

Overview of DNA

Primary Knowledge (PK)

Participant Guide

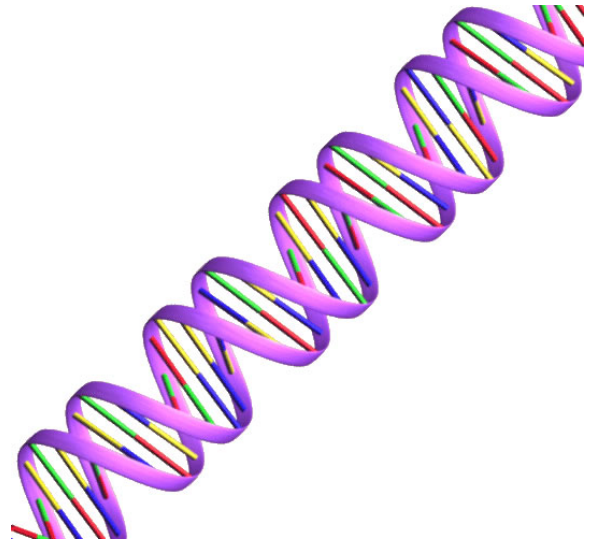
Description

The primary knowledge unit is part of the Overview of DNA Learning Module. This unit provides an overview of DNA (Deoxyribonucleic acid), its role as genetic material, its molecular components and structure, and DNA replication. This information is necessary to better understand the role of microelectromechanical systems (MEMS) in DNA analysis, disease diagnostics, and gene therapy.

Estimated Time to Complete

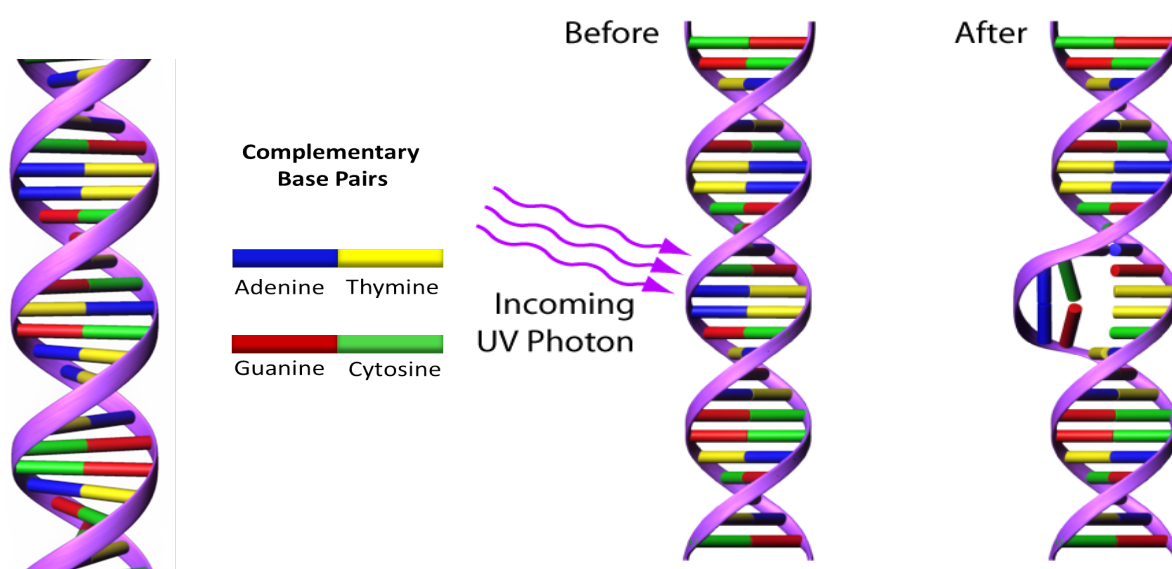
Allow approximately 15 minutes

DNA (Deoxyribonucleic acid)



Introduction

Deoxyribonucleic acid (DNA) is a long polymeric molecule found in most cells that functions as the carrier of genetic information. The genetic information carried in the various linear sequences of base pairs in DNA is what defines an organism. Changes in the linear sequence, sometimes called mutations or polymorphisms, explain the differences between individuals as well as identify diseases such as cancer. A number of factors cause these mutations, such as mistakes in DNA replication during cell division, radiation, chemical exposure, and ultraviolet (UV) light, just to name a few. The graphics below illustrate the base pair structures of a DNA molecule (left) and mutation formation (right). A mutation is a change in the DNA of one or two individuals, whereas a polymorphism is a change in a population.



Base Pairs Structure of DNA

Mutation formation: Ultraviolet (UV) photons harm the DNA molecules of living organisms in different ways. In one common damage event, adjacent bases bond with each other, instead of across the “ladder.” This makes a bulge, and the distorted DNA molecule does not function properly.²

(Illustration by an illustration by David Herring- courtesy of Earth Observatory, NASA)

Past and current studies of DNA identify how DNA can be used in biomedical applications. Such applications include the following:

- Improve diagnosis of a disease
- Test the best treatment options for a disease
- Execute rational drug design
- Create custom drugs
- Utilizing DNA in gene therapy

DNA Analysis and MEMS

The development of Microelectromechanical systems (MEMS) has helped in the analysis of the DNA molecule. Current MEMS applications involving DNA include

- the polymerase chain reaction (PCR),
- the Sanger dideoxy method of DNA sequencing,
- the use of DNA probes in DNA microarrays, and
- the use of single nucleotide polymorphisms (SNPs) in forensics.

Fields that can benefit from the DNA and MEMS partnership include

- energy sources and environmental screening,
- agriculture,
- livestock breeding,
- biomedical analysis, and
- risk assessment.

The application of MEMS devices in biomedical fields is huge, especially in the areas of therapeutics and diagnostics.

This unit answers the question, "What needs to be known about the DNA molecule to begin formulating new diagnostics and treatments?"

Objectives

- Describe the molecular components of DNA.
- Describe the structure and replication of DNA.
- Evaluate the role of DNA as genetic material.

Key Terms (Define in the glossary at the end of this unit)

Bacteriophage

Chromosome

Deoxyribonucleic acid (DNA)

DNA polymerase

DNA microarray

Gene

Mutation

Nitrogenous bases

Nucleic acid

Origin of Replication

Polymerase Chain Reaction (PCR)

Primase

Ribonucleic acid (RNA)

Semi-conservative replication

Transformation

Virulent

DNA exploration

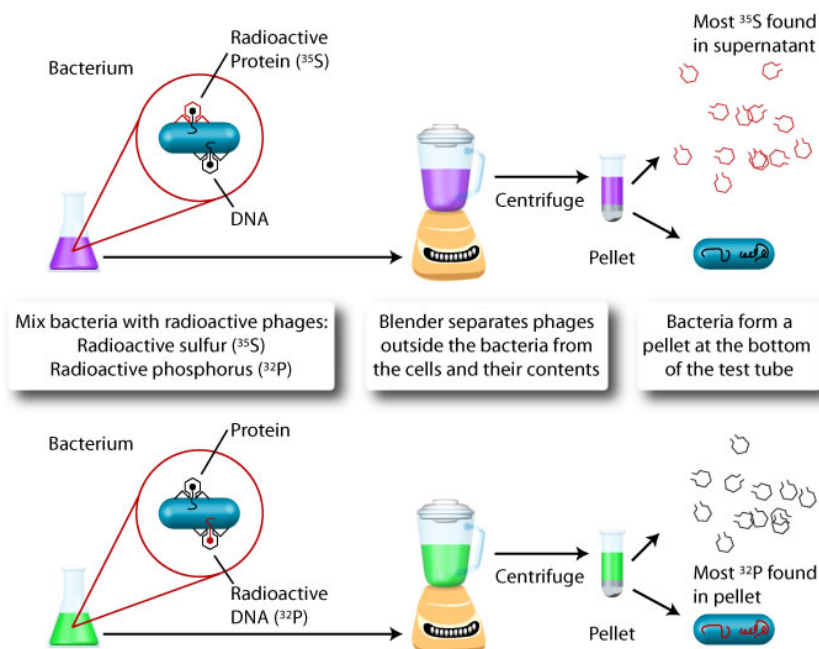
During the 1920's, scientists had resolved that chromosomes were composed of both DNA and protein. It remained to be resolved which of these molecules (DNA or protein) constituted the genetic material of the cell. In 1928, Frederick Griffith and colleagues discovered that a "chemical" from one type of bacteria was capable of transforming another bacterium from a non- virulent to virulent type. In 1952, a series of experiments by Alfred Hershey and Martha Chase conclusively demonstrated that the chemical was DNA.



Martha Chase and Alfred Hershey (1953)
[Photo by: Karl Maramorosch. Printed with permission.]

The Hershey-Chase Blender Experiments

In what is known as "The Hershey-Chase Blender Experiments", Hershey and Chase used ^{32}P - or ^{35}S -labeled T2 bacteriophage (a virus that infects bacteria) in separate experiments to infect bacteria (see graphic below). They followed the radioactive material through a productive infectious cycle. At the end of the experiment, they measured the radioactivity in the pellet and the liquid. Their findings ruled out protein as the genetic material and supported the role of DNA as the carrier of genetic information.



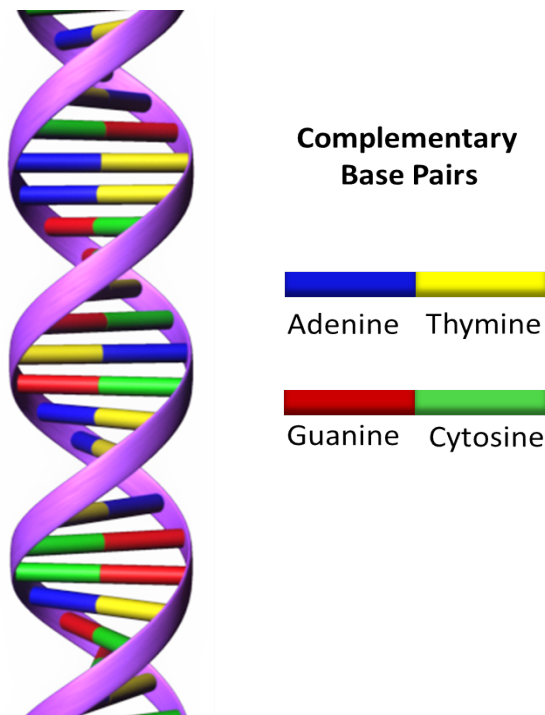
Hershey-Chase Blender Experiments

Chargaff's Rule

Early studies demonstrated that DNA was chemically composed of a repeating structure of the sugar deoxyribose linked to phosphate and four nitrogenous bases (adenine, thymine, cytosine and guanine). In 1950, Erwin Chargaff examined the nitrogenous base content and determined that the content of adenine equaled the content of thymine, and the content of guanine equaled the content of cytosine. This relationship became known as Chargaff's Rule.

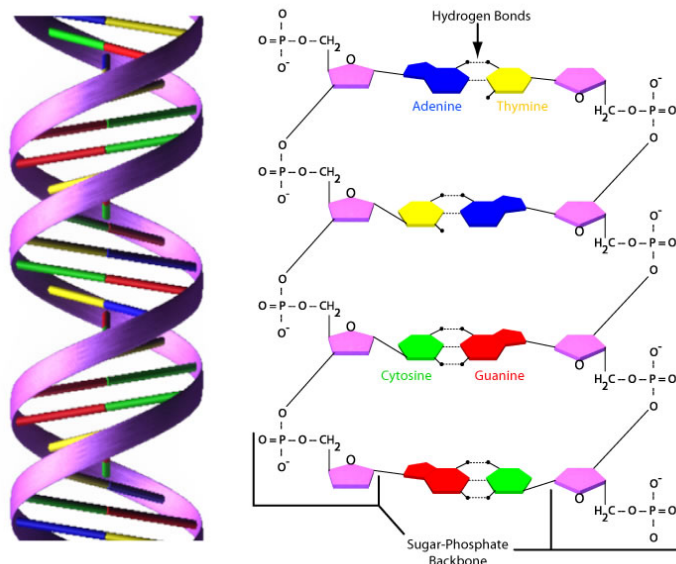
In 1953, James Watson and Francis Crick described the structure of DNA using the chemical description of the molecule in conjunction with the X-ray crystallography data from Rosalind Franklin and Maurice Wilkins. Four key features were noted:

- 1) DNA structure is a double-stranded (ds) helix
- 2) The molecule exhibits a uniform diameter
- 3) The molecule is right-handed
- 4) The two strands are anti-parallel and demonstrate complementary base-pairing (fulfilling Chargaff's Rule).



Sugar and phosphate groups form the rails or backbone of the DNA molecule and the nitrogenous bases form the steps. The bases are joined by weak hydrogen bonds (2 H bonds for A-T and 3 H bonds for C-G). (See graphic right)

A "nucleotide" is a single nitrogenous base with a sugar and phosphate attached. For example, a cytosine with a sugar/phosphate or a thymine with a sugar phosphate.



A complementary nitrogenous base pair consists of one of the following four combinations:

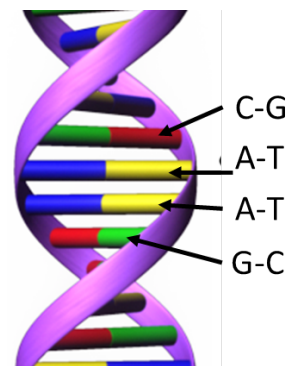
Adenine – Thymine (A-T)

Thymine – Adenine (T-A)

Guanine – Cytosine (G-C)

Cytosine – Guanine (C-G)

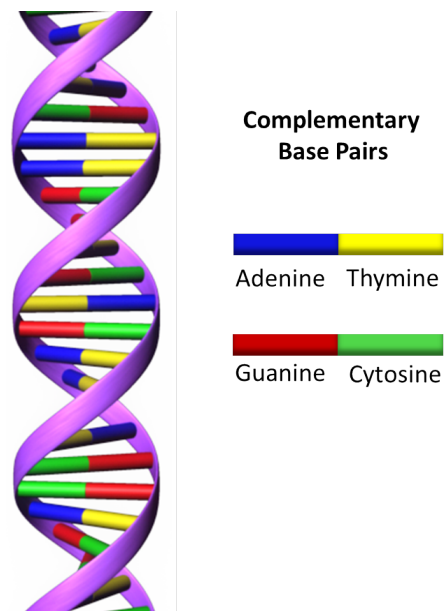
The base pairing provides a model for the precise replication of the DNA molecule. Genetic information in the molecule is stored in the linear sequences of the base pairs. For example and very simply, a specific gene might be identified by a linear sequence represented in this graphic (C-G, A-T, A-T, G-C). One DNA molecule can contain thousands of different sequences and thus thousands of genes.



The Human Genome Project³

In 2003, the Human Genome Project completed sequencing the entire human genome. The project goals were to sequence the 3 billion base pairs that make up the human genome, and identify all of the sequences of DNA that encoded genes.

One outcome of the project was the finding that the human genome encodes approximately 20-25,000 genes. The sequence data from this finding was able to be stored in databases because the information stored within the three-dimensional structure of the DNA molecule is digital and two-dimensional. Digital because each unit of information consists of a discrete nitrogenous base letter (A, T, G, or C), and there are only 4 of these in the DNA molecule (adenine, thymine, guanine and cytosine). Two-dimensional because the information is encoded as a linear specific sequence of these letters along the length of the molecule (e.g., T-A, C-G, A-T, etc.). All of this data is freely available.



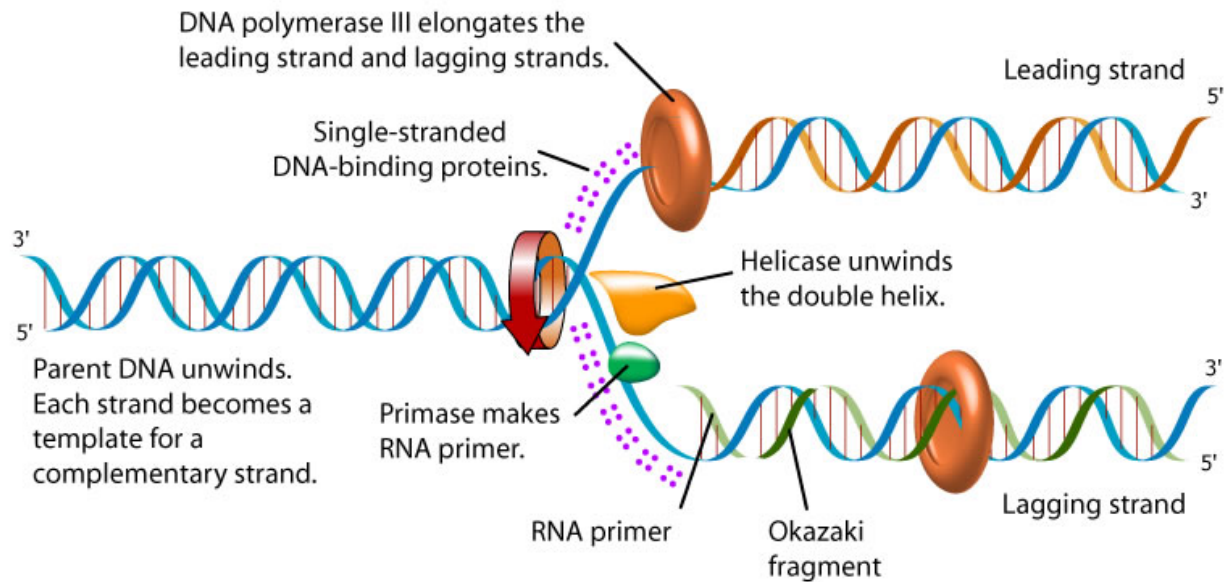
Another goal of the project was to transfer related information and technologies to the private sector. One transfer application, known as the DNA microarray, involves diagnosing and predicting disease and disease susceptibility. It is known that diseases have a genetic component, either inherited or as a result of exposure to an environmental stress such as chemicals or viruses. This has led to the development of the DNA microarray that is design to identify the presense or absenses of specific genes as well as the activity of genes under normal conditions or external factors such a chemical exposure, drugs, x-rays, or even stress.

DNA Replication

In 1957, Matthew Messelson and Franklin Stahl demonstrated that a double helix DNA molecule separated into two single strands could be replicated with each strand serving as a template on which its complementary strand is assembled.⁴ Subsequent work of Messelson's and Stahl's focused on the molecular mechanisms of DNA replication.

DNA replication is not as simple as splitting a double helix into two templates. It is a challenging process insuring the correct copying of the genetic information from the original DNA molecule to its replications. DNA replication involves numerous enzymes and proteins. It occurs in two basic steps:

- First step - the DNA helix is unwound at the site of replication
- Second step - new nucleotides are linked by covalent bonding to each growing new strand only at the 3' end which contains a free hydroxyl group.



DNA Replication

Replication begins at an origin of replication (ori). (*refer to previous graphic*)

- The hydrogen bonds that bind the base pairs are broken allowing the double helix to split or divide.
- A molecule of a DNA polymerase binds to one strand of the DNA and moves along the strand using it as a template for assembling a leading strand of nucleotides and reforming a double helix. In other words, the DNA polymerase “reads” a single DNA strand and uses what it “reads” as a template to synthesize a complementary strand that binds with the original single DNA strand.
- A molecule of a second type of DNA polymerase binds to the other template strand as the double helix opens. This molecule must synthesize discontinuous segments of polynucleotides (called Okazaki fragments).
- Another enzyme, stitches these together into the lagging strand.

While small circular DNA molecules may replicate from a single ori, large linear DNA molecules have many oris (hundreds of oris in human DNA). The enzyme that copies DNA is DNA polymerase.

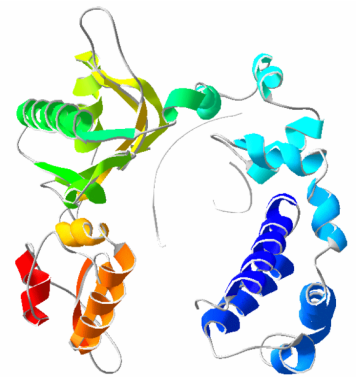
Replication Primer

DNA replication also requires a primer. The primer is a short stretch of Ribonucleic acid (RNA) synthesized by the enzyme, primase. During later steps in replication, the RNA primer is removed and filled in with DNA.

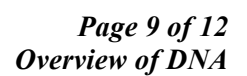
Cells contain several DNA polymerases (*see right*). One is involved in chromosome replication, and others are involved in primer removal and DNA repair.

DNA Polymerase

[Image courtesy of Magnus Manske: English Wikipedia Project]



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1. Reference the figure *Double strand DNA showing Base Pairing*. Based on this figure, what is the charge (positive or negative) on a molecule of DNA? Explain your answer.
2. The rungs or steps of the ladder are composed of the paired nitrogenous bases. What interactions hold the bases together? What implications does this have for unzipping the two strands of DNA?

Summary

- DNA is the genetic material with the genetic information stored in the linear array of nitrogenous bases.
- The DNA molecule is composed of the sugar deoxyribose, phosphate groups, and the nitrogenous bases.
- The molecular structure is a double-stranded helix with the sugar and phosphate groups forming the rail or backbone and the nitrogenous bases forming the steps of the ladder.
- DNA replication is a complex process that requires many enzymes and proteins.

An understanding of the DNA molecule, what it consists of, and how it replicates will help you to better understand the role of MEMS in DNA analysis, disease diagnostics, and gene therapy.

References

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- ⁹ List of Fluidic MEMS Companies. February 2016. <http://bit.ly/2wz1Lx5>

Glossary of Key Terms

Bacteriophage - A virus that specifically infects bacteria.

Chromosome - A single large macromolecule of DNA with associated proteins, which constitutes a physically organized form of DNA in a cell.

Deoxyribonucleic acid (DNA) - A long linear polymer formed from nucleotides and shaped like a double helix; associated with the transmission of genetic information.

DNA microarray - A microchip that holds single-stranded DNA probes and can recognize DNA strands from samples being tested. A complementary strand combines with the DNA probe forming a DNA hybrid.

DNA polymerase - An enzyme that mediates or acts as a catalyst for the replication of DNA.

Gene - A length of DNA sequence that contains information that is capable of being translated into a polypeptide product. This region is also usually associated with regulatory regions of DNA sequence.

Mutation - Heritable changes in the sequence of DNA bases.

Nitrogenous bases - Five bases are found in nucleic acids: two purine bases (adenine and guanine) and three pyrimidine bases (cytosine, uracil and thymine). Adenine and guanine occur in both DNA and RNA. Cytosine and thymine are the pair of pyrimidines in DNA, and cytosine and uracil are the pair in RNA.

Nucleic acid - Any of a group of long, linear macromolecules, either DNA or various types of RNA, that carry genetic information directing all cellular functions. Composed of linked nucleotides.

Origin of Replication - The specific site at which unwinding and initiation of DNA replication occurs.

Polymerase Chain Reaction (PCR) - A laboratory technique that can amplify the amount of DNA from a tiny sample to a large amount within just a few hours. PCR can take one molecule and produce measurable amounts of identical DNA in a short period of time.

Polymorphism – A change in DNA sequence which is common in a population.

Primase - The enzyme that mediates the replication of short stretches of RNA to initiate DNA replication by DNA polymerase.

Ribonucleic acid (RNA) - A polymer consisting of a long, usually single-stranded chain of alternating phosphate and ribose units with the bases adenine, guanine, cytosine, and uracil bonded to the ribose.

Semi-conservative replication - The process by which DNA is replicated. Each strand of the original molecule acts as a template for the synthesis of a new complementary DNA molecule.

Transformation - The genetic alteration of a cell resulting from the introduction, uptake and expression of foreign DNA in molecular biology.

Virulent - The relative ability of a microorganism to cause disease.

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