-P, PI-3,4-P_2 and PI-3,4,5-P_3 phosphoinositides which may, at elevated concentrations, facilitate endocytosis by affecting the membrane lipid bilayer curvature and which are able to uncap the barbed ends of actin filaments, potentially driving local cytoskeletal changes needed for the entry. In addition, the phosphoinositides may attract binding proteins with PH domains to the site of bacterial entry. Upon entry and vacuolar lysis mediated by the pore-forming toxin listeriolysin O and a phosphatidylinositol-specific phospholipase C, *Listeria* proliferates in the cytosol and moves from cell to cell in a manner similar to *Shigella*, on the tip of an actin tail polymerizing at one pole of the bacterium covered by the ActA protein. The bacterial system can be manipulated and studied more easily and a combination of immunofluorescence and videomicroscopy techniques, with purification of the actin polymerization complex and *in vitro* reconstitution of *Listeria* ActA-dependent actin-based mobility in cell extracts, was employed. This makes it now possible to define the composition and the mechanics of the machine. It appears that bacterial ActA binds the host cell protein VASP which itself binds another host protein, profilin, and together they recruit a complex of eight polypeptides, containing also villin, ezrin, talin, fimbrin, cofilin, tropomyosin and α-actinin, which initiates actin polymerization. The molecular basis of the actin-based *Listeria* propulsion at the tip of the growing actin tails is now being studied in detail because it is believed to reflect the same mechanisms as the movement of neutrophiles to the site of attraction or movement of cancer cells.

Actin polymerization-mediated movement is also crucial in the infectious cycle of many viruses; M. WAY demonstrated that on the example of vaccinia virus interactions with actin cytoskeleton (Way 1998). Rearrangements of actin cytoskeleton occur during vaccinia infection and actin tails strikingly similar to those seen in *Listeria*, *Shigella* and *Ricketsia* infections are formed. Intracellular, enveloped, vaccinia virus particles are propelled to the tip of these tails within cells and from cell to cell. Despite the apparent similarity of the actin tails, the vaccinia system of actin nucleation is different from the bacterial ones. The protein(s) involved in vaccinia tail nucleation remain unknown, but the nucleating membrane surface surrounding the virus appears to be derived from *trans*-Golgi network of the host cell and the nature of the nucleation complex probably reflects more truly the events occurring at the leading edge of a moving cell. The virus encodes, however, its one profilin and four other proteins similar to the actin-bundling host protein, scruin. The experimental system of vaccinia-induced actin tail formation is now being intensely used

as a model for dissection of actin-membrane interactions in the cell.

G. CORNELIS and H. WOLF-WATZ summarized their pioneering work on the plasmid-encoded Yop virulone, which is now becoming a paradigm of bacterial sophistication in manipulating host cells (Cornelis 1998; Falman *et al.* 1998). Its operation mode accounts, at least in part, for the unparalleled pathogenic potential of such natural-born killer, as the plague infectious agent *Yersinia pestis*. When yersinias establish an intimate attachment to the host cell, they turn on the setup of a complex dedicated type III secretion apparatus, made of some 22 proteins, whose molecular details of function are now being unraveled. It functions as a contact-dependent injection device which forms a path across both the bacterial cell envelope and the host cell membrane and delivers into the host cell cytosol the Yop effector proteins. These include an actin-depolymerizing cytotoxin (YopE), a eukaryote-like tyrosine phosphatase (YopH), a eukaryote-like serine-threonine kinase (YpkA), a host-transcription-modulating factor, suppressing IL-8 release (YopJ or YopP) and a protein of unknown mechanism of action (YopM). Coordinated intracellular action of these effector proteins subverts the target cell signaling networks and cytoskeleton organization, thereby allowing yersinias to tune down the early immune response, such as IL-8 release, to escape killing by phagocytosis and to proliferate in the lymphoid tissues. Analogous type-III secretion machinery for delivery of bacterial effector proteins into host cells is now being characterized and appears to play a key role in the virulence of a variety of pathogenic Gram-negative bacteria, including *Pseudomonas*, *Salmonella*, *Shigella*, and *Escherichia*.

This was documented by I. ROSENSHINE (Nisan *et al.* 1998), describing the molecular and cellular aspects of enteropathogenic *Escherichia coli* (EPEC) cross-talk with the host's intestinal epithelial cells. EPEC remains one of the leading causes of neonatal diarrhea and mortality in the developing world. Upon initial binding to the apical side of polarized epithelium *via* bundle-forming type-IV pili and formation of microcolonies, the EPEC bacteria deploy an arsenal of effectors delivered *via* the type-III secretion pathway into the epithelial cell. As a result, multiple signaling events take place, including calcium fluxes, phosphoinositol (PI_3) generation, serine/threonine phosphorylation and accumulation of translocated and tyrosine-phosphorylated bacterial intimin receptor beneath the cell membrane. Consequently, the actin filaments in the brush-border microvilli are depolymerized and the microvilli are effaced, resulting in decreased water and nutrient absorption capacity of the epithelium and severe diarrhea. The EPEC establish an intimate cell attachment, where only 10 nm separate the bacterial outer membrane from the host cell membrane and beneath the zone of attachment is a spectacular actin stalk, polymerized from actin filaments and actin binding proteins. This then propels the attached EPEC on an extending pedestal up to 10 μm above the cell surface into the gut lumen.

A quite different multi-faceted infectious model of *Neisseria*, which is heavily relying on cellular cross-talk, was presented by T.F. MEYER (Meyer 1998). Neisserias are pathogens apparently lacking exotoxins and their immune response escape under conditions of the infection is due to the production of a protective capsule, serum resistance and an exemplary capacity of antigenic variation of the surface proteins, such as pili and Opa. Besides that, a number of signaling mechanisms is currently being unraveled, which underlie the passage of meningococci and gonococci across the mucus membrane, entry into the host's epithelial cells, dissemination in the host, inhibition of the oxidative burst response and survival within professional phagocytes. These processes involve a primary pilus-mediated adhesion of the bacterium to host cells which allows a complex scheme of signaling involving Opa protein interaction with heparan-sulfate proteoglycans (the glypicans and syndecans) and CD66 receptors of the immunoglobulin superfamily. These interactions lead to activation of host signaling cascades involving calcium fluxes, phospholipase C and protein kinase C activation, eventually reaching the p21-activated kinase and the Jun-N-terminal kinase, which transmit stress signals to the host cell and its nucleus. A newly discovered *Neisseria* virulence mechanism involved in dramatic inhibition of oxidative burst response is emerging. It appears to be due to the capacity of *Neisseria* outer membrane porin PorB to translocate into membranes of human target cells. There it could operate as a nucleoside triphosphate-gated membrane channel altering cellular signaling. Moreover, PorB appears to account for a dramatic inhibition of the main component of oxidative burst in phagocytes, by specifically inhibiting the release of myeloperoxidase from secondary granules into the phagosome.

A new mode of toxin action was presented by E. PAPINI (Papini *et al.* 1998). In collaboration with the group of R. RAPPUOLI, Papini and colleagues have defined the action of the vacuolating cytotoxin VacA from the gastroduodenal ulcer and adenocarcinoma-provoking agent *Helicobacter pylori*. The biochemical basis of VacA action remains unknown. However, Papini and colleagues showed that it acts in the cytosol of host cells and causes the formation of large membrane-bound vacuoles derived from vesicles of the late endocytotic pathway, probably by inhibiting their acidification. These vacuoles resemble a hybrid compartment containing elements of late endosomes and lysosomes and their formation affects protein sorting and trafficking across this crucial cross-point. VacA action may, hence, have consequences for antigen processing and presentation in the infected tissue. This raises the possibility that VacA action might prevent antigen presentation, leading to reduced activation of specific T-cells in gastric mucosa, thereby contributing to *H. pylori* colonization. Furthermore, local suppression of antigen presentation might allow an

escape of transformed host cells from the surveillance of the immune system, thereby promoting formation of gastric carcinomas and lymphosarcomas which are associated with chronic infections by toxinogenic *H. pylori*.

The genetic setup required for production of active VacA by *H. pylori* was described by R. RAPPUOLI (Rappuoli *et al.* 1998). It was found that only type I strains of *H. pylori*, expressing the cytotoxin-associated antigen (CagA), are able to produce active vacuolization-inducing VacA and cause severe gastroduodenal disease, while the type II strains cause only a mild gastritis. Around the gene for CagA (*cagA*) on type I *H. pylori* chromosomes a 40-kb pathogenicity island (PAI) was found, containing a cluster of approximately 40 genes absent in type II strains. These genes resemble those encoding the type IV secretion machineries, involved in membrane translocation of large macromolecular complexes, such as transfer of the virulence plasmid DNA into plant cells by tumorigenic *Agrobacterium tumefaciens* or pertussis toxin secretion by the human whooping cough agent *Bordetella pertussis*. Moreover, the PAI contains genes resembling other known virulence factors and specific mutations in genes encoded in the PAI abolish many of the *in vitro* toxic activities of *H. pylori*, such as induction of IL-8 and protein tyrosine phosphorylation in host cells. It appears, hence, that the *cagA*-containing pathogenicity island of *H. pylori* encodes at least a part of a virulon, comprising effector proteins and an apparatus for their delivery into host cells.

The effects of a number of virulence factors from bacterial pathogens on host cell cytoskeleton are now being tracked down to the molecular level and P. BOQUET reported on the amazing new mechanism of action, employed by a recently identified family of dermonecrotic toxins from pathogenic strains of *Pasteurella*, *Bordetella* and *Escherichia* (Boquet 1998). One of them, the cytotoxic necrotizing factor (CNF1) of *E. coli*, was now shown to be a highly specific glutamine deaminase enzyme that converts the glutamine 63 of a small cytosolic GTP-binding protein Rho into glutamate. This biochemical "mutation" of the Rho protein, which is one of the key players in cellular regulation of cytoskeleton assembly, leads to its permanent activation and results in a multitude of effects, ranging from F-actin rearrangements, stress fiber formation, multiplication of focal contact points, block of cytokinesis, effacement of microvilli of intestinal brush-border cells, membrane ruffling, macropinocytosis and impairment of polymorphonuclear cell transmigration across the epithelial layer. These effects contribute to the pathogenesis of certain *E. coli* infections. Obviously, CNF1, together with other cytoskeleton-affecting toxins, such as clostridial exoenzyme C3 and toxin B, is now becoming a fine tool for dissection of the fundamental Rho-dependent cellular processes. Moreover, the mode of CNF1 action on Rho suggests that certain infectious processes might use similar mechanisms to induce tumors.

As documented in the above report, at the end of the exciting day of 6 October, 1997, the participants of the meeting were leaving for dinner with the firm conviction that bacteria still have a lot to teach us about ourselves. We hope that this conviction will be promoted further by the readers of this special issue of Folia Microbiologica.

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