Solving the structure of DNA

As long as the structure of DNA was unknown there was no meaningful way to think about the nature of the genetic code or how the genome is replicated. During the 1950's a considerable amount of evidence concerning the structure of DNA had accumulated an intense effort was underway to solve the structure of DNA. This was considered to be one of the paramount problems in biology.

Phosphodiester bonds

Biochemists had shown that DNA was composed of nucleotides and that each nucleotide was joined to the next by a phosphodiester bond.

Density

The density of the molecule much too high for DNA to be a single stranded molecule. The density of DNA could be achieved, however, by a double stranded molecule.

X-ray diffraction

Rosalind Franklin and Maurice Wilkins performed X ray diffraction experiments on pure DNA fibers. In these experiments, X rays are fired at the DNA and the molecule scatters them. The scattered X rays then strike a piece of film and expose it, producing black spots where they hit. The angle of scatter represented by each spot provides information on the position of atoms in the DNA.

Their data indicated that DNA:

1. formed a regular helix
2. that the helix made one turn every 34Å (3.4 nm)
3. that the diameter of the helix was about 20 Å (2 nm)
4. that there was one nucleotide every 3.4 Å (0.34 nm) and therefore there must be 10 nucleotides/turn of the helix

AT/GC ratio

In 1951, Chargaff analyzed the ratios of the different nitrogenous bases to one another in a large number of different organisms. What he saw was that the amount of A essentially equal to amount of T and that the amount of G was equal to the amount of C. That is [A] = [T] & [G] = [C]. Furthermore, [A & T] does not necessarily equal [G & C].

Here is a sample of Chargaff's data.

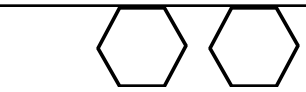
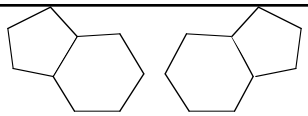
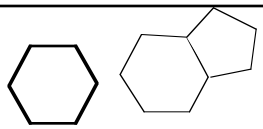
Organism	Tissue	Adenine	Thymine	Guanine	Cytosine
Escherichia coli		26.0	23.9	24.9	25.2
Diplococcus pneumoniae		29.8	31.6	20.5	18.0
Mycobacterium tuberculosis		15.1	14.6	34.9	35.4
Yeast		31.3	32.9	18.7	17.1
Sea Urchin	sperm	32.8	32.1	17.7	18.4
Herring	sperm	27.8	27.5	22.2	22.6
Rat	bone marrow	28.6	28.4	21.4	21.5
Human	thymus	30.9	29.4	19.9	19.8
Human	liver	30.3	30.3	19.5	19.9
Human	sperm	30.7	31.2	19.3	18.8

Chargaff saw that DNA from different animals or plants had different AT/GC ratios but the [A]=[T] & [G]=[C] rule still held. Furthermore, in a single organism it didn't matter which tissue you examined. All of the tissues, had the same AT/GC ratio.

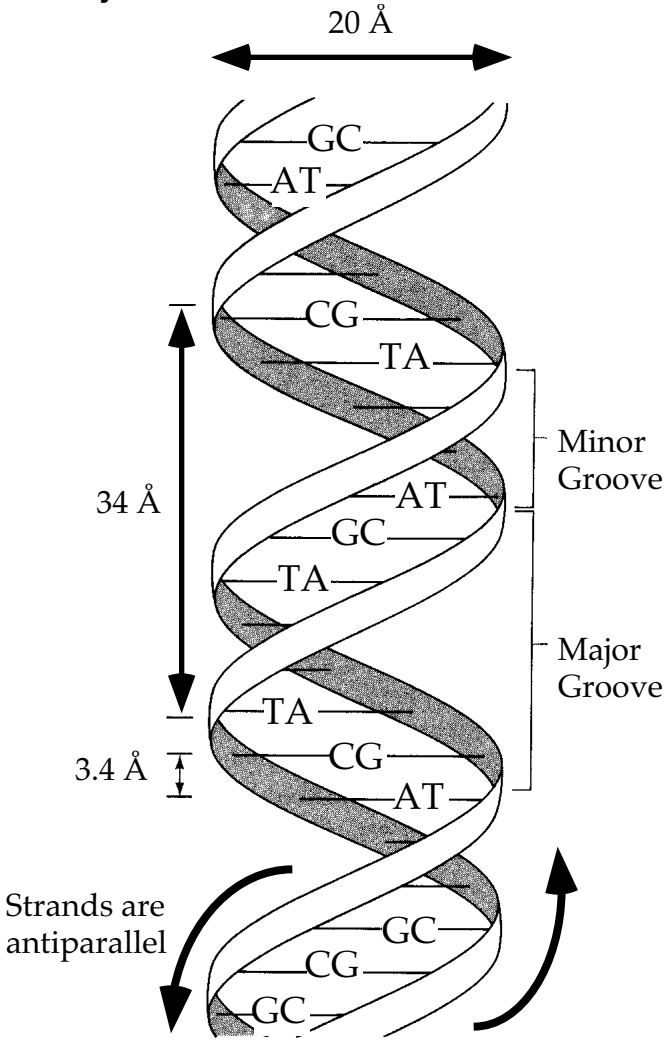
Watson & Crick's Model

All of the pieces of the puzzle were in place and many were racing to solve DNA's structure. The structure was solved in 1953 by James Watson and Francis Crick. They assembled all of the data given above and built stick figure molecules until they found one that fit all of the data.

1) Based on the buoyant density Watson & Crick knew that DNA must be a double helix.
2) Thermodynamically, they postulated that the most stable double helix would be one in which the sugar phosphate backbone interacted with water and the more hydrophobic nitrogenous bases were inside the helix hidden from the water.
3) Chargaff's data suggested that the different bases interacted with one another and that somehow the amount of A determined the amount of T (& vice versa) and the amount of G determined the amount of C (& vice versa). They postulated that the A on one strand H-bonded with T on the other and that the G on one strand H-bonded with C on the other. This pairing would stabilize the helix.
4) From Franklin and Wilkin's data they knew that there was one nucleotide every 3.4 Å (0.34 nm), that there were 10 nucleotides/turn of the helix and that the helix was 20 Å wide.
5) In support of 3) Watson & Crick's modeling showed that only pyrimidine:purine base pairs could produce a helix of 20 Å. See below

Pyrimidime:Pyrimidine base pairing would be <20Å.	 pyrimidine pyrimidine
Purine:Purine base pairing would produce a helix that was > 20 Å.	 purine purine
But Pyrimidine:Purine base pairing would produce a double helix of about 20 Å.	 pyrimidine purine

The only model that fit all of the data is the one with which you are already familiar. I present to you the Watson & Crick Double Helical Model of DNA.



They did this without performing any experiments. Theirs was a conceptual contribution that is perhaps the most important scientific breakthrough of this century. Although, the model has been refined it is still basically correct. *The structure suggested a theory that base-paired was used to both replicate and decode information.*

DNA Replication

Every time a cell divides the genome must be duplicated and passed on to the offspring. That is:

```
                                    5'--- TGCT ----3'
                                    3'--- ACGA ----5'
5'--- TGCT ----3'          REPLICATION
3'--- ACGA ----5'
                                    5'--- TGCT ----3'
                                    3'--- ACGA ----5'
```

Original molecule yields 2 molecules following DNA replication.

Our topic in this section is how is this done?

DNA replication must have high fidelity. Why? Well, if DNA replication was low fidelity the consequences would be:

1. dramatic and rapid random changes in the sequence of genes
2. which would cause an extreme reduction in viability

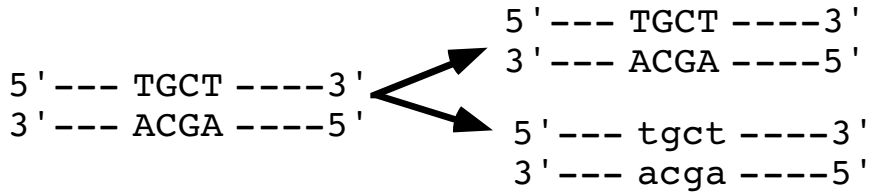
Because of this, in a complex organism, evolution will select against low fidelity DNA replication. The structure of DNA suggested a way that it could be replicated with high fidelity. Because the strands are complementary, one strand could specify the base on the opposite strand. This is actually what happens.

During the 1950's, three theories were proposed for how DNA might be replicated. They went by the names **Conservative**, **Dispersive** and **Semi-conservative** DNA replication. They are illustrated below.

Lowercase letters represent newly synthesized DNA and capital letters represent material from the original parental molecule.

Conservative Replication

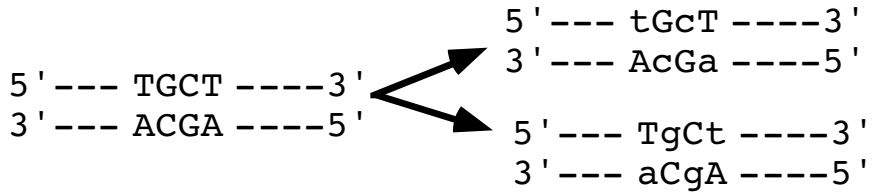
Following replication, one daughter molecule contains both of the parental strands. The other daughter molecule contains two newly synthesized DNA strands.



This theory was proven to be incorrect.

Dispersive Replication

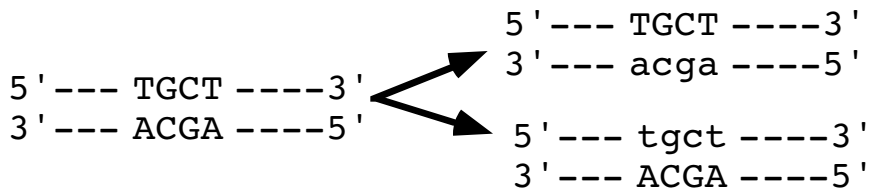
Both strands of each daughter molecule contain nucleotides derived from the parental molecule.



This theory was proven to be incorrect.

Semi-Conservative Replication

Each parental strand acts as a template for the synthesis of one new daughter strand. Therefore, in daughter molecules a newly synthesized strand is base paired to one of the original parental strands.



The semiconservative theory is correct !

How was it shown that DNA replication is Semi-Conservative?

In 1958, the *Meselson-Stahl experiment* proved that in bacteria DNA replication was semi-conservative. In the early 1960's the *Herbert Taylor Colchicine bean rootip experiment* demonstrated that a eukaryotic cell replicated by semiconservative replication.

Meselson and Stahl Experiment

To address this question this group used a way to isotopically label newly synthesized DNA. To do this they used two different isotopes of nitrogen (N).

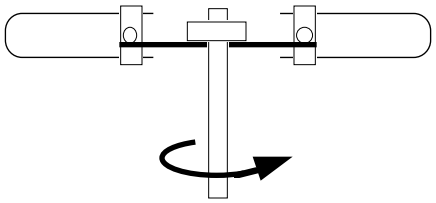
^{14}N most common form of nitrogen

^{15}N less common form and has greater mass than ^{14}N

DNA made using ^{15}N is about 1% denser than DNA made using ^{14}N. These two forms can be separated by equilibrium density gradient centrifugation (also called isopycnic centrifugation).

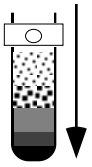
What is equilibrium density gradient centrifugation?

In this technique molecules mixed with a salt ($CsCl_2$), dissolved in water, and centrifuged at very high speed. The salt molecules form a density gradient.

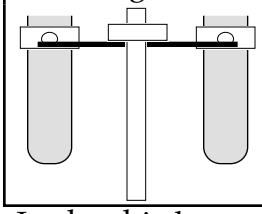
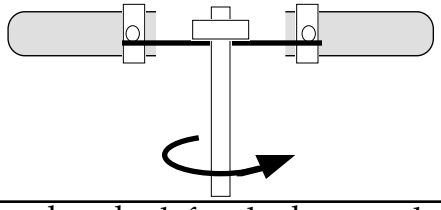
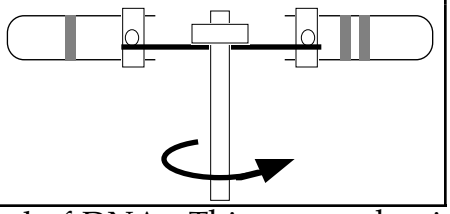


Centrifuge tubes spinning in a centrifuge

Prior to centrifugation the salt molecules are evenly distributed throughout the centrifuge tube. During centrifugation, the salt molecules are forced towards the bottom of the tube and a gradient of molecules is established. More of the salt is found at the bottom of the tube and less is at the top. Therefore, the density of the solution is smaller at the top and greater at the bottom.



Any DNA molecules in this centrifuge tube will also be forced towards the bottom of the tube. As the DNA travels down the tube the density of the surrounding salt solution gradually increases. The DNA stops moving relative to the salt solution when their two densities are equal. This is because of a physical property called buoyancy.
An example:

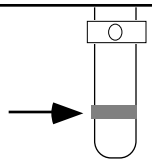
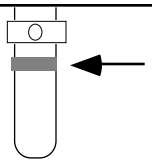
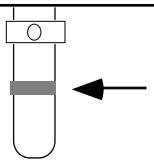
Prior to centrifugation, the DNA is evenly distributed throughout the centrifuge tubes.	Centrifugation begins and the salt and DNA begin sedimenting.	After prolonged centrifugation, the DNA has formed discrete bands reflecting its buoyant density.
		

In the third panel, notice that the left tube has one band of DNA. This means that in this tube all of the DNA molecules have the same buoyant density. Also notice that the right tube has two bands. This means that the DNA in this tube is a mixture of DNAs with two different buoyant densities.

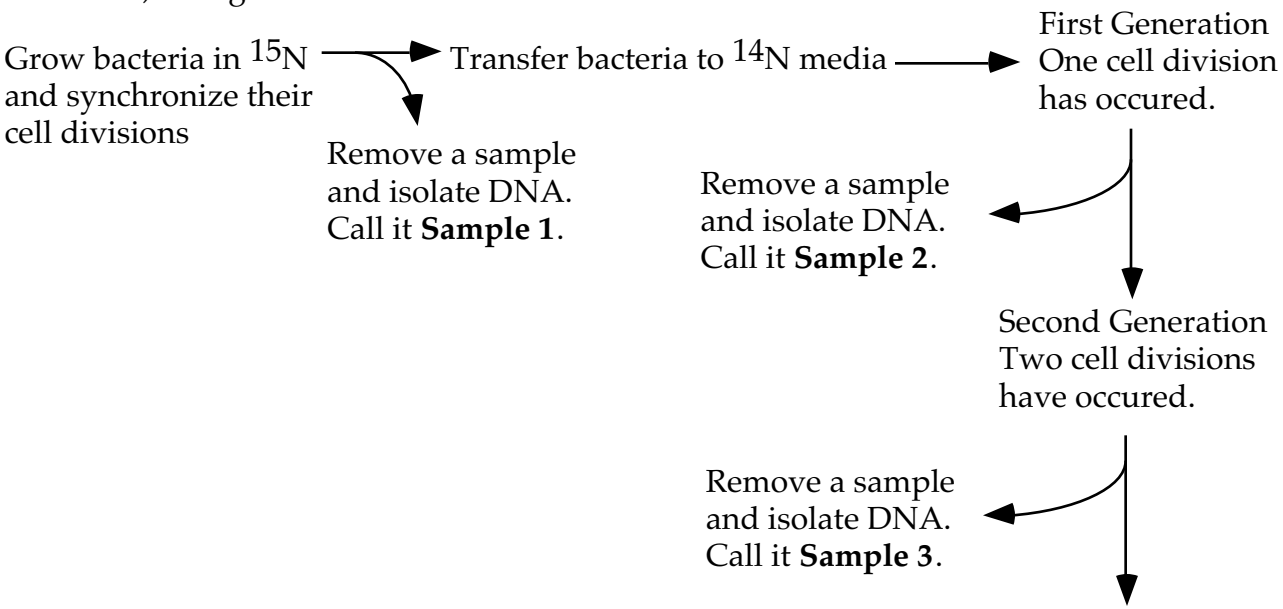
Back to the ^{15}N, ^{14}N stuff.

E. coli can be grown in media in which the sole source of nitrogen is ^{15}N or ^{14}N. Genomic DNA from bacteria grown in ^{15}N media will be about 1% denser than the DNA from bacteria grown in the ^{14}N media. This difference in density means that the ^{15}N and ^{14}N DNA can be separated by equilibrium density gradient centrifugation.

Previous experimentation had shown that:

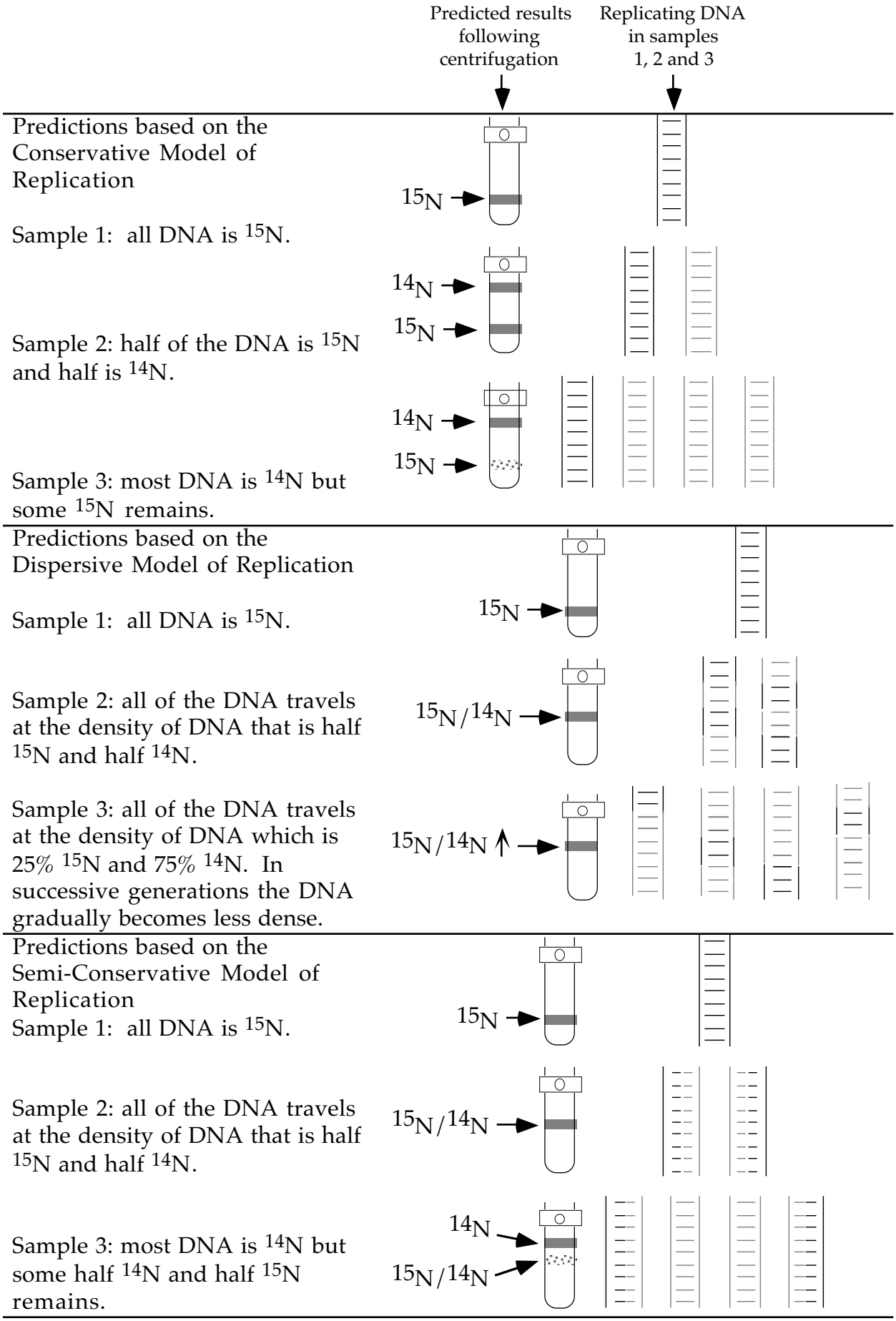
^{15}N labeled DNA sedimented at this position. 	 ^{14}N labeled DNA sedimented at this position	 If the DNA is 50% ^{15}N and 50% ^{14}N then it sediments at an intermediate position.

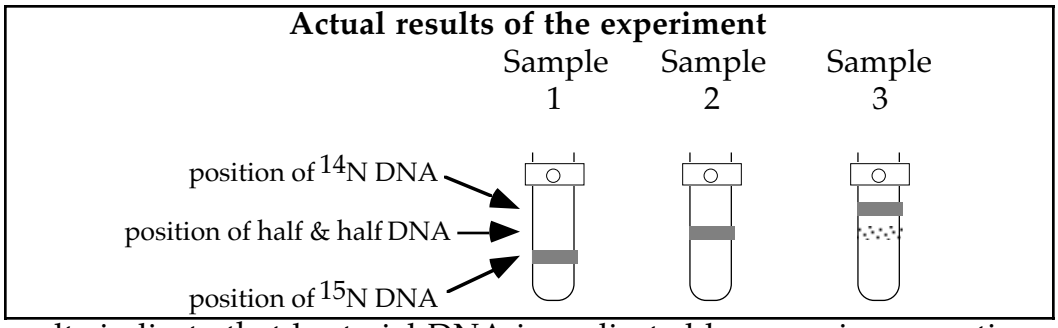
How was this used to determine which of the three DNA replication models is correct? Well, Meselson and Stahl grew bacteria in ^{15}N media until all of the DNA was uniformly labeled with ^{15}N. They then synchronized these cultures (in this context synchrony means that all of the bacteria in the culture are replicating their DNA and performing cell division in unison) and grew them in ^{14}N media. Here are the details.



Now samples 1, 2 and 3 are subjected to equilibrium density gradient centrifugation.

The three DNA replication models make specific predictions about the result of this experiment. These predictions are presented below. In this table $\overline{||||||||}$ represents DNA synthesized with ^{15}N and $\overline{||||||||}$ represents DNA synthesized with ^{14}N.


Predicted results following centrifugation
Replicating DNA in samples 1, 2 and 3
Predictions based on the Conservative Model of Replication
Sample 1: all DNA is 15N.
Sample 2: half of the DNA is 15N and half is 14N.
Sample 3: most DNA is 14N but some 15N remains.
Predictions based on the Dispersive Model of Replication
Sample 1: all DNA is 15N.
Sample 2: all of the DNA travels at the density of DNA that is half 15N and half 14N.
Sample 3: all of the DNA travels at the density of DNA which is 25% 15N and 75% 14N. In successive generations the DNA gradually becomes less dense.
Predictions based on the Semi-Conservative Model of Replication
Sample 1: all DNA is 15N.
Sample 2: all of the DNA travels at the density of DNA that is half 15N and half 14N.
Sample 3: most DNA is 14N but some half 14N and half 15N remains.



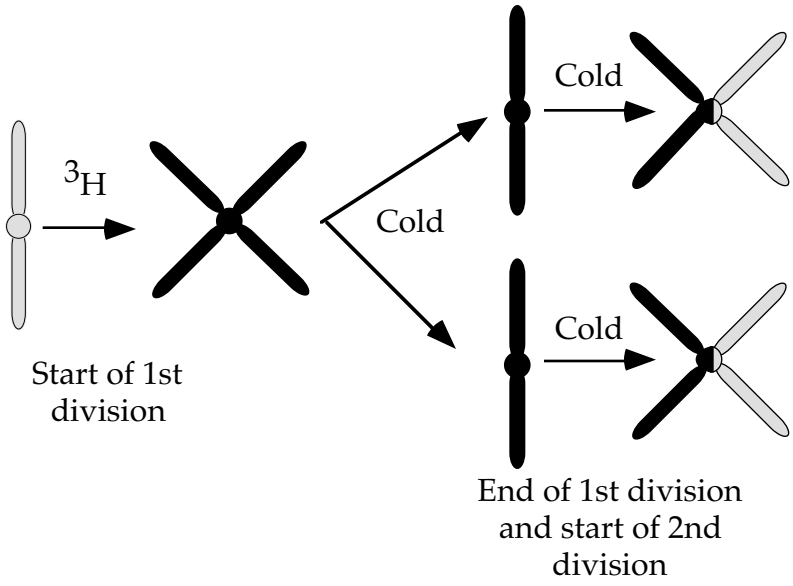
These results indicate that bacterial DNA is replicated by a semiconservative mechanism.

Now it's time for the Herbert Taylor Colchicine bean root tip experiment

The Meselson-Stahl experiment showed that bacteria used semi-conservative replication. But what about eukaryotic cells? For technical reasons, the Meselson-Stahl experiment could not be performed with eukaryotic cells. Herbert Taylor was the first to test whether eukaryotes use semi-conservative replication. He took root tip cells (plant cells) and allowed them to replicate in the presence of ^{3}H-thymidine. ^{3}H is a radioactive isotope of hydrogen. When it decays it releases β-particles. When β-particles strike a photographic emulsion they 'expose' it producing a black spot. This technique is called **autoradiography**.

During DNA replication, the newly synthesized DNA was radiolabeled with ^{3}H. After one round of replication the ^{3}H-thymidine was washed away and replaced with 'cold' thymidine. In this context, 'cold' means not radioactive. These cells were allowed to go through a second round of DNA replication. The cartoon below depicts a replicating chromosome. Black means that ^{3}H in the chromosome exposed the photographic emulsion. Gray means that the material is not radiolabeled and therefore does not expose the emulsion.

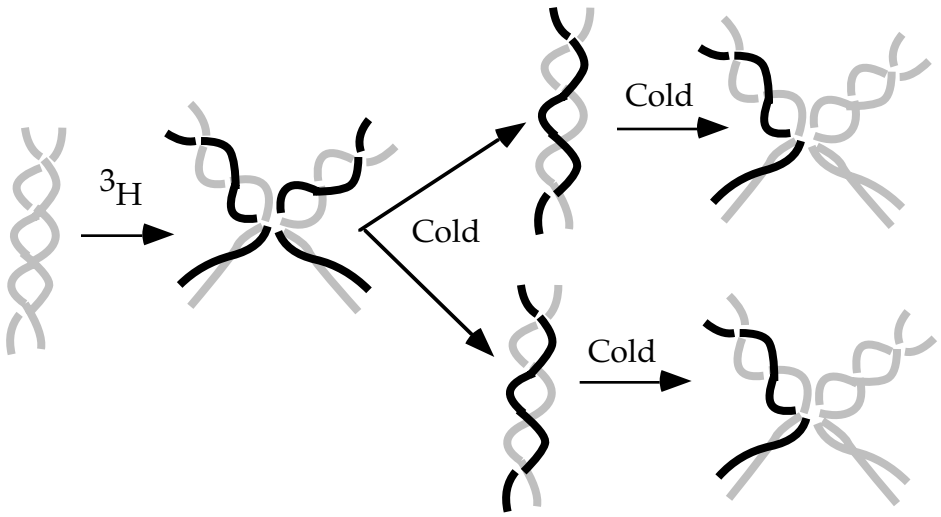
Experimental Results:



Interpretation of the results.

Each chromosome is shown as a double stranded DNA molecule.

Centromeres are not shown.



Definitions

autoradiography	A technique in which radioactive compounds are incorporated into a molecule or part of a cell. Either a piece of photographic film is placed against the sample or photographic emulsion is poured onto the sample. The decay of the radioactive isotope causes the emulsion to be exposed. After the 'film' is developed the exposed regions show up as black spots.
density	The quantity of matter in a given space based on the ration of mass to volume. This can be measured by centrifuging a molecule and measuring its rate of sedimentation.
isotope	One of several different forms of an atom with the same atomic number but differing atomic masses. Some isotopes are radioactive.
isotopically labeled	Refers to a compound that has been made using a rare isotope of an atom. For instance, the sulfur in proteins is usually the S isotope. But if a cell is grown in the presence of ^{35}S then the newly synthesized proteins will be isotopically labeled with ^{35}S.