2.4B: The Bacterial Chromosome and Nucleoid

Skills to Develop

1. Define genome.
2. Describe the composition of the bacterial chromosome.
3. Name the enzymes that enables bacterial DNA to become circular, supercoiled, and unwind during DNA replication.
4. Briefly describe the process of DNA replication.
5. State the function of the following enzymes in bacterial DNA replication:
   a. DNA polymerase III
   b. DNA polymerase II
   c. DNA helicase
   d. primase
   e. DNA ligase
6. State the function of DNA.
7. In terms of protein synthesis, briefly describe the process of transcription and translation.
8. Briefly state how the following antibacterial chemotherapeutic agents affect bacteria:
   a. fluoroquinolones (norfloxacin, lomefloxacin, fleroxacin, ciprofloxacin, enoxacin, trovafloxacin, etc.)
   b. trimethoprim and sulfamethoxazole

We will now look at the bacterial chromosome located in the nuclear region called the nucleoid.
A. Structure and Composition of the Bacterial Chromosome

The term genome refers to the sum of an organism’s genetic material. The bacterial genome is composed of a single molecule of chromosomal deoxyribonucleic acid or DNA and is located in a region of the bacterial cytoplasm visible when viewed with an electron microscope called the nucleoid. Unlike the eukaryotic nucleus, the bacterial nucleoid has no nuclear membrane or nucleoli.

In general it is thought that during DNA replication, each strand of the replicating bacterial DNA attaches to proteins at what will become the cell division plane. For example, Par proteins function to separate bacterial chromosomes to opposite poles of the cell during cell division. They bind to the origin of replication of the DNA and physically pull or push the chromosomes apart, similar to the mitotic apparatus of eukaryotic cells (Figure \(\PageIndex{1}\)).

Figure \(\PageIndex{1}\): Bacterial Division. In general it is thought that during DNA replication, each strand of the replicating bacterial DNA attaches to proteins at what will become the cell division plane. For example, Par proteins function to separate bacterial chromosomes to opposite poles of the cell during cell division. They bind to the origin of replication of the DNA and physically pull or push the chromosomes apart, similar to the mitotic apparatus of eukaryotic cells. In the center of the bacterium, a group of proteins called Fts (filamentous temperature sensitive) proteins interact to form a ring at the cell division plane. These proteins form the cell division apparatus known as the divisome and are directly involved in bacterial cell division by binary fission. The divisome is responsible for directing the synthesis of new cytoplasmic membrane and new peptidoglycan to form the division septum.

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Since bacteria are haploid, that is they have only one chromosome and only reproduce asexually, there is also no meiosis in bacteria.

The bacterial chromosome is one long, single molecule of double stranded, helical, supercoiled DNA. In most bacteria, the two ends of the double-stranded DNA covalently bond together to form both a physical and genetic circle. The chromosome is generally around 1000 µm long and frequently contains as many as 3500 genes (Figure \(\PageIndex{2}\)). \textit{E. coli}, a bacterium that is 2-3 µm in length, has a chromosome approximately 1400 µm long.
To enable a macromolecule this large to fit within the bacterium, histone-like proteins bind to the DNA, segregating the DNA molecule into around 50 chromosomal domains and making it more compact. A DNA topoisomerase enzyme called DNA gyrase then supercoils each domain around itself, forming a compacted mass of DNA approximately 0.2 µm in diameter. In actively growing bacteria, projections of the nucleoid extend into the cytoplasm. Presumably, these projections contain DNA that is being transcribed into mRNA. Supercoils are both inserted and removed by topoisomerases.

DNA topoisomerases are, therefore, essential in the unwinding, replication, and rewinding of the circular, supercoiled bacterial DNA. In order for the long molecule of DNA to fit within the bacterium, the DNA must be supercoiled. However, this supercoiled DNA must be uncoiled and relaxed in order for DNA polymerase to bind for DNA replication and RNA polymerase to bind for transcription of the DNA. For example, a topoisomerase called DNA gyrase catalyzes the negative supercoiling of the circular DNA found in bacteria. Topoisomerase IV, on the other hand, is involved in the relaxation of the supercoiled circular DNA, enabling the separation of the interlinked daughter chromosomes at the end of bacterial DNA replication.

### B. DNA Replication in Bacteria

In general, DNA is replicated by uncoiling of the helix, strand separation by breaking of the hydrogen bonds between the complementary strands, and synthesis of two new strands by complementary base pairing. Replication begins at a specific site in the DNA called the origin of replication (oriC).
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DNA replication is bidirectional from the origin of replication. To begin DNA replication, unwinding enzymes called DNA helicases cause short segments of the two parent DNA strands to unwind and separate from one another at the origin of replication to form two "Y"-shaped replication forks. These replication forks are the actual site of DNA copying (Figure \(\PageIndex{3}\)). All the proteins involved in DNA replication aggregate at the replication forks to form a replication complex called a replisome (Figure \(\PageIndex{4}\)).

Single-strand binding proteins bind to the single-stranded regions so the two strands do not rejoin. Unwinding of the double-stranded helix generates positive supercoils ahead of the replication fork. Enzymes called topoisomerases counteract this by producing breaks in the DNA and then rejoin them to form negative supercoils in order to relieve this stress in the helical molecule during replication.
As the strands continue to unwind and separate in both directions around the entire DNA molecule, new complementary strands are produced by the hydrogen bonding of free DNA nucleotides with those on each parent strand. As the new nucleotides line up opposite each parent strand by hydrogen bonding, enzymes called DNA polymerases join the nucleotides by way of phosphodiester bonds. Actually, the nucleotides lining up by complementary base pairing are deoxynucleotide triphosphates, composed of a nitrogenous base, deoxyribose, and three phosphates. As the phosphodiester bond forms between the 5’ phosphate group of the new nucleotide and the 3’ OH of the last nucleotide in the DNA strand, two of the phosphates are removed providing energy for bonding (see Figure 6). In the end, each parent strand serves as a template to synthesize a complementary copy of itself, resulting in the formation of two identical DNA molecules (see Figure 7). In bacteria, Par proteins function to separate bacterial chromosomes to opposite poles of the cell during cell division. They bind to the origin of replication of the DNA and physically pull or push the chromosomes apart, similar to the mitotic apparatus of eukaryotic cells. Fts proteins, such as FtsK in the divisome, also help in separating the replicated bacterial chromosome.

GIF animation illustrating DNA replication by complementary base pairing

In reality, DNA replication is more complicated than this because of the nature of the DNA polymerases. DNA polymerase enzymes are only able to join the phosphate group at the 5’ carbon of a new nucleotide to the hydroxyl (OH) group of the 3’ carbon of a nucleotide already in the chain. As a result, DNA can only be synthesized in a 5’ to 3’ direction while copying a parent strand running in a 3’ to 5’ direction.

Each DNA strand has two ends. The 5’ end of the DNA is the one with the terminal phosphate group on the 5’ carbon of the deoxyribose; the 3’ end is the one with a terminal hydroxyl (OH) group on the deoxyribose of the 3’ carbon of the deoxyribose (see Figure 8). The two strands are antiparallel, that is they run in opposite directions. Therefore, one parent strand - the one running 3’ to 5’ and called the leading strand - can be copied directly down its entire length (see Figure 9). However, the other parent strand - the one running 5’ to 3’ and called the lagging strand - must be copied discontinuously in short fragments (Okazaki fragments) of around 100-1000 nucleotides each as the DNA unwinds. This occurs, as mentioned above, at the replisome. The lagging DNA strand loops out from the leading strand and this enables the replisome to move along both strands pulling the DNA through as replication occurs. It is the actual DNA, not the DNA polymerase that moves during bacterial DNA replication (see Figure 5).

In addition, DNA polymerase enzymes cannot begin a new DNA chain from scratch. They can only attach new nucleotides onto 3’ OH group of a nucleotide in a preexisting strand. Therefore, to start the synthesis of the leading strand and each DNA fragment of the lagging strand, an RNA polymerase complex called a primase is required. The primase, which is capable of joining RNA nucleotides without requiring a preexisting strand of nucleic acid, first adds several complementary RNA nucleotides opposite the DNA nucleotides on the parent strand. This forms what is called an RNA primer (see Figure 10).

DNA polymerase III then replaces the primase and is able to add DNA nucleotides to the RNA primer (see Figure 11). Later, DNA polymerase II digests away the RNA primer and replaces the RNA nucleotides of the primer with the proper DNA nucleotides to fill the gap (see Figure 12). Finally, the DNA fragments themselves are hooked together by the enzyme DNA ligase (see Figure 9). Yet even with this complicated procedure, a 1000 micrometer-long macromolecule of tightly-packed, supercoiled DNA can make an exact copy of itself in only about 10 minutes time under optimum conditions, inserting nucleotides at a rate of about 1000 nucleotides per second!
C. Functions of the Bacterial Chromosome

The chromosome is the genetic material of the bacterium. Genes located along the DNA are transcribed into RNA molecules, primarily messenger RNA (mRNA), transfer RNA (tRNA, and ribosomal RNA (rRNA). Messenger RNA is then translated into protein at the ribosomes.

- **Transcription**: Ribonucleic acid (RNA) is synthesized by complementary base pairing of ribonucleotides with deoxyribonucleotides to match a portion of one strand of DNA called a gene. Although genes are present on both strands of DNA, only one strand is transcribed for any given gene. Following transcription of genes into mRNA, 30S and 50S ribosomal subunits attach to the mRNA and tRNA inserts the correct amino acids which are subsequently joined to form a polypeptide or a protein through a process called translation.

- **Translation**: During translation, specific tRNA molecules pick up specific amino acids, transfer those amino acids to the ribosomes, and insert them in their proper place according to the mRNA "message." This is done by the anticodon portion of the tRNA molecules complementary base pairing with the codons along the mRNA.

In general then, DNA determines what proteins and enzymes an organism can synthesize and, therefore, what chemical reactions it is able to carry out.

D. The Bacterial Epigenome

The epigenome refers to a variety of chemical compounds that modify the genome typically by adding a methyl (CH₃) group to the nucleotide base adenine at specific locations along the DNA molecule. This methylation can, in turn, either repress or activate transcription of specific genes. By basically turning genes on or off, the epigenome enables the bacterial genome to interact with and respond to the bacterium's environment. The epigenome can be inherited just like
All cells, including human cells, possess an epigenome. Just as the bacterial epigenome can affect the bacterial genome, bacteria can affect our epigenome and subsequently modify the function of our genome by causing either DNA methylation of nucleotides or by modifying our histone proteins. The resulting modification can either help activate various genes involved in immune defenses, or, in the case of some pathogens, suppress immune response genes.

E. Significance of the Chromosome to the Initiation of Body Defense

To protect against infection, one of the things the body must initially do is detect the presence of microorganisms. The body does this by recognizing molecules unique to microorganisms that are not associated with human cells. These unique molecules are called pathogen-associated molecular patterns or PAMPS. (Because all microbes, not just pathogenic microbes, possess PAMPs, pathogen-associated molecular patterns are sometimes referred to as microbe-associated molecular patterns or MAMPs.)

Bacterial and viral genomes contain a high frequency of unmethylated cytosine-guanine (CpG) dinucleotide sequences (a cytosine lacking a methyl or CH₃ group and located adjacent to a guanine). Mammalian DNA has a low frequency of cytosine-guanine dinucleotides and most are methylated. These unmethylated cytosine-guanine dinucleotide sequences in bacterial DNA are PAMPS that bind to pattern-recognition receptors on a variety of defense cells of the body and triggers innate immune defenses such as inflammation, fever, and phagocytosis.

F. Antimicrobial Agents that Inhibiting Normal Nucleic Acid Replication in Bacteria

Some antibacterial chemotherapeutic affect bacteria by inhibiting normal nucleic acid replication.

- The fluoroquinolones (norfloxacin, lomefloxacin, fleroxacin, ciprofloxacin, enoxacin, trovafloxacin, etc.) work by inhibiting one or more of the topoisomerases, the enzymes needed for bacterial nucleic acid synthesis.
- Co-trimoxazole, a combination of sulfamethoxazole and trimethoprim, block enzymes in the bacteria pathway required for the synthesis of tetrahydrofolic acid, a cofactor needed for bacteria to make the nucleotide bases thymine, guanine, uracil, and adenine. Without the tetrahydrofolic acid, the bacteria cannot synthesize DNA or RNA.

Antimicrobial chemotherapy will be discussed in greater detail later in Unit 2 under Control of Bacteria by Using Antibiotics and Disinfectants.

Exercise: Think-Pair-Share Questions

As we are learning, pathogen-associated molecular patterns (PAMPs) are microbial molecules many microbes share but are not found as a part of the human body and are able to initiate innate immune responses. Examples thus far include peptidoglycan fragments, lipopolysaccharide in the gram-negative cell wall, and lipoteichoic acids in the gram-positive cell wall, molecules that human cells lack. Bacterial and viral genomes also act as PAMPS.

Our cells also have DNA and RNA. How can bacterial and viral genomes initiate innate immunity when our genomes do not?
Summary

1. The genome is the sum of an organism’s genetic material.
2. Bacteria contain a single chromosome of double-stranded deoxyribonucleic acid (DNA).
3. The region of the bacterial cytoplasm where the chromosome is located and visible when viewed with an electron microscope called the nucleoid.
4. The bacterial chromosome is typically a physical and genetic circle, becomes supercoiled, and is not surrounded by a nuclear membrane.
5. Bacteria do not carry out mitosis or meiosis.
6. DNA topoisomerase enzymes are used to supercoil and relax the bacterial chromosome during DNA replication and transcription.
7. Like eukaryotic DNA, prokaryotic DNA replicates by sequential unwinding of the two DNA parent strands and the subsequent complementary base pairing of DNA nucleotides with each parent strand.
8. During DNA replication the nitrogenous base adenine forms hydrogen bonds with thymine and guanine forms hydrogen bonds with cytosine.
9. Genes located along the DNA are transcribed into RNA molecules, primarily messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA). Messenger RNA is then translated into protein at the ribosomes.
10. During transcription, ribonucleic acid (RNA) is synthesized by complementary base pairing of ribonucleotides with deoxyribonucleotides to match a portion of one strand of DNA called a gene.
11. During translation, specific tRNA molecules pick up specific amino acids, transfer those amino acids to the ribosomes, and insert them in their proper place according to the mRNA “message.”
12. Bacterial and viral genomes act as PAMPs to stimulate innate immunity.
13. Some antibacterial chemotherapeutic agents inhibiting normal nucleic acid replication in bacteria.

Questions

Study the material in this section and then write out the answers to these questions. Do not just click on the answers and write them out. This will not test your understanding of this tutorial.

1. The sum of an organism’s genetic material is called its___________. (ans)
2. Bacterial enzymes involved in the unwinding, replication, and rewinding of the circular, supercoiled bacterial DNA called _____________. (ans)
3. Describe the general composition of the chromosome in most bacteria. (ans)
4. Briefly describe the process of DNA replication. (ans)
5. State what enzyme carries out the following functions during DNA replication.
   a. Unwinds the helical DNA by breaking the hydrogen bonds between complementary bases. (ans)
   b. Synthesizes a short RNA primer at the beginning of each origin of replication. (ans)
   c. Adds DNA nucleotides to the RNA primer. (ans)
   d. Digests away the RNA primer and replaces the RNA nucleotides of the primer with the proper DNA nucleotides. (ans)
   e. Links the DNA fragments of the lagging strand together. (ans)
6. State the overall function of DNA. (ans)
7. Define transcription. (ans)
8. Define translation. (ans)
9. Ciprofloxacin (Cipro) is used to treat a variety of bacterial infections. How does it stop bacteria from growing? (ans)
10. Multiple Choice (ans)

Contributors

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