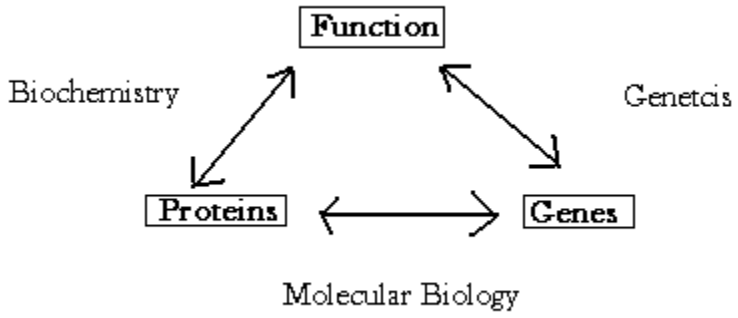
**Molecular Biology I**




Biochemistry – studying a single component in an organism
Genetics – studying an organism without that component

Biochemical Genetics

Look at the Arginine biosynthetic pathway:

A B C
→ → → Arginine

Intermediates: , , Enzymes: A,B,C

The pathway involves the conversion of via two intermediates, and to arginine. The three steps in the conversion of to arginine are catalyzed by the three enzymes, A, B and C (genes A, B and C)

Can have defects in any of the steps in the synthesis of arginine. With arginine in the medium, all arg mutants can grow on minimal medium.

Given a set of arg mutants, you can determine which mutants have mutations in which genes (steps) in the pathway by testing the growth of mutants and looking at accumulations of intermediates in the pathway.

Pathway and steps that are defective in each mutant:

A B C
→ → → Arginine

| Mutant | | | | |
|---|---|---|---|---|
| Arg1 | X | | | Arg1 has a mutation in gene A |
| Arg3 | | X | | Arg3 has a mutation in gene B |
| Arg5 | | | X | Arg5 has a mutation in geneC |
| Arg1/Arg3 | X | X | | |

(double mutant: blocked in two steps)

**Tests of Epistasis**

Epistasis is when the phenotype associated with an allele masks the expression of the phenotype associated with another allele.

Using mutant strains, you can provide intermediates and test mutants for growth under certain conditions. Using this type of information, you can order the genes in a pathway (if the order was not known)

Growth of strains under the following conditions:

Minimal media with:

| | | | | arginine |
|---|---|---|---|---|
| wt | + | + | + | + |
| Arg1 | - | - | + | + |
| Arg3 | - | - | - | + |
| Arg5 | - | - | + | + |
| Arg1/Arg3 | | | | |

"+" = growth, "-" = no growth Arg1/Arg3 is haploid with two mutations

Interpertations:

For example, given minimal medium plus " ", mutant Arg1 cannot grow because it has a defect in the step that converts → (mutation in gene A)

The Arg1 mutant can only grow if or or arginine is added to the medium.
The Arg1 mutant will build up because it cannot convert it to

The double mutant has defects in two steps of the pathway. This mutant has mutations in both genes A and B. IT is blocked in the earlier step of the pathway.

The Ar1/Arg3 double mutant has the growth requirements as the mutant in the later of the two steps (e.g. in this case, the Arg3 mutant).

The Arg1/Arg3 double mutant accumulates (or builds up) the intermediate like the mutant in the earlier of the two steps (e.g. in this case, the Arg1 mutant.

This is an example of epistasis. The phenotype of the Arg3 mutant masks the phenotype of the Arg1 mutant when testing for growth on different intermediates.

Information from this type of assay (testing for growth) allows one to determine the order of the steps (i.e. genes) in a biosynthetic pathway

**Molecular Biology**

Several key experiments were done in the early to mid 1900's to deduce the nature of the genetic material.

## §1. Discovery of the "Transfering Principle"

1928: Experiments done by F. Griffith with bacteris.
Griffith was studying the pneunococcus bacteria – infect mice and kill them.

2 strains:

1) Smooth –infectious (virulent) strain; grows as smooth colonies in petri dish (**S strain**) (it is encapsulated by a polsaccharide coat that makes it resistant to the immune system of the mouse)
2) Rough – noninfectious(non-virulent) strain, grow as rough colonies in petri dish (**R strain**) ( it lacks the polysaccharide coat so it is vulnerable to the mouse's immune system.

Griffith studied the effect of the se strains of bacteris on mice.

He infected mice with the pneumococcus bacteria

Experiments:

1) Injected the S strain into mice → mice die
2) Injected the R strain into the mice → no harmful effect
3) Injected heat killed S strain into mice → no harmful effect
4) Injected mice with a mixture of live R strain and heat killed S strain → mice died

These results from experiment 4 were surprising because neither the R strain nor the heat killed S strain was virulent when give independently. Girffith isolated the blood from the dead mouse and cultured it. He found smooth bacteria present in the dead mouse's blood!

He isolated live S strain from the blood of these mice. The heat killed S strain must have transformed the live R strain into S strain!

There must have been some material in the heat killed S strain that was able to convert a non-virulent R strain into a virulent S strain

In 1944: O. Avery, C. Macleod and M. McCarty: set out to discover the nature of the transforming principle

- Repeated the transformati8on experiments in a test tube
- Found that they could convert the R strain into and S strain just by adding a cell-free extract of the S strain to the R strain

But what was in the cell-free extract that could convert the R strain → S strain?
Was it a protein?
Was it a nucleic acid?
Was it a lipid?

In order to deduce this, they took the cell-free extract and treated it with various enzymes to assay whether the extract was still effective in transforming R→ S

1) Treated the cell-free extract with proteases (cuts proteins) → it was still active
   so its not a protein
2) Treated the cell-free extract with ribonuclease (cuts RNA) → it was still active
   so its not RNA
3) Treated the cell-free extract with deoxyribonuclease (cuts DNA) → it was NOT active
   MUST BE DNA

> The nature if the material that transformed the R strain to the S strain was DNA (deoxyribonucleic acid)

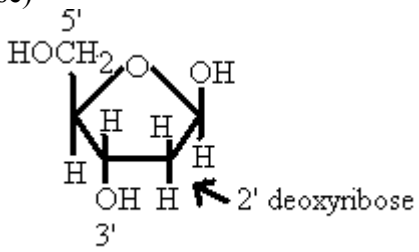
Despite these experiments, people were not convinced that DNA was the genetic material.

Why? Because DNA was considered to be an uninteresting molecule. Proteins were considered to be more interesting; many different types of proteins!

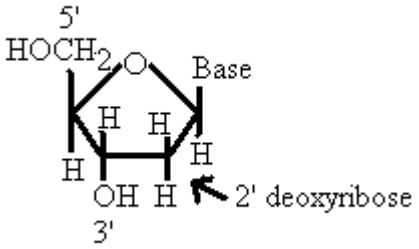
**§2. Structure of DNA**

DNA is made up of 3 components:

a) Sugar: (a pentose)



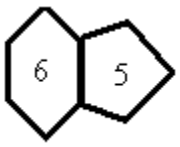

b) Base: 4 types in DNA




sugar + base = nucleoside

base is linked to C1' carbon of the sugar.

Bases in DNA: Purines: Adenine (A) and Guanine (G)



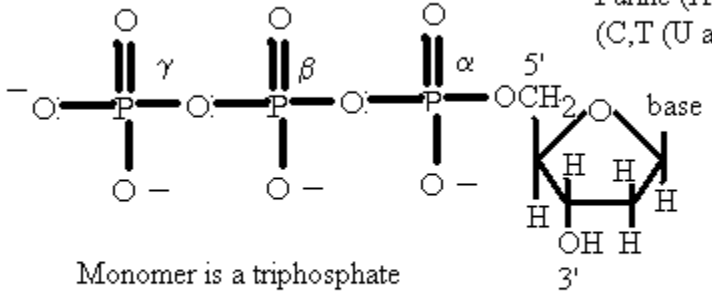

2 ring structure made up
of N, C, H and O atoms

<u>Pyrimidines:</u> Cytosine (C) and Thymine (T)


1 ring structure made up
of C, H, O and N atoms

c) <u>triphosphate</u>: 3 phosphate groups

sugar + base + phosphate group = <u>nucleotide</u>

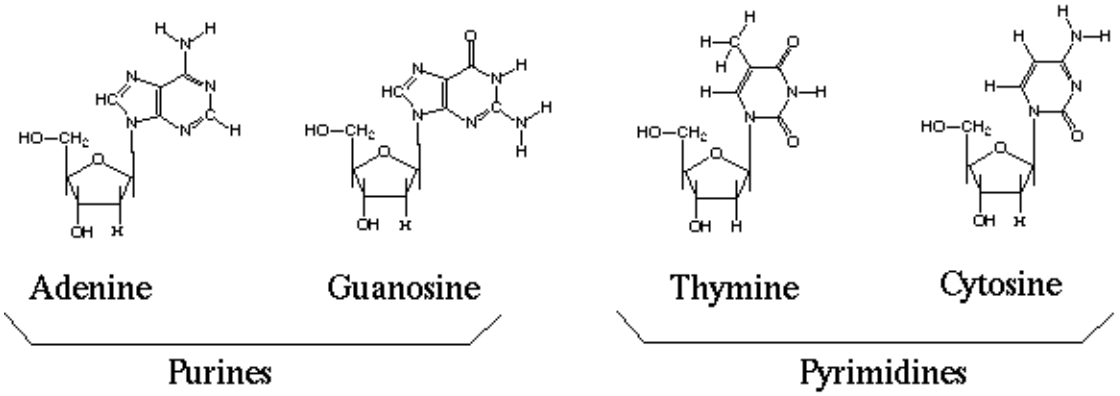



Purine (A,G) or Pyrimidine
(C,T (U and C in RNA)

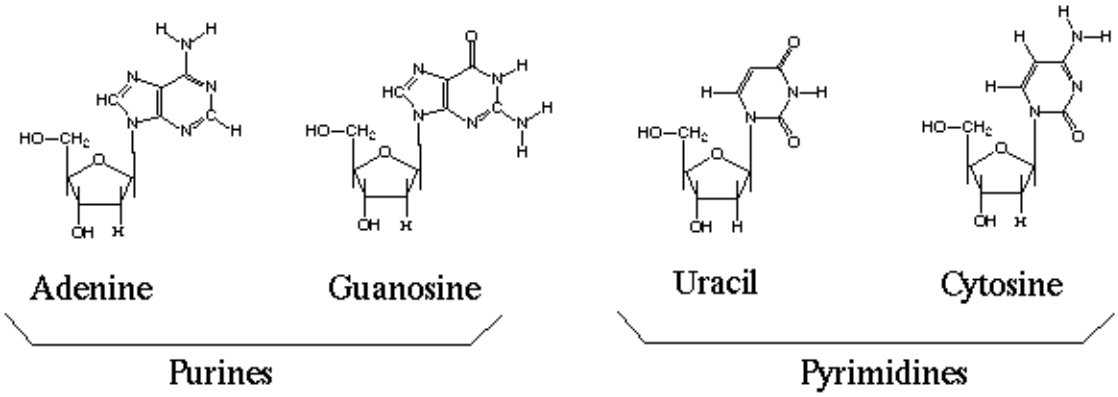
Monomer is a triphosphate

deoxyribose in DNA
ribose (has 2' OH) in RNA

## The Nucleosides of DNA



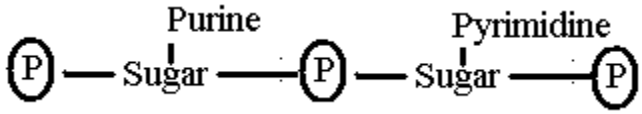

## The Nucleosides of RNA




Polymerization of Nucleotides:

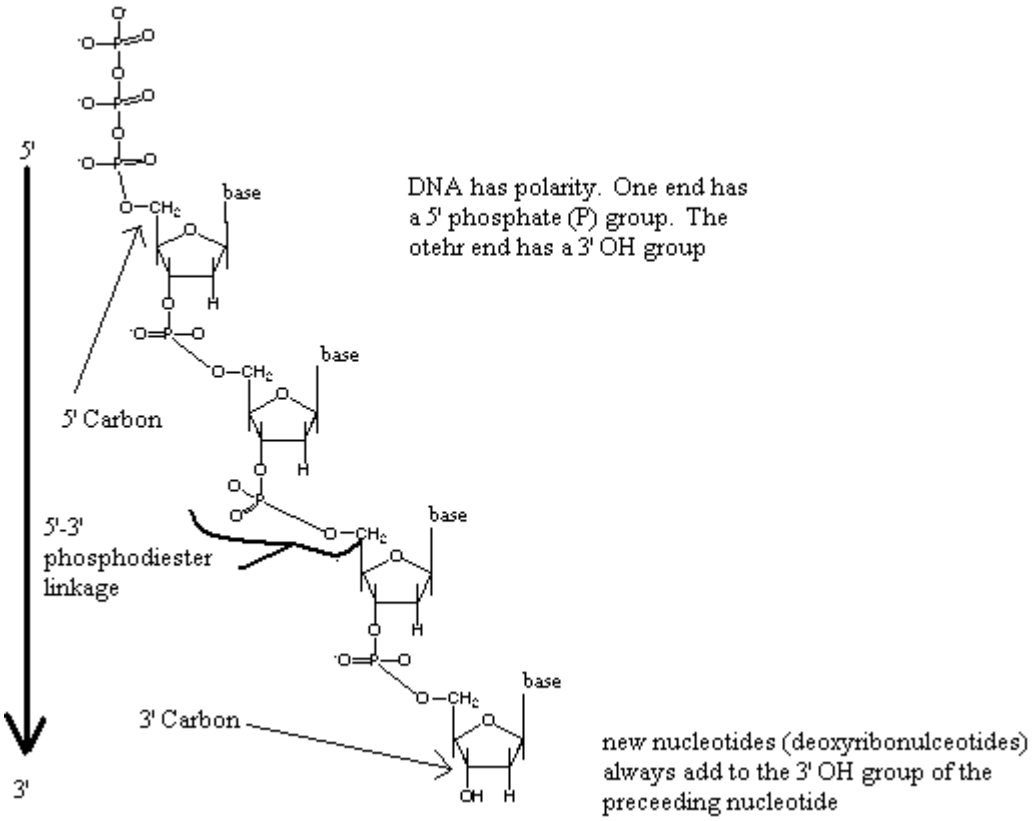
DNA is made up of monomers called deoxyribonucleotides (dNTPs)

The dNTPs are linked together through a sugar-phosphate linkage which makes up the backbone of DNA:




The sugar (deoxyribose) is covalently linked to a phosphate group via a phosphodiester linkage:

Sugar-phosphate backbone of DNA:




( from http://esg-www.mit.edu:8001/esgbio/lm/nucleicacids/dna.html )

DNA: Deoxyribonucleic Acid

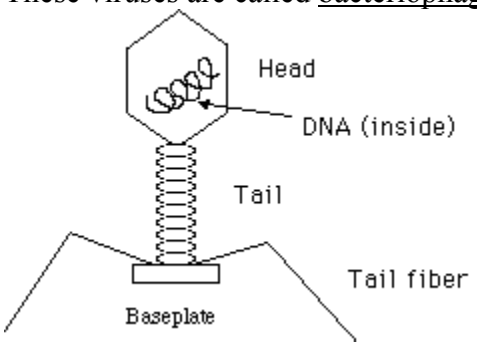
DNA is a nucleic acid because the pKa of the phosphate group is ~1.0

DNA is negatively charged at pH 7.0 due to the phosphate groups

## §3. Bacterial Viruses and the Hershey-Chase Experiment

1952: Further support for the genetic role of DNA come from studies done by A. Hershey and M. Chase with a virus that infects the E.coli bacteria.

These viruses are called bacteriophages (virus or pathogen that infects bacteria)




-The genetic material of a virus is stored inside a protein capsid.

-When the phage infects bacteria, it injects its viral DNA into the cell. The DNA is replicated; new progeny virions (virus particles) are made. The cell is lysed as the progeny of the virus are released, causing cell death.

How does the process of infection occur?

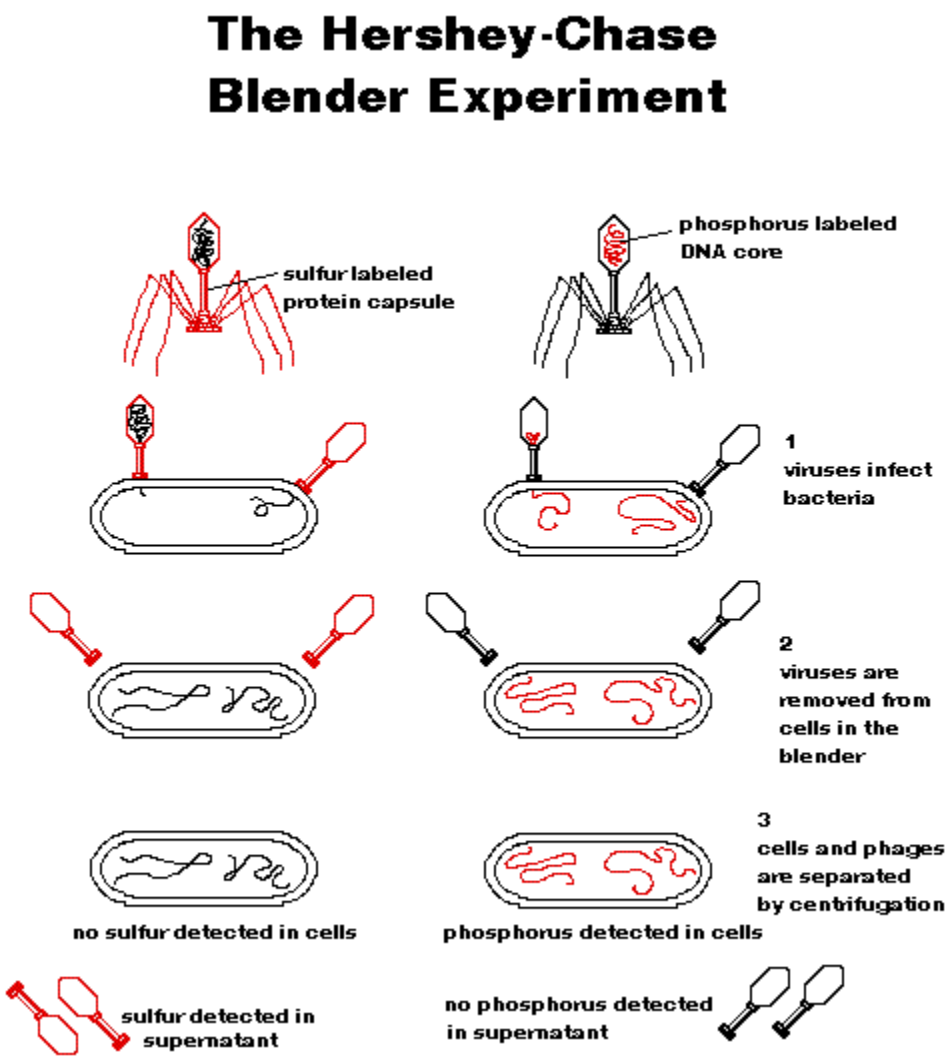
Does the bacteriophage inject its DNA, its protein capsid, or both?

To trace which material enters the cell, need to distinguish between DNA and Protein.

To trace where the protein and DNA go into the cell, they used radioisotopes

-used $^{32}P$ isotope to label viral DNA (labels only DNA – because phosphorus is not present in proteins)

-used $^{35}S$ isotope to label viral protein (labels only protein, because sulfur is not present in DNA)

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The virus particles, which are smaller then the bacteria remained in the supernatent – liquid part of the mixture in the test tube, after centrifugation. The bacteria made a pellet on the bottom of the test tube because they are heavier.

Result of Experiment:

1) Most of the $^{32}P$ label (phage DNA) was found inside the bacterial cells
2) Most of the $^{35}S$ label (phage protein) was found in the supernatent

Conclusion:

Since mainly DNA was found inside the cells, they concluded that DNA was the genetic material.

There was some $^{35}S$ label found associated with the bacterial cells – so some people believed that the genetic material laws the protein that was stuck to the cell

BUT, in general, most people believed the DNA was the genetic material

These experiments reinforced the notions proposed earlier by Avery, MacLeod and McCarty.

**§4. DNA Structure**

From studies done by E Chargaff (1950) and M. Wilkins (early 1950's) some information about the nature of DNA was determined.

From these results J. Watson and F. Crick deduced the structure of DNA in 1953

Model of the structure of DNA: 1953 J. Watson and F. Crick

1) DNA consists of 2 polynucleotides that are antiparallel, complementary and wrap around each other in a right handed double helix

2) The purine and pyrimidines bases are on the inside with sugar-phosphate backbone is on the outside of the helix

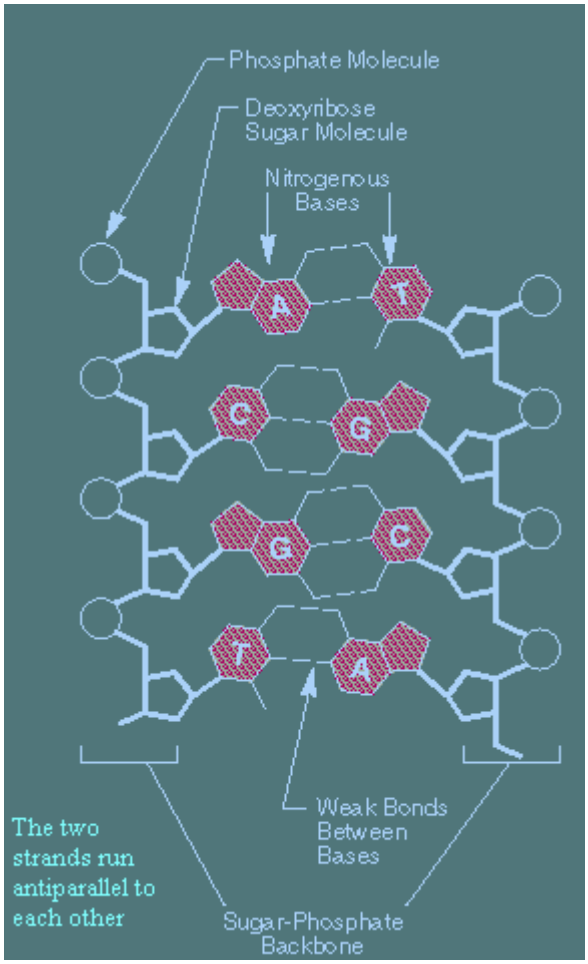
3) The two chains are held together by hydrogen bonds specifically formed between the bases:

A bonds with T 2 hydrogen bonds (A=T)
C bonds with 3 hydrogen bonds (C≡G)

4) There are 10 base pairs (bp) per every turn of the helix = 34 angstroms
   - Distance between the base pairs is 0.34 nm, ,width of the helix is 2nm (20 angstroms)

**The most important aspect of the Watson-Crick DNA model was the base-pairing found in DNA

Purines always were paired with pyrimidines, but only A could pair with T and only G can pair with C because of hydrogen bonding requirements.

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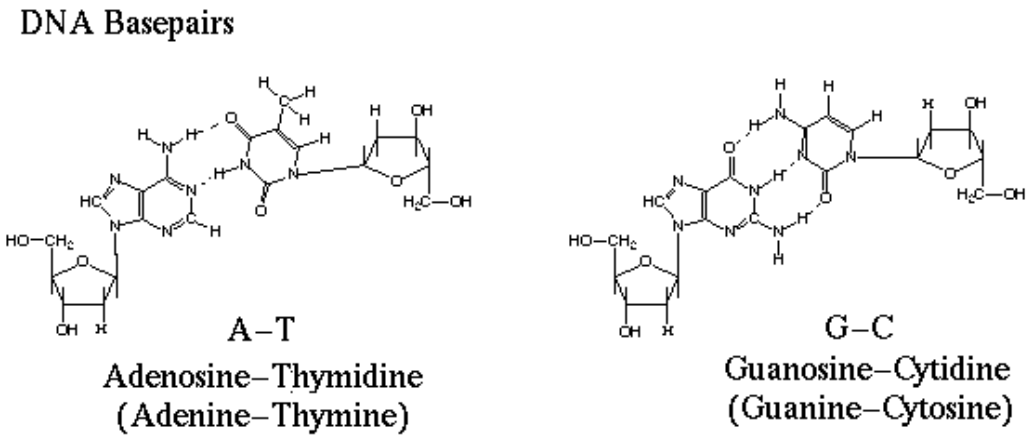
Base Pairs Found in DNA:




(from http://esg-www.mit.edu:8001/esgbio/lm/nucleicacids/nucleicacids.html)

-Earlier (in 1950, prior to Watson and Crick's findings), E Chargaff analyzed the purine and pyrimidines content of various DNAs from different sources: yeast, bacteria, humans etc.

-Chargaff found that the %of A was always equal to the %T and that the %G was always equal to %C.

Chargaff's rules

%A=%T

%G=%C

But Chargaff had no explanation for his findings. It was not until Watson and Crick proposed their model of the structure of DNA that Chargaff's rules "made sense"

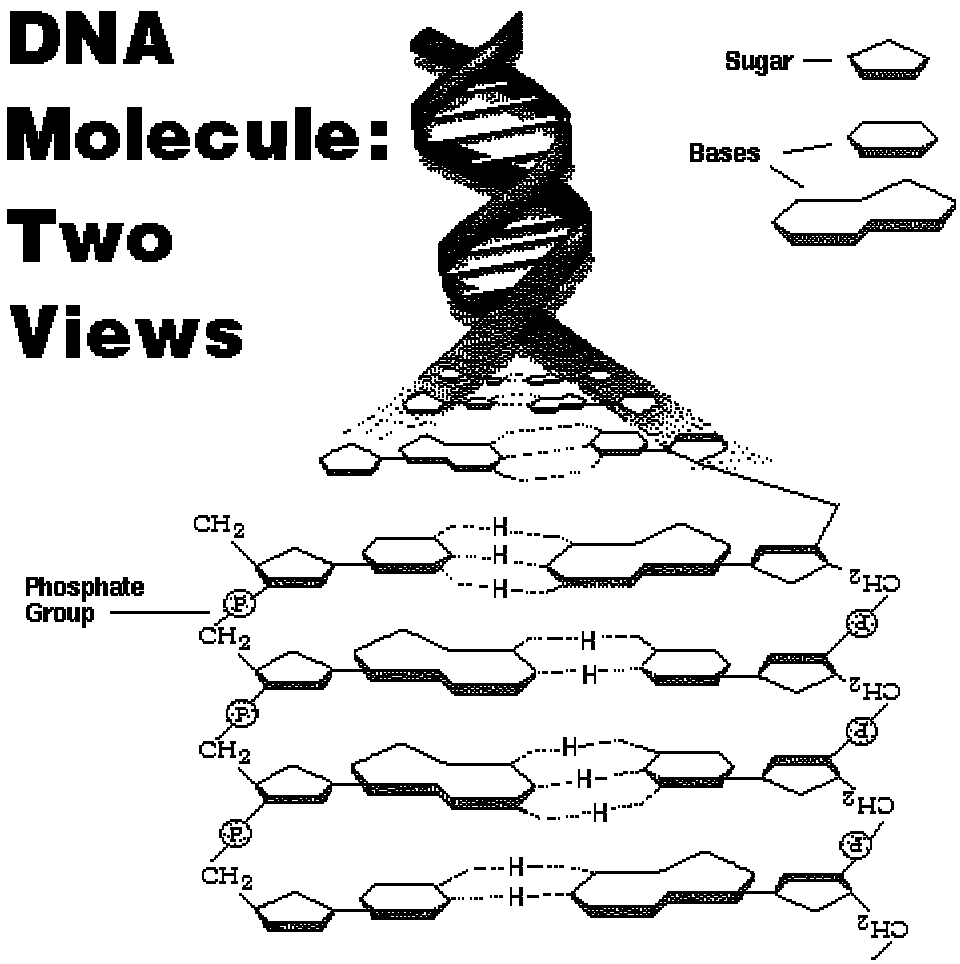
So, now that the model for DNA was deduced, what was so exciting about DNA?

What is the secret of life? How do you pass "heredity" to your offspring?

The structure of DNA enabled people to answer some of these questions.

Each strand of double stranded (ds) DNA helix is a complementary "copy" of the other strand.
Look at a small sequence of DNA:

```
5'-CGGTGA-3'
   | | | |
3'-GCCACT-5'
```

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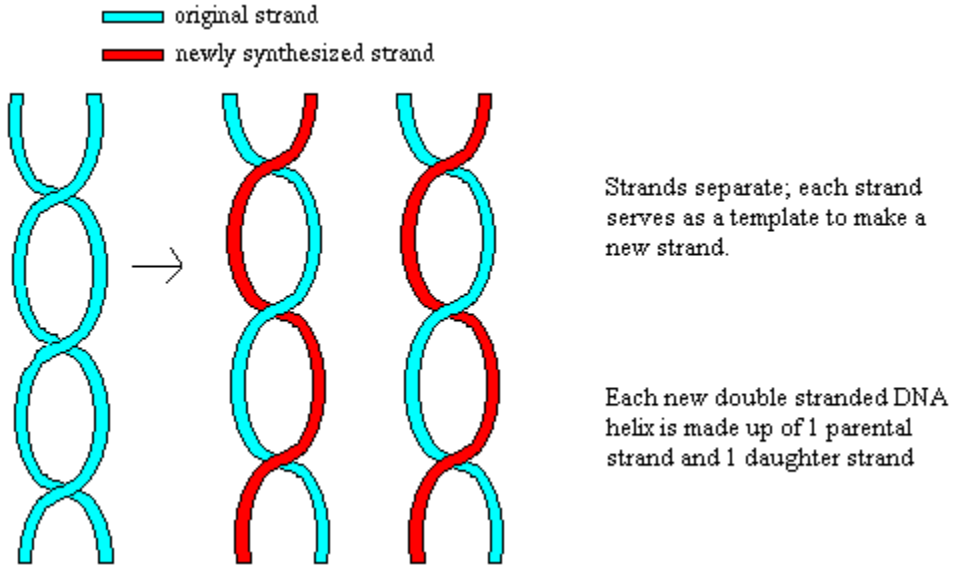
The double-helical model of DNA suggested a mechanism for DNA replication (copying of DNA)



The structure of DNA (two complementary strands of DNA) explains:

1)Process of DNA Replication

Each strand serves as a template for copying – satisfies model of heredity

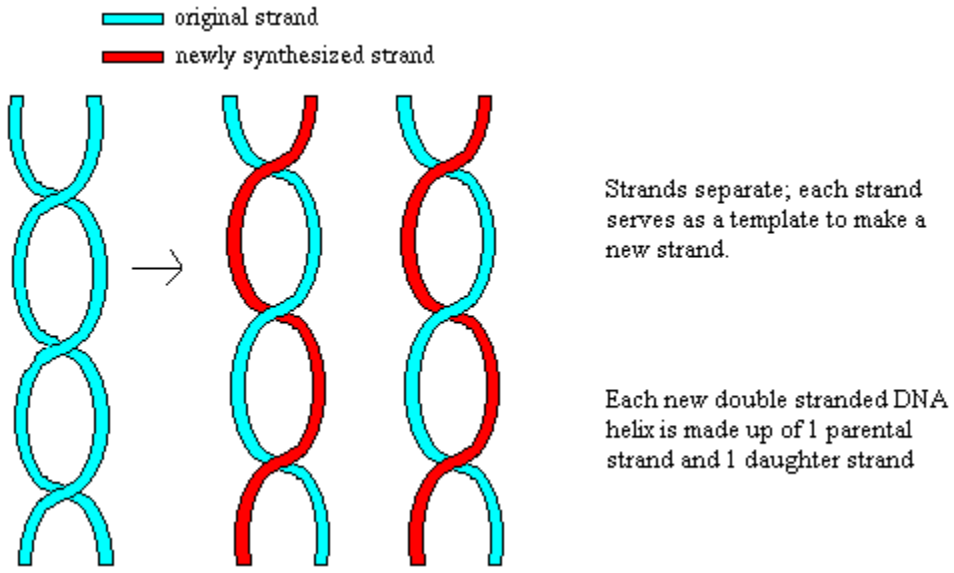
2) Occurrence of Mutations

Errors in DNA replication (insertion of a noncomplementary base during copying of the DNA) would lead to mutations (changes in the DNA sequence)

## §5. Proving the Crick- Watson Model

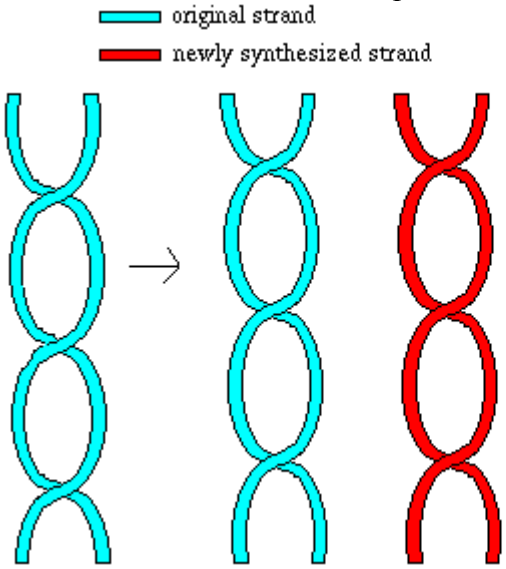
During DNA replication each strand acts as template for the synthesis of the other strand. Each new DNA molecule will consist of one parental (old) strand and one progeny (daughter, new) strand.

If each strand serves as a template for DNA replication, than DNA replication should occur in a semi-conservative mechanism i.e. each new DNA molecule is made up of one new strand and one old strand.




The semi-conservative mechanism of DNA replication was proposed by Watson and Crick.

Another model: Conservative Replication of DNA was suggested:




The original duplex somehow serves as a template for replication; however, after replication occurs, the original strands come back together.

1957 M. Meselson and F. Stahl wanted to confirm if DNA replication occurred by a semi-conservative manner.

They did this by using a heavy isotope ($^{15}N$) of $^{14}N$ (nitrogen)

Experiments:

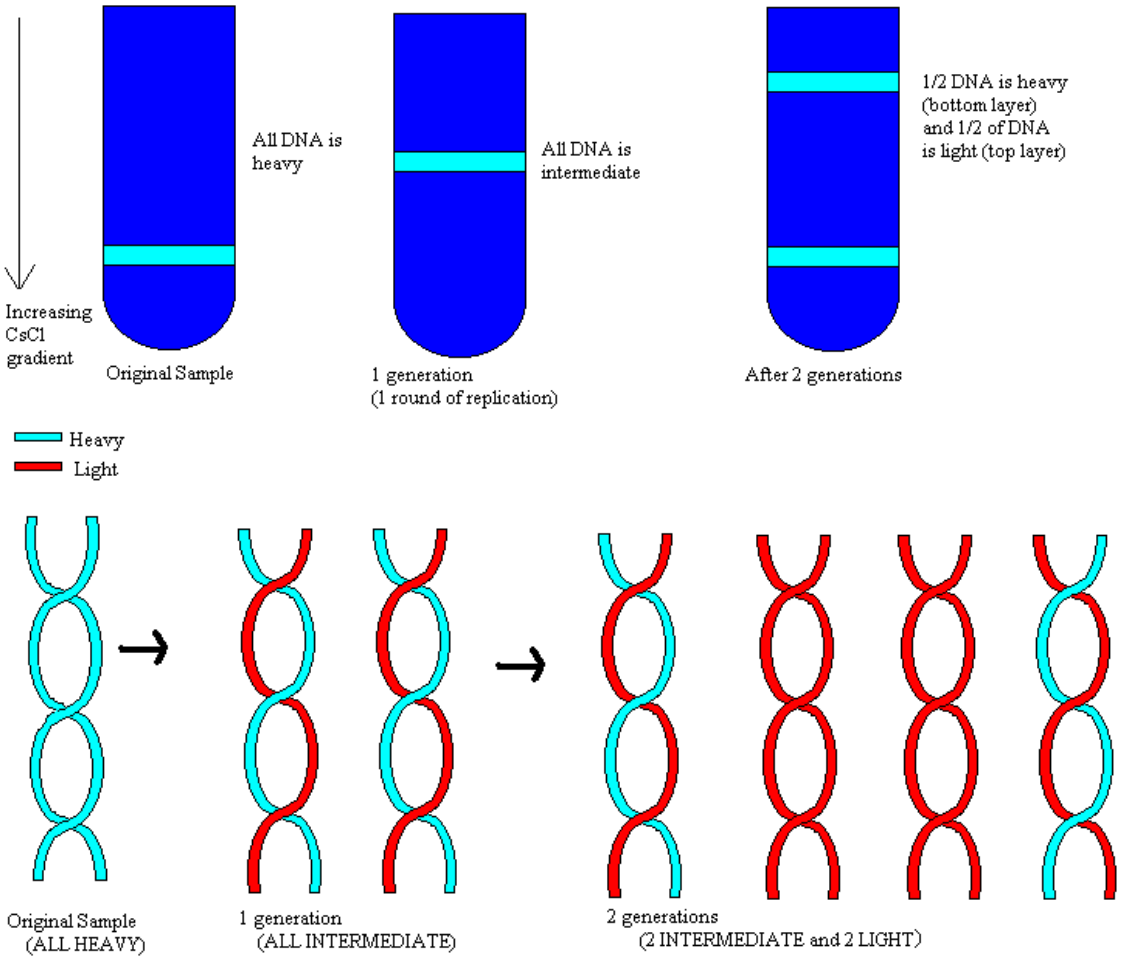
1) They grew E.Coli bacteria in a medium that contained $^{15}NH_4Cl$ as the sole nitrogen source
- The $^{15}N$ is incorporated into the pyrimidine and purine bases in DNA, therefore the DNA will be heavier then normal.
- They grew bacteria for many generations in $^{15}N$ so that the DNA strands would contain $^{15}N$. They isolated some of this heavy DNA.

2) They took the bacteria that was grown in $^{15}N$ and transferred it to a medium containing $^{14}NH_4Cl$. DNA was then isolated at various times after bacteria was in $^{14}N$ medium. They grew bacteria for two more generations and isolated DNA.
3) They used the technique of density gradient centrifugation (also called isopyenic centrifugation) which allows DNA molecules to be separated from each other on the basis of their density.

Density Gradient Centrifugation:

The DNA is mixed with a Cesium chloride (CsCl) solution. The CsCl-DNA mixture is centrifuged at a high G force in a centrifuge tube. The DNA will "band" in the CsCl gradient (formed by the centrifugation) at its corresponding density.

A small amount of the original $^{15}N/^{15}N$ DNA and the different DNA samples taken after 1 generation and 2 generation was centrifuged in the CsCl gradient. After centrifugation of the different samples:




Why does DNA form a "band" at a particular position in the CsCl gradient?

The DNA "settles" into a layer at a position in the tube where the density of the CsCl solution equals that of the DNA.

Meselson and Stahl's experiments proved that DNA was replicated in a semi-conservative method.

**The absence of a band at the heavy position in the gradient after 1 generation contradicted the conservative method, therefore replication was semi-conservative.

** Crick and Watson Model was correct – DNA replication by a semi-conservative method: 1 strand of each ds DNA molecule is conserved in the new daughter ds DNA molecule.