

Bacterial Growth and Cell Division: a Mycobacterial Perspective

Erik C. Hett and Eric J. Rubin*

Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts 02115

INTRODUCTION	127
CELL WALL COMPONENTS	127
What Is the Structure of the Mycobacterial Cell Wall?	127
PG	127
AG	129
MAs.....	129
Are Mycobacteria Gram Positive or Gram Negative?	129
Why Are Mycobacteria So Innately Resistant to Antibiotics?	129
If Mycobacteria Are So Impermeable, How Do They Access Nutrients?	130
How Do Mycobacteria Synthesize Cell Wall Components?	130
PG	130
AG	130
MAs.....	130
How Is PG Synthesis Controlled?	131
What Regulates the Activity of Enzymes Responsible for PG Synthesis?	132
Do Mycobacteria Recycle Cell Wall Material?	132
How Do Antibiotics Target the Cell Wall?	132
MAINTENANCE OF SHAPE	132
Why Are Mycobacteria Rods?	132
What Is the Role of PG in Determining Cell Shape?	132
Where Does Nascent PG Localize?	133
Is Inert PG Involved in Branching?	133
What Is the Role of the Cytoskeleton in Shape?	133
Tubulin	133
Intermediate filaments	134
Actin.....	134
Why Do Mycobacteria Grow at the Tips?	134
What About Inert PG at the Tips?	134
How Can Mycobacteria Maintain Shape without MreB?	135
What Is Different about Mycobacterial Growth?	135
BACTERIAL CELL DIVISION	135
What Is the Order of Events in Cell Division?	135
How Does the Chromosome Replicate?	135
What Segregates the Chromosome?	136
What Are the Divisome Components, and How Do They Assemble?	136
What Is Missing in Actinomycetes?	136
What Are the Negative Regulators of FtsZ Assembly?	137
Min system.....	137
Nucleoid occlusion	138
How Does FtsZ Constrict?	139
How Does FtsZ Polymerize, and Why Is Mycobacterial FtsZ So Slow?	139
Is Growth Rate Related to Mycobacterial Pathogenesis?	140
What Hydrolyzes the Septal PG?	140
What Is the V-Snapping Process of Dividing Mycobacteria?	140
What Is Different about Mycobacterial Cell Division?	140
REGULATION OF BACTERIAL GROWTH AND DIVISION	141
What Are STPKs, and How Do They Work?	141
PknA and PknB.....	141
Other STPKs	142
How Are WhiB Proteins Involved in Growth Regulation?	143
CELL DIVISION UNDER SPECIALIZED CONDITIONS	143
How Is Entry into and Escape from the Dormant States Regulated?	143

* Corresponding author. Mailing address: 200 Longwood Avenue, Armenise D2, Room 439, Boston, MA 02115. Phone: (617) 432-3337. Fax: (617) 432-3259. E-mail: erubin@hsph.harvard.edu.

TCSs	143
DosRS/T dormancy phosphorelay system.....	144
Other TCSs.....	144
Alternative sigma factors	144
What Else Is Regulated during Infection?	145
TA system.....	145
What Is the State of Dormancy Models?	145
Stringent response	146
How Do Mycobacteria Reactivate from Dormancy?	146
The Rpf story.....	146
How Is Rpf Regulated?	146
How Could Rpf Activate Bacteria?.....	146
Does Resuscitation Involve Normal Homeostatic Processes?	147
CONCLUSIONS	147
ACKNOWLEDGMENTS	147
REFERENCES	147

INTRODUCTION

The genus *Mycobacterium* is best known for its two major pathogenic species, *M. tuberculosis* and *M. leprae*, the causative agents of two of the world's oldest diseases, tuberculosis and leprosy, respectively. *M. tuberculosis* kills approximately two million people each year and is thought to latently infect one-third of the world's population. One of the most remarkable features of the nonsporulating *M. tuberculosis* is its ability to remain dormant within an individual for decades before reactivating into active tuberculosis. Thus, control of cell division is a critical part of the disease.

The mycobacterial cell wall has unique characteristics and is impermeable to a number of compounds, a feature in part responsible for inherent resistance to numerous drugs. The complexity of the cell wall represents a challenge to the organism, requiring specialized mechanisms to allow cell division to occur.

Besides these mycobacterial specializations, all bacteria face some common challenges when they divide. First, they must maintain their normal architecture during and after cell division. In the case of mycobacteria, that means synthesizing the many layers of complex cell wall and maintaining their rod shape. Second, they need to coordinate synthesis and breakdown of cell wall components to maintain integrity throughout division. Finally, they need to regulate cell division in response to environmental stimuli.

Here we discuss these challenges and the mechanisms that mycobacteria employ to meet them. Because these organisms are difficult to study, in many cases we extrapolate from gram-negative bacteria or more closely related GC-rich gram-positive organisms.

CELL WALL COMPONENTS

What Is the Structure of the Mycobacterial Cell Wall?

The mycobacterial cell wall consists of an inner layer and an outer layer that surround the plasma membrane (50). The outer compartment consists of both lipids and proteins. The lipids are often freely associated with the cell wall, with some long- and short-chain fatty acids complementing the short and long chains found in the inner layer. The lipid-linked polysaccharides associated with the outer cell wall consist of lipoarabi-

nomannan (LAM), lipomannan, phthiocerol-containing lipids such as phthiocerol dimycocerosate, dimycolyl trehalose (cord factor), sulfolipids specific to *M. tuberculosis*, and the phosphatidylinositol mannosides. In slow-growing, pathogenic mycobacteria, such as *M. tuberculosis* and *M. leprae*, the LAMs are capped at the terminal β -Ara residue with mannose residues and are referred to as ManLAMs (68, 69, 301, 324), whereas the fast-growing mycobacteria, such as *M. smegmatis* and *M. fortuitum*, have phosphoinositol-capped LAMs known as PILAMs (301). Furthermore, *M. chelonae* LAMs are devoid of caps and referred to as AraLAMs (68, 69, 301, 324). These outer proteins and lipids are soluble components of the cell wall and have been referred to as the signaling and effector molecules of mycobacteria (50) because of their known roles in interacting with the immune system.

The inner compartment consists of peptidoglycan (PG), arabinogalactan (AG), and mycolic acids (MA) covalently linked together to form a complex known as the MA-AG-PG complex that extends from the plasma membrane outward in layers, starting with PG and ending with MAs (Fig. 1). This complex, discussed in detail here, is insoluble and referred to as the essential core of the mycobacterial cell wall (50). Many of the drugs used to combat mycobacteria target the MA-AG-PG complex.

One caveat worth mentioning is that mycobacteria represent a diverse group of organisms. Much of the work on cell growth has been performed with two divergent members of the genus, the saprophytic bacterium *M. smegmatis* and the human pathogen *M. tuberculosis*. These two species have a number of important differences, and we do not know how many of the lessons learned with one apply to the other or, more generally, to other members of the genus.

PG. PG, or murein, is a versatile material, rigid enough to provide a scaffold for bacteria to maintain their shape and protect them from osmotic turgor pressure yet malleable enough for the bacteria to grow and expand. Almost all of the known species of eubacteria (hereafter referred to as bacteria) contain PG, with exceptions including the fascinating *Mycoplasma* and, arguably, *Chlamydia* (313). The archaea also have semirigid cell walls composed of compounds such as protein or pseudomurein that replace the role of PG (206, 222). The structure of PG is unique to bacteria and thus an excellent target for therapeutics. As its name implies, PG is made of

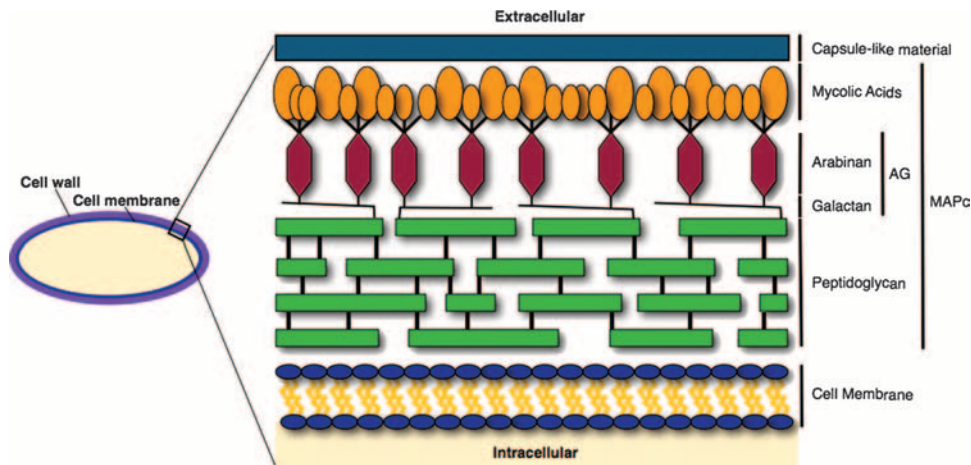


FIG. 1. Diagram of the basic components of the mycobacterial cell wall. MAPc, MA-AG-PG complex.

peptides and glycan strands. The long glycan strand typically consists of repeating *N*-acetylglucosamines (NAGs) linked to *N*-acetylmuramic acid (NAM). These strands are cross-linked by peptides bound to the lactyl group on NAMs from different glycan strands (22, 188). These peptide chains normally consist of *L*-alanyl-*D*-*iso*-glutaminy-*meso*-diaminopimelic acid (DAP) from one strand linked to the terminal *D*-alanine residue from *L*-alanyl-*D*-*iso*-glutaminy-*meso*-DAP-*D*-alanine from a different strand. While all bacteria require a dibasic residue for cross-linking PG, there are several residues from which to choose. Most gram-positive bacteria utilize lysine; however, DAP is commonly used instead by actinomycetes and gram-negative bacteria.

Mycobacteria have what is known as chemotype IV cell walls, containing PG with *meso*-DAP and AG (368). While similar to a standard PG structure, the mycobacterial structure has a few differences (Table 1). First, some of the muramic acid has an *N*-glycolyl group rather than an *N*-acetyl group. Of the known bacteria, only the actinomycetes have been shown to contain PG that has *N*-glycolylated muramic acid (186). The actual percentage of glycolylation is still debatable. Previous studies used the drug *D*-cycloserine, which inhibits *D*-Ala-*D*-Ala ligase, to block incorporation of PG precursors into the PG in order to generate sufficient precursor material for analysis. They found that all muramic acids were *N* glycolylated (11, 233, 315, 399). Re-

cently, using a technique that does not require blocking PG synthesis, researchers found that mycobacterial PG contains both *N*-glycolyl and *N*-acetyl modifications (254). The researchers noted that use of *D*-cycloserine resulted in all of the precursors becoming *N*-glycolylmuramic acid. Second, approximately 25% of cross-links in the PG of *M. smegmatis* (and other mycobacteria) are unique in that the bonds are between two DAP molecules (DAP-DAP), while the remaining cross-links are the typical DAP-alanine (446). In contrast to the 75% of PG cross-linked in mycobacteria, only 20 to 50% is cross-linked in *Escherichia coli* (268, 426). While DAP is thought to be essential to mycobacteria, a mutant that incorporates lanthionine in the place of DAP has been reported (77). Third, *L*-glutamate and DAP are amidated in mycobacterial PG (233). This is also the dominant form in *Bacillus subtilis* (5). Finally, some of the *D*-glutamate or *L*-alanine (*M. leprae*) residues are modified with glycine (116, 117, 224).

The biological significance of these differences is unknown (Table 1). It could be that the DAP-DAP linkage provides increased rigidity to the PG when bacteria are in certain stressful conditions (152). Interestingly, vegetatively growing *E. coli* typically lack DAP-DAP linkages, but DAP-DAP bonds appear during stationary phase, potentially in response to nutrient depletion or other stress (401). Also, the PG from mycobacteria is significantly more resistant to lysozyme than *E.*

TABLE 1. Attributes of mycobacterial PG and their biological significance

Mycobacterial PG attribute	Common PG attribute in other bacteria	Biological significance
DAP-DAP and DAP-Ala peptide cross-linkage	DAP-Ala peptide cross-linkage	May provide increased rigidity to the PG to help survive stressful conditions
<i>N</i> -Glycolylmuramic acid and NAM	NAM	Could tighten the PG sacculus by providing more opportunities for hydrogen bonding and increase resistance to β -lactams and lysozyme
Amidated <i>L</i> -Glu and DAP	Amidated <i>L</i> -Glu and DAP	May play a role in the regulation of cross-linking and could increase resistance to hydrolysis by specific endopeptidases, as seen with the amidation of PG sugar residues increasing resistance to muramidase
<i>D</i> -Glutamate or <i>L</i> -alanine modified with glycine	No modification	Unknown

coli PG (108). The amidation of L-Glu and DAP may play a role in the regulation of cross-linking and could also increase resistance to hydrolysis by specific endopeptidases, as seen with the amidation of PG sugar residues increasing resistance to muramidase (254). The N-glycolyl group on the muramic acid could provide more hydrogen-bonding energy and thus tighten the PG (51). An *M. smegmatis* strain lacking the enzyme for modifying the muramic acid to an N-glycolyl group was shown to have increased sensitivity to β -lactams and lysozyme (343).

The orientation of PG relative to the surface of the bacterium has been debated for over four decades (276). Traditionally, the PG layer has been thought of as being parallel to the surface of the bacterium (55, 111, 217, 218, 365, 426). Some argue that a parallel PG would better accommodate cell division (426). Others prefer a model where the PG is orthogonal or perpendicular to the surface membrane (111, 112, 123, 267, 319). Sufficient evidence to prove a particular model has not been forthcoming. Most recently, researchers reported the three-dimensional nuclear magnetic resonance structure of PG (276). Because the main limitation in solving PG structure is a lack of a pure extract, the researchers carried out the 37-step reaction required to synthesize a 2-kDa product *in vitro*. The product (NAG-NAM [pentapeptide]-NAG-NAM [pentapeptide]) was a replicate of typical gram-positive PG, containing L-lysine instead of *meso*-DAP, but should be similar to DAP-containing PG. The authors reported a PG structure that has an ordered, right-handed helical saccharide conformation with three NAG-NAM pairs per helix turn that is orthogonal to the bacterial surface (276). Understanding the structure of PG in relation to other cell wall components should provide insight into the biosynthesis of PG and may yield clues for novel therapeutics.

AG. AG is the major polysaccharide of the mycobacterial cell wall. It is important for cell wall integrity and for anchoring the impermeable MA layer to the PG layer. AG is composed of arabinan and galactan, both in the relatively uncommon furanose form (273). The galactose residues are arranged in alternating 1 \rightarrow 5, 1 \rightarrow 6 linkages of approximately 30 residues, with the arabinan attached to the 5 position of the galactan in 1 \rightarrow 5 linkages (84). Some of the galactan polymers may not have arabinan attached and thus are unbranched galactan chains (81). AG is covalently attached to the PG layer by a phosphoryl-N-acetylglucosaminosyl-rhamnosyl linkage (81, 272), which is related to the linker used to attach teichoic acid to PG in gram-negative bacteria (219).

MAs. MAs are the primary determinant for cell wall permeability and consist of a variety of short α -alkyl- and long β -hydroxyl-fatty acids ranging from 60 to 90 carbons per chain (27). The majority of MAs are covalently linked to the AG by ester links (81) and exist as tetramycolyl-pentaarabinofuranosyl clusters on the AG (67), although some have been found to be extractable from the membrane. The outer layer of lipids and proteins mentioned above intercalates into the MA layer. In *M. tuberculosis*, the meromycolate segments of the MAs can be, whereas the MAs of *M. smegmatis* are not, modified by cyclopropanation (67). Recent work has shown that *trans*-cyclopropanation of MAs suppresses inflammation caused by *M. tuberculosis* infection (334).

Are Mycobacteria Gram Positive or Gram Negative?

DNA-based molecular taxonomy groups mycobacteria with gram-positive bacteria, since most mycobacterial genes show high similarity to genes in other gram-positive organisms, such as *Bacillus*, compared to gram-negative organisms. However, there are potential functional distinctions between the mycobacterial cell wall and the standard gram-positive cell wall. The prototypical cell wall of gram-negative bacteria consists of an inner membrane formed by a lipid bilayer, a thin sheet of PG (one to three layers) contained within the periplasmic space, and an outer membrane. Often these bacterial cell walls include lipopolysaccharide, lipoproteins, and porins, and they are occasionally encapsulated by a carbohydrate capsule. In contrast, the prototypical gram-positive bacterial cell wall contains an inner membrane, a thick PG layer (10 to 20 layers), and no outer membrane. The PG layer of *M. leprae* is 4 to 5 nm thick, while the corresponding layer of *M. tuberculosis* is 10 to 15 nm (398). These cell walls are decorated with other compounds such as teichoic acids, lipoteichoic acids, and MAs. While mycobacteria are structurally gram-positive bacteria, lacking a true outer membrane and containing a thick layer of PG, they also share properties with gram-negative organisms, such as not retaining the Gram stain (instead they are known as acid fast for the retention of Carbol fuchsin dye even in the presence of acidic alcohol), containing porins in their outer lipid layer, and having a potential space that may function in a manner similar to the gram-negative periplasm.

Do mycobacteria actually have a periplasm? Although this is traditionally a gram-negative attribute, cryo-transmission electron micrographs of the gram-positive *B. subtilis* reveal a space similar to the periplasmic space (162). In mycobacteria, the outer layer of lipids and proteins mentioned above intercalates into the MA layer to form a lipid layer resembling the outer layer of gram-negative bacteria. This outer layer of lipids arguably forms a "pseudoperiplasm" that has functional consequences that we will discuss below.

Why Are Mycobacteria So Innately Resistant to Antibiotics?

While multidrug resistance has recently grabbed headlines, mycobacteria are inherently resistant to numerous antibiotics. Much of this resistance is attributable to the cell wall. Mycobacteria have unusually impermeable cell walls that are thought to be advantageous in stressful conditions of osmotic shock or desiccation as well as contributing to their considerable resistance to many drugs. The *M. chelonae* cell wall is 30 times less permeable to hydrophilic molecules than *E. coli* (200), while *M. smegmatis* was found to be about 20 times less permeable than *E. coli* (407). The permeation ability of a lipophilic molecule is inversely related to the fluidity of the cell wall, which decreases as the length of fatty acids in the MA layer increases (51). Lipophilic drugs, such as fluoroquinolones or rifamycins, pass more easily through the lipid-rich cell wall and thus are more active (51, 451).

However, the low permeability is not sufficient to explain the level of innate drug resistance seen in mycobacteria (199). The surface-to-volume ratio is extremely high for small organisms like bacteria, such that even when low permeability is factored in, mycobacteria must have some other characteristics that

make them resistant to antimicrobials (199). Experiments measuring the equilibrium diffusion for mycobacterial drugs show that the drugs reach cytotoxic levels inside the bacteria within a fraction of the generation time, yet the bacteria are able to survive (199). Strategies that mycobacteria use in combination with the low rate of diffusion through the cell wall likely include efflux pumps, response regulators, antibiotic-modifying or -degrading enzymes such as β -lactamase (62, 433), target-modifying enzymes, and decoys that mimic the drug target (300).

If Mycobacteria Are So Impermeable, How Do They Access Nutrients?

The MAs are the major determinants of cell wall fluidity and permeability of lipophilic molecules. The density and thickness of the MA layer directly determine the ability of lipophilic molecules to traverse the cell wall. Hydrophilic molecules, which are unable to cross, are able to pass through porins inserted into the cell wall and membrane (391, 408, 447). In contrast, lipophilic solutes are unable to efficiently pass through porins (302) and must diffuse through the membrane. The fast-growing *M. smegmatis* expresses the porin-like protein MspA, which appears to be important for nutrient uptake and is lacking in slow-growing *M. tuberculosis* (300). Expression of the *M. smegmatis* MspA porin in *M. bovis* BCG results in a twofold increase in glucose uptake and an higher growth rate (256), suggesting that access to extracellular nutrients may limit growth rates. The porin protein OmpATb, thought to be present only in pathogenic species of mycobacteria, shows a propensity to close its channel in low-pH conditions, which may help bacterial survival in the acidic conditions encountered in the macrophage phagosome (286).

How Do Mycobacteria Synthesize Cell Wall Components?

PG. As mentioned above, the structure of mycobacterial PG is quite similar to that of PG from other bacteria. While not yet confirmed, the same appears to be true for its biosynthesis. Mycobacteria possess several genes homologous to those that encode synthetic enzymes for PG in *E. coli* and *B. subtilis*. In other well-studied organisms, the precursors of PG are generated in the cytoplasm, translocated to the outside of the plasma membrane, and linked into the existing PG sacculus. PG synthesis has been thoroughly reviewed for *E. coli* (420–422) and for mycobacteria (81).

The first set of reactions takes place in the cytoplasm due to the requirement for nucleotide-activated sugars and soluble amino acids. (Fig. 2) Synthesis is initiated when UDP is linked to NAG (possibly catalyzed by FlmU) to form UDP-NAG. Next, MurA adds an enoyl pyruvate to the 3 position of NAG (107), which is then reduced by MurB to form muramic acid, resulting in UDP-NAM. A series of reactions then occur, adding, in order, L-alanine (catalyzed by MurC), D-glutamine (MurD), DAP (MurE), and D-alanyl-D-alanine (MurF) to form UDP-NAM-L-alanyl-D-iso-glutamyl-meso-DAP-D-alanyl-D-alanine (UDP-NAM-pentapeptide, also known as Park's nucleotide). Lipid I is generated when MurX transfers UDP-NAM-pentapeptide to decaprenyl phosphate, a long carbon chain uniquely used by mycobacteria rather than the standard

undecaprenyl phosphate (254). It has been suggested that NAM is glycolylated to become N-glycolylmuramic acid at some point after lipid I formation (254) by the enzyme NamH (343). Next, MurG links NAG to lipid I to synthesize lipid II. Lipid II is then translocated across the plasma membrane with the aid of bactoprenol (undecaprenol in *E. coli*), a carrier of cell wall subunits. FtsW or RodA might serve as a translocase or, possibly, as a flippase that assists in transporting the precursor across the membrane (104, 195, 214).

The second set of reactions occurs initially on the plasma membrane and then within the periplasmic or "pseudo-periplasmic" space. Initially, a transglycosylase reaction links the PG monomer to an existing strand of PG (458). Next, to further strengthen the PG, a transpeptidase reaction occurs that links the DAP of the acceptor peptide to either a DAP or the penultimate D-alanine on the donor peptide. The transpeptidase that forms the DAP-DAP linkage is likely resistant to β -lactams and thus represents a potential new drug target (152, 253). Unfortunately, the enzyme thought to catalyze this reaction has yet to be identified.

Cleavage of D-alanyl-D-alanine is thought to provide the energy necessary for the PG-cross-linking reaction, since no ATP is available in the periplasm. While the antibiotic penicillin mimics D-Ala-D-Ala, vancomycin binds to the terminal D-Ala-D-Ala, resulting in either drug blocking this cross-linking step. Researchers have shown that the dual-domain penicillin-binding protein encoded by *ponA1* of *M. smegmatis* is capable of both transglycosylase and transpeptidase reactions in vitro (30). Furthermore, an *M. smegmatis ponA1* null mutant has been reported to be more susceptible to β -lactams and has a reduced rate of growth (37). The *E. coli* PonA homologues PBP 1a and PBP 1b have been shown to attach lipid II to existing PG sacculi in vitro (46), and the PBP 1 proteins from *M. tuberculosis*, *M. leprae*, and *M. smegmatis* have all been shown to have similar functions (30, 239).

AG. The first step in AG synthesis (reviewed by Crick et al. [81] and Brennan and Nikaido [51]) is the formation of the "linker region." This linker is the site of attachment to PG and is related to that used for linkage of teichoic acid to PG (219). This linker is formed when UDP-NAG is transferred to a prenyl phosphate and rhamnose is added (279). The next two steps occur separately and result in the formation of units that will be polymerized in future steps. In these reactions, UDP-galactofuranose is generated from UDP-galactopyranose (444) and decaprenylphosphoryl-arabinofuranose is generated from the pentose phosphate pathway (366, 367). Galactofuranose is then added to the linker region and polymerized to form long, linear chains (281). Arabinofuranose moieties are added to the polymerized galactose in both linear and branched chains (20). Finally, the AG complex is ligated to the PG and decaprenyl phosphate is released. It is not yet known what enzyme ligates AG to PG.

MAs. There are two main means of synthesizing MAs (reviewed by Brennan [50] and Takayama et al. [400]). The first is through a multienzyme complex known as fatty acid synthase (FAS) type I. The complex is able to synthesize MA de novo, generating fatty acid acyl coenzyme A derivatives of C₁₄ to C₂₆ in length (67). The second is FAS type II, which requires an initial acylated product that can then be elongated by FAS type II.

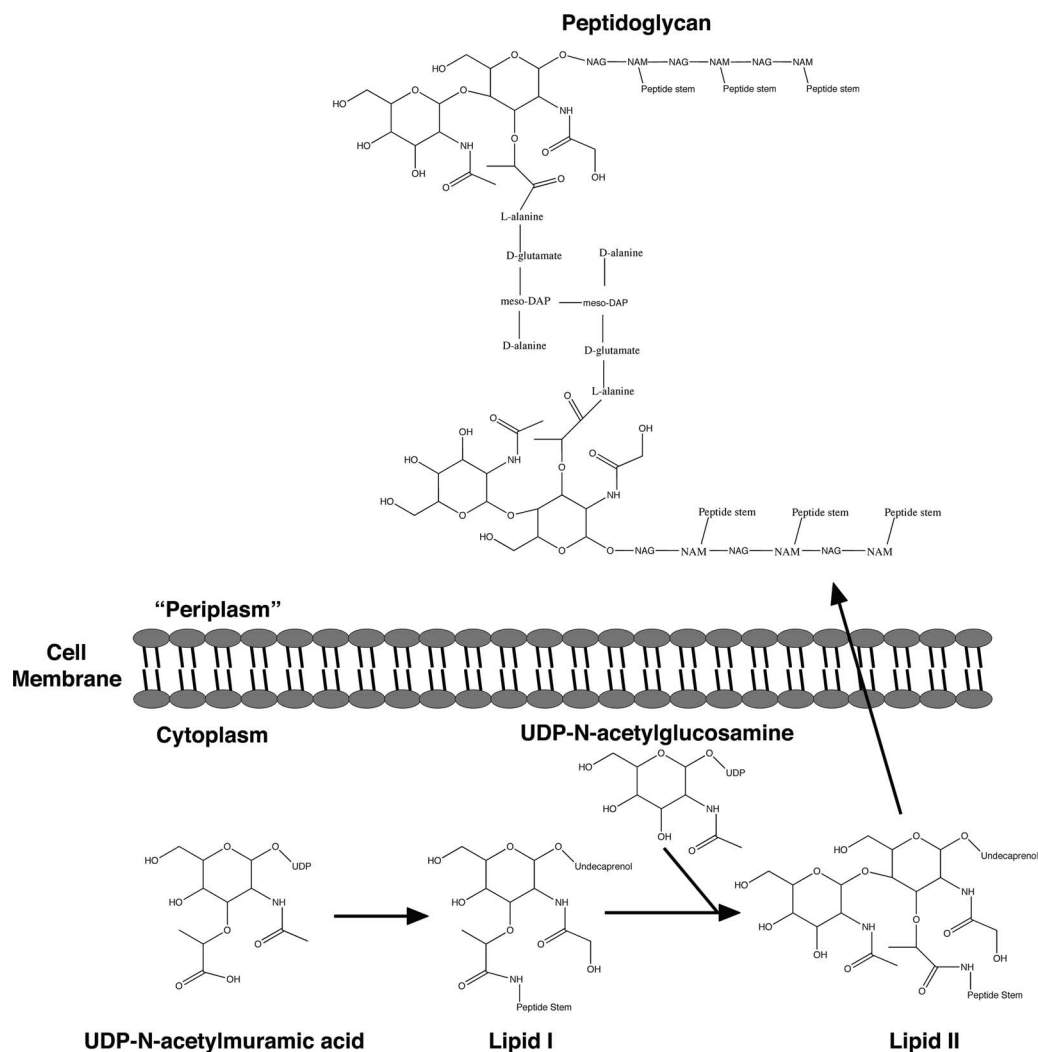


FIG. 2. Synthesis of PG. Major steps are shown, with precursors such as UDP-NAM, lipid I, and lipid II being synthesized within the cytoplasm. Lipid II is then transported across the lipid membrane, and periplasmic transglycosylases and endopeptidases link the PG monomer into existing PG sheets through β -1,4 glycosidic linkages and DAP-DAP peptide cross-linkages.

Recently, researchers found that a key component of FAS type II, the β -ketoacyl-acyl carrier protein KasA, physically associates with the chaperone GroEL1 (305). Mycobacteria encode two GroEL paralogues (GroEL1 and GroEL2), which is unusual. While GroEL2 is essential for normal growth (361), GroEL1 regulates MA formation and was shown to be required for the formation of mature biofilms (305). The transition to mature biofilms might require elevated levels of MAs, and this phenotypic switch could, therefore, be dependent on GroEL1 (305).

Lipid synthesis can also be regulated by Rv2869, a membrane-bound S2P metalloprotease that cleaves membrane-bound transcriptional regulators within their transmembrane segments (257). An Rv2869 null mutant produced less extractable mycolic lipid than the wild type, had decreased cording, and was found to have reduced virulence in mice (257). Additionally, the *pcaA* gene, encoding MA cyclopropane synthetase in *M. tuberculosis* and BCG, is required for proper in vitro cording of bacteria and in vivo pathogenesis (148). *M. smeg-*

matis does not make cyclopropanated MAs. A recent in silico systematic analysis of MA biosynthesis pathways used flux balance analysis to produce a comprehensive model of MA synthesis in *M. tuberculosis* (330). This model has yet to be fully investigated in the laboratory.

How Is PG Synthesis Controlled?

Penicillin-binding proteins (PBPs) have penicilloyl serine transferase activity and were first described for their ability to covalently bind penicillin. The DD-transpeptidase domain is what binds penicillin, an analogue of the enzyme's substrate D-Ala-D-Ala. The transglycosylase domain is insensitive to penicillin. PBPs are classified primarily by mass, from largest to smallest.

Enzymes involved in remodeling PG can be grouped as either biosynthetic or hydrolytic. Biosynthetic enzymes include transglycosylase and transpeptidase domains, often found on a single, bifunctional protein. Hydrolytic enzymes include mur-

amidase, glucosaminidase, lytic transglycosylase, amidase, endopeptidase, and carboxypeptidase. There is extensive overlap of function for many of the PBPs in most bacteria (102). An *E. coli* mutant lacking all but one synthetic PBP can still synthesize PG, suggesting that basic PG synthesis may not require hydrolytic enzymes and may be much simpler than presumed (102). However, that study focused on the known PBPs and thus did not consider other hydrolytic and biosynthetic PG enzymes.

While the reactions that biosynthetic and hydrolytic enzymes catalyze may be antagonistic, there is growing evidence that these two classes of enzymes physically interact to form complexes capable of breaking bonds to generate openings for new monomers, while also forming bonds necessary to unite PG strands. A complex in *E. coli* was found to include Slt70 (a soluble lytic transglycosylase), PBP 3 (a PG transpeptidase), and PBP 7/8 (a DD-endopeptidase) (352, 353). When combined, PBP7/8 and Slt70 were more stable and synergistically degraded PG, suggesting that protein-protein interactions are involved in regulating activity. Two other reported complexes include interactions between RpfB (lytic transglycosylase) and RipA (endopeptidase) in mycobacteria (183) and between PBP 1b (a bifunctional synthase), MltA (a lytic transglycosylase), and MipA (a scaffolding protein) in *E. coli* (426).

What Regulates the Activity of Enzymes Responsible for PG Synthesis?

If hydrolytic enzymes were not properly regulated, the bacterial cell wall would be degraded and the bacteria would lyse. There are several ways that these potentially lethal enzymes, some known as autolysins, could be governed. One method, mentioned above, is through the formation of complexes with other proteins. These proteins could suppress the activity of the enzyme, or they could be enzymes themselves with antagonistic reactions that join rather than degrade PG. Another possibility is that the enzymes are sequestered from their substrate until they are needed. A third method could be that the appropriate substrate is not made available until cleavage of it is required. For instance, a lytic transglycosylase could cleave the glycosidic bond in PG, making the cross-linking peptide accessible to an endopeptidase, or vice versa. This cooperation between antagonistic enzymes is in line with the current models of PG synthesis, where new bonds are made before old ones are broken. The question remains as to whether or not these PG enzyme complexes are the main mechanism for regulating their individual and overall activity.

Do Mycobacteria Recycle Cell Wall Material?

E. coli breaks down nearly 50% of its PG every generation (156, 157). If this material were lost each generation, a tremendous amount of energy and nutrients would be wasted. Instead, *E. coli* and many other gram-negative bacteria recycle the breakdown components. In the case of *E. coli*, the membrane protein AmpG is responsible for transporting the disaccharide peptides back into the cytoplasm (244). This process can also be used as a means of inducing β -lactamase, an enzyme capable of breaking down β -lactams, such as penicillin (197), and allows the bacterium to

monitor the status of its cell wall. The degree to which PG material is sloughed off by bacteria can greatly affect the host response to the infection. For instance, release of PG from *Bordetella pertussis* (153), *Neisseria gonorrhoeae* (274), *Helicobacter pylori* (423), and *Vibrio fischeri* (223) acts to stimulate inflammatory processes by binding specific pathogen recognition molecules.

It is traditionally thought that gram-positive bacteria do not recycle PG as it is broken down (312), since, without an outer membrane to hold in components, the released PG fragments diffuse away from the bacterium. However, in gram-positive bacteria like mycobacteria, with thick lipid-containing outer walls, the idea that they may have a functional "pseudo-periplasm" challenges the assumption that they cannot recycle PG fragments. A mycobacterial AmpG homologue has not yet been identified and characterized, but some transport of the PG material back into the cytosol would be needed. It should be noted that mycobacteria do have β -lactamases, enzymes associated with cell wall monitoring and remodeling (136, 137, 271, 434).

How Do Antibiotics Target the Cell Wall?

Most of the antibacterial therapeutics developed for mycobacteria target components of the cell wall. Ethambutol inhibits the polymerization step of AG synthesis (280). Isoniazid is a prodrug activated by mycobacterially encoded KatG catalase in the cell wall that, when active, inhibits MA synthesis (461). Ethionamide is a prodrug that, upon activation, inhibits fatty acid synthesis required for MA synthesis (14). The standard treatment for tuberculosis is 6 to 9 months of isoniazid, rifampin, pyrazinamide, and ethambutol, compounds developed in the 1950s and 1960s (300). A better understanding of mycobacterial cell wall synthesis will likely lead to new drug targets.

MAINTENANCE OF SHAPE

Why Are Mycobacteria Rods?

When choosing a shape, bacteria have a plethora of options, but why choose one shape over another? What are the physiological consequences of these different shapes, and what effect does shape have on the bacterium's biology? The decision as to why to assume one shape over another is likely guided by several factors, including nutrient acquisition (increased surface area-to-volume ratio increases diffusion of nutrients, which is important in a low-nutrient environment), motility (localization of flagella at one end of rod and recruitment of host proteins to form actin tail), attachment (localized adhesion molecules and structures), proper partitioning of genetic material (symmetry increases the likelihood of accurate division of chromosome and cytoplasm), and localization of complex structures (secretion apparatuses) (453). But what determines and maintains these shapes?

What Is the Role of PG in Determining Cell Shape?

The highly cross-linked glycan meshwork of PG that surrounds most bacteria is the primary agent that maintains bacterial shape. It is still debatable whether PG also determines

bacterial shape. That having been said, the mollicutes, which are related to gram-positive bacteria, have no cell wall or PG (they use cholesterol-containing cell membranes instead), yet they still maintain complex shapes (55), probably through use of several cytoskeletal proteins (227, 435).

PG isolated from *E. coli* retains its rod-like shape even in the absence of all other material (440, 441), confirming its role in shape maintenance. Also, treatment of bacteria with lysozyme, which degrades PG, results in rod-shaped cells becoming round spheroplasts (232, 439). Spheroplasts, or round bacteria lacking PG, can be formed in *M. smegmatis* through degradation of PG (417). Upon transfer to growth media, the spherical bacteria are able to regenerate wild-type rod-shaped cells. This occurs through elongation of bacteria that then branch, septate, and fragment (417). These data argue that shape and size are not simply governed by existing PG but that there is likely also a heritable, genetic shape and size determinant.

If PG maintains the shape of the bacterium, what determines the shape of PG? The logical answer is that the enzymes that synthesize the PG determine its shape. Much attention has been paid to the enzymes responsible for synthesizing and modifying PG, and many effective drugs target these proteins. Deletion or mutation of a number of these enzymes individually, but more often in combinations, can result in shape changes (55, 452). Also, loss or alteration of components attached to the PG can affect shape, such as in *B. subtilis*, where loss of teichoic acid or lipoteichoic acid leads to altered morphology (32).

How PG-synthesizing enzymes organize into complexes likely contributes to the resulting shape. There are many models for how this organization affects shape. The “two-competing sites” model advocates that, in rods, one complex of enzymes is involved in PG elongation, while another set generates the septum between two daughter cells before cell division (1). This model argues for the two reactions to be specialized and spatially exclusive, such that when elongating, septation would be inhibited and vice versa (245, 362). The “three-for-one” model and others predict the insertion of PG along a track, using an existing strand of PG as a template. This results in doubling the length in one direction, but since following a strand, no additional length is added in the direction perpendicular to the strand. Thus, width would stay constant (188). Another theory as to how cells maintain a constant width posits that the poles are capped with a type of PG that prevents rapid turnover or insertion of new PG (105). Thus, the caps would restrict the width of the bacterium.

Where Does Nascent PG Localize?

If synthesizing new PG determines the resulting shape of the bacterium, then it would be useful to know where PG is being synthesized and inserted (Fig. 3). One way to ascertain where new PG is being inserted into the cell wall is to measure the turnover of PG in the cell by pulsing cultures of bacteria with the abnormal amino acid D-cysteine, which will be incorporated into the newly generated PG, and then visualizing by immunodetection of the -SH groups throughout the entire cell. Measurement of PG turnover in *E. coli* revealed patches of inert, stable PG (105) at the poles and newly formed septa, as

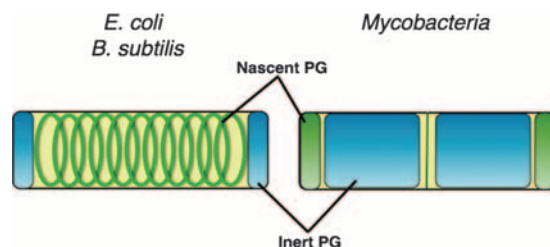


FIG. 3. Localization of nascent and inert PG in different bacteria.

if capped and protected from turnover. The switch from active to inert PG is thought to occur at the septum, as it is being produced, but it is unknown what causes this switch and the nature of the new, inert PG (105). A more recent technique, staining nascent PG in *B. subtilis* using the fluorescently labeled PG-binding antibiotic vancomycin (Van-FI) or ramoplanin, has been developed. Van-FI specifically binds D-Ala-D-Ala, which is found specifically in newly generated PG, while fluorescently labeled ramoplanin binds initiation sites of PG synthesis and lipid II. These techniques revealed helical patterns of nascent PG similar to those of localized MreB and Mbl (88, 406). It is conceivable that PG is not truly inert but that active enzyme complexes are restricted from certain areas and thus there is a lack of turnover in those areas.

Is Inert PG Involved in Branching?

Some mutations cause bacteria to form branches (170, 231, 299, 303), a phenomenon also seen in mycobacteria (121, 154). Depletion of a PG hydrolase in *M. smegmatis* results in long, branched chains of cells (182). In *E. coli*, PG at the tips of these branches is inert, similar to that of the original poles (106). The current model is that inert PG is inappropriately inserted into the side walls of mutant strains of bacteria, resulting in the inert patch being pushed out and eventually becoming another pole (106, 452). Some bacteria branch, while others, such as *B. subtilis*, have not been found to branch. It remains to be seen if there are patches of inert PG in mycobacteria similar to those seen in *E. coli* (105).

What Is the Role of the Cytoskeleton in Shape?

In eukaryotes, cytoskeletal proteins play a critical role in cell shape. This may also be true in bacteria, where recently discovered cytoskeleton-like proteins have been shown to be involved in DNA segregation, cell polarity, sporulation, and shape determination (Table 2).

Tubulin. The first cytoskeleton protein discovered in bacteria was the tubulin-like protein FtsZ, depletion of which from bacteria resulted in long filamentous cells (248). FtsZ was found to hydrolyze GTP (95, 293, 342), assemble into filaments in vitro (49, 294), and form a ring structure at the midcell in bacteria (34). Recent evidence suggests that FtsZ also forms dynamic helices in bacteria (403). The ring formed by FtsZ (Z ring) involves the highly ordered recruitment of both structural and enzymatic proteins involved in PG synthesis and thus formation of the septum (263). This recruitment has been suggested to be a determinant of cell width and thus of the shape of the cell (55). FtsZ might also direct the synthesis and local-

TABLE 2. Bacterial cytoskeletal elements

Cytoskeletal element	Protein	Function	<i>M. tuberculosis</i> homologue(s)
Tubulin	FtsZ	Forms a structural ring that recruits proteins necessary for the synthesis of septal PG	FtsZ (Rv2150c)
Actin	MreB (Mbl, MreBH) ParM MamK	Cell shape, guides PG synthesis DNA partitioning Organelle positioning	None ParA (Rv3918c), ParB (Rv3917c) ?
Intermediate filament	CreS	Determines the specialized crescent shape	None

ization of patches of inert, or stable, PG that give rise to specific morphologies (452). Thus, FtsZ guides the synthesis of septal PG and does this through the recruitment of membrane proteins that stabilize and bridge the interaction with periplasmic PG-synthesizing enzymes. PBP 3 and FtsW might be part of a septum-specific complex (48, 275). In *M. tuberculosis*, proteins FtsZ and FtsW have been shown to directly interact, an association mediated through cytoplasmic tails found uniquely in mycobacteria (92), suggesting that, in this genus, FtsW might play an important role.

Intermediate filaments. Recently, the first intermediate filament-like protein, CreS, was discovered in *Caulobacter crescentus* (6). This protein is localized to the inside curve of the crescent-shaped bacterium and plays a major role in determining the specialized shape (6). There is no evidence for the presence of intermediate filaments in mycobacteria.

Actin. Recently, much attention has been focused on the actin-like cytoskeletal proteins, including MreB (cell shape) (203), ParM (DNA partitioning) (142, 143), and MamK (organelle positioning) (221). MreB forms microfilament-like helical strands in vitro (419) and helical coils along the bacterial inner cell membrane (133, 203, 376, 390). Mbl and MreBH have been shown to be important for rod shape in *B. subtilis* (59, 88), theoretically directing the incorporation of PG along the lateral wall by recruiting PG-synthesizing enzymes. The recruitment of PG enzymes by MreB homologues has been shown in *B. subtilis*, where localization of the hydrolytic enzyme LytE was shown to be dependent on MreBH (59). How would the cytosolic MreB guide the periplasmic PG-synthesizing enzymes? It may be that, similar to the case for FtsZ, this recruitment of PG enzymes is mediated through transmembrane bridging proteins (55, 110). Most of the coccoid-shaped bacteria, such as *Streptococcus*, *Staphylococcus*, and *Lactococcus* spp., lack MreB family proteins, suggesting that these proteins are particularly important for bacteria with more complex shapes (203).

The MreB family of proteins are conspicuously absent from the rod-shaped actinomycetes, except for those species that generate spores through aerial hyphae (269). *Streptomyces coelicolor* encodes two orthologues of MreB. While formation of normal aerial hyphae and mature spores required the presence of MreB, vegetative growth was not affected in an MreB null mutant (269). The same is true for *S. coelicolor* FtsZ, which can be deleted without an effect on vegetative growth, but irregular aerial hyphae and immature spores result (164). *S. coelicolor* is unusual in that FtsZ and MreB are not essential for viability (164, 269, 270). The similar phenotypes in strains lacking either MreB or FtsZ suggest that these cytoskeletal

proteins are in a similar cooperative pathway, coordinating PG synthesis in *S. coelicolor* and possibly other bacteria.

Why Do Mycobacteria Grow at the Tips?

S. coelicolor has been shown to grow at the hyphal tips, which are generated by branching or germ tube emergence (135, 165, 369) and thus are different than those resulting from the separation of daughter cells during cell division. Similarly, staining of nascent PG suggests that several species of mycobacteria and *Corynebacterium glutamicum* might grow at the tips, rather than through lateral-wall extension (88, 404). When mycobacteria were stained with Van-FI to localize nascent PG, staining was seen at the tips and septa of bacteria. Because most other rod-shaped bacteria encode MreB proteins and show helical staining of nascent PG, researchers theorized that cytoskeletal scaffolds that would normally guide lateral-wall extension are missing in many of the actinomycetes. Thus, the PG-synthesizing enzymes are not recruited to the lateral walls to make nascent PG and end up at the poles by default (88, 404). If the localization of PG-synthesizing proteins in mycobacteria is truly a passive event, proteins would most likely appear diffuse, throughout the cell. Instead, similar to their recruitment to the septum by FtsZ during division, these proteins may be retained by FtsZ at the poles during elongation. Finally, the DivIVA protein (discussed below in detail) localizes to the tips of *S. coelicolor* and plays a role in directing growth at the tips (135). Mycobacteria also encode a DivIVA homologue that could be involved in guiding tip-oriented growth.

What About Inert PG at the Tips?

Why are the poles actively growing in mycobacteria, while they are inert in other bacteria (Fig. 3)? It has been suggested that FtsZ may guide synthesis of inert PG at the septum that then remains at the poles after division (105). This theory conflicts with observations that mycobacteria actively grow from their tips. A possible unifying theory is that PG-synthesizing enzymes are inactive unless in a complex that has been recruited by a cytoskeletal scaffold, such as FtsZ (or MreB in some bacteria). In a genetic background with both scaffold proteins, the activity of PG synthesis complexes will alternate between MreB (elongation) and FtsZ (division), depending on other regulatory factors. However, in mycobacteria, FtsZ does not need to compete with MreB. The FtsZ complex has a lower rate of disassembly (445), which may allow it to remain assembled after separation of daughter cells. Since there is no MreB to competitively recruit away proteins for elongation, PG con-

tinues to be generated at the poles (88). If there is no lateral wall extension in mycobacteria, then is there only one enzymatic complex for synthesizing PG in mycobacteria, compared to the two (elongation and division) hypothesized to exist in other bacteria?

How Can Mycobacteria Maintain Shape without MreB?

One theory of what governs cell width, mentioned above, is that the bacteria place inert PG at the poles such that the poles are functionally capped and prevented from expanding. Thus, the bacterium can extend along the sides while maintaining a constant width. What about bacteria that grow from the tips? If the only growth is at the poles, what maintains width? Perhaps the functionally inert lateral walls now guide the width of the bacterium.

Alternatively, there may be actin-like proteins encoded by mycobacteria that have not yet been described, due to a lack of sequence homology. This would not be surprising, considering the paltry homology between actin and MreB (15%) or tubulin and FtsZ (17%) (57). Also, it remains to be conclusively demonstrated that mycobacteria grow exclusively at their poles and septa. Whether there is an anomaly to be explained or a novel actin-like protein to be described, there remain exciting findings to be discovered regarding mycobacterial growth and shape determination.

What Is Different about Mycobacterial Growth?

In summary, in many bacteria PG maintains bacterial shape and PG-synthesizing enzymes determine where nascent PG is inserted. Recent evidence suggests that cytoskeletal-like proteins recruit or guide the PG-synthesizing enzymes, determining the location of PG expansion and thus determining shape. The facts that mycobacteria appear to lack actin-like homologues and that mycobacteria grow at their tips imply that perhaps the tubulin-like FtsZ protein controls both expansion and septation. The idea that FtsZ-generated inert PG exists and plays a role in determining shape is intriguing, yet it conflicts with current theories of mycobacterial growth and will need further experimentation. It will be interesting to see if there is a difference between septal and lateral PG and what determines that difference. Finally, growth from the tips results in a confined, small region from which to expand and elongate the cell compared to the large area along the lateral walls. Does this spatially restricted expansion also restrict elongation rates? Also, what about polarly located proteins? Does polar growth mean that polar proteins constantly relocate? It seems more likely that growth would occur near the tips, where the lateral wall begins to curve, but not immediately at the tips.

BACTERIAL CELL DIVISION

What Is the Order of Events in Cell Division?

At the most basic level, cell division involves segregating replicated DNA and dividing the cytoplasmic material in such a manner as to generate progeny with identical genetic material. To do this, bacteria must coordinate events spatially and temporally. That is, they must constantly decide where and when to divide and then coordinate those decisions (322).

After replication has begun, but before the chromosomes are completely partitioned, the divisome will assemble at midcell, assist in the final separation of DNA, synthesize PG de novo, constrict, and finally digest the layer of PG separating daughter cells, releasing two new cells. There are often multiple, complementary mechanisms in place to prevent assembly of the divisome too early or in the same location as the chromosome. It is critical that segregation and septation are tightly coordinated, since once the septum is completely formed, there is no chance to fix mistakes. While much has been elucidated regarding assembly of the divisome, the functions of many individual proteins have yet to be determined. In addition, the process of constriction and where the force for this process originates are still unknown. In mycobacteria, only a few division proteins have been studied, with the majority of work having been done with FtsZ. There are similarities and anomalies yet to be worked out.

How Does the Chromosome Replicate?

DNA replication and chromosomal segregation in *E. coli* have been thoroughly reviewed by Bartosik and Jagura-Burdzy (17). The basic mechanisms appear to be similar for mycobacteria. Replication begins at a specific stage of the cell cycle, stage C, which then progresses to cell division at stage D and then through stage G₁, which encompasses the time after cell division to DNA replication (180). Typically, the majority of bacterial DNA is contained within a single, circular chromosome, while the remainder exists in small plasmids. However, *Streptomyces* spp. contain one large linear chromosome (135).

The general steps of chromosomal replication occur as follows.

(i) The initiator protein, DnaA, binds to a defined site on the chromosome, known as *oriC* (251, 277). Conditional depletion of DnaA in *M. smegmatis* blocks cell division, while overexpression yields a loss of synchrony between DNA replication and division and thus multinucleoid cells (166).

(ii) Histone-like proteins such as HU and integration host factor help unwind the DNA (238).

(iii) DnaC delivers DnaB, a helicase, to the unwound DNA (442).

(iv) DnaG, a primase, forms the initial primer (354) and has been shown to be essential in *M. smegmatis* even though it resides in a different locus than in other bacteria (216).

(v) The replication machinery is sequentially loaded onto the unwound DNA, including the DNA polymerase holoenzyme, Pol III (13).

(vi) Replication proceeds bidirectionally from *oriC*, with two replication forks traveling in opposite directions. The replisomes are thought to be anchored near the cell center, with the DNA passing through the machinery (23, 234–236).

(vii) The replication machinery arrives at the termination sequence, *ter* (430), directly opposite *oriC*, or halfway around the chromosome, and the machinery disassembles.

(viii) The replicated chromosomes are often linked and must be resolved prior to complete partitioning. In *E. coli*, this is usually accomplished by topoisomerase IV decatenating the DNA (210). FtsK, or SpoIIIE in *B. subtilis*, binds topoisomerase IV and assists in decatenation (129). Also, dimers are

resolved by the XerC and XerD site-specific recombinases (40).

What Segregates the Chromosome?

Besides being replicated, the chromosome also needs to be separated into the two developing daughter cells. This process of chromosome segregation occurs concomitantly with replication, beginning soon after replication initiates. The new and old *oriC* regions of DNA are each rapidly moved to opposite poles, with the remaining DNA following, once replicated (161, 448). While some of the genes involved in chromosome segregation are known, the overall mechanism of DNA movement during cell division is not yet understood (57). There are multiple models for the generation of force needed to move the chromosome to the poles (120, 160, 234–236, 363).

While *E. coli* uses MreB, other bacteria have had to devise different solutions. For example, in *S. coelicolor*, the ParA/ParB system is involved in segregation of its linear chromosome (215). Mycobacteria encode the ParA/ParB homologues and thus may use this system to segregate replicating chromosomes to the poles.

Additionally, FtsK, which is targeted to FtsZ and the divisome by its N terminus (457), may use its cytoplasmic C-terminal Walker-type ATP-binding sites (126) to help translocate any residual DNA out of the septum to avoid dividing across unseparated DNA (7). The FtsK orthologue in *B. subtilis*, SpoIIIE, has been shown to translocate the replicated chromosome into newly forming spores (373, 374, 449).

What Are the Divisome Components, and How Do They Assemble?

Once the chromosomes have begun to be partitioned, the divisome begins assembling at the middle of the cell. The divisome consists of a set of 10 to 15 proteins that are recruited to the middle of the cell and are responsible for generating the septum that divides two daughter cells (Table 3). This is accomplished by synthesizing septal PG, constricting the cell wall to eventually close off the cytoplasmic compartments of each daughter cell, and finally hydrolyzing part of the PG that holds the two together in order to physically separate the cells. While assembly of this divisome has been thought to be strictly linear and hierarchical in *E. coli* (54), compared to a process of multiple proteins assembling at once in *B. subtilis* (128), recent evidence has blurred the distinction (53, 79, 109, 149–151, 209). In fact, of the 10 or so proteins essential for assembly, all of them interact with at least one other divisome component (109, 209, 424, 425). Because of the newly appreciated similarity between these two model species, this review will use *E. coli* for reference and mention differences that occur between other species, with special attention to the actinomycetes.

The tubulin-like protein FtsZ is the first protein known to assemble at midcell (34). Its formation of a ring around the cell, just under the plasma membrane, gives the assembled divisome the name Z ring. At least one of the two membrane-associated proteins ZipA (ZapA in *B. subtilis*) and FtsA (actin like) is required for proper assembly (173, 339). This “protoring” associates with the large multifunctional membrane protein, FtsK (SpoIIIE in *B. subtilis*), which is involved in Z-ring

stability as well as DNA segregation (35). Next, the members of a tripartite complex of integral membrane proteins, FtsQ, FtsB, and FtsL, are thought to first associate with each other and then assemble into the Z ring (53, 89, 150, 349, 380, 381). These proteins likely help bridge the cytoplasmic components with the periplasmic components (53, 424). Next, the periplasmic proteins FtsW (believed to transport PG precursors) and FtsI (septation-specific transpeptidase) assemble, possibly as a complex, and are thought to be critical for synthesis of the septal PG (188). Finally, FtsN, which has weak homology to an amidase (128) and binds PG (418), assembles (425). Besides these 10 proteins that are essential for a functional Z ring in *E. coli*, a number of other proteins localize to the septum and play various roles. The PG-degrading enzymes AmiC (amidase) and EnvC (endopeptidase) as well as other hydrolytic enzymes are recruited late to the septum to degrade septal PG and allow separation of daughter cells (24, 26). FtsE and FtsX belong to the ABC transporter family (98, 416) and are essential under conditions of high osmolarity (344), though they may play a role in assembly of the Z ring instead of transport (425). FtsE was found to localize to the membrane of *M. tuberculosis*; bound ATP *in vitro*; and, when coexpressed with FtsX, complemented an *E. coli* FtsE mutant (282). While not exclusive to cell division, the bifunctional PG-synthesizing enzymes PBP 1a and PBP 1b are important for septal PG synthesis (189).

What Is Missing in Actinomycetes?

The FtsZ-stabilizing proteins FtsA and ZipA were both thought to be absent from mycobacteria, though recent work identified two potential orthologues of ZipA in mycobacteria (386) (Table 3). In other bacteria, these proteins are thought to associate with both the membrane and FtsZ, helping to guide and stabilize polymerization (173, 304, 317, 339). In mycobacteria, FtsW localizes to the septum (329) and interacts with FtsZ through unique cytoplasmic tails (92). Furthermore, a mutant version of FtsZ, lacking these unique C-terminal residues required for interaction with FtsW, was not able to complement an FtsZ depletion strain, suggesting the importance for this interaction (329), although deletion of these residues could also have unintentional effects. It is possible that the interaction between FtsZ and FtsW is able to compensate for the lack of other FtsZ-stabilizing proteins. However, FtsW, unlike ZipA and FtsA, is recruited late to the septum (329). Therefore, it is unlikely that FtsW is able to stabilize the early formation of the Z ring. Interestingly, transcriptional profiling of *M. tuberculosis* treated with FtsZ inhibitors revealed several putative orthologues of *E. coli* cell division proteins: ZipA-like Rv2345 and Rv3835, originally annotated as conserved hypothetical proteins; MinD-like Rv1708, a putative initiation inhibitory protein; and Rv3660c, a putative septum site-determining protein (386). The function of these has yet to be confirmed.

FtsW has also been shown to interact with the transpeptidase protein FtsI in mycobacteria through two extracytoplasmic loops that are conserved across species of bacteria (93), suggesting the possibility that this interaction occurs in other bacteria. Depletion of FtsW in *M. smegmatis* results in a blockage of septation and inhibition of localization of FtsI to the

TABLE 3. Divisome proteins encoded in different bacterial genomes

Divisome ^a	Function	Homologue ^b in:			
		<i>E. coli</i>	<i>B. subtilis</i>	<i>S. coelicolor</i>	<i>M. tuberculosis</i>
FtsA	Positive regulator of FtsZ assembly, membrane associated, actin like	+	+	–	–
FtsB	Bridge cytoplasmic with periplasmic, transmembrane, forms tripartite complex with FtsL and FtsQ	+	DivIC	DivIC	?
FtsE	Unknown, ABC transporter family	+	+	–	+
FtsI	Synthesize septal PG, transpeptidase (PBP 3)	+	+	+	+
FtsK	DNA partitioning, large multifunctional membrane protein	+	SpoIIIE	+	+
FtsL	Bridge cytoplasmic with periplasmic, transmembrane, forms tripartite complex with FtsB and FtsQ	+	+	+	–
FtsN	Unknown, weak homology to amidase, binds PG	+	–	–	–
FtsQ	Bridge cytoplasmic with periplasmic, many protein-protein interactions, transmembrane	+	DivIB	+	+
FtsW	Predicted transporter of PG precursors, multitransmembrane protein	+	+	+	Interacts with FtsZ and FtsI and may substitute for lack of ZipA or FtsA
FtsX	Unknown, ABC transporter family	+	?	–	+
FtsZ	Initiates Z ring, helps constrict ring, tubulin like, GTPase	+	+	+	+
ZipA	Positive regulator of FtsZ assembly, membrane associated	+	ZapA	–	–
AmiC	Separates daughter cells by hydrolyzing septal PG, amidase	+	?	?	?
EnvC	Separates daughter cells by hydrolyzing septal PG, hydrolase	+	?	?	?

^a FtsH, FtsJ, and FtsY are not involved in cell division (FtsY has pleiotropic effects on cell division).

^b +, present in genome; –, not found in genome; ?, other homologues may exist.

midcell (93). FtsW may bridge FtsZ and FtsI, since the interaction between FtsW and FtsZ strengthens the interaction between FtsW and FtsI. They may form a ternary complex that likely regulates septal PG synthesis and Z-ring formation through stabilizing assembly in the absence of ZipA or FtsA (93).

The actinomycete *C. glutamicum* also lacks FtsA and MreB actin-like homologues important for shape determination. A 4-fold decrease in FtsZ was found to result in aberrant cells with buds, branches, and knots, but no filaments, while a 20-fold decrease yielded large, club-shaped cells (333). Promoter analysis of the *M. tuberculosis* *ftsZ* allele revealed up to six promoters, indicating a high level of transcriptional regulation available as well as the possible need for elevated basal levels of FtsZ in mycobacteria (355). Overexpression of FtsZ in *M. smegmatis* and *M. tuberculosis* interferes with cell division, with a 6-fold excess resulting in longer cells with multiple septa and a 20-fold excess yielding filaments without septa (121).

It is unclear what role actin-like proteins play in cell division. MreB condenses to the septum during cell division in an FtsZ-independent process in *B. subtilis* (57–59) and in an FtsZ-dependent process in *C. crescentus* (133, 147). This may signal a switch from lateral wall PG synthesis to synthesis of septal PG. Lacking MreB-like proteins and growing from the poles, mycobacteria may have only one complex for PG synthesis and thus would not require such a switch.

What Are the Negative Regulators of FtsZ Assembly?

Transcription of FtsZ has been shown to be temporally regulated in *E. coli* and mycobacteria (144), yet an excess of the cytosolic FtsZ is often present in bacteria without polymerization of FtsZ (351). Thus, transcriptional regulation alone is unlikely to temporally regulate Z-ring assembly. This may be different for *C. crescentus*, where FtsA and FtsQ have been shown to be specifically synthesized when required for assembly and then rapidly degraded when no longer needed (266). There are several negative regulatory systems described thus far (Table 4) that ensure that Z-ring assembly is neither at the poles, where unequal partitioning of DNA would occur, or on top of nucleoids, where chromosomes would be decapitated (340, 341).

Min system. The first discovered *ftsZ* regulatory system is named for the minicells, or cells without nucleoids, generated by a group of mutants (96, 345). *E. coli* encodes three proteins that work together, MinCDE. MinC inhibits FtsZ assembly and has been found to oscillate from pole to pole (193, 335, 336). MinC binds to MinD, an ATPase that tethers MinC to the polar membrane when bound to ATP (192, 194). MinE forms a ring that oscillates toward one pole and then to the other (337). It effectively sweeps the MinCD complex from one pole to the other by activating the ATPase of MinD, thereby causing MinD to hydrolyze ATP and thus release from the polar membrane (249, 462). The net result from this negative regulator oscillating from pole to pole is a gradient of inhibi-

TABLE 4. Inhibitors of FtsZ assembly

Inhibitor	Function	Homologue ^a in:			
		<i>E. coli</i>	<i>B. subtilis</i>	<i>S. coelicolor</i>	<i>M. tuberculosis</i>
EzrA	Negative regulator of FtsZ assembly, topology similar to that of ZapA	?	+	?	—
MinC	Negative regulator of FtsZ assembly at poles, binds and oscillates between poles with MinD	+	+	—	—
MinD	Oscillates MinC from pole to pole, ATPase, binds to membrane until MinE stimulates ATP hydrolysis	+	+	—	—
MinE	Sweeps MinCD complex from one pole to the other, stimulates MinD ATPase and release of MinCD from membrane	+	DivIVA; rather than oscillating between poles, remains at poles, tethering MinCD complex	DivIVA	DivIVA orthologue, Ag84, encoded by <i>wag31</i>
SulA	Negative regulator of FtsZ assembly, induced by SOS response	+	YneA, structurally unrelated to SulA but functionally similar	?	—
SepF	Unknown, interacts with FtsZ and localization depends on FtsZ	?	+	?	?
SlmA	Negative regulator of FtsZ assembly, prevents septum assembly over nucleoid (nucleoid occlusion)	+	—	?	?
Noc	Negative regulator of FtsZ assembly, prevents septum assembly over nucleoid (nucleoid occlusion)	?	+	?	?
CrgA	Negative regulator of FtsZ assembly	—	—	+	+ (Rv0011c)

^a +, present in genome; —, not found in genome; ?, other homologues may exist.

tion that is highest at the poles and least at the midcell (335, 336). Thus, the Min system directs the assembly of the divisome away from the poles, avoiding production of nonfunctional minicells. It has recently been appreciated that the FtsZ molecules that are not assembled into a ring form a helical pattern that may oscillate in a Min-dependent manner that is independent of MreB (403). Similarly, Min proteins form helical patterns that oscillate between poles (376).

B. subtilis lacks MinE but encodes DivIVA instead, which tethers MinCD complexes to each pole, rather than oscillating back and forth (265). *B. subtilis* also uses DivIVA to localize or attach the *oriC* to the poles during sporulation (127, 405). While many bacteria encode the Min system, mycobacteria, streptomycetes, and *C. crescentus* do not have orthologues of MinC or MinD (though recently MinD-like proteins have been suggested to exist in mycobacteria [386]). Mycobacteria and streptomycetes encode orthologues of DivIVA, likely the essential Ag84 in mycobacteria, encoded by *wag31* (181, 361). In *S. coelicolor*, DivIVA is essential for hyphal tip growth (134). Depletion results in irregular hyphae and branching, while overexpression gives rise to many new sites of PG synthesis and hyperbranching (134). DivIVA localizes to the tips and lateral branches of *S. coelicolor* (134).

As mentioned, both streptomycetes and mycobacteria are thought to synthesize PG de novo at the tips (165, 278, 404). For streptomycetes, cell division generates new poles, but unlike in mycobacteria, these poles do not elongate. Instead, new branches form in the lateral wall and extension occurs from these sites (134). This polar growth requires targeting of cell wall synthesis material and machinery to the tips, rather than

localizing them along the lateral wall, as in other bacteria. It appears that DivIVA functions differently in streptomycetes, and possibly mycobacteria, than in *B. subtilis*. It may play a role in determining cell polarity necessary for growth at the tips, providing scaffolding or cytoskeletal functions at the pole in the absence of other known cytoskeletal elements, or suppressing other branch formation during growth at the existing tips (134).

Nucleoid occlusion. Some bacteria also utilize a negative regulator of FtsZ assembly known as nucleoid occlusion. Specific proteins that are able to inhibit FtsZ assembly appear to bind to the nucleoid and thus prevent assembly of a functional divisome over a nucleoid (25, 450, 456). In *E. coli* SlmA and in *B. subtilis* Noc have been shown to prevent such guillotining of nucleoids. This system not only determines spatially where FtsZ (25, 450) will assemble (not over nucleoids) but also coordinates temporally when FtsZ will assemble, since the midcell is relatively free of nucleoids only after much of the DNA has been replicated and segregated (100).

Nucleoid occlusion is not seen in *S. coelicolor* and *C. glutamicum* during divisome assembly (164, 333, 369). Using fluorescence microscopy, the images seem to show overlapping nucleoid staining and FtsZ-green fluorescent protein (GFP) fluorescence; however, it could be that the resolution of such imaging is unable to detect the initiation of chromosome partitioning that may have already begun in the bacteria. Guillotining of the chromosome can result in termination of the bacterium. Therefore, it is likely that some mechanism of nucleoid occlusion occurs in all bacteria. That being said, sporulating *B. subtilis* begins generating a spore septum and then translocates the chromosome into the spore prior to comple-

tion of the septum via the action of the FtsK-like SpoIIIE (449). It is possible that the spore-forming streptomycetes also do this, although the generation of spores in aerial hyphae is quite different, since multiple septa are generated at once along a long hyphal branch (135, 369). It is also possible that mycobacteria use FtsK to accommodate for early formation of septa due to lack of inhibition of FtsZ assembly, but this has not been shown. *C. crescentus* lacks nucleoid-free zones until chromosome segregation, thus negating the need for the Min system (264, 325). It is unclear whether mycobacteria have a similar strategy to avoid forming minicells, since they too lack the complete Min system.

How Does FtsZ Constrict?

As division proceeds, the Z ring constricts in such a manner so as to be at the leading edge of the septum. During constriction of *E. coli* cells, the septal PG is degraded concurrently with invagination of the cell membrane, such that the bacteria appear to pinch at the site of division. In mycobacteria and other gram-positive bacteria, invagination is not visible with the constriction of the plasma membrane. The generation of a septum is completed first, sealing off daughter cells, and then at some later time PG hydrolases digest part of the septal PG, releasing the two daughter cells (139). It seems reasonable that invagination is visible in gram-negative bacteria, as septal PG must be hydrolyzed simultaneously with the pinching of the outer membrane. In gram-positive bacteria, there is not an outer membrane that needs to be constricted in concert with the inner membrane. Thus, the inner plasma membrane can constrict with the Z ring and form intact separate membranes for each daughter cell without the need for hydrolysis of PG. Furthermore, the PG layer of gram-positive bacteria is considerably thicker than that of gram-negative bacteria, likely requiring more hydrolysis and possibly more time to hydrolyze. Mycobacteria have a unique division morphology, which will be discussed below.

It is unclear what generates the force necessary for the Z ring to constrict (128). It could be an active process, with the force generated directly by FtsZ. Perhaps sufficient force could be generated through the sliding of FtsZ filaments past each other in a ratcheting process (357, 443). Alternatively, constriction could be a passive process, with the synthesis of septal PG pushing together the narrowing ring. This seems unlikely, due to the ability of bacteria lacking PG to also constrict during cell division, but the production of a different cell wall material in these organisms may suffice. It is also possible that the ring shrinks due to a net loss of FtsZ molecules, facilitated by a rapid turnover of FtsZ (396, 403). This mechanism would be similar to that seen with eukaryotic dynamins, large GTPases that have many functions, including organelle division in eukaryotes (323). At this point, the source of force required for constriction as well as the overall process of constriction is poorly understood.

How Does FtsZ Polymerize, and Why Is Mycobacterial FtsZ So Slow?

FtsZ contains variable regions of unspecified function in the N terminus and a spacer sequence just before the C terminus. The C terminus has been shown to interact with other proteins, such as ZipA, FtsA, and FtsW (92, 173, 339). The core of FtsZ,

between the N terminus and the variable region, is required for binding and hydrolyzing GTP as well as assembling the protofilament (95). FtsZ first binds GTP and then assembles head to tail in a linear manner (294), with GTP stabilizing the FtsZ polymer (443). These filaments then form bundles or sheets, unlike eukaryotic microtubule formation (125, 246, 455). Hydrolysis of GTP results in depolymerization and is thought to be the rate-limiting step for the cycle of FtsZ assembly and disassembly in the Z ring (443). The ring formed by FtsZ is highly dynamic, with FtsZ rapidly cycling on and off the ring in *E. coli* and *B. subtilis* (4). Maintaining assembly of the ring requires energy, as evident by the rapid depolymerization of FtsZ when ATP is depleted from the cell (356). This rapid turnover, or flux, of FtsZ may not be important for division, since a GTPase-defective mutant (FtsZ84) *E. coli* strain showed much slower turnover yet normal division at 30°C (316).

The *M. tuberculosis* FtsZ (FtsZ_{TB}) protein has been crystallized (240, 241) and shown to have slow GTP-dependent polymerization as well as weak GTPase activity compared to other bacteria (49, 445). Furthermore, polymers of FtsZ_{TB} were found to be very stable (445). Two FtsZ_{TB} point mutants have been generated and tested in vitro and in vivo (328). A mutant affected in GTP binding was found to be defective for GTP hydrolysis in vitro and polymerization both in vitro and in vivo. A mutant with the GTPase activity abolished was found to have normal GTP binding and diminished polymerization in vitro yet could associate with the Z ring in vivo (328).

In an attempt to explain why FtsZ_{TB} has a 20-fold-lower rate of polymerization than FtsZ in *E. coli*, researchers found that a mutant FtsZ_{TB} protein missing the C-terminal 169 residues, retaining the first 210 residues, formed long polymers at a rate similar to that of *E. coli* (3). They theorized that the C terminus of FtsZ_{TB} may inhibit polymerization, imposing a lower rate. Others responded to this result with concern that the mutant protein and its fast polymerization would not be physiologically relevant, since the C terminus is known to interact with other Z-ring proteins (45). They also worried that the polymerization seen in vitro could be aggregation and that the mutant might lack GTPase activity (45). The in vitro concerns were addressed when it was shown that the mutant FtsZ_{TB} polymer was not an aggregate and retained GTPase activity (171). However, though not the stated aim of the authors (171), the argument that this is not physiologically relevant still stands. In fact, alanine replacement of a mere four aspartic acid residues in the C terminus, which were shown to be important for interaction with FtsW (92), resulted in a mutant FtsZ_{TB} that was unable to yield viable bacteria when it was the sole source of FtsZ (329), even though this mutant was able to polymerize at wild-type rates in vitro and localize to the Z ring.

FtsZ is essential in mycobacteria (41, 122, 124). *M. smegmatis* expressing wild-type FtsZ_{TB} is viable, while strains expressing either of the above-mentioned mutations as the only source of FtsZ_{TB} are nonviable (328). This is in contrast to findings for *E. coli* that strains with corresponding mutations in FtsZ were viable (33, 247, 295, 394). The findings for *E. coli* were interpreted to mean that wild-type levels of GTP binding and hydrolysis were not required for viability in *E. coli* (247, 295, 328, 394). Using the same logic for mycobacteria, the slow polymerization and GTP hydrolysis of wild-type FtsZ_{TB} in vitro may

accurately approximate the rates *in vivo* for mycobacteria, with any further reduction in activity resulting in nonviability.

Is Growth Rate Related to Mycobacterial Pathogenesis?

16S RNA sequences divide mycobacteria into two taxonomic groups. Interestingly, these two sets of bacteria have markedly different growth rates (392). Fast-growing mycobacteria, a group that includes largely saprophytic organisms, generally form colonies within 7 days. Most of the pathogenic mycobacteria are slow growers, including *M. tuberculosis*, *M. leprae*, *M. bovis*, and *M. avium*, taking from weeks to months to form colonies.

This observation has led some researchers to hypothesize a possible causal link between growth rate and virulence. It is difficult to test this theory, since many of the known variables affecting growth rate also affect known mechanisms of virulence. For instance, researchers grew BCG in fast- and slow-growth conditions and then measured transcript levels under each condition (28), and they found an association between slow growth and induction of the dormancy survival regulon, which overlaps with a similar response by *M. tuberculosis* grown in macrophages. However, the conditions used to vary the growth rate included low carbon levels in the medium, which is already known to affect the dormancy regulon. Genetic modifications of strains or changes in the growth conditions will likely result in a variety of bacterial responses, thus confounding any conclusions.

Currently, it is not known which, if any, genes specifically limit the rate of growth of pathogenic mycobacteria. However, it is unlikely that a single gene will be found to control growth rate, since signaling and metabolic pathways are so interconnected that many of the components of a pathway likely have kinetics matched to cellular growth to prevent a buildup of unnecessary products and substrates in a pathway. Thus, mutations in these growth-regulatory genes would likely produce pleiotropic effects, and it would be difficult to disentangle changes in metabolism from alteration in virulence. For now, the relationship between growth rate and virulence will remain indirect, though intriguing.

What Hydrolyzes the Septal PG?

Once the septum is formed (or while it is forming, depending on the bacterium), a portion of the PG connecting the two daughter cells must be removed. This process is accomplished by several PG hydrolases with overlapping functions. For instance, in *E. coli*, there are 18 known hydrolases with clear redundancy. Given this large number, it has proven to be difficult to assign distinct functions to individual proteins (102). It is thought that most of the known hydrolases are involved in septum cleavage during division (177, 178, 187, 189).

Few hydrolases in mycobacteria have been characterized. The first characterized cell wall hydrolase of *M. tuberculosis*, known as CwlM, has been shown to hydrolyze PG substrates but awaits further analysis (101). A bicistronic operon encoding two predicted PG hydrolases was shown to be essential in *M. marinum* for virulence in zebra fish and invasion of macrophages (141). These proteins contain a C-terminal predicted endopeptidase domain that shares homology with the *Listeria*

monocytogenes p60 protein, which has been shown to hydrolyze cell wall material and to be important for separating daughter cells. On the basis of sequence homology, p60 is predicted to encode an LD-endopeptidase able to hydrolyze D-glutamyl-meso-DAP (43). The p60 domain is part of an NLPC-p60 family as well as the CHAP domain family (18), a family of PG hydrolases that contain two invariant cysteine and histidine residues. Strains of *Listeria* with mutations in p60 were also found to be deficient for invasion of macrophages, likely due to a growth defect and an inability to recruit actin (318). *M. tuberculosis* has a similar operon (Rv1477 and Rv1478), of which Rv1477 has been shown to be a cell wall hydrolase (183) and is essential for vegetative growth (361). The Rv1477 orthologue in *M. smegmatis* is also essential for growth, where depletion results in large chains of highly branched cells (182).

Rv2719c of *M. tuberculosis* has been shown to be a cell wall hydrolase that localizes to Van-Fl labeling of nascent PG (71). Rv2719c is highly induced in response to DNA-damaging agents and blocks cell division when overexpressed (71). It has weak homology to YneA of *B. subtilis*, which is an inhibitor of cell division that is induced upon DNA damage (211). Rv2719c does not inhibit FtsZ polymerization *in vitro*, but it may indirectly inhibit FtsZ polymerization *in vivo* (71).

What Is the V-Snapping Process of Dividing Mycobacteria?

Mycobacteria are known to form a V shape during the late stages of cell division (397), as shown more recently in outstanding micrographs (85). These V-shaped bacteria were found to have nascent PG deposited at the poles and the exterior side of the V (404). However, these sites may be more accessible to the Van-Fl stain used to probe for nascent PG and thus could be misleading. The final stage of mycobacterial cell division is referred to as snapping, since the cells form V shapes that then break into two daughter cells (Fig. 4). What causes this unusual morphology during division? It is thought that the plasma membrane and PG form the septa during Z-ring constriction, but the thick layers of AG and MA and other lipids remain intact during this stage. When the cell divides by hydrolyzing the PG linking the daughter cells together, the outer layers are still intertwined. The V-shaped splitting of cells results from an uneven snapping, or rupturing, of these outer layers (397, 404). This rupturing of outer material could also be aggravated polar growth occurring from the newly formed septum prior to completion of cell division (115).

What Is Different about Mycobacterial Cell Division?

In summary, mycobacteria undergo the same general processes during cell division as most bacteria. However, proteins that are key to these processes in other bacteria have not been identified in mycobacteria. For instance, FtsA (and perhaps ZipA) is not present to assist in early Z-ring formation, though the FtsZ-FtsW interaction may compensate for this deficiency. Also, the MinCD system seems to be absent, though a recent report suggests a possible homologue of MinD. It has been suggested that other actinomycetes lack a functional nucleoid occlusion mechanism. Do mycobacteria also lack this system, or can CrgA or other FtsZ negative regulators not yet identified prevent occlusion? Mycobacterial FtsZ polymerizes 20-

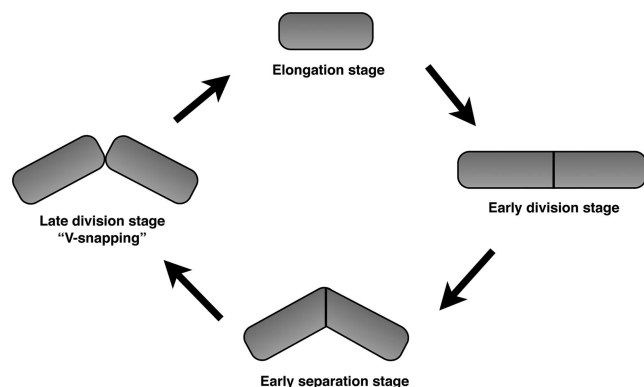


FIG. 4. V-snapping process of mycobacteria during cell division.

fold slower *in vitro* than *E. coli* FtsZ. Is this true *in vivo*? Does this slow polymerization allow FtsZ to remain at the poles after division to guide the unusual polar growth reported for mycobacteria? Understanding these fundamental questions may help us understand more complex processes such as pathogenesis and virulence.

REGULATION OF BACTERIAL GROWTH AND DIVISION

The mechanisms of eukaryotic cell cycle regulation are well described, yet bacterial cell cycle regulation remains poorly understood. This section will cover regulation of growth and division by serine/threonine protein kinases (STPKs) and WhiB proteins. Regulation of growth and division under specific conditions, such as nutrient stress, will be left for the following section.

What Are STPKs, and How Do They Work?

The reversible phosphorylation of proteins is an important mechanism for many organisms to receive and transfer signals from the environment (207). Once thought to exist primarily in eukaryotes, new bacterial protein kinases have been revealed with each genome sequenced. One of the most common kinase types found in mycobacteria is the STPKs (9, 237). It is clear that STPKs are sensors of environmental signals that regulate host-pathogen interactions and developmental changes (298), yet there is limited understanding of mycobacterial STPKs. *M. tuberculosis* encodes 11 Ser/Thr kinases, PknA to -L (Table 5) (9), all of which are from the PKN2 family (76) and three of which, PknA, PknB, and PknG, are thought to be essential (132, 361).

STPKs generally phosphorylate proteins containing fork-head-associated (FHA) domains. FHA domains mediate protein-protein interactions through recognition of phosphorylated threonine residues and often act as mediators of protein-protein interactions in STPK signal transduction (119). Most of the Pkn proteins also catalyze autophosphorylation and therefore are active without sensor domains (10, 61, 159, 225, 284, 285). Besides PknG and PknK, which are soluble proteins, all other STPKs of *M. tuberculosis* are predicted to be transmembrane, receptor-like proteins (76).

PknA and PknB. While *M. tuberculosis* has 11 STPKs, *Streptococcus pneumoniae* and *B. subtilis* have only one or two STPKs, respectively, and both are most similar to PknB (207). The structure of PknB was the first STPK structure of mycobacteria to be solved and was found to be similar to those of eukaryotic STPKs (306, 454). PknB has three to six autophosphorylation sites (42, 306, 454).

Both PknA and PknB have recently been shown to be involved in regulation of cell shape (207). These two kinases reside in an operon (Rv0014c-Rv0018c) that is conserved across mycobacteria and includes PstP, the cognate phosphatase of PknA and PknB (42, 73); RodA, involved in cell shape; and PBPA, a PG-synthesizing enzyme. While overexpression of the kinases resulted in slow growth and altered, bulbous shapes, depletion yielded narrow, elongated cells (207). These changes in morphology and the observation that the kinases are more highly expressed during exponential growth than in stationary phase suggest a role for the kinases in regulating active growth and shape determination (207). The authors identified the preferred (S/T)Q substrate for PknA and PknB using an *in vitro* peptide library screen and then used a phospho-(S/T)Q antibody to probe for *in vivo* substrates when either kinase was overexpressed (207). PknB, Wag31, and Rv1422 (a conserved hypothetical protein of unknown function) were identified as *in vivo* substrates. Overexpression of Wag31, an orthologue of *B. subtilis* DivIVA, resulted in altered shape (207). This phenotype required the expressed Wag31 allele to contain the phosphoacceptor residue, confirming regulation through phosphorylation.

PknB also phosphorylates PBPA, a PG-synthesizing enzyme localized to the septum and important for cell division, since a PBPA-null *M. smegmatis* strain is defective for septation (90). While the *M. tuberculosis* PBPA allele complements this mutant strain, a mutant allele lacking the phosphoacceptor residue is inactive (90). PstP dephosphorylates PBPA (90), PknA, and PknB (42, 73). These data suggest a model where PknB and PstP regulate the localization, and possibly the activity, of PBPA, thus regulating septal PG synthesis and cell division (90, 207). PknB has also been shown to phosphorylate GarA (Rv1827), a putative regulator of glycogen accumulation during exponential-phase growth of mycobacteria (21).

PknB has an extracellular sensor domain, with four slightly different PBP- and Ser/Thr kinase-associated (PASTA) domains (202). PASTA domains have a low affinity for penicillin and normally bind different PG stem peptides on un-cross-linked PG. Different PASTA domains can recognize slight changes in the stem peptide (202). PknB may use these different PASTA domains to sense different unlinked PG to determine where to direct PG synthesis (202).

The first evidence that PknA may regulate cell division came from data showing that overexpression of *M. tuberculosis* PknA in *E. coli* resulted in elongated cells (61). PknA has been found to autophosphorylate itself (61). Recently, the GTPase activity of FtsZ_{TB} was shown to be impaired when phosphorylated by PknA (402). PknA expression in *E. coli* phosphorylated both *E. coli* FtsZ and FtsZ_{TB}, resulting in filamentous cells (402). PknA was shown to interact with and phosphorylate FtsZ_{TB} *in vitro*. Phosphorylated FtsZ_{TB} had reduced GTPase activity and a decreased polymerization rate compared with unphosphorylated protein (402). The ability of PknA to phosphorylate FtsZ

TABLE 5. Mycobacterial growth regulatory systems

Growth regulatory system(s)	Mechanism	Mycobacterial protein(s)	Function
STPKs	Sensors of environmental signals that regulate host-pathogen interactions and developmental changes through signal transduction using reversible phosphorylation of proteins; <i>M. tuberculosis</i> encodes 12 STPKs	PknA	Regulates cell shape; essential; transmembrane
		PknB	Regulates cell shape; essential; transmembrane
		PknF	Phosphorylates putative ABC transporter of <i>M. tuberculosis</i> (Rv1747); transmembrane
		PknG	Modulates macrophage response to infection; essential; soluble
		PknH	Phosphorylates the OmpR-like EmbR transcription factor; transmembrane
		PknK PknC, -D, -E, -I, -J, -L	Soluble Transmembrane
WhiB genes	Thought to bind DNA and influence transcription as well as play a role in sensing intracellular redox state; <i>M. tuberculosis</i> encodes 7 WhiB homologues	WhiB2	Possibly involved in cell wall remodeling and cell division
		WhiB3	Interacts with RpoV and is important for pathogenesis within the host
		WhiB4	May be a sensor of oxidative stress
		WhiB1, -5, -6, -7	Unknown
TCSs	Transfers a phosphate from the histidine residue on the autophosphorylated sensor to the aspartate residue on the response regulator to relay signals from the environment; <i>M. tuberculosis</i> encodes 11 TCSs in total (some not listed)	DosRS/T MtrAB SenX3-RegX3 MprAB PhoPR	Dormancy survival Proliferation in macrophages May regulate phosphate-dependent gene expression Growth during persistent stage Implicated in regulating production of complex cell wall lipids
Alternative sigma factors	The largest subset are known as ECF sigma factors and are small regulatory proteins lacking some of the conserved regions of typical sigma factors; <i>M. tuberculosis</i> encodes 13 sigma factors, 10 of which are ECF sigma factors (some not listed)	SigB, -D, -E, -H	Regulate genes that allow bacteria to adapt to stress
TA system	Originally recognized as a mechanism for ensuring proper plasmid segregation, the TA loci have now been shown to have diverse roles; works by production of a stable toxin and an unstable antitoxin, thus requiring a constant supply of antitoxin; <i>M. tuberculosis</i> has 38 TA loci (some not listed)	3 RelBE homologues and 9 MazEF homologues	Toxins that cleave mRNA in response to nutrient stress or starvation
Stringent response	RelA is triggered by uncharged tRNAs at the ribosomal A site during carbon starvation and synthesizes P ₄ G, which binds RNA polymerase, reducing the promoter open-complex half-life and halting translation	RelA	Synthesizes P ₄ G
		SpoT	Hydrolyzes P ₄ G

and the subsequent decrease in the rate of polymerization reveals a potential mechanism for regulating a key player in bacterial cell division.

Other STPKs. PknF and PknH phosphorylate FHA domain-containing proteins (284, 285). PknF has been shown to phosphorylate *M. tuberculosis* Rv1747, a predicted ABC transporter (83, 169, 287). When PknF, which is absent in *M. smegmatis*, is overexpressed in *M. smegmatis*, the bacteria grow slowly and are short and swollen (103). Furthermore, when PknF is de-

pleted from *M. tuberculosis*, the cells grow more rapidly, are small, have aberrant septum formation, and have a 16-fold increase in glucose uptake (103). Phosphorylation of ABC transporters is known to affect activity and stability (97, 190), and thus PknF may regulate glucose uptake through phosphorylation of Rv1747. It remains to be determined whether PknF directly regulates glucose transport, possibly through regulation of the ABC transporter Rv1747, and how this is able to affect cellular growth and septation. PknH phosphorylates the

OmpR-like EmbR transcription factor thought to be involved in regulating genes coding for arabinosyl transferases (285, 372). Instead of regulating bacterial proteins, PknG modulates the response by macrophages to infection with *M. tuberculosis* (80, 431).

How Are WhiB Proteins Involved in Growth Regulation?

Strains of *S. coelicolor* carrying a mutation in one of eight loci, now named the *whiB* genes, were originally found to be mutants that were white, lacking the pigment that is added to aerial hyphae before spore formation (63, 65, 191). The WhiB family of proteins is exclusive to actinomycetes and is found in all actinomycetes sequenced (326). Common to all WhiB proteins are four conserved cysteines, which are thought to bind iron or iron-sulfur clusters (198), and a helix-turn-helix motif (326). *M. tuberculosis* encodes seven WhiB homologues, WhiB1 to -7 (Table 5) (76, 388). While the functional role of WhiB genes is poorly understood, they are thought to bind DNA and influence transcription as well as play a role in intracellular redox sensing (2, 94, 326). WhiB3 interacts with RpoV and is important for pathogenesis within the host (393). While WhiB3 is dispensable for *in vitro* and *in vivo* growth (393), it is essential for survival under nutrient starvation conditions (384). WhiB4 binds iron-sulfur clusters and is sensitive to redox changes such that it may be a sensor of oxidative stress (2). In *S. coelicolor*, WhiD binds iron-sulfur clusters under anaerobic conditions, similar to the case for SoxR and Fnr of *E. coli* (99, 198). Addition of cell wall inhibitors (cycloserine, ethambutol, or isoniazid) to *M. tuberculosis* resulted in a specific increase (2- to 2.5-fold) in WhiB2, suggesting a role for WhiB2 in cell wall remodeling and cell division (145). Furthermore, *whiB2* expression is downshifted upon entering stationary phase, when there is little cell growth or division (145).

The *M. tuberculosis* putative transcription factor WhiB2 has been shown to be syntenuous and functionally equivalent to WhmD of *M. smegmatis* (327). WhmD is also homologous to the *S. coelicolor* WhiB protein, which is not essential for growth but is required for proper aerial hyphae sporulation, where mutants fail to assemble Z rings and arrest without formation of septa (94). In *M. smegmatis*, the gene encoding WhmD is essential, with depletion resulting in irreversible branched filaments and multiple, unevenly distributed septa within 6 to 12 hours (154). Both *M. tuberculosis* WhiB2 and *S. coelicolor* WhiB are able to complement WhmD depletion in *M. smegmatis* (326). Overexpression of WhmD in *M. smegmatis* yielded small cells with multiple septa and significant lysis (154). No changes in levels of FtsZ were detected in the *M. smegmatis* WhmD depletion strain (154). WhmD contains conserved cysteine residues found in all WhiB homologues (326). The fact that WhmD is sensitive to reducing agents and has iron-specific staining suggests that WhmD contains a bound iron atom and may be important for coordinating iron (326). It is likely that the WhmD/WhiB2 protein is important for regulating genes involved in cell division, in particular, septum formation.

In streptomyces, six regulatory genes (encoding WhiA, -B, -G, -H, -I, and -J) are required for induction of the high levels of FtsZ essential for normal sporulation (64, 66). Streptomyces grow as long hyphal filaments that do not require FtsZ to grow and have nucleoids regularly spaced along the hyphal branch.

When sporulation is triggered, large amounts of FtsZ are required to form Z rings on each side of the developing spores to guide septum formation. This process appears to involve the actin-like MreB protein as well (269). The large number of regulators for FtsZ in streptomyces is a testament to the high levels of FtsZ required for sporulation.

In conclusion, there remain many unanswered questions about mycobacterial kinases. What are the metabolic functions they regulate? What are the protein substrates and effectors of each kinase? Are the kinases involved in switching from different stages of growth, including from dormant to active growth? WhiB2 appears to be important for regulating septum formation and cell division, but the mechanism of the regulation is unclear. Are all WhiB proteins transcription factors? If so, what genes do they individually regulate?

CELL DIVISION UNDER SPECIALIZED CONDITIONS

M. tuberculosis can survive for decades in humans as well as in hypoxic and nutrient-depleted media, yet mycobacteria do not form spores. So, what is the nature of this dormant, non-replicating bacterium? Shockingly little is known about the physiology or mechanism of entry into and out of this dormant state, even though an estimated one-third of the world's population is infected with latent *M. tuberculosis* that could reactivate at some point (78).

Tuberculosis is a complex disease, with bacteria found in several very different environmental niches within a single host. To study bacteria under each condition requires good models. No animal model, except possibly nonhuman primates, can replicate the diversity of disease seen in humans (47). In the Cornell model, the most widely used *in vivo* latency model, infected mice are treated with drugs to the point of sterility. After approximately 3 months, one-third of the mice will reactivate with drug-susceptible *M. tuberculosis*. Variations on this model use immunosuppressive therapy, such as aminoguanidine, to enhance reactivation (364). Other hosts, such as guinea pig and rabbit, more closely mimic human pathology (47).

Because bacteria are relatively inaccessible during infection, many investigators have tried to develop *in vitro* models of dormancy. While it is clearly debatable how representative these models are of *in vivo* conditions, they have allowed for the dissection of transcription profiles, which would not otherwise be possible. (For excellent reviews, see references 47, 410, 427, and 429.) The alternative sigma factors and the two-component systems (TCSs) are the major families of transcription regulators that are important for adaptation to *in vivo* conditions (176).

How Is Entry into and Escape from the Dormant States Regulated?

TCSs. The TCSs in bacteria transfer a phosphate from the histidine residue on the autophosphorylated sensor to the aspartate residue on the response regulator to relay signals from the environment (140, 185, 252). They are common among many different bacteria. *M. tuberculosis* encodes 11 sensor kinase/response regulator TCSs, as well as several orphan kinases and regulators (Table 5) (76). Evidence suggests that

many of the TCSs are involved in sensing the host environment and adjusting bacterial transcription to adapt to the new environment, including PrrA (130), DosRST/DevRS (258, 348), SenX3-RegX3 (310), MprAB (460), MtrAB (138), and PhoPR (314). Much of the research done on TCSs in *M. tuberculosis* has been on bacterial survival in macrophages and mice and suggests that TCSs are important for pathogenesis and virulence. The role of TCSs in the basic bacterial cell biology of mycobacteria is limited compared to that in other organisms. Regulation of the cell cycle in mycobacteria may be shown to utilize TCSs in such ways, or it may be that STPKs and WhiB proteins fulfill such homeostatic processes.

DosRS/T dormancy phosphorelay system. Originally identified as DevR (Rv3133c) and DevS (Rv3132c) in a screen for genes differentially expressed in the virulent strain (Dev) of *M. tuberculosis* Rv compared to the avirulent Ra strain (91), these proteins were found to be important in dormancy survival (Dos) and alternatively named DosR and DosS (44). DosR interacts with DosS in the mycobacterial two-hybrid system (385). In both *M. tuberculosis* and *M. smegmatis*, DosRS respond similarly to hypoxic conditions in vitro meant to simulate conditions in vivo (44, 307). A DosR-null strain of *M. tuberculosis* is hypervirulent in mice, growing and killing more rapidly than the wild type (309). These data suggest that mycobacteria may use the DosRS system to reduce growth while in the host and prepare for transition into a persistent state that does not destroy the host or bacteria (309). Similarly, in *M. smegmatis*, deletion of DosR results in a strain that is specifically sensitive to hypoxia and survives stationary phase poorly (307). Along with these findings, the protein alpha-crystallin (Acr) is repeatedly found to be upregulated in hypoxic conditions. *acr* (*rv2031c*), or *hspX*, encodes a heat shock chaperon protein that is also a major cell wall-associated protein during stationary phase and is one of approximately 50 genes in the DosR regulon (311, 360).

The orphan sensor kinase DosT does not have a cognate response regulator and instead signals through the DosR protein such that both DosS and DosT utilize the same response regulator (348). While DosS is a redox sensor, DosT is a hypoxia sensor (226). The transcription profile of a DosR-null strain of *M. tuberculosis* shows that most of the genes regulated during hypoxia are dependent on DosR for induction (311). Most of these genes induced by hypoxia have an upstream DNA consensus motif, which, in the case of *acr*, DosR has been shown to directly bind (311).

The mechanism used by the sensor kinases that detect environmental changes has not been well described for TCSs. It was known that exposure to NO induced the DosR regulon in *M. tuberculosis*, and other data suggested that heme may play a role in sensing NO (428). The N-terminal region of DosS has two tandem GAF domains (358–360), which are common small-molecule-binding regulatory domains. Recently, the GAF-A domain of DosS was shown to bind heme directly (196, 360), similar to the case for PAS domains (184). These data suggest a model where DosS is inactivated when oxygen is bound to the DosS heme group but becomes active in hypoxic conditions, in the absence of oxygen. Furthermore, NO binds heme but inhibits DosS approximately 50-fold less than oxygen (118, 409). When a macrophage is activated by the immune response and generates large amounts of NO, the NO com-

petes off the oxygen from heme, effectively activating DosS, which explains the findings that DosRS responds similarly to both hypoxic and increased-NO conditions (360). While still speculative, this model dovetails with the current findings on the DosRS mechanism. Other stresses besides hypoxia and NO induce DosR, including ethanol, H₂O₂, and 30 minutes of standing culture (213). The mechanism of these inductions has not been elucidated.

Other TCSs. The MtrAB system is required for proliferation in macrophages (460), but the mechanism is unknown. Overexpression of MtrA has no effect in vitro but results in decreased growth in vivo, likely due to impaired blockage of the phagosome maturation. DnaA, a key DNA replication protein, is upregulated upon overexpression of MtrA (138). Similarly, MtrA has been shown to bind the promoter of *dnaA* in vivo (138). MprAB, another TCS, is also required for in vivo growth, particularly during the persistent stage (459); this is possibly because of its response to stress through regulating stress-responsive determinants, including the sigma factors *sigB* and *sigE* (176). Likewise, MprAB is induced in nutrient starvation conditions that are thought to simulate in vivo conditions (29). Finally, disruption of the TCS PhoPR attenuates *M. tuberculosis* for growth in vivo and has been implicated in regulating production of complex cell wall lipids (155, 432). It is worth noting that the products of STPKs (phosphorylated Ser, Thr, and Tyr) are much more stable than the product of TCSs (phosphorylated Asp) (378, 379). This stability means that STPKs can produce long-term signals that require specific phosphatases to inactivate (167).

Alternative sigma factors. Alternative sigma factors help regulate expression of specific genes during stress or morphological development (52, 168). The largest, most heterogeneous subset of these, known as extracytoplasmic function (ECF) sigma factors, are small regulatory proteins lacking some of the conserved regions of typical sigma factors (168, 350). *M. tuberculosis* encodes 13 sigma factors, 10 of which are ECF sigma factors (Table 5) (260). At least four of the *M. tuberculosis* ECF sigma factors are cotranscribed with an anti-sigma factor, a negative regulator that binds the cognate sigma factor until an external signal causes release (19, 172, 331, 389). This pair functions analogously to the TCS in that external stimuli influence one partner to stimulate or free the other to then induce genes in response to the stimuli (179). To survive the changing environment within the host, *M. tuberculosis* uses alternative sigma factors to regulate expression of genes so as to adapt to the new conditions (56). Sigma factors are known to bind RNA polymerase and thus confer specificity to promoters of certain genes in the particular sigma factor's regulon (204, 205).

Most of the sigma factors regulate genes that allow bacteria to adapt to stress such as that seen in vivo, including SigB, SigD, SigE, and SigH (56, 131, 259–262, 331, 332). For instance, a strain of *M. tuberculosis* lacking SigD grows normally in vitro and during early stages of infection within mice but is attenuated and has a stationary-phase survival defect (309). Some of the genes reportedly controlled by SigD include a small set of ribosomal genes normally expressed in stationary phase (56) and the resuscitation-promoting factor C (RpfC) (331), which was shown to be important for mycobacterial regrowth from a dormant, nonreplicating state (292). There

are discrepancies between the two reported SigD regulons that may be due to a difference in strain background (350). SigD is part of the RelA regulon (87) (discussed below) and is induced under starvation conditions (29). In vitro inducing conditions for the alternative sigma factors, which are useful for determining regulons and assigning functions, have been defined for only three sigma factors so far (SigB, SigE, and SigH) (260, 350).

What Else Is Regulated during Infection?

The majority of genes required for survival within a macrophage were found to be constitutively expressed, rather than induced (346). Likewise, the genes encoding FtsZ, DosR, and Acr are expressed in *M. tuberculosis* within lung granulomas of guinea pigs, and the proteins are detectable throughout infection (371). To study the formation of Z rings in *M. tuberculosis* after growth within macrophages, the only copy of FtsZ was replaced with FtsZ-GFP (72). Bacteria were reported to be longer after growth in macrophages, and most had spiral-shaped FtsZ structures along the length of the cell. It is worth noting that FtsZ-GFP, though it will assemble into the Z ring, has been shown to be less functional than the wild type in *E. coli* (250, 395, 396), *B. subtilis* (242), and likely *S. coelicolor* (163), such that the stress of growth within macrophages could alter the activity of FtsZ-GFP enough to result in altered morphology.

TA system. Originally recognized as a mechanism for ensuring proper plasmid segregation, the toxin-antitoxin (TA) loci have now been shown to be abundant in prokaryotic chromosomes and to have diverse roles (308; see reference 243 for an excellent review of the TA loci and persister cells). The TA loci are abundant in free-living bacteria but are absent from most obligate intracellular bacteria (146).

There are no known environmental niches for *M. tuberculosis*, such that its evolution can be thought of as having been explicitly guided by a need to survive within humans, yet *M. tuberculosis* carries a plethora of TA loci. While *M. leprae* has no complete TA loci, *M. tuberculosis* carries 38 chromosomal TA loci (Table 5) (146, 308). Three of these encode RelBE and nine encode MazEF homologues, all of which are toxins that cleave mRNA in response to nutrient stress or starvation and may coordinate with the RelA response. TAs were thought to be involved in plasmid maintenance or programmed cell death, but evidence suggests that these proteins modulate translation and DNA replication under nutrient stress. This system works by production of a stable toxin and an unstable antitoxin, thus requiring a constant supply of antitoxin.

Persister cells that are tolerant to antibiotics and certain stresses have been described. When HipA is mutated in the HipAB locus in *E. coli*, more persisters appear (38), suggesting that HipA may suppress the formation of persisters. HipAB is a typical TA system (39) that is P₄G (described below) dependent; however, the mechanism of persistence is unknown (146). HipA may inhibit a cell process in a low percentage of the population of bacteria, which then become dormant persister cells (283).

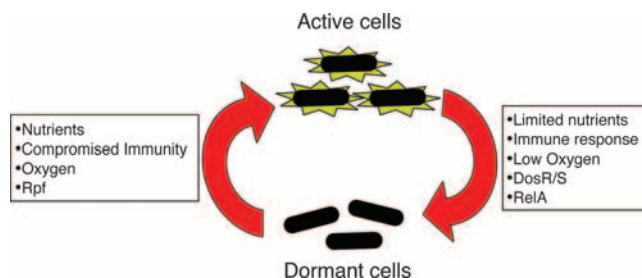


FIG. 5. Cycle of mycobacteria entering and exiting dormancy. Environmental conditions and proteins thought to be involved with inducing transitions between the two life cycle stages are listed.

What Is the State of Dormancy Models?

M. tuberculosis is thought to encounter nutrient deprivation and reduced oxygen levels, along with stresses produced by the immune response, once it is inside the host (47). Hypoxia could be one of the key signals for bacteria to enter into dormancy (Fig. 5) (438). Because it is difficult to know the precise conditions encountered by pathogens in the host, several in vitro model systems have been developed. These include a nonreplicative persistence oxygen depletion model (NRP) (297, 429, 437), where oxygen is slowly depleted; a steady-state reduced-oxygen model, which makes use of a chemostat (12); a static-culture oxygen depletion model (213), where cultures are left standing; an aerated stationary-phase model (429); a long-term nutritional depletion at high oxygen concentration model (174); a short-term oxygen depletion model (311, 375, 428); addition of nitric oxide (428); and a short course of complete starvation model (29).

M. tuberculosis incubated under many of these conditions, as well as *M. tuberculosis* isolated from infections, has thickened cell walls, an altered morphology (86), and duplicated chromosomes and stops replicating (427, 429, 436, 438). Persistent *M. tuberculosis* is thought to arrest its growth after completing chromosomal replication (437). In the case of *M. tuberculosis* cultured under the NRP conditions, where the culture is gently stirred in a sealed flask, resulting in a slow depletion of oxygen, the bacteria are able to resuscitate from bacteriostasis upon the addition of oxygen (438). Comparing transcription profiles from studies using the NRP model and from those using the steady-state growth in reduced oxygen model, the most striking commonality is the consistent upregulation of genes within the DosR regulon (311, 428), with little else in common (427). It is likely that the DosSR system is important for an evolutionary survival response, but it is still unclear whether it is involved in latency.

Deletion of *kasB* in *M. tuberculosis* causes loss of acid-fast staining and subclinical latent tuberculosis in immunocompetent mice. The most profound effect of *kasB* deletion is the ability of the mutant strain to persist in infected immunocompetent mice for up to 600 days without causing disease or mortality. This long-term persistence of the *kasB* mutant represents a possible new model for studying latency (31). Another intriguing potential model for studying chronic infections and, perhaps, latency involves the construction of artificial granulomas by implanting semidiffusible hollow fibers in mice (208).

Stringent response. When bacteria find themselves in an environment that is depleted of carbon sources, they down-regulate stable RNAs, stop DNA replication, halt translation, and then reset the rate of these homeostatic reactions to a slower pace (146, 220). This is known as the stringent response (Table 5) (146). To accomplish this, a protein known as RelA is triggered by uncharged tRNAs at the ribosomal A site and synthesizes ppGpp and pppGpp (collectively known as P₄G) (60). At the same time, the P₄G hydrolase, SpoT, is inhibited, resulting in accumulation of P₄G (296). (In *M. tuberculosis*, active domains from RelA and SpoT are found within a single RelA/SpoT protein [8].) P₄G binds RNA polymerase, reducing the promoter open-complex half-life (15, 16, 70). P₄G also inhibits exopolyphosphatase, resulting in an increase in polyphosphate (228, 230). This increase activates the Lon protease to degrade stalled ribosomal proteins, yielding new amino acids (229). P₄G is the master regulator during amino acid starvation, selectively affecting certain alternative sigma factors specialized in the stress response (201).

A *relA*-null strain of *M. smegmatis* forms clumpy, elevated colonies that have less pigment than the wild type but no detectable cell wall change or defect in growth (86). However, the mutant bacteria were longer and multiseptated, and some had a pear-like shape (86).

How Do Mycobacteria Reactivate from Dormancy?

The Rpf story. The addition of conditioned medium from *Micrococcus luteus*, or from species of mycobacteria, to cultures of in vitro-induced dormant mycobacteria results in resuscitation of growth of bacteria that normally would have an extended lag phase (377). Researchers isolated a protein secreted by these bacteria (288) that results in a similar growth-stimulating effect when added back as recombinant protein (36, 288, 289, 292, 463). *M. tuberculosis* has five of these resuscitation-promoting factors (RpfA to -E) (292), while *M. luteus* has only one. The single Rpf in *M. luteus* is essential for viability (291), while in *C. glutamicum*, a strain lacking both of its two *rpf* genes is viable, though it does have a lower growth rate and a longer lag phase (175). Strains with single deletions in each of the *M. tuberculosis* genes as well as in combinations of up to three genes are viable (114, 411). The bulk of the evidence suggests that Rpf proteins are not required for general viability. However, under certain conditions, Rpf does appear to be vital for growth. Strains of *M. tuberculosis* lacking combinations of three *rpf* genes were defective for growth in vivo and in an in vitro resuscitation assay (114). Furthermore, when strains of *M. tuberculosis* with single deletions of each Rpf were tested in a mouse model that attempts to simulate latent tuberculosis, a strain lacking RpfB failed to reactivate and caused a different histopathology (412). This implies that the Rpf proteins may be required to respond to conditions specific to a later stage of disease, in particular, dormancy.

How Is Rpf Regulated?

Rpf is glycosylated in *C. glutamicum* (175) by the *pmt* gene, a glycosyltransferase essential for glycosylating secreted proteins in *C. glutamicum* (255). It is unclear what affect glycosylation has on Rpf activity. In *M. tuberculosis*, RpfA was found

to be regulated by a cyclic AMP receptor-like transcription factor involved in the response to starvation (347), while RpfC may be regulated by SigD and possibly RelA and the stringent response (87, 331). We have recently shown that RpfB interacts with a predicted endopeptidase that is essential for growth and that the two proteins interact at the septum (183). Whether the relevant regulation of Rpf is transcriptional, through a modification, or through interaction with other proteins is unclear.

How Could Rpf Activate Bacteria?

The feature that defines Rpf proteins is an approximately 70-amino-acid region that is highly conserved across proteins within the Rpf family and found in many unrelated species (338). Recently, the structure of this conserved domain was found to resemble that of chicken-type lysozyme as well as the soluble lytic factor 70 (Slt70) of *E. coli* (74, 75). Rpf is able to hydrolyze PGs from different species of bacteria in several assays (290). The conserved active-site glutamate was found to be important for this hydrolytic activity but not essential (290). However, this glutamate was shown to be important for reactivation of dormant mycobacteria in vitro (74, 290). Interestingly, even though lysozyme cleaves a bond similar to that cleaved by Rpf, it does not have the same stimulatory effect (290). It is possible that Rpf needs to interact with at least one other protein to produce its effect, as suggested by the *C. glutamicum* data showing that strains lacking both Rpf proteins were not able to be stimulated by conditioned medium, indicating the need for at least one endogenously expressed Rpf to respond to the conditioned medium (175). A recent report that RpfB and RpfE interact with RipA, a PG hydrolase, provides further evidence that Rpf does not act alone during resuscitation (392).

Mycobacteria grown under hypoxic conditions have cell wall changes, including loss of pigmentation and increased thickness (82, 370, 387). *E. coli*, which can also enter a state of nonreplication, has been shown to rapidly modify its PG, making it more highly cross-linked with DAP-DAP bonds and less hydrolyzable by enzymes that typically break it down (158, 320, 413–415). Similarly, *Enterococcus faecalis* nonreplicating cells are twice as resistant to mechanical disruption as vegetative or stationary-phase cells and have a higher percentage of cross-linked PG (383), with more DAP-DAP cross-links and shortened glycan strands (382), and are O acetylated, which inhibits chicken egg white lysozyme (212). Considering the changes seen in the mycobacterial cell wall, as well as in the PGs of other bacteria when grown under hypoxic conditions, it may be that Rpf is required to degrade specific types of PG that arise under specific nutrient-limiting conditions. Hydrolytic enzymes are critical for breaking bonds in existing PG in order to allow insertion of new PG monomers. Without these enzymes, bacteria cannot grow. It may be that when bacteria are placed in hypoxic conditions, they go through a programmed shutdown, including more heavily cross-linking PG to survive stress. When nutrients become available, the bacteria will require hydrolases to nick PG to begin to grow out of this dormant state. While this is likely to prove true in general, evidence of this process in latent tuberculosis and Rpf involvement has yet to be conclusively demonstrated.

Interestingly, the mycobacteriophage TM4 requires the presence of an Rpf domain in its tape measure protein to effectively enter stationary-phase mycobacteria, while the domain is not needed for entry into vegetative bacteria (321). The changes in preparation for limited oxygen and nutrients during stationary phase may generate cell walls that are resistant to nonspecialized hydrolases.

Alternatively, the lytic activity of Rpf could release anhydromuropeptides from the PG, which have been shown to stimulate responses in other bacteria (197). These molecules could be released by Rpf alone or in concert with other enzymes. Sensors could then detect the released PG fragments and signal a response. Interestingly, PASTA domains found on the aforementioned PknB may be able to detect muropeptides released during hydrolysis of PG such that PknB could serve as a molecular switch between dormant and active bacteria (202). It could be that both processes of mechanical PG breakdown and signaling work in concert to effectively stimulate growth.

Does Resuscitation Involve Normal Homeostatic Processes?

In fact, the central role for Rpf proteins may actually be in normal homeostatic processes such as PG remodeling, bacterial growth, and cell division. The fact that strains lacking several of the Rpf proteins are viable is not at all surprising, as a strain of *E. coli* lacking eight PBPs remains viable (102). The high degree of homology among the Rpf proteins as well as the presence of other mycobacterial proteins predicted to be capable of hydrolyzing PG similarly suggest a requirement for this activity. Though multiple alleles of *rpf* can be deleted from *M. tuberculosis* without reduced in vitro viability, recall that *rpf* is essential in *M. luteus* (291) and important for normal growth in *C. glutamicum*, with a decreased growth rate and an increased log phase seen in the *rpf* null strain (175). The fact that Rpf peptides (E. Rubin lab, unpublished data) and encoding transcripts (113, 411) are found throughout all stages of growth suggests a role in normal growth (as well as a control mechanism beyond just transcriptional regulation). While the Rpf proteins may be redundant during normal growth (113), specific changes may occur, such as PG thickening, which then require Rpf, and possibly other enzymes, to initiate new growth and cell wall expansion. Thus, Rpf may not be specific for resuscitation.

In summary, mycobacteria have evolved to survive many different environments. In particular, *M. tuberculosis* is capable of adapting to many varied niches within a single host. Furthermore, *M. tuberculosis* can survive for decades before reactivating, a strategy that helps ensure a long-term reservoir of disease. While model systems have revealed several candidate mechanisms involved in the regulation of dormancy, how well these models reproduce important physiologic niches remains unclear. Validating these models and their predictions remains quite challenging.

CONCLUSIONS

It is striking how similar the processes of bacterial growth and division are among very different organisms. However, mycobacteria have developed many variations on these themes. For example, while PG structure is very well conserved

among bacteria, the many covalent modifications in mycobacteria create a cell wall that is strikingly different from those of most other gram-positive bacteria. In fact, these structures might well form the functional equivalent of a periplasmic space. In addition, while actin-like structures appear to be vital for maintaining cell shape, no mycobacterial proteins that serve this function have yet been identified. Perhaps FtsZ-guided polar growth and relatively inert lateral walls are sufficient for determining and maintaining shape in mycobacteria. Although other well-studied bacteria use similar sets of proteins to assemble the divisome, mycobacteria, which lack the initial FtsZ-stabilizing proteins, might use FtsQ and its unique cytoplasmic tails to resolve this deficiency. Also, while mycobacteria lack known Min and nucleoid occlusion proteins, some system for regulating FtsZ assembly that shares properties of those already known will likely be uncovered. For *M. tuberculosis*, not dividing can be as important as initiating cell division. The lack of growth during latency is probably intimately associated with the ability of the pathogen to survive host inflammatory mediators and antibiotic treatment. Establishing latency requires unique regulatory mechanisms, including signals to enter dormancy and to resume cell division. The participation of hydrolytic enzymes in this process suggests that dormant bacteria might alter cell wall structure and require unique metabolic pathways for regrowth to occur.

The cell wall remains the major target for antibiotic treatment of all bacterial infections. Antimycobacterial drugs are no exception, and this group of drugs is an integral part of standard tuberculosis therapy. The more we understand about the processes involved in cell wall metabolism and growth signaling, the more likely we are to be able to design a new generation of drugs that are effective against the recalcitrant organisms found during infection.

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REFERENCES

1. Alaedini, A., and R. A. Day. 1999. Identification of two penicillin-binding multienzyme complexes in *Haemophilus influenzae*. *Biochem. Biophys. Res. Commun.* **264**:191–195.
2. Alam, M. S., S. K. Garg, and P. Agrawal. 2007. Molecular function of WhiB4/Rv3681c of *Mycobacterium tuberculosis* H37Rv: a [4Fe-4S] cluster co-ordinating protein disulphide reductase. *Mol. Microbiol.* **63**:1414–1431.
3. Anand, S. P., H. Rajeswari, P. Gupta, R. Srinivasan, S. Indi, and P. Ajitkumar. 2004. A C-terminal deletion mutant of *Mycobacterium tuberculosis* FtsZ shows fast polymerization in vitro. *Microbiology* **150**:1119–1121.
4. Anderson, D. E., F. J. Gueiros-Filho, and H. P. Erickson. 2004. Assembly dynamics of FtsZ rings in *Bacillus subtilis* and *Escherichia coli* and effects of FtsZ-regulating proteins. *J. Bacteriol.* **186**:5775–5781.
5. Atrih, A., P. Zollner, G. Allmaier, M. P. Williamson, and S. J. Foster. 1998. Peptidoglycan structural dynamics during germination of *Bacillus subtilis* 168 endospores. *J. Bacteriol.* **180**:4603–4612.
6. Ausmees, N., and C. Jacobs-Wagner. 2003. Spatial and temporal control of differentiation and cell cycle progression in *Caulobacter crescentus*. *Annu. Rev. Microbiol.* **57**:225–247.
7. Aussel, L., F. X. Barre, M. Aroyo, A. Stasiak, A. Z. Stasiak, and D. Sherratt. 2002. FtsK is a DNA motor protein that activates chromosome dimer resolution by switching the catalytic state of the XerC and XerD recombinases. *Cell* **108**:195–205.
8. Avarbock, D., J. Salem, L. S. Li, Z. M. Wang, and H. Rubin. 1999. Cloning

- and characterization of a bifunctional RelA/SpoT homologue from *Mycobacterium tuberculosis*. *Gene* **233**:261–269.
9. Av-Gay, Y., and M. Everett. 2000. The eukaryotic-like Ser/Thr protein kinases of *Mycobacterium tuberculosis*. *Trends Microbiol.* **8**:238–244.
 10. Av-Gay, Y., S. Jamil, and S. J. Drews. 1999. Expression and characterization of the *Mycobacterium tuberculosis* serine/threonine protein kinase PknB. *Infect. Immun.* **67**:5676–5682.
 11. Azuma, I., D. W. Thomas, A. Adam, J. M. Ghuysen, R. Bonaly, J. F. Petit, and E. Lederer. 1970. Occurrence of N-glycolylmuramic acid in bacterial cell walls. A preliminary survey. *Biochim. Biophys. Acta* **208**:444–451.
 12. Bacon, J., B. W. James, L. Wernisch, A. Williams, K. A. Morley, G. J. Hatch, J. A. Mangan, J. Hinds, N. G. Stoker, P. D. Butcher, and P. D. Marsh. 2004. The influence of reduced oxygen availability on pathogenicity and gene expression in *Mycobacterium tuberculosis*. *Tuberculosis (Edinburgh)* **84**:205–217.
 13. Baker, R. P., and L. J. Reha-Krantz. 1998. Identification of a transient excision intermediate at the crossroads between DNA polymerase extension and proofreading pathways. *Proc. Natl. Acad. Sci. USA* **95**:3507–3512.
 14. Banerjee, A., E. Dubnau, A. Quemard, V. Balasubramanian, K. S. Um, T. Wilson, D. Collins, G. de Lisle, and W. R. Jacobs, Jr. 1994. *inhA*, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*. *Science* **263**:227–230.
 15. Barker, M. M., T. Gaal, and R. L. Gourse. 2001. Mechanism of regulation of transcription initiation by ppGpp. II. Models for positive control based on properties of RNAP mutants and competition for RNAP. *J. Mol. Biol.* **305**:689–702.
 16. Barker, M. M., T. Gaal, C. A. Josaitis, and R. L. Gourse. 2001. Mechanism of regulation of transcription initiation by ppGpp. I. Effects of ppGpp on transcription initiation in vivo and in vitro. *J. Mol. Biol.* **305**:673–688.
 17. Bartosik, A. A., and G. Jagura-Burdzy. 2005. Bacterial chromosome segregation. *Acta Biochim. Pol.* **52**:1–34.
 18. Bateman, A., and N. D. Rawlings. 2003. The CHAP domain: a large family of amidases including GSP amidase and peptidoglycan hydrolases. *Trends Biochem. Sci.* **28**:234–237.
 19. Beaucher, J., S. Rodrigue, P. E. Jacques, I. Smith, R. Brzezinski, and L. Gaudreau. 2002. Novel *Mycobacterium tuberculosis* anti-sigma factor antagonists control sigmaF activity by distinct mechanisms. *Mol. Microbiol.* **45**:1527–1540.
 20. Belanger, A. E., G. S. Besra, M. E. Ford, K. Mikusova, J. T. Belisle, P. J. Brennan, and J. M. Inamine. 1996. The embAB genes of *Mycobacterium avium* encode an arabinosyl transferase involved in cell wall arabinan biosynthesis that is the target for the antimycobacterial drug ethambutol. *Proc. Natl. Acad. Sci. USA* **93**:11919–11924.
 21. Belanger, A. E., and G. F. Hatfull. 1999. Exponential-phase glycogen recycling is essential for growth of *Mycobacterium smegmatis*. *J. Bacteriol.* **181**:6670–6678.
 22. Benson, T. E., C. T. Walsh, and J. M. Hogle. 1996. The structure of the substrate-free form of MurB, an essential enzyme for the synthesis of bacterial cell walls. *Structure* **4**:47–54.
 23. Berkmen, M. B., and A. D. Grossman. 2006. Spatial and temporal organization of the *Bacillus subtilis* replication cycle. *Mol. Microbiol.* **62**:57–71.
 24. Bernhardt, T. G., and P. A. de Boer. 2004. Screening for synthetic lethal mutants in *Escherichia coli* and identification of EnvC (YibP) as a periplasmic septal ring factor with murein hydrolase activity. *Mol. Microbiol.* **52**:1255–1269.
 25. Bernhardt, T. G., and P. A. de Boer. 2005. SlmA, a nucleoid-associated, FtsZ binding protein required for blocking septal ring assembly over chromosomes in *E. coli*. *Mol. Cell* **18**:555–564.
 26. Bernhardt, T. G., and P. A. de Boer. 2003. The *Escherichia coli* amidase AmiC is a periplasmic septal ring component exported via the twin-arginine transport pathway. *Mol. Microbiol.* **48**:1171–1182.
 27. Besra, G. S., and P. J. Brennan. 1997. The mycobacterial cell wall: biosynthesis of arabinogalactan and lipaarabinomannan. *Biochem. Soc. Trans.* **25**:845–850.
 28. Beste, D. J., E. Laing, B. Bonde, C. Avignone-Rossa, M. E. Bushell, and J. J. McFadden. 2007. Transcriptomic analysis identifies growth rate modulation as a component of the adaptation of mycobacteria to survival inside the macrophage. *J. Bacteriol.* **189**:3969–3976.
 29. Betts, J. C., P. T. Lukey, L. C. Robb, R. A. McAdam, and K. Duncan. 2002. Evaluation of a nutrient starvation model of *Mycobacterium tuberculosis* persistence by gene and protein expression profiling. *Mol. Microbiol.* **43**:717–731.
 30. Bhakta, S., and J. Basu. 2002. Overexpression, purification and biochemical characterization of a class A high-molecular-mass penicillin-binding protein (PBP), PBP1* and its soluble derivative from *Mycobacterium tuberculosis*. *Biochem. J.* **361**:635–639.
 31. Bhatt, A., N. Fujiwara, K. Bhatt, S. S. Gurcha, L. Kremer, B. Chen, J. Chan, S. A. Porcelli, K. Kobayashi, G. S. Besra, and W. R. Jacobs, Jr. 2007. Deletion of *kasB* in *Mycobacterium tuberculosis* causes loss of acid-fastness and subclinical latent tuberculosis in immunocompetent mice. *Proc. Natl. Acad. Sci. USA* **104**:5157–5162.
 32. Bhavsar, A. P., L. K. Erdman, J. W. Schertzler, and E. D. Brown. 2004. Teichoic acid is an essential polymer in *Bacillus subtilis* that is functionally distinct from teichuronic acid. *J. Bacteriol.* **186**:7865–7873.
 33. Bi, E., and J. Lutkenhaus. 1992. Isolation and characterization of *ftsZ* alleles that affect septal morphology. *J. Bacteriol.* **174**:5414–5423.
 34. Bi, E. F., and J. Lutkenhaus. 1991. FtsZ ring structure associated with division in *Escherichia coli*. *Nature* **354**:161–164.
 35. Bigot, S., J. Corre, J. M. Louarn, F. Cornet, and F. X. Barre. 2004. FtsK activities in Xer recombination, DNA mobilization and cell division involve overlapping and separate domains of the protein. *Mol. Microbiol.* **54**:876–886.
 36. Biketov, S., G. V. Mukamolova, V. Potapov, E. Gilenkov, G. Vostroknutova, D. B. Kell, M. Young, and A. S. Kaprelyants. 2000. Culturability of *Mycobacterium tuberculosis* cells isolated from murine macrophages: a bacterial growth factor promotes recovery. *FEMS Immunol. Med. Microbiol.* **29**:233–240.
 37. Billman-Jacobe, H., R. E. Haites, and R. L. Coppel. 1999. Characterization of a *Mycobacterium smegmatis* mutant lacking penicillin binding protein 1. *Antimicrob. Agents Chemother.* **43**:3011–3013.
 38. Black, D. S., A. J. Kelly, M. J. Mardis, and H. S. Moyed. 1991. Structure and organization of *hip*, an operon that affects lethality due to inhibition of peptidoglycan or DNA synthesis. *J. Bacteriol.* **173**:5732–5739.
 39. Black, R. C., A. N. Binns, C. F. Chang, and D. G. Lynn. 1994. Cell-autonomous cytokinin-independent growth of tobacco cells transformed by *Agrobacterium tumefaciens* strains lacking the cytokinin biosynthesis gene. *Plant Physiol.* **105**:989–998.
 40. Blakely, G., G. May, R. McCulloch, L. K. Arciszewska, M. Burke, S. T. Lovett, and D. J. Sherratt. 1993. Two related recombinases are required for site-specific recombination at *dif* and *cer* in *E. coli* K12. *Cell* **75**:351–361.
 41. Blokpoel, M. C., H. N. Murphy, R. O'Toole, S. Wiles, E. S. Runn, G. R. Stewart, D. B. Young, and B. D. Robertson. 2005. Tetracycline-inducible gene regulation in mycobacteria. *Nucleic Acids Res.* **33**:e22.
 42. Boitel, B., M. Ortiz-Lombardia, R. Duran, F. Pompeo, S. T. Cole, C. Cervenansky, and P. M. Alzari. 2003. PknB kinase activity is regulated by phosphorylation in two Thr residues and dephosphorylation by PstP, the cognate phospho-Ser/Thr phosphatase, in *Mycobacterium tuberculosis*. *Mol. Microbiol.* **49**:1493–1508.
 43. Boneca, I. G. 2005. The role of peptidoglycan in pathogenesis. *Curr. Opin. Microbiol.* **8**:46–53.
 44. Boon, C., and T. Dick. 2002. *Mycobacterium bovis* BCG response regulator essential for hypoxic dormancy. *J. Bacteriol.* **184**:6760–6767.
 45. Borhani, D. W., and E. L. White. 2004. Polymerization of C-terminally truncated *Mycobacterium tuberculosis* FtsZ is unlikely to be physiologically relevant. *Microbiology* **150**:3903–3906.
 46. Born, P., E. Breukink, and W. Vollmer. 2006. In vitro synthesis of cross-linked murein and its attachment to sacculi by PBPIA from *Escherichia coli*. *J. Biol. Chem.* **281**:26985–26993.
 47. Boshoff, H. I., and C. E. Barry III. 2005. Tuberculosis—metabolism and respiration in the absence of growth. *Nat. Rev. Microbiol.* **3**:70–80.
 48. Boyle, D. S., M. M. Khattar, S. G. Addinall, J. Lutkenhaus, and W. D. Donachie. 1997. *ftsW* is an essential cell-division gene in *Escherichia coli*. *Mol. Microbiol.* **24**:1263–1273.
 49. Bramhill, D., and C. M. Thompson. 1994. GTP-dependent polymerization of *Escherichia coli* FtsZ protein to form tubules. *Proc. Natl. Acad. Sci. USA* **91**:5813–5817.
 50. Brennan, P. J. 2003. Structure, function, and biogenesis of the cell wall of *Mycobacterium tuberculosis*. *Tuberculosis (Edinburgh)* **83**:91–97.
 51. Brennan, P. J., and H. Nikaido. 1995. The envelope of mycobacteria. *Annu. Rev. Biochem.* **64**:29–63.
 52. Browning, D. F., and S. J. Busby. 2004. The regulation of bacterial transcription initiation. *Nat. Rev. Microbiol.* **2**:57–65.
 53. Buddemeijer, N., and J. Beckwith. 2004. A complex of the *Escherichia coli* cell division proteins FtsL, FtsB and FtsQ forms independently of its localization to the septal region. *Mol. Microbiol.* **52**:1315–1327.
 54. Buddemeijer, N., and J. Beckwith. 2002. Assembly of cell division proteins at the *E. coli* cell center. *Curr. Opin. Microbiol.* **5**:553–557.
 55. Cabeen, M. T., and C. Jacobs-Wagner. 2005. Bacterial cell shape. *Nat. Rev. Microbiol.* **3**:601–610.
 56. Calamita, H., C. Ko, S. Tyagi, T. Yoshimatsu, N. E. Morrison, and W. R. Bishai. 2005. The *Mycobacterium tuberculosis* SigD sigma factor controls the expression of ribosome-associated gene products in stationary phase and is required for full virulence. *Cell Microbiol.* **7**:233–244.
 57. Carballido-Lopez, R. 2006. The bacterial actin-like cytoskeleton. *Microbiol. Mol. Biol. Rev.* **70**:888–909.
 58. Carballido-Lopez, R., and J. Errington. 2003. A dynamic bacterial cytoskeleton. *Trends Cell Biol.* **13**:577–583.
 59. Carballido-Lopez, R., and J. Errington. 2003. The bacterial cytoskeleton: in vivo dynamics of the actin-like protein Mbl of *Bacillus subtilis*. *Dev. Cell* **4**:19–28.
 60. Cashel, M., D. Gentry, V. J. Hernandez, and D. Vinella. 1996. The stringent response, p. 1458–1496. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M.

- Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. ASM Press, Washington, DC.
61. Chaba, R., M. Raje, and P. K. Chakraborti. 2002. Evidence that a eukaryotic-type serine/threonine protein kinase from *Mycobacterium tuberculosis* regulates morphological changes associated with cell division. *Eur. J. Biochem.* **269**:1078–1085.
 62. Chambers, H. F., D. Moreau, D. Yajko, C. Miick, C. Wagner, C. Hackbarth, S. Kocagoz, E. Rosenberg, W. K. Hadley, and H. Nikaido. 1995. Can penicillins and other beta-lactam antibiotics be used to treat tuberculosis? *Antimicrob. Agents Chemother.* **39**:2620–2624.
 63. Champness, W. C., and K. F. Chater. 1994. Regulation and integration of antibiotic production and morphological differentiation in *Streptomyces* spp., p. 61–94. In P. J. Piggot, P. Youngman, and C. Moran (ed.), *Regulation of bacterial differentiation*. ASM Press, Washington, DC.
 64. Chater, K. 1999. David Hopwood and the emergence of *Streptomyces* genetics. *Int. Microbiol.* **2**:61–68.
 65. Chater, K. F. 1972. A morphological and genetic mapping study of white colony mutants of *Streptomyces coelicolor*. *J. Gen. Microbiol.* **72**:9–28.
 66. Chater, K. F. 2001. Regulation of sporulation in *Streptomyces coelicolor* A3(2): a checkpoint multiplex? *Curr. Opin. Microbiol.* **4**:667–673.
 67. Chatterjee, D. 1997. The mycobacterial cell wall: structure, biosynthesis and sites of drug action. *Curr. Opin. Chem. Biol.* **1**:579–588.
 68. Chatterjee, D., S. W. Hunter, M. McNeil, and P. J. Brennan. 1992. Lipoarabinomannan. Multiglycosylated form of the mycobacterial mannolipophosphatidylinositols. *J. Biol. Chem.* **267**:6228–6233.
 69. Chatterjee, D., K. Lowell, B. Rivoire, M. R. McNeil, and P. J. Brennan. 1992. Lipoarabinomannan of *Mycobacterium tuberculosis*. Capping with mannosyl residues in some strains. *J. Biol. Chem.* **267**:6234–6239.
 70. Chatterji, M., S. Unniraman, S. Mahadevan, and V. Nagaraja. 2001. Effect of different classes of inhibitors on DNA gyrase from *Mycobacterium smegmatis*. *J. Antimicrob. Chemother.* **48**:479–485.
 71. Chauhan, A., H. Lofton, E. Maloney, J. Moore, M. Fol, M. V. Madiraju, and M. Rajagopalan. 2006. Interference of *Mycobacterium tuberculosis* cell division by Rv2719c, a cell wall hydrolase. *Mol. Microbiol.* **62**:132–147.
 72. Chauhan, A., M. V. Madiraju, M. Fol, H. Lofton, E. Maloney, R. Reynolds, and M. Rajagopalan. 2006. *Mycobacterium tuberculosis* cells growing in macrophages are filamentous and deficient in FtsZ rings. *J. Bacteriol.* **188**:1856–1865.
 73. Chopra, P., B. Singh, R. Singh, R. Vohra, A. Koul, L. S. Meena, H. Koduri, M. Ghildiyal, P. Deol, T. K. Das, A. K. Tyagi, and Y. Singh. 2003. Phosphoprotein phosphatase of *Mycobacterium tuberculosis* dephosphorylates serine-threonine kinases PknA and PknB. *Biochem. Biophys. Res. Commun.* **311**:112–120.
 74. Cohen-Gonsaud, M., P. Barthe, C. Bagneris, B. Henderson, J. Ward, C. Roumestand, and N. H. Keep. 2005. The structure of a resuscitation-promoting factor domain from *Mycobacterium tuberculosis* shows homology to lysozymes. *Nat. Struct. Mol. Biol.* **12**:270–273.
 75. Cohen-Gonsaud, M., N. H. Keep, A. P. Davies, J. Ward, B. Henderson, and G. Labesse. 2004. Resuscitation-promoting factors possess a lysozyme-like domain. *Trends Biochem. Sci.* **29**:7–10.
 76. Cole, S. T., R. Brosch, J. Parkhill, T. Garnier, C. Churcher, D. Harris, S. V. Gordon, K. Eiglmeier, S. Gas, C. E. Barry III, F. Tekaiia, K. Badcock, D. Basham, D. Brown, T. Chillingworth, R. Connor, R. Davies, K. Devlin, T. Feltwell, S. Gentles, N. Hamlin, S. Holroyd, T. Hornsby, K. Jagels, A. Krogh, J. McLean, S. Moule, L. Murphy, K. Oliver, J. Osborne, M. A. Quail, M. A. Rajandream, J. Rogers, S. Rutter, K. Seeger, J. Skelton, R. Squares, S. Squares, J. E. Sulston, K. Taylor, S. Whitehead, and B. G. Barrell. 1998. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* **393**:537–544.
 77. Consaul, S. A., L. F. Wright, S. Mahapatra, D. C. Crick, and M. S. Pavelka, Jr. 2005. An unusual mutation results in the replacement of diaminopimelate with lanthionine in the peptidoglycan of a mutant strain of *Mycobacterium smegmatis*. *J. Bacteriol.* **187**:1612–1620.
 78. Corbett, E. L., C. J. Watt, N. Walker, D. Maher, B. G. Williams, M. C. Raviglione, and C. Dye. 2003. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch. Intern. Med.* **163**:1009–1021.
 79. Corbin, B. D., B. Geissler, M. Sadasivam, and W. Margolin. 2004. Z-ring-independent interaction between a subdomain of FtsA and late septation proteins as revealed by a polar recruitment assay. *J. Bacteriol.* **186**:7736–7744.
 80. Cowley, S., M. Ko, N. Pick, R. Chow, K. J. Downing, B. G. Gordhan, J. C. Betts, V. Mizrahi, D. A. Smith, R. W. Stokes, and Y. Av-Gay. 2004. The *Mycobacterium tuberculosis* protein serine/threonine kinase PknG is linked to cellular glutamate/glutamine levels and is important for growth in vivo. *Mol. Microbiol.* **52**:1691–1702.
 81. Crick, D. C., S. Mahapatra, and P. J. Brennan. 2001. Biosynthesis of the arabinogalactan-peptidoglycan complex of *Mycobacterium tuberculosis*. *Glycobiology* **11**:107R–118R.
 82. Cunningham, A. F., and C. L. Spreadbury. 1998. Mycobacterial stationary phase induced by low oxygen tension: cell wall thickening and localization of the 16-kilodalton alpha-crystallin homolog. *J. Bacteriol.* **180**:801–808.
 83. Curry, J. M., R. Whalan, D. M. Hunt, K. Gohil, M. Strom, L. Rickman, M. J. Colston, S. J. Smerdon, and R. S. Buxton. 2005. An ABC transporter containing a forkhead-associated domain interacts with a serine-threonine protein kinase and is required for growth of *Mycobacterium tuberculosis* in mice. *Infect. Immun.* **73**:4471–4477.
 84. Daffe, M., P. J. Brennan, and M. McNeil. 1990. Predominant structural features of the cell wall arabinogalactan of *Mycobacterium tuberculosis* as revealed through characterization of oligoglycosyl alditol fragments by gas chromatography/mass spectrometry and by ¹H and ¹³C NMR analyses. *J. Biol. Chem.* **265**:6734–6743.
 85. Dahl, J. L. 2004. Electron microscopy analysis of *Mycobacterium tuberculosis* cell division. *FEMS Microbiol. Lett.* **240**:15–20.
 86. Dahl, J. L., K. Arora, H. I. Boshoff, D. C. Whiteford, S. A. Pacheco, O. J. Walsh, D. Lau-Bonilla, W. B. Davis, and A. G. Garza. 2005. The *relA* homolog of *Mycobacterium smegmatis* affects cell appearance, viability, and gene expression. *J. Bacteriol.* **187**:2439–2447.
 87. Dahl, J. L., C. N. Kraus, H. I. Boshoff, B. Doan, K. Foley, D. Avarbock, G. Kaplan, V. Mizrahi, H. Rubin, and C. E. Barry III. 2003. The role of RelMtb-mediated adaptation to stationary phase in long-term persistence of *Mycobacterium tuberculosis* in mice. *Proc. Natl. Acad. Sci. USA* **100**:10026–10031.
 88. Daniel, R. A., and J. Errington. 2003. Control of cell morphogenesis in bacteria: two distinct ways to make a rod-shaped cell. *Cell* **113**:767–776.
 89. Daniel, R. A., E. J. Harry, V. L. Katis, R. G. Wake, and J. Errington. 1998. Characterization of the essential cell division gene *ftsL*(yIIID) of *Bacillus subtilis* and its role in the assembly of the division apparatus. *Mol. Microbiol.* **29**:593–604.
 90. Dasgupta, A., P. Datta, M. Kundu, and J. Basu. 2006. The serine/threonine kinase PknB of *Mycobacterium tuberculosis* phosphorylates PBPA, a penicillin-binding protein required for cell division. *Microbiology* **152**:493–504.
 91. Dasgupta, N., V. Kapur, K. K. Singh, T. K. Das, S. Sachdeva, K. Jyothirsi, and J. S. Tyagi. 2000. Characterization of a two-component system, devR-devS, of *Mycobacterium tuberculosis*. *Tuber. Lung Dis.* **80**:141–159.
 92. Datta, P., A. Dasgupta, S. Bhakta, and J. Basu. 2002. Interaction between FtsZ and FtsW of *Mycobacterium tuberculosis*. *J. Biol. Chem.* **277**:24983–24987.
 93. Datta, P., A. Dasgupta, A. K. Singh, P. Mukherjee, M. Kundu, and J. Basu. 2006. Interaction between FtsW and penicillin-binding protein 3 (PBP3) directs PBP3 to mid-cell, controls cell septation and mediates the formation of a trimeric complex involving FtsZ, FtsW and PBP3 in mycobacteria. *Mol. Microbiol.* **62**:1655–1673.
 94. Davis, N. K., and K. F. Chater. 1992. The *Streptomyces coelicolor* whiB gene encodes a small transcription factor-like protein dispensable for growth but essential for sporulation. *Mol. Gen. Genet.* **232**:351–358.
 95. de Boer, P., R. Crossley, and L. Rothfield. 1992. The essential bacterial cell-division protein FtsZ is a GTPase. *Nature* **359**:254–256.
 96. de Boer, P. A., R. E. Crossley, and L. I. Rothfield. 1989. A division inhibitor and a topological specificity factor coded for by the *minicell* locus determine proper placement of the division septum in *E. coli*. *Cell* **56**:641–649.
 97. Decottignies, A., G. Owsianik, and M. Ghislaian. 1999. Casein kinase I-dependent phosphorylation and stability of the yeast multidrug transporter Pdr5p. *J. Biol. Chem.* **274**:37139–37146.
 98. de Leeuw, E., B. Graham, G. J. Phillips, C. M. ten Hagen-Jongman, B. Oudega, and J. Luirink. 1999. Molecular characterization of *Escherichia coli* FtsE and FtsX. *Mol. Microbiol.* **31**:983–993.
 99. Demple, B. 2002. Signal transduction by nitric oxide in cellular stress responses. *Mol. Cell. Biochem.* **234**–235:11–18.
 100. Den Blaauwen, T., N. Buddelmeijer, M. E. Aarsman, C. M. Hameete, and N. Nanninga. 1999. Timing of FtsZ assembly in *Escherichia coli*. *J. Bacteriol.* **181**:5167–5175.
 101. Deng, L. L., D. E. Humphries, R. D. Arbeit, L. E. Carlton, S. C. Smole, and J. D. Carroll. 2005. Identification of a novel peptidoglycan hydrolase CwlM in *Mycobacterium tuberculosis*. *Biochim. Biophys. Acta* **1747**:57–66.
 102. Denome, S. A., P. K. Elf, T. A. Henderson, D. E. Nelson, and K. D. Young. 1999. *Escherichia coli* mutants lacking all possible combinations of eight penicillin binding proteins: viability, characteristics, and implications for peptidoglycan synthesis. *J. Bacteriol.* **181**:3981–3993.
 103. Deol, P., R. Vohra, A. K. Saini, A. Singh, H. Chandra, P. Chopra, T. K. Das, A. K. Tyagi, and Y. Singh. 2005. Role of *Mycobacterium tuberculosis* Ser/Thr kinase PknF: implications in glucose transport and cell division. *J. Bacteriol.* **187**:3415–3420.
 104. de Pedro, M. A., W. D. Donachie, J. V. Holtje, and H. Schwarz. 2001. Constitutive septal murein synthesis in *Escherichia coli* with impaired activity of the morphogenetic proteins RodA and penicillin-binding protein 2. *J. Bacteriol.* **183**:4115–4126.
 105. de Pedro, M. A., J. C. Quintela, J. V. Holtje, and H. Schwarz. 1997. Murein segregation in *Escherichia coli*. *J. Bacteriol.* **179**:2823–2834.
 106. de Pedro, M. A., K. D. Young, J. V. Holtje, and H. Schwarz. 2003. Branching of *Escherichia coli* cells arises from multiple sites of inert peptidoglycan. *J. Bacteriol.* **185**:1147–1152.
 107. De Smet, K. A., K. E. Kempell, A. Gallagher, K. Duncan, and D. B. Young. 1999. Alteration of a single amino acid residue reverses fosfomicin resis-

- tance of recombinant MurA from *Mycobacterium tuberculosis*. *Microbiology* **145**:3177–3184.
108. **Dhople, A. M.** 1983. Effect of lysozyme on *Mycobacterium leprae*. *Lepr. India*. **55**:455–464.
 109. **Di Lallo, G., M. Fagioli, D. Barionovi, P. Ghelardini, and L. Paolozzi.** 2003. Use of a two-hybrid assay to study the assembly of a complex multicomponent protein machinery: bacterial septosome differentiation. *Microbiology* **149**:3353–3359.
 110. **Divakaruni, A. V., R. R. Loo, Y. Xie, J. A. Loo, and J. W. Gober.** 2005. The cell-shape protein MreC interacts with extracytoplasmic proteins including cell wall assembly complexes in *Caulobacter crescentus*. *Proc. Natl. Acad. Sci. USA* **102**:18602–18607.
 111. **Dmitriev, B., F. Toukach, and S. Ehlers.** 2005. Towards a comprehensive view of the bacterial cell wall. *Trends Microbiol.* **13**:569–574.
 112. **Dmitriev, B. A., F. V. Toukach, K. J. Schaper, O. Holst, E. T. Rietschel, and S. Ehlers.** 2003. Tertiary structure of bacterial murein: the scaffold model. *J. Bacteriol.* **185**:3458–3468.
 113. **Downing, K. J., J. C. Betts, D. I. Young, R. A. McAdam, F. Kelly, M. Young, and V. Mizrahi.** 2004. Global expression profiling of strains harbouring null mutations reveals that the five *rpf*-like genes of *Mycobacterium tuberculosis* show functional redundancy. *Tuberculosis (Edinburgh)* **84**:167–179.
 114. **Downing, K. J., V. V. Mischenko, M. O. Shleeva, D. I. Young, M. Young, A. S. Kaprelyants, A. S. Apt, and V. Mizrahi.** 2005. Mutants of *Mycobacterium tuberculosis* lacking three of the five *rpf*-like genes are defective for growth in vivo and for resuscitation in vitro. *Infect. Immun.* **73**:3038–3043.
 115. **Draper, P.** 1982. Bacteriology of *Mycobacterium leprae*: state of the art paper. *Ann. Microbiol. (Paris)* **133**:13–14.
 116. **Draper, P.** 1971. The walls of *Mycobacterium lepraemurium*: chemistry and ultrastructure. *J. Gen. Microbiol.* **69**:313–324.
 117. **Draper, P., O. Kandler, and A. Darbre.** 1987. Peptidoglycan and arabinogalactan of *Mycobacterium leprae*. *J. Gen. Microbiol.* **133**:1187–1194.
 118. **Dunham, C. M., E. M. Dioum, J. R. Tuckerman, G. Gonzalez, W. G. Scott, and M. A. Gilles-Gonzalez.** 2003. A distal arginine in oxygen-sensing heme-PAS domains is essential to ligand binding, signal transduction, and structure. *Biochemistry* **42**:7701–7708.
 119. **Durocher, D., and S. P. Jackson.** 2002. The FHA domain. *FEBS Lett.* **513**:58–66.
 120. **Dworkin, J., and R. Losick.** 2002. Does RNA polymerase help drive chromosome segregation in bacteria? *Proc. Natl. Acad. Sci. USA* **99**:14089–14094.
 121. **Dziadek, J., M. V. Madiraju, S. A. Rutherford, M. A. Atkinson, and M. Rajagopalan.** 2002. Physiological consequences associated with overproduction of *Mycobacterium tuberculosis* FtsZ in mycobacterial hosts. *Microbiology* **148**:961–971.
 122. **Dziadek, J., S. A. Rutherford, M. V. Madiraju, M. A. Atkinson, and M. Rajagopalan.** 2003. Conditional expression of *Mycobacterium smegmatis* ftsZ, an essential cell division gene. *Microbiology* **149**:1593–1603.
 123. **Egorov, S., S. V. Ulakhovich, V. V. Dmitriev, F. G. Kupriianova-Ashina, and I. B. Leshchinskaia.** 1999. Effect of the RNAase from *Bacillus intermedius* on growth and physiological characteristics of *Escherichia coli*. *Mikrobiologiya*. **68**:51–55.
 124. **Ehrt, S., X. V. Guo, C. M. Hickey, M. Ryou, M. Montealeone, L. W. Riley, and D. Schnappinger.** 2005. Controlling gene expression in mycobacteria with anhydrotetracycline and Tet repressor. *Nucleic Acids Res.* **33**:e21.
 125. **Erickson, H. P., D. W. Taylor, K. A. Taylor, and D. Bramhill.** 1996. Bacterial cell division protein FtsZ assembles into protofilament sheets and minirings, structural homologs of tubulin polymers. *Proc. Natl. Acad. Sci. USA* **93**:519–523.
 126. **Errington, J.** 2001. Septation and chromosome segregation during sporulation in *Bacillus subtilis*. *Curr. Opin. Microbiol.* **4**:660–666.
 127. **Errington, J., J. Bath, and L. J. Wu.** 2001. DNA transport in bacteria. *Nat. Rev. Mol. Cell Biol.* **2**:538–545.
 128. **Errington, J., R. A. Daniel, and D. J. Scheffers.** 2003. Cytokinesis in bacteria. *Microbiol. Mol. Biol. Rev.* **67**:52–65.
 129. **Espeli, O., C. Lee, and K. J. Marians.** 2003. A physical and functional interaction between *Escherichia coli* FtsK and topoisomerase IV. *J. Biol. Chem.* **278**:44639–44644.
 130. **Ewann, F., M. Jackson, K. Pethe, A. Cooper, N. Mielcarek, D. Ensergueix, B. Gicquel, C. Locht, and P. Supply.** 2002. Transient requirement of the PrtA-PrtB two-component system for early intracellular multiplication of *Mycobacterium tuberculosis*. *Infect. Immun.* **70**:2256–2263.
 131. **Fernandes, N. D., Q. L. Wu, D. Kong, X. Puyang, S. Garg, and R. N. Husson.** 1999. A mycobacterial extracytoplasmic sigma factor involved in survival following heat shock and oxidative stress. *J. Bacteriol.* **181**:4266–4274.
 132. **Fernandez, P., B. Saint-Joanis, N. Barilone, M. Jackson, B. Gicquel, S. T. Cole, and P. M. Alzari.** 2006. The Ser/Thr protein kinase PknB is essential for sustaining mycobacterial growth. *J. Bacteriol.* **188**:7778–7784.
 133. **Figge, R. M., A. V. Divakaruni, and J. W. Gober.** 2004. MreB, the cell shape-determining bacterial actin homologue, co-ordinates cell wall morphogenesis in *Caulobacter crescentus*. *Mol. Microbiol.* **51**:1321–1332.
 134. **Flardh, K.** 2003. Essential role of DivIVA in polar growth and morphogenesis in *Streptomyces coelicolor* A3(2). *Mol. Microbiol.* **49**:1523–1536.
 135. **Flardh, K.** 2003. Growth polarity and cell division in *Streptomyces*. *Curr. Opin. Microbiol.* **6**:564–571.
 136. **Flores, A. R., L. M. Parsons, and M. S. Pavelka, Jr.** 2005. Characterization of novel *Mycobacterium tuberculosis* and *Mycobacterium smegmatis* mutants hypersusceptible to beta-lactam antibiotics. *J. Bacteriol.* **187**:1892–1900.
 137. **Flores, A. R., L. M. Parsons, and M. S. Pavelka, Jr.** 2005. Genetic analysis of the beta-lactamases of *Mycobacterium tuberculosis* and *Mycobacterium smegmatis* and susceptibility to beta-lactam antibiotics. *Microbiology* **151**:521–532.
 138. **Fol, M., A. Chauhan, N. K. Nair, E. Maloney, M. Moomey, C. Jagannath, M. V. Madiraju, and M. Rajagopalan.** 2006. Modulation of *Mycobacterium tuberculosis* proliferation by MtrA, an essential two-component response regulator. *Mol. Microbiol.* **60**:643–657.
 139. **Foster, S. J.** 1991. Cloning, expression, sequence analysis and biochemical characterization of an autolytic amidase of *Bacillus subtilis* 168 trpC2. *J. Gen. Microbiol.* **137**:1987–1998.
 140. **Galperin, M. Y., A. N. Nikolskaya, and E. V. Koonin.** 2001. Novel domains of the prokaryotic two-component signal transduction systems. *FEMS Microbiol. Lett.* **203**:11–21.
 141. **Gao, L. Y., M. Pak, R. Kish, K. Kajihara, and E. J. Brown.** 2006. A mycobacterial operon essential for virulence in vivo and invasion and intracellular persistence in macrophages. *Infect. Immun.* **74**:1757–1767.
 142. **Garner, E. C., C. S. Campbell, and R. D. Mullins.** 2004. Dynamic instability in a DNA-segregating prokaryotic actin homolog. *Science* **306**:1021–1025.
 143. **Garner, E. C., C. S. Campbell, D. B. Weibel, and R. D. Mullins.** 2007. Reconstitution of DNA segregation driven by assembly of a prokaryotic actin homolog. *Science* **315**:1270–1274.
 144. **Garrido, T., M. Sanchez, P. Palacios, M. Aldea, and M. Vicente.** 1993. Transcription of ftsZ oscillates during the cell cycle of *Escherichia coli*. *EMBO J.* **12**:3957–3965.
 145. **Geiman, D. E., T. R. Raghunand, N. Agarwal, and W. R. Bishai.** 2006. Differential gene expression in response to exposure to antimycobacterial agents and other stress conditions among seven *Mycobacterium tuberculosis* *whiB*-like genes. *Antimicrob. Agents Chemother.* **50**:2836–2841.
 146. **Gerdes, K., S. K. Christensen, and A. Lobner-Olesen.** 2005. Prokaryotic toxin-antitoxin stress response loci. *Nat. Rev. Microbiol.* **3**:371–382.
 147. **Gitai, Z., N. Dye, and L. Shapiro.** 2004. An actin-like gene can determine cell polarity in bacteria. *Proc. Natl. Acad. Sci. USA* **101**:8643–8648.
 148. **Glickman, M. S., J. S. Cox, and W. R. Jacobs, Jr.** 2000. A novel mycolic acid cyclopropane synthetase is required for cording, persistence, and virulence of *Mycobacterium tuberculosis*. *Mol. Cell* **5**:717–727.
 149. **Goehring, N. W., and J. Beckwith.** 2005. Diverse paths to midcell: assembly of the bacterial cell division machinery. *Curr. Biol.* **15**:R514–R526.
 150. **Goehring, N. W., M. D. Gonzalez, and J. Beckwith.** 2006. Premature targeting of cell division proteins to midcell reveals hierarchies of protein interactions involved in divisome assembly. *Mol. Microbiol.* **61**:33–45.
 151. **Goehring, N. W., F. Gueiros-Filho, and J. Beckwith.** 2005. Premature targeting of a cell division protein to midcell allows dissection of divisome assembly in *Escherichia coli*. *Genes Dev.* **19**:127–137.
 152. **Goffin, C., and J. M. Ghuysen.** 1998. Multimodular penicillin-binding proteins: an enigmatic family of orthologs and paralogs. *Microbiol. Mol. Biol. Rev.* **62**:1079–1093.
 153. **Goldman, W. E., D. G. Klapper, and J. B. Baseman.** 1982. Detection, isolation, and analysis of a released *Bordetella pertussis* product toxic to cultured tracheal cells. *Infect. Immun.* **36**:782–794.
 154. **Gomez, J. E., and W. R. Bishai.** 2000. *whmD* is an essential mycobacterial gene required for proper septation and cell division. *Proc. Natl. Acad. Sci. USA* **97**:8554–8559.
 155. **Gonzalo Asensio, J., C. Maia, N. L. Ferrer, N. Barilone, F. Laval, C. Y. Soto, N. Winter, M. Daffe, B. Gicquel, C. Martin, and M. Jackson.** 2006. The virulence-associated two-component PhoP-PhoR system controls the biosynthesis of polyketide-derived lipids in *Mycobacterium tuberculosis*. *J. Biol. Chem.* **281**:1313–1316.
 156. **Goodell, E. W.** 1985. Recycling of murein by *Escherichia coli*. *J. Bacteriol.* **163**:305–310.
 157. **Goodell, E. W., and U. Schwarz.** 1985. Release of cell wall peptides into culture medium by exponentially growing *Escherichia coli*. *J. Bacteriol.* **162**:391–397.
 158. **Goodell, W., and A. Tomasz.** 1980. Alteration of *Escherichia coli* murein during amino acid starvation. *J. Bacteriol.* **144**:1009–1016.
 159. **Gopalaswamy, R., P. R. Narayanan, and S. Narayanan.** 2004. Cloning, overexpression, and characterization of a serine/threonine protein kinase pknI from *Mycobacterium tuberculosis* H37Rv. *Protein Expr. Purif.* **36**:82–89.
 160. **Gordon, G. S., D. Sitnikov, C. D. Webb, A. Teleman, A. Straight, R. Losick, A. W. Murray, and A. Wright.** 1997. Chromosome and low copy plasmid segregation in *E. coli*: visual evidence for distinct mechanisms. *Cell* **90**:1113–1121.
 161. **Gordon, G. S., and A. Wright.** 2000. DNA segregation in bacteria. *Annu. Rev. Microbiol.* **54**:681–708.

162. **Graham, L. L., T. J. Beveridge, and N. Nanninga.** 1991. Periplasmic space and the concept of the periplasm. *Trends Biochem. Sci.* **16**:328–329.
163. **Grantcharova, N., U. Lustig, and K. Flardh.** 2005. Dynamics of FtsZ assembly during sporulation in *Streptomyces coelicolor* A3(2). *J. Bacteriol.* **187**:3227–3237.
164. **Grantcharova, N., W. Ubhayasekera, S. L. Mowbray, J. R. McCormick, and K. Flardh.** 2003. A missense mutation in ftsZ differentially affects vegetative and developmentally controlled cell division in *Streptomyces coelicolor* A3(2). *Mol. Microbiol.* **47**:645–656.
165. **Gray, D. I., G. W. Gooday, and J. I. Prosser.** 1990. Apical hyphal extension in *Streptomyces coelicolor* A3(2). *J. Gen. Microbiol.* **136**:1077–1084.
166. **Greendyke, R., M. Rajagopalan, T. Parish, and M. V. Madiraju.** 2002. Conditional expression of *Mycobacterium smegmatis* dnaA, an essential DNA replication gene. *Microbiology* **148**:3887–3900.
167. **Greenstein, A. E., C. Grundner, N. Echols, L. M. Gay, T. N. Lombana, C. A. Miecowski, K. E. Pullen, P. Y. Sung, and T. Alber.** 2005. Structure/function studies of Ser/Thr and Tyr protein phosphorylation in *Mycobacterium tuberculosis*. *J. Mol. Microbiol. Biotechnol.* **9**:167–181.
168. **Gruber, T. M., and C. A. Gross.** 2003. Multiple sigma subunits and the partitioning of bacterial transcription space. *Annu. Rev. Microbiol.* **57**:441–466.
169. **Grundner, C., L. M. Gay, and T. Alber.** 2005. *Mycobacterium tuberculosis* serine/threonine kinases PknB, PknD, PknE, and PknF phosphorylate multiple FHA domains. *Protein Sci.* **14**:1918–1921.
170. **Gullbrand, B., T. Akerlund, and K. Nordstrom.** 1999. On the origin of branches in *Escherichia coli*. *J. Bacteriol.* **181**:6607–6614.
171. **Gupta, P., S. P. Anand, R. Srinivasan, H. Rajeswari, S. Indi, and P. Ajitkumar.** 2004. The C-terminally truncated MtFtsZ-DeltaC169 mutant of *Mycobacterium tuberculosis* FtsZ shows GTPase and GTP-induced, GTP-specific polymerization activities in vitro. *Microbiology* **150**:3906–3908.
172. **Hahn, M. Y., S. Raman, M. Anaya, and R. N. Husson.** 2005. The *Mycobacterium tuberculosis* extracytoplasmic-function sigma factor SigL regulates polyketide synthases and secreted or membrane proteins and is required for virulence. *J. Bacteriol.* **187**:7062–7071.
173. **Hale, C. A., and P. A. de Boer.** 1997. Direct binding of FtsZ to ZipA, an essential component of the septal ring structure that mediates cell division in *E. coli*. *Cell* **88**:175–185.
174. **Hampshire, T., S. Soneji, J. Bacon, B. W. James, J. Hinds, K. Laing, R. A. Stabler, P. D. Marsh, and P. D. Butcher.** 2004. Stationary phase gene expression of *Mycobacterium tuberculosis* following a progressive nutrient depletion: a model for persistent organisms? *Tuberculosis (Edinburgh)* **84**:228–238.
175. **Hartmann, M., A. Barsch, K. Niehaus, A. Puhler, A. Tauch, and J. Kalinowski.** 2004. The glycosylated cell surface protein Rpf2, containing a resuscitation-promoting factor motif, is involved in intercellular communication of *Corynebacterium glutamicum*. *Arch. Microbiol.* **182**:299–312.
176. **He, H., R. Hovey, J. Kane, V. Singh, and T. C. Zahrt.** 2006. MprAB is a stress-responsive two-component system that directly regulates expression of sigma factors SigB and SigE in *Mycobacterium tuberculosis*. *J. Bacteriol.* **188**:2134–2143.
177. **Heidrich, C., M. F. Templin, A. Ursinus, M. Merdanovic, J. Berger, H. Schwarz, M. A. de Pedro, and J. V. Holtje.** 2001. Involvement of N-acetylmuramyl-L-alanine amidases in cell separation and antibiotic-induced autolysis of *Escherichia coli*. *Mol. Microbiol.* **41**:167–178.
178. **Heidrich, C., A. Ursinus, J. Berger, H. Schwarz, and J. V. Holtje.** 2002. Effects of multiple deletions of murein hydrolases on viability, septum cleavage, and sensitivity to large toxic molecules in *Escherichia coli*. *J. Bacteriol.* **184**:6093–6099.
179. **Helmann, J. D.** 2002. The extracytoplasmic function (ECF) sigma factors. *Adv. Microb. Physiol.* **46**:47–110.
180. **Helmstetter, C. E., M. Thornton, A. Romero, and K. L. Eward.** 2003. Synchrony in human, mouse and bacterial cell cultures—a comparison. *Cell Cycle* **2**:42–45.
181. **Hermans, P. W., F. Abebe, V. I. Kuteyi, A. H. Kolk, J. E. Thole, and M. Harboe.** 1995. Molecular and immunological characterization of the highly conserved antigen 84 from *Mycobacterium tuberculosis* and *Mycobacterium leprae*. *Infect. Immun.* **63**:954–960.
182. **Hett, E. C., M. C. Chao, L. Deng, and E. J. Rubin.** A mycobacterial enzyme essential for cell division synergizes with resuscitation-promoting factor. *PLoS Pathog.*, in press.
183. **Hett, E. C., M. C. Chao, A. J. Steyn, S. M. Fortune, L. L. Deng, and E. J. Rubin.** 2007. A partner for the resuscitation-promoting factors of *Mycobacterium tuberculosis*. *Mol. Microbiol.* **66**:658–668.
184. **Ho, Y. S., L. M. Burden, and J. H. Hurley.** 2000. Structure of the GAF domain, a ubiquitous signaling motif and a new class of cyclic GMP receptor. *EMBO J.* **19**:5288–5299.
185. **Hoch, J. A.** 2000. Two-component and phosphorelay signal transduction. *Curr. Opin. Microbiol.* **3**:165–170.
186. **Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams (ed.).** 1994. *Bergey's manual of determinative bacteriology*, 9th ed. Williams & Wilkins, Baltimore, MD.
187. **Holtje, J. V.** 1995. From growth to autolysis: the murein hydrolases in *Escherichia coli*. *Arch. Microbiol.* **164**:243–254.
188. **Holtje, J. V.** 1998. Growth of the stress-bearing and shape-maintaining murein sacculus of *Escherichia coli*. *Microbiol. Mol. Biol. Rev.* **62**:181–203.
189. **Holtje, J. V., and C. Heidrich.** 2001. Enzymology of elongation and constriction of the murein sacculus of *Escherichia coli*. *Biochimie* **83**:103–108.
190. **Holyoak, C. D., S. Thompson, C. Ortiz Calderon, K. Hatzixanthis, B. Bauer, K. Kuchler, P. W. Piper, and P. J. Coote.** 2000. Loss of Cmk1 Ca(2+)-calmodulin-dependent protein kinase in yeast results in constitutive weak organic acid resistance, associated with a post-transcriptional activation of the Pdr12 ATP-binding cassette transporter. *Mol. Microbiol.* **37**:595–605.
191. **Hopwood, D. A., H. Wildermuth, and H. M. Palmer.** 1970. Mutants of *Streptomyces coelicolor* defective in sporulation. *J. Gen. Microbiol.* **61**:397–408.
192. **Hu, Z., and J. Lutkenhaus.** 2000. Analysis of MinC reveals two independent domains involved in interaction with MinD and FtsZ. *J. Bacteriol.* **182**:3965–3971.
193. **Hu, Z., and J. Lutkenhaus.** 1999. Topological regulation of cell division in *Escherichia coli* involves rapid pole to pole oscillation of the division inhibitor MinC under the control of MinD and MinE. *Mol. Microbiol.* **34**:82–90.
194. **Hu, Z., C. Saez, and J. Lutkenhaus.** 2003. Recruitment of MinC, an inhibitor of Z-ring formation, to the membrane in *Escherichia coli*: role of MinD and MinE. *J. Bacteriol.* **185**:196–203.
195. **Ikeda, M., T. Sato, M. Wachi, H. K. Jung, F. Ishino, Y. Kobayashi, and M. Matsuhashi.** 1989. Structural similarity among *Escherichia coli* FtsW and RodA proteins and *Bacillus subtilis* SpoVE protein, which function in cell division, cell elongation, and spore formation, respectively. *J. Bacteriol.* **171**:6375–6378.
196. **Ioanoviciu, A., E. T. Yukl, P. Moenne-Loccoz, and P. R. Montellano.** 2007. DevS, a heme-containing two-component oxygen sensor of *Mycobacterium tuberculosis*. *Biochemistry* **46**:4250–4260.
197. **Jacobs, C., J. M. Frere, and S. Normark.** 1997. Cytosolic intermediates for cell wall biosynthesis and degradation control inducible beta-lactam resistance in gram-negative bacteria. *Cell* **88**:823–832.
198. **Jakimowicz, P., M. R. Cheesman, W. R. Bishai, K. F. Chater, A. J. Thomson, and M. J. Buttner.** 2005. Evidence that the *Streptomyces* developmental protein WhiD, a member of the WhiB family, binds a [4Fe-4S] cluster. *J. Biol. Chem.* **280**:8309–8315.
199. **Jarlier, V., and H. Nikaido.** 1994. Mycobacterial cell wall: structure and role in natural resistance to antibiotics. *FEMS Microbiol. Lett.* **123**:11–18.
200. **Jarlier, V., and H. Nikaido.** 1990. Permeability barrier to hydrophilic solutes in *Mycobacterium chelonae*. *J. Bacteriol.* **172**:1418–1423.
201. **Jishage, M., K. Kvint, V. Shingler, and T. Nyström.** 2002. Regulation of sigma factor competition by the alarmone ppGpp. *Genes Dev.* **16**:1260–1270.
202. **Jones, G., and P. Dyson.** 2006. Evolution of transmembrane protein kinases implicated in coordinating remodeling of gram-positive peptidoglycan: inside versus outside. *J. Bacteriol.* **188**:7470–7476.
203. **Jones, L. J., R. Carballido-Lopez, and J. E. Irvington.** 2001. Control of cell shape in bacteria: helical, actin-like filaments in *Bacillus subtilis*. *Cell* **104**:913–922.
204. **Joo, D. M., N. Ng, and R. Calendar.** 1997. A sigma32 mutant with a single amino acid change in the highly conserved region 2.2 exhibits reduced core RNA polymerase affinity. *Proc. Natl. Acad. Sci. USA* **94**:4907–4912.
205. **Joo, D. M., A. Nolte, R. Calendar, Y. N. Zhou, and D. J. Jin.** 1998. Multiple regions on the *Escherichia coli* heat shock transcription factor σ 32 determine core RNA polymerase binding specificity. *J. Bacteriol.* **180**:1095–1102.
206. **Kandler, O., and H. Konig.** 1978. Chemical composition of the peptidoglycan-free cell walls of methanogenic bacteria. *Arch. Microbiol.* **118**:141–152.
207. **Kang, C. M., D. W. Abbott, S. T. Park, C. C. Dascher, L. C. Cantley, and R. N. Husson.** 2005. The *Mycobacterium tuberculosis* serine/threonine kinases PknA and PknB: substrate identification and regulation of cell shape. *Genes Dev.* **19**:1692–1704.
208. **Karakousis, P. C., T. Yoshimatsu, G. Lamichhane, S. C. Woolwine, E. L. Nuermberger, J. Grosset, and W. R. Bishai.** 2004. Dormancy phenotype displayed by extracellular *Mycobacterium tuberculosis* within artificial granulomas in mice. *J. Exp. Med.* **200**:647–657.
209. **Karimova, G., N. Dautin, and D. Ladant.** 2005. Interaction network among *Escherichia coli* membrane proteins involved in cell division as revealed by bacterial two-hybrid analysis. *J. Bacteriol.* **187**:2233–2243.
210. **Kato, J., Y. Nishimura, R. Imamura, H. Niki, S. Hiraga, and H. Suzuki.** 1990. New topoisomerase essential for chromosome segregation in *E. coli*. *Cell* **63**:393–404.
211. **Kawai, Y., S. Moriya, and N. Ogasawara.** 2003. Identification of a protein, YneA, responsible for cell division suppression during the SOS response in *Bacillus subtilis*. *Mol. Microbiol.* **47**:1113–1122.
212. **Keep, N. H., J. M. Ward, M. Cohen-Gonsaud, and B. Henderson.** 2006. Wake up! Peptidoglycan lysis and bacterial non-growth states. *Trends Microbiol.* **14**:271–276.
213. **Kendall, S. L., F. Movahedzadeh, S. C. Rison, L. Wernisch, T. Parish, K.**

- Duncan, J. C. Betts, and N. G. Stoker. 2004. The Mycobacterium tuberculosis dosRS two-component system is induced by multiple stresses. *Tuberculosis* (Edinburgh) **84**:247–255.
214. Khattar, M. M., S. G. Addinall, K. H. Stedul, D. S. Boyle, J. Lutkenhaus, and W. D. Donachie. 1997. Two polypeptide products of the *Escherichia coli* cell division gene *ftsW* and a possible role for FtsW in FtsZ function. *J. Bacteriol.* **179**:784–793.
215. Kim, H. J., M. J. Calcutt, F. J. Schmidt, and K. F. Chater. 2000. Partitioning of the linear chromosome during sporulation of *Streptomyces coelicolor* A3(2) involves an *oriC*-linked *parAB* locus. *J. Bacteriol.* **182**:1313–1320.
216. Klann, A. G., A. E. Belanger, A. Abanes-De Mello, J. Y. Lee, and G. F. Hatfull. 1998. Characterization of the *dnaG* locus in *Mycobacterium smegmatis* reveals linkage of DNA replication and cell division. *J. Bacteriol.* **180**:65–72.
217. Koch, A. L. 1998. Orientation of the peptidoglycan chains in the sacculus of *Escherichia coli*. *Res. Microbiol.* **149**:689–701.
218. Koch, A. L. 2000. Simulation of the conformation of the murein fabric: the oligoglycan, penta-muropeptide, and cross-linked nona-muropeptide. *Arch. Microbiol.* **174**:429–439.
219. Kojima, N., K. Uchikawa, Y. Araki, and E. Ito. 1985. A common linkage saccharide unit between teichoic acids and peptidoglycan in cell walls of *Bacillus coagulans*. *J. Biochem. (Tokyo)* **97**:1085–1092.
220. Kolter, R., D. A. Siegel, and A. Tormo. 1993. The stationary phase of the bacterial life cycle. *Annu. Rev. Microbiol.* **47**:855–874.
221. Komeli, A., Z. Li, D. K. Newman, and G. J. Jensen. 2006. Magnetosomes are cell membrane invaginations organized by the actin-like protein MamK. *Science* **311**:242–245.
222. Konig, H., and O. Kandler. 1979. The amino acid sequence of the peptide moiety of the pseudomurein from *Methanobacterium thermoautotrophicum*. *Arch. Microbiol.* **121**:271–275.
223. Koropatnick, T. A., J. T. Engle, M. A. Apicella, E. V. Stabb, W. E. Goldman, and M. J. McFall-Ngai. 2004. Microbial factor-mediated development in a host-bacterial mutualism. *Science* **306**:1186–1188.
224. Kotani, S., I. Yanagida, K. Kato, and T. Matsuda. 1970. Studies on peptides, glycopeptides and antigenic polysaccharide-glycopeptide complexes isolated from an L-11 enzyme lysate of the cell walls of *Mycobacterium tuberculosis* strain H37Rv. *Biken J.* **13**:249–275.
225. Koul, A., A. Choidas, A. K. Tyagi, K. Drlica, Y. Singh, and A. Ullrich. 2001. Serine/threonine protein kinases PknF and PknG of *Mycobacterium tuberculosis*: characterization and localization. *Microbiology* **147**:2307–2314.
226. Kumar, A., J. C. Toledo, R. P. Patel, J. R. Lancaster, Jr., and A. J. Steyn. 2007. *Mycobacterium tuberculosis* DosS is a redox sensor and DosT is a hypoxia sensor. *Proc. Natl. Acad. Sci. USA* **104**:11568–11573.
227. Kurner, J., A. S. Frangakis, and W. Baumeister. 2005. Cryo-electron tomography reveals the cytoskeletal structure of *Spiroplasma melliferum*. *Science* **307**:436–438.
228. Kuroda, A., H. Murphy, M. Cashel, and A. Kornberg. 1997. Guanosine tetra- and pentaphosphate promote accumulation of inorganic polyphosphate in *Escherichia coli*. *J. Biol. Chem.* **272**:21240–21243.
229. Kuroda, A., K. Nomura, R. Ohtomo, J. Kato, T. Ikeda, N. Takiguchi, H. Ohtake, and A. Kornberg. 2001. Role of inorganic polyphosphate in promoting ribosomal protein degradation by the Lon protease in *E. coli*. *Science* **293**:705–708.
230. Kuroda, A., S. Tanaka, T. Ikeda, J. Kato, N. Takiguchi, and H. Ohtake. 1999. Inorganic polyphosphate kinase is required to stimulate protein degradation and for adaptation to amino acid starvation in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **96**:14264–14269.
231. Latch, J. N., and W. Margolin. 1997. Generation of buds, swellings, and branches instead of filaments after blocking the cell cycle of *Rhizobium meliloti*. *J. Bacteriol.* **179**:2373–2381.
232. Lederberg, J. 1956. Bacterial protoplasts induced by penicillin. *Proc. Natl. Acad. Sci. USA* **42**:574–577.
233. Lederer, E. 1971. The mycobacterial cell wall. *Pure Appl. Chem.* **25**:135–165.
234. Lemon, K. P., and A. D. Grossman. 2000. Movement of replicating DNA through a stationary replisome. *Mol. Cell* **6**:1321–1330.
235. Lemon, K. P., and A. D. Grossman. 2001. The extrusion-capture model for chromosome partitioning in bacteria. *Genes Dev.* **15**:2031–2041.
236. Lemon, K. P., I. Kurtser, and A. D. Grossman. 2001. Effects of replication termination mutants on chromosome partitioning in *Bacillus subtilis*. *Proc. Natl. Acad. Sci. USA* **98**:212–217.
237. Leonard, C. J., L. Aravind, and E. V. Koonin. 1998. Novel families of putative protein kinases in bacteria and archaea: evolution of the “eukaryotic” protein kinase superfamily. *Genome Res.* **8**:1038–1047.
238. Leonard, T. A., J. Moller-Jensen, and J. Lowe. 2005. Towards understanding the molecular basis of bacterial DNA segregation. *Philos. Trans. R. Soc. London B* **360**:523–535.
239. Lepage, S., P. Dubois, T. K. Ghosh, B. Joris, S. Mahapatra, M. Kundu, J. Basu, P. Chakrabarti, S. T. Cole, M. Nguyen-Disteche, and J. M. Ghuyen. 1997. Dual multimodular class A penicillin-binding proteins in *Mycobacterium leprae*. *J. Bacteriol.* **179**:4627–4630.
240. Leung, A. K., E. Lucile White, L. J. Ross, R. C. Reynolds, J. A. DeVito, and D. W. Borhani. 2004. Structure of *Mycobacterium tuberculosis* FtsZ reveals unexpected, G protein-like conformational switches. *J. Mol. Biol.* **342**:953–970.
241. Leung, A. K., E. L. White, L. J. Ross, and D. W. Borhani. 2000. Crystallization of the *Mycobacterium tuberculosis* cell-division protein FtsZ. *Acta Crystallogr. D* **56**:1634–1637.
242. Levin, P. A., I. G. Kurtser, and A. D. Grossman. 1999. Identification and characterization of a negative regulator of FtsZ ring formation in *Bacillus subtilis*. *Proc. Natl. Acad. Sci. USA* **96**:9642–9647.
243. Lewis, K. 2007. Persister cells, dormancy and infectious disease. *Nat. Rev. Microbiol.* **5**:48–56.
244. Lindquist, S., K. Weston-Hafer, H. Schmidt, C. Pul, G. Korfmann, J. Erickson, C. Sanders, H. H. Martin, and S. Normark. 1993. AmpG, a signal transducer in chromosomal beta-lactamase induction. *Mol. Microbiol.* **9**:703–715.
245. Leo, M. M., P. Canepari, and G. Satta. 1990. Bacterial cell shape regulation: testing of additional predictions unique to the two-competing-sites model for peptidoglycan assembly and isolation of conditional rod-shaped mutants from some wild-type cocci. *J. Bacteriol.* **172**:3758–3771.
246. Lowe, J., and L. A. Amos. 1999. Tubulin-like protofilaments in Ca²⁺-induced FtsZ sheets. *EMBO J.* **18**:2364–2371.
247. Lu, C., J. Stricker, and H. P. Erickson. 2001. Site-specific mutations of FtsZ—effects on GTPase and in vitro assembly. *BMC Microbiol.* **1**:7.
248. Lutkenhaus, J. F., H. Wolf-Watz, and W. D. Donachie. 1980. Organization of genes in the *ftsA-envA* region of the *Escherichia coli* genetic map and identification of a new *fts* locus (*ftsZ*). *J. Bacteriol.* **142**:615–620.
249. Ma, L., G. F. King, and L. Rothfield. 2004. Positioning of the MinE binding site on the MinD surface suggests a plausible mechanism for activation of the *Escherichia coli* MinD ATPase during division site selection. *Mol. Microbiol.* **54**:99–108.
250. Ma, X., D. W. Ehrhardt, and W. Margolin. 1996. Colocalization of cell division proteins FtsZ and FtsA to cytoskeletal structures in living *Escherichia coli* cells by using green fluorescent protein. *Proc. Natl. Acad. Sci. USA* **93**:12998–13003.
251. Madiraju, M. V., M. Moomey, P. F. Neuenschwander, S. Muniruzzaman, K. Yamamoto, J. E. Grimwade, and M. Rajagopalan. 2006. The intrinsic ATPase activity of *Mycobacterium tuberculosis* DnaA promotes rapid oligomerization of DnaA on *oriC*. *Mol. Microbiol.* **59**:1876–1890.
252. Magasanik, B. 1995. Historical perspective, p. 1–5. *In* J. A. Hoch and T. J. Silhavy (ed.), *Two-component signal transduction*. ASM Press, Washington, DC.
253. Magnet, S., A. Arbeloa, J. L. Mainardi, J. E. Hugonnet, M. Fourgeaud, L. Dubost, A. Marie, V. Delfosse, C. Mayer, L. B. Rice, and M. Arthur. 2007. Specificity of L, D-transpeptidases from Gram-positive bacteria producing different peptidoglycan chemotypes. *J. Biol. Chem.* **282**:13151–13159.
254. Mahapatra, S., T. Yagi, J. T. Belisle, B. J. Espinosa, P. J. Hill, M. R. McNeil, P. J. Brennan, and D. C. Crick. 2005. Mycobacterial lipid II is composed of a complex mixture of modified muramyl and peptide moieties linked to decaprenyl phosphate. *J. Bacteriol.* **187**:2747–2757.
255. Mahne, M., A. Tauch, A. Puhler, and J. Kalinowski. 2006. The *Corynebacterium glutamicum* gene *pmt* encoding a glycosyltransferase related to eukaryotic protein-O-mannosyltransferases is essential for glycosylation of the resuscitation promoting factor (Rpf2) and other secreted proteins. *FEMS Microbiol. Lett.* **259**:226–233.
256. Mailaender, C., N. Reiling, H. Engelhardt, S. Bossmann, S. Ehlers, and M. Niederweis. 2004. The MspA porin promotes growth and increases antibiotic susceptibility of both *Mycobacterium bovis* BCG and *Mycobacterium tuberculosis*. *Microbiology* **150**:853–864.
257. Makinoshima, H., and M. S. Glickman. 2005. Regulation of *Mycobacterium tuberculosis* cell envelope composition and virulence by intramembrane proteolysis. *Nature* **436**:406–409.
258. Malhotra, V., D. Sharma, V. D. Ramanathan, H. Shakila, D. K. Saini, S. Chakravorty, T. K. Das, Q. Li, R. F. Silver, P. R. Narayanan, and J. S. Tyagi. 2004. Disruption of response regulator gene, *devR*, leads to attenuation in virulence of *Mycobacterium tuberculosis*. *FEMS Microbiol. Lett.* **231**:237–245.
259. Manganelli, R., L. Fattorini, D. Tan, E. Iona, G. Orefici, G. Altavilla, P. Cusatelli, and I. Smith. 2004. The extra cytoplasmic function sigma factor σ^E is essential for *Mycobacterium tuberculosis* virulence in mice. *Infect. Immun.* **72**:3038–3041.
260. Manganelli, R., R. Proveddi, S. Rodrigue, J. Beaucher, L. Gaudreau, and I. Smith. 2004. Sigma factors and global gene regulation in *Mycobacterium tuberculosis*. *J. Bacteriol.* **186**:895–902.
261. Manganelli, R., M. I. Voskuil, G. K. Schoolnik, E. Dubnau, M. Gomez, and I. Smith. 2002. Role of the extracytoplasmic-function sigma factor sigma(H) in *Mycobacterium tuberculosis* global gene expression. *Mol. Microbiol.* **45**:365–374.
262. Manganelli, R., M. I. Voskuil, G. K. Schoolnik, and I. Smith. 2001. The *Mycobacterium tuberculosis* ECF sigma factor sigmaE: role in global gene expression and survival in macrophages. *Mol. Microbiol.* **41**:423–437.
263. Margolin, W. 2005. FtsZ and the division of prokaryotic cells and organelles. *Nat. Rev. Mol. Cell Biol.* **6**:862–871.

264. Margolin, W. 2000. Themes and variations in prokaryotic cell division. *FEMS Microbiol. Rev.* **24**:531–548.
265. Marston, A. L., H. B. Thomaidis, D. H. Edwards, M. E. Sharpe, and J. Errington. 1998. Polar localization of the MinD protein of *Bacillus subtilis* and its role in selection of the mid-cell division site. *Genes Dev.* **12**:3419–3430.
266. Martin, M. E., M. J. Trimble, and Y. V. Brun. 2004. Cell cycle-dependent abundance, stability and localization of FtsA and FtsQ in *Caulobacter crescentus*. *Mol. Microbiol.* **54**:60–74.
267. Matias, V. R., and T. J. Beveridge. 2005. Cryo-electron microscopy reveals native polymeric cell wall structure in *Bacillus subtilis* 168 and the existence of a periplasmic space. *Mol. Microbiol.* **56**:240–251.
268. Matsushashi, M. 1994. Utilization of lipid-linked precursors and the formation of peptidoglycan in the process of cell growth and division: membrane enzymes involved in the final steps of peptidoglycan synthesis and the mechanism of their regulation, p. 55–72. *In* J. M. Ghuysen and R. Hakenbeck (ed.), *Bacterial cell wall*. Elsevier Science B.V., Amsterdam, The Netherlands.
269. Mazza, P., E. E. Noens, K. Schirner, N. Grantcharova, A. M. Mommaas, H. K. Koerten, G. Muth, K. Flardh, G. P. van Wezel, and W. Wohlleben. 2006. MreB of *Streptomyces coelicolor* is not essential for vegetative growth but is required for the integrity of aerial hyphae and spores. *Mol. Microbiol.* **60**:838–852.
270. McCormick, J. R., E. P. Su, A. Driks, and R. Losick. 1994. Growth and viability of *Streptomyces coelicolor* mutant for the cell division gene *ftsZ*. *Mol. Microbiol.* **14**:243–254.
271. McDonough, J. A., K. E. Hacker, A. R. Flores, M. S. Pavelka, Jr., and M. Braunstein. 2005. The twin-arginine translocation pathway of *Mycobacterium smegmatis* is functional and required for the export of mycobacterial beta-lactamases. *J. Bacteriol.* **187**:7667–7679.
272. McNeil, M., M. Daffe, and P. J. Brennan. 1990. Evidence for the nature of the link between the arabinogalactan and peptidoglycan of mycobacterial cell walls. *J. Biol. Chem.* **265**:18200–18206.
273. McNeil, M., S. J. Wallner, S. W. Hunter, and P. J. Brennan. 1987. Demonstration that the galactosyl and arabinosyl residues in the cell-wall arabinogalactan of *Mycobacterium leprae* and *Mycobacterium tuberculosis* are furanoid. *Carbohydr. Res.* **166**:299–308.
274. Melly, M. A., Z. A. McGee, and R. S. Rosenthal. 1984. Ability of monomeric peptidoglycan fragments from *Neisseria gonorrhoeae* to damage human fallopian-tube mucosa. *J. Infect. Dis.* **149**:378–386.
275. Mercer, K. L., and D. S. Weiss. 2002. The *Escherichia coli* cell division protein FtsW is required to recruit its cognate transpeptidase, FtsI (PBP3), to the division site. *J. Bacteriol.* **184**:904–912.
276. Merouch, S. O., K. Z. Bencze, D. Heseck, M. Lee, J. F. Fisher, T. L. Stemmler, and S. Mobashery. 2006. Three-dimensional structure of the bacterial cell wall peptidoglycan. *Proc. Natl. Acad. Sci. USA* **103**:4404–4409.
277. Messer, W. 2002. The bacterial replication initiator DnaA. DnaA and oriC, the bacterial mode to initiate DNA replication. *FEMS Microbiol. Rev.* **26**:355–374.
278. Miguez, E. M., C. Martin, M. B. Manzanal, and C. Hardisson. 1992. Growth and morphogenesis in *Streptomyces*. *FEMS Microbiol. Lett.* **79**:351–359.
279. Mikusova, K., M. Mikus, G. S. Besra, I. Hancock, and P. J. Brennan. 1996. Biosynthesis of the linkage region of the mycobacterial cell wall. *J. Biol. Chem.* **271**:7820–7828.
280. Mikusova, K., R. A. Slayden, G. S. Besra, and P. J. Brennan. 1995. Biogenesis of the mycobacterial cell wall and the site of action of ethambutol. *Antimicrob. Agents Chemother.* **39**:2484–2489.
281. Mikusova, K., T. Yagi, R. Stern, M. R. McNeil, G. S. Besra, D. C. Crick, and P. J. Brennan. 2000. Biosynthesis of the galactan component of the mycobacterial cell wall. *J. Biol. Chem.* **275**:33890–33897.
282. Mir, M. A., H. S. Rajeswari, U. Veeraraghavan, and P. Ajitkumar. 2006. Molecular characterisation of ABC transporter type FtsE and FtsX proteins of *Mycobacterium tuberculosis*. *Arch. Microbiol.* **185**:147–158.
283. Mittenhuber, G. 1999. Occurrence of mazEF-like antitoxin/toxin systems in bacteria. *J. Mol. Microbiol. Biotechnol.* **1**:295–302.
284. Molle, V., C. Girard-Blanc, L. Kremer, P. Doublet, A. J. Cozzone, and J. F. Prost. 2003. Protein PknE, a novel transmembrane eukaryotic-like serine/threonine kinase from *Mycobacterium tuberculosis*. *Biochem. Biophys. Res. Commun.* **308**:820–825.
285. Molle, V., L. Kremer, C. Girard-Blanc, G. S. Besra, A. J. Cozzone, and J. F. Prost. 2003. An FHA phosphoprotein recognition domain mediates protein EmbR phosphorylation by PknH, a Ser/Thr protein kinase from *Mycobacterium tuberculosis*. *Biochemistry* **42**:15300–15309.
286. Molle, V., N. Saint, S. Campagna, L. Kremer, E. Lea, P. Draper, and G. Molle. 2006. pH-dependent pore-forming activity of OmpATb from *Mycobacterium tuberculosis* and characterization of the channel by peptidic dissection. *Mol. Microbiol.* **61**:826–837.
287. Molle, V., D. Soulat, J. M. Jault, C. Grangeasse, A. J. Cozzone, and J. F. Prost. 2004. Two FHA domains on an ABC transporter, Rv1747, mediate its phosphorylation by PknF, a Ser/Thr protein kinase from *Mycobacterium tuberculosis*. *FEMS Microbiol. Lett.* **234**:215–223.
288. Mukamolova, G. V., A. S. Kaprelyants, D. I. Young, M. Young, and D. B. Kell. 1998. A bacterial cytokine. *Proc. Natl. Acad. Sci. USA* **95**:8916–8921.
289. Mukamolova, G. V., S. S. Korner, D. B. Kell, and A. S. Kaprelyants. 1999. Stimulation of the multiplication of *Micrococcus luteus* by an autocrine growth factor. *Arch. Microbiol.* **172**:9–14.
290. Mukamolova, G. V., A. G. Murzin, E. G. Salina, G. R. Demina, D. B. Kell, A. S. Kaprelyants, and M. Young. 2006. Muralytic activity of *Micrococcus luteus* Rpf and its relationship to physiological activity in promoting bacterial growth and resuscitation. *Mol. Microbiol.* **59**:84–98.
291. Mukamolova, G. V., O. A. Turapov, K. Kazarian, M. Telkov, A. S. Kaprelyants, D. B. Kell, and M. Young. 2002. The *rpf* gene of *Micrococcus luteus* encodes an essential secreted growth factor. *Mol. Microbiol.* **46**:611–621.
292. Mukamolova, G. V., O. A. Turapov, D. I. Young, A. S. Kaprelyants, D. B. Kell, and M. Young. 2002. A family of autocrine growth factors in *Mycobacterium tuberculosis*. *Mol. Microbiol.* **46**:623–635.
293. Mukherjee, A., K. Dai, and J. Lutkenhaus. 1993. *Escherichia coli* cell division protein FtsZ is a guanine nucleotide binding protein. *Proc. Natl. Acad. Sci. USA* **90**:1053–1057.
294. Mukherjee, A., and J. Lutkenhaus. 1994. Guanine nucleotide-dependent assembly of FtsZ into filaments. *J. Bacteriol.* **176**:2754–2758.
295. Mukherjee, A., C. Saez, and J. Lutkenhaus. 2001. Assembly of an FtsZ mutant deficient in GTPase activity has implications for FtsZ assembly and the role of the Z ring in cell division. *J. Bacteriol.* **183**:7190–7197.
296. Murray, K. D., and H. Bremer. 1996. Control of *spoT*-dependent ppGpp synthesis and degradation in *Escherichia coli*. *J. Mol. Biol.* **259**:41–57.
297. Muttucumar, D. G., G. Roberts, J. Hinds, R. A. Stabler, and T. Parish. 2004. Gene expression profile of *Mycobacterium tuberculosis* in a non-replicating state. *Tuberculosis (Edinburgh)* **84**:239–246.
298. Narayan, A., P. Sachdeva, K. Sharma, A. K. Saini, A. K. Tyagi, and Y. Singh. 2007. Serine threonine protein kinases of mycobacterial genus: phylogeny to function. *Physiol. Genomics* **29**:66–75.
299. Nelson, D. E., and K. D. Young. 2000. Penicillin binding protein 5 affects cell diameter, contour, and morphology of *Escherichia coli*. *J. Bacteriol.* **182**:1714–1721.
300. Nguyen, L., and C. J. Thompson. 2006. Foundations of antibiotic resistance in bacterial physiology: the mycobacterial paradigm. *Trends Microbiol.* **14**:304–312.
301. Nigou, J., M. Gilleron, and G. Puzo. 2003. Lipoarabinomannans: from structure to biosynthesis. *Biochimie* **85**:153–166.
302. Nikaïdo, H., and M. Vaara. 1985. Molecular basis of bacterial outer membrane permeability. *Microbiol. Rev.* **49**:1–32.
303. Nilsen, T., A. S. Ghosh, M. B. Goldberg, and K. D. Young. 2004. Branching sites and morphological abnormalities behave as ectopic poles in shape-defective *Escherichia coli*. *Mol. Microbiol.* **52**:1045–1054.
304. Ohashi, T., C. A. Hale, P. A. de Boer, and H. P. Erickson. 2002. Structural evidence that the P/Q domain of ZipA is an unstructured, flexible tether between the membrane and the C-terminal FtsZ-binding domain. *J. Bacteriol.* **184**:4313–4315.
305. Ojha, A., M. Anand, A. Bhatt, L. Kremer, W. R. Jacobs, Jr., and G. F. Hatfull. 2005. GroEL1: a dedicated chaperone involved in mycolic acid biosynthesis during biofilm formation in mycobacteria. *Cell* **123**:861–873.
306. Ortiz-Lombardia, M., F. Pompeo, B. Boitel, and P. M. Alzari. 2003. Crystal structure of the catalytic domain of the PknB serine/threonine kinase from *Mycobacterium tuberculosis*. *J. Biol. Chem.* **278**:13094–13100.
307. O'Toole, R., M. J. Smeulders, M. C. Blokpoel, E. J. Kay, K. Lougheed, and H. D. Williams. 2003. A two-component regulator of universal stress protein expression and adaptation to oxygen starvation in *Mycobacterium smegmatis*. *J. Bacteriol.* **185**:1543–1554.
308. Pandey, D. P., and K. Gerdes. 2005. Toxin-antitoxin loci are highly abundant in free-living but lost from host-associated prokaryotes. *Nucleic Acids Res.* **33**:966–976.
309. Parish, T., D. A. Smith, S. Kendall, N. Casali, G. J. Bancroft, and N. G. Stoker. 2003. Deletion of two-component regulatory systems increases the virulence of *Mycobacterium tuberculosis*. *Infect. Immun.* **71**:1134–1140.
310. Parish, T., D. A. Smith, G. Roberts, J. Betts, and N. G. Stoker. 2003. The *senX3-regX3* two-component regulatory system of *Mycobacterium tuberculosis* is required for virulence. *Microbiology* **149**:1423–1435.
311. Park, H. D., K. M. Guinn, M. I. Harrell, R. Liao, M. I. Voskuil, M. Tompa, G. K. Schoolnik, and D. R. Sherman. 2003. Rv3133c/dosR is a transcription factor that mediates the hypoxic response of *Mycobacterium tuberculosis*. *Mol. Microbiol.* **48**:833–843.
312. Park, J. T. 1995. Why does *Escherichia coli* recycle its cell wall peptides? *Mol. Microbiol.* **17**:421–426.
313. Pavelka, M. S., Jr. 2007. Another brick in the wall. *Trends Microbiol.* **15**:147–149.
314. Perez, E., S. Samper, Y. Bordas, C. Guilhot, B. Gicquel, and C. Martin. 2001. An essential role for *phoP* in *Mycobacterium tuberculosis* virulence. *Mol. Microbiol.* **41**:179–187.
315. Petit, J. F., A. Adam, J. Wietzerbin-Falszpan, E. Lederer, and J. M. Ghuysen. 1969. Chemical structure of the cell wall of *Mycobacterium smeg-*

- matic. I. Isolation and partial characterization of the peptidoglycan. *Biochem. Biophys. Res. Commun.* **35**:478–485.
316. **Phoenix, P., and G. R. Drapeau.** 1988. Cell division control in *Escherichia coli* K-12: some properties of the *ftsZ84* mutation and suppression of this mutation by the product of a newly identified gene. *J. Bacteriol.* **170**:4338–4342.
317. **Pichoff, S., and J. Lutkenhaus.** 2002. Unique and overlapping roles for ZipA and FtsA in septal ring assembly in *Escherichia coli*. *EMBO J.* **21**:685–693.
318. **Pilgrim, S., A. Kolb-Maurer, I. Gentschev, W. Goebel, and M. Kuhn.** 2003. Deletion of the gene encoding p60 in *Listeria monocytogenes* leads to abnormal cell division and loss of actin-based motility. *Infect. Immun.* **71**:3473–3484.
319. **Pink, D., J. Moeller, B. Quinn, M. Jericho, and T. Beveridge.** 2000. On the architecture of the gram-negative bacterial murein sacculus. *J. Bacteriol.* **182**:5925–5930.
320. **Pisabarro, A. G., M. A. de Pedro, and D. Vazquez.** 1985. Structural modifications in the peptidoglycan of *Escherichia coli* associated with changes in the state of growth of the culture. *J. Bacteriol.* **161**:238–242.
321. **Piuri, M., and G. F. Hatfull.** 2006. A peptidoglycan hydrolase motif within the mycobacteriophage TM4 tape measure protein promotes efficient infection of stationary phase cells. *Mol. Microbiol.* **62**:1569–1585.
322. **Popham, D. L., and K. D. Young.** 2003. Role of penicillin-binding proteins in bacterial cell morphogenesis. *Curr. Opin. Microbiol.* **6**:594–599.
323. **Praefcke, G. J., and H. T. McMahon.** 2004. The dynamin superfamily: universal membrane tubulation and fission molecules? *Nat. Rev. Mol. Cell Biol.* **5**:133–147.
324. **Prinzis, S., D. Chatterjee, and P. J. Brennan.** 1993. Structure and antigenicity of lipoarabinomannan from *Mycobacterium bovis* BCG. *J. Gen. Microbiol.* **139**:2649–2658.
325. **Quardokus, E. M., N. Din, and Y. V. Brun.** 2001. Cell cycle and positional constraints on FtsZ localization and the initiation of cell division in *Caulobacter crescentus*. *Mol. Microbiol.* **39**:949–959.
326. **Raghunand, T. R., and W. R. Bishai.** 2006. Mapping essential domains of *Mycobacterium smegmatis* WhmD: insights into WhiB structure and function. *J. Bacteriol.* **188**:6966–6976.
327. **Raghunand, T. R., and W. R. Bishai.** 2006. *Mycobacterium smegmatis* whmD and its homologue *Mycobacterium tuberculosis* whiB2 are functionally equivalent. *Microbiology* **152**:2735–2747.
328. **Rajagopalan, M., M. A. Atkinson, H. Lofton, A. Chauhan, and M. V. Madiraju.** 2005. Mutations in the GTP-binding and synergy loop domains of *Mycobacterium tuberculosis* ftsZ compromise its function in vitro and in vivo. *Biochem. Biophys. Res. Commun.* **331**:1171–1177.
329. **Rajagopalan, M., E. Maloney, J. Dziadek, M. Poplowska, H. Lofton, A. Chauhan, and M. V. Madiraju.** 2005. Genetic evidence that mycobacterial FtsZ and FtsW proteins interact, and colocalize to the division site in *Mycobacterium smegmatis*. *FEMS Microbiol. Lett.* **250**:9–17.
330. **Raman, K., P. Rajagopalan, and N. Chandra.** 2005. Flux balance analysis of mycolic acid pathway: targets for anti-tubercular drugs. *PLoS Comput. Biol.* **1**:e46.
331. **Raman, S., R. Hazra, C. C. Dascher, and R. N. Husson.** 2004. Transcription regulation by the *Mycobacterium tuberculosis* alternative sigma factor SigD and its role in virulence. *J. Bacteriol.* **186**:6605–6616.
332. **Raman, S., T. Song, X. Puyang, S. Bardarov, W. R. Jacobs, Jr., and R. N. Husson.** 2001. The alternative sigma factor SigH regulates major components of oxidative and heat stress responses in *Mycobacterium tuberculosis*. *J. Bacteriol.* **183**:6119–6125.
333. **Ramos, A., M. Letek, A. B. Campelo, J. Vaquera, L. M. Mateos, and J. A. Gil.** 2005. Altered morphology produced by ftsZ expression in *Corynebacterium glutamicum* ATCC 13869. *Microbiology* **151**:2563–2572.
334. **Rao, V., N. Fujiwara, S. A. Porcelli, and M. S. Glickman.** 2005. *Mycobacterium tuberculosis* controls host innate immune activation through cyclopropane modification of a glycolipid effector molecule. *J. Exp. Med.* **201**:535–543.
335. **Raskin, D. M., and P. A. de Boer.** 1999. MinDE-dependent pole-to-pole oscillation of division inhibitor MinC in *Escherichia coli*. *J. Bacteriol.* **181**:6419–6424.
336. **Raskin, D. M., and P. A. de Boer.** 1999. Rapid pole-to-pole oscillation of a protein required for directing division to the middle of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **96**:4971–4976.
337. **Raskin, D. M., and P. A. de Boer.** 1997. The MinE ring: an FtsZ-independent cell structure required for selection of the correct division site in *E. coli*. *Cell* **91**:685–694.
338. **Ravagnani, A., C. L. Finan, and M. Young.** 2005. A novel firmicute protein family related to the actinobacterial resuscitation-promoting factors by non-orthologous domain displacement. *BMC Genomics* **6**:39.
339. **RayChaudhuri, D.** 1999. ZipA is a MAP-Tau homolog and is essential for structural integrity of the cytokinetic FtsZ ring during bacterial cell division. *EMBO J.* **18**:2372–2383.
340. **RayChaudhuri, D., G. S. Gordon, and A. Wright.** 2000. How does a bacterium find its middle? *Nat. Struct. Biol.* **7**:997–999.
341. **RayChaudhuri, D., G. S. Gordon, and A. Wright.** 2001. Protein acrobatics and bacterial cell polarity. *Proc. Natl. Acad. Sci. USA* **98**:1332–1334.
342. **RayChaudhuri, D., and J. T. Park.** 1992. *Escherichia coli* cell-division gene ftsZ encodes a novel GTP-binding protein. *Nature* **359**:251–254.
343. **Raymond, J. B., S. Mahapatra, D. C. Crick, and M. S. Pavelka, Jr.** 2005. Identification of the namH gene, encoding the hydroxylase responsible for the N-glycosylation of the mycobacterial peptidoglycan. *J. Biol. Chem.* **280**:326–333.
344. **Reddy, M.** 2007. Role of FtsEX in cell division of *Escherichia coli*: viability of *ftsEX* mutants is dependent on functional SufI or high osmotic strength. *J. Bacteriol.* **189**:98–108.
345. **Reeve, J. N., N. H. Mendelson, S. I. Coyne, L. L. Hallock, and R. M. Cole.** 1973. Minicells of *Bacillus subtilis*. *J. Bacteriol.* **114**:860–873.
346. **Rengarajan, J., B. R. Bloom, and E. J. Rubin.** 2005. Genome-wide requirements for *Mycobacterium tuberculosis* adaptation and survival in macrophages. *Proc. Natl. Acad. Sci. USA* **102**:8327–8332.
347. **Rickman, L., C. Scott, D. M. Hunt, T. Hutchinson, M. C. Menendez, R. Whalan, J. Hinds, M. J. Colston, J. Green, and R. S. Buxton.** 2005. A member of the cAMP receptor protein family of transcription regulators in *Mycobacterium tuberculosis* is required for virulence in mice and controls transcription of the *rfpA* gene coding for a resuscitation promoting factor. *Mol. Microbiol.* **56**:1274–1286.
348. **Roberts, D. M., R. P. Liao, G. Wisedchaisri, W. G. Hol, and D. R. Sherman.** 2004. Two sensor kinases contribute to the hypoxic response of *Mycobacterium tuberculosis*. *J. Biol. Chem.* **279**:23082–23087.
349. **Robson, S. A., K. A. Michie, J. P. Mackay, E. Harry, and G. F. King.** 2002. The *Bacillus subtilis* cell division proteins FtsL and DivIC are intrinsically unstable and do not interact with one another in the absence of other septosomal components. *Mol. Microbiol.* **44**:663–674.
350. **Rodrigue, S., R. Provedvi, P. E. Jacques, L. Guadreau, and R. Manganelli.** 2006. The sigma factors of *Mycobacterium tuberculosis*. *FEMS Microbiol. Rev.* **30**:926–941.
351. **Romberg, L., and P. A. Levin.** 2003. Assembly dynamics of the bacterial cell division protein FtsZ: poised at the edge of stability. *Annu. Rev. Microbiol.* **57**:125–154.
352. **Romeis, T., and J. V. Holtje.** 1994. Penicillin-binding protein 7/8 of *Escherichia coli* is a DD-endopeptidase. *Eur. J. Biochem.* **224**:597–604.
353. **Romeis, T., and J. V. Holtje.** 1994. Specific interaction of penicillin-binding proteins 3 and 7/8 with soluble lytic transglycosylase in *Escherichia coli*. *J. Biol. Chem.* **269**:21603–21607.
354. **Rowen, L., and A. Kornberg.** 1978. Primase, the dnaG protein of *Escherichia coli*. An enzyme which starts DNA chains. *J. Biol. Chem.* **253**:758–764.
355. **Roy, S., and P. Ajitkumar.** 2005. Transcriptional analysis of the principal cell division gene, *ftsZ*, of *Mycobacterium tuberculosis*. *J. Bacteriol.* **187**:2540–2550.
356. **Rueda, S., M. Vicente, and J. Mingorance.** 2003. Concentration and assembly of the division ring proteins FtsZ, FtsA, and ZipA during the *Escherichia coli* cell cycle. *J. Bacteriol.* **185**:3344–3351.
357. **Ryan, K. R., and L. Shapiro.** 2003. Temporal and spatial regulation in prokaryotic cell cycle progression and development. *Annu. Rev. Biochem.* **72**:367–394.
358. **Saini, D. K., V. Malhotra, D. Dey, N. Pant, T. K. Das, and J. S. Tyagi.** 2004. DevR-DevS is a bona fide two-component system of *Mycobacterium tuberculosis* that is hypoxia-responsive in the absence of the DNA-binding domain of DevR. *Microbiology* **150**:865–875.
359. **Saini, D. K., V. Malhotra, and J. S. Tyagi.** 2004. Cross talk between DevS sensor kinase homologue, Rv2027c, and DevR response regulator of *Mycobacterium tuberculosis*. *FEBS Lett.* **565**:75–80.
360. **Sardiwal, S., S. L. Kendall, F. Movahedzadeh, S. C. Rison, N. G. Stoker, and S. Djordjevic.** 2005. A GAF domain in the hypoxia/NO-inducible *Mycobacterium tuberculosis* DosS protein binds haem. *J. Mol. Biol.* **353**:929–936.
361. **Sassetti, C. M., D. H. Boyd, and E. J. Rubin.** 2003. Genes required for mycobacterial growth defined by high density mutagenesis. *Mol. Microbiol.* **48**:77–84.
362. **Satta, G., R. Fontana, and P. Canepari.** 1994. The two-competing site (TCS) model for cell shape regulation in bacteria: the envelope as an integration point for the regulatory circuits of essential physiological events. *Adv. Microb. Physiol.* **36**:181–245.
363. **Sawitzke, J., and S. Austin.** 2001. An analysis of the factory model for chromosome replication and segregation in bacteria. *Mol. Microbiol.* **40**:786–794.
364. **Scanga, C. A., V. P. Mohan, H. Joseph, K. Yu, J. Chan, and J. L. Flynn.** 1999. Reactivation of latent tuberculosis: variations on the Cornell murine model. *Infect. Immun.* **67**:4531–4538.
365. **Scheffers, D. J., and M. G. Pinho.** 2005. Bacterial cell wall synthesis: new insights from localization studies. *Microbiol. Mol. Biol. Rev.* **69**:585–607.
366. **Scherman, M., A. Weston, K. Duncan, A. Whittington, R. Upton, L. Deng, R. Comber, J. D. Friedrich, and M. McNeil.** 1995. Biosynthetic origin of mycobacterial cell wall arabinosyl residues. *J. Bacteriol.* **177**:7125–7130.
367. **Scherman, M. S., L. Kalbe-Bournonville, D. Bush, Y. Xin, L. Deng, and M.**

- McNeil. 1996. Polyphosphatopentoses in mycobacteria are synthesized from 5-phosphoribose pyrophosphate. *J. Biol. Chem.* **271**:29652–29658.
368. Schleifer, K. H., and O. Kandler. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol. Rev.* **36**:407–477.
369. Schwedock, J., J. R. McCormick, E. R. Angert, J. R. Nodwell, and R. Losick. 1997. Assembly of the cell division protein FtsZ into ladder-like structures in the aerial hyphae of *Streptomyces coelicolor*. *Mol. Microbiol.* **25**:847–858.
370. Seiler, P., T. Ulrichs, S. Bandermann, L. Pradl, S. Jorg, V. Krenn, L. Morawietz, S. H. Kaufmann, and P. Aichele. 2003. Cell-wall alterations as an attribute of *Mycobacterium tuberculosis* in latent infection. *J. Infect. Dis.* **188**:1326–1331.
371. Sharma, D., A. Bose, H. Shakila, T. K. Das, J. S. Tyagi, and V. D. Ramanathan. 2006. Expression of mycobacterial cell division protein, FtsZ, and dormancy proteins, DevR and Acr, within lung granulomas throughout guinea pig infection. *FEMS Immunol. Med. Microbiol.* **48**:329–336.
372. Sharma, K., H. Chandra, P. K. Gupta, M. Pathak, A. Narayan, L. S. Meena, R. C. D'Souza, P. Chopra, S. Ramachandran, and Y. Singh. 2004. PknH, a transmembrane Hank's type serine/threonine kinase from *Mycobacterium tuberculosis*, is differentially expressed under stress conditions. *FEMS Microbiol. Lett.* **233**:107–113.
373. Sharp, M. D., and K. Pogliano. 2002. MinCD-dependent regulation of the polarity of SpoIIIE assembly and DNA transfer. *EMBO J.* **21**:6267–6274.
374. Sharpe, M. E., and J. Errington. 1995. Postseptational chromosome partitioning in bacteria. *Proc. Natl. Acad. Sci. USA* **92**:8630–8634.
375. Sherman, D. R., M. Voskuil, D. Schnappinger, R. Liao, M. I. Harrell, and G. K. Schoolnik. 2001. Regulation of the *Mycobacterium tuberculosis* hypoxic response gene encoding alpha-crystallin. *Proc. Natl. Acad. Sci. USA* **98**:7534–7539.
376. Shih, Y. L., T. Le, and L. Rothfield. 2003. Division site selection in *Escherichia coli* involves dynamic redistribution of Min proteins within coiled structures that extend between the two cell poles. *Proc. Natl. Acad. Sci. USA* **100**:7865–7870.
377. Shleeva, M., G. V. Mukamolova, M. Young, H. D. Williams, and A. S. Kaprelyants. 2004. Formation of 'non-culturable' cells of *Mycobacterium smegmatis* in stationary phase in response to growth under suboptimal conditions and their Rpf-mediated resuscitation. *Microbiology* **150**:1687–1697.
378. Sickmann, A., K. Marcus, H. Schafer, E. Butt-Dorje, S. Lehr, A. Herkner, S. Suer, I. Bahr, and H. E. Meyer. 2001. Identification of post-translationally modified proteins in proteome studies. *Electrophoresis* **22**:1669–1676.
379. Sickmann, A., and H. E. Meyer. 2001. Phosphoamino acid analysis. *Proteomics* **1**:200–206.
380. Sievers, J., and J. Errington. 2000. Analysis of the essential cell division gene *ftsL* of *Bacillus subtilis* by mutagenesis and heterologous complementation. *J. Bacteriol.* **182**:5572–5579.
381. Sievers, J., and J. Errington. 2000. The *Bacillus subtilis* cell division protein FtsL localizes to sites of septation and interacts with DivC. *Mol. Microbiol.* **36**:846–855.
382. Signoretto, C., M. M. Lleo, and P. Canepari. 2002. Modification of the peptidoglycan of *Escherichia coli* in the viable but nonculturable state. *Curr. Microbiol.* **44**:125–131.
383. Signoretto, C., M. M. Lleo, M. C. Tafi, and P. Canepari. 2000. Cell wall chemical composition of *Enterococcus faecalis* in the viable but nonculturable state. *Appl. Environ. Microbiol.* **66**:1953–1959.
384. Singh, A., L. Guidry, K. V. Narasimulu, D. Mai, J. Trombley, K. E. Redding, G. I. Giles, J. R. Lancaster, Jr., and A. J. Steyn. 2007. *Mycobacterium tuberculosis* WhiB3 responds to O₂ and nitric oxide via its [4Fe-4S] cluster and is essential for nutrient starvation survival. *Proc. Natl. Acad. Sci. USA* **104**:11562–11567.
385. Singh, A., D. Mai, A. Kumar, and A. J. Steyn. 2006. Dissecting virulence pathways of *Mycobacterium tuberculosis* through protein-protein association. *Proc. Natl. Acad. Sci. USA* **103**:11346–11351.
386. Slayden, R. A., D. L. Knudson, and J. T. Belisle. 2006. Identification of cell cycle regulators in *Mycobacterium tuberculosis* by inhibition of septum formation and global transcriptional analysis. *Microbiology* **152**:1789–1797.
387. Smeulders, M. J., J. Keer, R. A. Speight, and H. D. Williams. 1999. Adaptation of *Mycobacterium smegmatis* to stationary phase. *J. Bacteriol.* **181**:270–283.
388. Soliveri, J. A., J. Gomez, W. R. Bishai, and K. F. Chater. 2000. Multiple paralogous genes related to the *Streptomyces coelicolor* developmental regulatory gene *whiB* are present in *Streptomyces* and other actinomycetes. *Microbiology* **146**:333–343.
389. Song, T., S. L. Dove, K. H. Lee, and R. N. Husson. 2003. RshA, an anti-sigma factor that regulates the activity of the mycobacterial stress response sigma factor SigH. *Mol. Microbiol.* **50**:949–959.
390. Soufo, H. J., and P. L. Graumann. 2003. Actin-like proteins MreB and Mbl from *Bacillus subtilis* are required for bipolar positioning of replication origins. *Curr. Biol.* **13**:1916–1920.
391. Stahl, C., S. Kubetzko, I. Kaps, S. Seeber, H. Engelhardt, and M. Niederweis. 2001. MspA provides the main hydrophilic pathway through the cell wall of *Mycobacterium smegmatis*. *Mol. Microbiol.* **40**:451–464.
392. Stahl, D. A., and J. W. Urbance. 1990. The division between fast- and slow-growing species corresponds to natural relationships among the mycobacteria. *J. Bacteriol.* **172**:116–124.
393. Steyn, A. J., D. M. Collins, M. K. Hondalus, W. R. Jacobs, Jr., R. P. Kawakami, and B. R. Bloom. 2002. *Mycobacterium tuberculosis* WhiB3 interacts with RpoV to affect host survival but is dispensable for in vivo growth. *Proc. Natl. Acad. Sci. USA* **99**:3147–3152.
394. Stricker, J., and H. P. Erickson. 2003. In vivo characterization of *Escherichia coli ftsZ* mutants: effects on Z-ring structure and function. *J. Bacteriol.* **185**:4796–4805.
395. Sun, Q., and W. Margolin. 2004. Effects of perturbing nucleoid structure on nucleoid occlusion-mediated toporegulation of FtsZ ring assembly. *J. Bacteriol.* **186**:3951–3959.
396. Sun, Q., and W. Margolin. 1998. FtsZ dynamics during the division cycle of live *Escherichia coli* cells. *J. Bacteriol.* **180**:2050–2056.
397. Takade, A., K. Takeya, H. Taniguchi, and Y. Mizuguchi. 1983. Electron microscopic observations of cell division in *Mycobacterium vaccae* V1. *J. Gen. Microbiol.* **129**:2315–2320.
398. Takade, A., A. Umeda, M. Matsuoka, S. Yoshida, M. Nakamura, and K. Amako. 2003. Comparative studies of the cell structures of *Mycobacterium leprae* and *M. tuberculosis* using the electron microscopy freeze-substitution technique. *Microbiol. Immunol.* **47**:265–270.
399. Takayama, K., H. L. David, L. Wang, and D. S. Goldman. 1970. Isolation and characterization of uridine diphosphate-N-glycylmuramyl-L-alanyl-gamma-D-glutamyl-meso-alpha,alpha'-diaminopimelic acid from *Mycobacterium tuberculosis*. *Biochem. Biophys. Res. Commun.* **39**:7–12.
400. Takayama, K., C. Wang, and G. S. Besra. 2005. Pathway to synthesis and processing of mycolic acids in *Mycobacterium tuberculosis*. *Clin. Microbiol. Rev.* **18**:81–101.
401. Templin, M. F., A. Ursinus, and J. V. Holtje. 1999. A defect in cell wall recycling triggers autolysis during the stationary growth phase of *Escherichia coli*. *EMBO J.* **18**:4108–4117.
402. Thakur, M., and P. K. Chakraborti. 2006. GTPase activity of mycobacterial FtsZ is impaired due to its transphosphorylation by the eukaryotic-type Ser/Thr kinase, PknA. *J. Biol. Chem.* **281**:40107–40113.
403. Thanedar, S., and W. Margolin. 2004. FtsZ exhibits rapid movement and oscillation waves in helix-like patterns in *Escherichia coli*. *Curr. Biol.* **14**:1167–1173.
404. Thanky, N. R., D. B. Young, and B. D. Robertson. 2007. Unusual features of the cell cycle in mycobacteria: polar-restricted growth and the snapping-model of cell division. *Tuberculosis (Edinburgh)* **87**:231–236.
405. Thomaidis, H. B., M. Freeman, M. El Karoui, and J. Errington. 2001. Division site selection protein DivIVA of *Bacillus subtilis* has a second distinct function in chromosome segregation during sporulation. *Genes Dev.* **15**:1662–1673.
406. Tianont, K., T. Doan, M. B. Lazarus, X. Fang, D. Z. Rudner, and S. Walker. 2006. Imaging peptidoglycan biosynthesis in *Bacillus subtilis* with fluorescent antibiotics. *Proc. Natl. Acad. Sci. USA* **103**:11033–11038.
407. Trias, J., and R. Benz. 1994. Permeability of the cell wall of *Mycobacterium smegmatis*. *Mol. Microbiol.* **14**:283–290.
408. Trias, J., V. Jarlier, and R. Benz. 1992. Porins in the cell wall of mycobacteria. *Science* **258**:1479–1481.
409. Tuckerman, J. R., G. Gonzalez, E. M. Dioum, and M. A. Gilles-Gonzalez. 2002. Ligand and oxidation-state specific regulation of the heme-based oxygen sensor FixL from *Sinorhizobium meliloti*. *Biochemistry* **41**:6170–6177.
410. Tufariello, J. M., J. Chan, and J. L. Flynn. 2003. Latent tuberculosis: mechanisms of host and bacillus that contribute to persistent infection. *Lancet Infect. Dis.* **3**:578–590.
411. Tufariello, J. M., W. R. Jacobs, Jr., and J. Chan. 2004. Individual *Mycobacterium tuberculosis* resuscitation-promoting factor homologues are dispensable for growth in vitro and in vivo. *Infect. Immun.* **72**:515–526.
412. Tufariello, J. M., K. Mi, J. Xu, Y. C. Manabe, A. K. Kesavan, J. Drumm, K. Tanaka, W. R. Jacobs, Jr., and J. Chan. 2006. Deletion of the *Mycobacterium tuberculosis* resuscitation-promoting factor Rv1009 gene results in delayed reactivation from chronic tuberculosis. *Infect. Immun.* **74**:2985–2995.
413. Tuomanen, E., and R. Cozens. 1987. Changes in peptidoglycan composition and penicillin-binding proteins in slowly growing *Escherichia coli*. *J. Bacteriol.* **169**:5308–5310.
414. Tuomanen, E., Z. Markiewicz, and A. Tomasz. 1988. Autolysis-resistant peptidoglycan of anomalous composition in amino-acid-starved *Escherichia coli*. *J. Bacteriol.* **170**:1373–1376.
415. Tuomanen, E., J. Schwartz, S. Sande, K. Light, and D. Gage. 1989. Unusual composition of peptidoglycan in *Bordetella pertussis*. *J. Biol. Chem.* **264**:11093–11098.
416. Tyagi, J. S., T. K. Das, and A. K. Kinger. 1996. An *M. tuberculosis* DNA fragment contains genes encoding cell division proteins ftsX and ftsE, a basic protein and homologues of PemK and small protein B. *Gene* **177**:59–67.
417. Udou, T., M. Ogawa, and Y. Mizuguchi. 1982. Spheroplast formation of

- Mycobacterium smegmatis* and morphological aspects of their reversion to the bacillary form. *J. Bacteriol.* **151**:1035–1039.
418. Ursinus, A., F. van den Ent, S. Brechtel, M. de Pedro, J. V. Holtje, J. Lowe, and W. Vollmer. 2004. Murein (peptidoglycan) binding property of the essential cell division protein FtsN from *Escherichia coli*. *J. Bacteriol.* **186**:6728–6737.
419. van den Ent, F., L. Amos, and J. Lowe. 2001. Bacterial ancestry of actin and tubulin. *Curr. Opin. Microbiol.* **4**:634–638.
420. van Heijenoort, J. 1998. Assembly of the monomer unit of bacterial peptidoglycan. *Cell Mol. Life Sci.* **54**:300–304.
421. van Heijenoort, J. 2001. Formation of the glycan chains in the synthesis of bacterial peptidoglycan. *Glycobiology* **11**:25R–36R.
422. van Heijenoort, J. 2001. Recent advances in the formation of the bacterial peptidoglycan monomer unit. *Nat. Prod. Rep.* **18**:503–519.
423. Viala, J., C. Chaput, I. G. Boneca, A. Cardona, S. E. Girardin, A. P. Moran, R. Athman, S. Memet, M. R. Huerre, A. J. Coyle, P. S. DiStefano, P. J. Sansonetti, A. Labigne, J. Bertin, D. J. Philpott, and R. L. Ferrero. 2004. Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori* cag pathogenicity island. *Nat. Immunol.* **5**:1166–1174.
424. Vicente, M., and A. I. Rico. 2006. The order of the ring: assembly of *Escherichia coli* cell division components. *Mol. Microbiol.* **61**:5–8.
425. Vicente, M., A. I. Rico, R. Martínez-Arteaga, and J. Mingorance. 2006. Septum enlightenment: assembly of bacterial division proteins. *J. Bacteriol.* **188**:19–27.
426. Vollmer, W., and J. V. Holtje. 2004. The architecture of the murein (peptidoglycan) in gram-negative bacteria: vertical scaffold or horizontal layer(s)? *J. Bacteriol.* **186**:5978–5987.
427. Voskuil, M. I. 2004. *Mycobacterium tuberculosis* gene expression during environmental conditions associated with latency. *Tuberculosis (Edinburgh)* **84**:138–143.
428. Voskuil, M. I., D. Schnappinger, K. C. Visconti, M. I. Harrell, G. M. Dolganov, D. R. Sherman, and G. K. Schoolnik. 2003. Inhibition of respiration by nitric oxide induces a *Mycobacterium tuberculosis* dormancy program. *J. Exp. Med.* **198**:705–713.
429. Voskuil, M. I., K. C. Visconti, and G. K. Schoolnik. 2004. *Mycobacterium tuberculosis* gene expression during adaptation to stationary phase and low-oxygen dormancy. *Tuberculosis (Edinburgh)* **84**:218–227.
430. Wake, R. G. 1997. Replication fork arrest and termination of chromosome replication in *Bacillus subtilis*. *FEMS Microbiol. Lett.* **153**:247–254.
431. Walburger, A., A. Koul, G. Ferrari, L. Nguyen, C. Prescianotto-Baschong, K. Huygen, B. Klebl, C. Thompson, G. Bacher, and J. Pieters. 2004. Protein kinase G from pathogenic mycobacteria promotes survival within macrophages. *Science* **304**:1800–1804.
432. Walters, S. B., E. Dubnau, I. Kolesnikova, F. Laval, M. Daffe, and I. Smith. 2006. The *Mycobacterium tuberculosis* PhoPR two-component system regulates genes essential for virulence and complex lipid biosynthesis. *Mol. Microbiol.* **60**:312–330.
433. Wang, C., B. Hayes, M. M. Vestling, and K. Takayama. 2006. Transposome mutagenesis of an integral membrane transporter in *Corynebacterium matruchotii*. *Biochem. Biophys. Res. Commun.* **340**:953–960.
434. Wang, F., C. Cassidy, and J. C. Sacchettini. 2006. Crystal structure and activity studies of the *Mycobacterium tuberculosis* beta-lactamase reveal its critical role in resistance to beta-lactam antibiotics. *Antimicrob. Agents Chemother.* **50**:2762–2771.
435. Wang, X., and J. Lutkenhaus. 1996. FtsZ ring: the eubacterial division apparatus conserved in archaeobacteria. *Mol. Microbiol.* **21**:313–319.
436. Wayne, L. G. 1994. Dormancy of *Mycobacterium tuberculosis* and latency of disease. *Eur. J. Clin. Microbiol. Infect. Dis.* **13**:908–914.
437. Wayne, L. G., and L. G. Hayes. 1996. An in vitro model for sequential study of shift-down of *Mycobacterium tuberculosis* through two stages of nonreplicating persistence. *Infect. Immun.* **64**:2062–2069.
438. Wayne, L. G., and C. D. Sohaskey. 2001. Nonreplicating persistence of *Mycobacterium tuberculosis*. *Annu. Rev. Microbiol.* **55**:139–163.
439. Weibull, C. 1953. The isolation of protoplasts from *Bacillus megaterium* by controlled treatment with lysozyme. *J. Bacteriol.* **66**:688–695.
440. Weidel, W., H. Frank, and H. H. Martin. 1960. The rigid layer of the cell wall of *Escherichia coli* strain B. *J. Gen. Microbiol.* **22**:158–166.
441. Weidel, W., and H. Pelzer. 1964. Bagshaped macromolecules—a new outlook on bacterial cell walls. *Adv. Enzymol. Relat. Areas Mol. Biol.* **26**:193–232.
442. Weigel, C., and H. Seitz. 2002. Strand-specific loading of DnaB helicase by DnaA to a substrate mimicking unwound oriC. *Mol. Microbiol.* **46**:1149–1156.
443. Weiss, D. S. 2004. Bacterial cell division and the septal ring. *Mol. Microbiol.* **54**:588–597.
444. Weston, A., R. J. Stern, R. E. Lee, P. M. Nassau, D. Monsey, S. L. Martin, M. S. Scherman, G. S. Besra, K. Duncan, and M. R. McNeil. 1997. Biosynthetic origin of mycobacterial cell wall galactofuranosyl residues. *Tuber. Lung Dis.* **78**:123–131.
445. White, E. L., L. J. Ross, R. C. Reynolds, L. E. Seitz, G. D. Moore, and D. W. Borhani. 2000. Slow polymerization of *Mycobacterium tuberculosis* FtsZ. *J. Bacteriol.* **182**:4028–4034.
446. Wietzerbin, J., B. C. Das, J. F. Petit, E. Lederer, M. Leyh-Bouille, and J. M. Ghuyen. 1974. Occurrence of D-alanyl-(D)-meso-diaminopimelic acid and meso-diaminopimelyl-meso-diaminopimelic acid interpeptide linkages in the peptidoglycan of mycobacteria. *Biochemistry* **13**:3471–3476.
447. Wolschendorf, F., M. Mahfoud, and M. Niederweis. 2007. Porins are required for uptake of phosphates by *Mycobacterium smegmatis*. *J. Bacteriol.* **189**:2435–2442.
448. Wu, L. J. 2004. Structure and segregation of the bacterial nucleoid. *Curr. Opin. Genet. Dev.* **14**:126–132.
449. Wu, L. J., and J. Errington. 1994. *Bacillus subtilis* spoIIIE protein required for DNA segregation during asymmetric cell division. *Science* **264**:572–575.
450. Wu, L. J., and J. Errington. 2004. Coordination of cell division and chromosome segregation by a nucleoid occlusion protein in *Bacillus subtilis*. *Cell* **117**:915–925.
451. Yajko, D. M., C. A. Sanders, P. S. Nassos, and W. K. Hadley. 1990. In vitro susceptibility of *Mycobacterium avium* complex to the new fluoroquinolone sparfloxacin (CI-978; AT-4140) and comparison with ciprofloxacin. *Antimicrob. Agents Chemother.* **34**:2442–2444.
452. Young, K. D. 2003. Bacterial shape. *Mol. Microbiol.* **49**:571–580.
453. Young, K. D. 2006. The selective value of bacterial shape. *Microbiol. Mol. Biol. Rev.* **70**:660–703.
454. Young, T. A., B. Delagoutte, J. A. Endrizzi, A. M. Falick, and T. Alber. 2003. Structure of *Mycobacterium tuberculosis* PknB supports a universal activation mechanism for Ser/Thr protein kinases. *Nat. Struct. Biol.* **10**:168–174.
455. Yu, X. C., and W. Margolin. 1997. Ca²⁺-mediated GTP-dependent dynamic assembly of bacterial cell division protein FtsZ into asters and polymer networks in vitro. *EMBO J.* **16**:5455–5463.
456. Yu, X. C., and W. Margolin. 1999. FtsZ ring clusters in min and partition mutants: role of both the Min system and the nucleoid in regulating FtsZ ring localization. *Mol. Microbiol.* **32**:315–326.
457. Yu, X. C., A. H. Tran, Q. Sun, and W. Margolin. 1998. Localization of cell division protein FtsK to the *Escherichia coli* septum and identification of a potential N-terminal targeting domain. *J. Bacteriol.* **180**:1296–1304.
458. Yuan, Y., D. Barrett, Y. Zhang, D. Kahne, P. Sliz, and S. Walker. 2007. Crystal structure of a peptidoglycan glycosyltransferase suggests a model for processive glycan chain synthesis. *Proc. Natl. Acad. Sci. USA* **104**:5348–5353.
459. Zahrt, T. C., and V. Deretic. 2001. *Mycobacterium tuberculosis* signal transduction system required for persistent infections. *Proc. Natl. Acad. Sci. USA* **98**:12706–12711.
460. Zahrt, T. C., C. Wozniak, D. Jones, and A. Trevett. 2003. Functional analysis of the *Mycobacterium tuberculosis* MprAB two-component signal transduction system. *Infect. Immun.* **71**:6962–6970.
461. Zhang, Y., B. Heym, B. Allen, D. Young, and S. Cole. 1992. The catalase-peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. *Nature* **358**:591–593.
462. Zhou, H., and J. Lutkenhaus. 2005. MinC mutants deficient in MinD- and DicB-mediated cell division inhibition due to loss of interaction with MinD, DicB, or a septal component. *J. Bacteriol.* **187**:2846–2857.
463. Zhu, W., B. B. Plikaytis, and T. M. Shinnick. 2003. Resuscitation factors from mycobacteria: homologs of *Micrococcus luteus* proteins. *Tuberculosis (Edinburgh)* **83**:261–269.