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Review

Brucella abortus Cell Cycle and Infection Are Coordinated

Xavier De Bolle,[1,*] Sean Crosson,[2] Jean-Yves Matroule,[1] and Jean-Jacques Letesson[1]

Brucellae are facultative intracellular pathogens. The recent development of methods and genetically engineered strains allowed the description of cell-cycle progression of *Brucella abortus*, including unipolar growth and the ordered initiation of chromosomal replication. *B. abortus* cell-cycle progression is coordinated with intracellular trafficking in the endosomal compartments. Bacteria are first blocked at the G1 stage, growth and chromosome replication being resumed shortly before reaching the intracellular proliferation compartment. The control mechanisms of cell cycle are similar to those reported for the bacterium *Caulobacter crescentus*, and they are crucial for survival in the host cell. The development of single-cell analyses could also be applied to other bacterial pathogens to investigate their cell-cycle progression during infection.

Trends

Brucella abortus is a facultative intracellular pathogen that grows unipolarly and initiates the replication of its two chromosomes in a specific order.

Bacteria at the G1 stage of the cell cycle, that is, before the initiation of their chromosomal replication, are preferentially internalized in host cells.

Cell-cycle progression is coordinated with trafficking in the host cell, the endosomal stage being divided into two parts: a first, long, nongrowing part, and a second part in which growth and chromosomal replication are resumed.

A cell-cycle control network, conserved with the model bacterium *Caulobacter crescentus*, is essential for *B. abortus* virulence.

Brucella Infection and Intracellular Trafficking

Bacteria of the genus *Brucella* are responsible for a worldwide zoonosis called brucellosis [1]. Most bacteria in this genus form a cluster of strains that are very closely related phylogenetically [2]. *B. abortus*, *Brucella suis*, and especially *Brucella melitensis*, are the three species that have strains capable of infecting humans [1]. Human cases occur by direct contact with infected animals or through the consumption of raw milk-derived products. Livestock are the primary reservoir for these bacterial pathogens [1]. Within hosts, *Brucella* replicates in an intracellular niche [1], though it is easy to cultivate in rich bacteriological media [1]. *Brucella* virulence is generally tested with either cellular models of infection (i.e., Vero cells, HeLa cells, trophoblasts, immortalized or primary macrophages, and fibroblasts) or in animal models of infection, mainly mice [1,3].

Pioneering work by Anderson and Cheville, infecting goats, showed that *B. abortus* can replicate inside the endoplasmic reticulum (ER) of trophoblasts and suggested that *B. abortus* is first endocytosed by erythrophagocytic trophoblasts [4]. This correlates with the time course of infection with Vero cells, HeLa cells, and various macrophages, in which endosomal markers are associated with the ***Brucella*-containing vacuole (BCV)** (see Glossary) in the first stage of the infection. The bacteria subsequently reach the ER for massive proliferation (see [5] for a recent review on cellular trafficking of *Brucella*). Interestingly, several studies have reported that this trafficking can differ depending on host cell type and bacterial species [6,7]. The course of HeLa cell infection is also characterized by two stages: in the first stage the number of colony-forming units (CFUs) is stable for about 8 h while afterwards, in the second stage, this number starts to increase. This roughly correlates with the transition between endosomal BCV (eBCV) and replicative BCV (rBCV), the latter being characterized by ER markers such as calreticulin [8]. In macrophages (e.g., bone-marrow-derived macrophages), the first stage also correlates with a bacterial killing phase [8]. The eBCV stage of intracellular trafficking is crucial for the success of the infection because it is characterized by a low number of CFUs, and thus a high survival rate is necessary to successfully complete cellular infection. In these eBCVs, *Brucella* must adapt its

[1]University of Namur, 61 rue de Bruxelles, 5000 Namur, Belgium
[2]University of Chicago, Gordon Center for Integrative Science W125, 929 E. 57th Street, Chicago, IL 60637, USA

*Correspondence:
xavier.debolle@unamur.be (X. De Bolle).

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growth and its replication, in other words, control its cell cycle. In this review, we describe the main steps of the cell cycle of the *B. abortus* pathogen, in culture and inside host cells. We also highlight the molecular mechanisms that are involved in the control of cell-cycle progression.

Brucella Growth

The cell cycle of *B. abortus* is rather unusual compared to 'classical' intracellular pathogens such as *Salmonella*. First, *Brucella* exhibits an asymmetric division, like other Rhizobiales and other *Alphaproteobacteria* [9]. Second, growth is also asymmetric since it occurs through one pole and at the division site, but there is no sign of lateral growth along the main axis of the cell [10]. Asymmetry of growth has been reported in many different bacteria [11], but asymmetric growth inside intracellular niches has been poorly investigated until now. Unipolar growth is now easy to detect in time-lapse experiments via labelling of bacteria with Texas Red succinimidyl ester (TRSE), which covalently binds to amine groups at the surface of the bacteria. Since this label is basically immobile on the cell surface, growth is easily detected by monitoring the increasing fraction of unlabelled cell body during growth. The immobility of TRSE is likely due to the fact that labelled amine groups are found mainly on exposed peptides of major outer-membrane proteins of *B. abortus* that could be directly or indirectly anchored to immobile peptidoglycan [12]. Incorporation of fluorescent D-amino acids can also mark growth zones on the bacterial peptidoglycan [13], but this method was not applied to *B. abortus* until very recently. These labelling systems allow growth at the single-cell level to be easily followed, as well as its potential heterogeneity. Interestingly, the polar growth of *B. abortus* correlates with the polar localization of the systems that produce cyclic β-1,2-glucan (Cgs) and transport (Cgt) it to the periplasmic space [14]. Cyclic β-1,2-glucan is a cyclic polymer of 17–25 glucose residues [15] that is important for resistance to osmotic shock and for virulence [16], which likely functions by disrupting lipid rafts of host cells [17]. The nonpolar distribution of the modifying enzyme of cyclic β-1,2-glucan Cgm [14] suggests that cyclic β-1,2-glucan can diffuse in the periplasm to be succinidylated by Cgm. The colocalization of the Cgs–Cgt complex with poles suggests that other complexes involved in periplasmic or outer-membrane components could also be produced at the pole(s). Indeed, it is likely that peptidoglycan synthesis machinery, as well as lipoprotein and lipopolysaccharide (LPS) export systems, can be found at the growing pole and at the division site. These hypotheses remain to be tested.

Replication and Segregation of the *Brucella* Chromosomes

Growth and division of *B. abortus* must be accompanied by the replication of its genome. The number and size of chromosomes vary between *Brucella* strains [18]. In *B. abortus*, chromosome I (chr. 1) is 2.1 Mb long, circular, and its predicted replication origin (*oriI*) is located 115 kb from the *dnaA* gene, close to the *parAB* operon. Chromosome II (chr. 2) is 1.2 Mb long and circular, and can be classified as a chromid [19]. Indeed, chr. 2 GC content is very similar to chr. 1, but its replication–segregation system is different. Chr. 2 contains genes coding for a RepABC system, in which RepC is thought to be the initiator of DNA replication, while RepA and RepB would cooperate to segregate the duplicated replication origin of chr. 2 (*oriII*) that is predicted to be located within the *repABC* genes [20]. Intriguingly, several *Brucella* strains have a rearranged genome compared to this situation, the most surprising being *B. suis* biovar 3, in which the two chromosomes are fused [18].

B. abortus belongs to the *Alphaproteobacteria*, like the model organism *C. crescentus*. *C. crescentus* is a well-established model for studying the main steps of the cell cycle, including growth, division, and DNA replication. It is also a model for the study of bacterial cell differentiation, and for the regulation of cell-cycle progression. Although *C. crescentus* and *B. abortus* exhibit distinct lifestyles, they share many features at the molecular level (Table 1). The ParB partitioning protein of *C. crescentus* was found to be associated with the replication origin of its unique chromosome (*oriC*), at both poles of the cell upon replication initiation, and at the *old pole* when bacteria are at the G1 stage of their cell cycle [21].

Glossary

***Brucella*-containing vacuole (BCV):** contains the bacterium during intracellular trafficking. There is usually one bacterium per vacuole, and this vacuole harbours different markers, for example, Lamp1 and calnexin, that respectively allow the discrimination between endosomal BCV (eBCV) and replicative BCV (rBCV).

Cell cycle: a key stage of the cell cycle is division. After division, the newly generated cells grow. Prior to initiation of DNA replication, cells are classified as G1 bacteria or newborns. After initiation of DNA replication, cells are at the S stage of the cell cycle. The G2 stage corresponds to bacteria that have completed DNA replication but have not completed cell division. Since replication termination (*ter*) sites can remain associated even after the completion of their duplication [70], it is difficult to distinguish S from G2 phases at the experimental level.

Old pole vs. new pole: the new poles are produced by cell division, while old poles exist before cell division [71]. In *Brucella abortus*, two proteins (PdhS and FumC) are known to be associated with the old pole [51,72], while others, such as IfoP, PopZ, or AidB, are mainly localized at the new pole [22,73].

Two-component systems: are signal transduction systems composed of a least two proteins: (i) a histidine kinase (HK) that autophosphorylates on a conserved histidine residue in response to a signal, and (ii) a response regulator (RR) that catalyzes phosphotransfer from the phosphohistidine of the HK to itself, on a conserved aspartate residue. In phosphorelays, hybrid HK proteins such as CckA are frequently involved. They are composed of a classical HK domain followed by a domain typical of RR, with a conserved aspartate residue involved in phosphotransfer. A histidine phosphotransferase (like ChpT) acts as an intermediate to provide a phosphoryl group to an RR, that is often fused to a DNA-binding domain, like CtrA.

Table 1. Similarities and Differences between *Caulobacter crescentus* and *Brucella abortus*

	Caulobacter crescentus	*Brucella abortus*
Lifestyle	Free living	Facultative intracellular pathogen
Cell shape	Crescent	Straight short bacillus
Division	Asymmetric	Asymmetric
Number of chromosomes	1	2
Polar appendices	Flagellum, pili, holdfast, and stalk	Flagellum[a]
Growth mode	Lateral elongation and division	Polar elongation and division
MreB (bacterial actin homolog), TipN (cell polarity determinant)	Present	Absent
Orientation of main chromosome (*ori* to *ter*)	Old to new pole	Old to new pole
Segregation system of the main chromosome	ParAB	ParAB
PopZ localization in S/G2 phases	Bipolar	Unipolar (new pole)
DivK–CtrA pathway	Present	Present
PdhS	Absent	Present
GcrA, CcrM, MucR, SciP	Present[b]	Present

[a]The polar flagellum of *B. abortus* is detected only under very specific conditions [74], and it is involved in the control of infection since a Δ*fliC* mutant (lacking flagellin) is characterized by a high CFU number per spleen in mice [75]. *B. abortus* is nonmotile in all conditions tested so far. Intriguingly, a CtrA binding site is detected close to the *fliC* promoter, encoding flagellin.
[b]There are two paralogs of MucR in *C. crescentus* [58].

In *B. abortus*, ParB and *oriI* are found either at the old pole in newborn bacteria, or at both poles in intermediate and constricting (i.e., dividing) bacteria (Figure 1A) [22]. This distribution closely resembles that of ParB and *oriC* in *C. crescentus*, and is consistent with an ordered chromosomal positioning in *B. abortus*, as in *C. crescentus* [23]. In agreement with this, the terminator of chromosome I (*terI*) is close to the *new pole* in newborn *B. abortus* [22].

According to the data available for other *Alphaproteobacteria*, RepB is predicted to bind near *oriII* to promote the segregation of the two *oriII*s shortly after their replication [20]. In agreement with the observations made for megaplasmids pSymA and pSymB replication origins in *Sinorhizobium meliloti* [24], YFP-RepB foci are not very strongly associated with the poles in *B. abortus* [22]. Interestingly, the proportion of *B. abortus* with a single YFP-RepB focus is twofold more frequent than cells with a single mCherry-ParB focus, suggesting that replication and segregation of *oriI* occur before replication and segregation of *oriII* (Figure 1A). This is further supported by the fact that *B. abortus* with two *oriI*s and a single *oriII* are 18-fold more frequent than bacteria with a single *oriI* and a duplicated *oriII*.

In exponentially growing cells in rich medium, it is expected that the G1 stage of the cell cycle would be very short and thus difficult to detect. Therefore, it is notable that a fraction of 20–25% *B. abortus* is at the G1 stage of the cell cycle in an exponentially growing culture [22]. This suggests that there is a delay between cell division and the initiation of chr. 1 replication. Such a delay is observed with *C. crescentus* swarmer cells, which remain at the G1 stage of their cell cycle for a about 20 min in rich medium (Figure 1B); this G1 stage is prolonged in starvation conditions [25], their motility and their chemotactic system allowing them to find a new niche for growth. Time-lapse experiments can determine whether both *Brucella* daughter cells display an equal delay between cell division and the initiation of S phase, unlike in *C. crescentus*, in which the stalked cell immediately resumes chromosome replication after cytokinesis, while the

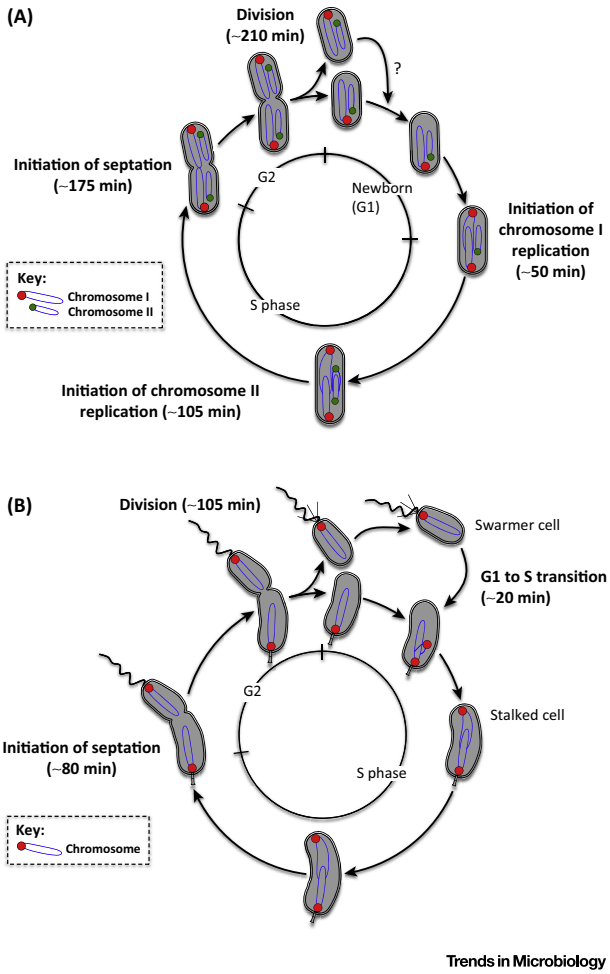

Figure 1. Cell Cycle of *Brucella abortus* and *Caulobacter crescentus*. (A) Cell cycle of *B. abortus*. Cell division generates two daughter cells. The *B. abortus* generation time is approximately 210 min during exponential phase in rich medium, on average these bacteria duplicate and segregate *oriI* ~50 min after division. This corresponds to the G1/S transition of the cell cycle. The *oriII* is duplicated and segregated from ~105 min post-division, while a constriction is detected ~35 min before cell division. These constricting bacteria are also called predivisional. The red dot represents the *oriI*, and the green dot represents *oriII*. The proposed timing is an average generated from the proportion of cell types in a mixed population, all attempts to synchronize *B. abortus* cell cycle having failed to this point. The question mark indicates that a differentiation event is probably occurring at this step of the cell cycle [51]. (B) The cell cycle of *C. crescentus* [76]. Cell division generates two specialized daughter cells: a stalked cell able to rapidly reinitiate chromosome replication, and a swarmer cell going through a G1 phase before differentiating into a stalked cell. The duration of the cell cycle is given for a culture of *C. crescentus* in rich medium. The cell cycle time is about 105 min for swarmer cells, while it is about 85 min for stalked cells.

newborn swarmer cell must differentiate into a stalked cell before entering S phase. An additional remaining question in *Brucella* spp. is whether the two daughter cells have distinct functions.

In *C. crescentus*, *oriC* is anchored to both poles by a matrix-like complex containing the polymerized PopZ protein [26,27]. *B. abortus* PopZ forms a unique focus at the new pole [22]. The role of PopZ in *B. abortus* is unknown, but its function is not likely to be perfectly conserved between *C. crescentus* and *B. abortus* with respect to *oriI*, which is retained at the old pole through an anchor other than PopZ. Moreover, in *C. crescentus*, a new pole protein named TipN contributes to the segregation of *oriC* [28]; there is no homolog for *tipN* in the *B. abortus* genomes, suggesting that its function is fulfilled by another protein. In summary, we now have a description of the main chromosomal replication steps, and the molecular tools to localize *ori* and *ter* regions. This opens the door to other investigations, for example to understand how *oriI* is localized at the poles, or to decipher the mechanisms allowing the ordered initiation of replication of chromosomes. In *B. abortus*, being an intracellular pathogen, it is also interesting to analyze how the cell cycle could be coordinated with the trafficking inside host cells.

Growth and Replication inside Host Cells

In order to understand how the *Brucella* cell cycle progresses inside a host cell during infection, fluorescently labelled bacterial origins of replication [22] have been monitored in *B. abortus*

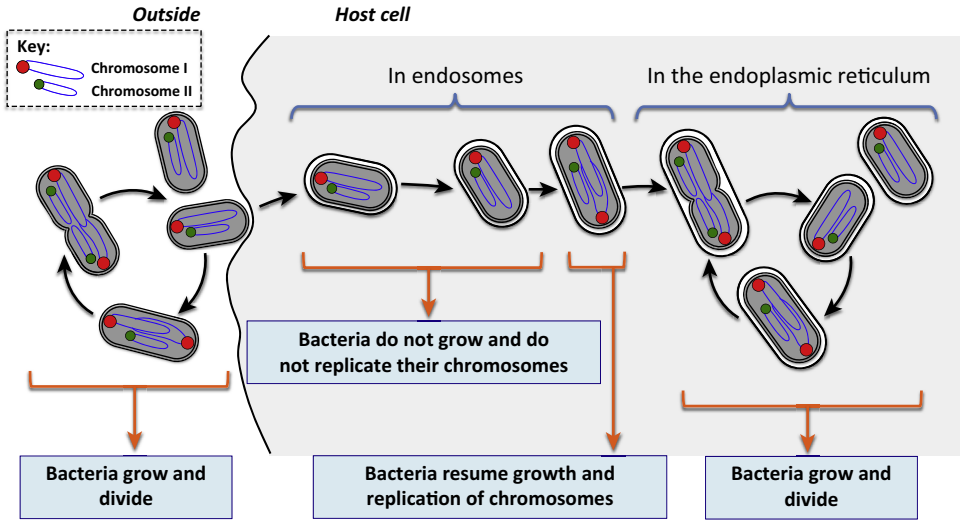

Figure 2. Coordination of the Cell Cycle of *Brucella* with Intracellular Trafficking. From a mixed population in culture, the G1 bacteria have a disproportionately high probability of entry into host cells (HeLa cells or RAW264.7 macrophages). Once inside the host cells, these bacteria remain at the G1 stage of the cell cycle for several hours, depending on the host cell type. When they are still in endosomes (endosomal *Brucella*-containing vacuoles, eBCVs), these bacteria resume growth and replication of chromosomes. Shortly after DNA replication initiation they are found in their replicative niche (relicative *Brucella*-containing vacuoles, rBCVs), that is, in the endoplasmic reticulum (ER). The asymmetric nature of *Brucells abortus* division is not shown here since the type of daughter (small or large) cell internalized was not determined.

strains infecting HeLa cells (Figure 2). In fact, due to their flat morphology, HeLa cells are an ideal model for localizing fluorescent markers in bacteria during intracellular trafficking. As early as 15 min post-infection, 73% of the intracellular bacteria are at the G1 stage of their cell cycle (i.e., cells have only one copy of *oriI*) [22]. Six hours later, the majority of the bacteria (about 75–80%) remain in G1, and have not apparently grown [22]. This is entirely consistent with the oligotrophic [29] and acidic [30] conditions of the BCV, which affect cellular growth. Notably, a large fraction of *B. abortus* cells resumes growth and replication of chr. 1 before eBCVs mature into rBCVs [22] (Figure 2), suggesting that the conditions sensed by the bacterium shift just before leaving eBCV to mature into rBCV. This initial growth could be necessary to permit insertion of the type IV secretion system (VirB) into the bacterial envelope. VirB is required for the maturation of eBCV into rBCV [31], and its production is induced by the conditions thought to occur in the eBCV, that is, starvation and acidic pH [32], and is modulated by the presence of long-chain *N*-acyl homoserine lactones [33].

One may predict that cell types other than HeLa could differently modulate cell-cycle progression of intracellular *B. abortus*. Indeed, it is likely that production of cationic peptides and reactive oxygen species or reactive nitrogen species [34–36] could also block the *B. abortus* cell cycle. The same could be true if the bacterium has to face deprivation of iron [37–39] or other essential compounds, or if the host cell metabolism is modified, as a result of the recognition of the bacterium by the innate immune system. Future studies directed toward investigation of cell-cycle progression in different types of host cell, such as trophoblasts or macrophages (activated or not), will provide a deeper understanding of the interface between host cell biology, *Brucella* cell cycle, and infection.

Cell-Cycle Control

The control of *C. crescentus* cell-cycle progression is achieved, at least in part, by the DivK–CtrA regulation network (Box 1). This network is composed mainly of two-component regulators [40],

Box 1. Core of the *Caulobacter crescentus* DivK–CtrA Regulation Network Conserved in *Brucella abortus*

The histidine kinases (HK) DivJ and PleC control the phosphorylation level of DivK and PleD response regulators (RR) (Figure I) [77,78]. By interacting with HK-like protein, DivL, DivK~P interrupts CckA-ChpT-CpdR/CtrA phosphorylation flow [40]. Moreover, cyclic di-GMP (cdG) also controls CckA activity by stimulating its phosphatase activity [42]. By dephosphorylating DivK~P at the flagellated pole, PleC protects DivL from DivK~P, thereby stimulating the phosphorylation flow from CckA. If active, CckA phosphorylates ChpT, which transfers phosphoryl groups to CtrA and CpdR [45]. CpdR induces CtrA and PdeA degradation through the ClpXP proteolysis machinery but, when it is phosphorylated, this induction is impaired [44,46]. By degrading cdG, PdeA reinforces the phosphorelay, starting from CckA. PopA, a cdG effector recruiting RcdA to the cell pole to direct CtrA degradation by ClpXP in *C. crescentus* [54], is not conserved in *B. abortus*. CtrA binds to 95 promoters in *C. crescentus* [48], including the chromosomal replication origin *oriC* [79]. The *B. abortus oril* is not bound by phosphorylated CtrA, at least *in vitro* [80]. In *B. abortus*, the PleC–DivJ subfamily comprises a clear homolog for PleC, a membrane HK currently annotated as DivJ, and a large soluble HK named PdhS [51].

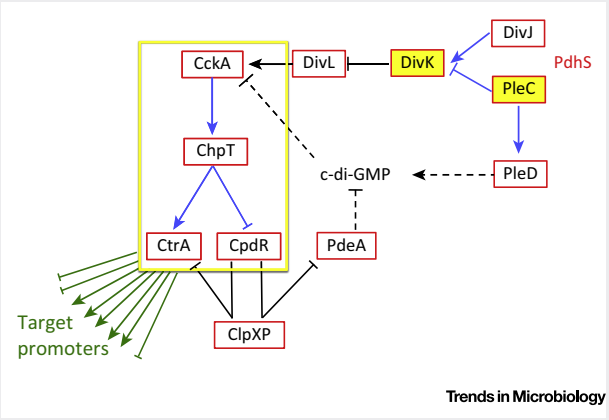

Figure I. A Conserved Cell-Cycle Regulatory Network in *Alphaproteobacteria*. Protein–protein interactions are shown as black lines, pathways of phosphoryl transfer in blue, and transcriptional control in green. Cyclic di-GMP (cdG) is produced by PleD and hydrolyzed by PdeA, and it controls CckA activity (dashed black lines). The corresponding genes conserved in *Brucella abortus* are shown with a red frame, and partial heterocomplementation of *Caulobacter crescentus divK* and *pleC* mutants by homologous *B. abortus* genes [51] is shown in yellow. The yellow frame around the CckA-ChpT-CpdR-CtrA phosphorelay indicates that it is functional in *B. abortus* [49].

a conserved protease (ClpXP) [41], and a secondary messenger molecule, cyclic di-GMP (cdG) [42] being synthesized by diguanylate cyclases (DGCs, like PleD) [43] and degraded by phosphodiesterases (PDEs, like PdeA) [44]. At the heart of this network, the CckA-ChpT-CpdR phosphorelay controls the proteolysis and the phosphorylation of the CtrA transcriptional regulator [45–47]. CtrA controls transcription of many cell-cycle genes (59% of its direct targets are cell-cycle regulated – such as *ftsZ*, *ccrM*, and *ftsW* [48]), and it is, itself, embedded in a network of transcriptional regulators described below.

In *B. abortus*, the CckA-ChpT-CpdR phosphorelay has been reconstituted and is fully functional *in vitro*, suggesting that this conserved signaling network is functional in *Brucella* [49]. Moreover, overexpression of the dominant *cpdR(D52A)* allele results in a strong division defect, suggesting that this network controls at least cell division [49]. Division defects are also observed in *B. abortus* encoding a thermosensitive *ctrA* allele, providing evidence that *ctrA* plays a key role in *B. abortus* cell-cycle progression [49]. These regulatory mutants are specifically impaired for survival in a macrophage model of infection, further suggesting that cell-cycle control is essential for a successful cellular infection [49].

In *C. crescentus*, the DivK–CtrA network is controlled by the PleC and DivJ histidine kinases (HK), respectively acting as phosphatase and kinase of DivK in predivisional cells [50]. In *B. abortus*, besides the PleC and DivJ HKs, there is a third PleC-DivJ homolog named PdhS

[51] (Box 1). This large cytoplasmic HK is associated with the old (nongrowing) pole, where DivK is recruited [51]. The presence and the phosphorylation of PdhS and DivK are essential, and the analysis of a thermosensitive *pdhS* mutant suggests that this HK could control growth and cell division [52]. This is again consistent with a main role for the DivK–CtrA pathway in the control of cell-cycle progression.

The role of cdG has not been deeply investigated in *B. abortus.* However, a pioneering study has revealed that controlled cdG production and degradation is required for *Brucella melitensis* virulence in a mouse infection model [53]. The 11 genes encoding DGCs and PDEs of *B. melitensis* were separately deleted, and the residual virulence of the mutant has been evaluated [53]. Two PDE genes and one DGC gene were found to be required for full virulence [53]. The function of cdG in *Brucella* cell-cycle progression remains undefined, though the PleD and PdeA enzymes involved in the cell-cycle-regulated production and degradation of cdG in *C. crescentus* (Box 1) are conserved in *B. abortus*. Notably, *B. abortus* lacks PopA, suggesting that CtrA regulation differs from that in *C. crescentus*. Briefly, PopA is a PleD paralog that binds cdG and ultimately delivers an RcdA–CtrA complex to ClpXP for degradation in *C. crescentus* [54]. Although *B. abortus* encodes an RcdA homolog, the absence of PopA raises questions regarding the functional role of *B. abortus* RcdA. Certainly, a lack of full conservation of this regulatory system in *B. abortus* does not mean that the CtrA activity/stability is independent of cdG since CckA activity relies on cdG in *C. crescentus*, and the binding site for cdG on CckA is conserved in Rhizobiales [42]. Among Rhizobiales, the bacterium *Sinorhizobium meliloti* is a very interesting case of cell-cycle progression coordinated with interaction with a host. Indeed, this symbiotic bacterium has also a functional CtrA homolog controlling cell division, whose proteolysis is dependent on a CpdR homolog as well as RcdA [55,56]. Depletion of CtrA generates branching morphologies mimicking bacteroïds found inside the nodules induced on infected plants [56]. These data suggest that the CtrA proteolysis mechanism is conserved in Rhizobiales.

In *C. crescentus*, CtrA controls transcription of a network of genes including MucR1/2, SciP, and GcrA [57–60]. The MucR homolog of *B. abortus* is a crucial determinant of *B. melitensis* virulence [61]. In *C. crescentus*, GcrA is a cell-cycle regulator that has the ability to discriminate between hemimethylated DNA [57] and DNA that has been fully methylated by the CcrM methylase [62]. In brief, the fully methylated genome at G**A**nTC sites is progressively converted to the hemimethylated state by the semiconservative replication of DNA. At the end of DNA replication, CcrM methylates hemimethylated GAnTC sites to convert the chromosome into a fully methylated state [62]. The P1 promoter of *ctrA* is controlled by GcrA and its methylation status [62]. Thus, genes located near the *oriC* or the *ter* site spend different amounts of time in the hemimethylated state across the cell cycle; this correlates with differential transcriptional control by GcrA at some promoters on the chromosome [57]. CcrM is conserved and essential in *B. abortus*, and perturbation of its function results in attenuation in a macrophage infection model; this phenotype is independent of cell division defects [63]. It is likely that the *B. abortus* GcrA homolog is involved in CcrM-dependent regulation of gene expression, though this hypothesis remains to be tested.

Beyond the core genes in the CtrA transcriptional network, cell-cycle progression is thought to be controlled by other regulatory mechanisms. For example, the GdhZ glutamate dehydrogenase, which permits entry of carbon into the tricarboxylic acid cycle in *C. crescentus* by catabolizing glutamate, controls dynamics of the essential cell division protein FtsZ by stimulating its GTPase activity [64]. Deletion of *gdhZ* in *B. abortus* generates bacteria with aberrant morphology that are attenuated in a cellular model of infection. This result suggests that metabolic control of the cell cycle is also mediated, at least in part, by this system in *Brucella* [64].

In summary, the deciphering of the molecular mechanisms of cell-cycle control in *B. abortus* strongly benefits from the deep studies performed on the *C. crescentus* model. Additional and original knowledge is expected to arise from the comparison of *B. abortus* and *C. crescentus*.

Concluding Remarks

Recent data presented in this review show that cell-cycle progression and its control are intimately linked to the virulence of *B. abortus*, and in particular its intracellular trafficking. New tools available for studying the molecular mechanisms of *Brucella* cell-cycle control are providing new insight into this aspect of its biology. Besides deciphering these mechanisms in *B. abortus*, a central question for future research is to understand how *Brucella* utilizes its cell-cycle control system to adapt to conditions encountered inside and outside the host intracellular environment (see Outstanding Questions). More generally, it will be interesting to investigate how pathogens other than *Brucella* integrate cell-cycle progression with host cell infection and trafficking. Indeed, studies of *Ehrlichia chaffeensis* suggest that it is important to investigate the intersection of the cell cycle and infection in other bacterial pathogens [65,66].

The broad conservation of genes encoding the cell-cycle regulation network in *Alphaproteobacteria* [67] suggests that a sophisticated control system evolved in an early ancestor. Over the course of evolutionary time, each group likely adapted cell-cycle control features that favour replication and survival in the diverse niches inhabited by bacteria of this clade. The comparison of *C. crescentus*, *B. abortus*, and other free-living or host-associated bacteria [56,68] should provide an opportunity to analyze how modifications of their regulation networks provide adaptive advantage in their ecological niches. Such studies could be conducted in parallel with genomic analyses identifying the selection of pathogenicity or fitness islands by horizontal transfer [69]. This would advance a deeper understanding of how *B. abortus*, whose recent ancestor was probably a soil bacterium, became such a successful pathogen.

Outstanding Questions

Are machineries necessary for the biosynthesis and/or the incorporation of peptidoglycan, lipopolysaccharide, and outer-membrane proteins localized at growth zones?

How are *oril* replication origins so closely associated to the poles? What is the function of PopZ regarding this anchoring?

How is the cell cycle adapted in the different strains of *Brucella*? Does this differ in fast-growing strains of *Brucella* (e.g., *Brucella microti* and *Brucella suis* biovar 5), and differ in those with fused chromosomes (e.g., *B. suis* biovar 3)?

What is the global function of the CtrA and GcrA transcriptional networks in *Brucella abortus*?

What is the role of cyclic di-GMP in the *B. abortus* cell cycle?

How are changes in the host niche (such as starvation, acidic pH, and the presence of reactive oxygen and/or nitrogen species) integrated into the cell-cycle control system?

How do carbon and nitrogen metabolic fluxes control the cell cycle?

What are the distinct functions of the two cells generated by cell division, given the asymmetric distribution of regulatory proteins at the old cell poles?

How is the cell cycle controlled in trophoblasts and primary macrophages? Is cell-cycle control also observed in mouse models of infection?

Do other important pathogens, such as *Salmonella*, *Mycobacterium*, or *Shigella*, control their cell cycle during infection?

References

1. Moreno, E. and Moriyon, I. (2006) The genus *Brucella*. *Prokaryotes* 5, 315–456
2. Wattam, A.R. *et al.* (2014) Comparative phylogenomics and evolution of the *Brucellae* reveal a path to virulence. *J. Bacteriol.* 196, 920–930
3. Archambaud, C. *et al.* (2010) Contrasting roles of macrophages and dendritic cells in controlling initial pulmonary *Brucella* infection. *Eur. J. Immunol.* 40, 3458–3471
4. Anderson, T.D. *et al.* (1986) Pathogenesis of placentitis in the goat inoculated with *Brucella abortus*. II. Ultrastructural studies. *Vet. Pathol.* 23, 227–239
5. Celli, J. (2015) The changing nature of the *Brucella*-containing vacuole. *Cell. Microbiol.* 17, 951–958
6. Bellaire, B.H. *et al.* (1988) Opsonized virulent *Brucella abortus* replicates within nonacidic, endoplasmic reticulum-negative, LAMP-1-positive phagosomes in human monocytes. *Infect. Immun.* 73, 3702–3713
7. Salcedo, S.P. *et al.* (2013) Pathogenic *brucellae* replicate in human trophoblasts. *J. Infect. Dis.* 207, 1075–1083
8. Starr, T. *et al.* (2008) *Brucella* intracellular replication requires trafficking through the late endosomal/lysosomal compartment. *Traffic* 9, 678–694
9. Hallez, R. *et al.* (2004) Morphological and functional asymmetry in alpha-proteobacteria. *Trends Microbiol.* 12, 361–365
10. Brown, P.J. *et al.* (2012) Polar growth in the Alphaproteobacterial order Rhizobiales. *Proc. Natl. Acad. Sci. U.S.A.* 109, 1697–1701
11. Brown, P.J. *et al.* (2011) Polarity and the diversity of growth mechanisms in bacteria. *Semin. Cell Dev. Biol.* 22, 790–798
12. Moriyon, I. and Berman, D.T. (1983) Isolation, purification, and partial characterization of *Brucella abortus* matrix protein. *Infect. Immun.* 39, 394–402
13. Kuru, E. *et al.* (2015) Synthesis of fluorescent D-amino acids and their use for probing peptidoglycan synthesis and bacterial growth in situ. *Nat. Protoc.* 10, 33–52
14. Guidolin, L.S. *et al.* (2015) Interaction network and localization of *Brucella abortus* membrane proteins involved in the synthesis, transport, and succinylation of cyclic β-1,2-glucans. *J. Bacteriol.* 197, 1640–1648
15. Bundle, D.R. *et al.* (1988) Characterization of *Brucella* polysaccharide B. *Infect. Immun.* 56, 1101–1106
16. Roset, M.S. *et al.* (2014) *Brucella* cyclic β-1,2-glucan plays a critical role in the induction of splenomegaly in mice. *PLoS ONE* 9, e101279
17. Arellano-Reynoso, B. *et al.* (2005) Cyclic beta-1,2-glucan is a *Brucella* virulence factor required for intracellular survival. *Nat. Immunol.* 6, 618–625
18. Jumas-Bilak, E. *et al.* (1998) Differences in chromosome number and genome rearrangements in the genus *Brucella*. *Mol. Microbiol.* 27, 99–106
19. Harrison, P.W. *et al.* (2010) Introducing the bacterial 'chromid': not a chromosome, not a plasmid. *Trends Microbiol.* 18, 141–148
20. Pinto, U.M. *et al.* (2012) The ABCs of plasmid replication and segregation. *Nat. Rev. Microbiol.* 10, 755–765
21. Mohl, D.A. and Gober, J.W. (1997) Cell cycle-dependent polar localization of chromosome partitioning proteins in *Caulobacter crescentus*. *Cell* 88, 675–684
22. Deghelt, M. *et al.* (2014) G1-arrested newborn cells are the predominant infectious form of the pathogen *Brucella abortus*. *Nat. Commun.* 5, 4366
23. Viollier, P.H. *et al.* (2004) Rapid and sequential movement of individual chromosomal loci to specific subcellular locations during bacterial DNA replication. *Proc. Natl. Acad. Sci. U.S.A.* 101, 9257–9262

24. Kahng, L.S. and Shapiro, L. (2003) Polar localization of replicon origins in the multipartite genomes of *Agrobacterium tumefaciens* and *Sinorhizobium meliloti*. *J. Bacteriol.* 185, 3384–3391
25. Lesley, J.A. and Shapiro, L. (2008) SpoT regulates DnaA stability and initiation of DNA replication in carbon-starved *Caulobacter crescentus*. *J. Bacteriol.* 190, 6867–6880
26. Bowman, G.R. *et al.* (2008) A polymeric protein anchors the chromosomal origin/ParB complex at a bacterial cell pole. *Cell* 134, 945–955
27. Ebersbach, G. *et al.* (2008) A self-associating protein critical for chromosome attachment, division, and polar organization in *Caulobacter*. *Cell* 134, 956–968
28. Schofield, W.B. *et al.* (2010) Cell cycle coordination and regulation of bacterial chromosome segregation dynamics by polarly localized proteins. *EMBO J.* 29, 2068–2081
29. Dozot, M. *et al.* (2006) The stringent response mediator Rsh is required for *Brucella melitensis* and *Brucella suis* virulence, and for expression of the type IV secretion system virB. *Cell. Microbiol.* 8, 1791–1802
30. Porte, F. *et al.* (1999) Early acidification of phagosomes containing *Brucella suis* is essential for intracellular survival in murine macrophages. *Infect. Immun.* 67, 4041–4047
31. Comerci, D.J. *et al.* (2001) Essential role of the VirB machinery in the maturation of the *Brucella abortus*-containing vacuole. *Cell. Microbiol.* 3, 159–168
32. Boschiroli, M.L. *et al.* (2002) The *Brucella suis virB* operon is induced intracellularly in macrophages. *Proc. Natl. Acad. Sci. U. S.A.* 99, 1544–1549
33. Terwagne, M. *et al.* (2013) Quorum sensing and self-quorum quenching in the intracellular pathogen *Brucella melitensis*. *PLoS ONE* 8, e82514
34. Gross, A. *et al.* (1998) Expression and bactericidal activity of nitric oxide synthase in *Brucella suis*-infected murine macrophages. *Infect. Immun.* 66, 1309–1316
35. Jiang, X. *et al.* (1993) Macrophage control of *Brucella abortus*: role of reactive oxygen intermediates and nitric oxide. *Cell. Immunol.* 151, 309–319
36. Sola-Landa, A. *et al.* (1998) A two-component regulatory system playing a critical role in plant pathogens and endosymbionts is present in *Brucella abortus* and controls cell invasion and virulence. *Mol. Microbiol.* 29, 125–138
37. González Carreró, M.I. *et al.* (2002) *Brucella abortus* strain 2308 produces brucebactin, a highly efficient catecholic siderophore. *Microbiology* 148, 353–360
38. Leonard, B.A. *et al.* (1997) *Brucella abortus* siderophore 2,3-dihydroxybenzoic acid protects brucellae from killing by macrophages. *Vet. Res.* 28, 87–92
39. Lopez-Goni, I. *et al.* (1992) Identification of 2,3-dihydroxybenzoic acid as a *Brucella abortus* siderophore. *Infect. Immun.* 60, 4496–4503
40. Tsokos, C.G. *et al.* (2011) A dynamic complex of signaling proteins uses polar localization to regulate cell-fate asymmetry in *Caulobacter crescentus*. *Dev. Cell* 20, 329–341
41. Jenal, U. and Fuchs, T. (1998) An essential protease involved in bacterial cell-cycle control. *EMBO J.* 17, 5658–5669
42. Lori, C. *et al.* (2015) Cyclic di-GMP acts as a cell cycle oscillator to drive chromosome replication. *Nature* 523, 236–239
43. Paul, R. *et al.* (2004) Cell cycle-dependent dynamic localization of a bacterial response regulator with a novel di-guanylate cyclase output domain. *Genes Dev.* 18, 715–727
44. Abel, S. *et al.* (2011) Regulatory cohesion of cell cycle and cell differentiation through interlinked phosphorylation and second messenger networks. *Mol. Cell* 43, 550–560
45. Biondi, E.G. *et al.* (2006) Regulation of the bacterial cell cycle by an integrated genetic circuit. *Nature* 4444, 899–904
46. Iniesta, A.A. *et al.* (2006) A phospho-signaling pathway controls the localization and activity of a protease complex critical for bacterial cell cycle progression. *Proc. Natl. Acad. Sci. U.S.A.* 103, 10935–10940
47. Jacobs, C. *et al.* (1999) Cell cycle-dependent polar localization of an essential bacterial histidine kinase that controls DNA replication and cell division. *Cell* 97, 111–120
48. Laub, M.T. *et al.* (2002) Genes directly controlled by CtrA, a master regulator of the *Caulobacter* cell cycle. *Proc. Natl. Acad. Sci. U.S. A.* 99, 4632–4637
49. Willett, J.W. *et al.* (2015) Structural asymmetry in a conserved signaling system that regulates division, replication, and virulence of an intracellular pathogen. *Proc. Natl. Acad. Sci. U.S.A.* 112, E3709–E3718
50. Matroule, J.Y. *et al.* (2004) Cytokinesis monitoring during development; rapid pole-to-pole shuttling of a signaling protein by localized kinase and phosphatase in *Caulobacter*. *Cell* 118, 579–590
51. Hallez, R. *et al.* (2007) The asymmetric distribution of the essential histidine kinase PdhS indicates a differentiation event in *Brucella abortus*. *EMBO J.* 26, 1444–1455
52. Van der Henst, C. *et al.* (2012) The histidine kinase PdhS controls cell cycle progression of the pathogenic alphaproteobacterium *Brucella abortus*. *J. Bacteriol.* 194, 5305–5314
53. Petersen, E. *et al.* (2011) *Brucella melitensis* cyclic di-GMP phosphodiesterase BpdA controls expression of flagellar genes. *J. Bacteriol.* 193, 5683–5691
54. Duering, A. *et al.* (2009) Second messenger-mediated spatiotemporal control of protein degradation regulates bacterial cell cycle progression. *Genes Dev.* 23, 93–104
55. Kobayashi, H. *et al.* (2009) *Sinorhizobium meliloti* CpdR1 is critical for co-ordinating cell cycle progression and the symbiotic chronic infection. *Mol. Microbiol.* 73, 586–600
56. Pini, F. *et al.* (2015) Cell cycle control by the master regulator CtrA in *Sinorhizobium meliloti*. *PLoS Genet.* 11, e1005232
57. Fioravanti, A. *et al.* (2013) DNA binding of the cell cycle transcriptional regulator GcrA depends on N6-adenosine methylation in *Caulobacter crescentus* and other *Alphaproteobacteria*. *PLoS Genet.* 9, e1003541
58. Fumeaux, C. *et al.* (2014) Cell cycle transition from S-phase to G1 in *Caulobacter* is mediated by ancestral virulence regulators. *Nat. Commun.* 5, 4081
59. Gora, K.G. *et al.* (2010) A cell-type-specific protein-protein interaction modulates transcriptional activity of a master regulator in *Caulobacter crescentus*. *Mol. Cell* 39, 455–467
60. Holtzendorff, J. *et al.* (2004) Oscillating global regulators control the genetic circuit driving a bacterial cell cycle. *Science* 304, 983–987
61. Mirabella, A. *et al.* (2013) *Brucella melitensis* MucR, an orthologue of *Sinorhizobium meliloti* MucR, is involved in resistance to oxidative, detergent, and saline stresses and cell envelope modifications. *J. Bacteriol.* 195, 453–465
62. Reisenauer, A. and Shapiro, L. (2002) DNA methylation affects the cell cycle transcription of the CtrA global regulator in *Caulobacter*. *EMBO J.* 21, 4969–4977
63. Robertson, G.T. *et al.* (2000) The *Brucella abortus* CcrM DNA methyltransferase is essential for viability, and its overexpression attenuates intracellular replication in murine macrophages. *J. Bacteriol.* 182, 3482–3489
64. Beaufay, F. *et al.* (2015) A NAD-dependent glutamate dehydrogenase coordinates metabolism with cell division in *Caulobacter crescentus*. *EMBO J.* 34, 1786–1800
65. Cheng, Z. *et al.* (2011) Insights into the CtrA regulon in development of stress resistance in obligatory intracellular pathogen *Ehrlichia chaffeensis*. *Mol. Microbiol.* 82, 1217–1234
66. Kumagai, Y. *et al.* (2006) Biochemical activities of three pairs of *Ehrlichia chaffeensis* two-component regulatory system proteins involved in inhibition of lysosomal fusion. *Infect. Immun.* 74, 5014–5022
67. Brilli, M. *et al.* (2010) The diversity and evolution of cell cycle regulation in alpha-proteobacteria: a comparative genomic analysis. *BMC Syst. Biol.* 4, 52
68. Pini, F. *et al.* (2013) The DivJ, CbrA and PleC system controls DivK phosphorylation and symbiosis in *Sinorhizobium meliloti*. *Mol. Microbiol.* 90, 54–71
69. Czibener, C. and Ugalde, J.E. (2012) Identification of a unique gene cluster of *Brucella* spp. that mediates adhesion to host cells. *Microbes Infect.* 14, 79–85

70. Deghorain, M. *et al.* (2011) A defined terminal region of the *E. coli* chromosome shows late segregation and high FtsK activity. *PLoS ONE* 6, e22164
71. Van der Henst, C. *et al.* (2013) The *Brucella* pathogens are polarized bacteria. *Microbes Infect.* 15, 998–1004
72. Mignolet, J. *et al.* (2010) PdhS, an old-pole-localized histidine kinase, recruits the fumarase FumC in *Brucella abortus*. *J. Bacteriol.* 192, 3235–3239
73. Dotreppe, D. *et al.* (2011) The alkylation response protein AidB is localized at the new poles and constriction sites in *Brucella abortus*. *BMC Microbiol.* 11, 257
74. Fretin, D. *et al.* (2005) The sheathed flagellum of *Brucella melitensis* is involved in persistence in a murine model of infection. *Cell. Microbiol.* 7, 687–698
75. Terwagne, M. *et al.* (2013) Innate immune recognition of flagellin limits systemic persistence of *Brucella*. *Cell. Microbiol.* 15, 942–960
76. Curtis, P.D. and Brun, Y.V. (2010) Getting in the loop: regulation of development in *Caulobacter crescentus*. *Microbiol. Mol. Biol. Rev.* 74, 13–41
77. Jacobs, C. *et al.* (2001) Dynamic localization of a cytoplasmic signal transduction response regulator controls morphogenesis during the *Caulobacter* cell cycle. *Proc. Natl. Acad. Sci. U.S.A.* 98, 4095–4100
78. Wheeler, R.T. and Shapiro, L. (1999) Differential localization of two histidine kinases controlling bacterial cell differentiation. *Mol. Cell* 4, 683–694
79. Quon, K.C. *et al.* (1998) Negative control of bacterial DNA replication by a cell cycle regulatory protein that binds at the chromosome origin. *Proc. Natl. Acad. Sci. U.S.A.* 95, 120–125
80. Bellefontaine, A.F. *et al.* (2002) Plasticity of a transcriptional regulation network among alpha-proteobacteria is supported by the identification of CtrA targets in *Brucella abortus*. *Mol. Microbiol.* 43, 945–960