

# FARM MICROBIOLOGY 2008

## PART 2: BASIC STRUCTURE AND GENETICS OF BACTERIA

### I. Basic Morphology (Shape) of Vegetative Cells.

#### A. Microscopic.

Example – *Escherichia coli* (aka *E. coli*) is 1.3  $\mu\text{m}$  (= 0.000052 inch) wide by 4.0  $\mu\text{m}$  (= 0.00016 inch) long. It would take 6250 cells strung end-to-end to make an inch. (1,000,000 nanometers (nm) = 1000 micrometers ( $\mu\text{m}$ ) = 1 millimeter (mm) = 0.04 inch.)

#### B. Extremes of size.

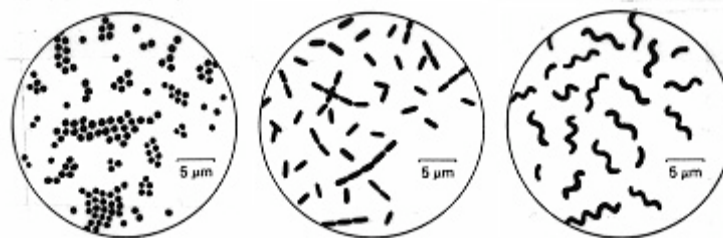
1. *Epulopiscium fishelsoni* and *Thiomargarita namibiensis*. *Epulopiscium* is a long, rod-shaped organism, visible to the naked eye at half a millimeter in length and found in fish in the Red Sea and Australia. *Thiomargarita* (which means “sulfur jewel”) is a spherical organism that was found in the ocean off Africa and is also visible to the naked eye.

2. **Nanobacteria.** (Do they really exist?) Ultra-small at 200nm, these structures resemble bacteria superficially but have not been shown to have any signs of metabolism or replication. So far they are generally considered hypothetical.

C. **What do we mean by vegetative cell and “growth” of bacteria?** Bacteria actively metabolize as “vegetative cells,” and “growth” of bacteria refers to an increasing population of living vegetative cells – generally by binary fission. For some genera of bacteria, vegetative cells can give rise to specialized cells which have various degrees of survival capability in the environment.

#### D. Shapes of vegetative cells.

1. **Coccus.** Spherical, berry-shaped.
2. **Bacillus.** Cylindrical, rod-shaped.
3. **Spirillum.** Spiral, helical-shaped.



Shapes of bacterial cells.

### II. Other Types of Cells (besides vegetative cells).

#### A. Endospore.

1. **Features.** These are the most durable and long-lived of all types of cells, possessing many resistant outer layers and very little (if any) water. When endospore-forming bacteria cause diseases in animals, such as anthrax, tetanus and botulism, the spores are usually involved in transmission and survival of the organism.

**2. Formation.** Formed mainly by vegetative cells of the genera *Bacillus* and *Clostridium*. As a population of cells grows by binary fission, a signal triggered by decreasing nutrients can cause a cell to divide into two cells – but this time, one of the cells (i.e. the endospore) is formed inside of the other. Eventually the outer cell breaks apart and the endospore is “freed.”

**B. Reproductive spore.** Formed by branched bacteria in the soil such as *Streptomyces*. Not as hardy as endospore. Used mainly for reproduction.

**C. Cyst.** Highly desiccation-resistant cell formed by certain soil bacteria.

**III. Some Structural Components.** Compared to eukaryotic cells, procaryotes have a relatively simple internal architecture and organization. Unicellular organisms without a true nucleus or nuclear membrane.

**A. Cell wall and cell membrane = cell envelope.**

**1. Cell wall.** The cell wall prevents osmotic rupture or lysis of the cells. Most bacteria have a “gram-positive” or a “gram-negative” type of cell wall. The former is made up of one thick rigid layer made of peptidoglycan, and the latter is made up of two relatively thin layers – a peptidoglycan layer and a cell membrane on the outside. This is a topic gone over more thoroughly in lab during the gram-staining exercise. Bergey’s Manual starts classifying bacteria with shape and gram reaction.

**2. Cell membrane.** Composed of phospholipids and proteins, it completely surrounds and encloses the protoplasm of the cell. In procaryotes the membrane is a permeability barrier that is used to take up nutrients and to expel waste products. It is also a site for various types of energy generation including respiration.

**B. Capsule.** A coherent slime layer which forms on the outside of the cell. Composed of protein or polysaccharide and is not part of the cell wall or envelope. Produced by some species of bacteria. Used mainly for protection against engulfment by other organisms and for attachment to surfaces.

**C. Ribosomes.** These are the sites of protein synthesis. Ribosomes are composed of ribosomal RNA (rRNA) and protein. The ribosomes of procaryotes are smaller in size and slightly different than eukaryotic ribosomes.

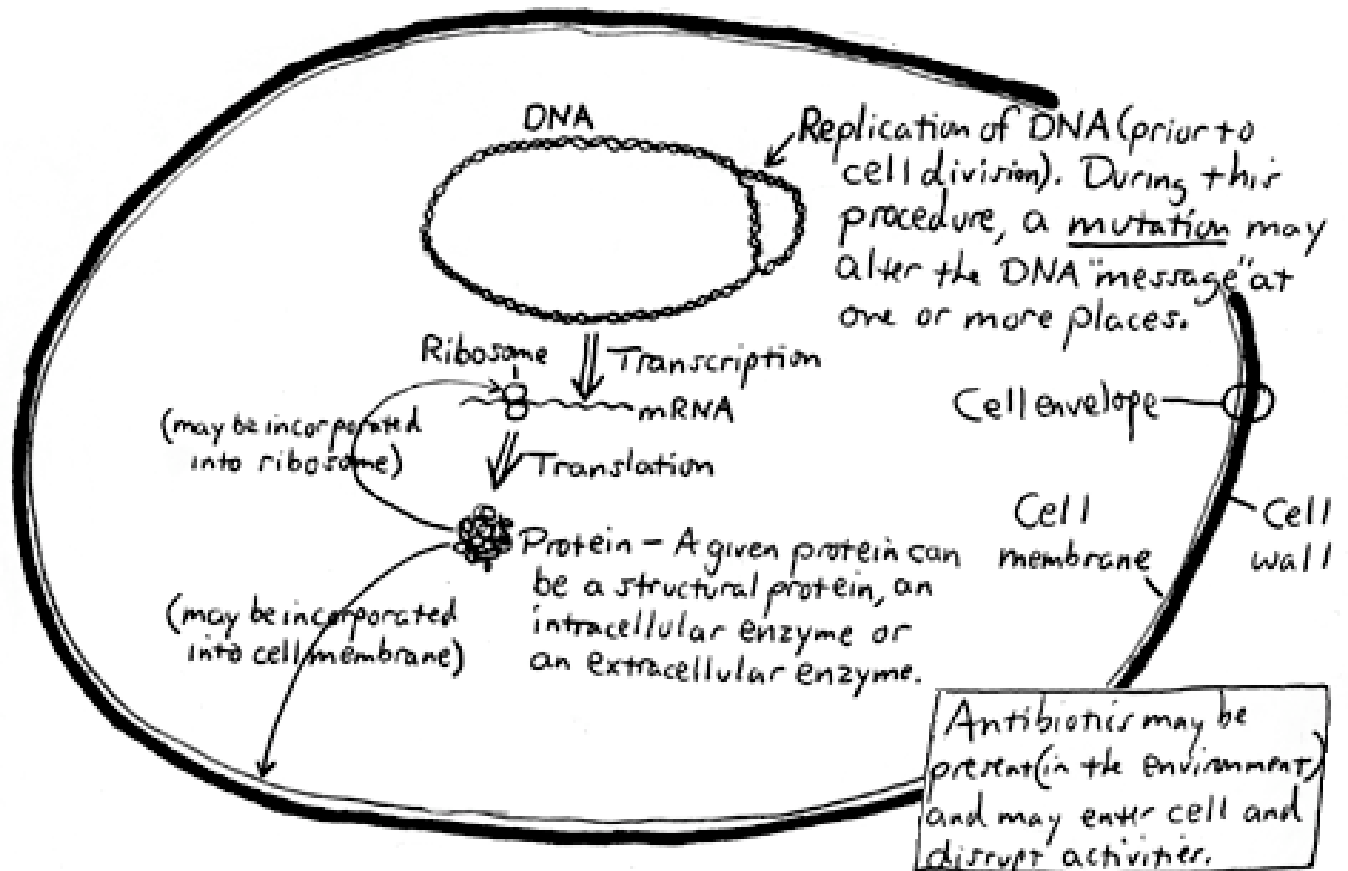
**D. Storage granules.** These are distinct granules or inclusions in the cells. Usually they are reserve materials of some sort, analogous to starch or fat bodies in higher organisms.

**E. Flagella.** Whip-like filaments that are responsible for swimming movement.

**F. Fimbriae (also called Pili).** Hair-like structures on the covering the cell surface. Fimbriae have similar functions to capsules in that they are involved in attachment to surfaces or protection against engulfment by higher organisms.

#### IV. DNA Replication, Transcription and Translation.

Following is a simplified scheme of a typical bacterial cell showing DNA replication, transcription and translation – processes which occur simultaneously and continuously:



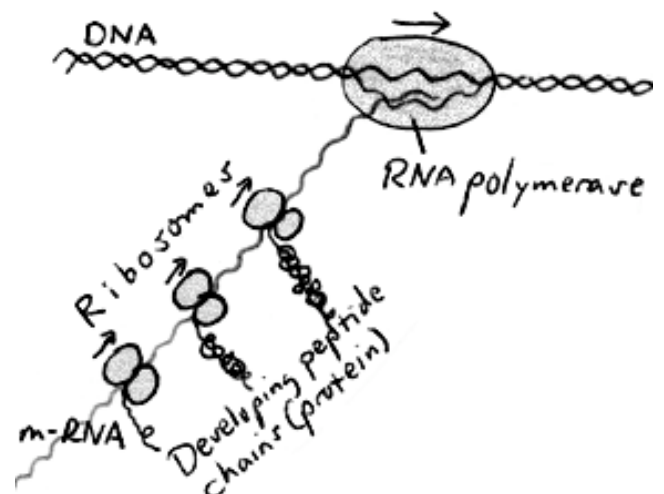
The diagrams at right and below show transcription and translation in a little more detail.

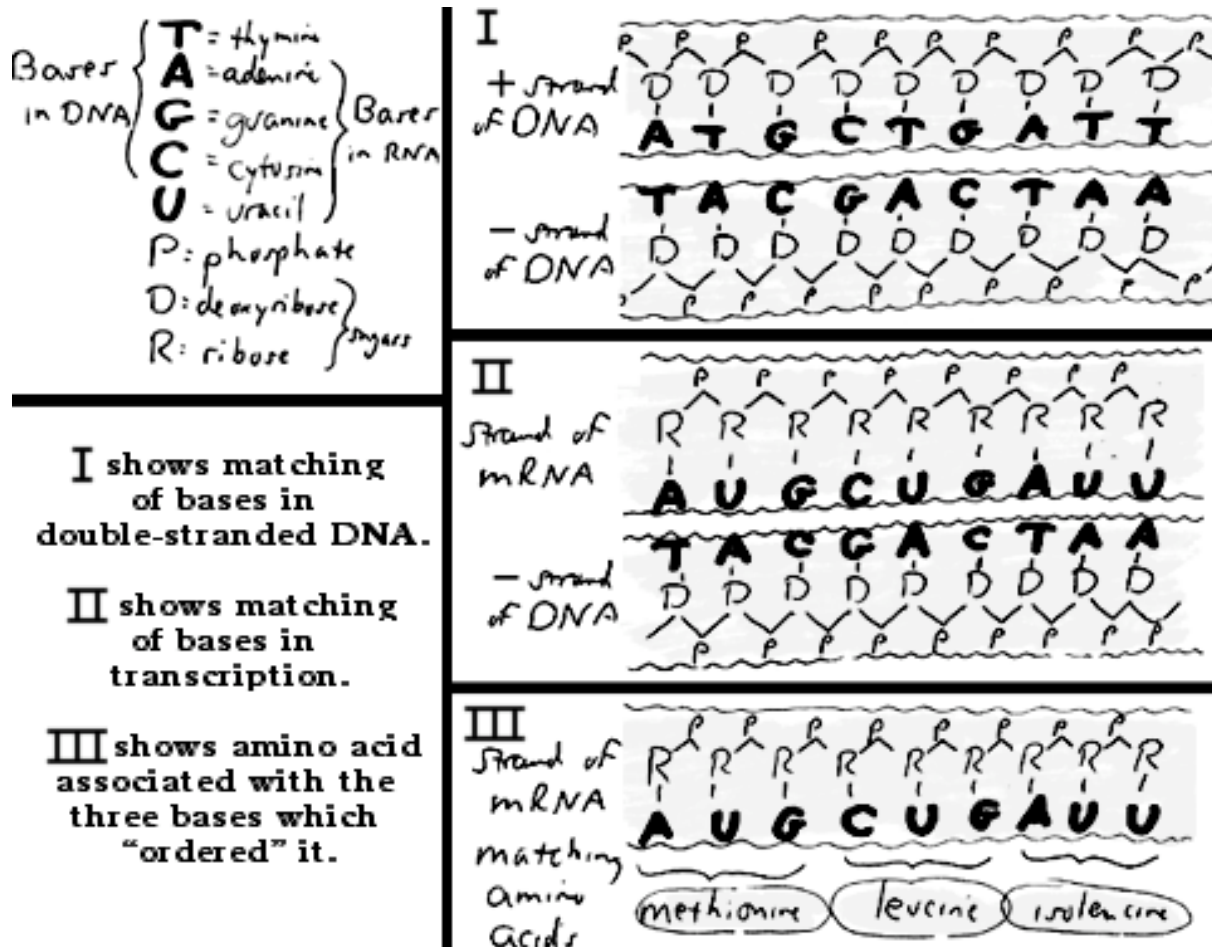
Transcription (synthesis of mRNA):

As RNA polymerase moves along the DNA, a strand of mRNA is synthesized from nucleic acid bases and other compounds in the cytoplasm. The sequence of bases on the DNA serves as a template upon which mRNA is made.

Translation (synthesis of protein):

As the ribosome moves along the mRNA, a strand of peptide (protein) is synthesized from amino acids in the cytoplasm. the sequence of bases on the mRNA dictates the order in which the amino acids are added to form the particular protein coded for.





## V. Review of Genetic Material and Associated Activities.

**A. Nucleic acids – DNA and RNA.** DNA=deoxyribonucleic acid. The procaryotic chromosome is usually a single circular molecule of DNA. RNA=ribonucleic acid.

### B. Structure of DNA.

**1. Basic unit = nucleotide – composed of:**

- a. **Base molecule: adenine, guanine, cytosine or thymine.**
- b. **Sugar molecule = deoxyribose.** Hooked to a sugar and phosphate.
- c. **Phosphate.**

**2. Linkage of nucleotides = strand of DNA.** The nucleotides are linked together by the phosphate molecules to form a strand of the DNA molecule.

### C. Replication of DNA.

### D. The genetic code.

**1. Bases in groups of 3.** Each group codes for a specific amino acid.

**2. Genes.** About two thousand different genes code for various structures and enzymes. Each gene is composed of many nucleotides that (in groups of 3) code for the various amino acids that eventually get strung together to produce a certain protein.

### E. RNA.

**1. Differences from DNA.**

- a. **Base molecule: adenine, guanine, cytosine or uracil.**
- b. **Sugar molecule = ribose.**

2. **Synthesis = transcription.** Strands of messenger RNA (mRNA) are synthesized according to the DNA code.

F. **Protein synthesis = translation.** The code on the mRNA orders up the various amino acids which are ultimately strung together in the precise order to produce a specific protein. This happens at the site of the ribosome.

G. **Activities (DNA replication, transcription, translation) accomplished in any order?** These activities occur simultaneously and constantly in actively-metabolizing cells.

## VI. Alteration of DNA.

DNA may be changed by **mutation** or **recombination**. When either happens, the phenotype may be altered (i.e., a change in a visible characteristic may be seen), or the cell may die due to a lack of a needed function. The following discussion gives a brief overview of the **mutation** concept.

A **mutation** is a spontaneous change in the DNA sequence which is passed on to the "offspring." This change, generally occurring during DNA replication, may be accomplished by substitution, deletion or insertion of one or more bases such that the base sequence is altered, leading to other than the intended protein being constructed. Most mutations are deleterious, leading to death of the cell. Those mutations which are not lethal may cause a visible alteration of the phenotype and may confer a special survival benefit to the organism, possibly allowing the organism to colonize a previously-hostile environment.

A mutation may lead to one or more of the following changes in the phenotype of the cell:

**Structural component mutation** – altering a part of the cell – for example, the cell membrane, ribosome, flagella or capsule.

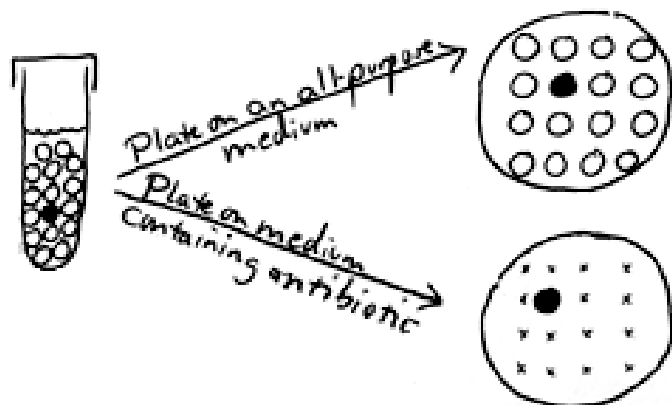
**Biochemical mutation** – losing the ability to utilize certain nutrients or to synthesize certain needed organic compounds such as vitamins, fatty acids, nucleic acid bases, amino acids, and siderophores. The loss of the ability to synthesize a certain organic compound thus requires that the organism be provided with that organic compound which is now considered a growth factor.

**Resistance mutation** – making the cell resistant to an agent which affects other cells in the population negatively. A resistance mutation can confer resistance to particular antibiotics, viruses (bacteriophages) or antibodies which would otherwise inhibit or kill the organism.

**Silent mutation** – leading to no observable phenotypic change. An altered triplet code may still code for the same amino acid.

**Lethal mutation** – leading to death of the cell.

The following over-simplified example shows selection of antibiotic-resistant cells from a culture of a species generally thought of as being sensitive to the antibiotic. A test tube culture containing sixteen cells is shown on the left in this diagram. All of the cells are sensitive to a particular antibiotic except for one cell which – because of a mutation – is resistant. If the culture is dumped onto a plate of an all-purpose medium, all of the cells will



form colonies. One cannot distinguish colonies of antibiotic-resistant cells from colonies of antibiotic-sensitive cells. If the culture is dumped instead onto a plate of the same medium to which is added the antibiotic, the sensitive cells will be inhibited or killed by the antibiotic – the selective agent in the medium – while the resistant cell will form a colony.

A take-home lesson: Suppose that this test tube is your body and the bacterial cells are infecting it. If you are given this antibiotic to control the infection, will it be totally effective? Might the resistant mutant escape your body's own natural defenses and then multiply and continue the infection? This is an important way in which antibiotic-resistant strains of a pathogen can arise. In the antibiotic disc sensitivity test, we test various species of bacteria (potential pathogens) with a number of different antibiotics, and we can determine the best antibiotics to use against a given organism – where there is no indication of any growth of resistant cells.

## VII. Genetic Differentiation and Identification of Bacteria – Base-Sequencing.

### A. Differentiating between species and establishing relationships.

#### An Example of Genotypic Differentiation.

Here is a selected 22-base segment of the gene that codes for 16S rRNA:

Pseudomonas fluorescens: ... ccgcat[acg+cctacg]ggagaa...  
Aquimonas haywardensis: ... ccgcata[acg+cgcaag]accaa...  
Budvicia squatica: ... ccgegta[acg+cgaaag]accaa...  
Edwardiella tarda: ... ccgcata[acg+cgcaag]accaa...

Similarities and differences can be seen when different organisms are compared – as shown above. The segment within brackets ( [ ] ) appears "shifted" when P. fluorescens is compared to the others.

When 1308 bases of this gene are compared between the 4 species, one can note the % difference in base sequence between any two of the species:

	PF	AH	BA	ET
PF	0			
AH	14.5	0		
BA	14.5	3.2	0	
ET	14.9	4.3	5.0	0

The 4 species can be connected by lines which correspond to the % difference in base sequence. This is then a "phylogenetic tree" which can suggest evolutionary relationships.



Series of diagrams from Bacteriology 102.

### B. Identification of unknown organisms and establishing new species.