port, Sanchez *et al.* demonstrate that the damage-dependent phosphorylation changes to Rad53 are lost in *mec1* mutant cells and can be restored by overexpression of the functionally overlapping *TEL1* gene (*4*).

In mammalian cells, a similar analysis of the stabilization of p53 (a biochemical marker for the G_1-S checkpoint) has indicated that the ATM protein functions in either a detector or signaling pathway that is upstream of the p53 effector for the G_1-S checkpoint (*10*). Furthermore, the involvement of ATM but not p53 in the G_2-M checkpoint indicates that the ATM pathway sends signals to distinct effectors for each cell cycle transition. Walworth and Bernards' data indicate that Chk1 is a cell cycle transition–specific effector at mitosis for the Rad3-dependent DNA damage checkpoint pathway. The data of Sanchez *et al.* show that Rad53, which has a more pleiotropic influence on several checkpoints, probably acts downstream of Mec1 and Tel1 in a pathway that signals to the different cell cycle transition–specific effectors such as Chk1. No checkpoint genes are identified as lying downstream of Chk1, consistent with its role as an effector. However, the data of Sanchez *et al.* suggest that *S. cerevisiae* Rad9 may be downstream of Rad53 because Rad9 does not appear to influence Rad53 phosphorylation. This result amply demonstrates the utility of a biochemical assay for the checkpoints because Rad9, along with Rad17 and Rad24, is involved in the processing of single-strand breaks into regions of single-stranded DNA. This property of Rad9 led to the suggestion that it is involved in the initiation of the checkpoint signal (*11*). But the new data suggest that the relation between checkpoint signal initiation and lesion processing may be more complicated.

The *ATM* gene encodes a human checkpoint protein related to Rad3 and Mec1; it is likely that human homologs of Chk1 and Rad53 also exist and that their activity will be influenced by the ATM pathway. The identification of a biochemical marker for checkpoints by Walworth and Bernards (*3*) and Sanchez *et al.* (*4*) will allow the analysis of the DNA structure checkpoints and their upstream activators in human cells, where genetic approaches are not available.

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Cellular Microbiology Emerging

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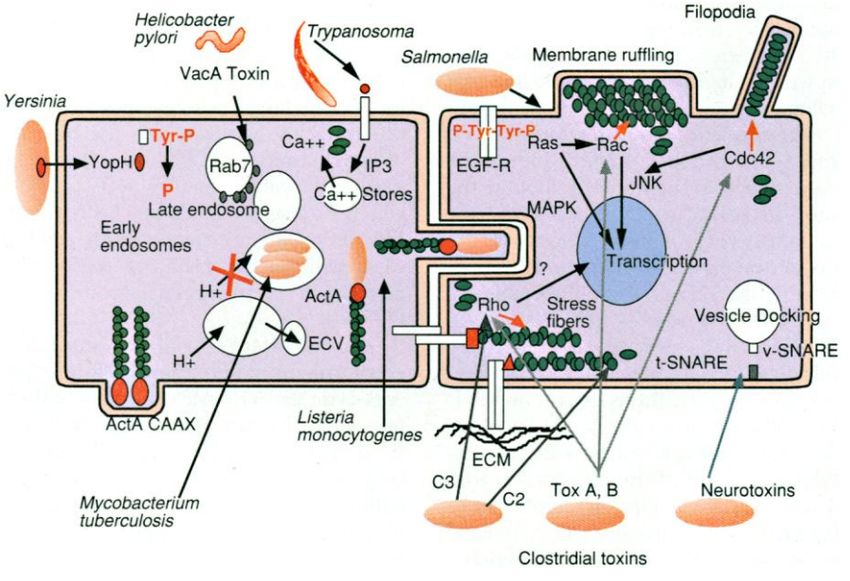
A new discipline, cellular microbiology, is emerging at the interface between cell biology and microbiology. Traditional cell biological approaches are already widely used to unravel the tactics microbes utilize to infect their hosts, but the use of pathogens to tackle questions in cell biology is just now yielding promising approaches and elegant results. Two meetings, in 1989 and 1991 (*1*), laid the groundwork for the field, and a third meeting in 1995 highlighted recent progress (*2*).

A major focus of this new field is the actin network, which together with intermediate filaments and microtubules constitute the cytoskeleton. The rapid assembly and disassembly of actin microfilaments is essential for phagocytosis, motility, cell division, and adhesion to a substratum or to another cell. Yet, the signaling pathways that control actin dynamics are poorly understood. Bacteria that can be genetically manipulated and parasites can provide tools to dissect these control pathways. When certain bacteria, such as *Salmonella* and *Shigella*, infect cells, they mimic the action of epidermal growth factor (EGF), inducing membrane ruffling and active actin polymerization (*3*–*5*) (see figure). The ruffling leads to internalization of the bacteria.

The internalization of other pathogens occurs without membrane ruffling or even actin polymerization. The parasite *Trypanosoma cruzi* enters cells by triggering a combination of events—a transient increase in cytosolic free calcium, rapid rearrangement of the cortical actin cytoskeleton, and lysosome recruitment and clustering at the invasion site (*6*, *7*). Lysosomes contribute membrane for the formation of the parasitophorous vacuole. Disruption of cortical actin by the increase in local calcium allows lysosomes to migrate and fuse, a phenomenon also regulated by calcium. Phospholipase C



Pathogenic bacteria interfere with numerous eukaryotic cell functions, providing a sophisticated tool kit for cell biologists.

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(PLC) and inositol 1, 4, 5-trisphosphate (IP_3) formation are responsible for the calcium release from intracellular stores and the subsequent microfilament rearrangement under the plasma membrane. Thus, *T. cruzi* can be used to probe PLC (phosphoinositide) and calcium control of cytoskeleton rearrangements and subsequent downstream events in mammalian cells.

Several intracellular bacteria (*Listeria*, *Shigella*, and *Rickettsia*) induce actin polymerization at one pole to generate movement (*8*). This process is similar to the formation of lamellipodia at the leading edge of moving cells and to the formation of microvilli during development. In the bacteria, a surface protein—the ActA protein of *Listeria* and IcsA of *Shigella*—acts to nucleate actin polymerization. The search for mammalian homologs of these two nucleating proteins is now underway. Expression of ActA at the inner face of the eukaryotic plasma membrane induces membrane extensions and cell shape changes (*9*). Clearly, motile bacteria have much to teach mammalian cell biologists.

Clostridia and some *Escherichia coli* strains produce toxins that alter the microfilament network. The toxins either directly cap the barbed ends of actin filaments (for example, the C2 toxin of *Clostridium botulinum*) or interfere with a signaling cascade containing small guanosine triphosphate (GTP)–binding proteins, namely Rho, Rac, and Cdc42 (for example, toxins A and B of *Clostridium difficile*, C3 of *C. botulinum*, and CNF of pathogenic *E. coli*). The specificity of some of these toxins (C3 acts on Rho but not on Rac or Cdc42 in vivo) make them valuable tools for dissection of the pathways regulated by the Rho family members, which control various cytoskeleton rearrangements (*10*, *11*). Tight junctions are disrupted upon treatment with *C. difficile* toxins, indicating that Rho family members also participate in cell-cell adhesion and tissue formation (*12*). Toxins A and B from *C. difficile* induce the glycosylation of a threonine residue in the effector domain of the Rho guanosine triphosphatase (*13*). The specificity of the toxins' action is puzzling because this threonine is highly conserved in all members of the five classes of small GTP-binding proteins (Ras, Rab, Arf, Ran, and Rho). Zonula occludens toxin (ZOT), a toxin produced together with cholera toxin by *Vibrio cholerae*, also disrupts tight junctions of intestinal epithelium, albeit by a mechanism different from that of the *C. difficile* toxin. ZOT increases the amount of protein kinase C–α in the cytosol, leading to cytoskeletal rearrangements mediated by phosphorylation (*14*).

The inositol phospholipid pathway, like the cyclic AMP pathway, is under the control of large heterotrimeric G proteins, which interact with receptors when they are stimulated by ligand. The functions of these G proteins have long been addressed through the use of bacterial toxins. The two that have proved most useful are pertussis and cholera toxin, two nicotinamide adenine dinucleotide–ribosylating enzymes secreted by *Bordetella pertussis* and *V. cholerae*, the bacteria responsible for whooping cough and cholera (*15*).

When some bacteria—*Shigella*, *Salmonella*, *E. coli* (*EPEC*), and *Yersinia*—interact with mammalian cells, they secrete proteins or directly inject proteins into the mammalian cell by a recently discovered, type III secretion system (*16*). These proteins often aid in the establishment of a succesful infection by disrupting the cellular cytoskeleton or internal communications.

Toxins have also been useful in dissecting intracellular compartments. The discovery of the mode of action of the clostridial neurotoxins—proteolysis—and their targets— SNAP-25, VAMP/synaptobrevin, and syntaxin (*17*)—has been crucial for the vSNARE-tSNARE model for the docking of vesicles to membranes. This work has been a breakthrough in the understanding of neurotransmission and in solving the riddle of intracellular compartment formation.

Helicobacter pylori, the bacterium responsible for stomach ulcers, produces an intriguing toxin, VacA. This toxin provokes the formation of vacuoles coated with large quantities of Rab7, a small GTP-binding protein thought to be involved in the homotypic fusion of late endosomal compartments. Accumulation of Rab7 on the vacuoles suggests that a downstream effector of Rab7, probably one controlling membrane fusion, is the target of this *H. pylori* toxin (*18*). VacA may therefore be a tool for another fast-moving field of cell biology, the endocytic pathway and the regulation of vesicle trafficking. Likewise, the study of apparently stable vacuoles, such as those in which *Toxoplasma gondii* or a bacterium like *Mycobacterium tuberculosis* reside and replicate, will further facilitate the study of maturation of intracellular compartments (*19*, *20*).

An important signal for intracellular compartment maturation, the formation of endocytic carrier vesicles, or the ability to fuse with other compartments is their acidification by vacuolar type H^+–adenosine triphosphatases, which are sensitive to bafilomycin A1 (*21*). Some pathogens may inhibit vacuolar acidification to block compartment maturation and allow their own long-term residency within the cell.

A final area of cell biology in which pathogens are proving instructive is the signaling pathways linking external cues to nuclear transcription, resulting in cell differentiation or cell division. Here again, *C. botulinum* C3 toxin has been instrumental in finding that Rho family members, in addition to regulating the cytoskeleton, also operate in mitogen-activated protein kinase (MAPK) cascades and the control of transcriptional activity (*22*). Whereas the cytoskeletal effects of Rac and Cdc42 are distinct, both proteins activate the same kinase cascade, the c-Jun kinase (JNK) cascade. Rho does not regulate the JNK pathway; rather, it is required for signaling to the serum response factor (SRF) by serum, lysophosphatidic acid, and external agents that act through a pertussis toxin–sensitive heterotrimeric G protein. Because the next decade will see intense study of the regulatory mechanisms controlling coupling between cell division and cell shape, there is no doubt that the Ras and Rho guanosine triphosphatases and their inhibitors or activators produced by pathogens will also be important players in this field.

This new discipline—cellular microbiology—gains its creative power from the coevolution of mammalian cells and microbes, which has ensured that virulence factors are extremely well adapted to the study of mammalian cells and tissues.

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