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Review Article

REVIEW ARTICLE

The role of peptidoglycan in chlamydial cell division: towards resolving the chlamydial anomaly

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One sentence summary: This review summarizes recent advances regarding the chlamydial division, including detection of chlamydial peptidoglycan and discovery of mid-cell localized division proteins, which allow a better understanding of the division of *Chlamydiales* in absence of an FtsZ homologue.
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ABSTRACT

Chlamydiales are obligate intracellular bacteria including some important pathogens causing trachoma, genital tract infections and pneumonia, among others. They share an atypical division mechanism, which is independent of an FtsZ homologue. However, they divide by binary fission, in a process inhibited by penicillin derivatives, causing the formation of an aberrant form of the bacteria, which is able to survive in the presence of the antibiotic. The paradox of penicillin sensitivity of chlamydial cells in the absence of detectable peptidoglycan (PG) was dubbed the chlamydial anomaly, since no PG modified by enzymes (Pbps) that are the usual target of penicillin could be detected in *Chlamydiales*. We review here the recent advances in this field with the first direct and indirect evidences of PG-like material in both Chlamydiaceae and *Chlamydia*-related bacteria. Moreover, PG biosynthesis is required for proper localization of the newly described septal proteins RodZ and NlpD. Taken together, these new results set the stage for a better understanding of the role of PG and septal proteins in the division mechanism of *Chlamydiales* and illuminate the long-standing chlamydial anomaly. Moreover, understanding the chlamydial division mechanism is critical for the development of new antibiotics for the treatment of chlamydial chronic infections.

Key words: *Waddlia chondrophila*; cell division; contractile ring; MreB; RodZ; peptidoglycan; cell wall

INTRODUCTION

Chlamydiales is an order of obligate intracellular bacteria comprising the Chlamydiaceae and the *Chlamydia*-related bacteria. Members of the Chlamydiaceae family include the well-known pathogens *Chlamydia trachomatis*, which causes genital tract infections and trachoma, and the causative agents of pneumonia, *C. pneumoniae* and *C. psittacci* (Greub 2009b). This family also contains important animal pathogens like *C. abortus*, which causes abortion in cattle (Vretou *et al.*, 2001). *Chlamydia*-related bacteria were described more recently and there is increasing evidence that at least some of them are indeed pathogenic. *Waddlia chondrophila*, for example, is thought to play a role in abortion in animals and miscarriage in humans (Rurangirwa *et al.*, 1999; Baud *et al.*, 2007, 2011; Baud, Regan and Greub 2008; de Barsy and Greub 2013). There are also good indications that *Parachlamydia acanthamoebae*, *Simkania negevensis* and *Protochlamydia naegleriophila* are agents of human pneumonia (Corsaro and Greub 2006; Casson *et al.*, 2008; Greub 2009a; Lamoth and Greub 2010).

Members of the *Chlamydiales* order are characterized by a particular infection cycle, involving two developmental stages: the elementary bodies (EBs) that are infectious but have

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reduced metabolic activity (Sixt *et al.*, 2013), and the non-infectious reticulate bodies (RBs) that are able to divide (Friis 1972; Greub and Raoult 2002; Abdelrahman and Belland 2005). EBs enter the cell generally through phagocytosis or endocytosis and are thus engulfed in an endosome. Release of effectors modify the properties of the surrounding membrane by modification of protein and cytoskeleton recruitment and allow escape of the endocytic pathway. The resulting vacuole is called an inclusion (Abdelrahman and Belland 2005). In this environment, they differentiate into RBs, proliferate and finally re-differentiate into EBs that are expelled from the cells either by exocytosis or by cell lysis (Todd and Caldwell 1985; Hybiske and Stephens 2007). In some cases, in the presence of stress or nutrient deprivation, *Chlamydiales* can enter a persistent stage, in which the bacteria becomes enlarged and polyploid (described in the section 'box 2: aberrant bodies and persistence').

Chlamydiales rely on an atypical cell division mechanism, which is still not well understood. First, *Chlamydiales* do not possess a homologue of FtsZ, the typical organizer of the cytokinetic machinery in bacteria (Stephens *et al.*, 1998; Margolin 2005; Bertelli *et al.*, 2010). Secondly, *Chlamydiales* do not possess a peptidoglycan (PG) cell wall with a conventional structure (Fox *et al.*, 1990; Moulder 1993; Ghuysen and Goffin 1999). This appears contradictory to the effect of penicillin on chlamydial division (Matsumoto and Manire 1970; Skilton *et al.*, 2009) and with previous findings showing that the enzymes involved in the PG biosynthesis pathway are indeed functional (McCoy and Maurelli 2006; Henrichfreise *et al.*, 2009). This apparent contradiction was called the 'chlamydial anomaly' (Moulder 1993).

In this review, we summarize the recent progress on understanding the division of *Chlamydiales* and the chlamydial anomaly. Indeed, recent studies showed (i) that an intact PG polymer surrounding chlamydial cells and of PG-like chemical composition is indeed extractable from at least one *Chlamydiales* member (Pilhofer *et al.*, 2013), (ii) that typical peptide components of PG accumulate at the division site as in other bacteria and may be incorporated into a PG-like polymer by chlamydial cells (Liechti *et al.*, 2013) and (iii) that PG precursors are required for proper localization of PG- and bacterial actin-homologue-binding proteins at the division septum (Frandi *et al.*, 2014; Jacquier *et al.*, 2014). This new knowledge allows us to draw an updated model of chlamydial division.

DIVISION IN GRAM-NEGATIVE BACTERIA, A SHORT OVERVIEW

The Gram-negative bacteria possess two lipid bilayers, the inner membrane (IM) and the outer membrane (OM). Between them, a layer of PG maintains the cellular integrity and allows tolerance to osmotic pressure. During division, all these three layers have to be produced and subsequently split between the daughter cells: IM invagination is followed by modification of the PG layer (new synthesis and subsequent hydrolysis), to allow the new formation of PG layers between the two daughter cells. Finally, invagination of the OM is tightly linked to the IM invagination and PG splitting through a transenvelope structure linking all three layers, the Tol–Pal complex (reviewed in Gerding *et al.*, 2007; Egan and Vollmer 2013).

Bacteria usually divide by complex mechanisms organized by the bacterial tubulin homologue FtsZ, which assembles into short protofilaments that encircle the division site in a structure called the Z-ring (Margolin 2005). The driving force for constriction by FtsZ seems to stem primarily from septal PG synthesis (Adams and Errington 2009). In addition, FtsZ recruits more than 10 essential proteins (the Fts proteins) into the divisome complex at mid-cell. More accessory proteins also localize to the division septum during division, but are not essential for invagination or septal PG synthesis, likely regulating the proper placement and/or timing of divisome recruitment (Kirkpatrick and Viollier 2011; Natale, Pazos and Vicente 2013). Z-ring formation requires stabilization of FtsZ polymerization and anchoring of the polymers to the membrane via the action of the proteins ZipA and FtsA, which are essential for bacterial division (Haney *et al.*, 2001; Pichoff and Lutkenhaus 2005). Four Z-ring-associated proteins, ZapA, ZapB, ZapC and ZapD are associated with FtsZ, but not essential for *Escherichia coli* division, but likely support efficient Z-ring formation or maturation (reviewed in Egan and Vollmer 2013). Several proteins are then recruited to this nascent Z-ring. One of them, FtsK, has a bifunctional role in cell division and in chromosome segregation (Grenga *et al.*, 2008). FtsK recruits FtsQ, FtsL and FtsI to the division septum (Chen and Beckwith 2001). This results into further recruitment of late division factors such as FtsW (Mohammadi *et al.*, 2011). The fact that FtsZ not only recruits PG-biosynthetic (PBP3) and -precursor enzymes (MurG, see below Aaron *et al.*, 2007; Mohammadi *et al.*, 2007) but also proteins (FtsEX) that indirectly regulate PG-hydrolyzing enzymes (AmiA/B) highlights the importance of tightly coordinated PG remodelling during division (Yang *et al.*, 2011).

THE ROLE OF PG IN DIVISION OF GRAM-NEGATIVE BACTERIA

PG is a polymer of glycan chains composed of N-acetylmuramic acid (MurNAc) and N-acetylglucosamine (GlcNAc) connected by short peptides. These peptides are usually synthesized as pentapeptides containing both L- and D-aminoacids. The first steps of PG biosynthesis occur in the cytosol by the enzymes MurA and MurB. These two enzymes synthesize MurNAc using GlcNAc as a substrate. Amino acids are sequentially added to MurNAc by MurC, D, E and F (Fig. 1, reviewed in Barreteau *et al.*, 2008). The resulting monosaccharide–pentapeptide is then attached to the IM by addition of a lipid by the enzyme MraY to form undecaprenyl-pyrophosphoryl-MurNAc-pentapeptide, also called lipid I. Lipid I is then further modified by MurG, which adds a GlcNAc, forming the disaccharide-pentapeptide unit, lipid II (Bouhss *et al.*, 2008). Lipid II is then flipped across the IM by the MurJ flippase (Sham *et al.*, 2014; Ruiz 2008). Glycosyltransferases (penicillin-binding proteins class A) then accept this functional unit of lipid II, incorporating it into a growing glycan strand. Glycan strands are then cross-linked by pentapeptide bridges introduced by the transpeptidation activity of penicillin-binding proteins class A and B (PBP2/3) (Sauvage *et al.*, 2008).

PG biosynthesis enzymes are recruited to the divisome at a later stage of division, the Z-ring maturation, being followed by the initiation of cell-wall constriction. This starts with the recruitment of FtsK. FtsQ is then recruited in an FtsK-dependent manner. Subsequently, FtsL, FtsB, FtsW and PBP3 are recruited to the divisome (Begg *et al.*, 1990; Aarsman *et al.*, 2005; Mohammadi *et al.*, 2011). The *de novo*-synthesized septal PG separates the daughter compartments, but the cells remain attached until the septal PG is cleaved by amidases (Yang *et al.*, 2011). Amidase activity should be tightly regulated, as uncontrolled activity can lead to cell lysis. Specific activation of amidases at the septum is regulated by EnvC and NlpD (Uehara *et al.*, 2010). These proteins are recruited to the division septum by the ABC-transporter complex FtsEX and the SPOR-domain protein FtsN, respectively (Uehara *et al.*, 2010; Yang *et al.*, 2011).

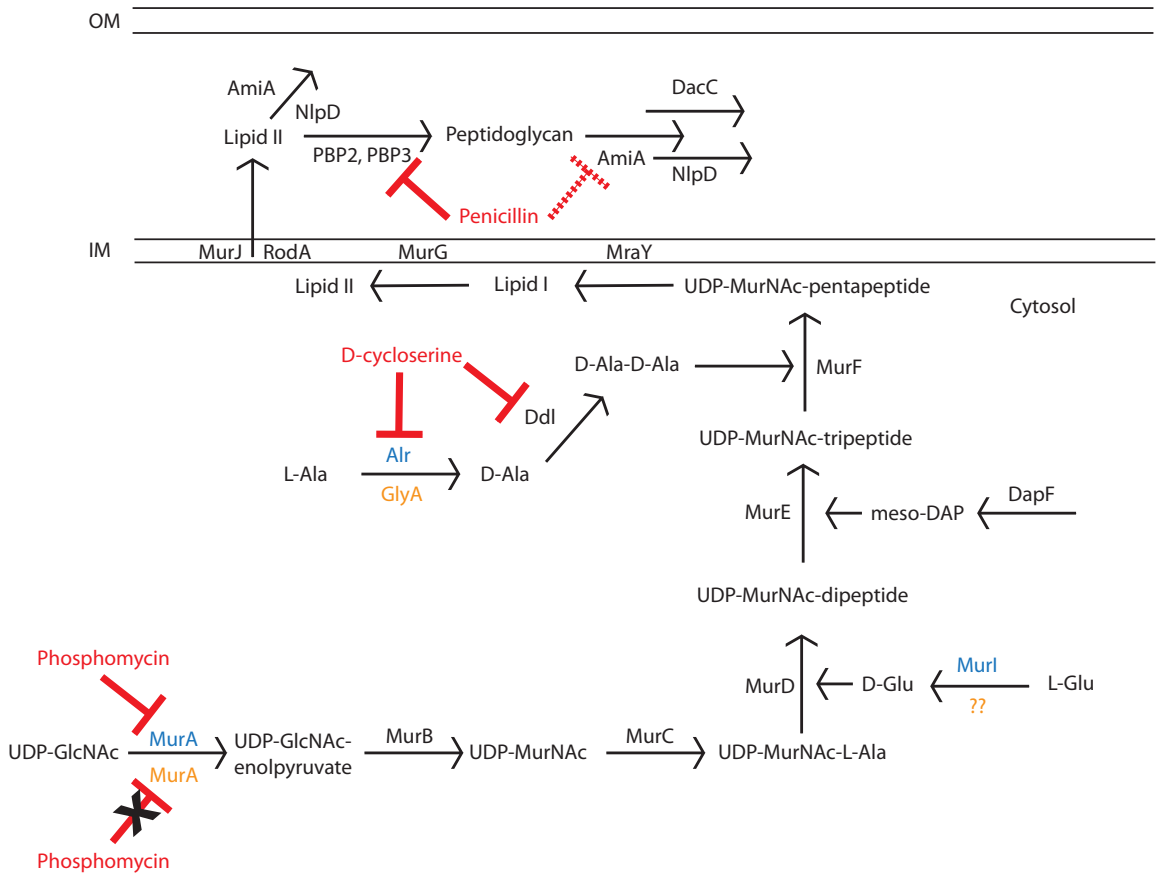

Figure 1. PG biosynthesis pathway comparison between *W. chondrophila* and Chlamydiaceae. Enzymes present only in *W. chondrophila* (Alr and MurI) are depicted in blue, as enzyme replacing Alr in Chlamydiaceae (GlyA) is depicted in orange. Orange question marks highlight the unknown source of D-Glu in Chlamydiaceae. Steps inhibited by D-cycloserin, phosphomycin and penicillin are shown in red. MurA is depicted in blue for *W. chondrophila*, which is sensitive to phosphomycin and in orange for Chlamydiaceae, which are resistant to phosphomycin (illustrated by a black cross).

OM INVAGINATION DURING CYTOKINESIS

The Tol–Pal complex is composed of five conserved proteins, TolQ, TolR and TolA, which are transmembrane proteins inserted in the IM, TolB, a soluble periplasmic protein and Pal, a lipoprotein localized at the inner leaflet of the OM. Pal directly interacts with PG (Mizuno 1981) and through its transient interaction with TolB is thought to coordinate invagination of the OM-PG-IM layers, drawing the OM inwards once septal PG splitting commences (for review, see Godlewska *et al.*, 2009). Other complexes might also play a role in coordinating OM invagination as the PBP1B–LpoB complex (Typas *et al.*, 2010) or other lipoproteins that can bind PG covalently or non-covalently such as Lpp or OmpA.

REGULATION OF BACTERIAL DIVISION

Bacterial division has to be tightly regulated to ensure a division resulting in two viable daughter cells in an efficient way. Moreover, PG splitting mediated by potentially lethal hydrolytic enzymes (amidases, endopeptidases) is carefully controlled in time and space to prevent lysis of cells. Several conditions have to be met before division is initiated: (i) control of the energetic status of the bacteria to allow complete replication of the chromosome, (ii) identification of mid-cell for the assembly of the septum and (iii) complete segregation of the chromosome to clear the division site so that no DNA is trapped during invagination of the envelope and does not get bisected at cell separation.

The energetic status of the bacteria is measured mainly by the availability of ATP. DnaA is the replication initiator protein and its active form is bound to an ATP molecule. In contrast, ADP bound DnaA is inactive. Active DnaA binds to the origin of replication OriC and induces the recruitment of the replication machinery (for review, see Leonard and Grimwade 2011). It has also to be mentioned that DNA replication by DNA primase in *Bacillus subtilis* is regulated by ppGpp availability, thus controlled by the nutrient availability (Wang, Sanders and Grossman 2007).

Several mechanisms are involved in the correct positioning of the divisome at mid-cell. The best-known regulatory system is the MinCD(E/J) (Lutkenhaus 2007). MinC inhibits the polymerization of FtsZ and blocks its interaction with other divisomal proteins. MinC is recruited to the membrane and activated by binding to MinD. A polar gradient of MinCD induces polymerization of FtsZ at mid-cell only and allows a correct spatial assembly of the Z-ring (Lutkenhaus 2007; Kirkpatrick and Viollier 2011). Anionic phospholipids like cardiolipins seem to also be important for correct Z-ring assembly (Kawai *et al.*, 2004). They are enriched at the division site and at the poles and may influence localization of the Z-ring (reviewed in Mileykovskaya and Dowhan 2005). During delays in DNA replication, septation is inhibited by a process called nucleoid occlusion. In this process, septation over nucleoid is inhibited by the proteins Noc in *B. subtilis* (Wu and Errington 2004) or SlmA in *E. coli* (Bernhardt and de Boer 2005). This process prevents an uneven partition of the chromosomes between the daughter cells and delays the

Table 1. Conservation and percentage of identity of chlamydial division proteins compared to *E. coli*.

	Cpn	Wch	Comments
FtsZ	–	–	Tubulin homologue, bacterial division organizer
ZapA/B/C/D	–	–	
FtsA	–	–	
ZipA	–	–	
FtsE	–	–	
FtsX	–	–	
FtsK	39.29	40.91	Putative chromosome segregation protein
FtsB	–	–	
FtsQ	–	29.82	
FtsL	–	11.40	
FtsW	31.25	36.11	Putative lipid II flippase
FtsN	–	–	
FtsP	–	–	
MreB	57.40	59.17	Actin homologue
RodZ	28.57	23.81	MreB interactor

Table 2. Percentage of identity and coverage (in brackets) of *W. chondrophila* division proteins compared to *C. pneumoniae* (Cpn), *C. trachomatis* (Ctr), *P. acanthamoebae* (Pac) and *S. negevensis* (Sne).

	Cpn	Ctr	Pac	Sne
FtsK	44.84 (95)	46.04 (97)	59.24 (96)	65.92 (59)
FtsQ	25.87 (76)	21.85 (90)	28.57 (87)	30.95 (92)
FtsL	49.21 (63)	44.26 (61)	57.83 (83)	43.75 (80)
FtsW	51.40 (95)	51.96 (95)	59.28 (97)	51.58 (94)
MreB	85.55 (96)	86.92 (94)	89.84 (100)	90.90 (93)
RodZ	40.15 (93)	42.11 (93)	60.61 (97)	60.15 (97)

cytokinesis until the chromosomes are properly separated (reviewed in Rothfield, Taghbalout and Shih 2005).

ROLE OF MreB ACTIN IN DIVISION OF THE CHLAMYDIALES

Chlamydial division is mysterious, since it occurs in the absence of a sequence homologue of FtsZ, the usual organizer of bacterial division (Ghuysen and Goffin 1999). Nevertheless, chlamydial cells divide by binary fission, in a process that highly resembles FtsZ-dependent coccoid division (Brown and Rockey 2000; Greub and Raoult 2002; Abdelrahman and Belland 2005). Other key components of division are also not encoded in chlamydial genomes. Chlamydiaceae only possess annotated homologues of FtsK, FtsW and FtsI (PBP3, Tables 1 and 3). *Waddlia chondrophila* possesses homologues of FtsK, FtsW, FtsI (PBP3), FtsQ and FtsL (Table 1). Interestingly, Chlamydiaceae possess homologues of all these *W. chondrophila* proteins, even if FtsQ and FtsL homologues were not annotated by alignment against the *E. coli* proteins (Tables 1 and 2) (Ouellette *et al.*, 2012; Jacquier *et al.*, 2014). The replacement of the homologue of tubulin FtsZ by MreB, an actin homologue that assembles into filaments that line the cytoplasmic membrane (Dominguez-Escobar *et al.*, 2011; Garner *et al.*, 2011; van Teeffelen *et al.*, 2011; Reimold *et al.*, 2013), was discussed by several groups (Gaballah *et al.*, 2011; Ouellette *et al.*, 2012; Jacquier *et al.*, 2014). An important role of MreB for chlamydial division could be shown, as MreB inhibition by its known inhibitors A22 and MP265 blocked chlamydial proliferation in *C. trachomatis* (Ouellette *et al.*, 2012), *C. pneumoniae* and *W. chondrophila* (Jacquier *et al.*, 2014). However, MreB localizes at the division septum only at a late stage of division in *W. chondrophila* (Jacquier *et al.*, 2014). This indicates that, even if MreB plays an important role in chlamydial division, it does not seem to be an early cell division protein such as FtsZ. Interestingly, the MreB-binding protein RodZ localizes to the septum earlier than MreB, but the MreB-inhibitor MP265 prevents the localization of RodZ to mid-cell (Jacquier *et al.*, 2014). An unknown organizer of chlamydial division (if not RodZ itself) seems thus to recruit RodZ to the division septum in an MreB-dependent manner. Given that RodZ and MreB interact and co-localize in *E. coli* and at the *C. crescentus* division septum (described in the section 'Box 3: MreB and RodZ'), septal RodZ might subsequently recruit MreB to the chlamydial division septum. This is consistent with the recent identification by two hybrid of RodZ as an MreB interactor in *C. trachomatis* (Ouellette *et al.*, 2014; Kemege *et al.*, 2014). However, it is unclear why MreB should only concentrate at the deeply constricted division furrow and not earlier. It is possible that the direct interaction between chlamydial MreB and RodZ is required for the positioning of RodZ at the future division site, for example by MreB-facilitated movement of the bitopic membrane protein RodZ within the cytoplasmic membrane. Alternatively, the dependence of RodZ positioning on MreB may be indirect, relying instead on the recruitment of MurG and/or MurF (both involved in lipid II synthesis, see below) to the division septum for the production of lipid II. In this model, inhibition of MreB via A22/MP265 would interfere with lipid II biosynthesis. Thus, MurG/F or other biosynthetic enzymes would remain in the cytoplasm and could no longer participate in lipid II biosynthesis at the membrane. A link has been proposed between MreB and PG biosynthesis enzymes through direct interactions between MreB and MurF, and possibly MurG (Gaballah *et al.*, 2011), suggesting that an intact MreB-based cytoskeleton is required for PG biosynthesis in chlamydial cells (see the section 'Box 3: MreB and RodZ').

SPATIAL CONTROL OF THE CHLAMYDIAL DIVISION SEPTUM BY LIPID II

The important role of lipid II in localization of RodZ to the chlamydial division septum was unearthed by recent experiments using the antibiotic phosphomycin (Frandi *et al.*, 2014; Jacquier *et al.*, 2014). This antibiotic inhibits MurA, the first enzyme in the lipid I and lipid II biosynthesis pathways, and thereby prevents chlamydial division, presumably because lipid II (and/or lipid I) is no longer available for septal PG synthesis. While RodZ is no longer septal in phosphomycin-treated *W. chondrophila* cells, in the presence of penicillin the accumulation of RodZ at the division septum is increased. This suggests that PG biosynthetic enzymes can also affect the dispersion of proteins from the division septum (Jacquier *et al.*, 2014).

Why lipid II (and/or lipid I) is required for septal localization of RodZ is unclear. However, labelling of chlamydial cell with fluorescent PG precursors provides evidence that lipid II (and/or lipid I) is concentrated at the septum. Thus, it is conceivable that lipid II (and/or lipid I) acts as a spatial cue by being itself confined to the division site. Alternatively, spatial control could be governed indirectly for example through stabilization of the septal PG biosynthetic apparatus even if PG is dispersed throughout the envelope. Perhaps, the PG biosynthetic machine along with RodZ disintegrates in the absence of the enzymatic

substrate. Such a reverse-instability effect was reported for FtsZ in *Caulobacter crescentus* where depletion of the later-acting division protein FtsK causes the collapse of the FtsZ-ring (Wang, West and Shapiro 2006). RodZ and MreB are known to localize to the division plane in *Caulobacter*, but in this case this localization is dependent on FtsZ (Alyahya *et al.*, 2009). It has been reported that certain epsilonproteobacteria encode an MreB homologue, but not RodZ (Alyahya *et al.*, 2009). This finding is consistent with the interpretation that RodZ and MreB do not always have to be functionally linked, and thus that RodZ or MreB may adopt functions that are independent of the other. Moreover, the chlamydial RodZ is truncated, lacking a C-terminal periplasmic domain and cannot complement an *E. coli rodZ* mutant (Ouellette *et al.*, 2014). It is therefore plausible that RodZ has acquired a new function in division control of *Chlamydiales* that is independent of MreB.

ORGANIZATION OF THE CHLAMYDIAL DIVISOME

Other potential components of the septum, such as FtsK, AmiA, FtsW, FtsQ and FtsL, are highly conserved in the *Chlamydiales* order (Tables 1 and 2). Immunolocalization experiments with antibodies to FtsK or AmiA did not provide evidence for overt and pervasive septal localization (Frandi *et al.*, 2014; Jacquier *et al.*, 2014). However, evidence indicates that some PG-biosynthetic and remodelling enzymes are located at the chlamydial division septum (Frandi *et al.*, 2014). FtsI (PBP3), a protein that is localized to the division septum in other bacteria where it promotes septal PG synthesis, is also encoded in *Chlamydiales* (Tables 3 and 4). Localization of FtsI (PBP3) in *C. trachomatis* was punctuate, with no more than one focus per cell (Ouellette *et al.*, 2012), a result consistent with a septal localization. With the recent identification of the LysM-domain (Buist *et al.*, 2008) protein NlpD as a 'late' septal protein, three proteins are now known to reside at the division septum: RodZ and NlpD are early and intermediate septal recruits, while MreB is a late recruit (Frandi *et al.*, 2014; Jacquier *et al.*, 2014). NlpD was indeed shown to localize at mid-cell primarily in constricted cells and this localization was inhibited by prior addition of penicillin or phosphomycin, suggesting that NlpD recognizes a septal PG-like polymer. In support of this idea, NlpD binds *E. coli* PG *in vitro* and in a manner that depends on an intact LysM-domain (Frandi *et al.*, 2014). Cell division can be inhibited in *Chlamydiales* exposed to beta-lactams (targeting PBPs) suggesting that septal PG synthesis drives chlamydial division but a classical PG was not detected in Chlamydiaceae (Fox *et al.*, 1990; Moulder 1993; Ghuysen and Goffin 1999). Nevertheless, a non-proteinaceous antigen, which might be part of a PG-like structure, was observed at the chlamydial division septum (Brown and Rockey 2000). In addition, *C. trachomatis* activates the Nod1 receptor, which recognizes PG fragments (muropeptides containing meso-diaminopimelate, see below) during cell infection (Welter-Stahl *et al.*, 2006). These results suggest that the Chlamydiaceae either assemble classical PG, but only in trace amounts, and/or that PG is of an atypical (modified) form that at least carries the muramyltripeptide that is recognized by Nod1. As intracellular bacteria and in strong contrast to free-living bacteria that rely on the PG-based cell wall for osmo- and chemical protection, there is less need for osmoprotection for *Chlamydiales* when intracellularly located. Furthermore, PG fragments have the capacity to alert the innate immune system of a bacterial infection. Thus, PG production could translate in a fitness cost to invading bacterial pathogens, a cost that is limited for *Chlamydiales* because of the limited production of a PG-like component. This might explain why *Chlamydiales* may remain nearly undetected (Welter-Stahl *et al.*, 2006). Potentially this pressure for reduced PG material is counterbalanced by the advantage of having PG synthesis at the division plane, which is thought to facilitate constriction of bacterial cells, a process that *Chlamydiales* should also rely on and that can be inhibited by penicillin. Indeed, it has been proposed that chlamydial shape and osmotic pressure resistance can be maintained, at least in EBs, by OM proteins highly cross-linked by disulphide bridges (Hatch 1996).

Recently, a modified form of PG was detected biochemically in the *Chlamydia*-related bacteria *Pr. amoebophila* by cell-wall extraction and HPLC/MS (Pilhofer *et al.*, 2013). Moreover, PG or lipid II was also indirectly detected by fluorescent labelling in a ring-like structure at the division septum in *C. trachomatis* (Liechti *et al.*, 2013). In this study, Liechti *et al.* (2013) developed a novel metabolic labelling technique using D-amino acids dipeptide probes and showed their integration in replicating *C. trachomatis*.

Table 3. Conservation and percentage of identity of chlamydial PG biosynthesis enzymes compared to *E. coli*.

	C. pneumoniae	*W. chondrophila*	Remarks
Alr	–	29.00	
Ddl	33.05	28.53	Fused to MurC in *C. pneumoniae*
MurI	–	28.57	
DacC	29.52	28.31	Homologue of PBP6 of *E. coli*
DapA	28.37	39.64	
DapB	28.10	37.85	
DapL	26.97	26.61	Homologous to *E. coli* aspartate aminotransferase
DapF	33.33	30.47	
MurA	34.63	35.63	Target of phosphomycin, Cpn MurA is resistant
MurB	27.22	30.79	
MurC	33.26	34.88	
MurD	31.24	33.26	
MurE	36.95	34.52	
MurF	27.46	30.36	Fused to Alr in *W. chondrophila*
MraY	38.26	43.08	
MurJ	24.56	29.03	
MurG	30.56	31.14	
Pbp1	–	–	
Pbp2	22.55	26.16	Target of penicillin and mecillinam
Pbp3/FtsI	27.72	29.42	Target of penicillin and piperacillin
Pbp4	–	–	
Pbp5	–	–	
Pbp7	–	–	
Pbp8	–	–	
AmiA	28.93	27.13	Target of penicillin
AmiB	18.54	22.05	Contains a LysM domain, which binds PG
AmiC	–	–	
NlpD	25.75	17.24	
EnvC	–	–	

Table 4. Percentage of identity and coverage (in brackets) of *W. chondrophila* PG biosynthesis enzymes compared to *C. pneumoniae* (Cpn), *C. trachomatis* (Ctr), *P. acanthamoebae* (Pac) and *S. negevensis* (Sne).

	Cpn	Ctr	Pac	Sne
Ddl	25.85 (98)	29.41 (87)	28.35 (98)	32.47 (98)
MurI	–	–	47.67 (93)	–
DacC	32.73 (88)	33.05 (82)	43.81 (97)	41.64 (88)
DapA	28.95 (90)	31.62 (86)	49.09 (93)	45.07 (97)
DapB	25.87 (77)	27.35 (93)	49.56 (97)	47.60 (96)
DapL	46.11 (96)	47.30 (96)	55.98 (97)	50.13 (97)
DapF	32.30 (86)	35.23 (95)	47.21 (93)	37.17 (94)
MurA	56.30 (93)	55.40 (92)	76.50 (96)	67.12 (93)
MurB	44.80 (94)	46.74 (97)	56.29 (96)	50.17 (97)
MurC	40.32 (99)	40.32 (99)	52.38 (98)	44.07 (100)
MurD	37.83 (97)	34.38 (98)	45.62 (99)	43.61 (99)
MurE	43.32 (100)	40.85 (99)	54.41 (99)	47.75 (99)
MurF-Alr	30.21 (43)	28.32 (40)	49.22 (99)	28.36 (39)
MraY	40.00 (92)	38.29 (90)	66.50 (100)	62.84 (100)
MurJ	32.78 (94)	34.26 (94)	50.46 (94)	42.76 (93)
MurG	32.66 (96)	30.84 (96)	39.50 (98)	43.90 (94)
Pbp2	39.11 (99)	37.41 (99)	49.78 (99)	40.66 (96)
Pbp3/FtsI	44.68 (99)	46.44 (99)	59.24 (99)	51.67 (99)
AmiA	40.84 (82)	43.09 (78)	48.48 (96)	42.47 (95)
AmiB	30.51 (83)	28.29 (94)	33.65 (98)	30.64 (83)
NlpD	35.00 (100)	37.21 (100)	40.32 (100)	38.17 (100)

These two key articles provided the first evidence for the presence of a PG-like polymer in *Chlamydiales*. This makes *Chlamydiales*, the first bacterial order known to use a functional PG pathway for division without a sequence homologue of FtsZ.

STRUCTURE OF THE CHLAMYDIAL PG

Following the recent advances in the detection of PG in *Chlamydiales* along with several clues from the predicted coding sequences in chlamydial genomes, we have learned several things about chlamydial PG. Gram-negative bacteria typically have unconventional amino acids, meso-diaminopimelate (m-DAP), D-Alanine (D-Ala) and D-Glutamate (D-Glu). These amino acids should be synthesized by *Chlamydiales* (which are Gram-negative bacteria), because there is no evidence of mDAP, D-Ala or D-Glu in mammalian cells. A specific aminotransferase pathway conserved in *Chlamydiales* and plants is required for m-DAP biosynthesis and Nod1 indeed detects the m-DAP-containing muramyltripeptide. In *Chlamydiales*, a specific conserved enzyme DapL, a L,L-diaminopimelate aminotransferase, bypasses the usual pathway present in other bacteria (McCoy *et al.*, 2006). D-Ala biosynthesis is usually performed by the alanine racemase (Alr). A predicted Alr coding sequence is present in the genomes of *P. acanthamoebae*, *Pr. amoebophila* and *W. chondrophila*, but not in Chlamydiaceae and in *S. negevensis* (Tables 3 and 4) Recent evidences involve the serine hydroxymethyltransferase GlyA as an alternative source of D-Ala in *C. pneumoniae*, as chlamydial GlyA can partially complement an *E. coli* racemase double mutant and has a weak racemase activity *in vitro* (De Benedetti *et al.*, 2014). D-Glu biosynthesis involves MurI, a glutamate racemase (Doublet *et al.*, 1993). A *murI*-like gene is indeed encoded in the genomes of *P. acanthamoebae*, *Pr. amoebophila* and *W. chondrophila*, but not in Chlamydiaceae and in *S. negevensis* (Tables 3 and 4). There is no clear evidence of presence of D-Glu in Chlamydiaceae and no D-Glu biosynthesis pathways encoded by their genomes, suggesting that there is no D-Glu in chlamydial PG. However, absence of D-Glu in chlamydial PG is highly improbable (see below), because PG biosynthesis enzymes are conserved in all *Chlamydiales* and have a normal *in vitro* activity on PG precursors containing D-Glu. Most importantly, D-Glu was recently identified as a constituent of PG-like building blocks (Pilhofer *et al.*, 2013).

m-DAP, D-Ala and D-Glu are used by enzymes involved in lipid II biosynthesis. Genes encoding MurA, MurB, MurC, MurD, MurE, MurF, MurG, MraY and Ddl orthologues are conserved among the *Chlamydiales* (Henrichfreise *et al.*, 2009) (Fig. 1 and Tables 3 and 4) and present in the genome of the *Chlamydia*-related bacterium *W. chondrophila* (Jacquier *et al.*, 2014). Requirement of MurA for growth of *W. chondrophila* and *Pr. amoebophila* was demonstrated by use of the MurA inhibitor phosphomycin (Pilhofer *et al.*, 2013; Jacquier *et al.*, 2014). Interestingly, Chlamydiaceae are naturally resistant to phosphomycin. This resistance is caused by an amino acid exchange in MurA (McCoy, Sandlin and Maurelli 2003). *In vitro* and *in vivo* activity of the Chlamydiaceae enzymes was demonstrated for MurA (McCoy, Sandlin and Maurelli 2003), MurC (Hesse *et al.*, 2003), MurE (Patin *et al.*, 2009), MurF (Patin *et al.*, 2012), MurG and MraY (Henrichfreise *et al.*, 2009). Ddl is active and part of a MurC-DdI fusion protein (McCoy and Maurelli 2005). Activity of MurB and MurD was not demonstrated yet, even if mRNA of MurB was detected in *C. trachomatis* (McCoy, Sandlin and Maurelli 2003). Taken together, these studies strongly indicate that *Chlamydiales* synthesize a classical lipid II (Henrichfreise *et al.*, 2009). *In vitro* studies based on substrate specificities of chlamydial MurC, MurE, MurF and Ddl enzymes indicate that the possible structure of the pentapeptide in *Chlamydiales* might be L-Ala/L-Ser/Gly-D-Glu-m-DAP-D-Ala-D-Ala (Fig. 1) (Patin *et al.*, 2012). Biochemical analysis by HPLC of sacculi extracted from *Pr. amoebophila*, a *Chlamydia*-related bacterium and digested with cellosyl, a glycan strand-cleaving PG muramidase, showed the presence of PG-like fragments, including a canonical disaccharide unit harbouring D-Glu (GlcNAc-MurNAc(r)-L-Ala- D-Glu) along with an undetermined 314 Da modification in the PG unit and fluorescent labelling provided evidence of the incorporation of D-Ala (Liechti *et al.*, 2013; Pilhofer *et al.*, 2013). Intriguingly, no PG-like material could be detected in *S. negevensis*, another *Chlamydia*-related bacterium. Moreover, *S. negevensis* is completely resistant to penicillin and phosphomycin (Pilhofer *et al.*, 2013). This seems to be specific to *S. negevensis* as other *Chlamydia*-related species are at least partially sensitive to penicillin, despite the presence of a beta-lactamase (Bertelli *et al.*, 2010; de Barsy, Bottinelli and Greub 2014; Jacquier *et al.*, 2014). The *Chlamydiales* family is highly diverse and divergent and we thus cannot exclude that PG presence and structure can be different between its members. Nevertheless, the relatively high conservation of the large majority of the PG biosynthesis enzymes indicates that PG biosynthesis plays an important role in the *Chlamydiales* for which genome sequences are available.

Chlamydiales possess only few PG cross-linking enzymes. No class A (bifunctional) PBPs are encoded by members of the *Chlamydiales* order. Only class B (monofunctional) PBPs are present, resembling PBP2 and PBP3 of *E. coli* (Ouellette *et al.*, 2012) (Tables 3 and 4). A low molecular weight PBP is also conserved among *Chlamydiales*, with homologies to PBP6 of *E. coli* (McCoy and Maurelli 2006). Nevertheless, these PBPs play an important role, as *Chlamydiales* are sensitive to penicillin, which induces the formation of aberrant bodies (Matsumoto and Manire 1970; Ouellette *et al.*, 2012). They also impede the dispersion of RodZ from the division septum (Jacquier *et al.*, 2014). Interestingly, PBP3/FtsI is involved in transpeptidation of the pentapeptide stem in lipid II at the *E. coli* division septum and is required

for proper assembly of the Z-ring (Pogliano *et al.*, 1997). In contrast, PBP2 is involved in lateral PG biosynthesis and thus required for rod-shape maintenance in *E. coli* (Bendezu and de Boer 2008). Moreover, PBP2 and PBP3 have distinct and non-redundant functions in *Chlamydiales*, because specific inhibition of PBP2 by mecillinam and of PBP3/FtsI by piperacillin caused different morphologies of aberrant bodies (Ouellette *et al.*, 2012; Jacquier *et al.*, 2014). PBP3 inhibition induced the accumulation of RodZ at the division septum and PBP2 inhibition abolished the septum formation and caused the formation of similar aberrant bodies as with MurA inhibition (Jacquier *et al.*, 2014). This might indicate that PBP2 is required for the PG biosynthesis in a process that affects MreB or depends on it and that PBP3 is also required at a later stage for PG modification during septum dispersion, while PBP2 acts very early in division. Alternatively, PBP2 and PBP3 might simply introduce different types of PG modifications.

In bacteria with PG, daughter cell separation involves PG remodelling by lytic transglycosylases, amidases (N-acetylmuramoyl-L-alanine hydrolases) and peptidases (LD-carboxypeptidases and DD-endopeptidases). *Chlamydiales* possess at least a functional amidase (AmiA) (Frandi *et al.*, 2014; Klockner *et al.*, 2014) and a PG peptidase (NlpD) (Frandi *et al.*, 2014), but no lytic transglycosylase homologues. When expressed in *E. coli*, AmiA of *W. chondrophila* causes an increase in cell lysis, apparently due to ectopic amidase activity (Frandi *et al.*, 2014). While *E. coli* amidases are by default inactive enzymes and need to be stimulated, it appears that chlamydial AmiAs are synthesized as active (lytic) variants that are not autoinhibited (Klockner *et al.*, 2014). Moreover, AmiA from *C. pneumoniae* possesses a carboxypeptidase activity, a function generally carried out by separate proteins in other organisms (Klockner *et al.*, 2014). AmiA regulation seems to be less important for *Chlamydiales* compared to other bacteria and might help to reduce the PG thickness to dampen innate immune detection, akin to the role of staphylococcal autolysins that prevent detection by the Drosophila innate immune system (Atilano *et al.*, 2014).

OM INVAGINATION

In *E. coli*, several PG-binding lipoproteins (Lpp, OmpA, Pal) that are localized to the inner leaflet of the OM are used to coordinate PG remodelling with OM invagination. Of these, a Pal-like protein seems to be encoded in the chlamydial genomes. Pal interacts with the *cis*-encoded TolABQR proteins, which together with Pal assembled into the Tol–Pal transenvelope complex at the *E. coli* division plane. Pal, TolQ, TolR, TolA and TolB (Tables 5 and 6) are conserved among *Chlamydiales* suggesting that the Tol–Pal complex is functional in *Chlamydiales*. By analogy to *E. coli*, chlamydial Pal might interact with chlamydial PG to draw in the OM during constriction. This model has yet to be investigated and might help to illuminate how chlamydial divisome extends into the OM.

Table 5. Conservation and percentage of identity of chlamydial proteins possibly involved in OM septation compared to *E. coli*.

	C. pneumoniae	*W. chondrophila*	Comments
Pal	32.80	48.72	PG associated lipoprotein
TolQ	24.88	28.25	
TolR	25.89	24.09	
TolA	17.94	20.18	
TolB	26.76	23.87	
LpoA	–	–	
LpoB	–	–	
Lpp	–	–	

Table 6. Conservation and percentage of identity of chlamydial proteins possibly involved in mid-cell determination compared to *E. coli*.

	C. pneumoniae	*W. chondrophila*	Comments
MinC	–	–	
MinD/ParA	27.10	26.37	Similar homology to MinD and ParA
MinE	–	–	
Noc/ParB	31.73	35.26	Similar homology to Noc and ParB
SlmA	–	–	

DISCUSSION AND PERSPECTIVES

The recent discoveries regarding chlamydial PG synthesis and divisome composition help us to refine the model of chlamydial division. The first detection of PG-related material indicates that, even in absence of an FtsZ homologue, division might occur in a similar way as in other bacteria. *Chlamydiales* thus need to control coordinated invagination of (i) the OM, (ii) the PG layer, which might be present as a sacculus or only at the division septum and (iii) the IM (Fig. 2A). Rod-shape determining proteins MreB and RodZ seem to play an important role in this process. As they are able to control PG biosynthesis in other species, they might act similarly in *Chlamydiales*, by bringing together the PG biosynthesis and remodelling enzymes to the divisome (Fig. 2B).

The structure of chlamydial PG has still to be determined to better understand its exact role in these bacteria. Moreover, a structural difference between the PG of Chlamydiaceae and *Chlamydia*-related species cannot be excluded. Chlamydia-related bacteria apparently possess a larger amount of PG, which is able to form a sacculus. In contrast, labelling of PG in Chlamydiaceae is only observed at the division septum, indicating that new synthesis of PG occurs only at this localization. It has still to be confirmed (i) if PG is really present only at the division septum and (ii) if the observed septal localization of PG is not due to restrained diffusion of the new synthesized PG in Chlamydiaceae, even though at least one member of the *Chlamydia*-related bacteria is clearly able to synthesize PG throughout the cell.

Division is tightly regulated in *Chlamydiales*, as only RBs are able to divide. This implies a differentiation program, which allows expression of division genes. Comparison of transcriptional pattern in EBs and RBs was performed in *C. trachomatis* (Albrecht *et al.*, 2010) and *C. pneumoniae* (Albrecht *et al.*, 2011). Expression of several division genes is induced in RBs compared to EBs, including also the PG biosynthesis genes. It thus seems that expression of components of the division machinery is inhibited in EBs and induced during differentiation of EBs into RBs. DnaA is conserved among *Chlamydiales* and was shown to be expressed in *C. pneumoniae* (Byrne *et al.*, 2001). It is thus likely that it has the same function in the regulation of replication initiation as in other bacteria (Ozaki and Katayama 2009). The MinC/D(E/J) system is an important division regulator in many bacteria (see above). A homologue of MinD is conserved in *Chlamydiales*, but it might also be a homologue of ParA, a protein involved in chromosome partitioning (Tables 7 and Box1). Virtually nothing is known about the presence or not of nucleoid occlusion mechanisms in *Chlamydiales*. A potential homologue of the *B. subtilis*

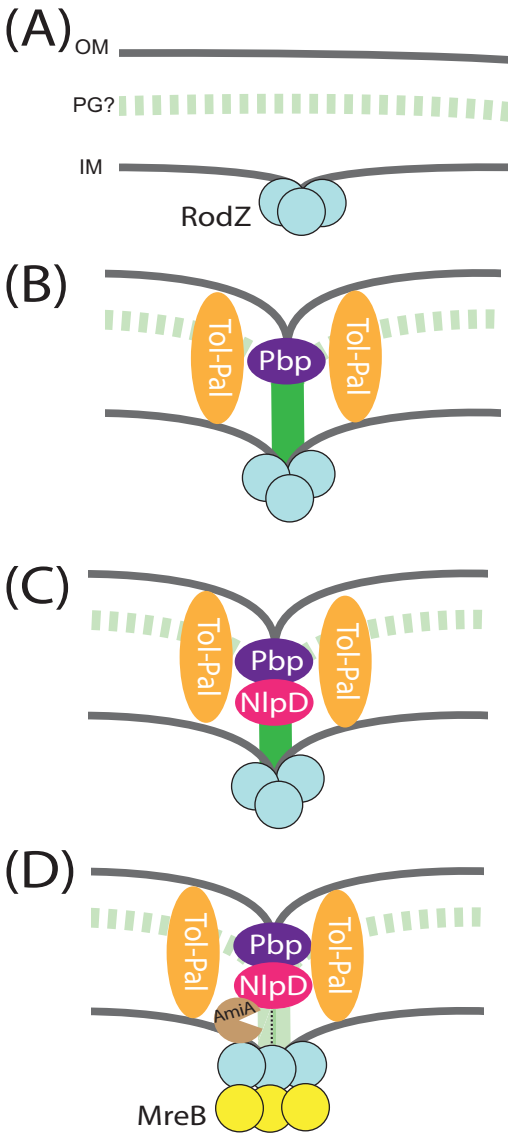

Figure 2. Models of chlamydial division. (**A**) RodZ accumulates at the division septum (OM: outer membrane, PG: putative peptidoglycan layer, IM: inner membrane). (**B**) PBPs synthesize new PG at the division site and Tol–Pal complex maintains the cohesion between the OM and IM. (**C**) NlpD is recruited at the septum where it modifies the PG (and/or lipidII). (**D**) AmiA degrades the PG (and/or lipidII) and MreB is recruited and induces the cytokinesis.

Table 7. Percentage of identity and coverage (in brackets) of *W. chondrophila* proteins possibly involved in OM septation and mid-cell determination compared to *C. pneumoniae* (Cpn), *C. trachomatis* (Ctr), *P. acanthamoebae* (Pac) and *S. negevensis* (Sne).

	Cpn	Ctr	Pac	Sne
Pal	47.46 (47)	51.38 (43)	54.51 (97)	39.92 (99)
TolQ	33.61 (95)	29.11 (95)	46.77 (99)	45.53 (93)
TolR	37.59 (94)	35.71 (89)	49.25 (95)	53.66 (87)
TolA	21.80 (35)	23.24 (83)	25.85 (97)	26.54 (68)
TolB	40.37 (96)	39.38 (94)	46.21 (96)	41.80 (86)
MinD/ParA	67.48 (95)	68.70 (95)	78.95 (96)	66.80 (95)
Noc/ParB	43.40 (96)	47.64 (93)	54.83 (97)	22.22 (66)

Noc is conserved among the *Chlamydiales* but is annotated as ParB (Tables 7 and Box 1). Further studies have to be performed to study the division regulation of *Chlamydiales*.

Recent advances in the field of chlamydial transformation might give us in a near future new tools to study the chlamydial division (Binet and Maurelli 2009; Wang *et al.*, 2011; Mishra *et al.*, 2012). For this purpose, we need to genetically modify *Chlamydiales* to place the genes of interest under conditional promoters, allowing their repression. This would help to understand the role of the gene products in the division mechanism. This needs further development of the transformation techniques in *Chlamydiales* to allow insertion of DNA in the chlamydial genome. Another option is the transformation of *Chlamydiales* with antisense DNA, which can modulate the gene expression or to exploit the CRISPR/Cas9 technology for targeted genetic manipulation (Mishra *et al.*, 2012).

In conclusion, recent advances in the field of chlamydial division allow a better understanding of this process, but many questions remain open on the exact composition of the divisome and on the chronology and the regulation of its assembly and disassembly. *Chlamydiales* might have evolved novel mechanisms of division to overcome the absence of an FtsZ homologue. This possibly involves additional unknown proteins, which might interact with components of the divisome as RodZ or PG biosynthetic enzymes. Describing in details these processes is of course of general interest to better understand the particular evolution of these obligate intracellular bacteria. Moreover, this might lead to the discovery of specific antibiotics targeting the chlamydial division, allowing the specific destruction of *Chlamydiales* without side effects on the genital flora, for example and without inducing antibiotic resistance in other bacterial orders. Finally, a better understanding of the persistence mechanisms, which are tightly related to division mechanisms (see the section 'Box 2: aberrant bodies and persistence'), would improve the strategies to treat chronic chlamydial diseases.

BOX1: PHYLOGENY, ECOLOGY AND BIOLOGY OF CHLAMYDIALES

The *Chlamydiales* order is composed of the Chlamydiaceae and of the *Chlamydia*-related bacteria. Whole genome sequencing was performed for several species of Chlamydiaceae: *C. trachomatis* (Stephens *et al.*, 1998), *C. pneumoniae* (Kalman *et al.*, 1999), *C. muridarum* (Read *et al.*, 2000) *C. caviae* (Read *et al.*, 2003), *C. abortus* (Thomson *et al.*, 2005), *C. felis* (Azuma *et al.*, 2006), *C. pecorum* (Mojica *et al.*, 2011), *C. psittaci* (Voigt *et al.*, 2011), *C. avium*, *C. gallinacea* (Sachse *et al.*, 2014) and *C. suis* (Donati *et al.*, 2014) (Fig. Box1). Their genome size is strongly reduced (between 1.04 and 1.23 Mb, Table Box1). *Chlamydia*-related bacteria were discovered more recently and less genomes are available: *Pr. amoebophila* (Horn *et al.*, 2004), *W. chondrophila* (Bertelli *et al.*, 2010), *P. acanthamoebae* (Greub *et al.*, 2009; Collingro *et al.*, 2011), *S. negevensis* (Collingro *et al.*, 2011) and *Neochlamydia* sp. (Ishida *et al.*, 2014). Genomes of *Chlamydia*-related bacteria are bigger than Chlamydiaceae genomes (between 2.12 and 3.19 Mb, Table Box 1). Divergence between these two groups is estimated to have occurred 1 billion years ago (Fig. Box1) (Greub and Raoult 2003). Nevertheless, genome comparisons indicate that major virulence mechanisms are conserved between these two groups (for review, see Nunes and Gomes 2014). 700 genes have homologues in both groups, but synteny is poorly conserved (Horn *et al.*, 2004). Chlamydiaceae genome reduction seems to be linked to its

Table Box1. Genome size, pathogenicity, hosts and references of chlamydial species for which genomes are available (n.d. not determined, * provisional designations).

Species	Genome size (Mb)	Pathogenicity	Host	Reference
C. trachomatis	1.04	Trachoma, genital infections	Human	Stephens et al. (1998)
C. pneumoniae	1.23	Pneumonia	Human, horse, marsupials, frogs	Kalman *et al.* (1999)
C. muridarum	1.07	Pneumonitis in mouse	Mouse	Read *et al.* (2000)
C. caviae	1.17	Conjuctivitis and genital tract infections in guinea pig	Guinea pig	Read *et al.* (2003)
C. abortus	1.14	Zoonotic infections, miscarriage	Ruminants	Thomson *et al.* (2005)
C. felis	1.17	Conjuctivitis and respiratory disease in cat	Cat	Azuma *et al.* (2006)
C. pecorum	1.11	Asymptomatic chronic infections, metritis, arthritis, conjunctivitis and mastitis in animals	Ruminants, swine, birds, koala	Mojica *et al.* (2011)
C. psittaci	1.17	Zoonotic infections, pneumonia	Human, birds	Voigt *et al.* (2011)
*C. avium**	1.04	n.d.	Pigeon	Sachse *et al.* (2014)
*C. gallinacea**	1.05	n.d.	Poultry	Sachse *et al.* (2014)
C. suis	1.08	Conjuctivitis, pneumonia, enteritis, reproductive disorders in swine	Swine	Donati *et al.* (2014)
W. chondrophila	2.12	Miscarriage	Human, ruminants	Bertelli *et al.* (2010)
S. negevensis	2.50	Respiratory diseases	Human, possibly amoebae	Collingro *et al.* (2011)
P. acanthamoebae	3.07	Pneumonia	Human, amoebae	Greub *et al.* (2009), Collingro *et al.* (2011)
Pr. amoebophila	2.41	n.d.	Amoebae	Horn *et al.* (2004)
Neochlamydia sp.	3.19	n.d.	Amoebae	Ishida *et al.* (2014)

evolution within animal hosts. In comparison, *Chlamydia*-related bacteria can infect protists, which may serve as a melting pot for genes exchanges (Greub 2009a; Moliner, Fournier and Raoult 2010) and several of them are agents of human and/or animal diseases (Table Box1). Chlamydiaceae lost several amino acid biosynthesis enzymes, consequence of their parasitic life style. *Chlamydia*-related bacteria lost less enzymes, perhaps because their environment contains a lower concentration of nutrients and they thus need to synthesize more molecules. For example, *W. chondrophila* possesses the complete pathways to synthesize 11 essential amino acids, whereas *C. trachomatis* only 3 (Bertelli *et al.*, 2010). *Chlamydia*-related bacteria are thus able to develop in different niches (from protists to human cells). In contrast, Chlamydiaceae are more specialized and can infect only limited species. Thus, *C. trachomatis* can infect only human (Table Box1).

BOX2: ABERRANT BODIES AND PERSISTENCE

When the proliferation cycle of *Chlamydiales* is blocked, enlarged bacteria called aberrant bodies accumulate (Wyrick 2010). Aberrant bodies can be induced by diverse stimuli including the addition of penicillin (Matsumoto and Manire 1970; Lambden, Pickett and Clarke 2006), activation of interferon-gamma (Shemer and Sarov 1985; Pantoja *et al.*, 2001), starvation of iron or nutrient (Coles *et al.*, 1993) as well as coinfection of the host with herpes or other viruses (Deka *et al.*, 2006; Borel *et al.*, 2010) in a process that is not mediated by any known persistence inducer (Vanover *et al.*, 2008). Aberrant bodies apparently replicate continuously their DNA in absence of division. This is indicated by the expression of DNA replication genes, but not cytokinesis genes during persistence in *C. pneumoniae* (Byrne *et al.*, 2001; Abdelrahman, Rose and Belland 2011). This is consistent with the accumulation of a minimum of 16 copies of the genome in the aberrant bodies in *C. trachomatis* treated with penicillin (Lambden, Pickett and Clarke 2006). Aberrant bodies are considered a persistent stage since they de-differentiate in RBs and subsequently into infectious EBs when the stress is relieved (Matsumoto and Manire 1970). This persistence mechanism allows *Chlamydiales* to survive in presence of penicillin and is believed to account for the observed recurrence of infection when the beta-lactam treatment is stopped. Persistent aberrant bodies formation is a conserved feature among *Chlamydiales* as they are also observed in *Chlamydia*-related *W. chondrophila* (Fig. Box2) (Kebbi-Beghdadi, Cisse and Greub 2011). Persistent aberrant bodies were also observed *in vivo* (Borel *et al.*, 2008; Phillips Campbell *et al.*, 2012) and presence of chlamydial DNA and RNA in patients with chronic chlamydial infections was a strong indication for persistence of viable but not proliferating *Chlamydiae* (Gerard *et al.*, 1998). Aberrant bodies are thus believed to play an important role in the onset of chronic infections by *Chlamydiales* (Mpiga and Ravaoarinoro 2006). Nevertheless, a direct role of aberrant bodies in the onset of chronic infections has still to be demonstrated. It is thus important to better understand the mechanisms that cause the formation of aberrant bodies to improve the treatment of chronic chlamydial infections.

BOX3: MreB AND RodZ

MreB is an actin homologue, which, already in the eighties, was demonstrated to be required for the rod-shape maintenance in *E. coli* and which is involved in mecillinam resistance

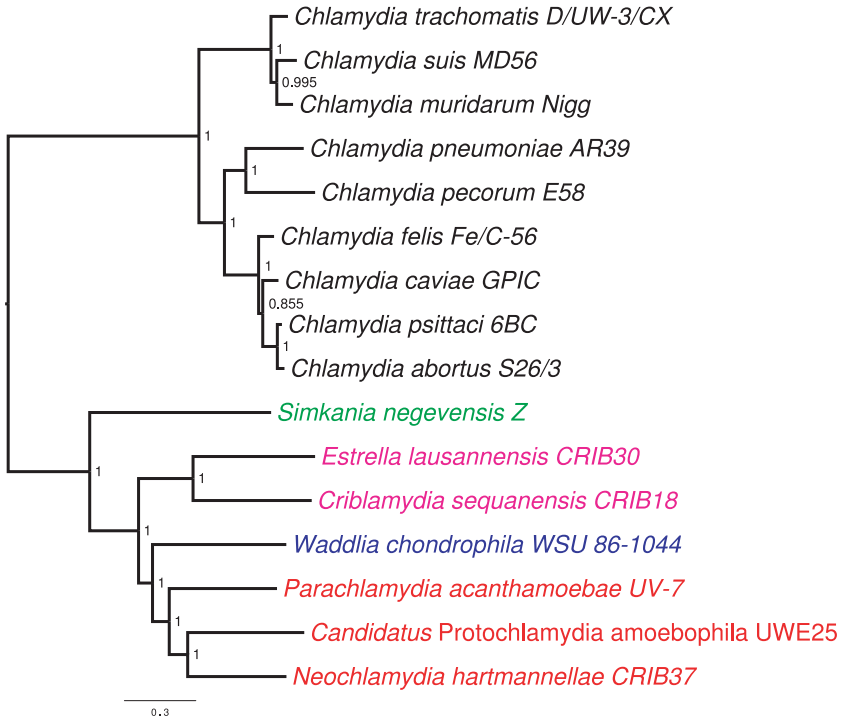

Figure Box 1. Phylogenic tree of *Chlamydiales* with available genomes. Mid-point-rooted phylogenetic tree of the *Chlamydiales* order based on concatenated alignments of 470 core proteins was reconstructed using PhyML with LG + Γ+I model of substitution. It includes representatives of five main clades: the *Chlamydiaceae* family (black), the *Simkaniaceae* family (green), the *Criblamydiaceae* family (pink), the *Waddliaceae* family (blue) and the *Parachlamydiaceae* family (red).

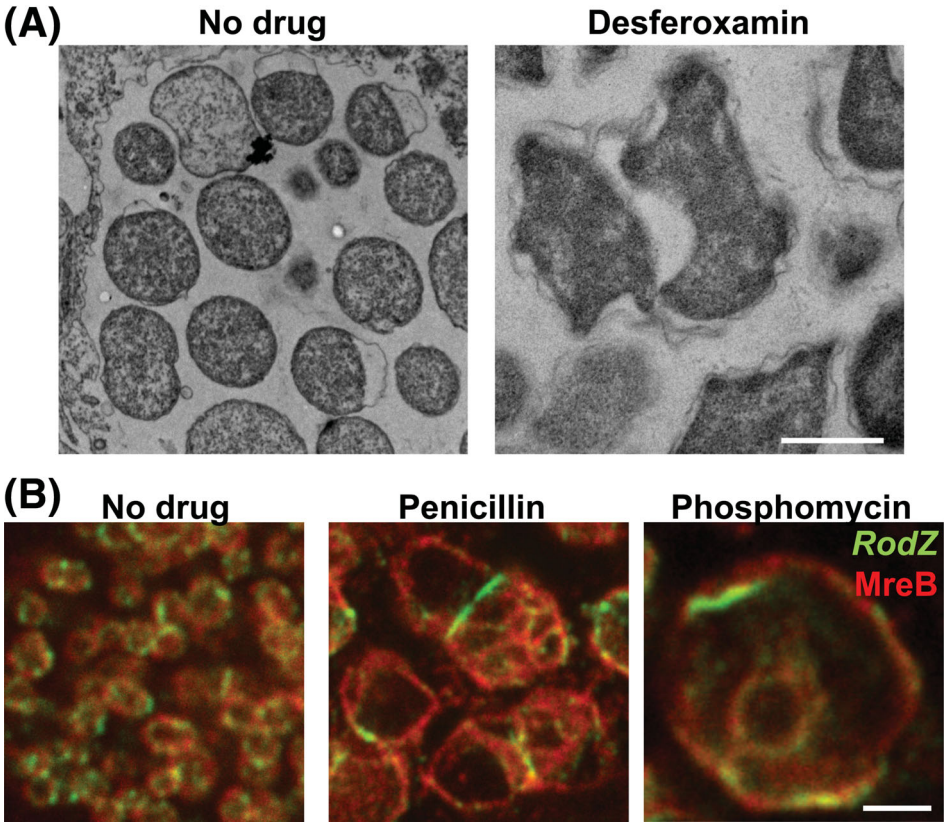

Figure Box 2. Aberrant bodies caused by different stimuli. (**A**) Electron microscopy pictures of *W. chondrophila* infecting Vero cells in absence or in presence of 400 μM of the iron chelator deferoxamine (scale bar 2 μm). (**B**) Immunofluorescence micrographs of Vero cells infected by *W. chondrophila* in presence or in absence of 500 μg ml^{-1} penicillin or phosphomycin (scale bar 2 μm).

(Doi *et al.*, 1988). Only later, in 2001, MreB was shown to polymerize in a filamentous and helical actin-like structure (Jones, Carballido-Lopez and Errington 2001). MreB possess a similar tertiary structure as actin, when compared by crystallography even if the primary structures are divergent (van den Ent, Amos and Lowe 2001). MreB is conserved among most of rod-shape bacteria. Some bacteria, including *B. subtilis*, possess several homologues of MreB producing distinct kinds of filaments (Jones, Carballido-Lopez and Errington 2001). Localization studies of MreB by immunofluorescence or by fluorescent proteins fusion first indicated a helicoidal localization around the cell (Jones, Carballido-Lopez and Errington 2001; Figge, Divakaruni and Gober 2004; Gitai *et al.*, 2005; reviewed in Carballido-Lopez 2006). Recently, use of total internal reflection fluorescence microscopy could determine that MreB forms discontinuous patches (Dominguez-Escobar *et al.*, 2011; Garner *et al.*, 2011; reviewed in White and Gober 2012). These patches are believed to be sites of PG biosynthesis, as MreB interacts with several PG biosynthesis enzymes (MurB, MurC, MurE, MurF and MurG) (Divakaruni *et al.*, 2007; Mohammadi *et al.*, 2007; Varma and Young 2009; White, Kitich and Gober 2010; Gaballah *et al.*, 2011). Moreover, MreB requires the membrane proteins MreC and MreD for the organization of the PG biosynthesis and modification (White, Kitich and Gober 2010). MreB filaments are anchored to the plasma membrane by several interactions: direct hydrophobic interactions with the plasma membrane through an N-terminal amphipathic helix and a membrane insertion loop (Salje *et al.*, 2011), and interactions with integral membrane proteins like RodZ and FtsK (Alyahya *et al.*, 2009; van den Ent *et al.*, 2010; Ouellette *et al.*, 2012). RodZ is required to get a normal assembly of the MreB cytoskeleton (Bendezu *et al.*, 2009). It is hypothesized that RodZ might be the link between the cytoplasmic MreB and periplasmic PG modifying enzymes PBPs. This is consistent with the fact that RodZ interacts with RodA and MreD in a two hybrid assay in *C. crescentus* (White, Kitich and Gober 2010). Moreover, RodZ and MreB localize at the division septum in *C. crescentus* and *W. chondrophila* (Alyahya *et al.*, 2009; Jacquier *et al.*, 2014). Thus, MreB and RodZ likely localize to the places where active PG biosynthesis occurs (reviewed in White and Gober 2012).

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Conflict of Interest Statement. None declared.

REFERENCES

Aaron M, Charbon G, Lam H, *et al.* The tubulin homologue FtsZ contributes to cell elongation by guiding cell wall precursor synthesis in Caulobacter crescentus. *Mol Microbiol* 2007;**64**:938–52.

Aarsman ME, Piette A, Fraipont C, *et al.* Maturation of the *Escherichia coli* divisome occurs in two steps. *Mol Microbiol* 2005;**55**:1631–45.

Abdelrahman YM, Belland RJ. The chlamydial developmental cycle. *FEMS Microbiol Rev* 2005;**29**:949–59.

Abdelrahman YM, Rose LA, Belland RJ. Developmental expression of non-coding RNAs in *Chlamydia trachomatis* during normal and persistent growth. *Nucleic Acids Res* 2011;**39**:1843–54.

Adams DW, Errington J. Bacterial cell division: assembly, maintenance and disassembly of the Z ring. *Nat Rev Microbiol* 2009;**7**:642–53.

Albrecht M, Sharma CM, Dittrich MT, *et al.* The transcriptional landscape of *Chlamydia pneumoniae*. *Genome Biol* 2011;**12**:R98.

Albrecht M, Sharma CM, Reinhardt R, *et al.* Deep sequencing-based discovery of the *Chlamydia trachomatis* transcriptome. *Nucleic Acids Res* 2010;**38**:868–77.

Alyahya SA, Alexander R, Costa T, *et al.* RodZ, a component of the bacterial core morphogenic apparatus. *P Natl Acad Sci USA* 2009;**106**:1239–44.

Atilano ML, Pereira PM, Vaz F, *et al.* Bacterial autolysins trim cell surface peptidoglycan to prevent detection by the Drosophila innate immune system. *eLife* 2014;**3**:e02277.

Azuma Y, Hirakawa H, Yamashita A, *et al.* Genome sequence of the cat pathogen, *Chlamydophila felis*. *DNA Res* 2006;**13**:15–23.

Barreteau H, Kovac A, Boniface A, *et al.* Cytoplasmic steps of peptidoglycan biosynthesis. *FEMS Microbiol Rev* 2008;**32**:168–207.

Baud D, Goy G, Osterheld MC, *et al.* *Waddlia chondrophila*: from bovine abortion to human miscarriage. *Clin Infect Dis* 2011;**52**:1469–71.

Baud D, Regan L, Greub G. Emerging role of Chlamydia and Chlamydia-like organisms in adverse pregnancy outcomes. *Curr Opin Infect Dis* 2008;**21**:70–6.

Baud D, Thomas V, Arafa A, *et al.* *Waddlia chondrophila*, a potential agent of human fetal death. *Emerg Infect Dis* 2007;**13**:1239–43.

Begg KJ, Takasuga A, Edwards DH, *et al.* The balance between different peptidoglycan precursors determines whether *Escherichia coli* cells will elongate or divide. *J Bacteriol* 1990;**172**:6697–703.

Bendezu FO, de Boer PA. Conditional lethality, division defects, membrane involution, and endocytosis in mre and mrd shape mutants of *Escherichia coli*. *J Bacteriol* 2008;**190**:1792–811.

Bendezu FO, Hale CA, Bernhardt TG, *et al.* RodZ (YfgA) is required for proper assembly of the MreB actin cytoskeleton and cell shape in *E. coli*. *EMBO J* 2009;**28**:193–204.

Bernhardt TG, de Boer PA. SlmA, a nucleoid-associated, FtsZ binding protein required for blocking septal ring assembly over Chromosomes in *E. coli*. *Mol Cell* 2005;**18**:555–64.

Bertelli C, Collyn F, Croxatto A, *et al.* The Waddlia genome: a window into chlamydial biology. *PloS One* 2010;**5**:e10890.

Binet R, Maurelli AT. Transformation and isolation of allelic exchange mutants of *Chlamydia psittaci* using recombinant DNA introduced by electroporation. *P Natl Acad Sci USA* 2009;**106**:292–7.

Borel N, Dumrese C, Ziegler U, *et al.* Mixed infections with Chlamydia and porcine epidemic diarrhea virus—a new in vitro model of chlamydial persistence. *BMC Microbiol* 2010;**10**:201.

Borel N, Summersgill JT, Mukhopadhyay S, *et al.* Evidence for persistent *Chlamydia pneumoniae* infection of human coronary atheromas. *Atherosclerosis* 2008;**199**:154–61.

Bouhss A, Trunkfield AE, Bugg TD, *et al.* The biosynthesis of peptidoglycan lipid-linked intermediates. *FEMS Microbiol Rev* 2008;**32**:208–33.

Brown WJ, Rockey DD. Identification of an antigen localized to an apparent septum within dividing chlamydiae. *Infect Immun* 2000;**68**:708–15.

Buist G, Steen A, Kok J, *et al.* LysM, a widely distributed protein motif for binding to (peptido)glycans. *Mol Microbiol* 2008;**68**:838–47.

Byrne GI, Ouellette SP, Wang Z, *et al*. *Chlamydia pneumoniae* expresses genes required for DNA replication but not cytokinesis during persistent infection of HEp-2 cells. *Infect Immun* 2001;**69**:5423–9.

Carballido-Lopez R. The bacterial actin-like cytoskeleton. *Microbiol Mol Biol R* 2006;**70**:888–909.

Casson N, Michel R, Muller KD, *et al*. *Protochlamydia naegleriophila* as etiologic agent of pneumonia. *Emerg Infect Dis* 2008;**14**:168–72.

Chen JC, Beckwith J. FtsQ, FtsL and FtsI require FtsK, but not FtsN, for co-localization with FtsZ during *Escherichia coli* cell division. *Mol Microbiol* 2001;**42**:395–413.

Coles AM, Reynolds DJ, Harper A, *et al*. Low-nutrient induction of abnormal chlamydial development: a novel component of chlamydial pathogenesis? *FEMS Microbiol Lett* 1993;**106**:193–200.

Collingro A, Tischler P, Weinmaier T, *et al*. Unity in variety–the pan-genome of the Chlamydiae. *Mol Biol Evol* 2011;**28**:3253–70.

Corsaro D, Greub G. Pathogenic potential of novel Chlamydiae and diagnostic approaches to infections due to these obligate intracellular bacteria. *Clin Microbiol Rev* 2006;**19**:283–97.

de Barsy M, Bottinelli L, Greub G. Antibiotic susceptibility of Estrella lausannensis, a potential emerging pathogen. *Microb Infect/ Institut Pasteur* 2014;**16**:746–54.

de Barsy M, Greub G. *Waddlia chondrophila*: from biology to pathogenicity. *Microbes Infect/ Institut Pasteur* 2013;**15**:1033–41.

De Benedetti S, Buhl H, Gaballah A, *et al*. Characterization of serine hydroxymethyltransferase GlyA as a potential source of D-alanine in *Chlamydia pneumoniae*. *Front Cell Infect Microbiol* 2014;**4**:19.

Deka S, Vanover J, Dessus-Babus S, *et al*. Chlamydia trachomatis enters a viable but non-cultivable (persistent) state within herpes simplex virus type 2 (HSV-2) co-infected host cells. *Cell Microbiol* 2006;**8**:149–62.

Divakaruni AV, Baida C, White CL, *et al*. The cell shape proteins MreB and MreC control cell morphogenesis by positioning cell wall synthetic complexes. *Mol Microbiol* 2007;**66**:174–88.

Doi M, Wachi M, Ishino F, *et al*. Determinations of the DNA sequence of the mreB gene and of the gene products of the mre region that function in formation of the rod shape of *Escherichia coli* cells. *J Bacteriol* 1988;**170**:4619–24.

Dominguez-Escobar J, Chastanet A, Crevenna AH, *et al*. Processive movement of MreB-associated cell wall biosynthetic complexes in bacteria. *Science* 2011;**333**:225–8.

Donati M, Huot-Creasy H, Humphrys M, *et al*. Genome sequence of Chlamydia suis MD56, isolated from the conjunctiva of a weaned piglet. *Genome Announc* 2014;**2**:e00425-14.

Doublet P, van Heijenoort J, Bohin JP, *et al*. The murI gene of *Escherichia coli* is an essential gene that encodes a glutamate racemase activity. *J Bacteriol* 1993;**175**:2970–9.

Egan AJ, Vollmer W. The physiology of bacterial cell division. *Ann NY Acad Sci* 2013;**1277**:8–28.

Figge RM, Divakaruni AV, Gober JW. MreB, the cell shape-determining bacterial actin homologue, co-ordinates cell wall morphogenesis in Caulobacter crescentus. *Mol Microbiol* 2004;**51**:1321–32.

Fox A, Rogers JC, Gilbart J, *et al*. Muramic acid is not detectable in *Chlamydia psittaci* or *Chlamydia trachomatis* by gas chromatography-mass spectrometry. *Infect Immun* 1990;**58**:835–7.

Frandi A, Jacquier N, Theraulaz L, *et al*. FtsZ-independent septal recruitment and function of cell wall remodelling enzymes in chlamydial pathogens. *Nat Commun* 2014;**5**:4200.

Friis RR. Interaction of L cells and *Chlamydia psittaci*: entry of the parasite and host responses to its development. *J Bacteriol* 1972;**110**:706–21.

Gaballah A, Kloeckner A, Otten C, *et al*. Functional analysis of the cytoskeleton protein MreB from *Chlamydophila pneumoniae*. *PloS One* 2011;**6**:e25129.

Garner EC, Bernard R, Wang W, *et al*. Coupled, circumferential motions of the cell wall synthesis machinery and MreB filaments in *B. subtilis*. *Science* 2011;**333**:222–5.

Gerard HC, Kohler L, Branigan PJ, *et al*. Viability and gene expression in *Chlamydia trachomatis* during persistent infection of cultured human monocytes. *Med Microbiol Immun* 1998;**187**:115–20.

Gerding MA, Ogata Y, Pecora ND, *et al*. The trans-envelope Tol-Pal complex is part of the cell division machinery and required for proper outer-membrane invagination during cell constriction in *E. coli*. *Mol Microbiol* 2007;**63**:1008–25.

Ghuysen JM, Goffin C. Lack of cell wall peptidoglycan versus penicillin sensitivity: new insights into the chlamydial anomaly. *Antimicrob Agents Ch* 1999;**43**:2339–44.

Gitai Z, Dye NA, Reisenauer A, *et al*. MreB actin-mediated segregation of a specific region of a bacterial chromosome. *Cell* 2005;**120**:329–41.

Godlewska R, Wisniewska K, Pietras Z, *et al*. Peptidoglycan-associated lipoprotein (Pal) of Gram-negative bacteria: function, structure, role in pathogenesis and potential application in immunoprophylaxis. *FEMS Microbiol Lett* 2009;**298**:1–11.

Grenga L, Luzi G, Paolozzi L, *et al*. The *Escherichia coli* FtsK functional domains involved in its interaction with its divisome protein partners. *FEMS Microbiol Lett* 2008;**287**:163–7.

Greub G. *Parachlamydia acanthamoebae*, an emerging agent of pneumonia. *Clin Microbiol Infec* 2009a;**15**:18–28.

Greub G. The medical importance of Chlamydiae. *Clin Microbiol Infec* 2009b;**15**:2–3.

Greub G, Kebbi-Beghdadi C, Bertelli C, *et al*. High throughput sequencing and proteomics to identify immunogenic proteins of a new pathogen: the dirty genome approach. *PloS One* 2009;**4**:e8423.

Greub G, Raoult D. Crescent bodies of *Parachlamydia acanthamoeba* and its life cycle within Acanthamoeba polyphaga: an electron micrograph study. *Appl Environ Microb* 2002;**68**:3076–84.

Greub G, Raoult D. History of the ADP/ATP-translocase-encoding gene, a parasitism gene transferred from a Chlamydiales ancestor to plants 1 billion years ago. *Appl Environ Microb* 2003;**69**:5530–5.

Haney SA, Glasfeld E, Hale C, *et al*. Genetic analysis of the Escherichia coli FtsZ.ZipA interaction in the yeast two-hybrid system. Characterization of FtsZ residues essential for the interactions with ZipA and with FtsA. *J Biol Chem* 2001;**276**:11980–7.

Hatch TP. Disulfide cross-linked envelope proteins: the functional equivalent of peptidoglycan in chlamydiae? *J Bacteriol* 1996;**178**:1–5.

Henrichfreise B, Schiefer A, Schneider T, *et al*. Functional conservation of the lipid II biosynthesis pathway in the cell wall-less bacteria Chlamydia and Wolbachia: why is lipid II needed? *Mol Microbiol* 2009;**73**:913–23.

Hesse L, Bostock J, Dementin S, *et al*. Functional and biochemical analysis of *Chlamydia trachomatis* MurC, an enzyme displaying UDP-N-acetylmuramate: amino acid ligase activity. *J Bacteriol* 2003;**185**:6507–12.

Horn M, Collingro A, Schmitz-Esser S, *et al*. Illuminating the evolutionary history of chlamydiae. *Science* 2004;**304**:728–30.

Hybiske K, Stephens RS. Mechanisms of host cell exit by the intracellular bacterium Chlamydia. *P Natl Acad Sci USA* 2007;**104**:11430–5.

Ishida K, Sekizuka T, Hayashida K, *et al*. Amoebal endosymbiont Neochlamydia genome sequence illuminates the bacterial role in the defense of the host amoebae against *Legionella pneumophila*. *PloS One* 2014;**9**:e95166.

Jacquier N, Frandi A, Pillonel T, *et al*. Cell wall precursors are required to organize the chlamydial division septum. *Nat Commun* 2014;**5**:3578.

Jones LJ, Carballido-Lopez R, Errington J. Control of cell shape in bacteria: helical, actin-like filaments in *Bacillus subtilis*. *Cell* 2001;**104**:913–22.

Kalman S, Mitchell W, Marathe R, *et al*. Comparative genomes of *Chlamydia pneumoniae* and *C. trachomatis*. *Nat Genet* 1999;**21**:385–9.

Kawai F, Shoda M, Harashima R, *et al*. Cardiolipin domains in *Bacillus subtilis* marburg membranes. *J Bacteriol* 2004;**186**:1475–83.

Kebbi-Beghdadi C, Cisse O, Greub G. Permissivity of Vero cells, human pneumocytes and human endometrial cells to *Waddlia chondrophila*. *Microbes Infect/ Institut Pasteur* 2011;**13**:566–74.

Kemege KE, Hickey JM, Barta ML, *et al*. *Chlamydia trachomatis* protein CT009 is a structural and functional homolog to the key morphogenesis component RodZ and interacts with division septal plane localized MreB. *Mol Microbiol* 2014, DOI: 10.1111 /mmi.12855.

Kirkpatrick CL, Viollier PH. New(s) to the (Z-)ring. *Curr Opin Microbiol* 2011;**14**:691–7.

Klockner A, Otten C, Derouaux A, *et al*. AmiA is a penicillin target enzyme with dual activity in the intracellular pathogen *Chlamydia pneumoniae*. *Nat Commun* 2014;**5**:4201.

Lambden PR, Pickett MA, Clarke IN. The effect of penicillin on *Chlamydia trachomatis* DNA replication. *Microbiology* 2006;**152**:2573–8.

Lamoth F, Greub G. Fastidious intracellular bacteria as causal agents of community-acquired pneumonia. *Expert Rev Anti-Infe* 2010;**8**:775–90.

Leonard AC, Grimwade JE. Regulation of DnaA assembly and activity: taking directions from the genome. *Ann Rev Microbiol* 2011;**65**:19–35.

Liechti GW, Kuru E, Hall E, *et al*. A new metabolic cell-wall labelling method reveals peptidoglycan in *Chlamydia trachomatis*. *Nature* 2013;**506**:507–10.

Lutkenhaus J. Assembly dynamics of the bacterial MinCDE system and spatial regulation of the Z ring. *Ann Rev Biochem* 2007;**76**:539–62.

McCoy AJ, Adams NE, Hudson AO, *et al*. L,L-diaminopimelate aminotransferase, a trans-kingdom enzyme shared by Chlamydia and plants for synthesis of diaminopimelate/lysine. *P Natl Acad Sci USA* 2006;**103**:17909–14.

McCoy AJ, Maurelli AT. Characterization of Chlamydia MurC-Ddl, a fusion protein exhibiting D-alanyl-D-alanine ligase activity involved in peptidoglycan synthesis and D-cycloserine sensitivity. *Mol Microbiol* 2005;**57**:41–52.

McCoy AJ, Maurelli AT. Building the invisible wall: updating the chlamydial peptidoglycan anomaly. *Trends Microbiol* 2006;**14**:70–7.

McCoy AJ, Sandlin RC, Maurelli AT. In vitro and in vivo functional activity of Chlamydia MurA, a UDP-N-acetylglucosamine enolpyruvyl transferase involved in peptidoglycan synthesis and fosfomycin resistance. *J Bacteriol* 2003;**185**:1218–28.

Margolin W. FtsZ and the division of prokaryotic cells and organelles. *Nat Rev Mol Cell Bio* 2005;**6**:862–71.

Matsumoto A, Manire GP. Electron microscopic observations on the effects of penicillin on the morphology of *Chlamydia psittaci*. *J Bacteriol* 1970;**101**:278–85.

Mileykovskaya E, Dowhan W. Role of membrane lipids in bacterial division-site selection. *Curr Opin Microbiol* 2005;**8**:135–42.

Mishra MK, Gerard HC, Whittum-Hudson JA, *et al*. Dendrimer-enabled modulation of gene expression in *Chlamydia trachomatis*. *Mol Pharm* 2012;**9**:413–21.

Mizuno T. A novel peptidoglycan-associated lipoprotein (PAL) found in the outer membrane of Proteus mirabilis and other Gram-negative bacteria. *J Biochem* 1981;**89**:1039–49.

Mohammadi T, Karczmarek A, Crouvoisier M, *et al*. The essential peptidoglycan glycosyltransferase MurG forms a complex with proteins involved in lateral envelope growth as well as with proteins involved in cell division in *Escherichia coli*. *Mol Microbiol* 2007;**65**:1106–21.

Mohammadi T, van Dam V, Sijbrandi R, *et al*. Identification of FtsW as a transporter of lipid-linked cell wall precursors across the membrane. *EMBO J* 2011;**30**:1425–32.

Mojica S, Huot Creasy H, Daugherty S, *et al*. Genome sequence of the obligate intracellular animal pathogen Chlamydia pecorum E58. *J Bacteriol* 2011;**193**:3690.

Moliner C, Fournier PE, Raoult D. Genome analysis of microorganisms living in amoebae reveals a melting pot of evolution. *FEMS Microbiol Rev* 2010;**34**:281–94.

Moulder JW. Why is Chlamydia sensitive to penicillin in the absence of peptidoglycan? *Infect Agent Dis* 1993;**2**:87–99.

Mpiga P, Ravaoarinoro M. *Chlamydia trachomatis* persistence: an update. *Microbiol Res* 2006;**161**:9–19.

Natale P, Pazos M, Vicente M. The *Escherichia coli* divisome: born to divide. *Environ Microbiol* 2013;**15**:3169–82.

Nunes A, Gomes JP. Evolution, phylogeny, and molecular epidemiology of Chlamydia. *Infect Genet Evol* 2014;**23**: 49–64.

Ouellette SP, Karimova G, Subtil A, *et al*. Chlamydia co-opts the rod shape-determining proteins MreB and Pbp2 for cell division. *Mol Microbiol* 2012;**85**:164–78.

Ouellette SP, Rueden KJ, Gauliard E, *et al*. Analysis of MreB interactors in Chlamydia reveals a RodZ homolog but fails to detect an interaction with MraY. *Front Microbiol* 2014;**5**:279.

Ozaki S, Katayama T. DnaA structure, function, and dynamics in the initiation at the chromosomal origin. *Plasmid* 2009;**62**:71–82.

Pantoja LG, Miller RD, Ramirez JA, *et al*. Characterization of Chlamydia pneumoniae persistence in HEp-2 cells treated with gamma interferon. *Infect Immun* 2001;**69**:7927–32.

Patin D, Bostock J, Blanot D, *et al*. Functional and biochemical analysis of the *Chlamydia trachomatis* ligase MurE. *J Bacteriol* 2009;**191**:7430–5.

Patin D, Bostock J, Chopra I, *et al*. Biochemical characterisation of the chlamydial MurF ligase, and possible sequence of the chlamydial peptidoglycan pentapeptide stem. *Arch Microbiol* 2012;**194**:505–12.

Phillips-Campbell R, Kintner J, Whittimore J, *et al*. Chlamydia muridarium enters a viable but non-infectious state in amoxicillin-treated BALB/c mice. *Microbes Infect* 2012;**14**:1177–85

Pichoff S, Lutkenhaus J. Tethering the Z ring to the membrane through a conserved membrane targeting sequence in FtsA. *Mol Microbiol* 2005;**55**:1722–34.

Pilhofer M, Aistleitner K, Biboy J, *et al*. Discovery of chlamydial peptidoglycan reveals bacteria with murein sacculi but without FtsZ. *Nat Commun* 2013;**4**:2856.

Pogliano J, Pogliano K, Weiss DS, *et al*. Inactivation of FtsI inhibits constriction of the FtsZ cytokinetic ring and delays the assembly of FtsZ rings at potential division sites. *P Natl Acad Sci USA* 1997;**94**:559–64.

Read TD, Brunham RC, Shen C, *et al*. Genome sequences of *Chlamydia trachomatis* MoPn and *Chlamydia pneumoniae* AR39. *Nucleic Acids Res* 2000;**28**:1397–406.

Read TD, Myers GS, Brunham RC, *et al*. Genome sequence of Chlamydophila caviae (Chlamydia psittaci GPIC): examining the role of niche-specific genes in the evolution of the Chlamydiaceae. *Nucleic Acids Res* 2003;**31**:2134–47.

Reimold C, Defeu Soufo HJ, Dempwolff F, *et al*. Motion of variable-length MreB filaments at the bacterial cell membrane influences cell morphology. *Mol Biol Cell* 2013;**24**:2340–9.

Rothfield L, Taghbalout A, Shih YL. Spatial control of bacterial division-site placement. *Nat Rev Microbiol* 2005;**3**: 959–68.

Ruiz N. Bioinformatics identification of MurJ (MviN) as the peptidoglycan lipid II flippase in *Escherichia coli*. *P Natl Acad Sci USA* 2008;**105**:15553–7.

Rurangirwa FR, Dilbeck PM, Crawford TB, *et al*. Analysis of the 16S rRNA gene of micro-organism WSU 86–1044 from an aborted bovine foetus reveals that it is a member of the order Chlamydiales: proposal of Waddliaceae fam. nov., Waddlia chondrophila gen. nov., sp. nov. *Int J Syst Bacteriol* 1999;**49**(Pt 2):577–81.

Sachse K, Laroucau K, Riege K, *et al*. Evidence for the existence of two new members of the family Chlamydiaceae and proposal of Chlamydia avium sp. nov. and Chlamydia gallinacea sp. nov. *Syst Appl Microbiol* 2014;**37**:79–88.

Salje J, van den Ent F, de Boer P, *et al*. Direct membrane binding by bacterial actin MreB. *Mol Cell* 2011;**43**:478–87.

Sauvage E, Kerff F, Terrak M, *et al*. The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. *FEMS Microbiol Rev* 2008;**32**:234–58.

Sham LT, Butler EK, Lebar MD, *et al*. Bacterial cell wall. MurJ is the flippase of lipid-linked precursors for peptidoglycan biogenesis. *Science* 2014;**345**:220–2.

Shemer Y, Sarov I. Inhibition of growth of *Chlamydia trachomatis* by human gamma interferon. *Infect Immun* 1985;**48**:592–6.

Sixt BS, Siegl A, Muller C, *et al*. Metabolic features of *Protochlamydia amoebophila* elementary bodies—a link between activity and infectivity in Chlamydiae. *PLoS Pathog* 2013;**9**:e1003553.

Skilton RJ, Cutcliffen LT, Barlow D, *et al*. Penicillin induced persistence in *Chlamydia trachomatis*: high quality time lapse video analysis of the developmental cycle. *PloS One* 2009;**4**:e7723.

Stephens RS, Kalman S, Lammel C, *et al*. Genome sequence of an obligate intracellular pathogen of humans: *Chlamydia trachomatis*. *Science* 1998;**282**:754–9.

Thomson NR, Yeats C, Bell K, *et al*. The Chlamydophila abortus genome sequence reveals an array of variable proteins that contribute to interspecies variation. *Genome Res* 2005;**15**: 629–40.

Todd WJ, Caldwell HD. The interaction of *Chlamydia trachomatis* with host cells: ultrastructural studies of the mechanism of release of a biovar II strain from HeLa 229 cells. *J Infect Dis* 1985;**151**:1037–44.

Typas A, Banzhaf M, van den Berg van Saparoea B, *et al*. Regulation of peptidoglycan synthesis by outer-membrane proteins. *Cell* 2010;**143**:1097–109.

Uehara T, Parzych KR, Dinh T, *et al*. Daughter cell separation is controlled by cytokinetic ring-activated cell wall hydrolysis. *EMBO J* 2010;**29**:1412–22.

van den Ent F, Amos LA, Lowe J. Prokaryotic origin of the actin cytoskeleton. *Nature* 2001;**413**:39–44.

van den Ent F, Johnson CM, Persons L, *et al*. Bacterial actin MreB assembles in complex with cell shape protein RodZ. *EMBO J* 2010;**29**:1081–90.

van Teeffelen S, Wang S, Furchtgott L, *et al*. The bacterial actin MreB rotates, and rotation depends on cell-wall assembly. *P Natl Acad Sci USA* 2011;**108**:15822–7.

Vanover J, Sun J, Deka S, *et al*. Herpes simplex virus co-infection-induced *Chlamydia trachomatis* persistence is not mediated by any known persistence inducer or anti-chlamydial pathway. *Microbiology* 2008;**154**:971–8.

Varma A, Young KD. In *Escherichia coli*, MreB and FtsZ direct the synthesis of lateral cell wall via independent pathways that require PBP 2. *J Bacteriol* 2009;**191**:3526–33.

Voigt A, Schofl G, Heidrich A, *et al*. Full-length de novo sequence of the *Chlamydophila psittaci* type strain, 6BC. *J Bacteriol* 2011;**193**:2662–3.

Vretou E, Psarrou E, Kaisar M, *et al*. Identification of protective epitopes by sequencing of the major outer membrane protein gene of a variant strain of *Chlamydia psittaci* serotype 1 (*Chlamydophila abortus*). *Infect Immun* 2001;**69**:607–12.

Wang JD, Sanders GM, Grossman AD. Nutritional control of elongation of DNA replication by (p)ppGpp. *Cell* 2007;**128**:865–75.

Wang SC, West L, Shapiro L. The bifunctional FtsK protein mediates chromosome partitioning and cell division in Caulobacter. *J Bacteriol* 2006;**188**:1497–508.

Wang Y, Kahane S, Cutcliffe LT, *et al*. Development of a transformation system for *Chlamydia trachomatis*: restoration of glycogen biosynthesis by acquisition of a plasmid shuttle vector. *PLoS Pathog* 2011;**7**:e1002258.

Welter-Stahl L, Ojcius DM, Viala J, *et al*. Stimulation of the cytosolic receptor for peptidoglycan, Nod1, by infection with *Chlamydia trachomatis* or *Chlamydia muridarum*. *Cell Microbiol* 2006;**8**:1047–57.

White CL, Gober JW. MreB: pilot or passenger of cell wall synthesis? *Trends Microbiol* 2012;**20**:74–9.

White CL, Kitich A, Gober JW. Positioning cell wall synthetic complexes by the bacterial morphogenetic proteins MreB and MreD. *Mol Microbiol* 2010;**76**:616–33.

Wu LJ, Errington J. Coordination of cell division and chromosome segregation by a nucleoid occlusion protein in *Bacillus subtilis*. *Cell* 2004;**117**:915–25.

Wyrick PB. Chlamydia trachomatis persistence in vitro: an overview. *J Infect Dis* 2010;**201** (Suppl 2):S88–95.

Yang DC, Peters NT, Parzych KR, *et al*. An ATP-binding cassette transporter-like complex governs cell-wall hydrolysis at the bacterial cytokinetic ring. *P Natl Acad Sci USA* 2011;**108**:E1052–60.