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Rho GTPases as pathogen targets: Focus on curable sexually transmitted infections

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Pathogens have evolved highly specialized mechanisms to infect hosts. Several microorganisms modulate the eukaryotic cell surface to facilitate their engulfment. Once internalized, they hijack the molecular machinery of the infected cell for their own benefit. At different stages of phagocytosis, particularly during invasion, certain pathogens manipulate pathways governed by small GTPases. In this review, we focus on the role of Rho proteins on curable, sexually transmitted infections caused by *Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis* and *Treponema pallidum*. Despite the high, worldwide frequencies of these sexually-transmitted diseases, very little is known about the strategies developed by these microorganisms to usurp key eukaryotic proteins that control intracellular signaling and actin dynamics. Improved knowledge of these molecular mechanisms will contribute to the elucidation of how these clinically important pathogens manipulate intracellular processes and parasitize their hosts.

Introduction

Rho GTPases constitute one of the 5 distinct families, Ras, Arf, Ran, Rab and Rho, of the Ras superfamily of small GTP-binding proteins.[1] These GTPases act as molecular switches, cycling between a GDP-bound form (inactive state) and a GTP-bound form (active state). This cycle is carefully regulated by more than 60 activators (guanine nucleotide exchange factors, GEFs) and over 70 inhibitors (GTPase-activating proteins, GAPs). Multiple and diverse processes of eukaryotic cells are regulated by these GTPases, and more than 100 target/effector proteins have been described.[2] Ras (rat sarcoma) oncoproteins are mainly involved in the control of gene expression and cell proliferation; Arf (ADP-ribosylation factor) members participate in cargo sorting and in the formation of vesicle coats along different transport pathways; Ran (Ras-related nuclear) GTPase functions in the nucleo-cytoplasmic transport of both RNA and proteins and regulates mitotic spindle and nuclear envelope assemblies; Rab (Ras-related proteins in brain) proteins are master controllers of intracellular vesicular transport; and the Rho (Ras homologous) GTPases serve as key regulators of extracellular stimulus-mediated signaling networks that regulate actin organization, cell cycle progression and gene expression.[3,4]

After the discovery of Rho as a Ras-related protein in 1985, 20 genes were subsequently identified as Rho family members, RhoA, Rac1 and Cdc42 being the best characterized.[5,6] Rho GTPases participate in many essential cellular processes; the most important ones are the regulation of the assembly and organization of the actin cytoskeleton. Additionally, they regulate cell polarity, microtubule dynamics, vesicular transport pathways, G1 cell cycle progression and a multiplicity of enzymatic activities.[7] Interestingly, Rho GTPases also play critical roles in the interaction between pathogens and host cells by controlling innate and adaptive immune responses.[8,9]

Pathogens have evolved different strategies to alter host cell functions. By modifying eukaryotic GTPases, microorganisms can manipulate a variety of cellular pathways to favor microbial colonization and/or proliferation, thereby parasitizing and even killing the host cell. Rho GTPases are involved in actin reprogramming at the underlying site of adhesion of microbes to the cell surface, in the entry or invasion of host cells, in the intracellular survival of microbes, or in the polymerization of actin to propel microorganisms into adjacent cells. A large number of bacterial virulence factors target Rho GTPases. These virulence factors can be classified into 2 distinct categories: the first group includes injected effectors, in which bacteria must be in contact with their host cells to deploy their effectors via secretion apparatuses that are similar to molecular syringes;[10] and the second group includes exotoxins secreted by bacteria into their environs, which does not require direct contact with the target cell.[8,11] Once the toxin reaches the cell surface, it binds to specific receptors and enters the cell by endocytosis. Both injected effectors and exotoxins alter GTPase function through direct chemical modifications of the molecule or by interfering with regulatory elements of the GTPase cycle.

This review focuses on the relationship between Rho GTPases and 4 sexually transmitted pathogens, 3 bacteria (*Chlamydia trachomatis, Neisseria gonorrhoeae, and Treponema pallidum)* and the parasite *Trichomonas vaginalis.* A recent World Health Organization (WHO) study (http://www.who.int/reproductivehealth/publications/rtis/stisestimates/en/) estimates that 498 million adults, aged between 15 and 49, are infected with at least one of these

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microorganisms. Therefore, knowledge of the interplay between these pathogens and human cells is of great importance.

Chlamydia trachomatis

C. trachomatis is the major causative agent of bacterial sexually transmitted diseases and preventable blindness worldwide. Infections can result in urethritis, cervicitis, epididimitis to trachoma, lymphogranuloma venereum, pelvic inflammatory disease, tubal obstruction, ectopic pregnancy and infertility.[12] Persistent infections have recently been linked to severe chronic inflammatory diseases and cancer.[13]

C. trachomatis is a Gram-negative, obligate intracellular bacterium that is restricted to humans. It has developed diverse strategies to invade, survive and multiply within eukaryotic cells.[14,15] *Chlamydia* resides in a vacuole, called "inclusion," where it avoids intracellular degradation and acquires nutrients and structural molecules from host cells.[16–19] *Chlamydia* displays a unique biphasic lifecycle that starts when the infectious bacterial form, the elementary body (EB), enters the cell. Then, the EB differentiates into a larger, metabolically active, but non-infectious form, the reticulate body (RB), which multiplies by binary fission. After numerous rounds of replication, RBs undergo transformation back into infectious EBs to disseminate to adjacent cells.[20–22]

Rho GTPases in *Chlamydia* Entry

Invasion starts with the attachment of EBs to the plasma membrane of host cells. This binding is highly specific and efficient, and has been termed parasite-specific phagocytosis.[23] Despite the importance of this early event in chlamydial pathogenesis, the specific receptor-ligand interaction involved in bacterial entry remains elusive. The diversity of chlamydial strains and eukaryotic cells used in different studies, the variability in the experimental setups, and the difficulty of genetically manipulating these bacteria are the main reasons for this lack of consensus regarding the adhesins and ligands involved in *Chlamydia* entry.[24–28] Independently of host cell types or bacterial serovars, the unifying feature of chlamydial entry is actin remodelling at attachment sites, an event controlled by Rho GTPases.

Rho GTPases involved in the internalization step appear to be species-specific; only Rac1 is involved in *C. trachomatis* entry,[29,30] whereas both Cdc42 and Rac1 are activated during *C. caviae* invasion.[31] Basically, Rac1, which is recruited to the entry sites where actin polymerizes,[30] is rapidly and transiently activated after the binding of *C. trachomatis* to host cells. It is likely that *C. trachomatis* activates a cascade involving both bacterial and host proteins, which results in the rearrangement of the actin cytoskeleton and leads to successful colonization of the host cell. In fact, after bacterial attachment, EBs secrete a protein called Translocated Actin Recruiting Protein (TARP), which is injected into the host cell cytosol through a type III secretion system (a multiprotein needle-like delivery system).[32,33] Once on the cytosolic face of the plasma membrane, TARP is phosphorylated on its N-terminal, tyrosine-rich tandem repeats by host Src[34] and Abelson (Abl) kinases.[29] This phosphorylation allows TARP to recruit the GEFs Sos1 and Vav2, which in turn activate Rac1.[35] Subsequently, Rac1 recruits WAVE2 and Abl interactor 1 (Abi-1), leading to actin-related protein (Arp2/3) complex activation and actin recruitment and polymerization at the bacterial binding site.[36] It has been proposed that a synergistic action between both bacterial and host cell proteins promotes invasion. In addition, other host tyrosine kinases, such as platelet derived growth factor receptor (PDFGR) and feline Gardner-Rasheed sarcoma viral oncogene homolog (FGR), are phosphorylated upon infection and recruited to the *Chlamydia* attachment site. It is possible that these kinases might function redundantly in the internalization step.[29,37] The last stage in the cascade is probably regulated by another bacterial protein, CT166, which inactivates Rac1 (but not Rho A) via glucosylation,[38] thus completing the activation/inactivation cycle of Rac1. In summation, this is a complex and tightly regulated process in which diverse bacterial and host proteins play essential roles in the attachment and entry of *Chlamydia*, in which Rac1 plays an important function throughout the process (**Fig. 1A**).

Rho GTPases in *Chlamydia* Exit

Paradoxically, another member of the Rho family is involved in the release of the bacteria from infected cells. After their intracellular multiplication, *Chlamydia* disseminate and colonize neighboring cells. Two different mutually exclusive mechanisms have been described. The first one involves the lysis of the infected cells in an ordered sequence of membrane ruptures, beginning with the inclusion membrane, followed by the nuclear envelope and finally the plasma membrane. The second mechanism, known as extrusion, consists of pinching off the inclusion, protrusion out of the cell within a membrane compartment, and ultimately detachment from the host cell. The extrusion mechanism requires actin polymerization, neuronal Wiskott–Aldrich syndrome protein (WASP), myosin II and the GTPase Rho A[39] (**Fig. 1A**).

Rho GTPases at Other Stages of Chlamydial Infection

Infection-triggered arthritis

The triggering of joint inflammation by infectious agents has been well established in the case of reactive arthritis.[40] Infection with the obligate intracellular pathogen *C. trachomatis* is a common antecedent for reactive arthritis, as the synocyte is a suitable host cell for these bacteria.[41] In this regard, Zhang et al. demonstrated that Rac proteins have a dual role in the outcome of *Chlamydia*-triggered arthritis.[42] In the acute phase, Rac functions to exacerbate joint inflammation by promoting neutrophil migration, whereas during the chronic phase, Rac expression serves to alleviate arthritis, probably aiding the host clearance of *Chlamydia* and coordinating the appropriate expression of Toll-like receptor 4 (TLR-4) in neutrophils.[43]

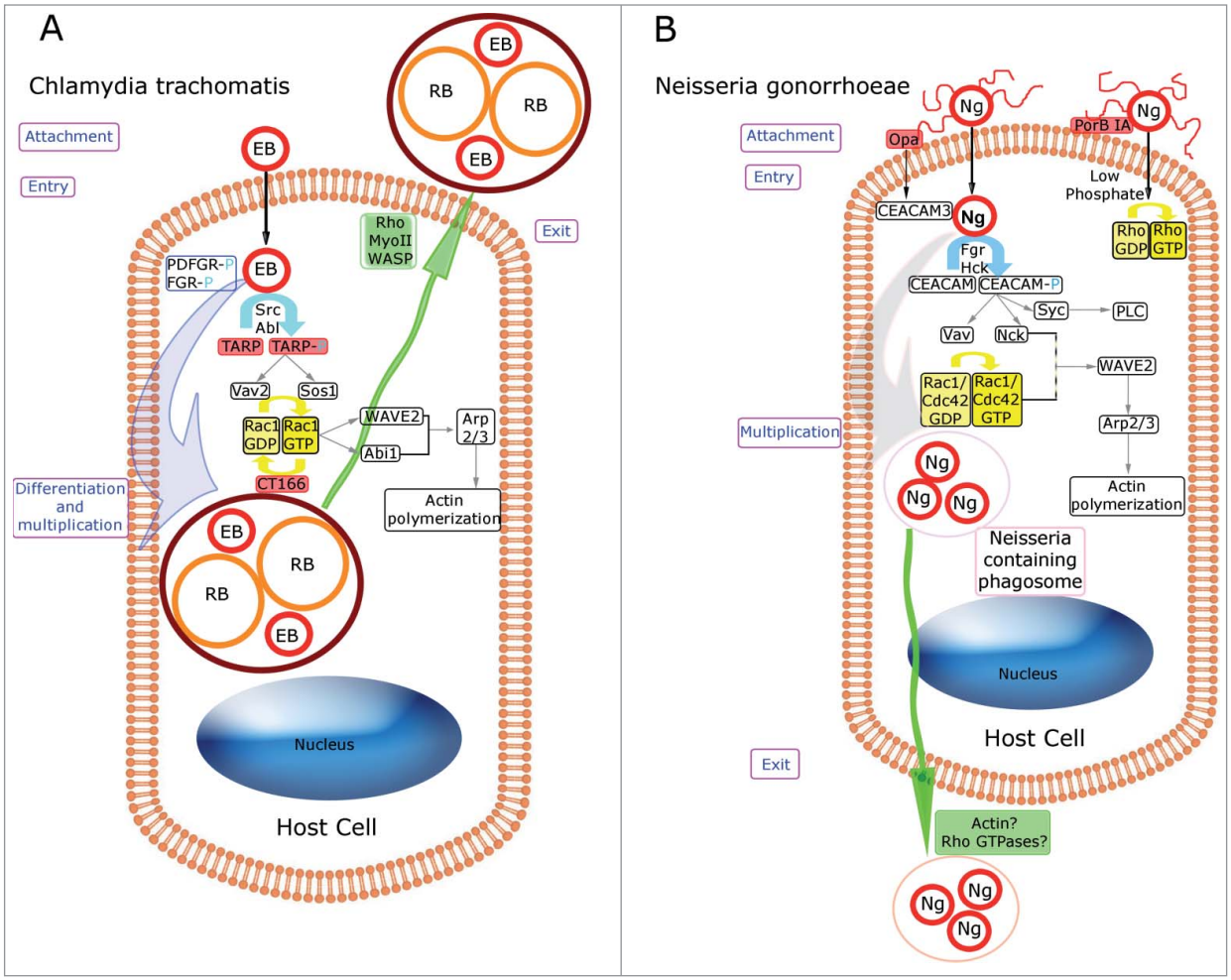

Figure 1. Schematic comparison of Rho GTPases involved in *Chlamydia trachomatis* and *Neisseria gonorrhoeae*-host cell interplay. Bacterial proteins are shown in red boxes. Yellow arrows indicate Rho GTPase cycling. Phosphorylation events are shown in light blue. Putative roles of Rho GTPases are indicated in purple boxes. Bacterial exit processes are represented in green. (**A**) Rho GTPases and *Chlamydia trachomatis*. The bacterial infective form (Elementary Body, EB), through the injection of the bacterial factor TARP (Translocated Actin Recruitment Protein), triggers phosphorylation events and the recruitment of the guanine-nucleotide exchange factors (GEFs), Sos1 and Vav2, leading to Rac1 activation. Then, Rac1-GTP recruits WAVE2 and Abi-1, promoting Arp2/3 complex activation and actin polymerization at the bacterial entry site. After chlamydial differentiation into the replicative form (Reticulate Body, RB) and numerous rounds of multiplication, the *Chlamydia*-containing vacuole is extruded from the infected host cell by a mechanism that involves WASP, myosin II and RhoA. (**B**) Rho GTPases and *Neisseria gonorrhoeae*. Opa_{CEA}-expressing *Neisseria gonorrhoeae* (Ng), through Opa-CEACAM3 recognition, triggers Rac1 and Cdc42 activation, and the downstream recruitment of WAVE2 and the actin recruiting protein Arp2/3, which ends in actin cytoskeleton rearrangements and bacterial internalization. In contrast, Rho-mediated entry triggered by the bacterial porin $PorB_{IA}$ drives actin polymerization that promotes the internalization of piliated gonococci into non-phagocytic cells. The release of *Neisseria*-containing phagosomes might involve Rho GTPases and actin reorganization.

Neisseria gonorrhoeae

Neisseria gonorrhoeae (the gonococcus) is the causative agent of gonorrhoeae, which is exclusively a human disease. These bacteria colonize the mucosae of the urethra, endocervix, fallopian tube, rectum, conjunctiva and pharynx. Neisserial infections range in severity from acute infections with good prognoses to severe diseases, such as purulent arthritis, pelvic inflammatory disease, endocarditis and meningitis.[44] In addition, chronic infections can lead to ectopic pregnancies and infertility.[45]

N. gonorrhoeae is a Gram-negative bacterium with a typical signature: a high capacity to modulate its antigenic surface via phase variations that result from changes in gene expression. Additionally, it rapidly accumulates point mutations, acquiring antibiotic resistance, which are the basis of its ability to colonize host cells and evade immune system surveillance.[46,47] Gonococci can reside inside several human cell types (neutrophils, monocytes, mucosal epithelial cells, endothelial cells) or act as extracellular pathogens.

Rho GTPases in *Neisseria* Entry

The infection begins with the attachment of the bacteria to the apical surface of mucosal cells via the gonococcal pilus.[48,49]

N. gonorrhoeae approach the cell host by extending their pili, and this is followed by their retraction or disassembly. Pilus retraction[50] triggers a tight association between the bacterial opacity-associated (Opa) outer membrane proteins and the host cell receptors, which promotes invasion.[48,50–53] A single gonococcal strain can harbour 11 *opa genes* that can be switched on and off independently. These genes contain 5′ tandem repeats [CTCTT]n that cause high-frequency, phase-variable expression,[54] in addition to intra- and inter-genomic recombination events.[55,56] Opa adhesins are classified into 2 main groups, Opa_{HS} or Opa_{CEA}, according to their host cell receptor. The heparan-sulfate proteoglycan (HSPG) receptors present in epithelial cells interact with one particular Opa_{HS} variant, Opa_{50},[57] while the carcinoembryonic antigen cell adhesion molecules (CEACAM) expressed by a variety of cell types recognize Opa_{CEA}.[58–60] The CEACAM family belongs to the immunoglobulin superfamily of adhesion molecules,[61] and 4 members are receptors for Opa proteins: CEA, CEACAM1, CEACAM3 and CEACAM6.[62,63] Even when the first cell-cell contact prior to host-pathogen adherence is carried out by pili, the specificity of the binding is controlled by Opa proteins. In addition to proteoglycan-mediated adherence that involves Opa_{50}, several CEACAM receptors are differentially recognized by gonococcal Opa_{CEA} variants. The strategies targeting these receptors might constitute an interesting alternative for drug design against gonorrhoea, especially to overcome the antigenic variability of *Neisseria* and its high capacity to generate antibiotic resistance.

Rho GTPases in CEACAM-mediated *neisseria* entry

The involvement of Rho GTPases varies depending on the receptor used for internalization. Invasion mediated by CEACAM1 and CEACAM6 does not require actin cytoskeleton reorganization; however, entry involving CEACAM3 triggers a high and localized reorganization of the host cell surface that is regulated by Rac1 and Cdc42, but not Rho A.[64] The most distinctive feature of CEACAM3 is the presence of a sequence in its cytoplasmic domain that is reminiscent of an immunoreceptor tyrosine-based activation motif (ITAM) characterized by 2 precisely spaced tyrosine residues in a particular sequence context. Of note, CEACAM3 is exclusively expressed in neutrophils, and the majority of Opa-expressing *Neisseria* interact with neutrophils, leading to non-opsonic phagocytosis.[59,65,66] Recent studies indicate that the CEACAM3-ITAM sequence associates directly with Vav, a Rac GEF, thus promoting the phagocytosis and elimination of CEACAM-bound bacteria.[67] Interestingly, Vav directly associates via its Src homology 2 (SH2) domain with a phosphorylated tyrosine residue within the ITAM-like sequence of the receptor. Homologous SH2 domains are also found in the adaptor molecules Nck1 and Nck2, and mediate their interaction with CEACAM3 in a complex with the Rac effector WAVE2. Finally, these steps lead to F-actin polymerization during *Neisseria* uptake[68] (**Fig. 1B**).

Rac1 and Cdc42 also participate in the activation of proinflammatory cytokines via a cascade of cellular stress response kinases, leading to the activation of JNK/AP1 via p21-activated kinase1 (PAK),[69] in addition to their roles in bacteria internalization.[70–74]

Rho GTPases in porin-mediated *neisseria* entry

Porins are the major component of the outer membrane of pathogenic *Neisseria* species. In primary cervical epithelial cells, bacterial entry involves the binding of PorB, pili and lipooligosacharide to complement receptor type 3.[75,76] In non-professional phagocytes, bacterial internalization is mediated by the PorB porin subtype A ($PorB_{IA}$).[77] $PorB_{IA}$ is a bacterial GTP binding protein that forms a voltage-gated channel that translocates into mammalian cell membranes and modulates host cell signaling events. This porin-mediated mechanism of entry is independent of the Opa proteins.[77] In addition, this invasion process is not dependent on host microtubules, PI3K, or Src kinases, but requires low intracellular phosphate levels.[78] It is noteworthy that the signaling cascades triggered by $PorB_{IA}$ in epithelial cells involve Rho GTPases and actin (**Fig. 1B**).

Rho GTPases in caveolin-mediated impairment of *neisseria* entry

Interestingly, type IV piliated *N. gonorrhoeae* induce the recruitment of host cell caveolin-1 (Cav1) and trigger Cav1 phosphorylation and downstream phosphotyrosine signaling. These events lead to cytoskeletal rearrangements that impair bacterial internalization. In brief, Cav1 interacts directly with Vav2, a Rho-family GEF, and both Vav2 and its substrate, RhoA, play a major role in the Cav1-mediated prevention of bacterial uptake.[79]

Rho GTPases in *Neisseria* Exit

After invading the host cell through the apical region, *N. gonorrhoeae* traverses the cell by transcytosis, exiting through the basal region.[80] Host dynein and kinesin play a role in microtubule-mediated *Neisseria* transit across the cell. Conversely, myosin I is involved in the movement along the actin filament network.[81] At present, the mechanisms used by this bacterium to disrupt the plasma membrane and disseminate into surrounding cells are not clear. This process may require actin reorganization, and it could be orchestrated by the activity of Rho GTPases (**Fig. 1B**). The exit process requires further study to unravel the molecular participants and their functions.

Rho GTPases at Other Stages of Gonococcal Infection

Rho GTPases and apoptosis

The PorB porin may induce apoptosis in epithelial cells and phagocytes.[82] It is the only neisserial factor identified thus far that induces apoptosis in infected cells. Two host pro-apoptotic proteins, Bim and Bmf, act as crucial regulators of neisserial-induced apoptosis. Both eukaryotic proteins belong to the Bcl-2 family, and they function synergistically. Bim and Bmf are

associated with the cytoskeleton and are released in a Rac-1-dependent manner after gonococcal infection.[83]

Trichomonas vaginalis

Trichomonas vaginalis, an extracellular protozoan parasite, is the etiological causative agent of trichomonosis, a widely disseminated, non-viral, sexually transmitted disease in humans.[84] The symptoms associated with infection in women include abdominal pain, vulvar itching, purulence, the presence of a frothy malodorous discharge with leucorrhoea, signs of colpitis macularis, and vulvar and vaginal erythema.[84] In men, it is usually asymptomatic; however, occasionally it can cause pruritus, urethritis with or without purulent secretions, epididymitis and prostatitis.[85] Additionally, *T. vaginalis* can cause infertility in both men and women,[84,86] and has been associated with an increased risk of acquiring HIV, cervical cancer and aggressive prostate cancer.[84,87] Despite the high incidence of trichomonosis, the exact mechanism of infection used by *T. vaginalis* is not well established.

Trichomonas Attachment: Participation of Rho-Related GTPases in Actin Remodeling

Despite its extracellular nature, this protozoan must adhere to epithelial cells in the urogenital tract to survive. Several adhesins have been implicated, including multifunctional metabolic proteins, lipophosphoglycans and membrane proteins.[88–92] Recent studies proposed a novel strategy for the adherence of *T. vaginalis* to host cells that involves the secretion of exosomes that have similar physical characteristics and protein compositions to mammalian exosomes. These exosomes promote both host-parasite and parasite-parasite interactions, and they play a role in the attachment of these protozoa to host epithelial cells.[93]

Actually, the binding to host cells causes the transition of *T. vaginalis* from an ovoid, free-swimming parasite into an amoeboid, highly adherent form.[94] This process involves a transcriptomic response in the parasite that is triggered by contact with the host cell surface, which increases the expression of genes related to protein synthesis, phenotypic plasticity and host cell degradation. In fact, a major up-regulation of actin and actin-associated genes is evident, suggesting a role for the cytoskeleton in the amoeboid transition.[95] Interestingly, TvRsp, a Rac1 homolog gene, has been found in *T. vaginalis.*[96] The relationship between the Rac-related genes of this protozoan and actin cytoskeleton rearrangements remains to be further explored (**Fig. 2**).

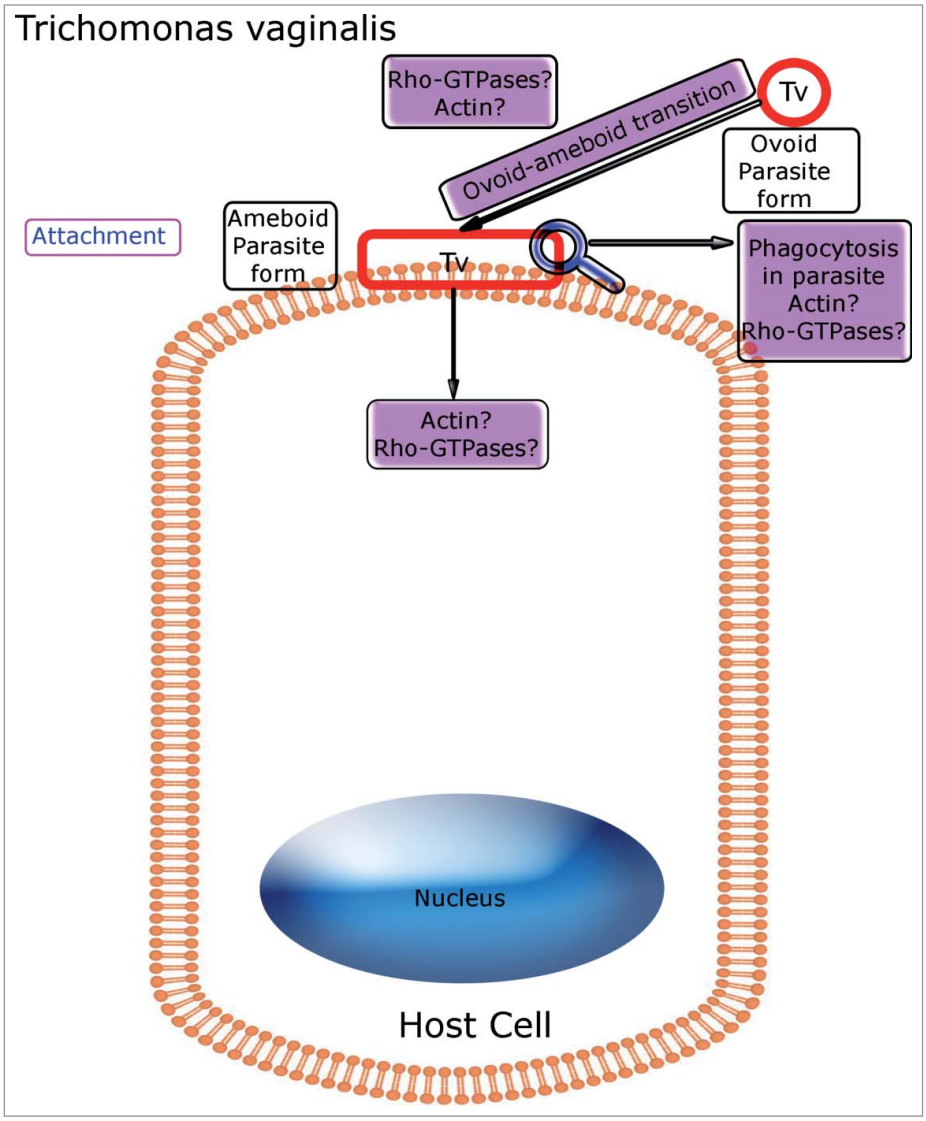

Figure 2. Schema depicting Rho GTPases involved in *Trichomonas vaginalis*-host cell interplay. Putative roles for Rho GTPases are indicated in purple boxes. Rho GTPase homologues are probably involved in the actin reorganization that occurs inside the parasite during the transition from the ovoid, free-swimming form to the amoeboid form, after its attachment to the host cell. In addition, Rho-related GTPases and actin polymerization apparently play a role in phagocytic processes that take place within the parasite.

Putative Role for Rho-Related GTPases in Nutrient Uptake by *T. Vaginalis*

T. vaginalis attachment to the host is important not only for establishing infection, but also for nutrient acquisition. However, a crucial source of nutrients comes from phagocytic processes occurring within these parasites. It is now clear that *T. vaginalis*

is a phagocytic cell, able to efficiently ingest particles, bacteria and even mammalian cells.[97] In immune cells, phagocytosis involves the reorganization of the cytoskeleton and relies on the control of actin dynamics by Rho GTPases.[97,98] In contrast, the precise functions of actin and Rho-related GTPases during the phagocytic processes occurring in *T. vaginalis* remain elusive (**Fig. 2**).

Treponema pallidum

Treponema pallidum is the causative agent of syphilis, a chronic and multi-stage disease, with a diverse and wide range of clinical manifestations.[99] The lack of treatment can lead to the development of primary, secondary and tertiary syphilis. The first stage is characterized by the appearance of a single, painless lesion (the chancre) at the site of inoculation. This pathogen is sexually transmitted through microabrasions in mucosal membranes where *T. pallidum* adheres to epithelial cells and extracellular matrix components of the skin and mucosa. In the second stage, characterized by a disseminated maculopapular rash, *T. pallidum* trespasses intercellular junctions of the endothelium, resulting in haematogenous dissemination and the seeding of the central nervous system and the remainder of the body.[100–102] This pathogen may also use transcytosis to spread through the endothelium.[100] In the tertiary and last stage, the disease may progress to cardiovascular disease, lesions of the skin, bones or viscera (gummata), or neurosyphilis.[103]

T. pallidum belongs to the *Spirochaetaceae* family of spiral-shaped bacteria. It is exclusively a human pathogen, able to survive for decades within the host, but extremely fragile *ex vivo*. This fragility represents one of the main challenges for its study. At present, it can only be transiently cultured in rabbit testes.[104] Analysis of the *T. pallidum* genome led to the identification of numerous transport proteins and a limited number of genes encoding metabolic and biosynthetic enzymes, indicating that *Treponema* relies heavily on scavenging compounds from the human host.[105]

Treponema pallidum attachment to host cells

Infection starts with the attachment of *T. pallidum* to a wide variety of cell types, including epithelial, fibroblast-like and endothelial cells.[106] After binding to the epithelial surface, this microorganism traverses the tissue barrier and enters the circulation by passing through the tight junctions between endothelial cells.[100] *T. pallidum* invasion results in widespread dissemination.

The host cell components involved in *Treponema* attachment include host serum, cell membrane compounds, and the extracellular matrix.[107] In particular, host cell fibronectin has been identified as the main target for bacterial adhesion. Actually, several proteins expressed in *T. pallidum* have been described as adhesins. Among them, Tp0155 binds to matrix fibronectin, whereas Tp0483 binds to both the soluble and matrix forms of fibronectin of host cells.[108] To enter the circulatory system and penetrate cells, Tp0155 is required for attachment in the bloodstream, and Tp0483 is involved in tissue attachment; in both cases, attachment is promoted via binding to fibronectin. Tp0136 is another bacterial adhesin that binds to fibronectin and laminin, 2 host extracellular matrix glycoproteins.[109] In addition, Tp0750 has a dual function, as it binds specifically to a variety of laminin isoforms,[110] and it is able to degrade host molecules, suggesting that it plays a role

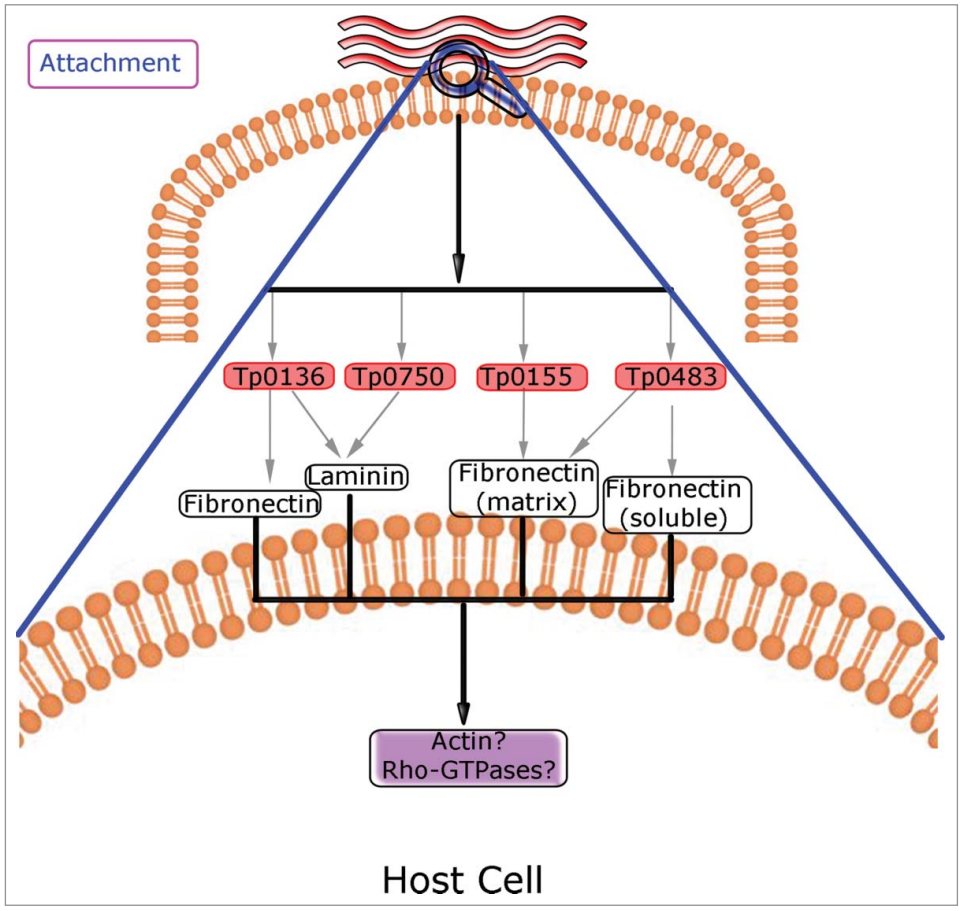

Figure 3. Schema depicting Rho GTPases involved in *Treponema pallidum*-host cell interplay. Putative roles for Rho GTPases are indicated in purple boxes. The bacterial adhesins Tp0136, Tp0155, Tp0483 and Tp0750, which binds to host fibronectin and/or laminin, are involved in the attachment to host cells. *Treponema* binding to cell surface might trigger actin polymerization that is regulated by Rho GTPases. The details of the events occurring at the plasma membrane are enlarged in the lower part of the panel.

Table 1 Rho GTPases in sexually-transmitted infections. The table summarizes the main characteristics of certain sexually-transmitted pathogens, plus the role of Rho GTPases in several stages of the infectious processes. In dark gray are indicated the proteins belonging to the pathogen. nd: not determined.

Pathogen	***Chlamydia trachomatis***		***Neisseria gonorrhoeae***		***Trichomonas vaginalis***	***Treponema pallidum***
Pathogen type	Bacteria Gram (-)		Bacteria Gram (-) (diplococci)		Flagellated protozoan	Bacteria Gram (-) (spirochetae)
Niche	Intracellular obligate		Intracellular facultative		Extracellular	Extracellular
Host cell type	Epithelial cells		Epithelial cells		Epithelial cells	Epithelial, fibroblast-like and endotelial cells
Step	Entry	Exit	CEACAM-mediated entry	PorinA-mediated entry	Ovoid-ameboid transition	Attachment to host cell
Member of the Rho family	Rac1	RhoA	Rac1 – Cdc42	RhoA	nd	nd
Rho activity regulators	Vav2- Sos1 (GEFs)	nd	Vav (GEF) - Nck	nd	nd	nd
Rho effectors	WAVE2- Abi1	MyoII- WASP	WAVE2-Arp2/3	nd	nd	nd
Pathogen molecules	*TARP (actin recruiting protein)*	*CT 166 (Rac1 inactivator)*	*nd*	*$PorB_{IA}$ (GTP-binding protein)*	*TvRSP (Rac1 homolog)*	*nd*

in the proteolytic mechanisms involved in tissue damage, destruction and dissemination of *T. pallidum* during infection[111] (**Fig. 3**).

Fibronectin and laminin may act in concert with Rho GTPases in a variety of physiological and pathological processes in eukaryotic cells.[112–115] In non-infected cells, Rac1, RhoA and Cdc42, in connection with fibronectin and laminin, are involved in polarization, cell cycle progression[117] and cell-cell adhesion.[118] Interestingly, some microorganisms, through the release of adhesion molecules, bind to fibronectin and activate Rho GTPases to promote host cell invasion.[119] For instance, the bacterial fibronectin-binding protein of *Campylobacter jejuni,* CadF, and the intact flagellum are involved in eukaryotic Rac1 GTPase activation and host cell invasion.[116,120] It has been postulated that CadF binding to fibronectin results in integrin clustering and activation. These events lead to the activation of signaling cascades that involves epidermal growth factor receptor (EGFR) and focal adhesion kinase (FAK), resulting in association of c-Src and phosphorylation of paxillin. Finally, the recruitment of Dock180, a Rac1-specific guanine nucleotide exchange factor, provokes the activation of Rac1, leading to a local restructuring of the actin cytoskeleton and the engulfment of the bacteria.[116,120] Similarly, in infections caused by *T. pallidum*, the bacterial adhesins might target fibronectin and laminin not only to enhance the binding to host cells[108–110] but also to trigger intracellular signaling pathways that might promote Rac1 activation and consequently actin remodelling at the attachment site, facilitating the invasion of host tissues. Nevertheless, the role of Rho GTPases and the actin cytoskeleton in treponemal infections remains almost unexplored.

Conclusion

In pathogen-host cell interactions, the cytoskeleton plays a central role. It is necessary for epithelial and endothelial barrier integrity, and for preventing and limiting the invasion and dissemination of the pathogen to other tissues.[121] It also participates actively in several processes in immune cells: migration,[122] phagocytosis,[9] immune synapsis and cell signaling.[123,124] Actin dynamics are critical for phagocytosis and endocytosis in a wide variety of cell types. Conveniently, several pathogens, through diverse mechanisms, manipulate the cytoskeleton to infect host cells.[125]

Rho GTPases participate in multiple and essential cellular functions, acting as the most important controllers of the assembly and organization of the actin cytoskeleton. Therefore, it is not surprising that pathogens target Rho GTPases to promote their entry into host cells and to manipulate actin-dependent processes of infected cells. Here, we update the actual knowledge about the role played by Rho GTPases in infections caused by 4 different pathogens that cause the most important (non-viral), curable, sexually transmitted diseases, and the main data are summarized in **Table 1**.

C. trachomatis, a Gram-negative, obligate intracellular bacterium, utilizes Rac1 to facilitate its entry into host cells, and it

uses RhoA to exit host cells and to disseminate to neighboring tissues.

N. gonorrhoeae, a Gram-negative, facultative intracellular bacterium, utilizes Rac1 and Cdc42 to promote Opa-CEACAM3 mediated entry into phagocytic host cells, and Rho for $PorB_{IA}$-mediated entry for piliated gonococci in non-phagocytic host cells. In both cases, a signaling cascade involving phosphorylation events, kinases, GTPases and GEFs leads to the final actin cytoskeleton rearrangement at the sites of entry of the bacteria.

T. vaginalis, an extracellular protozoan, does not provoke significant cytoskeletal rearrangements in host cells. Instead, actin reorganization occurs in the parasite itself. The role of Rho GTPases is not fully unravelled, but the parasite has genes that encode Rho homologues. Further investigation of *T. vaginalis* Rho-like GTPases and their relationship with the actin cytoskeleton of the parasite is necessary.

Finally, in the case of *T. pallidum*, a spiral-shaped bacterium, the role of the actin cytoskeleton and Rho GTPases during infection remains unexplored. This is largely due to the difficulties in the culturing and manipulation of *Treponema*, which hinder a full description of the infection process.

Knowledge of the mechanisms of invasion, proliferation and dissemination of these pathogens should contribute to more effective treatments of these sexually transmitted diseases. Rho GTPases, which are likely involved in many stages of the infection, such as the attachment, engulfment and spread of these pathogens, are molecular targets that require further studies to determine their usefulness in the control of these sexually transmitted infectious diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Rojas AM, Fuentes G, Rausell A, Valencia A. The ras protein superfamily: evolutionary tree and role of conserved amino acids. J Cell Biol 2012; 196:189-201; PMID:22270915; http://dx.doi.org/10.1083/jcb.201103008
2. Bishop AL, Hall A. Rho GTPases and their effector proteins. Biochem J 2000; 348 Pt 2:241-55; PMID:10816416; http://dx.doi.org/10.1042/0264-6021:3480241
3. Thumkeo D, Watanabe S, Narumiya S. Physiological roles of rho and rho effectors in mammals. Eur J Cell Biol 2013; 92(10-11):303-15; PMID:24183240; http://dx.doi.org/10.1016/j.ejcb.2013.09.002
4. Wennerberg K, Rossman KL, Der CJ. The ras superfamily at a glance. J Cell Sci 2005; 118:843-6; PMID:15731001; http://dx.doi.org/10.1242/jcs.01660
5. Hall A. Rho GTPases and the actin cytoskeleton. Science 1998; 279:509-14; PMID:9438836; http://dx.doi.org/10.1126/science.279.5350.509
6. Madaule P, Axel R. A novel ras-related gene family. Cell 1985; 41:31-40; PMID:3888408; http://dx.doi.org/10.1016/0092-8674(85)90058-3
7. Etienne-Manneville S, Hall A. Rho GTPases in cell biology. Nature 2002; 420:629-35; PMID:12478284; http://dx.doi.org/10.1038/nature01148
8. Boquet P, Lemichez E. Bacterial virulence factors targeting rho GTPases: parasitism or symbiosis? Trends Cell Biol 2003; 13:238-46; PMID:12742167; http://dx.doi.org/10.1016/S0962-8924(03)00037-0
9. Bokoch GM. Regulation of innate immunity by rho GTPases. Trends Cell Biol 2005; 15:163-71; PMID:15752980; http://dx.doi.org/10.1016/j.tcb.2005.01.002
10. Galan JE, Cossart P. Host-pathogen interactions: a diversity of themes, a variety of molecular machines. Curr Opin Microbiol 2005; 8:1-3; PMID:15694849; http://dx.doi.org/10.1016/j.mib.2004.12.015
11. Aktories K, Barbieri JT. Bacterial cytotoxins: targeting eukaryotic switches. Nat Rev Microbiol 2005; 3:397-410; PMID:15821726; http://dx.doi.org/10.1038/nrmicro1150
12. Carey AJ, Beagley KW. Chlamydia trachomatis, a hidden epidemic: effects on female reproduction and options for treatment. Am J Reprod Immunol 2010; 63:576-86; PMID:20192953; http://dx.doi.org/10.1111/j.1600-0897.2010.00819.x
13. Knowlton AE, Brown HM, Richards TS, Andreolas LA, Patel RK, Grieshaber SS. Chlamydia trachomatis infection causes mitotic spindle pole defects independently from its effects on centrosome amplification. Traffic 2011; 12:854-66; PMID:21477082; http://dx.doi.org/10.1111/j.1600-0854.2011.01204.x
14. Scidmore MA. Recent advances in chlamydia subversion of host cytoskeletal and membrane trafficking pathways. Microbes Infect 2011; 13:527-35; PMID:21334451; http://dx.doi.org/10.1016/j.micinf.2011.02.001
15. Fields KA, Hackstadt T. The chlamydial inclusion: escape from the endocytic pathway. Annu Rev Cell Dev Biol 2002; 18:221-45; PMID:12142274; http://dx.doi.org/10.1146/annurev.cellbio.18.012502.105845
16. Robertson DK, Gu L, Rowe RK, Beatty WL. Inclusion biogenesis and reactivation of persistent chlamydia trachomatis requires host cell sphingolipid biosynthesis. PLoS Pathog 2009; 5:e1000664; PMID:19936056; http://dx.doi.org/10.1371/journal.ppat.1000664
17. Cocchiaro JL, Valdivia RH. New insights into chlamydia intracellular survival mechanisms. Cell Microbiol 2009; 11:1571-8; PMID:19673891; http://dx.doi.org/10.1111/j.1462-5822.2009.01364.x
18. Leiva N, Capmany A, Damiani MT. Rab11-family of interacting protein 2 associates with chlamydial inclusions through its rab-binding domain and promotes bacterial multiplication. Cell Microbiol 2013; 15:114-29; PMID:23006599; http://dx.doi.org/10.1111/cmi.12035
19. Capmany A, Damiani MT. Chlamydia trachomatis intercepts golgi-derived sphingolipids through a rab14-mediated transport required for bacterial development and replication. PLoS One 2010; 5:e14084; PMID:21124879; http://dx.doi.org/10.1371/journal.pone.0014084
20. Valdivia RH. Chlamydia effector proteins and new insights into chlamydial cellular microbiology. Curr Opin Microbiol 2008; 11:53-9; PMID:18299248; http://dx.doi.org/10.1016/j.mib.2008.01.003
21. Capmany A, Leiva N, Damiani MT. Golgi-associated rab14, a new regulator for chlamydia trachomatis infection outcome. Commun Integr Biol 2011; 4:590-3; PMID:22046472; http://dx.doi.org/10.4161/cib.16594
22. Damiani MT, Gambarte Tudela J, Capmany A. Targeting eukaryotic rab proteins: a smart strategy for chlamydial survival and replication. Cell Microbiol 2014; 16(9):1329-38; PMID:24948448.
23. Byrne GI, Moulder JW. Parasite-specified phagocytosis of chlamydia psittaci and chlamydia trachomatis by L and HeLa cells. Infect Immun 1978; 19:598-606; PMID:344217.
24. Su H, Raymond L, Rockey DD, Fischer E, Hackstadt T, Caldwell HD. A recombinant chlamydia trachomatis major outer membrane protein binds to heparan sulfate receptors on epithelial cells. Proc Natl Acad Sci U S A 1996; 93:11143-8; PMID:8855323; http://dx.doi.org/10.1073/pnas.93.20.11143
25. Zhang JP, Stephens RS. Mechanism of C. trachomatis attachment to eukaryotic host cells. Cell 1992; 69:861-9; PMID:1591780; http://dx.doi.org/10.1016/0092-8674(92)90296-O
26. Abromaitis S, Stephens RS. Attachment and entry of chlamydia have distinct requirements for host protein disulfide isomerase. PLoS Pathog 2009; 5:e1000357; PMID:19343202; http://dx.doi.org/10.1371/journal.ppat.1000357
27. Conant CG, Stephens RS. Chlamydia attachment to mammalian cells requires protein disulfide isomerase.

Cell Microbiol 2007; 9:222-32; PMID:16925789; http://dx.doi.org/10.1111/j.1462-5822.2006.00783.x
28. Puolakkainen M, Kuo CC, Campbell LA. Chlamydia pneumoniae uses the mannose 6-phosphate/insulin-like growth factor 2 receptor for infection of endothelial cells. Infect Immun 2005; 73:4620-5; PMID:16040974; http://dx.doi.org/10.1128/IAI.73.8.4620-4625.2005
29. Elwell CA, Ceesay A, Kim JH, Kalman D, Engel JN. RNA interference screen identifies abl kinase and PDGFR signaling in Chlamydia trachomatis entry. PLoS Pathog 2008; 4:e1000021; PMID:18369471; http://dx.doi.org/10.1371/journal.ppat.1000021
30. Carabeo RA, Grieshaber SS, Hasenkrug A, Dooley C, Hackstadt T. Requirement for the rac GTPase in chlamydia trachomatis invasion of non-phagocytic cells. Traffic 2004; 5:418-25; PMID:15117316; http://dx.doi.org/10.1111/j.1398-9219.2004.00184.x
31. Subtil A, Wyplosz B, Balana ME, Dautry-Varsat A. Analysis of chlamydia caviae entry sites and involvement of cdc42 and rac activity. J Cell Sci 2004; 117:3923-33; PMID:15265988; http://dx.doi.org/10.1242/jcs.01247
32. Beeckman DS, Vanrompay DC. Bacterial secretion systems with an emphasis on the chlamydial type III secretion system. Curr Issues Mol Biol 2010; 12:17-41; PMID:19605938.
33. Clifton DR, Fields KA, Grieshaber SS, Dooley CA, Fischer ER, Mead DJ, Carabeo RA, Hackstadt T. A chlamydial type III translocated protein is tyrosine-phosphorylated at the site of entry and associated with recruitment of actin. Proc Natl Acad Sci U S A 2004; 101:10166-71; PMID:15199184; http://dx.doi.org/10.1073/pnas.0402829101
34. Jewett TJ, Dooley CA, Mead DJ, Hackstadt T. Chlamydia trachomatis tarp is phosphorylated by src family tyrosine kinases. Biochem Biophys Res Commun 2008; 371:339-44; PMID:18442471; http://dx.doi.org/10.1016/j.bbrc.2008.04.089
35. Lane BJ, Mutchler C, Al Khodor S, Grieshaber SS, Carabeo RA. Chlamydial entry involves TARP binding of guanine nucleotide exchange factors. PLoS Pathog 2008; 4:e1000014; PMID:18383626; http://dx.doi.org/10.1371/journal.ppat.1000014
36. Carabeo RA, Dooley CA, Grieshaber SS, Hackstadt T. Rac interacts with abi-1 and WAVE2 to promote an arp2/3-dependent actin recruitment during chlamydial invasion. Cell Microbiol 2007; 9:2278-88; PMID:17501982; http://dx.doi.org/10.1111/j.1462-5822.2007.00958.x
37. Kim JH, Jiang S, Elwell CA, Engel JN. Chlamydia trachomatis co-opts the FGF2 signaling pathway to enhance infection. PLoS Pathog 2011; 7:e1002285; PMID:21998584; http://dx.doi.org/10.1371/journal.ppat.1002285
38. Thalmann J, Janik K, May M, Sommer K, Ebeling J, Hofmann F, Genth H, Klos A. Actin re-organization induced by chlamydia trachomatis serovar D–evidence for a critical role of the effector protein CT166 targeting rac. PLoS One 2010; 5:e9887; PMID:20360858; http://dx.doi.org/10.1371/journal.pone.0009887
39. Hybiske K, Stephens RS. Mechanisms of host cell exit by the intracellular bacterium chlamydia. Proc Natl Acad Sci U S A 2007; 104:11430-5; PMID:17592133; http://dx.doi.org/10.1073/pnas.0703218104
40. Hyrich KL, Inman RD. Infectious agents in chronic rheumatic diseases. Curr Opin Rheumatol 2001; 13:300-4; PMID:11555732; http://dx.doi.org/10.1097/00002281-200107000-00010
41. Inman RD, Chiu B. Synoviocyte-packaged chlamydia trachomatis induces a chronic aseptic arthritis. J Clin Invest 1998; 102:1776-82; PMID:9819362; http://dx.doi.org/10.1172/JCI2983
42. Zhang X, Glogauer M, Zhu F, Kim T, Chiu B, Inman RD. Innate immunity and arthritis neutrophil rac and toll-like receptor 4 expression define outcomes in infection-triggered arthritis. Arthritis Rheum 2005; 52:1297-304; PMID:15818670; http://dx.doi.org/10.1002/art.20984
43. Zhang X, Glogauer M, Zhu F, Kim TH, Chiu B, Inman RD. Innate immunity and arthritis: neutrophil rac and toll-like receptor 4 expression define outcomes in infection-triggered arthritis. Arthritis Rheum 2005; 52:1297-304; PMID:15818670; http://dx.doi.org/10.1002/art.20984
44. Skerlev M, Čulav-Košćak I. Gonorrhea: new challenges. Clin Dermatol 2014; 32:275-81; PMID:24559563; http://dx.doi.org/10.1016/j.clindermatol.2013.08.010
45. Nolan GH, Osborne N. Gonococcal infections in the female. Obstet Gynecol 1973; 42:156-64; PMID:4198268.
46. Segal E, Hagblom P, Seifert HS, So M. Antigenic variation of gonococcal pilus involves assembly of separated silent gene segments. Proc Natl Acad Sci U S A 1986; 83:2177-81; PMID:2870495; http://dx.doi.org/10.1073/pnas.83.7.2177
47. Stern A, Brown M, Nickel P, Meyer TF. Opacity genes in neisseria gonorrhoeae: control of phase and antigenic variation. Cell 1986; 47:61-71; PMID:3093085; http://dx.doi.org/10.1016/0092-8674(86)90366-1
48. Merz AJ, Rifenbery DB, Arvidson CG, So M. Traversal of a polarized epithelium by pathogenic neisseriae: facilitation by type IV pili and maintenance of epithelial barrier function. Mol Med 1996; 2:745-54; PMID:8972489.
49. Virji M, Heckels JE. The role of common and type-specific pilus antigenic domains in adhesion and virulence of gonococci for human epithelial cells. J Gen Microbiol 1984; 130:1089-95; PMID:6147388.
50. Merz AJ, So M, Sheetz MP. Pilus retraction powers bacterial twitching motility. Nature 2000; 407:98-102; PMID:10993081; http://dx.doi.org/10.1038/35024105
51. Meyer TF, Pohlner J, van Putten JP. Biology of the pathogenic neisseriae. Curr Top Microbiol Immunol 1994; 192:283-317; PMID:7859510.
52. Swanson J. Studies on gonococcus infection. IV. pili: their role in attachment of gonococci to tissue culture cells. J Exp Med 1973; 137:571-89; PMID:4631989; http://dx.doi.org/10.1084/jem.137.3.571
53. Swanson J. Colony opacity and protein II compositions of gonococci. Infect Immun 1982; 37:359-68; PMID:6809633.
54. Stern A, Brown M, Nickel P, Meyer TF. Opacity genes in neisseria gonorrhoeae: control of phase and antigenic variation. Cell 1986; 47:61-71; PMID:3093085; http://dx.doi.org/10.1016/0092-8674(86)90366-1
55. Bilek N, Ison CA, Spratt BG. Relative contributions of recombination and mutation to the diversification of the opa gene repertoire of Neisseria gonorrhoeae. J Bacteriol 2009; 191:1878-90; PMID:19114493; http://dx.doi.org/10.1128/JB.01518-08
56. Blake MS, Gotschlich EC. Purification and partial characterization of the opacity-associated proteins of neisseria gonorrhoeae. J Exp Med 1984; 159:452-62; PMID:6420502; http://dx.doi.org/10.1084/jem.159.2.452
57. Van Putten JP, Paul SM. Binding of syndecan-like cell surface proteoglycan receptors is required for neisseria gonorrhoeae entry into human mucosal cells. EMBO J 1995; 14:2144-54; PMID:7774572.
58. Gray-Owen SD, Dehio C, Haude A, Grunert F, Meyer TF. CD66 carcinoembryonic antigens mediate interactions between opa-expressing neisseria gonorrhoeae and human polymorphonuclear phagocytes. EMBO J 1997; 16:3435-45; PMID:9218786; http://dx.doi.org/10.1093/emboj/16.12.3435
59. Gray-Owen SD, Lorenzen DR, Haude A, Meyer TF, Dehio C. Differential opa specificities for CD66 receptors influence tissue interactions and cellular response to neisseria gonorrhoeae. Mol Microbiol 1997; 26:971-80; PMID:9426134; http://dx.doi.org/10.1046/j.1365-2958.1997.6342006.x
60. Virji M, Makepeace K, Ferguson DJ, Watt SM. Carcinoembryonic antigens (CD66) on epithelial cells and neutrophils are receptors for opa proteins of pathogenic neisseriae. Mol Microbiol 1996; 22:941-50; PMID:8971715; http://dx.doi.org/10.1046/j.1365-2958.1996.01551.x
61. Obrink B. CEA adhesion molecules: multifunctional proteins with signal-regulatory properties. Curr Opin Cell Biol 1997; 9:616-26; PMID:9330864; http://dx.doi.org/10.1016/S0955-0674(97)80114-7
62. Kuespert K, Pils S, Hauck CR. CEACAMs: their role in physiology and pathophysiology. Curr Opin Cell Biol 2006; 18:565-71; PMID:16919437; http://dx.doi.org/10.1016/j.ceb.2006.08.008
63. Sadarangani M, Pollard AJ, Gray-Owen SD. Opa proteins and CEACAMs: pathways of immune engagement for pathogenic neisseria. FEMS Microbiol Rev 2011; 35:498-514; PMID:21204865; http://dx.doi.org/10.1111/j.1574-6976.2010.00260.x
64. Billker O, Popp A, Brinkmann V, Wenig G, Schneider J, Caron E, Meyer TF. Distinct mechanisms of internalization of neisseria gonorrhoeae by members of the CEACAM receptor family involving rac1- and cdc42-dependent and -independent pathways. EMBO J 2002; 21:560-71; PMID:11847104; http://dx.doi.org/10.1093/emboj/21.4.560
65. Belland RJ, Chen T, Swanson J, Fischer SH. Human neutrophil response to recombinant neisserial opa proteins. Mol Microbiol 1992; 6:1729-37; PMID:1630313; http://dx.doi.org/10.1111/j.1365-2958.1992.tb01345.x
66. Virji M, Heckels JE. The effect of protein II and pili on the interaction of neisseria gonorrhoeae with human polymorphonuclear leucocytes. J Gen Microbiol 1986; 132:503-12; PMID:2872268.
67. Schmitter T, Pils S, Sakk V, Frank R, Fischer KD, Hauck CR. The granulocyte receptor carcinoembryonic antigen-related cell adhesion molecule 3 (CEACAM3) directly associates with vav to promote phagocytosis of human pathogens. J Immunol 2007; 178:3797-805; PMID:17339478; http://dx.doi.org/10.4049/jimmunol.178.6.3797
68. Pils S, Kopp K, Peterson L, Delgado Tascon J, Nyffenegger-Jann NJ, Hauck CR. The adaptor molecule nck localizes the WAVE complex to promote actin polymerization during CEACAM3-mediated phagocytosis of bacteria. PLoS One 2012; 7:e32808; PMID:22448228; http://dx.doi.org/10.1371/journal.pone.0032808
69. Naumann M, Rudel T, Wieland B, Bartsch C, Meyer TF. Coordinate activation of activator protein 1 and inflammatory cytokines in response to neisseria gonorrhoeae epithelial cell contact involves stress response kinases. J Exp Med 1998; 188:1277-86; PMID:9763607; http://dx.doi.org/10.1084/jem.188.7.1277
70. Nagel G, Grunert F, Kuijpers TW, Watt SM, Thompson J, Zimmermann W. Genomic organization, splice variants and expression of CGM1, a CD66-related member of the carcinoembryonic antigen gene family. Eur J Biochem 1993; 214:27-35; PMID:8508798; http://dx.doi.org/10.1111/j.1432-1033.1993.tb17892.x
71. Hauck CR, Gulbins E, Lang F, Meyer TF. Tyrosine phosphatase SHP-1 is involved in CD66-mediated phagocytosis of opa52-expressing neisseria gonorrhoeae. Infect Immun 1999; 67:5490-4; PMID:10496937.
72. Sarantis H, Gray-Owen SD. The specific innate immune receptor CEACAM3 triggers neutrophil bactericidal activities via a syk kinase-dependent pathway. Cell Microbiol 2007; 9:2167-80; PMID:17506820; http://dx.doi.org/10.1111/j.1462-5822.2007.00947.x
73. Buntru A, Kopp K, Voges M, Frank R, Bachmann V, Hauck CR. Phosphatidylinositol 3'-kinase activity is critical for initiating the oxidative burst and bacterial

destruction during CEACAM3-mediated phagocytosis. J Biol Chem 2011; 286:9555-66; PMID:21216968; http://dx.doi.org/10.1074/jbc.M110.216085

74. Chen T, Bolland S, Chen I, Parker J, Pantelic M, Grunert F, Zimmermann W. The CGM1a (CEACAM3/CD66d)-mediated phagocytic pathway of neisseria gonorrhoeae expressing opacity proteins is also the pathway to cell death. J Biol Chem 2001; 276:17413-9; PMID:11278708; http://dx.doi.org/10.1074/jbc.M010609200
75. Edwards JL, Brown EJ, Uk-Nham S, Cannon JG, Blake MS, Apicella MA. A co-operative interaction between neisseria gonorrhoeae and complement receptor 3 mediates infection of primary cervical epithelial cells. Cell Microbiol 2002; 4:571-84; PMID:12390350; http://dx.doi.org/10.1046/j.1462-5822.2002.t01-1-00215.x
76. Edwards JL, Apicella MA. The role of lipooligosaccharide in neisseria gonorrhoeae pathogenesis of cervical epithelia: lipid a serves as a C3 acceptor molecule. Cell Microbiol 2002; 4:585-98; PMID:12390351; http://dx.doi.org/10.1046/j.1462-5822.2002.00212.x
77. Van Putten JP, Duensing TD, Carlson J. Gonococcal invasion of epithelial cells driven by P.IA, a bacterial ion channel with GTP binding properties. J Exp Med 1998; 188:941-52; PMID:9730895; http://dx.doi.org/10.1084/jem.188.5.941
78. Kuhlewein C, Rechner C, Meyer TF, Rudel T. Low-phosphate-dependent invasion resembles a general way for neisseria gonorrhoeae to enter host cells. Infect Immun 2006; 74:4266-73; PMID:16790801; http://dx.doi.org/10.1128/IAI.00215-06
79. Boettcher JP, Kirchner M, Churin Y, Kaushansky A, Pompaiah M, Thorn H, Brinkmann V, Macbeath G, Meyer TF. Tyrosine-phosphorylated caveolin-1 blocks bacterial uptake by inducing vav2-rhoa-mediated cytoskeletal rearrangements. PLoS Biol 2010; 8 (8): e1000457. PMID:20808760; http://dx.doi.org/10.1371/journal.pbio.1000457
80. Criss AK, Seifert HS. Gonococci exit apically and basally from polarized epithelial cells and exhibit dynamic changes in type IV pili. Cell Microbiol 2006; 8:1430-43; PMID:16922862; http://dx.doi.org/10.1111/j.1462-5822.2006.00722.x
81. Wang J, Meyer TF, Rudel T. Cytoskeleton and motor proteins are required for the transcytosis of neisseria gonorrhoeae through polarized epithelial cells. Int J Med Microbiol 2008; 298:209-21; PMID:17683982; http://dx.doi.org/10.1016/j.ijmm.2007.05.004.
82. Muller A, Gunther D, Dux F, Naumann M, Meyer TF, Rudel T. Neisserial porin (PorB) causes rapid calcium influx in target cells and induces apoptosis by the activation of cysteine proteases. EMBO J 1999; 18:339-52; PMID:9889191; http://dx.doi.org/10.1093/emboj/18.2.339
83. Kepp O, Gottschalk K, Churin Y, Rajalingam K, Brinkmann V, Machuy N, Kroemer G, Rudel T. Bim and bmf synergize to induce apoptosis in neisseria gonorrhoeae infection. PLoS Pathog. 2009;5(3): e1000348; PMID:19300516; http://dx.doi.org/10.1371/journal.ppat.1000348
84. Petrin D, Delgaty K, Bhatt R, Garber G. Clinical and microbiological aspects of trichomonas vaginalis. Clin Microbiol Rev 1998; 11:300-17; PMID:9564565.
85. Krieger JN. Trichomoniasis in men: old issues and new data. Sex Transm Dis 1995; 22:83-96; PMID:7624817; http://dx.doi.org/10.1097/00007435-199503000-00003
86. Lloyd G, Case JR, De Frias D, Brannigan RE. Trichomonas vaginalis orchitis with associated severe oligoasthenoteratospermia and hypogonadism. J Urol 2003; 170:924; PMID:12913736; http://dx.doi.org/10.1097/01.ju.0000080375.18547.cc
87. Sutcliffe S, Giovannucci E, Alderete JF, Chang TH, Gaydos CA, Zenilman JM, De Marzo AM, Willett WC, Platz EA. Plasma antibodies against trichomonas vaginalis and subsequent risk of prostate cancer. Cancer Epidemiol Biomarkers Prev 2006; 15:939-45; PMID:16702374; http://dx.doi.org/10.1158/1055-9965.EPI-05-0781
88. Lehker MW, Sweeney D. Trichomonad invasion of the mucous layer requires adhesins, mucinases, and motility. Sex Transm Infect 1999; 75:231-8; PMID:10615308; http://dx.doi.org/10.1136/sti.75.4.231
89. Hirt RP, Noel CJ, Sicheritz-Ponten T, Tachezy J, Fiori PL. Trichomonas vaginalis surface proteins: a view from the genome. Trends Parasitol 2007; 23:540-7; PMID:17962075; http://dx.doi.org/10.1016/j.pt.2007.08.020
90. Lama A, Kucknoor A, Mundodi V, Alderete JF. Glyceraldehyde-3-phosphate dehydrogenase is a surface-associated, fibronectin-binding protein of trichomonas vaginalis. Infect Immun 2009; 77:2703-11; PMID:19380472; http://dx.doi.org/10.1128/IAI.00157-09
91. Fichorova RN, Trifonova RT, Gilbert RO, Costello CE, Hayes GR, Lucas JJ, Singh BN. Trichomonas vaginalis lipophosphoglycan triggers a selective upregulation of cytokines by human female reproductive tract epithelial cells. Infect Immun 2006; 74:5773-9; PMID:16988255; http://dx.doi.org/10.1128/IAI.00631-06
92. Okumura CY, Baum LG, Johnson PJ. Galectin-1 on cervical epithelial cells is a receptor for the sexually transmitted human parasite trichomonas vaginalis. Cell Microbiol 2008; 10:2078-90; PMID:18637021; http://dx.doi.org/10.1111/j.1462-5822.2008.01190.x
93. Twu O, de Miguel N, Lustig G, Stevens GC, Vashisht AA, Wohlschlegel JA, Johnson PJ. Trichomonas vaginalis exosomes deliver cargo to host cells and mediate host : parasite interactions. PLoS Pathog. 2013; 9(7): e1003482; PMID:23853596; http://dx.doi.org/10.1371/journal.ppat.1003482
94. Arroyo R, Gonzalez-Robles A, Martinez-Palomo A, Alderete JF. Signalling of trichomonas vaginalis for amoeboid transformation and adhesion synthesis follows cytoadherence. Mol Microbiol 1993; 7:299-309; PMID:8446032; http://dx.doi.org/10.1111/j.1365-2958.1993.tb01121.x
95. De Miguel N, Lustig G, Twu O, Chattopadhyay A, Wohlschlegel JA, Johnson PJ. Proteome analysis of the surface of trichomonas vaginalis reveals novel proteins and strain-dependent differential expression. Mol Cell Proteomics 2010; 9:1554-66; PMID:20467041; http://dx.doi.org/10.1074/mcp.M000022-MCP201
96. Xu M-Y, Liu J-L, Zhang R-L, Fu Y. Isolation of a novel ras gene from trichomonas vaginalis: a possible evolutionary ancestor of the ras and rap genes of higher eukaryotes. Biochem Cell Biol 2007; 85:239-45; PMID:17534405; http://dx.doi.org/10.1139/O07-008
97. Rendon-Maldonado JG, Espinosa-Cantellano M, Gonzalez-Robles A, Martinez-Palomo A. Trichomonas vaginalis: in vitro phagocytosis of lactobacilli, vaginal epithelial cells, leukocytes, and erythrocytes. Exp Parasitol 1998; 89:241-50; PMID:9635448; http://dx.doi.org/10.1006/expr.1998.4297
98. Twu O, de Miguel N, Lustig G, Stevens GC, Vashisht AA, Wohlschlegel JA, Johnson PJ. Trichomonas vaginalis exosomes deliver cargo to host cells and mediate hostratioparasite interactions. PLoS Pathog 2013; 9: e1003482; PMID:23853596; http://dx.doi.org/10.1371/journal.ppat.1003482
99. Farhi D, Dupin N. Origins of syphilis and management in the immunocompetent patient: facts and controversies. Clin Dermatol 2010; 28:533-8; PMID:20797514; http://dx.doi.org/10.1016/j.clindermatol.2010.03.011
100. Thomas DD, Navab M, Haake DA, Fogelman AM, Miller JN, Lovett MA. Treponema pallidum invades intercellular junctions of endothelial cell monolayers. Proc Natl Acad Sci U S A 1988; 85:3608-12; PMID:3285346; http://dx.doi.org/10.1073/pnas.85.10.3608
101. Juanpere-Rodero N, Martin-Ezquerra G, Fernandez-Casado A, Magan-Perea L, Garcia-Alguacil MA, Barranco-Sanz C, Serrano-Figueras S, Pujol-Vallverdu RM, Lloreta-Trull J. Cell and tissue interactions of treponema pallidum in primary and secondary syphilitic skin lesions: an ultrastructural study of serial sections. Ultrastruct Pathol 2013; 37:36-42; PMID:21736426; http://dx.doi.org/10.3109/01913123.2011.584498
102. Chapel TA. The signs and symptoms of secondary syphilis. Sex Transm Dis 1980; 7:161-4; PMID:7455863; http://dx.doi.org/10.1097/00007435-198010000-00002
103. Kent ME, Romanelli F. Reexamining syphilis: an update on epidemiology, clinical manifestations, and management. Ann Pharmacother 2008; 42(2):226-36; PMID:18212261; http://dx.doi.org/10.1345/aph.1K086
104. Tight RR, Perkins RL. Treponema pallidum infection in subcutaneous polyethylene chambers in rabbits. Infect Immun 1976; 13:1606-12; PMID:786879.
105. Fraser CM, Norris SJ, Weinstock GM, White O, Sutton GG, Dodson R, Gwinn M, Hickey EK, Clayton R, Ketchum KA, et al. Complete genome sequence of treponema pallidum, the syphilis spirochete. Science 1998; 281:375-88; PMID:9665876; http://dx.doi.org/10.1126/science.281.5375.375
106. Fitzgerald TJ, Johnson RC, Miller JN, Sykes JA. Characterization of the attachment of treponema pallidum (nichols strain) to cultured mammalian cells and the potential relationship of attachment to pathogenicity. Infect Immun 1977; 18:467-78; PMID:336548.
107. Hayes NS, Muse KE, Collier AM, Baseman JB. Parasitism by virulent treponema pallidum of host cell surfaces. Infect Immun 1977; 17:174-86; PMID:328394.
108. Cameron CE, Brown EL, Kuroiwa JM, Schnapp LM, Brouwer NL. Treponema pallidum fibronectin-binding proteins. J Bacteriol 2004; 186:7019-22; PMID:15466055; http://dx.doi.org/10.1128/JB.186.20.7019-7022.2004
109. Brinkman MB, McGill MA, Pettersson J, Rogers A, Matejkova P, Smajs D, Weinstock GM, Norris SJ, Palzkill T. A novel treponema pallidum antigen, TP0136, is an outer membrane protein that binds human fibronectin. Infect Immun 2008; 76:1848-57; PMID:18332212; http://dx.doi.org/10.1128/IAI.01424-07
110. Cameron CE. Identification of a treponema pallidum laminin-binding protein. Infect Immun 2003; 71:2525-33; PMID:12704124; http://dx.doi.org/10.1128/IAI.71.5.2525-2533.2003
111. Houston S, Hof R, Francescutti T, Hawkes A, Boulanger MJ, Cameron CE. Bifunctional role of the treponema pallidum extracellular matrix binding adhesin Tp0751. Infect Immun 2011; 79:1386-98; PMID:21149586; http://dx.doi.org/10.1128/IAI.01083-10
112. Watanabe T, Takahashi Y. Tissue morphogenesis coupled with cell shape changes. Curr Opin Genet Dev 2010; 20:443-7; PMID:20677359; http://dx.doi.org/10.1016/j.gde.2010.05.004
113. Calì G, Mazzarella C, Chiacchio M, Negri R, Retta SF, Zannini M, Gentile F, Tarone G, Nitsch L, Garbi C. RhoA activity is required for fibronectin assembly and counteracts beta1B integrin inhibitory effect in FRT epithelial cells. J Cell Sci 1999; 112 (Pt 6):957-65; PMID:10036245.
114. Dubash AD, Wennerberg K, García-Mata R, Menold MM, Arthur WT, Burridge K. A novel role for lsc/p115 rhoGEF and LARG in regulating rhoa activity downstream of adhesion to fibronectin. J Cell Sci 2007; 120:3989-98; PMID:17971419; http://dx.doi.org/10.1242/jcs.003806
115. O'Brien LE, Jou TS, Pollack AL, Zhang Q, Hansen SH, Yurchenco P, Mostov KE. Rac1 orientates epithelial apical polarity through effects on basolateral laminin assembly. Nat Cell Biol 2001; 3:831-8;

PMID:11533663; http://dx.doi.org/10.1038/ncb0901-831
116. Eucker TP, Konkel ME. The cooperative action of bacterial fibronectin-binding proteins and secreted proteins promote maximal campylobacter jejuni invasion of host cells by stimulating membrane ruffling. Cell Microbiol. 2012; 14(2):226-38; PMID:21999233; http://dx.doi.org/10.1111/j.1462-5822.2011.01714.x
117. Bourdoulous S, Orend G, MacKenna DA, Pasqualini R, Ruoslahti E. Fibronectin matrix regulates activation of RHO and CDC42 GTPases and cell cycle progression. J Cell Biol 1998; 143:267-76; PMID:9763437; http://dx.doi.org/10.1083/jcb.143.1.267
118. Arthur WT, Noren NK, Burridge K. Regulation of rho family GTPases by cell-cell and cell-matrix adhesion. Biol Res 2002; 35:239-46; PMID:12415742; http://dx.doi.org/10.4067/S0716-97602002000200016
119. Chung JW, Hong SJ, Kim KJ, Goti D, Stins MF, Shin S, Dawson VL, Dawson TM, Kim KS. 37-kda laminin receptor precursor modulates cytotoxic necrotizing factor 1-mediated rhoa activation and bacterial uptake. J Biol Chem 2003; 278:16857-62; PMID:12615923; http://dx.doi.org/10.1074/jbc.M301028200
120. Boehm M, Krause-Gruszczynska M, Rohde M, Tegtmeyer N, Takahashi S, Oyarzabal OA, Backert S. Major host factors involved in epithelial cell invasion of campylobacter jejuni: role of fibronectin, integrin beta1, FAK, tiam-1, and DOCK180 in activating rho GTPase rac1. Front Cell Infect Microbiol 2011; 1:17; PMID:22919583; http://dx.doi.org/10.3389/fcimb.2011.00017
121. Lemichez E, Lecuit M, Nassif X, Bourdoulous S. Breaking the wall: targeting of the endothelium by pathogenic bacteria. Nat Rev Microbiol 2010; 8:93-104; PMID:20040916.
122. Insall RH, Machesky LM. Actin dynamics at the leading edge: from simple machinery to complex networks. Dev Cell 2009; 17:310-22; PMID:19758556; http://dx.doi.org/10.1016/j.devcel.2009.08.012
123. Beemiller P, Krummel MF. Mediation of T-cell activation by actin meshworks. Cold Spring Harb Perspect Biol 2010; 2:a002444; PMID:20702599; http://dx.doi.org/10.1101/cshperspect.a002444
124. Harwood NE, Batista FD. The cytoskeleton coordinates the early events of B-cell activation. Cold Spring Harb Perspect Biol 2011; 3:a002360. PMID:21047917; http://dx.doi.org/10.1101/cshperspect.a002360
125. Frischknecht F, Way M. Surfing pathogens and the lessons learned for actin polymerization. Trends Cell Biol 2001; 11:30-8; PMID:11146296; http://dx.doi.org/10.1016/S0962-8924(00)01871-7