

The Genetic Material

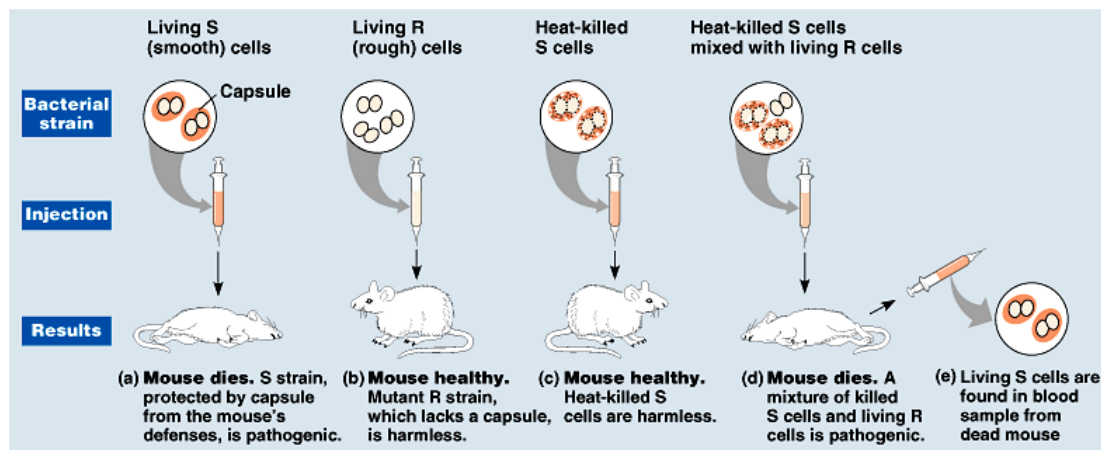
A. Genetic material must be:

1. able to store information used to control both the development and the metabolic activities of cells;
2. stable so it can be replicated accurately during cell division and be transmitted for generations; and
3. able to undergo mutations providing genetic variability required for evolution.

B. Previous Knowledge About DNA

1. Knowing the chemistry of DNA was essential to discovery that DNA is genetic material.
2. 1869, Swiss chemist **Friedreich Miescher** removed nuclei from pus cells and isolated DNA "nuclein"; it was rich in phosphorus and lacked sulfur.
3. Nuclein was analyzed by other scientists who found that it contained an acid: **nucleic acid**.
4. Two types of nucleic acids were discovered: **DNA (deoxyribonucleic acid)** and **RNA (ribonucleic acid)**.
5. Early in the twentieth century, discovery that nucleic acids contain four types of **nucleotides. (PA Levene)**
 - a. DNA was composed of repeating units, each of which always had just one of each of four different nucleotide (A, T, G, or C).
 - b. In this model, DNA could not vary between species and therefore could not be the genetic material; therefore some other protein component was expected to be the genetic material.

C. Transformation of Bacteria



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1. In 1931, bacteriologist **Frederick Griffith** experimented with *Streptococcus pneumoniae* (pneumococcus) that causes pneumonia in mammals.

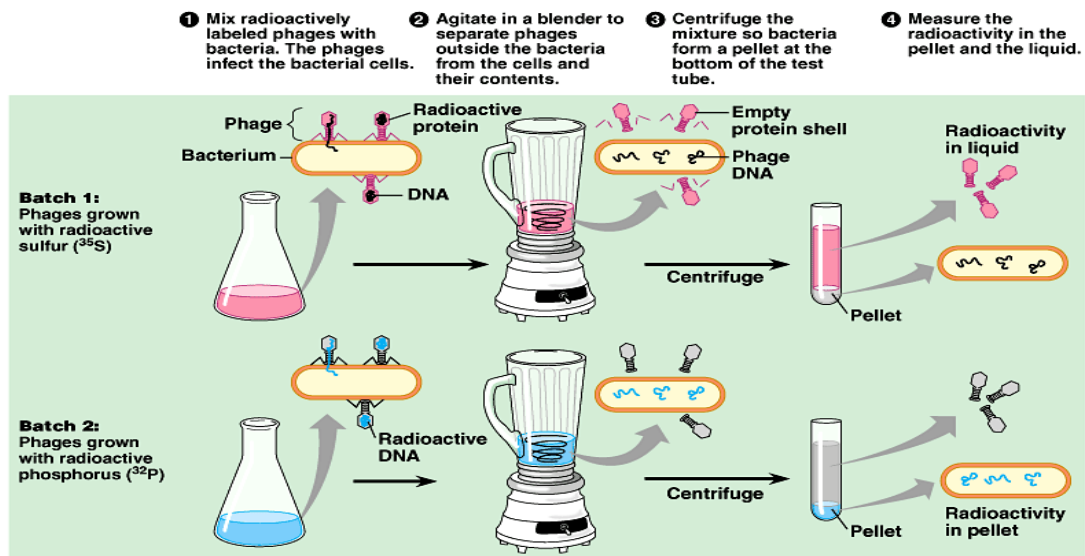
2. Griffith injected mice with two strains of pneumococcus: an encapsulated (S) strain and a non-encapsulated (R) strain.
 - a. The S strain is virulent (the mice died); it has a mucous capsule and forms shiny colonies.
 - b. The R strain is not virulent (the mice lived); it has no capsule and forms dull colonies.
3. In an effort to determine if the capsule alone was responsible for the virulence of the S strain, he injected the mice with heat-killed S strain bacteria; the mice lived.
4. Finally, he injected mice with a mixture of heat-killed S strain and live R strain bacteria.
 - a. The mice died and living S strain pneumococcus were recovered from their bodies.
 - b. Griffith concluded some substance necessary to synthesis of the capsule and, therefore, virulence must pass from dead S strain bacteria to living R strain bacteria so the R strain were *transformed*.
 - c. This change in phenotype of the R strain bacteria must be due to a change in their genotype, which suggested that the transforming substance may have passed from S strain to R strain.

D. DNA: The Transforming Substance

1. **Oswald Avery, Maclyn McCarty and Colin MacLeod** reported that the transforming substance was DNA. Avery was born in Halifax and MacLeod in Port Hastings.
2. Purified DNA is capable of bringing about the transformation; their evidence included the following:
 - a. DNA from S strain pneumococcus causes R strain bacteria to be transformed.
 - b. Enzymes that degrade proteins cannot prevent transformations, nor do enzymes that digest RNA.
 - c. Digestion of the transforming substance with enzyme that digests DNA prevents transformation.
 - d. Molecular weight of the transforming substance is great enough for some genetic variability.
3. Their experimental results demonstrated DNA is genetic material and DNA controls biosynthetic properties of a cell.
4. Modern experiments with bacteria show some can take up DNA to gain penicillin resistance.

E. Reproduction of Viruses

1. Bacteriophage is a virus that infects bacteria; consists only of a protein coat surrounding a nucleic acid core.
2. Bacteriophage T2 is a virus that infects the bacterium *Escherichia coli* (*E. coli*), a species of intensely studied bacteria that normally lives within the human gut.
3. In 1952, **Hershey and Chase** used bacteriophage T2 in their experiments.
 - a. The purpose of their experiments was to see which of the bacteriophage components -- the protein coat or the DNA -- entered bacterial cells and directed reproduction of the virus.
 - b. In two separate experiments, they labeled the protein coat with ^{35}S and the DNA with ^{32}P .
 - c. Viral coats are sheared away from bacterial cells; they are separated by centrifugation.
 - d. Results: radioactive ^{32}P alone is taken up by bacterial host and incorporated in virus reproduction. This result reinforced the notion that DNA is the genetic material.



(b) The experiment showed that T2 proteins remain outside the host cell during infection, while T2 DNA enters the cell.

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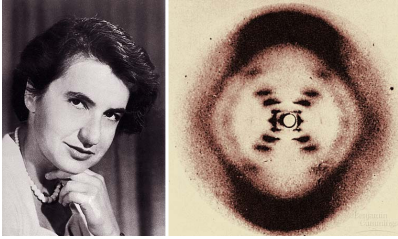
The Structure of DNA

A. Nucleotide Data

- In 1940's, Erwin Chargaff analyzed base content of DNA using new chemical techniques.
- It was known DNA contained four different nucleotide:
 - two with *purine* bases, **adenine (A)** and **guanine (G)**; a **purine** is a type of nitrogen-containing base having a double-ring structure.
 - two with *pyrimidine* bases, **thymine (T)** and **cytosine (C)**; a **pyrimidine** is a type of nitrogen-containing base having a single-ring structure.
- The results of his analysis proved DNA does have the variability necessary to code genetic material.
- Chargaff discovered that for a species, DNA has the *constancy* required of genetic material.
- This constancy is given in Chargaff's rules:
 - The amount of A, T, G, and C in DNA varies from species to species.**
 - In each species, the amount of A=T and the amount of G=C.**
- The tetranucleotide hypothesis proposing DNA has repeating units of one of these four bases was disapproved.

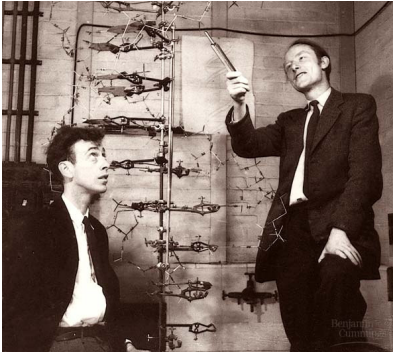
B. Variation in Base Sequence

- The variability is staggering; a human chromosome contains about 140 million base pairs.
- Since any of the four possible nucleotide can be present at each nucleotide position, the total number of possible nucleotide sequence is $4^{140 \times 10^6} = 4^{140,000,000}$.



C. Diffraction Data

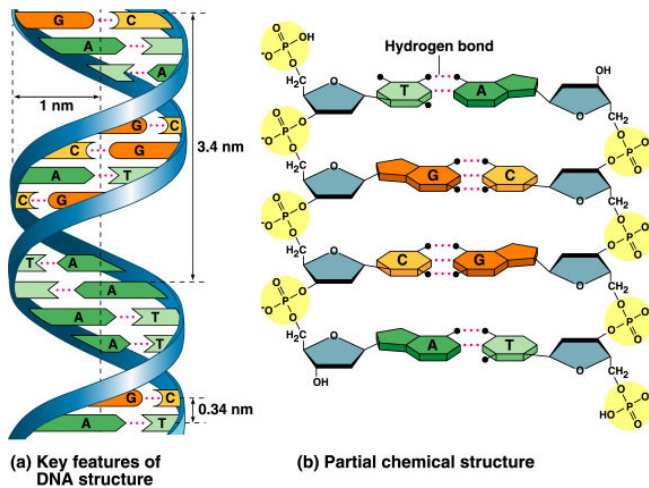
1. Rosalind Franklin, a student at King's College, produced X-ray diffraction photographs.
2. Franklin's work provided evidence that DNA has the following features:
 - a. DNA is a helix.
 - b. One part of the helix is repeated.



D. The Watson and Crick Model

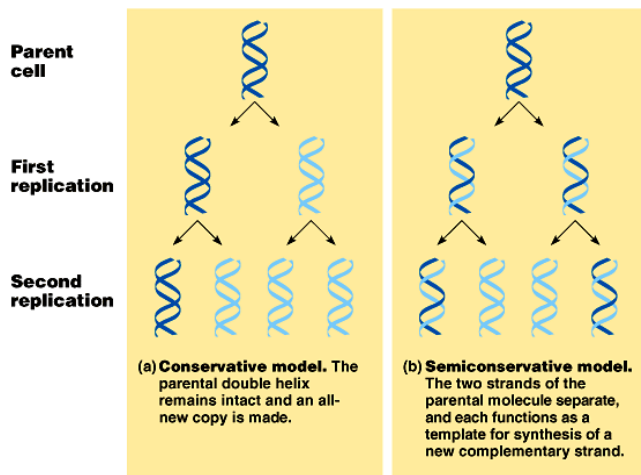
1. American James Watson joined with Francis H.C. Crick in England to work on structure of DNA.
2. Watson and Crick received the Nobel Prize in 1962 for their model of DNA.
3. Using information generated by Chargaff and Franklin, Watson and Crick built a model of DNA as double helix; sugar-phosphate molecules on outside, paired bases on inside.
4. Their model was consistent with both Chargaff's rules and dimensions of DNA polymer provided by Franklin's photograph of X-ray diffraction of DNA.
5. **Complementary base pairing** is the paired relationship between purines and pyrimidines in DNA, such that A is hydrogen-bonded to T and G is hydrogen-bonded to C.

Replication of DNA



A. DNA replication is the process of copying a DNA molecule.

1. **Unwinding:** old strands of the parent DNA molecule are unwound as weak hydrogen bonds between the paired bases are unzipped and broken by the enzyme **helicase**.
2. **Complementary base pairing:** free nucleotide present in nucleus bind with complementary bases on unzipped portions of the two strands of DNA; process is catalyzed by **DNA polymerase**.
3. **Joining:** complimentary nucleotide bond to each other to form new strands; each daughter DNA molecule contains and old strand and a new strand; process is also catalyzed by DNA polymerase.
4. DNA replication must occur before a cell can divide; in cancer, drugs with molecules similar to the four nucleotide are used to stop replication.
5. Advanced class students should refer to their text for a deeper look into this topic. **Key words:** primers, anti-parallel, Okazaki fragments, 5'-3' alignment, lagging and continuous strands. Use **Thinkwell Replication Animation** In Molecular Genetics Unit on my website to learn the advanced details of DNA Replication.



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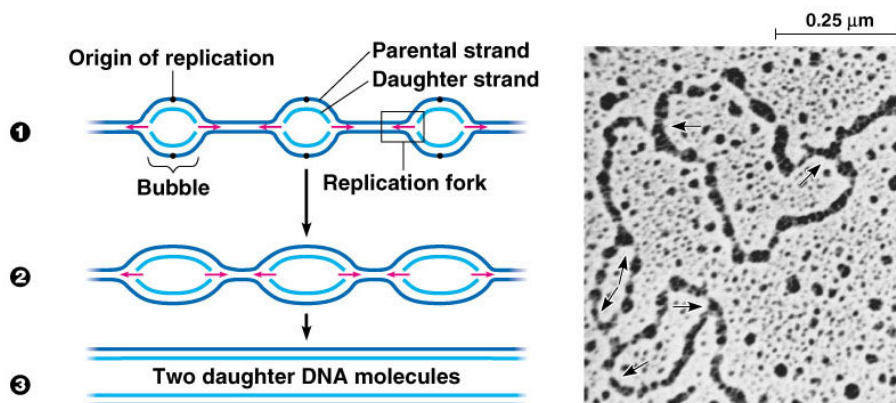
B. Replication is Semiconservative

1. DNA replication is semiconservative because each daughter double helix has one parental strand and one new strand.
2. 1958, Matthew Meselson and Franklin Stahl confirmed a model of DNA replication.
 - a. They grew bacteria in medium with heavy nitrogen (^{15}N), then switched to light nitrogen (^{14}N).

- b. Density of DNA following replication is intermediate as measure by centrifugation of molecules.
 - c. After one division, only hybrid DNA molecules were in the cells.
 - d. After two divisions, half the DNA molecules were light and half were hybrid.
3. These were exactly the results to be expected if DNA replication is semiconservative.

C. Prokaryotic Versus Eukaryotic Replication

1. Prokaryotic Replication
 - a. Bacteria have a single loop of DNA that must replicate before the cell divides.
 - b. Replication in prokaryotes may be bidirectional from one point of origin or in only one direction.
 - c. Replication only proceeds in one direction, from 5' to 3'.
 - d. Bacterial cells are able to replicate their DNA at a rate of about 10^6 base pairs per minute.
 - e. Bacterial cells can complete DNA replication in 40 minutes; eukaryotes take hours.



(a) In eukaryotes, DNA replication begins at many sites along the giant DNA molecule of each chromosome.

(b) In this micrograph, three replication bubbles are visible along the DNA of cultured Chinese hamster cells. The arrows indicate the direction of DNA replication at the two ends of each bubble (TEM).

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2. Eukaryotic Replication

- Replication in eukaryotes starts at many points of origin and spreads with many replication bubbles -- places where the DNA strands are separating and replication is occurring.
- Replication forks** are the V-shaped ends of the replication bubbles; the sites of DNA replication.
- Eukaryotes replicate their DNA at a slower 500 - 5,000 base pairs per minute.
- Eukaryotes take hours to complete DNA replication.

D. Replication Errors

- A genetic mutation is a permanent change in the sequence of bases.
- Base changes during replication are one way mutations occur.
- A mismatched nucleotide may occur one per 100,000 base pairs, causing a pause in replication.
- DNA repair enzymes perform a proofreading function and reduce the error rate to one per billion.
- Incorrect base pairs that survive the proofreading process contribute to gene mutations.

Images: Campbell, Neil and Reece, Jane. *Biology* (6th ed.) San Francisco: Benjamin Cummings

Modified Notes: Mader, Sylvia. *Biology* (7th ed) New York: McGraw Hill Publishing