# Lack of Cell Wall Peptidoglycan versus Penicillin Sensitivity: New Insights into the Chlamydial Anomaly

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Intracellular bacterial pathogens enter their hosts surrounded by a membrane-bound vacuole and use a panel of tricks to exploit or evade eukaryotic cell functions (9, 12). *Chlamydia* inhabits vesicles that do not fuse with lysosomes and remains within these parasitophorous vacuoles (termed inclusions) for the duration of its replication cycle. Although the biogenesis of these vacuoles is still poorly understood, it is becoming clear that the parasites which multiply within vacuoles modify those vesicles that arrest their maturation at discrete stages of the endocytic pathway, indicating more of a continuum along the endocyclic and lysosomal pathway than has been suspected in the past (18, 21, 30).

*Chlamydia pneumoniae* is the causative agent of about 10% of pneumonia cases in children. *Chlamydia trachomatis* is still a major cause of blindness in developing countries and is one of the most commonly encountered pathogens in sexually transmitted diseases. *Chlamydia* is a gram-negative bacterium and occurs in two forms. Well-shaped elementary bodies (EBs) are adapted to extracellular survival. The entry of EBs into host cells launches metabolic activity, i.e., the transformation of EBs into pleomorphic reticulate bodies (RBs), RB division by binary fission, back-differentiation of RBs to EBs, and EB release through the lysis of the host cells or by a process in which the inclusion membrane fuses with the plasma membrane. On infected monolayers, the cycle takes about 40 h. Electron micrographs highlight the multilayer structure of the cell envelope of EBs (44) and RBs (18, 36).

The bacterial cell wall peptidoglycan is a covalently closed, net-like polymer in which glycan strands made of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid residues are cross-linked by peptides. In contrast to the vast majority of eubacteria, *Chlamydia* lacks detectable amounts of this essential polymer (20, 31). Yet, *Chlamydia* is susceptible to D-cy-closerine, bacitracin, and penicillin, which are wall peptidoglycan inhibitors, and it produces three penicillin-binding proteins (PBPs) which are the molecular targets of penicillin action (4). This paradox is known as the chlamydial anomaly. In light of the results of the *C. trachomatis* genome sequencing project (40), Chopra et al. (8) have proposed that *C. trachomatis* has the information for the entire pathway of peptidoglycan synthesis. At variance with this view, we propose that in *Chlamydia*, a glycanless wall polymer whose synthesis is penicillin sensitive might substitute for a wall peptidoglycan.

## PREDICTIVE STUDIES

This proposal relies, at least in part, on predictive studies involving 12 bacterial species whose genomes have also been sequenced. *Escherichia coli, Haemophilus influenzae, Bacillus subtilis, Rickettsia prowazekii, Treponema pallidum, Mycobacterium tuberculosis, Helicobacter pylori, Borrelia burgdorferi, Aquifex aeolicus,* and *Synechocystis* PCC6803 each possess a wall peptidoglycan. In contrast, *Mycoplasma genitalium and Mycoplasma pneumoniae* are wallless and peptidoglycanless bacteria. A search of similarity in amino acid sequence was carried out with the National Center for Biotechnology Information's new gapped BLAST algorithm with the tblastn program (2). This program compares a protein query sequence against a nucleotide sequence database dynamically translated in all six reading frames (both strands). The probability that structural relatedness occurs by chance is expressed by an index *P* value. Values equal to or smaller than $10^{-3}$ are indicative of a statistically significant similarity.

## LIPID II IN *E. COLI*

Lipid II (Fig. 1) is the immediate precursor of the wall peptidoglycan. Its synthesis involves an interchange of carriers that are compatible with the environments of the cell. In *E. coli* (42), UDP-*N*-acetylglucosamine is converted into UDP-*N*-acetylglucosamine-enolpyruvate by MurA and from this into UDP-*N*-acetylmuramic acid by MurB. The Ddl (ATP:ADP + $P_i$) ligase catalyzes the formation of a D-alanyl-D-alanine dipeptide, and the MurC, MurD, MurE, and MurF (ATP: ADP + $P_i$) ligases catalyze the formation of the UDP-*N*-acetylmuramoyl

pentapeptide by sequential additions to UDP-*N*-acetylmuramic acid of L-alanine, D-glutamic acid, mesodiaminopimelic acid, and the preformed D-alanyl-D-alanine dipeptide. Then, MraY transfers the phospho-N-acetylmuramoyl pentapeptide from its uridylic carrier to the phosphate group of a membrane-anchored $C_{55}$-isoprenoid alcohol phosphate, and in turn, MurG transfers the N-acetylglucosamine from its uridylic carrier to the lipid-linked N-acetylmuramic acid residue. Somehow, lipid II flips over the membrane bilayer, and the disaccharide pentapeptide moiety is exposed on the outer face of the membrane. In *E. coli, ddl, murC, murD, murE, murF, murG, and mraY* reside in a cluster (*dcw*) at the 2-min region of the chromosome.

## LIPID II IN C. TRACHOMATIS

*C. trachomatis possesses genes* that are homologous *to the E. coli ddl, murA, murB, murC, murD, murE, murF, and mraY* genes. In particular, Table 1 gives the extent of homology between the MurA enolpyruvate transferases and MurB reductases which catalyze the first committed steps of lipid II synthesis and between the MraY transferases and MurG transglycosylases which catalyze the terminal steps of the pathway.

The *C. trachomatis ddl, murC, murD, murF, murG, and mraY* genes cluster in a particular region of the genome (open reading frames [ORFs] D756 to D762), but *murE*, which encodes the *meso*-diaminopimelic-acid-adding enzyme, is outside the cluster (ORF D269). *C. trachomatis* has no L-Ala→D-Ala racemase-encoding gene, but it possesses *dag*A homologues for D-alanine-glycine permeases, suggesting that the synthesis of lipid II depends on an exogenous source of D-alanine (8). Peptidyl D-amino acids are present in rat liver tissues (32), and D-amino acids, including D-alanine, occur in tissues and body fluids of humans and other vertebrates (3, 22). Consistently, *C. trachomatis* is not susceptible to alaphosphine which is directed against the L-Ala→D-Ala racemase, and it is susceptible to D-cycloserine, which inhibits the Ddl ligase.

**FIG. 1.** *Lipid II as the immediate biosynthetic precursor of wall peptidoglycan in E. coli and putative glycanless wall polypeptide in C. trachomatis. G, N-acetylglucosamine; M, N-acetylmuramic acid; Dpm, diaminopimelic acid; thick bar, transmembrane $C_{55}$ lipid. Reaction 1, transglycosylase-catalyzed synthesis of a glycosidic bond. Reaction 2, N-acetylmuramoyl-L-alanine-catalyzed hydrolysis of a D-lactoyl-L-alanine bond. Reactions 3 and 4, acylserine transferase-catalyzed transpeptidations at the expense of the D-alanyl-D-alanine bond of pentapeptide units. The amino acceptors of the transfer reactions are the amino group at the D center of meso-diaminopimelic acid in reaction 3 and the amidase-released L-alanine amino group in reaction 4. The product of reactions 1 and 3 is the wall peptidoglycan. The product of reactions 2, 3, and 4 is the putative wall polypeptide.*
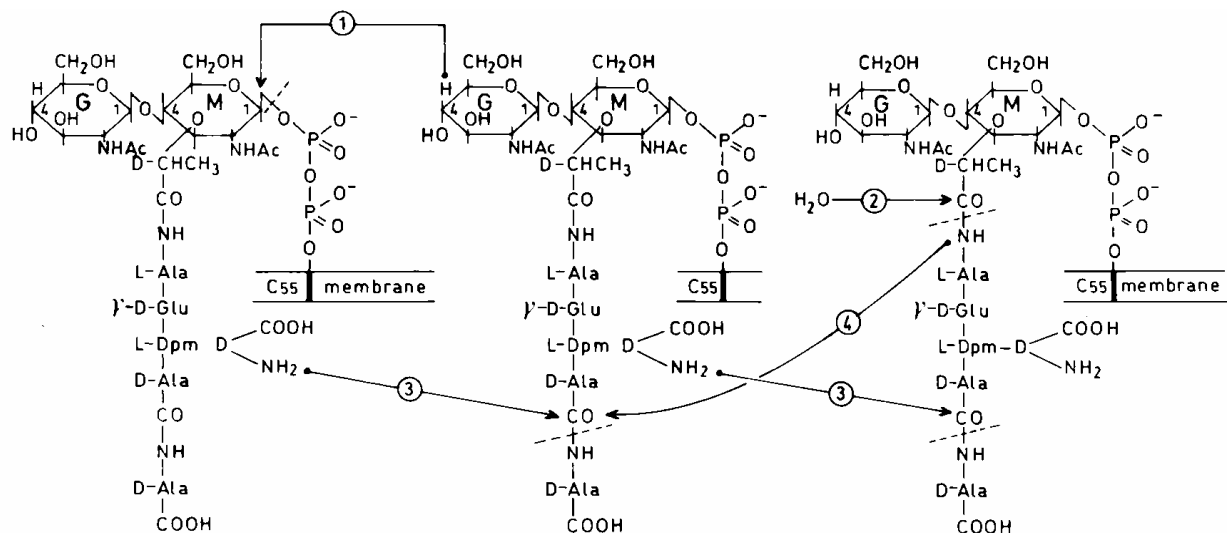


## PEPTIDOGLYCAN ASSEMBLY IN E. COLI

The assembly of the lipid 11-transported disaccharide pentapeptide units into peptidoglycan and the remodelling of the polymer throughout the bacterial cell cycle are carried out by specialized transferases (14, 17). Glycosyltransferases catalyze glycan chain elongation (transglycosylation) by displacing the pyrophosphate

linked to C-1 of *N*-acetylmuramic acid of a disaccharide unit by the 4-hydroxyl group of *N*-acetylglucosamine of another disaccharide unit (Fig. 1, reaction 1). Acylserine transferases working as transpeptidases catalyze peptide cross-linking between glycan strands (Fig. 1, reaction 3). The rupture of the D-alanyl-D-alanine bond at the carboxy end of a pentapeptide unit and the attack of the penultimate D-alanyl by the amino group at the D center of a meso-diaminopimelic acid of another peptide proceeds via the formation of a peptidyl enzyme in which the D-alanyl moiety is linked as an ester to a serine residue at the enzyme's active site. Other acylserine transferases catalyze the hydrolytic breakdown of serine ester-linked peptidyl enzymes. The hydrolysis of the carboxy-terminal D-alanyl-D-alanine bonds by DD-carboxypeptidases limits the number of pentapeptides available for transpeptidation, and the hydrolysis of the interpeptide D-ala-nyl-(D)-meso-diaminopimelic bonds by DD-endopeptidases allows the wall peptidoglycan to undergo remodelling.

Penicillin is a mechanism-based inactivator of the DD(trans-, carboxy-, endo-) peptidases. The interaction produces stable serine ester-linked penicilloyl enzymes, and the inactivated enzymes can be detected as PBPs. The low-$M_r$ PBPs are monofunctional DD-(carboxy-, endo-)peptidases. They do not seem to be essential. The high-$M_r$ PBPs are, globally, the primary targets of penicillin action.

**TABLE 1.** *Homologous E. coli and C. trachomatis enzymes of the lipid II synthesis pathway*

| Enzyme | Size (amino acids) of: | | | $P^a$ |
|---|---|---|---|---|
| | *E. coli enzyme* | *C. trachomatis* enzyme | Homologous segment | |
| MurA | 419 | 444 | 400 | $10^{-62}$ |
| MurB | 342 | 290 | 200 | $10^{-6}$ |
| MraY | 360 | 335 | 230 | $10^{-60}$ |
| MurG | 355 | 352 | 320 | $10^{-32}$ |

[a] *P* values smaller than $10^{-3}$ are indicative of significant amino acid sequence similarities.

## HIGH-$M_r$ PBPs

The high-$M_r$ PBPs are multimodular (17). A transmembrane spanner is linked to the amino end of a non-penicillin-binding (n-PB) module which is linked to the amino end of an acylserine transferase PB module (Fig. 2). A conserved junction site links the n-PB and PB modules. The PB modules carry the three active-site-defining motifs SerXXLys (where Ser is the active serine residue and X is a variable amino acid residue), SerXAsn or an analogue, and LysThrGly or an analogue, which are characteristic of the penicilloyl serine transferases superfamily. Occasionally, adducts occur at various places along the polypeptide chains.

In spite of these common structural features, the high-$M_r$ PBPs fall into two classes, A and B, which are recognizable by the distinctive motifs borne by the n-PB modules of class A versus class B (17). The n-PB modules of the class A PBPs have an extended signature in the form of five motifs (Fig. 2). *E. coli* PBP 1a and PBP 1b of class A have been identified biochemically as transglycosylase (n-PB module)-transpeptidase (PB module) enzymes. They catalyze the conversion of lipid 11 into peptidoglycan in in vitro assays, and the conserved dicarboxylic amino acid residues Glu and Asp of motif 1 and Glu of motif 3 are important components of the transglycosylase catalytic center of the n-PB module (40a).

The n-PB modules of the class B PBPs have a less extended signature in the form of three motifs (Fig. 2). Motifs 1 to 3 of the class B PBP 2x of *Streptococcus pneumoniae*, whose structure has been determined (35), occupy positions that are likely to be sites of interaction between the n-PB and PB modules (17). Consistently, motifs 1 and 3 are important elements of the amino acid sequence folding information of the class B PBP 3 of *E. coli* (16). PBP 2x and PBP 3 each catalyze peptide bond formation (PB module) on properly structured thiolesters (1). However, PBP 3 does not perform transglycosylation on lipid II in in vitro assays. Consistently, the inactivation of the PBP 3-encoding *ftsI* (*E. coli ftsI* 63 mutant) does not induce a significant change in glycan chain lengths in the peptidoglycan of *E. coli* (23), indicating that the n-PB module of the class B PBPs fulfills functions other than glycan chain elongation in peptidoglycan synthesis. It has been proposed (17) that the class B PBPs perform peptide cross-linking (PB module) and that this activity is regulated by the associated n-PB module itself in interaction with components of morphogenetic networks involved in cell shape maintenance and cell septation.

**FIG. 2.** *Modular design of the multimodular PBPs of classes A and B. Indicated are the positions of the five motifs of the transglycosylase n-PB module of class A PBPs (· ) and the three motifs of the cell cycle n-PB module of class B PBPs (E). The intermodule junction sites (, ) are common to the PBPs of classes A and B.*
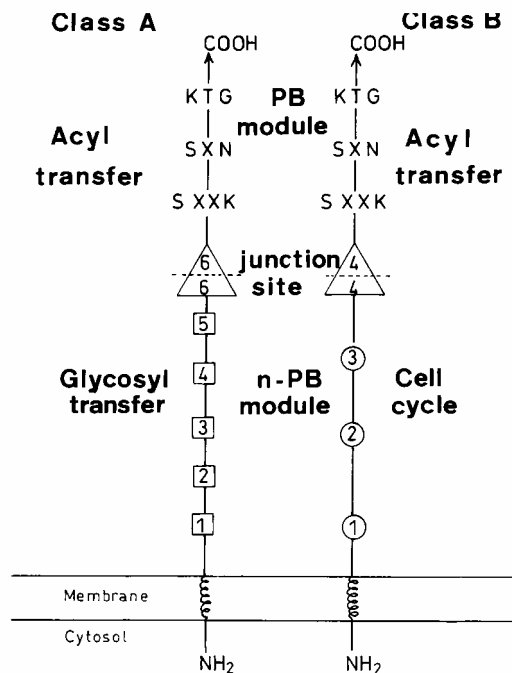


## PBPs IN C. TRACHOMATIS

*C. trachomatis* produces three PBPs of varying molecular masses (4). ORF D551 codes for a low-$M_r$ PBP that is 343 amino acid residues long. ORFs D682 and D270 code for two high-$M_r$ PBPs that are 1,080 and 647 amino acid residues long, respectively. Based on a long-lasting belief first formulated by Ishino et al. in the early 1980's that the high-$M_r$ PBPs are bifunctional transglycosylase-transpeptidase enzymes (24, 25), Chopra et al. (8) concluded that *C. trachomatis* has the required PBPs to manufacture a typical peptidoglycan from lipid II. The assignment of distinct functions to the class A and class B PBPs leads to a different conclusion. The two *C. trachomatis* high-$M_r$ PBPs are both of class B, and the *C. trachomatis* genome has no ORFs that would code for proteins having the characteristic amino acid sequence signature of the transglycosylase (n-PB) module of the class A PBPs or the monofunctional transglycosylases known to be present in several bacterial species (39). Therefore, *C. trachomatis* does not synthesize a wall peptidoglycan because it lacks the required glycosyltransferases for glycan chain elongation from lipid II.

**FIG. 3.** *Occurrence of motifs characteristic of class B PBPs along amino acid sequences of the 647-amino-acid (ORF D270-encoded) PBP and the 1,080-amino-acid (ORFD682-encoded) PBP of C. trachomatis (Ctr). E. coli (Eco) PBP 3 and PBP 2 are the prototypes of PBPs of subclasses B3 and B2, respectively (17). The intermotif distances are in numbers of amino acid residues.*

| PBPs | | n-PB module | | | | | | PB module | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 ⋏ 4 | | | 5 | 6 | 7 | COOH |
| *Eco3* | 71<br>RGx₃DRSG | 87 | 167<br>RxYPxG | 15 | 188<br>Gx₂GxEx₃D | 31 | 229<br>AxNx₃SxDx₃Q | 27 | 269<br>TGExLAx₄PSxNP | 23 | 307<br>Sx₂K | 359<br>48 SSN | 494<br>132 KTG | 91 | **588** |
| *Ctr(D270)* | 54<br>RGx₃ANTS | 102 | 165<br>RxYPxG | 22 | 193<br>Px₂GxEx₃N | 31 | 234<br>GxDx₃TxDx₃Q | 27 | 274<br>TGExLAx₄PFxNP | 28 | 317<br>Sx₂K | 382<br>61 SSN | 540<br>155 KTG | 104 | **647** |
| *Ctr(D682)* | 62<br>RAx₃DRFG | 110 | 181<br>RxYSxG | 71 | 258<br>Gx₂GxEx₃D | 32 | 300<br>GxKx₃TxSx₃Q | | 457 | | 770<br>Sx₂K | 850<br>76 SSN | 1004<br>151 KTS | 73 | **1080** |
| *Eco2* | 68<br>RGx₃DRNG | 87 | 164<br>RxYPxG | 36 | 206<br>Gx₂GxEx₃E | 31 | 247<br>GxDx₃TxDx₃Q | 20 | 280<br>TGGxLAx₄PSxDP | 35 | 330<br>Sx₂K | 387<br>53 SAD | 544<br>154 KSG | 86 | **633** |

The identification of the class B-specific motifs borne by the two *C. trachomatis* high-$M_r$ PBPs allows the n-PB and PB modules to be identified (Fig. 3). The motifs borne by the 647-amino-acid PBP occur with the expected spacing along the polypeptide chain. The motifs borne by the 1,080-amino-acid PBP occur in the correct order. However, this PBP has peculiar features. The n-PB module contains an extended polypeptide between motifs 2 and 3. A 457-amino-acid polypeptide is inserted downstream from the intermodule junction site. This insert is large enough to have its own fold, and it lacks amino acid sequence similarity with known proteins. Motif 7 of the PB module, LysThrSer, is somewhat unusual in that a serine residue substitutes for a glycine residue.

The hierarchical analysis of 34 high-$M_r$ class B PBPs and their constitutive modules has led to several observations (17). The n-PB and PB modules of PBPs of gram-positive bacteria fall into subclasses B1, B4 (prototype: *S. pneumoniae* PBP 2x), and B5. The n-PB and PB modules of gram-negative bacteria fall into subclasses B2 (prototype: *E. coli* PBP 2) and B3 (prototype: *E. coli* PBP 3). An n-PB module of a given subclass is linked, almost invariably, to a PB module of the same subclass. In all likelihood, the PBPs of subclasses B1, B4, and B5 in the gram-positive bacteria are paralogs, i.e., they perform different functions; the PBPs of subclasses B2 and B3 in the gram-negative bacteria are also paralogs; and the PBPs of subclasses B4 and B5 (gram-positive bacteria) and the PBPs of subclasses B2 and B3 (gram-negative bacteria) may be orthologs, i.e., they perform similar functions. Few bacterial species do not obey these rules. The n-PB and PB modules of PBP VD of the gram-positive organism *B. subtilis*, which is involved in sporulation, belong to subclass B3. The PB module of PBB 2 of the gram-negative organism *B. burgdorferi* belongs to subclass B2, but the n-PB module to which the PB module is associated is an outlier distantly related to the same subclass.

As derived from predictive studies, the 647-amino-acid PBP of C. trachomatis belongs to subclass B3. The n-PB module, from motif 2 to the intermodule junction site, has structural relatedness with the n-PB module of B. subtilis PBP VD ($P = 7 \times 10^{-4}$), and the associated PB module has close similarity with the PB module of *E. coli* PBP 3 throughout the entire sequence ($P = 5 \times 10^{-33}$). In turn, the 1,080-amino-acid PBP of C. trachomatis belongs, most likely, to subclass B2. The n-PB module, from motif 1 to the intermodule junction site, has structural relatedness with the n-PB module of *E. coli* PBP 2 ($P = 7 \times 10^{-6}$), and the associated PB module, from motif 5 to motif 7, has structural relatedness with the PB modules of E. coli PBP 2 ($P = 3 \times 10^{-5}$) and B. burgdorferi PBP 2 ($P = 1 \times 10^{-7}$). One may note that in *E. coli*, the paralogous PBP 2 of subclass B2 and PBP 3 of subclass B3 perform different functions. They cannot substitute for each other.

**TABLE 2.** *Occurrence of E. coli protein homologues in bacterial species with known genomic data*

| E. coli protein | Presence of homology with protein of: | | | | | | | | | | | $P^c$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group A[a] | | | | | | | | | Group B[b] | | |
| | *H. influenzae* | *B. subtilis* | *R. prowazekii* | *T. pallidum* | *M. tuberculosis* | *H. pylori* | *B. burgdorferi* | *A. aeolicus* | *Synechocystis PCC 6803* | *M. pneumoniae* | *C. trachomatis* | |
| FtsH | + | + | + | + | + | + | + | + | + | + | + | $< 1 \times 10^{-112}$ |
| FtsY | + | + | + | + | + | + | + | + | + | + | + | $< 9 \times 10^{-44}$ |
| MraW | + | + | + | + | + | + | + | + | + | + | + | $<1 \times 10^{-32}$ |
| FtsW | + | + | + | + | + | + | + | + | + | - | + | $<7 \times 10^{-21}$ |
| RodA | + | + | + | + | + | + | + | + | + | - | + | $< 3 \times 10^{-17}$ |
| FtsZ | + | + | + | + | + | + | + | + | + | + | - | $< 7 \times 10^{-10}$ |
| FtsK | + | + | + | + | + | + | + | + | + | - | + | $< 4 \times 10^{-87}$ |
| FtsA | + | + | + | + | - | + | + | - | - | - | - | $< 7 \times 10^{-10}$ |
| MraZ | + | + | + | + | + | - | - | + | - | + | - | $< 7 \times 10^{-8}$ |
| FtsN | + | + | - | - | - | - | - | - | - | - | - | $< 7 \times 10^{-4}$ |
| FtsQ | + | - | - | - | - | - | - | - | - | - | - | $6 \times 10^{-38}$ |
| FtsL | + | - | - | - | - | - | - | - | - | - | - | $1 \times 10^{-5}$ |
| ZipA | + | - | - | - | - | - | - | - | - | - | - | $3 \times 10^{-16}$ |

[a] The bacterial species of group A have a wall peptidoglycan.
[b] The bacterial species of group B are wall-less (*M. pneumoniae*) or wall peptidoglycan-less (C. *trachomatis*).
[c] *P* values smaller than $10^{-3}$ are indicative of significant amino acid sequence similarities. When protein homologues occur in two or more bacterial species, the *P* value with the lowest significance is given.

## A GLYCANLESS WALL POLYPEPTIDE IN CHLAMYDIA?

In the wall peptidoglycans of chemotype III found in a number of species of the family *Micrococcaceae*, the disaccharide and peptide units occur in the expected 1 to 1 molar ratio, but a large proportion of the *N*-acetylmuramic acid residues are not peptide substituted, and polypeptides consisting of peptides with the same amino acid sequences as the peptide units cross-link the glycan chains (13, 15). Admittedly, the presence of these polypeptides, which are made of peptide repeats, implies a tight coordination between *N*-acetylmuramoyl-L-ala-nine amidase and transpeptidase activities.

*C. trachomatis* possesses ORFs (D268, D601, and perhaps D759) which code for *N*-acetylmuramoyl-L-alanine amidases (8). Therefore, there is a possibility that the lipid II-transported L-Ala-γ-D-Glu-(L)-mesodiaminopimelic acid-(L)-D-Ala-D-Ala pentapeptides are released from their carrier by amidase action (Fig. 1, reaction 2) and then polymerized by the two class B PBPs into a cross-linked covalently closed wall polypeptide. D-Alanyl-D-alanine sequences could serve as carbonyl donors for two types of penicillin-sensitive transpeptidation reactions. Transpeptidation involving the L-alanine residue at the amino end of the amidase-released peptides as an acceptor would result in a head-to-tail assembly of linear polypeptide chains (Fig. 1, reaction 4). Transpeptidation involving the amino group at the D center of meso-diaminopimelic residues as the acceptor would create cross-linkages between linear polypeptide chains (Fig. 1, reaction 3).

This glycanless wall polypeptide, together with the lipoproteins to which it might be linked covalently (6), the lipopolysaccharides (37), and the highly disulfide cross-linked proteins (20) of the outer membrane, could provide *Chlamydia* with a cell envelope of sufficient mechanical strength. Moreover, the wall polypeptide could be remodelled throughout the chlamydial cell cycle by the low-$M_r$-PBP-catalyzed hydrolysis of the carboxy-terminal D-alanyl-D-alanine bonds of the pentapeptide units and the D-alanyl-(D)-meso-diaminopimelic acid cross-linkages at various places in the polymer.

## LIPID II RECYCLING

In *E. coli,* the delivery of the disaccharide pentapeptide from lipid II generates an undecaprenyl pyrophosphate which is dephosphorylated, and then the $C_{55}$ isoprenoid alcohol phosphate turns over the membrane bilayer so that the phosphate group faces the cytosol, thus allowing a new cycle to start. In *Chlamydia*, the $C_{55}$ isoprenoid-pyrophosphate-disaccharide which results from the delivery of the pentapeptide might turn over the membrane bilayer, and a new cycle could start directly by ligase-catalyzed additions to the *N*-acetylmuramic acids of the lipid-borne disaccharide units of L-alanine, D-glutamic acid, meso-diaminopimelic acid, and the dipeptide D-alanyl-D-alanine. Attributing to the disaccharide moiety of lipid II the role of pentapeptide unit carrier would explain why *N*-acetylmuramic acid is not biochemically detected in *Chlamydia* or is detected in very small amounts. Alternatively, hydrolysis of the pyrophosphate bond might occur with the release of the $C_{55}$ isoprenoid alcohol phosphate.

Bacitracin is a wall peptidoglycan inhibitor because it complexes the pyrophosphate group of lipid II before dephosphorylation occurs (38). Bacitracin also inhibits the synthesis of the outer membrane lipopolysaccharides whose polysaccharide chains are assembled on the same undecaprenyl pyrophosphate as that utilized in peptidoglycan synthesis and then are transferred as whole entities to the lipid A core of the molecule (37). In the absence of a typical wall peptidoglycan, the inhibition of the lipopolysaccharide synthesis might destabilize the chlamydial cell envelope. Following this view, the lipopolysaccharide of the outer membrane could be the target of bacitracin in *Chlamydia.*

## CELL CYCLE PROTEINS

Cell cycle proteins channel PBP-catalyzed peptidoglycan assembly into wall expansion and septum formation in a cell cycle-dependent fashion. In recent years, the catalogue of these proteins has grown considerably. They are discussed in recent reviews (26, 33). Suffice it to say that in E. coli, a cell division dcw cluster at the 2-min region of the chromosome contains genes for the synthesis of lipid II and PBP 3 of subclass B3. It also contains genes encoding the cell division proteins MraZ (YABB), MraW (YABC), FtsL, FtsW, FtsQ, FtsA, and the ring-shaped FtsZ. A cell shape cluster at the 14-min region of the chromosome contains the gene encoding PBP 2 of subclass B2. It also contains genes encoding the low-$M_r$ PBP5 and RodA.

Genes located outside these clusters are also devoted to cell division. Although essential to the process, some of them encode proteins which are not components of the morphogenetic apparatus itself. FtsK (encoded by a gene at 21 min) performs a septation function (N-terminal domain) and a chromosome partition function (C-terminal

domain) (11, 43). ZipA (encoded by a gene at 52 min) is an integral membrane protein which interacts with the ring-shaped FtsZ (19). FtsH (encoded by a gene at 69 min) is a membrane-bound, ATP-dependent protease which degrades the heat shock transcription factor $\sigma^{-32}$ (41). FtsY (encoded by a gene at 78 min) is a functional homologue of a signal recognition particle protein involved in the reception and insertion of a subset of proteins at the plasma membrane (28). FtsN (encoded by a gene at 88 min) suppresses certain missense mutations in other fts genes (10).

In view of these advances, the question of which assortment of proteins in *Chlamydia* is involved in cell morphogenesis arises. To begin to solve the problem, one should note that the *E. coli* proteins are conserved, to various degrees, in those bacterial species that make wall peptidoglycan (Table 2, group A). FtsH, FtsY, the cell division MraW, FtsW, FtsZ, and the cell shape RodA are ubiquitous. MraW bears a putative S-adenosylmethionine-binding motif (7). FtsW and RodA are homologous integral membrane proteins with loops exposed on both faces of the plasma membrane (5). FtsZ is a GTPase related to eukaryotic tubulins. It localizes early at the division site, where it forms a ring-shaped structure that allows cell envelope constriction to take place (27, 29, 34).

Proteins functionally equivalent to the E. coli FtsK, FtsA, MraZ, FtsN, FtsQ, FtsL, and ZipA proteins (Table 2, group A) might also be ubiquitous, but then one has to assume that, depending on the proteins and the bacterial species, they have diverged so far from the corresponding E. coli proteins that similarity is marginal or almost nonexistent ($P > 10^{-3}$). One may also note that, as result of diverging evolution, similarities between homologous proteins may be restricted to segments of the polypeptide chains only. Examples are given in Table 3.

The strict conservation of MraW, FtsW, RodA, and FtsZ among the peptidoglycan-containing bacterial species of group A is of particular significance when the wall-less mycoplasmas and the peptidoglycanless C. trachomatis are taken into consideration (Table 2, group B). The mycoplasmas do not synthesize lipid II. They produce MraW and FtsZ but lack FtsW and RodA, suggesting that FtsW and RodA might be connected to the flipping of lipid II through the membrane. *C. trachomatis* synthesizes lipid II. It produces MraW, FtsW, and RodA, and similarities with the corresponding *E. coli* proteins extend throughout the entire sequences (Table 3). But *C. trachomatis* lacks FtsZ, suggesting that in the bacterial species that manufacture a wall peptidoglycan, there is a link, direct or indirect, between FtsZ and the transglycosylase (n-PB module) of the class A PBPs. Consistently, the inactivation of FtsZ in *E. coli* (*ftsZ* 84 mutant) is associated with a significant change in the length distribution of glycan strands in newly synthesized peptidoglycan, with a shift from longer to shorter chain lengths (23).

**TABLE 3.** *Homologous E. coli and C. trachomatis proteins*[a]

| Protein | E. coli | | C. trachomatis | | Homologous segment in the E. coli protein | | P |
|---|---|---|---|---|---|---|---|
| | Gene location (min) | Size (amino acids) | Gene location (ORF) | Size (amino acids) | NH$_2$ | COOH | |
| FtsZ | 2 | 383 | | | | | |
| MraW | 2 | 313 | D272 | 300 | 3 | 311 | $2 \cdot 10^{-35}$ |
| FtsW | 2 | 414 | D760 | 403 | 48 | 397 | $1 \cdot 10^{-28}$ |
| RodA | 14 | 370 | D726 | 379 | 47 | 363 | $2 \cdot 10^{-21}$ |
| FtsK | 21 | 1,329 | D739 | 799 | 861 | 1,283 | $4 \cdot 10^{-87}$ |
| FtsH | 69 | 644 | D841 | 913 | 140 | 593 | $1 \cdot 10^{-142}$ |
| FtsY | 78 | 497 | D820 | 284 | 247 | 492 | $3 \cdot 10^{-52}$ |

[a] The homologies between the C. trachomatis and E. coli FtsK, FtsH, and FtsY proteins are limited to segments of the polypeptide chains.

**CONCLUSIONS**

*Chlamydia* is a peptidoglycanless bacterium because it does not have the information for the synthesis of class A PBPs (or monofunctional transglycosylases). The proposal that *Chlamydia* utilizes one or several *N*-acetylmuramoyl-L-alanine amidases and one bifunctional (cell cycle transpeptidase) PBP each of subclasses B2 and B3 to manufacture from lipid II a covalently closed, glycanless wall polypeptide made of peptide repeats whose synthesis is penicillin sensitive offers a clue to the chlamydial anomaly. This model has alternative possibilities. In particular, a combination of the identified enzymatic activities could lead to the synthesis of a wall polypeptide bearing few disaccharide units. Moreover, the question of what connections may exist between the

presumed wall polypeptide, the presence of one additional domain in the PBP of subclass B2, the likely absence of a ring-shaped FtsZ-like protein, and the process of cell division in *Chlamydia* remains unanswered. These possibilities are presented here as a basis for future research on and an interpretation of a problem of great biological interest.

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