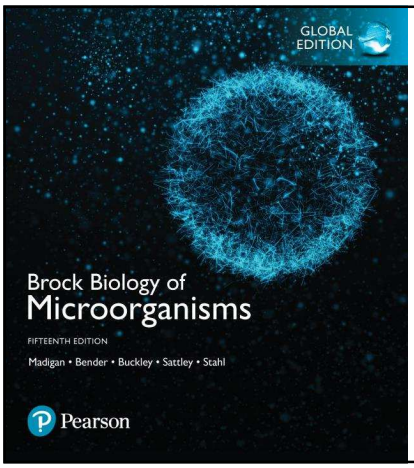

PowerPoint® Lecture Presentations

CHAPTER 7

Molecular Biology of Microbial Growth

I. Bacterial Cell Division

- ~~7.1 Visualizing Molecular Growth~~
- 7.2 Chromosome Replication and Segregation
- 7.3 Cell Division and Fts Proteins
- 7.4 MreB and Cell Morphology
- 7.5 Peptidoglycan Biosynthesis

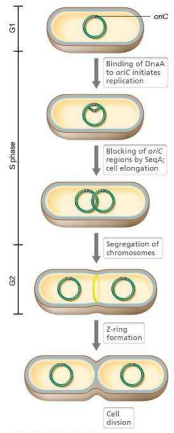

Figure 7.1

7.2 Chromosome Replication and Segregation

- Regulation of chromosome replication initiation
  - Several proteins initiate and inhibit chromosome replication in *E. coli.*
  - DnaA binding to specific sequences within *oriC* region leads to unwinding and loading of replisome.
    - most active when linked to ATP (DnaA-ATP)
  - Mechanisms to inactivate DnaA-ATP include competition for *oriC* binding, repression of *dnaA* expression, titration of DnaA-ATP away from *oriC*, and inactivation of DnaA-ATP.
  - After replication initiation, only parental strand is methylated, yielding *hemimethylated DNA* facilitation competition for origin binding between DnaA-ATP and SeqA protein. (Figure 7.4)
    - hemimethylated *oriC* strongly bound by SeqA, blocking DnaA-ATP

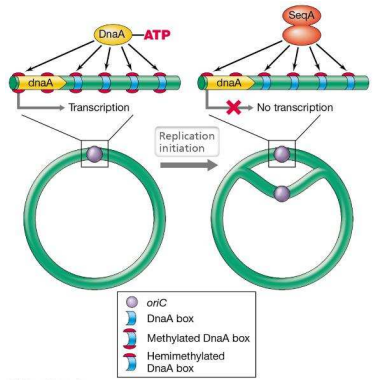

Figure 7.4

7.2 Chromosome Replication and Segregation

- Genome replication in fast-growing cells
  - circular genome replication *bidirectional* from origin
  - *E. coli*'s genome replication takes 40 minutes but is independent of generation time, which can be as little as 20 minutes.
  - *multiple DNA replication forks* present in each cell if doubling time shorter than genome replication time, so a new round begins before the previous round completed (Figure 7.5)
    - some genes present in multiple copies

7.2 Chromosome Replication and Segregation

- Chromosome segregation
  - required so daughter cell gets copy of genome and for septum formation
  - In many bacteria, *Par* (partitioning) *system* distributes chromosomes and plasmids equally.
  - *E. coli* lacks Par but has *structural maintenance of chromosome* complex (topoisomerase IV + MukBEF proteins).
    - *decatenation* (separation) of replicated sister chromosomes
  - Plasmids replicated similarly (Figure 7.7)
    - Replication at poles helps ensure efficient transfer to daughter cells.
    - partitioning systems

7.3 Cell Division and Fts Proteins

- The Divisome
  - several essential proteins called *Fts proteins*
  - FtsZ protein crucial in binary fission
    - related to tubulin (eukaryotic cell-division protein)
    - also found in virtually all *Archaea*
  - other Fts proteins found only in *Bacteria*
  - Fts proteins interact to form the *divisome* (cell division apparatus).
    - In rod-shaped cells, formation begins with attachment of FtsZ molecules around center of cell in a ring that becomes cell-division plane.
    - Ring attracts other divisome proteins including FtsA and ZipA.
    - *ZipA:* anchor that connects FtsZ ring to cytoplasmic membrane
    - *FtsA:* related to actin; recruits FtsZ and other divisome proteins and helps connect FtsZ ring to membrane
  - Divisome forms about ¾ of the way into cell division.

7.3 Cell Division and Fts Proteins

- The divisome
  - also contains Fts proteins needed for peptidoglycan synthesis
    - FtsI: penicillin-binding protein (activity inhibited by penicillin antibiotic)
  - orchestrates synthesis of new cytoplasmic membrane and cell wall material (*division septum*), then cell divides

7.3 Cell Division and Fts Proteins

- Min proteins and cell division
  - DNA replicates before the FtsZ ring forms (Figure 7.9) because ring forms between nucleoids.
    - Before nucleoids segregate, they block formation of FtsZ ring (nucleoid occlusion).
  - MinC, MinD, and MinE proteins guide FtsZ to cell midpoint instead of poles.
  - FtsK and other proteins mediate separation of chromosomes to daughter cells.
  - FtsZ depolymerizes, triggering inward growth of wall materials to form septum.
  - FtsZ also hydrolyzes GTP to provide energy for polymerization and depolymerization of FtsZ ring.

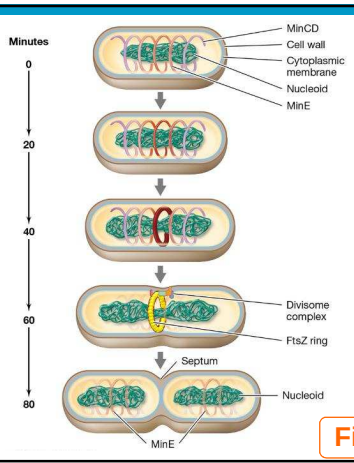

Figure 7.9

7.4 MreB and Cell Morphology

- Prokaryotes contain a cell cytoskeleton that is dynamic and multifaceted.
- Cell shape and MreB
  - major shape-determining factor in *Bacteria* and a few *Archaea*
  - forms simple cytoskeleton with patchlike filaments around inside of cell just below cytoplasmic membrane (Figure 7.10)
  - recruits other proteins for cell wall growth to group into a specific pattern
  - inactivation causes cells to become cocci
  - Most coccoid bacteria lack MreB.
  - Filaments move from one side to another, localizing synthesis of peptidoglycan and allowing new cell wall to form at several points.

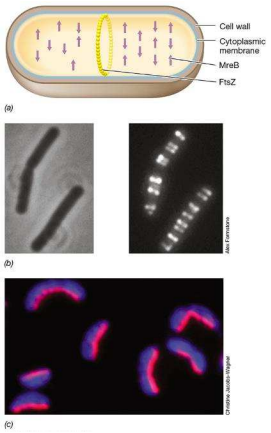

Figure 7.10

7.4 MreB and Cell Morphology

- Evolution of cell division and cell shape
  - MreB functions similarly to eukaryotic actin.
    - Actin forms *microfilaments* that function as scaffolding in cytoskeleton and cell division.
  - Diversity of morphologies exists, especially in gram-negative *Alphaproteobacteria.* (Figure 7.11)
    - can synthesize peptidoglycan at poles (polar elongation)
    - Some grow by budding.
  - cannot predict phylogeny from morphology or vice versa

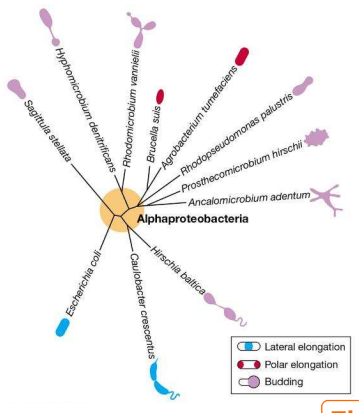

Figure 7.11

7.5 Peptidoglycan Biosynthesis

- Preexisting peptidoglycan needs to be temporarily severed to allow newly synthesized peptidoglycan to form.
- In cocci, cell walls grow in opposite directions outward from the FtsZ ring. (Figure 7.12)
- In rod-shaped cells, cell wall growth occurs at several points along length of the cell.
- In cells that grow by budding, cell wall growth localized
- Must synthesize new peptidoglycan and export it outside the cytoplasmic membrane.

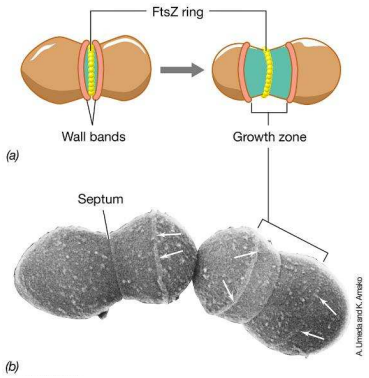

Figure 7.12

7.5 Peptidoglycan Biosynthesis

- Insertion of new peptidoglycan
  - requires controlled cutting of existing peptidoglycan and simultaneous insertion of precursors
  - *Bactoprenol* has a major role in precursor insertion.
    - hydrophobic C_{55} alcohol
    - bonds to *N*-acetylglucosamine/*N*-acetylmuramic acid/pentapeptide peptidoglycan precursor (Figure 7.13)
    - transports precursors across membrane
  - Outside cell, bactoprenol complex interacts with *transglycosylases* that insert peptidoglycan precursors into growing points and catalyze glycosidic bond formation. (Figure 7.14)

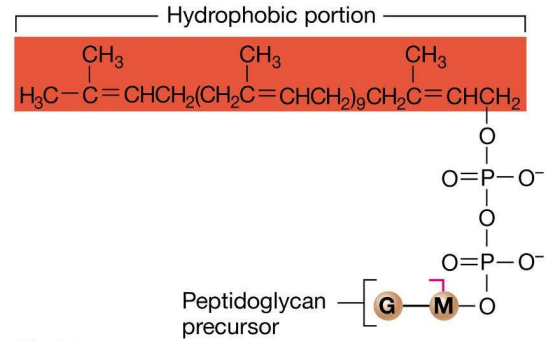

Figure 7.13

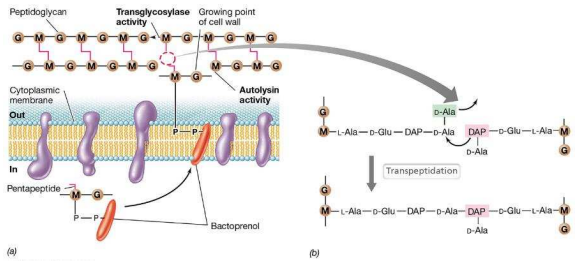

Figure 7.14

7.5 Peptidoglycan Biosynthesis

- Insertion of new peptidoglycan
  - Small gaps for growing points are created by *autolysins* that hydrolyze bonds between *N*-acetylglucosamine and *N*-acetylmuramic acid.
  - new cell wall material added across gaps
  - *wall band:* junction between new and old peptidoglycan on surface of gram-positive bacteria

7.5 Peptidoglycan Biosynthesis

- Transpeptidation
  - final step (Figure 5.8b)
  - forms the peptide cross-links between muramic acid residues in adjacent glycan chains
  - In gram-negative bacteria, cross-links form between diaminopimelic acid (DAP) on one peptide and D-alanine on adjacent peptide.
  - Removal of extra D-alanine from precursor is exergonic and drives transpeptidation forward.
  - In *E. coli,* FtsI is a transpeptidase.
  - inhibited by the antibiotic penicillin
    - Continued activity of autolysins weakens peptidoglycan, and cell bursts.

III. Antibiotics and Microbial Growth

- 7.10 Antibiotic Targets and Antibiotic Resistance

7.10 Antibiotic Targets and Antibiotic Resistance

- Antibiotics are antimicrobials naturally produced by microbes.
  - kill or inhibit bacterial growth
  - target essential molecular processes

7.10 Antibiotic Targets and Antibiotic Resistance

- Antibiotics that target major molecular processes
  - Many antibiotics target DNA replication, RNA synthesis, and translation. (Figure 7.21*a*)
    - Quinolones target DNA gyrase and topoisomerase IV by interfering with DNA unwinding and replication.
    - Rifampin and actinomycin prevent RNA synthesis by blocking RNA polymermase active site or RNA elongation.
  - inhibition of protein synthesis
  - Ribosomes in *Bacteria* are 70S; eukaryotic are 80S.
    - Puromycin binds to A site in 70S ribosome, inducing chain termination and inhibition protein synthesis.
    - Aminoglycoside antibiotics (*e.g.,* streptomycin) target 16S rRNA of 30S ribosome, leading to error-filled proteins.

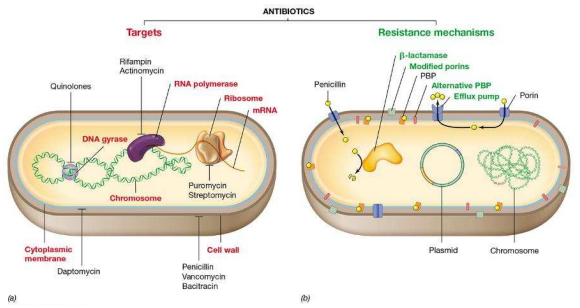

Figure 7.21

7.10 Antibiotic Targets and Antibiotic Resistance

- Antibiotics that target the cell membrane and wall
  - Daptomycin specifically binds to phosphatidylglycerol residues of bacterial plasmid membrane, leading to pore formation, depolarization, and death.
  - Polymyxins are cyclic peptides whose long tails target LPS layer, disrupting membrane and causing leakage and death
  - targeting peptidoglycan synthesis
    - β-lactams (penicillin, cephalosporin, derivatives) interfere with transpeptidation (formation of cross-links)
    - Vancomycin binds to pentapeptide precursor and prevents interbridge formation.
    - Bacitracin binds to bactoprenol and prevents new peptidoglycan precursors from reaching site of synthesis.

7.10 Antibiotic Targets and Antibiotic Resistance

- Antibiotic resistance: spontaneous mutations and antibiotic modification
  - resistance mechanisms genetically encoded in four classes: modification of drug target, enzymatic inactivation, removal via efflux pumps, metabolic bypasses
  - Random chromosomal mutations can lead to resistance.
    - Example: Spontaneous mutants resistant to antibiotic rifampin can be obtained by exposing a large population.
  - Resistance genes can exist on mobile genetic elements and be transferred by horizontal gene flow.
    - Many mobile resistance genes encode enzymes that inactivate antibiotic (*e.g.,* β-lactamase cleaves a ring structure; an acetylating enzyme adds acetyl groups to chloramphenicol).

7.10 Antibiotic Targets and Antibiotic Resistance

- Antibiotic resistance: efflux pumps and metabolic bypasses
  - *Efflux pumps* are ubiquitous and transport various molecules, including antibiotics, out of the cell.
    - lowers intracellular concentration, allowing cell to survive at higher external concentrations
    - Many act promiscuously and transport different classes outside cell.
    - contribute to multidrug resistance
    - AcrAB-TolC of *E. coli* is one of the best characterized and pumps out rifampicin, chloramphenicol, fluoroquinolones.
  - Biofilm growth leads to increased resistance.
    - makes infections difficult to treat
    - AcrAB-TolC efflux pump genes upregulated when cells enter biofilm growth mode
    - *Pseudomonas aeruginosa* encodes several multidrug efflux pumps that are more active when cells grow in an attached state.

7.10 Antibiotic Targets and Antibiotic Resistance

- Antibiotic resistance: efflux pumps and metabolic bypasses
  - Antibiotic target is no longer essential.
    - example: Methicillin-resistant *Staphylococcus aureus* (MRSA)
      - Methicillin is a β-lactam resistant to β-lactamase cleavage.
      - MRSA strains contain a DNA island called *Staphylococcus chromosomal cassette for methicillin resistance (SCCmec)* that encodes MecA, an *alternative* penicillin-binding protein that is not recognized by β-lactams.
      - MRSA synthesize MecA only in the presence of β-lactams due to repressor MecI and β-lactam sensor MecR1.