

Lecture 6 - DNA

The Search to Identify the Genetic Material

Discovery of Nucleic Acids - Friedrich Miescher, 1869

Miescher isolated the nuclei of white blood cells obtained from pus cells. His experiments revealed that nuclei contained a chemical that contained nitrogen and phosphorus but no sulfur. He called the chemical nuclein because it came from nuclei. It later became known as nucleic acid.

Proteins Produce Genetic Traits - Archibald Garrod, 1909

Garrod noticed that people with certain genetic abnormalities (inborn errors of metabolism) lacked certain enzymes. This observation linked proteins (enzymes) to genetic traits.

Genetic Material can Transform Bacteria - Frederick Griffith, 1931

When *Streptococcus pneumoniae* (pneumococcus) bacteria are grown on a culture plate, some produce smooth shiny colonies (S) while others produce rough colonies (R). This is because the S strain bacteria have a mucous (polysaccharide) coat, while R strain does not.

Mice infected with the S strain die from pneumonia infection but mice infected with the R strain do not develop pneumonia.

S strain → Inject into mice → Mice die

R strain → Inject into mice → Mice live

Griffith was able to kill bacteria by heating them. He observed that heat-killed S strain bacteria injected into mice did not kill them. When he injected a mixture of heat-killed S and live R bacteria, the mice died. Moreover, he recovered living S bacteria from the carcasses.

S strain (heat killed) → Inject into mice → Mice live

S strain (heat killed)
+
R strain (live) → Inject into mice → Mice die

He concluded that some substance needed to produce the mucous coat was passed from the dead bacteria (S strain) to the live ones (R strain); they became *transformed*.

This must be due to a change in the genotype associated with the transfer of the genetic material.

The transforming material is DNA - Oswald Avery, Colin MacLeod, and Maclyn McCarty, 1944

Prior to the work of Avery, MacLeod, and McCarty, the genetic material was thought to be protein. Avery, MacLeod, and McCarty worked to determine what the transforming substance was in Griffith's experiment (above).

They purified chemicals from the heat-killed S cells to see which ones could transform live R cells into S cells. They discovered that DNA alone from S bacteria caused R bacteria to become transformed.

They also discovered that protein-digesting enzymes (proteases) and RNA-digesting enzymes (RNase) did not affect transformation, so the transforming substance was not a protein or RNA. Digestion with *DNase* did inhibit transformation, so DNA caused transformation.

They concluded that DNA is the hereditary material, but not all biologists were convinced.

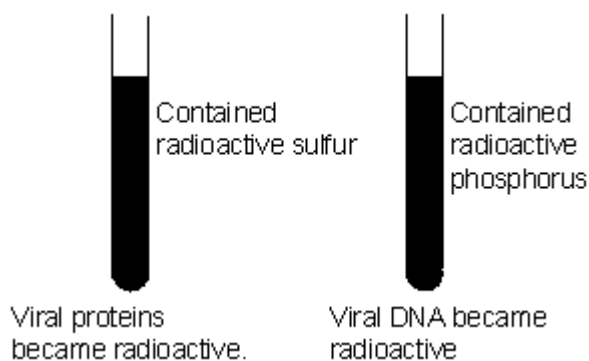
More Evidence: The Genetic Material is DNA - Alfred D. Hershey and Martha Chase, 1952

Hershey and Chase worked with viruses that infect bacteria called bacteriophages.

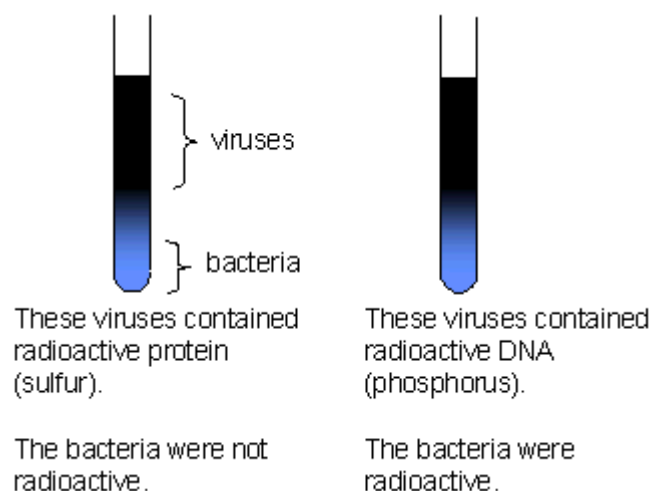
The bacteriophage becomes attached to the bacteria and its genetic material then enters the bacterial cell. The bacterial cell treats the viral

genetic material as if it was its own and subsequently manufactures more virus particles. Hershey and Chase worked to discover whether it was protein or DNA from the viruses that entered the bacteria.

They grew a virus population in medium that contained radioactive phosphorus and another in medium that contained radioactive sulfur. Viruses grown in the presence of radioactive phosphorus contained radioactive DNA but not radioactive protein because DNA contains phosphorus but protein does not. Similarly, viruses grown on radioactive sulfur contained radioactive protein but not radioactive DNA because DNA does not contain sulfur.

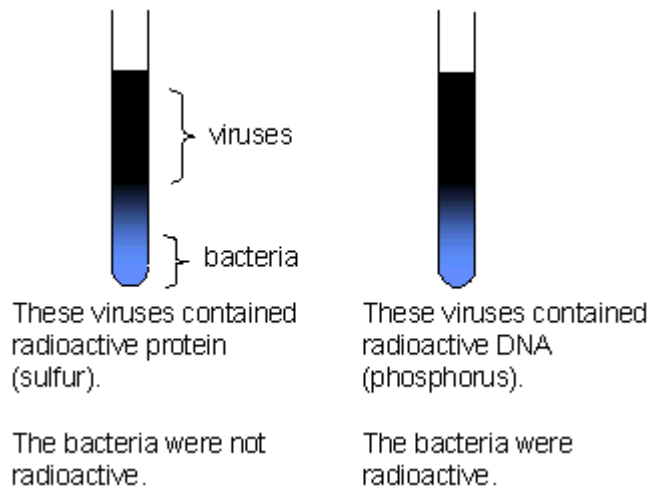


Radioactive bacteriophages were allowed to attach to *E. coli* bacteria. Then as the infection proceeded, the viral coats were removed from the bacteria by agitating them in a blender. The viruses particles were separated from the bacteria by spinning them in a centrifuge.



Bacteria that were infected with viruses that had radioactive DNA were radioactive, indicating that DNA was the material that passed from the virus to the bacteria. Bacteria that were infected with viruses that had radioactive proteins were not radioactive. This indicates that proteins did

not enter the bacteria from the viruses. DNA is therefore the genetic material that is passed from virus to bacteria.



Discovery of the Structure of DNA

Erwin Chargaff, 1940's and early 50's

DNA was thought to contain equal amounts of A, T, T, and C. Chargaff found that the base composition of DNA differs among species.

His data showed that in each species, the percent of A equals the percent of T, and the percent of G equals the percent of C. so that 50% of the bases were purines (A + G) and 50% were pyrimidines (T + C)

Chargaff's rule: The amount of A = T and the amount of G = C.

M.H.F. Wilkins and Rosalind Franklin, early 50's

Wilkins and Franklin studied the structure of DNA crystals using X-rays.

They found that the crystals contain regularly repeating subunits.

Structures that are close together cause the x-ray to bend more than structures that are further apart. The X pattern produced by DNA suggested that DNA contains structures with dimensions of 2 nm, 0.34 nm, and 3.4 n. The dark structures at the top and bottom indicate that some structure was repeated, suggesting a helix.

James Watson and Francis H.C. Crick, 1953

Watson and Crick used Chargaff's base data and Franklin's X-ray diffraction data to construct a model of DNA.

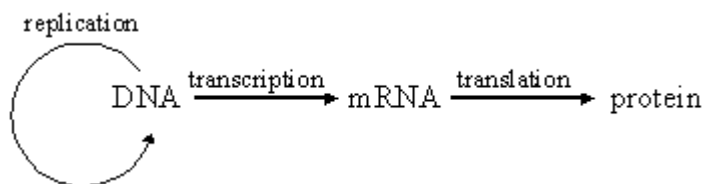
The model showed that DNA is a double helix with sugar-phosphate backbones on the outside and the paired nucleotide bases on the inside, in a structure that fit the spacing estimates from the X-ray diffraction data.

Chargaff's rules showed that $A = T$ and $G = C$, so there was complementary base pairing of a purine with a pyrimidine, giving the correct width for the helix.

The paired bases can occur in any order, giving an overwhelming diversity of sequences.

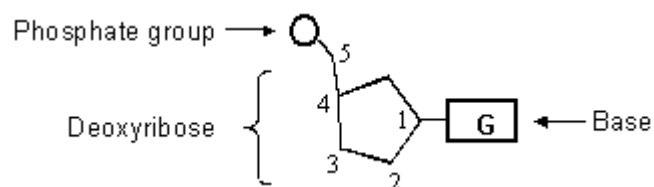
Properties of Genetic Material

DNA is an ideal genetic material because it can store information, is able to replicate, and is able to undergo changes (mutate).

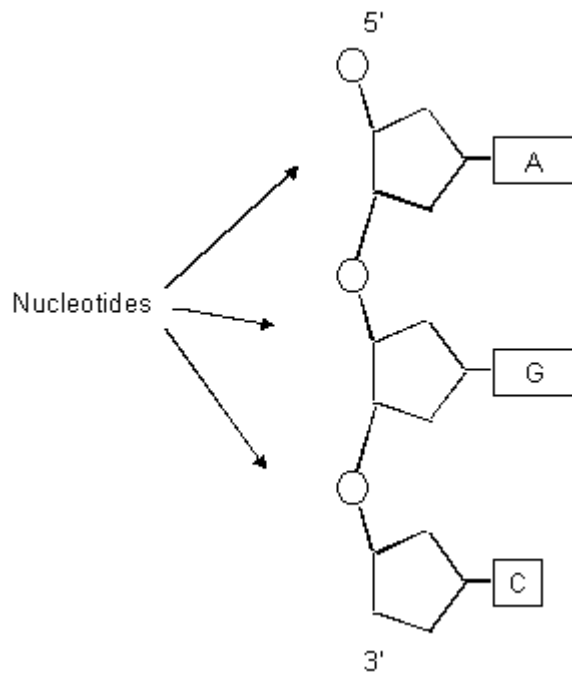


Structure of DNA

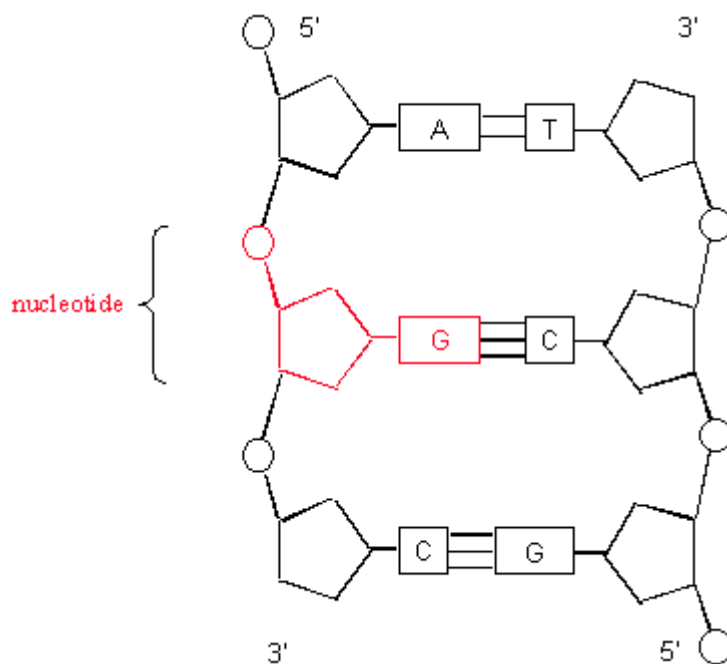
DNA is composed of units called nucleotides. Each nucleotide contains a phosphate group, a deoxyribose sugar, and a nitrogenous base.



The nucleotides joined together to form a chain. The phosphate end of the chain is referred to as the 5' end. The opposite end is the 3' end.



DNA is composed of two chains of nucleotides linked together in a ladder-like arrangement with the sides composed of alternating deoxyribose sugar and phosphate groups and the rungs being the nitrogenous bases as indicated by the diagram below.



The "A" of one strand is always paired with a "T" on the other. Similarly, the "G" of one strand is paired with a "C" on the other.

The two strands are held together by [hydrogen bonds](#) (electrostatic attraction). Two hydrogen bonds hold adenine to thymine. Three bonds attach cytosine to guanine as indicated in the diagram above.

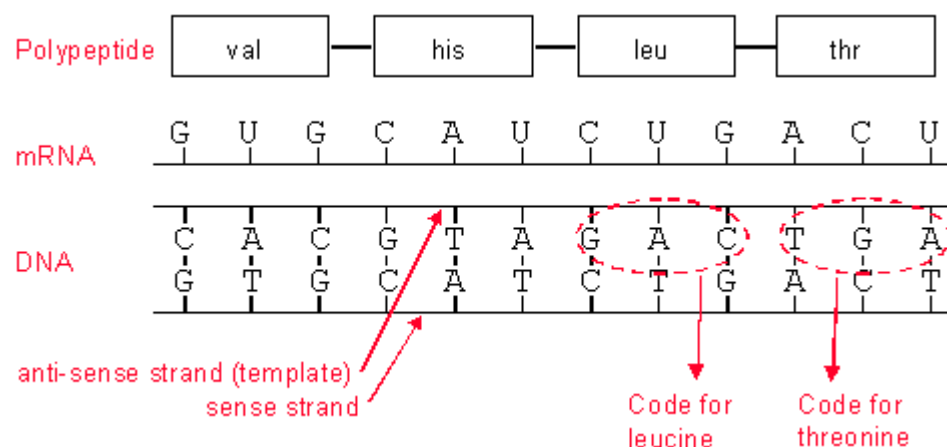
During the process of cell division, the DNA becomes tightly coiled, forming structures called *chromosomes*. The diagram below is a portion of a double-stranded chromosome showing the centromere and a portion of the base sequence. The diagram does not show the extensive looping and coiling and the proteins associated with coiling. Notice that the base sequence in the two chromatids is identical.



How is Information Stored?

The diagram below shows that one strand of the DNA double-helix serves as a template for the construction of mRNA. The sequence of nucleotides in this DNA strand is complimentary (opposite) the sequence in mRNA. The diagram also shows that the sequence of nucleotides in mRNA determines the amino acids in the protein. For example GUG in mRNA (or CAC in DNA) codes for valine (see below).

The strand of DNA that contains the genetic code is called the anti-sense. It is often referred to as the coding strand or the template strand. The other strand (the sense strand) is not used. Notice that the sense strand has the same base sequence as mRNA except that mRNA has U instead of T.



The codes in DNA are copied to produce mRNA. Each three-letter code in mRNA (called a *codon*) codes for one amino acid. The sequence of amino acids in proteins is therefore most directly determined by the sequence of codons in mRNA, which in turn, are determined by the sequence of bases in DNA.

There are four letters in the genetic alphabet (A, T, G, and C) and each codon contains three letters. It is therefore possible to have 64 different codons. Because there are only 20 different amino acids and 64 possible codons, some amino acids have several different codons.

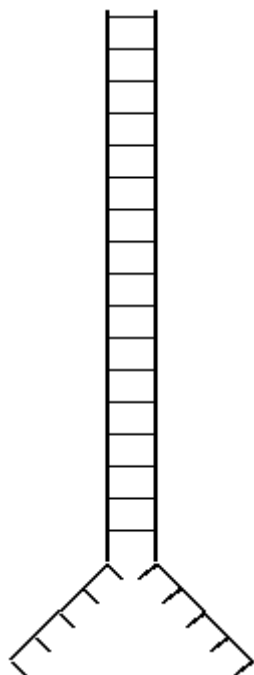
Terminators are codes that indicate the end of a genetic message (gene).

An initiator codon (usually AUG) indicates where the genetic information begins.

DNA replication

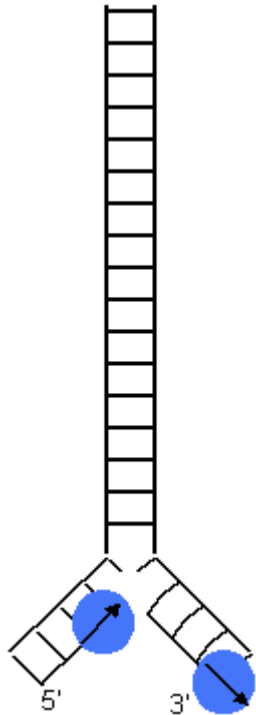
DNA replication involves:

Unwinding



The DNA must be unwound and bonds between the bases broken so that the two strands become separated.

Complimentary Base Pairing

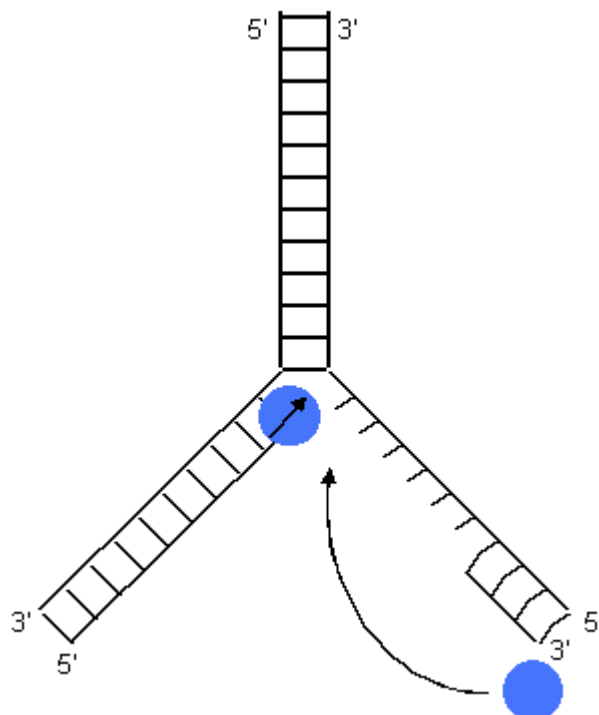


Each strand serves as a template for the synthesis of a new strand.

DNA polymerase adds nucleotides to match to the nucleotide present on the template strand. A is paired with T and G with C. Because each molecule of DNA contains one strand from the original strand, the replication process is *semiconservative replication*.

The nucleotides used for synthesis are ATP, GTP, CTP and TTP. Each of these DNA nucleotides has three phosphate groups. Two of the phosphates will be removed when the nucleotide is attached to the growing chain of new DNA.

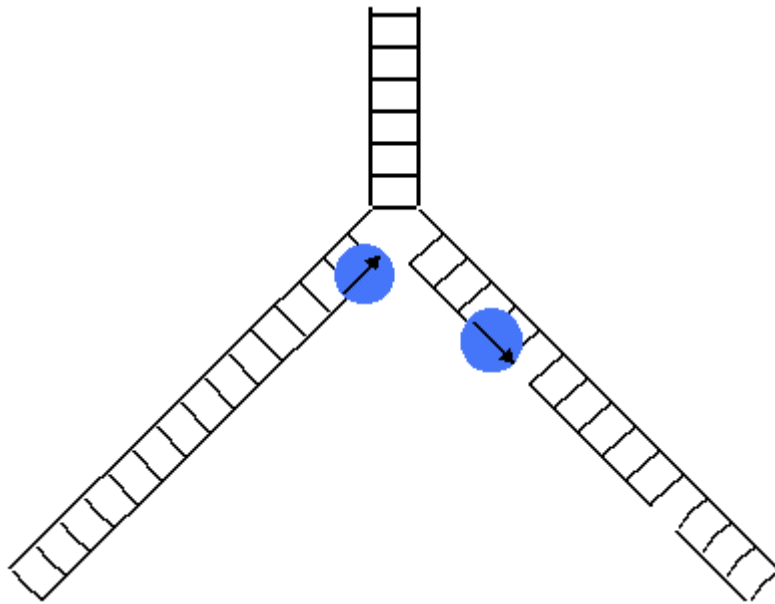
5' to 3'



The strand shown on the right side of the diagram must be synthesized in fragments because DNA polymerase always moves in the direction of 5' to 3'.

The area in a DNA molecule where unwinding is occurring is called a *replication fork*. In the diagram, it looks like an upside-down Y.

Okazaki Fragments

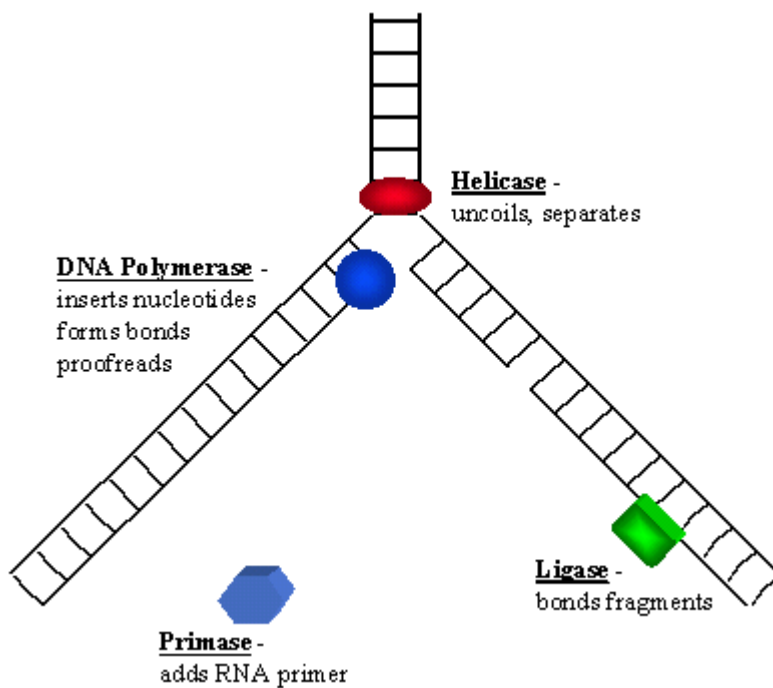


The resulting fragments are called *Okazaki fragments*.

Joining

Covalent bonds must be formed between the newly-added nucleotides.

Enzymes



DNA Helicase

DNA helicase unwinds the DNA molecule by breaking hydrogen bonds.

DNA Polymerase

DNA polymerase lengthens the strand that is being synthesized by adding nucleotides that are complimentary to those on the template strand (A paired with T and G paired with C).

It proofreads the new strand as it synthesizes it. Incorrectly paired bases are removed and the correct one is inserted ([discussed below](#)).

Primase

DNA polymerase cannot initiate a new strand, it can only elongate a strand that is already present. Synthesis of new DNA therefore cannot begin until a short strand of nucleotides is added. This short strand is called a primer. Primase creates an RNA primer. DNA polymerase can extend this strand by adding DNA nucleotides. The RNA primer will be removed and replaced by DNA.

DNA Ligase

DNA ligase catalyzes the formation of the covalent bonds between the Okazaki fragments.

Replication Forks

DNA synthesis occurs at numerous different locations on the same DNA molecule (hundreds in a human chromosome).

These form bubbles of replication with a ***replication fork*** at the growing edge.

The replication rate of eukaryotic DNA is 500 to 5000 base pairs per minute.

A human cell typically requires a few hours to duplicate the 6 billion base pairs.

Repair of damaged DNA

Changes in the DNA code are called ***mutations***. Repair enzymes repair most of the errors that occur in DNA. There are three different classes of repair mechanisms.

1. Proofreading corrects errors made during the DNA replication process.
2. Mismatch repair corrects base pair mismatching (A-T and G-C).
3. Excision repair removes and replaces small segments of damaged DNA.

Organization of DNA

Chromosome Structure

Chromosomes are structures composed of condensed DNA and associated proteins. When DNA condenses, the molecule becomes wrapped around proteins called *histones*. The histones are then arranged in a coiled pattern to produce a larger fiber. This larger fiber is further compacted by looping to produce looped domains. The looped domains are coiled and compacted to produce chromosomes.