Exploiting host microtubule dynamics: a new aspect of bacterial invasion

Sei Yoshida[1,2] and Chihiro Sasakawa[1]

[1]Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, 4-6-1, Shirokanedai, Minato-Ku, Tokyo 108-8639, Japan
[2]PRESTO, Japan Science and Technology Corporation (JST), Japan

During infection, many pathogenic bacteria modulate the actin cytoskeleton of eukaryotic host cells to facilitate various infectious processes such as the attachment to or invasion of epithelial cells. Additionally, some pathogenic bacteria are capable of modulating the dynamics of host microtubule (MTs). Although the molecular basis for this is still poorly understood, a recent study of the *Shigella* VirA effector protein, which is delivered via a type III secretion system, suggests that MT destabilization plays an important role in *Shigella* infection.

During the process of infection, many pathogenic bacteria have the ability to remodel the structure of the actin cytoskeleton within the host cell. This ability facilitates bacterial attachment to, or invasion of, host cells, intra- and intercellular trafficking of bacteria and evasion from macrophage killing [1]. In addition to this pivotal activity, some pathogenic bacteria can also modulate the dynamics of microtubules (MTs) present in the host cell [2]. Each MT is composed of rows of tubulin molecules; the length of MTs can be altered by the addition or removal of tubulin from one or both ends. MT networks constitute a major component of the cytoskeleton in most eukaryotic cells, and play essential roles in chromosome segregation during mitosis, organelle movement, stimulating signal transduction and modulating actin dynamics [3].

Although the role of MT dynamics in bacterial infection varies between different bacterial species (Table 1), and the molecular basis of any role remains largely unclear, a recent study of the *Shigella* effector protein VirA has provided the first clues to how bacteria can modulate eukaryotic MT dynamics, a prerequisite for stimulating *Shigella* entry into epithelial cells [4]. Here, we describe several bacterial infection processes that exploit host MT networks, and then focus on *Shigella*-directed MT instability.

The role of MTs in establishing infection

MT-dependent internalization

The internalization of most pathogenic bacteria by epithelial cells is dependent on the rearrangement of the actin cytoskeleton, however, it has been indicated that the entry of some pathogenic bacteria into host cells is dependent on host MT structures. Although the molecular basis of bacterial invasion of epithelial cells is poorly understood, the ability to infect cultured epithelial cells is decreased by the pharmacologically induced destabilization of host MTs. In fact, invasion of epithelial cells by *Citrobacter freundii* [5], *Campylobacter jejuni* [5], *Haemophilus influenzae* [6], *Klebsiella pneumoniae* [7], *Listeria monocytogenes* [8], *Mycoplasma penetrans* [9], *Neisseria gonorrhoeae* [10], *Porphyromonas gingivalis* [11] and *Vibrio hollisae* [12] was reported to be strongly inhibited when the host cells were treated with an MT-destabilizing agent such as nocodazole.

Kopecko and co-workers have shown that *C. jejuni* strain 81-176 enters epithelial cells via an MT-dependent, cytochalasin-D-insensitive internalization process (cytochalasin D is an actin depolymerizing agent) [5,13,14]. In addition, they demonstrated that the invasion of cultured monolayers of INT407 or Caco-2 cells by the 81-176 strain was markedly decreased when the host cells were treated with the MT-depolymerizing agents nocodazole, colchicine or demecolcine [5]. Immunofluorescence microscopy revealed that *C. jejuni* 81-176 binds initially to the tips of host cell membrane protrusions, which are formed by localized disruption of actin and extension of MTs. During the

Table 1. Bacterial infection and MT dynamics[a]

Species	Ref.
Bacterial invasion inhibited by MT destruction	
Campylobacter jejuni	[5]
Citrobacter freundii	[5]
Haemophilus influenzae	[6]
Klebsiella pneumoniae	[7]
Listeria monocytogenes	[8]
Mycoplasma penetrans	[9]
Neisseria gonorrhoeae	[10]
Porphyromonas gingivalis	[11]
Vibrio hollisae	[12]
Bacterial invasion stimulated by MT destruction	
Actinobacillus actinomycetemcomitans	[17]
Salmonella enterica sv. Typhi	[5]
Shigella flexneri	[4]
Bacterial trafficking associated with MT networks	
Actinobacillus actinomycetemcomitans	[17]
Campylobacter jejuni	[5]
Chlamydia trachomatis	[18]
Orientia tsutsugamushi	[19]

[a]Abbreviation: MT, microtubule.

Corresponding author: Chihiro Sasakawa (sasakawa@ims.u-tokyo.ac.jp).

http://timi.trends.com 0966-842X/03/$ - see front matter doi:10.1016/S0966-842X(03)00023-4

invasion process, as they are being surrounded by membrane vacuoles the bacteria align in parallel with the MTs, before finally moving towards the perinuclear region of the host cell [13].

The endocytosis of *C. jejuni* 81-176 is thought to be powered by dynein, an MT-associated motor protein that migrates to the minus (i.e. non-growing) ends of MTs, which are orientated towards the MT-organizing center (MTOC) in the perinuclear region of host cells [15]. When dynein activity is inhibited by orthovanadate, the invasive efficiency of *C. jejuni* 81-176 is greatly decreased, suggesting that this molecular motor is involved in the bacterial invasion of, and vacuole trafficking in, epithelial cells [13]. Recently, it was reported that the *Campylobacter* protein Cjp29, which is secreted via a type IV secretion system, was required for *C. jejuni* invasion of eukaryotic cells [16]. Whether Cjp29 is involved in the MT-dependent cell entry phenomenon remains an open question; however, a protein or proteins secreted via the type IV system could fulfill this role.

MT-dependent intracellular movement

Intracellular movement of other pathogens including *Actinobacillus actinomycetemcomitans* [17], *Chlamydia trachomatis* [18] and *Orientia tsutsugamushi* [19] appears to be MT- and dynein-dependent. The family *Chlamydiaceae* comprises the obligate intracellular pathogens [20] *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *Chlamydia psittaci* and *Chlamydia pecorum*. *C. trachomatis* is responsible for major diseases of the eye and genitourinary tract in humans and also causes respiratory infections. The life cycle of the Chlamydiae involves two morphologically distinct forms, the infectious form, elementary bodies (EB) and the reproductive form, reticulate bodies (RB). EBs are non-dividing, osmotically stable cells that attach to and invade eukaryotic target cells. Following internalization, EBs become localized in the host cytoplasm, and are surrounded by membranous endosomes; EBs then differentiate into RBs. During this stage, *C. trachomatis*-containing membrane-bound vesicles have been shown to co-localize with the MT network [18]; the vesicles are then redistributed along the network of MTs. Dynein has been shown to co-localize with perinuclear aggregates of *C. trachomatis* [18] and, in agreement with this observation, it has been shown that inhibiting dynein activity had serious effects on *Chlamydia* infectivity [18].

O. tsutsugamushi, another obligate intracellular pathogen, enters host cells by inducing phagocytosis but, unlike *Chlamydia*, it can disrupt the phagocytic membrane and escape to the cytoplasm, where it can multiply [19]. Using immunofluorescence microscopy in the presence or absence of colchicine or nocodazole, the position of the pathogen was shown to be dependent on MT networks [19]. When dynamitin, a component of the dynein-associated dynactin complex, which is required for MT-dependent minus-end-directed transport processes, [21], is overexpressed in *O. tsutsugamushi*-infected host cells, bacterial movement towards the MTOC is inhibited. This observation suggests that *O. tsutsugamushi* uses a dynein–dynactin complex for intracellular movement [19].

MT-dependent movement has also been observed in the trafficking of some bacterial effector proteins such as YopM and $IpaH_{9.8}$, which are secreted by *Yersinia pestis* [22] and *Shigella flexneri* [23], respectively. Both YopM and $IpaH_{9.8}$ are members of the leucine-rich-repeat protein family secreted by type III secretion machinery. Their movement along host MT networks towards the perinuclear region is an important prerequisite for translocation into the nucleus, as indicated by the fact that destruction of the MT networks by nocodazole blocks nuclear accumulation [22,23]. Intracellular trafficking of these proteins appears to occur in the absence of vesicles, suggesting that they associate directly with an MT motor protein.

Although the molecular basis of bacterial (and effector protein) movement along the MT network remains to be elucidated, this process is important for establishing infection. In addition, the study of bacterial movement along MT networks will also provide a useful tool to help us understand the trafficking of membranous and non-membranous organelles in mammalian cells.

MT-dependent cell-to-cell spreading

A. actinomycetemcomitans can adhere to and invade epithelial and endothelial cells. This ability is believed to be crucial for this pathogen to evade the host defense response and disseminate from the initial site of infection [24]. It has been shown that *A. actinomycetemcomitans* (strain SUNY 465) enters epithelial cells, escapes from the phagocytic vacuole and eventually spreads intra- and intercellularly via an MT-dependent mechanism and the formation of intercellular protrusions [17]. Unlike *Shigella* cell-to-cell spreading, internalized *A. actinomycetemcomitans* seem to be released from infected cells into the medium via the formation of membrane protrusions. Although the mechanism is unclear, when the host cells are treated with colchicine, *Actinobacillus* invasiveness is enhanced to a level three- or fourfold greater than that of the non-treated control [17]. Immunofluorescence microscopy shows that the extending protrusions that mediate bacterial spreading contain an MT structure, and that the pathogen associates with the plus ends (i.e. growing ends) of the MTs [17]. Thus, *Actinobacillus* is presumed to express and secrete factor(s) that can associate with the MT networks, although nothing is yet known about the bacterial proteins or the MT-associated host factors involved in bacterial entry or cell-to-cell spreading.

MTs and *Shigella*

Shigella spp. deliver a subset of effector proteins into eukaryotic host cells via a type III secretion system. These effectors stimulate host cell signal-transduction pathways to modulate the dynamics of host actin to allow *Shigella* invasion of epithelial cells [25]. The invading bacterium is surrounded by a phagocytic vacuole; however, it immediately disrupts the vacuolar membrane to escape into the cytoplasm. Once in the cytoplasm, the bacterium can multiply and move by inducing actin polymerization at one pole of the bacterial cell. Interestingly, a confocal microscopic examination of the cytoskeletal architecture proximal to invading *Shigella* revealed that the local MT networks beneath the protruding membrane ruffles

undergo a remarkable process of destruction [4]. This finding, together with a marked increase in the ability of *Shigella* to invade epithelial cells after treatment with MT-destabilizing agents, suggests that the bacteria can modulate the dynamism of both actin and tubulin [4].

Shigella VirA and MTs

Shigella potentially secretes >20 effectors, including IpaA, IpaB, IpaC, IpgD and VirA, via a type III secretion system, and these proteins have been shown to contribute to stimulating the uptake of bacteria by epithelial cells via the modulation of actin [25–32]. VirA, a 45-kDa protein comprising 401 amino acids, has recently been shown to trigger MT instability *in vitro* and *in vivo*, leading to stimulation of the activity of the Rho GTPase Rac1 and membrane ruffling [4,32]. VirA can bind αβ-tubulin dimers but not MTs stabilized using an MT-stabilizing drug such as Taxol, suggesting that VirA can specifically interact with tubulin heterodimers [4]. In an *in vitro* tubulin-polymerization assay, purified VirA inhibited the polymerization of tubulin and stimulated MT destabilization [4] (Fig. 1). Interestingly, the region of VirA involved in interaction with tubulin heterodimers (residues 224–315) shares significant (21%) amino acid identity with a region of EspG, a protein which is encoded by the *espG* gene present at the locus of enterocyte effacement (LEE) in enteropathogenic *Escherichia coli* (EPEC) [33] and enterohemorrhagic *E. coli* (EHEC). Homology is also shared with other, uncharacterized bacterial proteins including NMB0928 from *Neisseria meningitidis* and Cj1457c from *C. jejuni*. Intriguingly, the expression of EPEC EspG in a *Shigella virA* mutant can rescue the decreased invasiveness phenotype, suggesting that the function of EspG is similar to that of VirA [33].

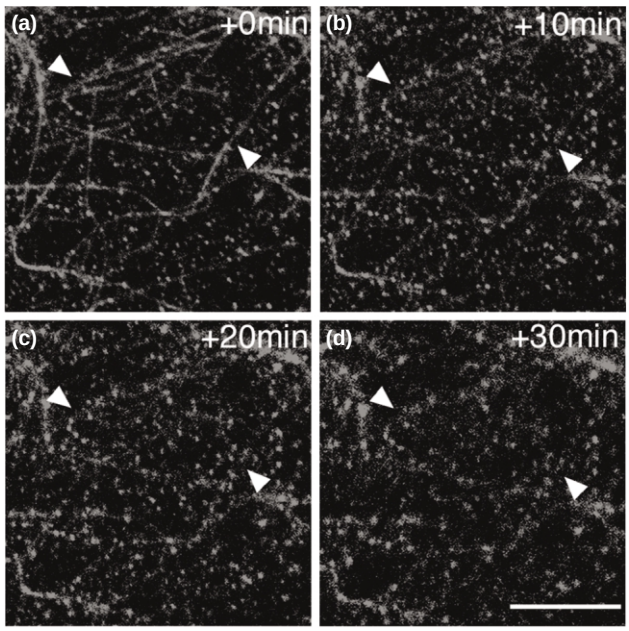

Fig. 1. VirA-induced destabilization of X-rhodamine-labelled microtubules (MTs). The X-rhodamine-labelled MTs almost completely disappeared when incubated with VirA for 30 min (arrowheads), but was still intact when incubated for 30 min with PM buffer alone (not shown). Scale bar = 10 μm.

The expression of VirA in mammalian cells such as HeLa, COS-7 and Swiss3T3 cells induces the formation of membrane ruffles [4]. Indeed, microinjection of purified VirA into HeLa cells induces localized membrane ruffling within a few minutes; overexpression of VirA causes MT destruction and protrusion of membrane ruffles [4]. Importantly, VirA-induced membrane ruffling appears to depend on Rac1 activity, as when VirA is coexpressed with a dominant-negative Rac1 mutant in host cells, ruffles are not usually observed [4]. In agreement with this finding, wild-type *S. flexneri* (but not a *virA* mutant) activates Rac1, including the formation of membrane ruffles in infected HeLa cells. These results, together with other studies [34,35], strongly suggest that MT destabilization in host cells, induced by *Shigella*-secreted VirA, provokes the formation of membrane ruffles via the stimulation of Rac1 activity, thus promoting bacterial entry into host cells (Fig. 2) [4]. It is still unclear whether other invasive bacteria such as *A. actinomycetemcomitans* can also direct host MT instability to promote bacterial entry.

MT dynamic instability, Rho GTPases and actin

In migrating and growing cells, MT networks undergo a lengthening and shortening process that is generally referred to as MT dynamic instability. This process is mediated by various MT-stabilizing and -destabilizing factors [3]. For migrating cells, the interplay between the MT and actin cytoskeletal systems is crucial [35]. Indeed, recent studies have strongly indicated that MT dynamic instability is involved in activating Rac1 and RhoA signaling [34,36], thus controlling actin dynamics. Waterman-Storer *et al.* revealed that when the MT growth was stimulated by the removal of nocodazole from drug-treated fibroblast cells, Rac1 activity was increased, leading to formation of lamellipodial protrusions in the cells [34]. In 1996, it was demonstrated for the first time that MT disruption by colcemid or vinblastine rapidly and reversibly induced the formation of actin stress fibers and focal adhesions, accompanied by the activation of cell motility [37]. In agreement with this, it was also observed that, in fibroblasts, RhoA activity could be stimulated by colchicine treatment [36]. These studies suggest that depolymerization or shortening of MTs can somehow trigger the stimulation of Rho activity, possibly by releasing into the cytosol MT-bound factors that are required for the activation of Rho GTPases at the plasma membrane [35].

This notion has recently been reinforced by the discovery that guanine nucleotide-exchange factors such as GEF-H1 are involved in regulating Rho activity [38]. Although its importance has only recently been highlighted, the association of MTs with Rac1 or GEF-H1 was noted previously [39,40]. Furthermore, recent studies strongly suggest that Rac1 and RhoA are functionally linked to each other, where the enhancement of the activity of one protein downregulates the activity of the other [41,42]. This 'cross-talk' between Rac1 and RhoA activities provides a first clue as to how MT destabilization, which affects Rac1 activity, can lead to the actin

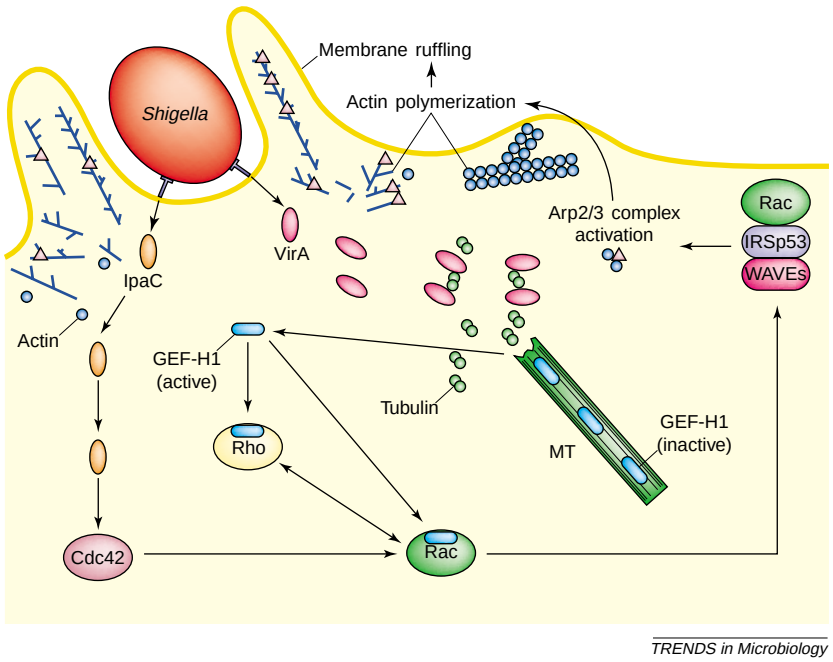

Fig. 2. A possible role for *Shigella* VirA in inducing membrane ruffling in epithelial cells. VirA, which is secreted into epithelial cells using a type III secretion system, induces local microtubule (MT) destabilization. This destabilization results in depolymerization or shortening of MTs, which triggers the stimulation of RhoA and Rac1 as MT-bound factors such as GEF-H1 are released into the cytsol. The activated Rho GTPases beneath the point of *Shigella* entry might be involved in the formation of a Rac1–IRSp53–WAVE2 complex, which in turn recruits and activates the Arp2/3 complex, ultimately leading to the induction of large-scale membrane ruffling, thus promoting the entry of *Shigella*. For the induction actin rearrangements and membrane ruffling on a scale sufficient to engulf several bacterial particles simultaneously, cellular signals evoked by *Shigella* effectors such as IpaA, IpaC and IpgD are also involved. IpaA binds directly to the vinculin head and promotes the depolymerization of F-actin required for the modification of *Shigella*-induced membrane protrusions (not shown in this figure, see [26,27]). IpaC is integrated into the host plasma membrane, and is probably engaged in actin polymerization in a mechanism that involves stimulating Cdc42 activity, which in turn activates Rac1 (see [28,29]). IpgD has phosphatidylinositol 4-phosphatase activity, which facilitates detachment of the plasma membrane from the actin cytoskeleton, thus probably promoting extension of membrane filopodia and ruffles around invading *Shigella* (not shown in this figure, see [30,31]).

polymerization required for the formation of membrane ruffles. In the case of *Shigella*-mediated MT destabilization, however, it is unclear whether GEF-H1 plays a pivotal role in stimulating Rac1 activity via the deactivation of RhoA. One can postulate that Rac1 activated via MT destabilization might elicit membrane ruffling via the formation of a Rac1–IRSp53–WAVE2 complex, which, in turn, recruits the Arp2/3 complex together with profilin, which are required for efficient actin polymerization (Fig. 2) [43].

Questions for Future Research

- How does MT destabilization in host cells affect bacterial invasion?
- How do pathogenic bacteria such as *C. trachomatis* and *O. tsutsugamushi* use dynein to move within the infected cells?
- What invasion mechanism does *C. jejuni* use?
- Does *A. actinomycetemcomitans* use a similar system to that of *Shigella* VirA-induced MT destabilization to invade host cells?
- What is the exact molecular basis of VirA-induced MT destabilization?
- Does *S. flexneri* have other factor(s) able to induce MT destabilization?
- Does *S. flexneri* require VirA-induced MT destabilization in other stages of the infection process?
- How does MT dynamic instability regulate the cross-talk between RhoA and Rac1 activity?

Conclusions

Recent studies strongly suggest that the physical and functional coordination of the MT and actin cytoskeletons is an important issue in understanding various dynamic cellular processes including cell movement, cell polarity, proliferation, cell–cell interactions, vesicular trafficking and macropinocytic events [35]. Although the molecular basis of MT-dependent internalization of bacteria is still poorly understood, it has become evident that MT networks are an important host target for various pathogens to exploit during infection. As shown by the activities of *Shigella* VirA, the modulation of the actin cytoskeleton via induction of dynamic instability in MT networks results in the activation of Rho GTPases. This is the first clue to the bacterial strategy that links the ability to stimulate MT dynamics with the induction of local actin polymerization required for invasion of host cells. Although we must await further study on the mechanisms underlying the MT-dependent strategies used by various pathogenic bacteria, we believe that the ability of pathogenic bacteria to modulate MT dynamics plays an important role in their ability to infect eukaryotic host cells.

Acknowledgements

We are grateful to Toshihiko Suzuki, Hitomi Mimuro and Reiko Akakura for critical review of the manuscript. We thank all the other members of the Sasakawa laboratory. This work was supported by a grant-in-aid for Scientific Research on Priority Areas and a grant-in-aid for Young Scientists from the Japanese Ministry of Education, Science, Sports, and Culture, and PRESTO, Japan Science and Technology Corporation (JST).

References

1 Finlay, B.B. and Cossart, P. (1997) Exploitation of mammalian host cell functions by bacterial pathogens. *Science* 276, 718–725
2 Finlay, B.B. and Falkow, S. (1997) Common themes in microbial pathogenicity revisited. *Microbiol. Mol. Biol. Rev.* 61, 136–169
3 Desai, A. and Mitchison, T.J. (1997) Microtubule polymerization dynamics. *Annu. Rev. Cell Dev. Biol.* 13, 83–117
4 Yoshida, S. *et al.* (2002) Shigella deliver an effector protein to trigger host microtubule destabilization, which promotes Rac1 activity and efficient bacterial internalization. *EMBO J.* 21, 2923–2935
5 Oelschlaeger, T.A. *et al.* (1993) Unusual microtubule-dependent endocytosis mechanisms triggered by *Campylobacter jejuni* and *Citrobacter freundii*. *Proc. Natl. Acad. Sci. U.S.A.* 90, 6884–6888
6 St Geme, J.W. III and Falkow, S. (1990) *Haemophilus influenzae* adheres to and enters cultured human epithelial cells. *Infect. Immun.* 58, 4036–4044
7 Oelschlaeger, T.A. and Tall, B.D. (1997) Invasion of cultured human epithelial cells by *Klebsiella pneumoniae* isolated from the urinary tract. *Infect. Immun.* 65, 2950–2958
8 Kuhn, M. (1998) The microtubule depolymerizing drugs nocodazole and colchicines inhibit the uptake of *Listeria monocytogenes* by P388D1 macrophages. *FEMS Microbiol. Lett.* 160, 87–90
9 Borovsky, Z. *et al.* (1998) Protein kinase C activation and vacuolation in HeLa cells invaded by *Mycoplasma penetrans*. *J. Med. Microbiol.* 47, 915–922
10 Grassme, H.U.C. *et al.* (1996) Gonococcal opacity protein promotes bacterial entry-associated rearrangements of the epithelial cell actin cytoskeleton. *Infect. Immun.* 64, 1621–1630
11 Lamont, R.J. *et al.* (1995) *Porphyromonas gingivalis* invasion of gingival epithelial cells. *Infect. Immun.* 63, 3878–3885
12 Miliotis, M.D. *et al.* (1995) Adherence to and invasion of tissue culture cells by *Vibrio hollisae*. *Infect. Immun.* 63, 4959–4963
13 Hu, L. and Kopecko, D.J. (1999) *Campylobacter jejuni* 81-176 associates with microtubules and dynein during invasion of human intestinal cells. *Infect. Immun.* 67, 4171–4182
14 Kopecko, D.J. *et al.* (2001) *Campylobacter jejuni*-microtubule-dependent invasion. *Trends Microbiol.* 9, 389–396
15 Hirokawa, N. *et al.* (1998) Kinesin and dynein superfamily proteins in organelle transport and cell division. *Curr. Opin. Cell Biol.* 10, 60–73
16 Bereswill, S. and Kist, M. (2002) Molecular microbiology and pathogenesis of *Helicobacter* and *Campylobacter* updated: a meeting report of the 11th conference on *Campylobacter*, *Helicobacter* and related organisms. *Mol. Microbiol.* 45, 255–262
17 Meyer, D.H. *et al.* (1999) Microtubules are associated with intracellular movement and spread of the periodontopathogen *Actinobacillus actinomycetemcomitans*. *Infect. Immun.* 67, 6518–6525
18 Clausen, J.D. *et al.* (1997) *Chlamydia trachomatis* utilizes the host cell microtubule network during early events of infection. *Mol. Microbiol.* 25, 441–449
19 Kim, S-W. *et al.* (2001) Microtubule- and dynein-mediated movement of *Orientia tsutsugamushi* to the microtubule organizing center. *Infect. Immun.* 69, 494–500
20 Wyrick, P.B. (2000) Intracellular survival by *Chlamydia*. *Cell. Microbiol.* 2, 275–282
21 Burkhardt, J.K. *et al.* (1997) Overexpression of the dynamitin (p50) subunit of the dynactin complex disrupts dynein-dependent maintenance organelle disruption. *J. Cell Biol.* 137, 113–129
22 Skrzypek, E. *et al.* (1998) Targeting of the *Yersinia pestis* YopM protein into HeLa cells and intracellular trafficking to the nucleus. *Mol. Microbiol.* 30, 1051–1065
23 Toyotome, T. *et al.* (2001) *Shigella* protein $IpaH_{9.8}$ is secreted from bacteria within mammalian cells and transported to the nucleus. *J. Biol. Chem.* 276, 32071–32079
24 Kachlany, S.C. *et al.* (2001) Genes for tight adherence of *Actinobacillus actinomycetemcomitans*: from plaque to plague to pond scum. *Trends Microbiol.* 9, 429–437
25 Sansonetti, P.J. (1999) *Shigella* plays dangerous games. *ASM News* 65, 611–617
26 Tran Van Nhieu, G. *et al.* (1997) Modulation of bacterial entry into epithelial cells by association between vinculin and the *Shigella* IpaA invasin. *EMBO J.* 16, 2717–2729
27 Bourdet-Sicard, R. *et al.* (1999) Binding of the *Shigella* protein IpaA to vinculin induces F-actin depolymerization. *EMBO J.* 18, 5853–5862
28 Tran Van Nhieu, G. *et al.* (1999) IpaC induces actin polymerization and filopodia formation during *Shigella* entry into epithelial cells. *EMBO J.* 18, 3249–3262
29 Kuwae, A. *et al.* (2001) *Shigella* invasion of macrophage requires the insertion of IpaC into the host plasma membrane. *J. Biol. Chem.* 276, 32230–32239
30 Niebuhr, K. *et al.* (2000) IpgD, a protein secreted by the type III secretion machinery of *Shigella flexneri*, is chaperoned by IpgE and implicated in entry focus formation. *Mol. Microbiol.* 38, 8–19
31 Niebuhr, K. *et al.* (2002) Conversion of PtdIns (4,5) P2 into PtdIns (5) P by the *S. flexneri* effector IpgD reorganizes host cell morphology. *EMBO J.* 21, 5069–5078
32 Uchiya, K. *et al.* (1995) Identification of a novel virulence gene, *virA*, on the large plasmid of *Shigella*, involved in invasion and intercellular spreading. *Mol. Microbiol.* 17, 241–250
33 Elliott, S. *et al.* (2001) EspG, a novel type III system-secreted protein from enteropathogenic *Escherichia coli* with similarities to VirA of *Shigella flexneri*. *Infect. Immun.* 69, 4027–4033
34 Waterman-Storer, C. *et al.* (1999) Microtubule growth activates Rac1 to promote lamellipodial protrusion in fibroblasts. *Nature Cell Biol.* 1, 45–50
35 Wittmann, T. and Waterman-Storer, C. (2001) Cell motility: can Rho GTPase and microtubules point the way? *J. Cell. Sci.* 114, 3795–3803
36 Ren, X-D. *et al.* (1999) Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. *EMBO J.* 18, 578–585
37 Enomoto, T. (1996) Microtubule disruption induces the formation of actin stress fibers and focal adhesions in cultured cells: possible involvement of Rho signal cascade. *Cell Struct. Func.* 21, 317–326
38 Krendel, M. *et al.* (2002) Nucleotide exchange factor GEF-H1 mediates cross-talk between microtubules and the actin cytoskeleton. *Nature Cell Biol.* 4, 294–301
39 Best, A. *et al.* (1996) The Ras-related GTPase Rac1 binds tubulin. *J. Biol. Chem.* 271, 3756–3762
40 Ren, Y. *et al.* (1998) Cloning and characterization of GEF-H1, a microtubule-associated guanine nucleotide exchange factor for Rac and Rho GTPases. *J. Biol. Chem.* 273, 34954–34960
41 Rottner, K. *et al.* (1999) Interplay between Rac and Rho in the control of substrate contact dynamics. *Curr. Biol.* 9, 640–648
42 Li, Z. *et al.* (2002) Regulation of Rho GTPase by crosstalk and neuronal activity *in vivo*. *Neuron* 33, 741–750
43 Takenawa, T. and Miki, H. (2001) WASP and WAVE family proteins: key molecules for rapid rearrangement of cortical actin filaments and cell movement. *J. Cell. Sci.* 114, 1801–1809