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Rickettsial genomics and the paradigm of genome reduction associated with increased virulence

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Abstract

Rickettsia species are arthropod endosymbiotic α -proteobacteria that can infect mammalian hosts during their obligate intracellular lifecycle, and cause a range of mild to severe diseases in humans. Paradoxically, during their adaptation to a bottleneck lifestyle, rickettsial genomes have undergone an evolution marked by a progressive chromosomal and plasmidic degradation resulting in a genome reduction from 1.5 to 1.1 Mb, with a coding capacity of 69–84%. A striking finding of rickettsial genomics has been that the most virulent species had genomes that were drastically reduced and degraded when compared to closely related less virulent or nonpathogenic species. This paradoxical evolution, which is not unique to members of the genus *Rickettsia* but has been identified as a convergent evolution of several major human pathogenic bacteria, parallels a selected loss of genes associated with transcriptional regulators, but with a high preservation of toxin-antitoxin (TA) modules and recombination and DNA repair proteins. In addition, these bacteria have undergone a proliferation of genetic elements, notably short palindromic elements, whose role remains unknown. Recent proteomic and transcriptomics analyses have revealed a differential level or degradation of gene expression that may, at least partially, explain differences in virulence among *Rickettsia* species. However, future investigations are mandatory to provide novel insights into the mechanisms by which genomic reductive evolution contributes to an emergence of pathogenesis. © 2017 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.

Keywords: Rickettsial genomics; Reductive evolution; Virulence; Pathogenesis

1. Introduction

The genus *Rickettsia* (order *Rickettsiales*, family *Rickettsiaceae*) is currently made of obligate intracellular α -proteobacteria mostly associated to arthropods worldwide [1,2]. These bacteria can also infect mammalian hosts, mostly through arthropod bites or feces, and were initially phylogenetically classified into two major groups on the basis of their pathogenicity for humans. These groups include the spotted fever group (SFG) that currently contains 16 pathogenic agents (Table 1) causing spotted fevers, as well as numerous species of as-yet unknown pathogenicity, associated with

ticks, fleas and mites; and the typhus group (TG) that is made of *Rickettsia prowazekii* and *Rickettsia typhi* causing typhus and associated with human body lice and rat fleas, respectively. However, the SFG group was later demonstrated to be divided into distinct phylogenetic subgroups on the basis of gene sequence comparisons [3–5]. *Rickettsia* species cause a range of mild to severe diseases, the most common being scalp eschar and neck lymphadenopathy (SENLAT), also named tick-borne lymphadenopathy (TIBOLA) or Dermacentor-borne necrosis, erythema and lymphadenopathy (DEBONEL), murine typhus, Mediterranean spotted fever (MSF), Rocky Mountain spotted fever (RMSF), and epidemic typhus [6–8]. In addition to spotted fever and typhus group rickettsiae, two species, *Rickettsia bellii* and *Rickettsia canadensis*, associated with ticks but not causing to date any recognized human

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Table 1
Classification, vectors, reservoirs and diseases of *Rickettsia* species with known pathogenicity to humans.

Antigenic group	Species	Strain_name	Rickettsiosis	Vector	Reservoirs
Spotted fever group	<i>R. aeschlimannii</i>	MC16	Rickettsiosis	Ticks: <i>Hyalomma m. sp.</i>	Unknown
	<i>R. africae</i>	ESF-5	African tick-bite fever	Ticks: <i>Amblyomma variegatum</i>	Ruminants
	<i>R. akari</i>	Hartford	Rickettsialpox	Mites: <i>Allodermanyssus sanguineus</i>	Mice, rodents
	<i>R. australis</i>	Cutlack	Queensland tick typhus	Ticks: <i>Ixodes sp.</i>	Rodents
	<i>R. conorii</i>	Malish 7	Mediterranean spotted fever	Ticks: <i>Rhipicephalus sp.</i>	Dogs, rodents
	<i>R. felis</i>	URRWXCal2	Flea spotted fever	Flea: <i>Ctenocephalides felis</i>	Cats, rodents, opossums
	<i>R. heilongjiangensis</i>	O54	Far Eastern tick borne rickettsiosis	Ticks: <i>Dermacentor silvarum</i>	Rodents
	<i>R. helvetica</i>	C9P9	Aneruptive fever/Unnamed rickettsiosis	Ticks: <i>Ixodes ricinus</i>	Rodents
	<i>R. honei</i>	RB	Flinders Island spotted fever, Thai tick typhus	Ticks: <i>Aponomma hydrosauri</i>	Rodents, reptiles
	<i>R. japonica</i>	YH	Japanese spotted fever or Oriental spotted fever	Ticks: <i>Haemaphysalis sp.</i>	Rodents
	<i>R. massiliae</i>	MTU5	Mediterranean spotted fever	Tck: <i>Rhipicephalus turanicus</i>	Unknown
	<i>R. parkeri</i>	Portsmouth	Unnamed rickettsiosis	Ticks	Rodents
	<i>R. raoultii</i>	Khabarovsk	scalp eschar and neck lymphadenopathy (SENLAT)	Ticks: <i>Dermacentor sivarum</i>	Unknown
	<i>R. rickettsii</i>	Sheila Smith	Rocky Mountain spotted fever	Ticks: <i>Dermacentor sp.</i>	Rodents
	<i>R. sibirica</i>	246	North Asian tick typhus, Siberian tick typhus	Ticks: <i>Dermacentor sp.</i>	Rodents
	<i>R. sibirica</i>	HA-91	Lymphangitis-associated rickettsiosis	Ticks: <i>Dermacentor sp.</i>	Rodents
	<i>R. slovaca</i>	13-B	scalp eschar and neck lymphadenopathy (SENLAT)	Ticks: <i>Dermacentor sp.</i>	Lagomorphes, rodents
Typhus group	<i>R. prowazekii</i>	Brein1	Epidemic typhus, Brill-Zinsser disease	Louse: <i>Pediculus humanus</i>	Humans, flying squirrels
	<i>R. prowazekii</i>	Rp22	Epidemic typhus	Louse: <i>Pediculus humanus</i>	Humans, flying squirrels
	<i>R. typhi</i>	Wilmington	Murine typhus	Fleas: <i>Xenopsylla cheopis</i>	Rodents

disease, diverged early from these two groups. Furthermore, recent studies have reported the association of other *Rickettsia* lineages with other reservoirs including protozoa, algae, leeches plants or insects [4,9,10].

In 1995, the complete genome sequencing of *Haemophilus influenzae* (the first sequenced genome) [11] marked the beginning of the genomic era. Over the past two decades, the completion of the genome sequences of most *Rickettsia* species, starting with that of *R. prowazekii*, allowed better knowledge about the molecular mechanisms involved in their pathogenicity [12] (see Fig. 1).

2. Characteristics and genome architecture of *Rickettsia* species

Rickettsia species have genome sizes ranging from 1.1 to 2.3 Mbp and exhibit a G + C content of 29–33% (Table 2). *Rickettsia hoogstraalii* and *Rickettsia* endosymbiont of *Ixodes scapularis* [13] have the largest genomes sequenced to date but exhibit no known pathogenic effects. Rickettsial genomes are also characterized by a high degree of synteny (Fig. 2) [4] despite the presence of numerous pseudogenes and a large fraction of non-coding DNA, reaching 24% in *R. prowazekii* [14,15]. This genomic degradation likely results from their

endosymbiotic lifestyle that has allowed them to discard genes involved in metabolisms supplied by their eukaryotic host cells [16,17]. This genomic downsizing has occurred through a progressive gene degradation, from complete functional genes to functional pseudogenes to non functional pseudogenes to gene remnants to discarded genes [18–21]. Generally, rickettsial genomes are typical of those of symbiotic bacteria, which are obligate intracellular and are characterized by a reduced genome, relatively small, made of a single circular chromosome, evolving slowly, and maintaining a near perfect colinearity between species [22]. However, in parallel to this reduction phenomenon, rickettsial genomes exhibit a paradoxical expansion of genetic elements, including plasmids, short palindromic elements named rickettsia palindromic elements (RPEs) [23], ankyrin and tetratricopeptide repeats, toxin-antitoxin modules, ADP-ATP translocases, type IV secretion system (T4SS), as well as *sca*, *spoT*, *proP* and *ampG* genes. Moreover, the presence of plasmids in *Rickettsia* genomes was first detected in *Rickettsia felis*, demonstrating that these bacteria were able to exchange genetic material by conjugation, a mechanism that was thought to be absent from obligate intracellular and allopatric bacteria [16,17,24]. To date, 20 plasmids have been identified in 11 species, some species having 1 to 4 distinct plasmids [25–27].

Table 2
Main characteristics of rickettsial genomes available in Genbank.

Species	Strain	Genome size (Mbp)	G + C content (%)	Presence of plasmid (s)	Protein-coding genes	% coding sequences	Rickettsia palindromic elements	Chromosome accession number
<i>R. aeschlimannii</i>	MC16	1.31	32.2	Plasmid 1, Plasmid 2	1051	—	—	CCER01000000
<i>R. africae</i>	ESF-5	1.28	32.4	pRaf	1219	78.26	—	CP001612
<i>R. akari</i>	Hartford	1.23	32.3		1259	77.4	—	CP000847
<i>R. amblyommatidis</i>	Ac37	1.46	32.4	pRAMAC18, pRAMAC23	1511	—	—	NZ_CP012420
<i>R. amblyommatidis</i>	AcPa	1.44	32.4		1123	—	—	LANR01000001
<i>R. amblyommatidis</i>	Darkwater	1.44	32.8		1060	—	—	LAOH01000001
<i>R. amblyommatidis</i>	GAT-30V	1.48	32.4	pMCE1, pMCE2, pMCE3	1550	—	—	NC_017028
<i>“R. argasii”</i>	T170-B	1.44	32.3		1187	—	—	LAOQ01000006
<i>R. asembonensis</i>	NMRCii	1.36	32.3	pRAS01	1212	—	—	JWSW01000001
<i>R. australis</i>	Phillips	1.32	32.2	pRau01	1099	—	—	AKVZ01000001
<i>R. australis</i>	Cutlack	1.33	32.3	pMC5_1	1136	—	—	NC_017058
<i>R. bellii</i>	RMLAn4	1.54	31.6		1311	—	—	LAOI01000001
<i>R. bellii</i>	RMLMog	1.62	31.5		1336	—	—	LAOJ01000001
<i>R. bellii</i>	OSU 85-389	1.52	31.6		1476	—	—	NC_009883
<i>R. bellii</i>	RML369-C	1.52	31.7		1429	85.2%	525	NC_007940
<i>R. endosymbiont of Ixodes scapularis</i>	REIS	1.82	33.0	pReis1, pReis2, pReis3, pReis4	2309	—	—	CM000770
<i>R. canadensis</i>	CA410	1.15	31.1		1016	—	—	NC_016929
<i>R. canadensis</i>	McKiel	1.16	31.1		902	75.2%	—	NC_009879
<i>R. conorii</i>	Malish 7	1.27	32.4		1227	81.5	559	NC_003103
<i>R. conorii</i>	A-167	1.26	32.5		1210	—	—	AJUR01000001
<i>R. conorii</i>	ITTR	1.25	32.4		1157	—	—	AJHC01000001
<i>R. conorii</i>	ISTT CDC1	1.25	32.5		1200	—	—	AJVP01000001
<i>R. endosymbiont of Ixodes pacificus</i>	Humboldt	1.56	32.2		1294	—	—	LAOP01000001
<i>“R. felis”</i>	LSU	1.54	32.4	pRF	1970	—	—	JSEM01000001
<i>“R. felis”</i>	LSU lb	1.58	32.4	pRF, pLbaR	1691	—	—	JSEL01000001
<i>“R. felis”</i>	Pedreira	1.49	32.5		1594	—	—	LANQ01000001
<i>“R. felis”</i>	URRWXCal2	1.49	32.5	pRF, pRFδ	1444	83.6%	726	NC_007109
<i>R. gravesii</i>	BW1-1	1.37	32.2	pRgr	1158	—	—	AWXL01000001
<i>R. heilongjiangensis</i>	O54	1.28	32.3		1140	—	—	CP002912
<i>R. helvetica</i>	C9P9	1.37	32.2	pRhe	1114	—	—	CM001467
<i>R. honei</i>	RB	1.27	32.4		1171	—	—	AJTT01000001
<i>R. hoogstraalii</i>	Croatia	1.48	32.4		1250	—	—	CCXM01000001
<i>R. hoogstraalii</i>	RCCE3	2.3	32.4		2479	—	—	LAOB01000001
<i>R. japonica</i>	YH	1.28	32.4		1142	—	—	NC_016050
<i>R. massiliae</i>	AZT80	1.28	32.5	pRmaB	1207	—	—	NC_016931
<i>R. massiliae</i>	MTU5	1.37	32.5	pRma	1152	—	565	NC_009900
<i>“R. monacensis”</i>	IrR/Munich	1.35	32.4	pRM	1447	—	—	NZ_LN794217
<i>R. montanensis</i>	OSU 85-930	1.28	32.6		1125	—	—	CP003340
<i>R. parkeri</i>	AT#24	1.3	32.4		1226	—	—	LAOL01000001
<i>R. parkeri</i>	GrandBay	1.31	32.4		1223	—	—	LAOK01000001
<i>R. parkeri</i>	Portsmouth	1.3	32.4		1228	—	—	NC_017044
<i>R. parkeri</i>	TatesHell	1.3	32.4		1227	—	—	LAOO01000001
<i>R. peacockii</i>	Rustic	1.29	32.6	pRpe	927	—	—	CP001227
<i>“R. philipii”</i>	364D	1.29	32.5		1218	—	—	CP003308
<i>R. prowazekii</i>	Breiln	1.11	29		842	—	—	NC_020993
<i>R. prowazekii</i>	BuV67-CWPP	1.11	29		843	—	—	NC_017056
<i>R. prowazekii</i>	Cairo3	1.11	29		842	—	—	APMO01000001
<i>R. prowazekii</i>	Chernikova	1.11	29		845	—	—	NC_017049
<i>R. prowazekii</i>	Dachau	1.11	29		839	—	—	NC_017051
<i>R. prowazekii</i>	GvV257	1.11	29		829	—	—	NC_017048
<i>R. prowazekii</i>	Katsinyan	1.11	29		844	—	—	NC_017050
<i>R. prowazekii</i>	Madrid E	1.11	29		834	75.4%	120	NC_000963
<i>R. prowazekii</i>	NMRC Madrid E	1.11	29		830	—	—	NC_020992
<i>R. prowazekii</i>	Rp22	1.11	29		864	76.2%	—	NC_017560
<i>R. prowazekii</i>	RpGvF24	1.11	29		870	—	—	NC_017057
<i>R. raoultii</i>	Khabarovsk	1.34	32.8	pRa1, pRa2, pRa3, pRa4	1334	—	—	CP010969
<i>R. rhipicephali</i>	3-7-female 6-CWPP	1.31	32.4	pRrh	1117	—	—	NC_017042
<i>R. rhipicephali</i>	Ect	1.27	32.6		1067	—	—	LAOC01000001
<i>R. rhipicephali</i>	HJ#5	1.45	32.3	pHJ51, pHJ52	1200	—	—	NZ_CP013133

(continued on next page)

Table 2 (continued)

Species	Strain	Genome size (Mbp)	G + C content (%)	Presence of plasmid (s)	Protein-coding genes	% coding sequences	Rickettsia palindromic elements	Chromosome accession number
<i>R. rickettsii</i>	Arizona	1.27	32.4		1343	—	—	NC_016909
<i>R. rickettsii</i>	Brazil	1.25	32.4		1339	—	—	NC_016913
<i>R. rickettsii</i>	Colombia	1.27	32.4		1342	—	—	NC_016908
<i>R. rickettsii</i>	Hauke	1.27	32.4		1347	—	—	NC_016911
<i>R. rickettsii</i>	Hino	1.27	32.4		1346	—	—	NC_016914
<i>R. rickettsii</i>	Hlp#2	1.27	32.4		1339	—	—	NC_016915
<i>R. rickettsii</i>	Iowa	1.27	32.4		1384	—	—	NC_010263
<i>R. rickettsii</i>	Morgan	1.27	32.4		1343	—	—	NZ_CP006010
<i>R. rickettsii</i>	R	1.26	32.4		1334	—	—	NZ_CP006009
<i>R. rickettsii</i>	Sheila Smith	1.26	32.5		1345	78.5%	—	NC_009882
<i>R. sibirica</i>	246	1.25	32.5		1227	77.8%	—	AABW01000001
<i>R. sibirica</i>	HA-91	1.25	32.4		1175	—	—	AHQB01000001
<i>R. sibirica</i>	BJ-90	1.25	32.4		1217	—	—	AHQB01000001
<i>R. slovaca</i>	D-CWPP	1.27	32.5		1261	—	—	NC_017065
<i>R. slovaca</i>	13-B	1.27	32.5		1260	—	—	NC_016639
<i>R. tamurae</i>	AT-1	1.44	32.4	Plasmid 1, Plasmid 2	1200	—	—	CCMG01000008
<i>R. typhi</i>	B9991CWPP	1.11	28.9		819	—	—	NC_017062
<i>R. typhi</i>	TH1527	1.11	28.9		819	—	—	NC_017066
<i>R. typhi</i>	Wilmington	1.11	28.9		817	76.3%	121	NC_006142

Species with as yet no standing in nomenclature are written with quotation marks (—) = no available data.

Fig. 1. Phylogenetic tree of 31 *Rickettsia* species with validly published names based on the alignment of 450 concatenated core proteins using the Maximum Likelihood method with JTT and GAMMA models and display only topology. Values at the nodes represent the percentages of bootstrap values obtained by repeating analysis 500 times to generate a majority consensus tree. Only values greater than 70% were indicated.

3. Comparative analysis of rickettsial genomes

The first genomic comparison of *Rickettsia* species was that of the first two sequenced genomes from *Rickettsia conorii* and *R. prowazekii* [22]. This study showed a near perfect colinearity between both species (Fig. 2) but the latter species had a smaller genome and a higher proportion of non coding DNA, including many pseudogenes. Further comparisons confirmed this trend in genomic reduction (1.5–1.1 Mb, coding capacity 69–84%) through progressive gene degradation until complete disappearance [28]. Degraded genes include mostly those coding for amino-acid, ATP, LPS and cell wall component biosynthesis [14,22,29].

Comparative genomic analysis of *Rickettsia* species revealed variations in chromosome size and plasmid number and size (Table 2), despite a common ongoing reductive evolution [30] by progressive gene loss and concomitant gene gain by gene duplication, proliferation of RPEs and horizontal gene transfer [4]. Gene family duplication is frequent in rickettsial genomes and is thought to enable adaptation to environmental changes in the host. The two most duplicated genes encode ADP/ATP translocases, often found in several copies and enabling energy exploitation produced by host cells [29,31], and *spoT* genes found in 4–14 copies and involved in the microbial response to environmental stress [24,29,32]. Other duplicated gene families include proline/betaine transporters, toxin/antitoxin modules, T4SS, *sca* and *ampG* involved in rickettsial pathogenesis as previously described [17,22,29,33]. Rickettsiae possess an incomplete P-T4SS system that is characterized by the lack of *virB5* but the duplication of the *virB4*, *virB6*, *virB8* and *virB9* genes [34]. Surface cell antigen (Sca) proteins are a family of 17 orthologous autotransporters diversely detected in all rickettsial genomes [33]. They were demonstrated to be localized at the

Fig. 2. Genomic alignment showing the high degree of conserved genomic synteny between *Rickettsia* species.

surface of bacteria and play roles in mammalian cell infection as well as infecting their arthropod hosts' cells, notably by promoting actin-based motility [35]. In addition, many repetitive elements are distributed in intergenic regions (tandem repeats) [36] or both intergenic and coding regions (RPEs) [37,38]. RPEs are at least five times more numerous in SFG rickettsia than in TG rickettsia (Table 2). They are assumed to play a role in the evolution of rickettsial genomes by promoting the emergence of new proteins [39]. Twenty-two copies of ankyrin and 11 copies of tetratricopeptide repeats (TPR-repeat), frequently found in endosymbionts [40–43] are found in *R. felis* [44]. Finally, plasmids are less abundant in virulent than less virulent species [8,24,45]. They were most likely acquired vertically from *Orientia/Rickettsia* chromosome ancestors [27]. The genome from REIS, the largest rickettsial genome to date, is characterized by a remarkable proliferation of mobile genetic elements (35% of the entire genome) including a RAGE module considered as a genetic exchange facilitators [46] and resulting from multiplied genomic invasion events [13]. It was also described in *Orientia tsutsugamushi*, *Rickettsia massiliae* [25], *R. bellii* [47] and in the pLbaR plasmid of *R. felis* strain LSU-Lb [48]. Several genes including *tra* cluster, T4SS, ADP/ATP translocases and

patatin-encoding genes found in *Rickettsia* spp. are phylogenetically close to those found in many amoeba-associated bacteria, suggesting their acquisition by horizontal transfer events between *Rickettsia* and non-rickettsial bacteria [4,47].

Other lessons from rickettsial genome comparison are the identification of 15–191 small non-coding RNAs (sRNAs) in intergenic sequences, depending on species [49]. These post-transcriptional regulators are assumed to influence virulence and adaptation depending on the host niche through transcriptomic regulation [49]. Their presence may explain why early comparative studies had identified highly conserved intergenic spacers [22]. A total of 1785 sRNAs were detected from 13 species spanning all rickettsial groups, and the expression of sRNAs was demonstrated in *R. prowazekii* [49]. In addition, all five genes required for the *de novo* folate biosynthesis were demonstrated to be present in 15 *Rickettsia* species, including both human pathogens and non pathogens but excluding the typhus group [50].

Finally, comparative genomics at the intraspecies level enabled identification of variable situations [51]. In *Rickettsia japonica*, 31 strains from the three major lineages exhibited only 112 single nucleotide polymorphisms (SNPs) and 44 InDels, thus suggesting a long generation time in nature or a

recent clonal expansion [51]. In *R. prowazekii*, similar findings were identified, with 81 SNPs observed among 3 strains [51]. In *Rickettsia rickettsii*, the comparison of 4 strains, two eastern and two western strains showed geographic divergences but an overall high genetic homology with few differences in coding regions [52]. This study also demonstrated that the avirulent strain Iowa only diverged from virulent strains by 29 SNPs in addition to a 891-bp insertion in the *ompA* gene [52]. In contrast, the comparison of 3 *R. felis* strains, including two from cat fleas and one from book lice, demonstrated that not only was the book louse strain divergent, with a unique plasmid and SNPs occurring in intergenic regions, RPEs and conserved *Rickettsia* genes, but also were both cat flea strains which exhibited SNPs in genes associated to the *Rickettsia* mobilome [48]. These data suggest that the observed difference may result from spatial isolation for cat flea strains and host specialization in the case of the book louse strain.

4. Paradigm of genome reduction associated with increased virulence

For long, it was believed that bacteria gain virulence by the acquisition of foreign genetic material. However, the comparison of the *R. prowazekii* and *R. conorii* genomes demonstrated that the former species, which is the most virulent, has a drastically degraded genome [19]. Further studies demonstrated that, in *Rickettsia* spp., some speculated virulence factors were found in both pathogenic and nonpathogenic bacteria, and genomes from the most pathogenic species were found to have few or no additional genes when compared to closely related but lesser pathogens. In addition, no association was found between virulence and the presence of plasmids or gene acquisition [45]. *R. prowazekii*, the most pathogenic *Rickettsia* species and agent of epidemic typhus has the smallest genome and an inverse correlation exists between genome size and degree of pathogenicity [21]. These findings suggested a new paradigm in rickettsial pathogenicity that linked increased virulence to genome reductive evolution rather than virulence gene acquisition. Comparative genomics showed a loss of nonessential genes including genes coding for the amino acid synthesis and biosynthetic pathway components during reductive evolution [53]. The most virulent *R. prowazekii* has lost transcriptional regulator genes with a decreased translational capacity [54], but conserved genes coding for toxins, toxin-antitoxin (TA) modules and recombination and DNA repair proteins most likely needed for protection against host immune response [55]. In addition, recent multi-omics data showed a link between reductive evolution and differential gene expression between two virulent and two less virulent SFG rickettsiae. The two virulent *R. conorii* (MSF) and *Rickettsia slovaca* (SENLAT) agents exhibit less up-regulated than down-regulated genes and than the less virulent *R. massiliae* (MSF) and *Rickettsia raoultii* (SENLAT) agents [8]. The former two species have more reduced genomes with plasmid loss than the latter two, suggesting that reductive genomic evolution associated with increased virulence may not be only a question of presence or

lack of a specific protein but may also result from differential level or degradation of expression of common proteins [8]. It was speculated that loss of regulator genes, as observed in several intracellular pathogens, is a critical cause of virulence [45].

This phenomenon was also observed in other human pathogens not genetically related to *Rickettsia* species such as *Treponema* spp., *Mycobacterium* spp. or *Yersinia* spp. [16,20,56]. As examples, *Mycobacterium leprae*, *Treponema pallidum* and *Yersinia pestis* have smaller genomes than closely related but less virulent species in their respective genera. Thus, genomic reductive evolution with alteration of the regulation of invasion, replication and transmission processes, in addition to a differential level or degradation of expression of common proteins may result in an emergence of high pathogenicity.

5. Identified virulence factors in rickettsial genomes

Predicting virulence factors from genome sequences has been among the first objectives of genomics, especially for intracellular bacteria expressing few phenotypic characters. Therefore, several studies were conducted to compare rickettsial species or strains exhibiting diverse virulence phenotypes in order to identify pathogenesis factors. Surprisingly, no association was found between pathogenesis and the acquisition of novel virulence genes [17,21,45]. In contrast, outer membrane proteins, notably Sca2 in *R. rickettsii*, and ankyrin repeat-coding genes were demonstrated to be essential virulence determinants [43,57]. However, RelA/SpoT responsible for the synthesis and hydrolysis of (p)ppGpp [58] and RickA, involved in actin-based bacterial motility [22] were found in both avirulent and virulent *R. rickettsii* strains and were thus ruled out as essential pathogenesis determinants [57]. In *R. prowazekii*, three virulence markers were identified through genome comparison, including *recO*, involved in DNA repair, *metK* and *adr1* encoding a S-adenosyl-methionine synthase and an adhesin, respectively, which are mutated in avirulent strains [53]. In addition, the RalF protein, a T4SS effector coded by genes conserved in all species, was demonstrated to play a role in host cell invasion in *R. typhi*, in contrast with SFG species in which it is pseudogenized [59].

6. Role of rickettsial plasmids in virulence

The presence of plasmids in *Rickettsia* genomes was first detected in that of *R. felis* [24]. To date, plasmids have been detected in 11 *Rickettsia* species [27]. Rickettsial plasmids result from vertical inheritance, mainly from *Orientia/Rickettsia* chromosome ancestors [27]. However, plasmids vary in number within and between species [27,60,61]. A variable plasmid content was observed in strains of *Rickettsia africanae*, *R. bellii*, *Rickettsia akari*, *Rickettsia amblyommatis* and *R. felis* [21,60,61]. In addition, plasmid loss was demonstrated in cell culture [61]. As plasmids were present in several pathogenic species and contained protein-encoding genes necessary for recognition, invasion and pathogenicity, their role in rickettsial

virulence was questioned [27]. However, the unstable plasmid content of *R. africae* did not support a role of plasmid in virulence in this species [21]. Furthermore, a strong correlation was observed between plasmid and genome sizes, with a paralleling decrease existing between plasmid size, number, and chromosome size. As examples, several species causing mild or no disease, such as the SFG *Rickettsia helvetica*, *R. felis*, and *Rickettsia peacockii*, possess one or more plasmids [21,24,43] whereas the most virulent species *R. prowazekii* and *R. typhi* that exhibit the most reduced genomes are plasmidless [15,52,53]. Furthermore, a recent multi-omics-study that compared four SFG rickettsiae showed that *R. conorii* and *R. slovaca*, the agents of MSF and SENLAT, respectively, were plasmidless but *R. massiliae* and *R. raoultii*, two less virulent agents of these diseases, harbor one and three plasmids, respectively [8]. Moreover, plasmids were also shown to undergo reductive evolutionary events similar to those affecting rickettsial chromosomes [27]. These findings support the absence of association between the presence of plasmids and difference in virulence in *Rickettsia* species.

7. Transcriptomic and proteomic investigation of rickettsial virulence

To date, several studies have demonstrated that transcriptomic and proteomic results are complementary to genomic analyses for analyzing bacterial virulence. A proteomic analysis of *Rickettsia parkeri* revealed that 91 proteins, including mostly virulence-related surface proteins (OmpA, OmpB, β -peptide, RickA), were differentially expressed during human infection [62]. Proteomic profile comparison of *R. prowazekii* grown in different cell lines, revealed an up-regulation of stress-related proteins in L929 murine fibroblasts [63]. In addition, proteins involved in protein synthesis, especially enoyl-(acyl carrier protein) reductase, a protein involved in fatty acid biosynthesis, were highly expressed when grown in *I. scapularis* ISE6 cells, suggesting that this rickettsia has the ability to regulate differentially its proteome according to the host [63]. Using transcriptomic and proteomic analyses of virulent and avirulent *R. prowazekii* strains, we identified four phenotypes that differed in virulence depending on the regulation of anti-apoptotic genes or the interferon I pathway in host cells [53]. Furthermore, *R. prowazekii* protein methylation (overproduced in virulent strains) and surface protein expression (Adr1 altered in avirulent Madrid E) varied with virulence, supporting the assumption that methylation of surface-exposed protein plays a role in the virulence of *R. prowazekii* [53]. In addition, in a recent proteomic and transcriptomic study, we compared two virulent agents, *R. conorii* and *R. slovaca*, causing MSF and SENLAT diseases, respectively, to two less virulent agents of the same diseases (*R. massiliae* and *R. raoultii*, respectively) [8]. Virulent species differed from less virulent ones by exhibiting mainly less up-regulated (8) than down-regulated (61) proteins. These included proteins associated mainly with translation, ribosomal structure and biogenesis, post-translational modification, protein turnover, chaperones, energy production

and conversion [8]. In addition, virulent agents had rarely specifically expressed proteins [8]. This provides novel insights into the pathogenesis of *Rickettsia* species and suggests that virulence may not only be a question of presence or lack of a specific protein but may also result from a differential level or degradation of expression of a common protein.

8. Conclusion and perspective

Rickettsia spp., living mainly intracellularly in various arthropods, have undergone a particular paradoxical evolution marked by an evolutive chromosomal and plasmidic degradation resulting in a progressive genome reduction from 1.5 to 1.1 Mb with a coding capacity of 69–84%. This reductive evolution is marked by a selected loss of genes such as those associated with ATP, amino-acid and LPS metabolism or with synthesis of cell wall molecular components. In addition, a loss of regulatory genes and a high preservation of toxin-associated proteins and toxin-antitoxin modules are correlated to a rise in pathogenicity. However, paradoxically, these bacteria have undergone a proliferation of genetic elements whose role remains to be determined. As proteomic and transcriptomic analyses have just started to unveil the molecular mechanisms explaining the differences in virulence among *Rickettsia* species, and because the phenomenon of genome reduction associated with increased virulence seems to occur in other major human pathogens, these being examples of convergent evolution, *i. e.* natural selection leading to a similar biological outcome occurring independently in more than one unrelated biological group, future studies should identify which of the differences in rickettsial genomes account for their phenotypes.

Conflict of interest

The authors declare no conflict of interest.

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