Advances in Rickettsia Pathogenicity

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One century after the first description of rickettsiae as human pathogens, the rickettsiosis remained poorly understood diseases. These microorganisms are indeed characterized by a strictly intracellular location which has, for long, prohibited their detailed study. Within the last ten years, the completion of the genome sequences of several strains allowed gaining a better knowledge about the molecular mechanisms involved in rickettsia pathogenicity. Here, we summarized available data concerning the critical steps of rickettsia-host cell interactions that should contribute to tissue injury and diseases, that is, adhesion, phagosomal escape, motility, and intracellular survival of the bacteria.

***Key words*: actin-based motility; adhesins; phagosomal escape; PLD; rickettsia; T4SS**

Introduction

One century elapsed since Ricketts provided the first experimental evidence for involvement of a transmissible microbe causing the Rocky Mountain spotted fever.[1] His investigations highlighted most of the major characteristics of the responsible infectious agent, including its intracellular nature and its arthropod transmission.[2] In honor to Ricketts's contribution to the description of a new class of pathogens that were not fulfilling the Koch's postulate, as uncultivable,[3] the genus *Rickettsia* was born. These gram-negative and rod-shaped bacterial species were divided in two main groups, the spotted fever group (SFG) and the typhus group (TG).[4] During the 80 years following their initial description, only a few rickettsiae were conclusively associated with human diseases.[4] The introduction of modern diagnostic tools including culture, PCR, and serological analysis[5] largely contributed to the recent expansion of rickettsia identification from human samples. This is exemplified by the fact that among the 20 pathogenic strains yet established, 13 new tick-borne rickettsiae were identified since 1991.[6] Significant progresses have also been achieved concerning both epidemiology[6] and genotyping.[7] In contrast, questions regarding the underlying mechanisms of their pathogenicity remained for long poorly understood.

The main steps of rickettsial infections can be depicted as follows: Rickettsiae are usually inoculated in human beings by arthropods that transmit bacteria through salivary secretion (ticks) or infected feces (flea and louse).[4] Indirect routes of transmission were also described, such as contamination of the eyes following crushing of ticks with the fingers and contamination of abraded skin by tick-borne rickettsiae excreted in tick feces.[6] In addition, *R. prowazekii* that is stable in dried louse feces can be transmitted through aerosols.[4] The risk of disease transmission results from a complex process that relies on various parameters including, for example, abundance of infected arthropod vectors, the prevalence of their infection rate, and increased attachment time (for tick-transmitted rickettsiosis).[4,6] Bacteria then invade human endothelial cells, via the process of induced phagocytosis,[8]

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Rickettsiology and Rickettsial Diseases-Fifth International Conference: Ann. N.Y. Acad. Sci. 1166: 94–105 (2009).
doi: 10.1111/j.1749-6632.2009.04517.x © 2009 New York Academy of Sciences.

and rapidly escape from the phagosome into the host cytoplasm[9] where they replicate. Historically, besides growth temperature and antigenicity, the actin-based motility was depicted as a major feature; allowing to differentiate SFG and TG rickettsiae. While actin polymerization promotes cell-to-cell spreading, TG rickettsiae that are devoid of motility replicate until the cell bursts. The clinical features mainly correspond to pulmonary and brain endothelial cell damages.[10]

The strictly intracellular location of bacteria from the *Rickettsia* genus has for long prohibited their study,[11] explaining that the detailed picture of their interaction with host cells was lacking. During the last few years, the completion of the genome sequence of several strains of rickettsiae,[12] starting from that of *R. prowazekii* (published in 1998),[13] allowed gaining a better understanding of the molecular mechanisms involved in tissue injury and disease caused by these pathogens. Based on the premise that differences or similarities in the ability of two microbes to promote disease is reflected in genomic sequence data, the comparison of two closely related genomes can theoretically allow the finding of differences that correlate with virulence. Therefore, due to the lack of nonpathogenic rickettsial mutants, the computational prediction and characterization of putative virulence factors is a hard task. In fact, to our knowledge, such a comparative analysis has been made possible only twice. Thus, identification of genes involved in the virulence of *R. rickettsii* was carried out from the genome sequences of the virulent strain *R. rickettsii* Sheila Smith and of *R. rickettsii* Iowa; the later being depicted as avirulent in a guinea pig model of infection.[14] Similarly, the comparative analysis of *R. prowazekii* Madrid E (avirulent) and BreinL (virulent) has been reported.[15–19] However, we have to keep in mind that the nonpathogenic phenotype of these strains has been established on experimental animal models, and extrapolation from such model studies to the human diseases is somewhere highly speculative.

Here, we review recent data concerning the main steps of such a bacteria-host cell interaction, namely adhesion, phagosomal escape, and motility. Most of the bacterial virulence factors are proteic compounds either secreted at the bacterial cell surface or released into the external environment. Gram-negative bacteria have developed only a small number of secretion systems by which such proteins pass through their outer membranes and exert their function. Rickettsial secretion system and putatively secreted virulence proteins will also be discussed. All these steps were depicted in Figure 1.

Rickettsial Ligands and Host Cell Receptors

A major constituent of the outer membrane of gram-negative bacteria is lipopolysaccharide, but in contrast to what was observed for several pathogens,[20] its role as rickettsial adhesin was discarded.[10,21] Because rickettsiae lost their ability to bind to host cells after heating or incubation with trypsin, involvement for a proteic ligand was suggested.[22] The proteins susceptible to play a role in binding and entry of the bacteria into their host cells are listed above.

Sca Family Proteins

It was long evidenced that rickettsiae were surrounded by a regularly arrayed surface structure (S-layer)[23] containing proteins able to elicit protective immune responses.[24,25] Prior to *rickettsial* genome sequencing, only two surface protein antigens (SPAs), namely rOmpA and rOmpB, were identified. Analysis of their amino acid sequence highlighted the presence of a conserved autotransporter β-barrel domain otherwise called autotransporter domain (AT) at their C-terminal end. Availability of *R. prowazekii* and *R. conorii* genomes[13,26] further allowed evidence of three additional genes encoding such an AT domain. These genes were annotated as "surface cell antigen" (*sca*) genes

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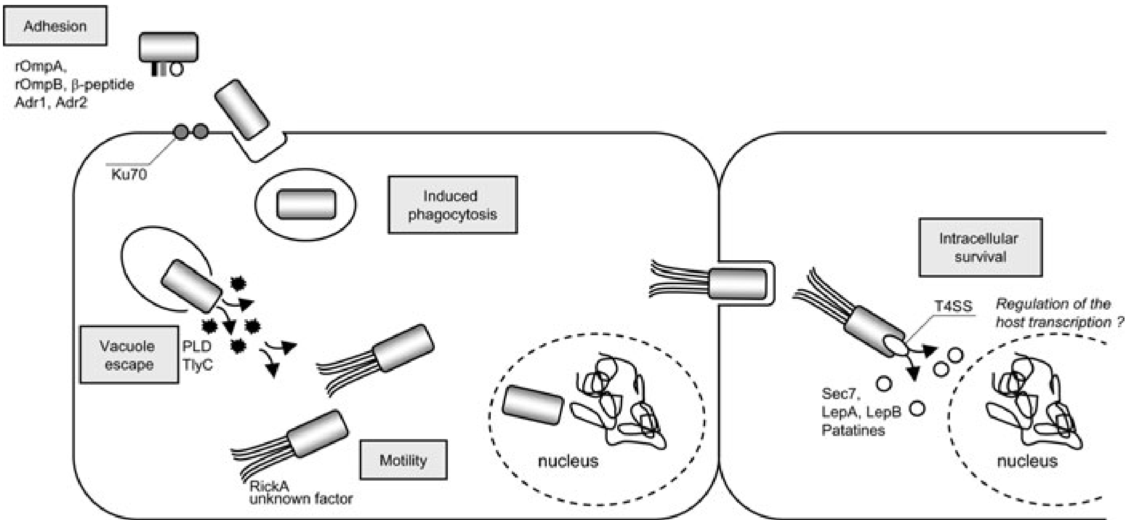


Figure 1. Schematic representation of rickettsia-endothelial cell interaction. Following rickettsia entry into host cells through induced phagocytosis, bacteria rapidly escape from the vacuole to gain the cytosolic compartment and possibly the eucaryotic nucleus where they replicate. For rickettsiae exhibiting a motile phenotype, cell-to-cell spreading is observed. T4SS translocate effectors that should contribute to the intracellular survival of the rickettsiae.

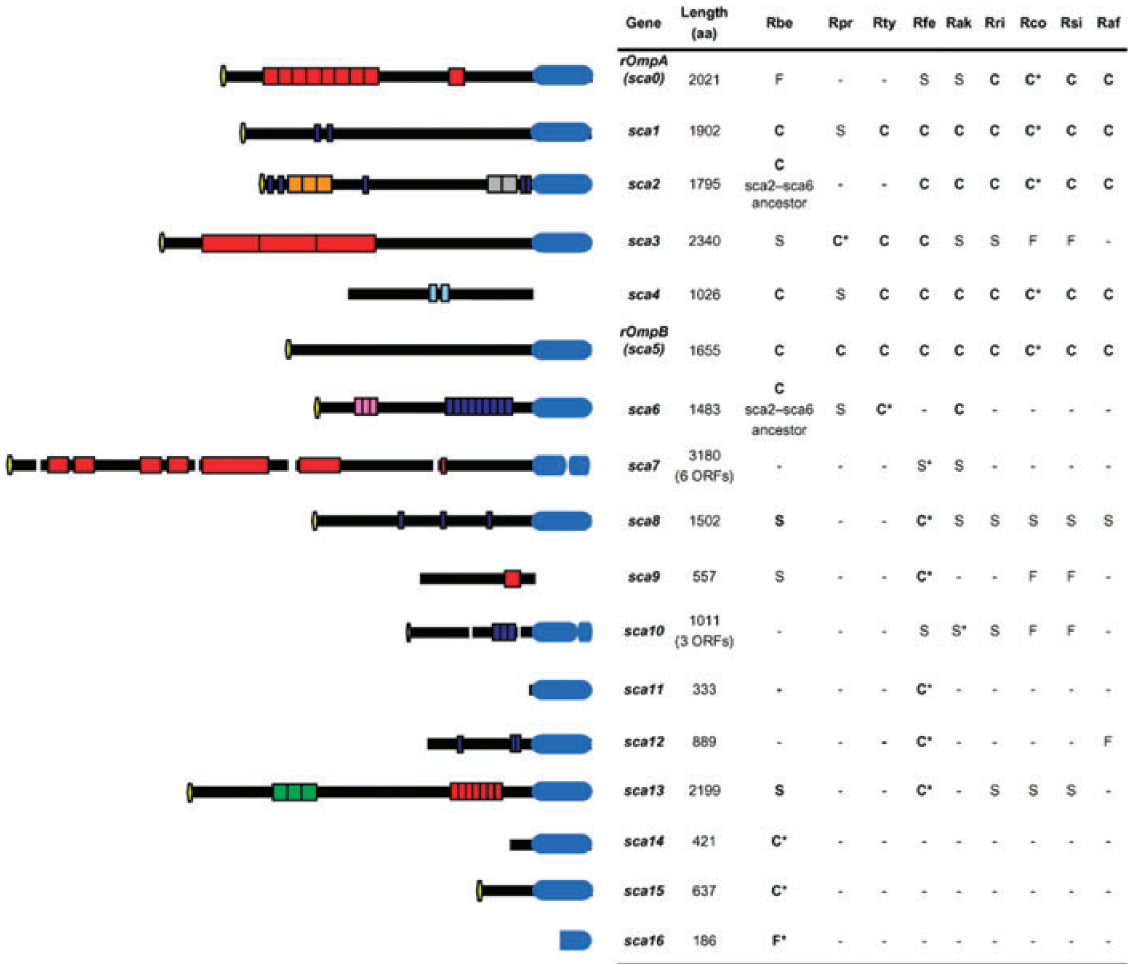
(i.e., *sca1*, *2*, *3*) together with *rOmpA* (*sca0*) and *rOmpB* (*sca5*), while *geneD* was renamed *sca4*. Using nine rickettsial genomic sequences, the "Sca protein family" was then extensively characterized and 17 subfamilies were established.[27] Their typical architecture (Fig. 2) comprises a N-terminal signal peptide and a C-terminal AT domain that promotes the export of the central passenger domain to the outside of the bacteria. With a few exceptions, including rOmpA and rOmpB (further detailed below), the function of these Sca proteins remains unknown. However, it was hypothesized that they should contribute to the specific recognition of different sets of host receptors.[27]

rOmpA

This 190 kDa immunodominant surface-exposed protein was thought to be involved in adhesion of rickettsiae to host cells, based on the protective effect against rickettsial infections in animal models immunized with the recombinant truncated rOmpA or DNA plasmid encoding this protein.[28–32] The critical role displayed by rOmpA in the attachment of rickettsiae to host cells was further confirmed *ex vivo*.[33] Moreover, the recent comparative genomic analysis of *R. rickettsii* Sheila Smith (virulent) and Iowa (avirulent) strains highlighted a deletion resulting in defect of rOmpA expression in the latter.[14] Immunoblotting and immunofluorescence confirmed the absence of rOmpA from *R. rickettsii* Iowa. While this gene was initially present within *R. prowazekii* genome, its evolution led to its complete disappearance by degradation, leaving only traces of short homologous sequences.[34] Thus, and as confirmed from rickettsial genome sequencing, rOmpA is exclusive for the SFG rickettsiae.[12,27]

rOmpB

As rOmpA, rOmpB was long associated with both antigenicity and pathogenicity of these bacteria.[35] The gene *omp*B coding for this autotransporter belongs to the core genes of *Rickettsia*.[12] In fact, among the 17 members of rickettsial cell surface antigens (*sca* family) identified within available rickettsial genomes, *rompB* was the unique conserved one.[27] This



Gene	Length (aa)	Rbe	Rpr	Rty	Rfe	Rak	Rri	Rco	Rsi	Raf
rOmpA (sca0)	2021	F	-	-	S	S	C	C*	C	C
sca1	1902	C	S	C	C	C	C	C*	C	C
sca2	1795	C sca2–sca6 ancestor	-	-	C	C	C	C*	C	C
sca3	2340	S	C*	C	C	S	S	F	F	-
sca4	1026	C	S	C	C	C	C	C*	C	C
rOmpB (sca5)	1655	C	C	C	C	C	C	C*	C	C
sca6	1483	C sca2–sca6 ancestor	S	C*	-	C	-	-	-	-
sca7	3180 (6 ORFs)	-	-	-	S*	S	-	-	-	-
sca8	1502	S	-	-	C*	S	S	S	S	S
sca9	557	S	-	-	C*	-	-	F	F	-
sca10	1011 (3 ORFs)	-	-	-	S	S*	S	F	F	-
sca11	333	-	-	-	C*	-	-	-	-	-
sca12	889	-	-	-	C*	-	-	-	-	F
sca13	2199	S	-	-	C*	-	S	S	S	-
sca14	421	C*	-	-	-	-	-	-	-	-
sca15	637	C*	-	-	-	-	-	-	-	-
sca16	186	F*	-	-	-	-	-	-	-	-

Figure 2. The 17 *sca* gene families identified in nine *Rickettsia* genomes. See[27]; reprinted with permission, Oxford Journals, Oxford University Press licence number 1954711133562. The structural domains of the Sca proteins are schematized on the left. The yellow diamonds, thick black lines, and the blue ovals represent the predicted peptide signals, passenger domains, and AT domains, respectively. Interruptions in the thick black lines or the blue ovals indicate the occurrence of in-frame stop codons in the coding sequence. Colored boxes show the presence of repeated peptide motifs. Boxes of same color refer to similar peptide motifs. The table on the right summarizes the names, lengths of the peptide products, and the presence or absence of the *sca* genes in the *Rickettsia* genomes. The characters "C," "S," "F," and "-" indicate whether the gene is complete, split, fragment, or absent, respectively, in a given genome. Genes were defined as split if the entire protein is encoded by successive ORFs and as fragment if the gene size was less than 50% of that of the longest ortholog. Asterisks indicate the genes from which the length and the structural domains of the peptide products are shown. For Sca7 and Sca10, the protein lengths were obtained after concatenating the successive ORFs.

gene encodes a large precursor subsequently processed into the mature 120-kDa rOmpB and the 32 kDa β-peptide, respectively.[36,37] Recent experiments showed that expression of rOmpB in *Escherichia coli* allowed adherence and entry of transformed bacteria in epithelial cells.[38] The comparative analysis of virulent and avirulent strains of *R. prowazekii* suggested that rOmpB methylation could be associated with the virulent phenotype.[39] A relationship was further evidenced between the defect of methylation observed in the avirulent MadridE

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and the presence of a mutation within the gene encoding the lysine methyltransferase,[18] impairing expression of this enzyme.[19] Through overlay assays carried out with biotinylated endothelial cells, we recently evidenced that the β-peptide was a putative rickettsial ligand. This interaction was not observed with the avirulent *R. rickettsii* Iowa strain[40] that is defective in the processing of rOmpB.[14,37] While such interaction was somewhere in contradiction with the theoretical location of the β-peptide,[41] a positive immunofluorescence staining of rickettsiae outer membrane was observed with anti-β-peptide antibodies (unpublished data).

Adr1 and Adr2 Rickettsial Adhesins

Overlay assays coupled with high resolution 2D-PAGE and mass spectrometry analysis also permitted to point out two putative rickettsial adhesins of 30 kDa encoded by the paralogous genes RC1281 and RC1282 in *R. conorii* and, respectively, designed as Adr1 and Adr2.[40] As rOmpB, these proteins were found ubiquitously present within the *Rickettsia* genus.[12] While initially annotated of unknown function, BLAST analysis further evidenced homologies between these proteins and other bacterial adhesins including the adhesin/virulence factor Hek of *Escherichia coli* UTI89 (ZP_00726099), the putative invasin of *Lawsonia intracellularis* (YP_595216), and the possible outer membrane adhesin of *Salmonella enterica* subsp. (ZP_02675271). Moreover, we observed that polyclonal antibodies raised against the recombinant *R. conorii* Adr1 inhibited the bacterial entry into the host cells, estimated by plaque formation (unpublished data).

Eukaryotic Ligands

Only a few experiments were aimed at identifying the eukaryotic receptor of rickettsiae. It was demonstrated that the Ku70 subunit of DNA-dependent protein kinase, a protein present in the nucleus, cytoplasm, plasma membrane, and lipid raft microdomains might be involved in rickettsia entry within non-phagocytic mammalian cells.[42] While the cascade of signalling events leading to rickettsial phagocytosis remains to be characterized, the rOmpB protein was identified as the rickettsial ligand that interacted with Ku70.

Regulation of Rickettsial Ligand Expression

During their life cycle, bacteria from the *Rickettsia* genus *may* adapt to diverse environments in the ticks and mammals. Their adaptation strategy most probably results from a selective gene expression, as depicted for *Borrelia burgdorferi*.[43] Accordingly, we observed that rOmpA expression can undergo major changes, as being strongly detected when rickettsiae propagated within Vero cells while being poorly expressed in bacteria that was collected from tick hemolymph.[44] Variation in rOmpA, but not in rOmpB expression, was also evidenced in *R. massiliae* during the *Rhipicephalus turanicus* life cycle.[45] When inoculated from arthropod vectors to human beings, rickettsiae most probably exhibit a proteic profile different to that observed from bacteria grown in culture. *Ex-vivo* experiments aimed at characterizing this host-pathogen interaction should thus be analyzed with caution.

Phagosomal Escape

As several other pathogens of the genus *Listeria*, *Shigella*, and *Mycobacterium*, *Rickettsia* rapidly gain access to the cytosol of infected cells through phagosomal vacuole escape. The involvement for a phospholipase A_2 (PLA_2) in the entry vesicle lysis has for long been proposed for *R. rickettsii*[46,47] and then extended to both *R. conorii* and *R. prowazekii*.[48] Therefore, and despite the completion of corresponding genomes, any PLA_2-encoding gene was found. The first phospholipase identified within a rickettsial genome was the *R. conorii* phospholipase

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D (PLD).[49] This dimeric protein belongs to a new clade of PLD superfamily and is characterized by the presence, on each monomer, of only one HKD motif that is critical for the biochemical activity.[50] Recent published data demonstrating that Salmonella isolates transformed with the *R. prowazekii* PLD genes were able to escape phagosomal vacuoles confirmed that PLD is likely to be the major effector of rickettsial phagosomal escape.[51] Of note is that this gene is conserved in all species of the *Rickettsia* yet sequenced.

Besides PLD ortholog, the analysis of *R. typhi* genome highlighted the presence of another putative phospholipase-like protein exhibiting significant similarity to the patatin family of proteins.[52] Patatin is the major storage glycoprotein found in potatoes and is endowed for phospholipase A_2 (PLA_2) activity.[53] While only one patatin-like protein gene (*pat1)* is present in rickettsial genomes including *R. conorii, R. rickettsii, R. prowazekii, R. bellii, R. akari, R. sibirica, R. africae,* and *R. akari,*[54] three copies 100% identical were found in *R. felis.*[55] The gene *pat1* is located on the chromosome, and the two others, designed *pat2A* and *pat2B,* are located on the two *R. felis* plasmids. Alignment of all the rickettsial patatin-like proteins with characterized patatins from plants evidenced that the amino acid residues accounting for phospholipase A_2 activity are highly conserved.[54] Accordingly, McLeod and colleagues[52] hypothesized that patatin-like proteins might be responsible for the PLA_2 activity and thus involved in escape of the organism from the phagolysosome. Therefore, in contrast to what was observed for *pld*, the *pat1* gene is not transcriptionally expressed in *R. prowazekii* during the peak time of escape.[51] Another candidate with potential membranolytic activities and susceptible to intervene in such a process is tlyC.[51]

Actin-Based Motility

Exploitation of the host-cell actin cytoskeleton is crucial for several microbial pathogens to enter and to disseminate within cells, thus avoiding the host immune response.[56,57] The capacity of rickettsiae to use the actin-based motility system for promoting cell-to-cell spreading was evidenced for several SFG rickettsiae including *R. conorii*, *R. rickettsii, R. montanensis, R. parkeri, R. australis,* and *R. monacensis.*[58–60] It was long considered that actin-based motility was confined to the SFG rickettsiae. Accordingly, the rickettsial factor susceptible to be responsible for actin polymerization was identified through the comparative analysis of *R. conorii* (SFG) and *R. prowazekii* (TG) genomes.[26] The so-called RickA protein contains a domain with homologies with Wiskott-Aldrich Syndrome Protein (WASP)-family proteins and was thought to function as a nucleation-promoting factor that directly activates the Arp2/3 complex.[61,62] In this respect, rickettsiae have evolved a strategy similar to that described for *Listeria monocytogenes.*[63] From *in vitro* actin branching assay performed with recombinant RickA, the involvement of additional bacterial or eukaryotic factors in reorganizing Arp2/3 complex generated Y-branched networks into parallel arrays was also suggested.[62]

Because genetic manipulations are still unfeasible, the role of RickA in the motility of rickettsiae has not been formerly demonstrated. Its function was in part supported by the absence of motility of *R. peacockii,* a strain for which *rickA* is disrupted by an insertion sequence of 1,095 nucleotides termed IRSpe1.[64] Therefore, some points remain unclear. Thus, and while RickA was found to be expressed on the bacterial surface,[65] both signal sequence and hydrophobic domain that are respectively required for secretion and membrane anchorage of this protein are lacking.[56,57] Thus, the putative involvement of the type IV secretion factor (T4SS) in the secretion of RickA to the rickettsial membrane was evoked.[61] Other bacterial components known to be responsible for intracellular motility as IcsA, ActA, or BimA, exhibited a polarized distribution.[66–68] Such polarization was not observed for RickA that was found to be expressed over the entire bacterial surface of

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R. conorii,[61] a result consistent with the fact that stationary rickettsiae are usually surrounded by a uniform actin coat.[69] It can be speculated that a polarized distribution of RickA is sequentially achieved through the activity of a cofactor. For example, the unipolar distribution of IcsA that acts as the actin nucleation factor for the gram-negative bacterium *Shigella flexneri* relies on both a membrane protease and also onto the LPS structure.[70] Another key element came from *R. typhi* analysis. Short and occasional actin tails (<1%) were observed for these TG rickettsiae,[58,59] which were able to move approximately at the same rate as *R. rickettsii*, at least some of them, while others exhibited rather erratic movements.[71] However, *R. typhi* genome sequencing confirmed the absence of *rickA,*[52] thus reinforcing the role for another protein in actin-based motility. Whether rickettsial motility is facilitated by a bacterial or a host factor remains to be elucidated. Experiments achieved on *R. raoultii*, a newly isolated rickettsiae classified within the SFG,[72] evidenced that the motile phenotype could be dependent on the host cells and unrelated to the level of RickA expression.[73]

Specific Features of Motile Rickettsiae

The propulsive force generated by actin tail assembly could favor entry of SFG rickettsiae within the nucleus of eucaryotic cells. Accordingly, TG rickettsiae cannot penetrate in this cellular compartment.[4] Once inside the nucleus, the bacteria then become trapped, a phenomenon that should result from the lack of Arp2 and Arp3 proteins. In this respect, within the nucleus of Vero cells, *R. rickettsii* displayed irregular actin tails that was not accompanied with bacterial motility and tends to form high-density microcolonies.[69] The intranuclear growth of *R. bellii* was also observed, resulting in an intranuclear colony growth that should be favored by a nutrient enriched environment.[74]

As described for other microbial pathogens, including *Listeria monocytogenes* and *Shigella flexneri*, the intracellular actin-based motility *is also exploited by rickettsiae* to escape the host cell.[10,59,65] The filaments of actin push the rickettsiae to the surface of the host cell, where the host cell membrane is deformed outward and invaginates into the adjacent cell. Disruption of both cell membranes then enables the rickettsia to enter the adjoining cell without being exposed to the extracellular environment. The ability to spread from cell to cell without passing through the intercellular space enable bacteria to evade the immune response and thus contribute to the development of the infection. Through such long and thin cell projections, rickettsiae can exit via the luminal surface of blood vessels into the bloodstream without lysing the host cell. In constrast, TG rickettsiae devoid of motility escape the host cell by multiplying in such large numbers that they cause endothelial cell burst with subsequent release of bacteria into the blood.[10]

The reductive evolution of rickettsial genomes led to the lost of *rickA* from TG rickettsiae, namely *R. typhi* and *R. prowazekii,*[12] the latter being the most deadly of the rickettsiae infecting humans.[75–77] This observation somewhere contradicts the recent classification of RickA as a virulence factor.[78] We believe that this protein might rather play a critical role under specific situations, including long-term survival in arthropods, while not being a main actor of bacterial pathogenicity.

Type IV Secretion System

Several ORFs related to the T4SS[79] belong to the core gene set of rickettsiae.[12] T4SS are complex multiprotein structures spanning the bacterial envelope and composed of up to 12 individual protein building blocks classified into three groups,[80] each represented in this bacterial genus. Thus, proteins exposed to the cytoplasm of the cells and providing the energy for the biogenesis of the transporter (VirB4, VirB11, VirD4) are conserved in all *Rickettsia* sequenced genomes as those involved in the

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building of the translocation channel (VirB6, VirB8, VirB9, VirB10). Among the surface-exposed proteins, we noticed the presence of the genes encoding the major pilus component VirB2 and the pilus-associated protein VirB3. In contrast, VirB5 and VirB7 are lacking. The periplasmic $VirB5_{Ti}$ subunits contribute to the translocation of DNA substrates to the cell surface, and VirB7 are small lipoproteins only found in a subset of T4SS.[79]

The precise function of these transporters, which are ancestrally related to bacterial conjugation systems, have diverged during evolution. Three T4SS subfamilies are yet described.[80] Two of them, namely the conjugation system and the recently discovered "DNA uptake and release system" ensure genetic exchanges between bacteria, the latter functioning independently of contact with a target cell. The third T4SS subfamily corresponds to the "effector translocator systems" also called "injectisomes" and has been adopted by several bacterial pathogens for the delivery of virulence factors targeting eukaryotic host cells.[80,81] Since T4SS components are conserved in *Rickettsia* genomes that lack plasmids, their primary suspected role was the secretion of virulence factors rather than conjugation.[55] The T4SS were divided into two distinct subgroups, type IVA and type IVB, respectively.[82] Based on homologies with the *virB/virD4* secretion system of the plant pathogen *Agrobacterium tumefaciens* the T4SS conserved within the genus *Rickettsia* belongs to the type IVA.

What are the rickettsial substrates susceptible to, to be exported by such a secretion system? A high homology between *L. pneumophila* RalF and the Sec7 proteins of *R. prowazekii* (RP374) and *R. typhi* (RT0362) was reported.[52,83] The precise role of an effector known to contribute to the establishment of a replicative organelle by inhibiting the phagosome-lysosome fusion[84] is questionable in rickettsiae, since these bacteriae are growing into the cytoplasm of infected cells.[4] This protein is not conserved within all rickettsial genomes. Thus, besides *R. typhi* and *R. prowazekii*, *sec7* is also present in *R. felis* and *R. akari*[85] (http://www.igs.cnrs-mrs.fr/mgdb/Rickettsia/rig/). Interestingly, two orthologous genes were found in *R. bellii*, a bacterium able to survive within ameba.[74] Miscroscopy examination of *Acanthameba polyphaga* coinfected by *R. bellii* and L. pneumophila revealed a colocalization of both bacteria within the amebal vacuole. In other SFG rickettsiae, Sec7 is either split or degraded.

We also noticed that other translocated effectors of the *L. pneumophila* Dot/Icm secretion system (Type IVB secretion system), namely LepA (RC0359) and LepB (RC0156), are present in all *Rickettsia* genome sequenced. These peptides are not required for *L. pneumophila* replication in mammalian cells, but rather promote the release of bacteria from protozoa through an exocytic pathway, thus favoring the pathogen dissemination via "faecal" or "respirable" infectious vesicles.[86] Again, this observation fits well with the probable ancestral location of rickettsiae within ameba.[74] As mentioned above, *R. bellii* can replicate within protozoan hosts, but this is not the case for other rickettsia species. Accordingly, whether these proteins are associated to the pathogenicity or parasitic lifestyle of rickettsiae during their interaction with eukaryotic host is not yet established.

VipD is patatin domain-containing protein that perturbs membrane trafficking and was recently identified as another effector of *L. pneumophila*.[87] This protein was found to modulate the intracellular bacterial growth.[88] As previously mentioned, *Rickettsia* also possesses patatin-like proteins with a conserved consensus phospholipase domain[54] and exhibits homologies with VipD including oxyanion hole (GGGXK/R), the active site serine (GXSXG), and the active site aspartate (DXG) regions. Similarly to what was observed in the L. pneumophila Philadelphia-1 genome,[88] three paralogs were found in *R. felis*.[55] The role of rickettsial patatin in the vacuole escape was discarded.[51] However, these proteins should be required in other steps of the infectious process.

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While increasing number of T4SS effectors are described,[80] putative rickettsial effectors identified through the examination of available genomes are exclusively related to *Legionella* effectors. In contrast with other bacterial pathogens, including rickettsiae that possess a type IVA secretion system, *L. pneumophila* uses a type IVB secretion system, a specificity also observed for *Coxiella burnetii*.[98] Since relationships were evidenced between type IVA and IVB secretion systems,[89] it is conceivable that Dot/Icm-like substrates could be translocated through VirB/D4 system. The rationale for why effectors known to pervert the host cell endosomal pathways and promote growth of bacteria into intracellular compartments found in *Rickettsia* is not explained.

To date, the regulation of T4SS in intracellular pathogens has been poorly studied. It was shown that expression of *Brucella suis* and *Bartonella henselae* T4SS were specifically induced when the bacteria were located within their host cells.[90,91] Due to the obligate intracellular nature of rickettsiae, such a transcriptomic comparison cannot be achieved. However, from a global proteomic analysis conducted on whole *R. felis* extract, we identified 134 proteins among which 4 components were of the T4SS.[92] Detection of these proteins indicated that they were majoritary expressed, suggesting their role as main actors of the rickettsia life. Moreover, we recently evidenced the up-regulation of the *virB* operon in *R. conorii* maintained in Vero cells and exposed to a nutrient stress.[93] This result is consistent with what was reported for *Brucella suis* when incubated in minimal medium.[90] Accordingly, it could be hypothesized that factors secreted by the T4SS should promote rickettsia survival by triggering synthesis of nutrients from the host cell or used for another purpose allowing adaptation of rickettsiae to intracellular or extracellular environments. A detailed understanding of the regulation of Rickettsia transcriptome would facilitate the identification of the effector functions, which enable this pathogen to parasitize eukaryotic cells.

Conclusions

The post-genomic analysis of bacteria from the *Rickettsia* genus achieved the last few years allowed to identify several proteins susceptible to be involved in their interaction with host cells. Functional studies of these factors would provide a better knowledge about the strategies devised by such obligate intracellular pathogens in colonizing their hosts.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Ricketts, H.T. 1906. The transmission of Rocky Mountain spotted fever by the bite of the wood-tick (*Dermacentor occidentalis*). *J. Am. Med. Assoc.* **47:** 358.
2. Walker, D.H. 2004. Ricketts creates rickettsiology, the study of vector-borne obligately intracellular bacteria. *J. Infect. Dis.* **189:** 938–955.
3. Harden, V.A. 1987. Koch's postulates and the etiology of rickettsial diseases. *J. Hist. Med. Allied Sci.* **42:** 277–295.
4. Raoult, D. & V. Roux. 1997. Rickettsioses as paradigms of new or emerging infectious diseases. *Clin. Microbiol. Rev.* **10:** 694–719.
5. La Scola, B. & D. Raoult. 1997. Laboratory diagnosis of rickettsioses: current approaches to diagnosis of old and new rickettsial diseases. *J. Clin. Microbiol.* **35:** 2715–2727.
6. Parola, P., C.D. Paddock & D. Raoult. 2005. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. *Clin. Microbiol. Rev.* **18:** 719–756.
7. Fournier, P.E., Y. Zhu, H. Ogata & D. Raoult. 2004. Use of highly variable intergenic spacer sequences for multispacer typing of *Rickettsia conorii* strains 264. *J. Clin. Microbiol.* **42:** 5757–5766.
8. Walker, T.S. & H.H. Winkler. 1978. Penetration of cultured mouse fibroblasts (L cells) by *Rickettsia prowazekii*. *Infect. Immun.* **22:** 200–208.
9. Teysseire, N., J.A. Boudier & D. Raoult. 1995. *Rickettsia conorii* entry into Vero cells. *Infect. Immun.* **63:** 366–374.
10. Valbuena, G., H.M. Feng & D.H. Walker. 2002. Mechanisms of immunity against rickettsiae. New perspectives and opportunities offered by unusual intracellular parasites. *Microbes. Infect.* **4:** 625–633.

17496632, 2009, 1, Downloaded from https://nyaspubs.onlinelibrary.wiley.com/doi/10.1111/j.1749-6632.2009.04517.x by Test, Wiley Online Library on [25/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

11. Wood, D.O. & A.F. Azad. 2000. Genetic Manipulation of *Rickettsiae*: a Preview. *Infect. Immun.* **68:** 6091–6093.
12. Blanc, G. *et al*. 2007. Reductive genome evolution from the mother of *Rickettsia*. *PloS Genet.* **3:** e14.
13. Andersson, S.G. *et al*. 1998. The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. *Nature* **396:** 133–140.
14. Ellison, D.W. *et al*. 2008. Genomic comparison of virulent *Rickettsia rickettsii* Sheila Smith and avirulent *Rickettsia rickettsii* Iowa. *Infect. Immun.* **76:** 542–550.
15. Winkler H.H. & R.M. Daugherty. 1983. Cytoplasmic distinction of avirulent and virulent *Rickettsia prowazekii*: fusion of infected fibroblasts with macrophage-like cells. *Infect. Immun.* **40:** 1245–1247.
16. Balayeva, N.M., M. Eremeeva & D. Raoult. 1994. Genomic identification of *Rickettsia slovaca* among spotted fever group rickettsia isolates from *Dermacentor marginatus* in Armenia. *Acta. Virol.* **38:** 321–325.
17. Ge, H. *et al*. 2004. Comparative genomics of *Rickettsia prowazekii* Madrid E and Breinl Strains. *J. Bacteriol.* **186:** 556–565.
18. Zhang, J.Z., J.F. Hao, D.H. Walker & X.J. Yu. 2006. A mutation inactivating the methyltransferase gene in avirulent Madrid E strain of *Rickettsia prowazekii* reverted to wild type in the virulent revertant strain. *Evir. Vaccine* **24:** 2317–2323.
19. Chao, C.C., *et al*. 2007. Insight into the virulence of Rickettsia prowazekii by proteomic analysis and comparison with an avirulent strain. *Biochim. Biophys. Acta.* **1774:** 373–381.
20. Jacques, M. 1996. Role of lipo-oligosaccharides and lipopolysaccharides in bacterial adherence. *Trends Microbiol.* **4:** 408–409.
21. Feng, H.-M., T. Whitworth, V.L. Popov & D.H. Walker. 2004. Fc-dependent polyclonal antibodies and antibodies to outer membrane proteins A and B, but not to lipopolysaccharide, protect SCID mice against fatal *Rickettsia conorii* infection. *Infect. Immun.* **72:** 2222–2228.
22. Li, H. & D.H. Walker. 1992. Characterization of rickettsial attachment to host cells by flow cytometry. *Infect. Immun.* **60:** 2030–2035.
23. Palmer, E.L., M.L. Martin & L. Mallavia. 1974. Ultrastucture of the surface of *Rickettsia prowazeki* and *Rickettsia akari*. *Appl. Microbiol.* **28:** 713–716.
24. Ching, W.-M., M. Carl & G.A. Dasch. 1992. Mapping of monoclonal antibody binding sites on CNBr fragments of the S-layer protein antigens of *Rickettsia typhi* and *Rickettsia prowazekii*. *Mol. Immunol.* **29:** 95–105.
25. Teysseire, N. & D. Raoult. 1992. Comparison of Western immunoblotting and microimmunofluorescence for diagnosis of Mediterranean spotted fever. *J. Clin. Microbiol.* **30:** 455–460.
26. Ogata, H. *et al*. 2001. Mechanisms of evolution in *Rickettsia conorii* and *R. prowazekii*. *Science* **293:** 2093–2098.
27. Blanc, G., M. *et al*. 2005. Molecular evolution of Rickettsia surface antigens: Evidence of positive selection. *Mol. Biol. Evol.* **22:**2073–2083.
28. McDonald, G.A., R.L. Anacker & K. Garjian. 1987. Cloned gene of *Rickettsia rickettsii* surface antigen: candidate vaccine for Rocky Mountain spotted fever. *Science* **235:** 83–85.
29. Li, H., B. Lenz & D.H. Walker. 1988. Protective monoclonal antibodies recognize heat-labile epitopes on surface proteins of spotted fever group rickettsiae. *Infect. Immun.* **56:** 2587–2593.
30. Vishwanath, S., G.A. McDonald & N.G. Watkins. 1990. A recombinant *Rickettsia conorii* vaccine protects guinea pigs from experimental boutonneuse fever and Rocky Mountain spotted fever. *Infect. Immun.* **58:** 646–653.
31. Sumner, J.W., K.G. Sims, D.C. Jones & B.E. Anderson. 1995. Protection of guinea-pigs from experimental Rocky Mountain spotted fever by immunization with baculovirus-expressed *Rickettsia rickettsii* rOmpA protein. *Vaccine* **13:** 29–35.
32. Crocquet-Valdes, P.A. *et al*. 2001. Immunization with a portion of rickettsial outer membrane protein A stimulates protective immunity against spotted fever rickettsiosis. *Vaccine* **20:** 979–988.
33. Li, H. & D.H. Walker. 1998. rOmpA is a critical protein for the adhesion of Rickettsia rickettsiito host cells. *Microbial. Pathogenesis* **24:** 289–298.
34. Renesto, P. *et al*. 2005. Some lessons from Rickettsia genomics. *FEMS Microbiol. Rev.* **29:** 99–117.
35. Bourgeois, A.L. & G.A. Dasch. 1981. The species-specific surface protein antigen in *Rickettsia typhi* : immunogenicity and protecting efficacy in guinea-pigs. In *Rickettsiae and Rickettsial Diseases*. W. Burgdorfer & R.L. Anacker, Eds.: 71–80. New York.
36. Gilmore, R.D., Jr., W.J. Cieplak, P.F. Policastro & T. Hackstadt. 1991. The 120 kilodalton outer membrane protein (rOmp B) of *Rickettsia rickettsii* is encoded by an unusually long open reading frame: evidence for protein processing from a large precursor. *Mol. Microbiol.* **5:** 2361–2370.
37. Hackstadt, T., R. Messer, W. Cieplak & M.G. Peacock. 1992. Evidence for proteolytic cleavage of the 120-kilodalton outer membrane protein of rickettsiae: identification of an avirulent mutant deficient in processing. *Infect. Immun.* **60:** 159–165.
38. Uchiyama, T., H. Kawano & Y. Kusuhara. 2006. The major outer membrane protein rOmpB of spotted fever group rickettsiae functions in the rickettsial adherence to and invasion of Vero cells. *Microbes. Infect.* **8:** 801–809.

17496632, 2009, 1, Downloaded from https://nyaspubs.onlinelibrary.wiley.com/doi/10.1111/j.1749-6632.2009.04517.x by Test, Wiley Online Library on [25/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

39. Ching, W.M., H. Wang, J. Davis & G.A. Dasch. 1993. Amino acid analysis and multiple methylation of lysine rsidues in the surface protein antigen of *Rickettsia prowazekii*. In *Techniques in Protein Chemistry*. R.H. Angeletti, Ed.: 307–314.
40. Renesto, P. *et al*. 2006. Identification of two putative rickettsial adhesins by proteomic analysis. *Res. Microbiol.* **158:** 605–612.
41. Wells, T.J., J.J. Tree, G.C. Ulett & M.A. Schembri. 2007. Autotransporter proteins: novel targets at the bacterial cell surface. *FEMS Microbiol. Lett.* **274:** 163–172.
42. Martinez, J.J. *et al*. 2005. Ku70, a component of DNA-dependent protein kinase, is a mammalian receptor for *Rickettsia conorii*. *Cell* **123:** 1016–1023.
43. Fikrig, E. & S. Narasimhan. 2006. *Borrelia burgdorferi* – Traveling incognito? *Microbes Infection* **8:** 1390–1399.
44. Rovery, C. *et al*. 2005. Preliminary transcriptional analysis of *spoT* gene family and of membrane proteins in *Rickettsia conorii* and *Rickettsia felis*. *Ann. N.Y. Acad. Sci.* **1063:** 79–82.
45. Ogawa, M., K. Matsumoto, P. Parola & P. Brouqui. 2006. Expression of rOmpA and rOmpB protein in *Rickettsia massiliae* during the *Rhipicephalus turanicus* life cycle. *Ann. N.Y. Acad. Sci.* **1078:** 352–356.
46. Winkler, H.H. & E.T. Miller. 1982. Phospholipase A and the interaction of *Rickettsia prowazekii* and mouse fibroblasts (L-929 cells). *Infect. Immun.* **38:** 109–113.
47. Silverman, D.J., L.A. Santucci, N. Meyers & Z. Sekeyova. 1992. Penetration of host cells by *Rickettsia rickettsii* appears to be mediated by a phospholipase of rickettsial origin. *Infect. Immun.* **60:** 2733–2740.
48. Walker, D.H., H.M. Feng & V.L. Popov. 2001. Rickettsial phospholipase A2 as a pathogenic mechanism in a model of cell injury by typhus and spotted fever group rickettsiae. *Am. J. Trop. Med. Hyg.* **65:** 936–942.
49. Renesto, P. *et al*. 2003. Identification and characterization of a phospholipase D-superfamily gene in rickettsiae. *J. Infect. Dis.* **188:** 1276–1283.
50. Ponting, C.P. & I.D. Kerr. 1996. A novel family of phospholipase D homologues that includes phospholipid synthases and putative endonucleases: identification of duplicated repeats and potential active site residues. *Protein Sci.* **5:** 914–922.
51. Whitworth, T. *et al*. 2005. Expression of the *Rickettsia prowazekii* pld or tlyC gene in *Salmonella enterica* serovar Typhimurium mediates phagosomal escape. *Infect. Immun.* **73:** 6668–6673.
52. McLeod, M.P. *et al*. 2004. Complete genome sequence of *Rickettsia typhi* and comparison with sequences of other rickettsiae 282. *J. Bacteriol.* **186:** 5842–5855.
53. Hirschberg, H.J., J.W. Simons, N. Dekker & M.R. Egmond. 2001. Cloning, expression, purification and characterization of patatin, a novel phospholipase A. *Eur. J. Biochem.* **268:** 5037–5044.
54. Blanc, G., P. Renesto & D. Raoult. 2005. Phylogenic analysis of rickettsial patatin-like protein with conserved phospholipase A2 active sites. *Ann. N.Y. Acad. Sci.* **1063:** 83–86.
55. Ogata, H. *et al.* 2005. The genome sequence of *Rickettsia felis* identifies the first putative conjugative plasmid in an obligate intracellular parasite. *PLoS Biology* **3:** e248.
56. Stevens, J.M., E.E. Galyov & M.P. Stevens. 2006. Actin-dependent movement of bacterial pathogens. *Nat. Rev. Microbiol.* **4:** 91–101.
57. Carlsson, F. & E.J. Brown. 2006. Actin-based motility of intracellular bacteria, and polarized surface distribution of the bacterial effector molecules. *J. Cell Physiol.* **209:** 288–296.
58. Teysseire, N. & D. Raoult. 1992. Comparison of Western immunoblotting and microimmunofluorescence for diagnosis of Mediterranean spotted fever. *Infect. Immun.* **30:** 455–460.
59. Heinzen, R.A., S.F. Hayes, M.G. Peacock & T. Hackstadt. 1993. Directional actin polymerization associated with spotted fever group Rickettsia infection of Vero cells. *Infect. Immun.* **61:** 1926–1935.
60. Baldridge, G.D. *et al*. 2005. Analysis of fluorescent protein expression in transformants of *Rickettsia monacensis*, an obligate intracellular tick symbiont. *Appl. Environ. Microbiol.* **71:** 2095–2105.
61. Gouin, E. *et al*. 2004. The RickA protein of *Rickettsia conorii* activates the Arp2/3 complex. *Nature* **427:** 457–461.
62. Jeng, R.L. *et al*. 2004. A Rickettsia WASP-like protein activates the Arp2/3 complex and mediates actin-based motility. *Cell Microbiol.* **6:** 761–769.
63. Gouin, E., M.D. Welch & P. Cossart. 2005. Actin-based motility of intracellular pathogens. *Curr. Opin. Microbiol.* **8:** 35–45.
64. Simser, J.A., M.S. Rahman, S.M. Dreher-Lesnick & A.F. Azad. 2005. A novel and naturally occurring transposon, ISRpe1 in the *Rickettsia peacockii* genome disrupting the *rickA* gene involved in actin-based motility. *Mol. Microbiol.* **58:** 71–79.
65. Gouin, E., H. Gantelet, C. Egile, *et al*. 1999. A comparative study of the actin-based motilities of the pathogenic bacteria *Listeria monocytogenes*, *Shigella flexneri* and *Rickettsia conorii*. *J. Cell Sci.* **112:** 1697–1708.
66. Goldberg, M.B., O. Barzu, C. Parsot & P.J. Sansonetti. 1993. Unipolar localization and ATPase activity of IcsA, a *Shigella flexneri* protein involved in intracellular movement. *J. Bacteriol.* **175:** 2189–2196.
67. Kocks, C. *et al*. 1993. Polarized distribution of *Listeria monocytogenes* surface protein ActA at the site of directional actin assembly. *J. Cell Sci.* **105:** 699–710.

17496632, 2009, 1, Downloaded from https://nyaspubs.onlinelibrary.wiley.com/doi/10.1111/j.1749-6632.2009.04517.x by Test, Wiley Online Library on [25/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

68. Stevens, M.P. *et al*. 2005. Identification of a bacterial factor required for actin-based motility of *Burkholderia pseudomallei*. *Mol. Microbiol.* **56:** 40–53.
69. Heinzen, R.A., S.S. Grieshaber, L.S. Van Kirk & C.J. Devin. 1999. Dynamics of actin-based movement by *Rickettsia rickettsii* in vero cells. *Infect. Immun.* **67:** 4201–4207.
70. Monack, D.M. & J.A. Theriot. 2001. Actin-based motility is sufficient for bacterial membrane protrusion formation and host cell uptake. *Cell Microbiol.* **3:** 633–647.
71. Heinzen, R.A. 2003. Rickettsial Actin-Based Motility: Behavior and Involvement of Cytoskeletal Regulators. *Ann. N. Y. Acad. Sci.* **990:** 535–547.
72. Mediannikov, O. *et al*. 2008. *Rickettsia raoultii* sp. nov., a new spotted fever group rickettsia associated with *Dermacentor* ticks in Europe and Russia. *Int. J. Syst. Evol. Microbiol.*
73. Balraj, P. *et al*. 2008. RickA expression is not sufficient to promote actin-based motility of *Rickettsia raoultii*. *PLoS ONE* **3:** e2582.
74. Ogata, H. *et al*. 2006. Genome sequence of *Rickettsia bellii* illuminates the role of amoebae in gene exchanges between intracellular pathogens. *PLoS Genet.* **2:** e76.
75. Duma, R.J. *et al*. 1981. Epidemic typhus in the United States associated with flying squirrels. *JAMA* **245:** 2318–2323.
76. Raoult, D. *et al*. 1998. Outbreak of epidemic typhus associated with trench fever in Burundi. *Lancet* **352:** 353–358.
77. Netesov, S.V. & J.L. Conrad. 2001. Emerging infectious diseases in Russia, 1990–1999. *Emerg. Infect. Dis.* **7:** 1–5.
78. Hybiske, K. & R.S. Stephens. 2008. Exit strategies of intracellular pathogens. *Nat. Rev. Microbiol.* **6:** 99–110.
79. Christie, P.J. *et al*. 2005. Biogenesis, architecture, and function of bacterial type IV secretion systems. *Annu. Rev. Microbiol.* **59:** 451–85.
80. Cascales, E. & P.J. Christie. 2003. The versatile bacterial type IV secretion systems. *Nat. Rev. Microbiol.* **1:** 137–149.
81. Burns, D.L. 2003. Type IV transporters of pathogenic bacteria. *Curr. Opin. Microbiol.* **6:** 29–34.
82. Christie, P.J. & J.P. Vogel. 2000. Bacterial type IV secretion: conjugation systems adapted to deliver effector molecules to host cells. *Trends Microbiol.* **8:** 354–360.
83. Sexton, J.A. & J.P. Vogel. 2002. Type IVB secretion by intracellular pathogens. *Traffic* **3:** 178–185.
84. Nagai, H., J.C. Kagan, R.A. Kahn & C.R. Roy. 2002. A bacterial guanine nucleotide exchange factor activates ARF on Legionella phagosomes. *Science* **295:** 679–682.
85. http://www.igs.cnrs-mrs.fr/mgdb/Rickettsia/rig/
86. Chen, J. *et al*. 2004. *Legionella* effectors that promote nonlytic release from protozoa. *Science* **303:** 1358–1361.
87. Shohdy, N., J.A. Efe, S.D. Emr & H.A. Shuman. 2005. Pathogen effector protein screening in yeast identifies Legionella factors that interfere with membrane trafficking. *Proc Natl Acad Sci USA* **102:** 4866–4871.
88. Vanrheenen, S.M., Z.Q. Luo, T. O'Connor & R.R. Isberg. 2006. Members of a *Legionella pneumophila* family of proteins with ExoU (phospholipase A) active sites are translocated to target cells. *Infect. Immun.* **74:** 3597–3606.
89. Segal, G., M. Feldman & T. Zusman. 2005. The Icm/Dot type-IV secretion systems of *Legionella pneumophila* and *Coxiella burnetii*. *FEMS Microbiol. Rev.* **29:** 65–81.
90. Boschiroli, M.L. *et al*. 2002. The *Brucella suis virB* operon is induced intracellularly in macrophages. *Proc. Natl. Acad. Sci. USA* **99:** 1544–1549.
91. Schmiederer, M. *et al*. 2001. Intracellular induction of the *Bartonella henselae virB* operon by human endothelial cells. *Infect. Immun.* **69:** 6495–6502.
92. Ogawa, M. *et al*. 2007. Proteome analysis of *Rickettsia felis* highlights the expression profile of intracellular bacteria. *Proteomics* **8:** 1232–1248.
93. La, M.V. *et al*. 2007. Development of a method for recovering rickettsial RNA from infected cells to analyze gene expression profiling of obligate intracellular bacteria. *J. Microbiol. Methods* **71:** 292–297.

17496632, 2009, 1, Downloaded from https://nyaspubs.onlinelibrary.wiley.com/doi/10.1111/j.1749-6632.2009.04517.x by Test, Wiley Online Library on [25/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License