DNA Replication

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

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

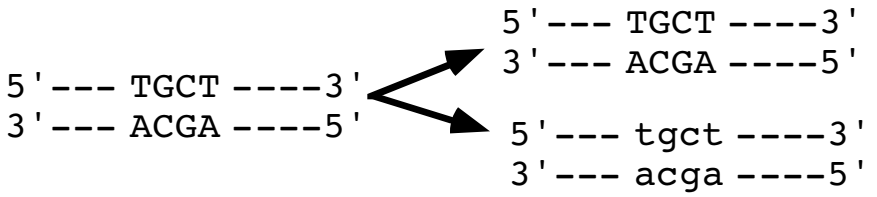
Because of this, in a complex organism, evolution will select against low fidelity DNA replication. The structure of DNA suggested a way that it could be replicated with high fidelity. Because the strands are complementary, one strand could specify the base on the opposite strand. This is actually what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

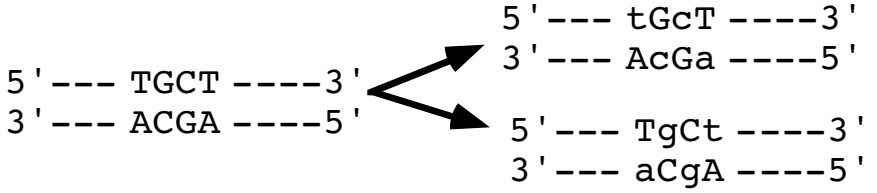
Following replication, one daughter molecule contains both of the parental strands. The other daughter molecule contains two newly synthesized DNA strands.



This theory was proven to be incorrect.

Dispersive Replication

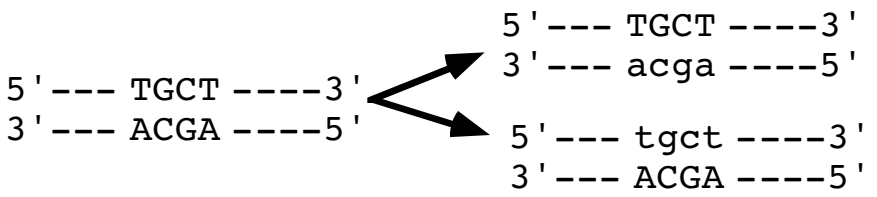
Both strands of each daughter molecule contain nucleotides derived from the parental molecule.



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).

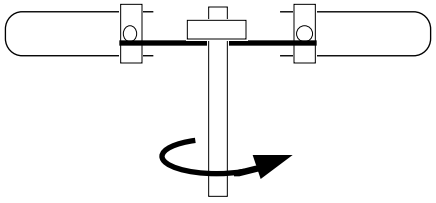
^{14}N most common form of nitrogen

^{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).

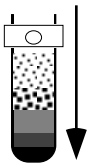
What is equilibrium density gradient centrifugation?

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.

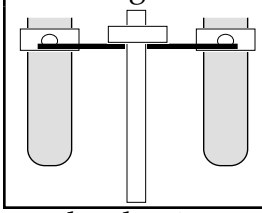
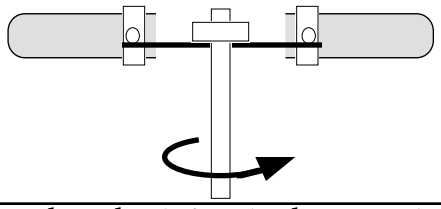
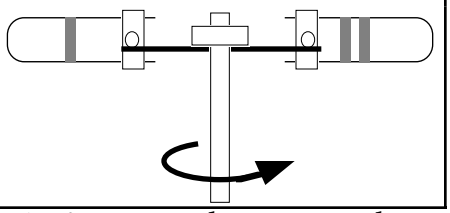


Centrifuge tubes spinning in a centrifuge

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:

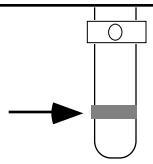
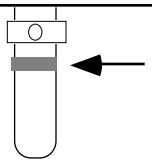
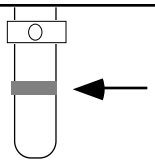
Prior to centrifugation, the DNA is evenly distributed throughout the centrifuge tubes.	Centrifugation begins and the salt and DNA begin sedimenting.	After prolonged centrifugation, the DNA has formed discrete bands reflecting its buoyant density.
		

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.

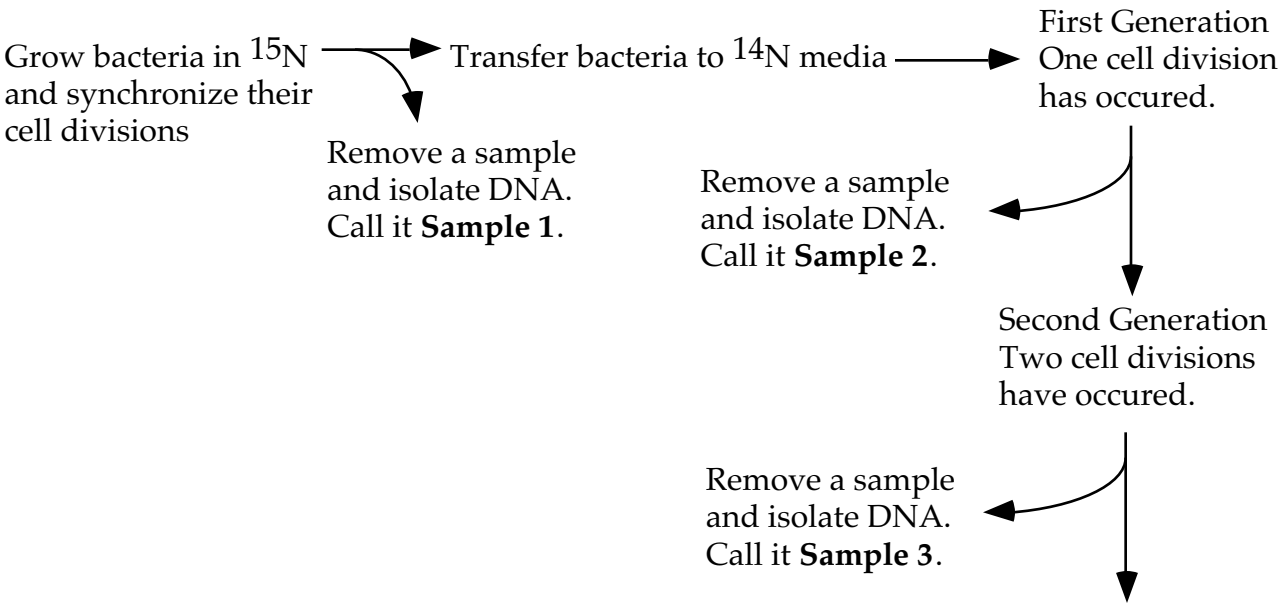
Back to the ^{15}N, ^{14}N stuff.

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:

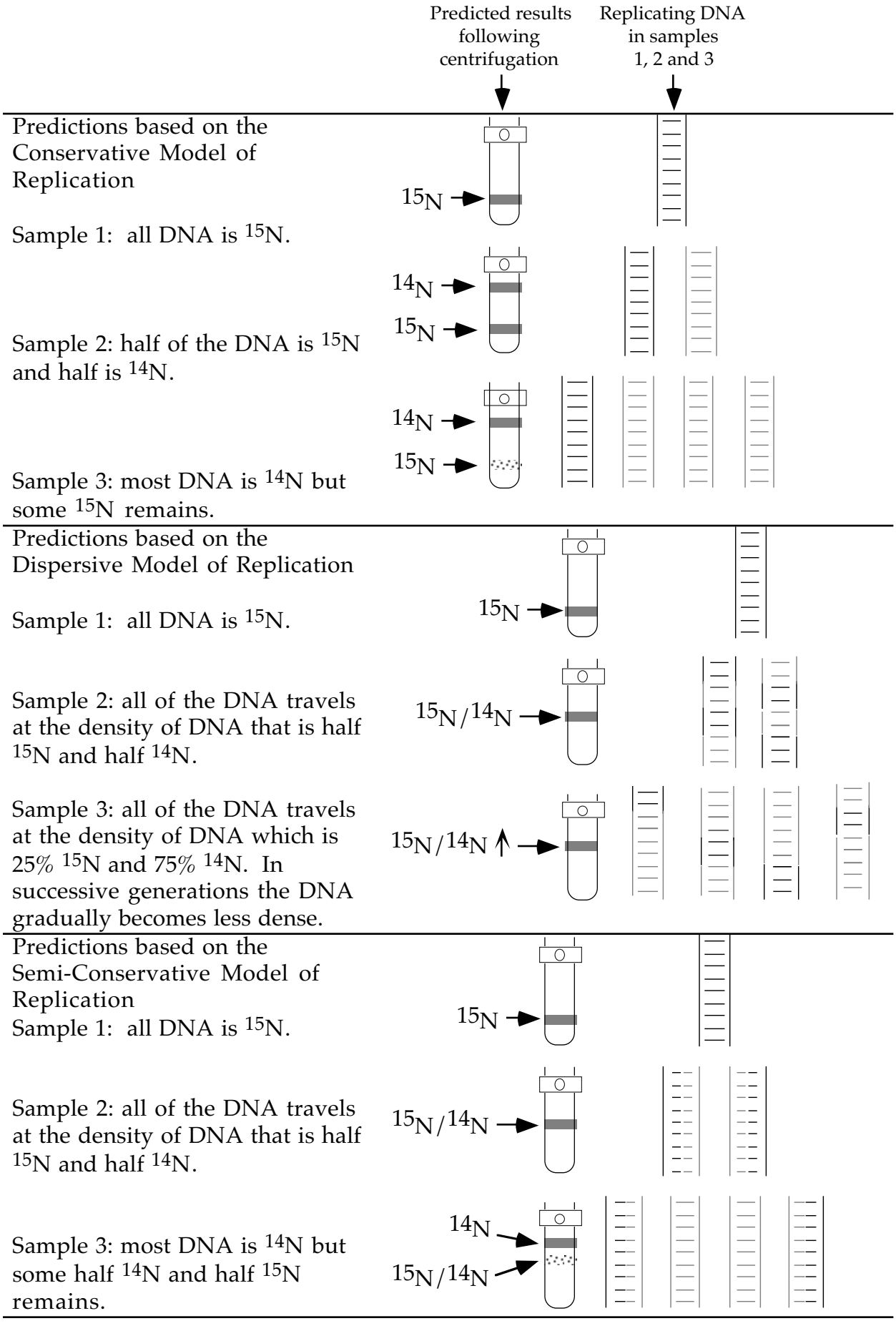
^{15}N labeled DNA sedimented at this position.	^{14}N labeled DNA sedimented at this position	If the DNA is 50% ^{15}N and 50% ^{14}N then it sediments at an intermediate position.
		

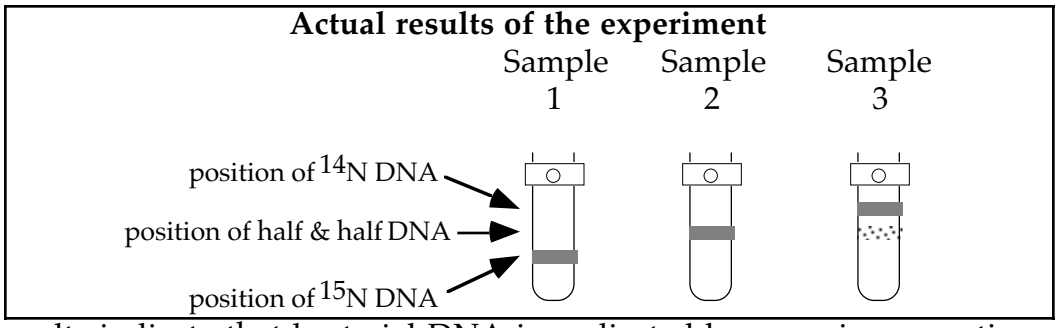
How was this used to determine which of the three DNA replication models is correct? Well, Meselson and Stahl grew bacteria in ^{15}N media until all of the DNA was uniformly labeled with ^{15}N. They then synchronized these cultures (in this context synchrony means that all of the bacteria in the culture 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 ||||||||| represents DNA synthesized with ^{15}N and ||||||||| 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.
15N
14N
15N/14N



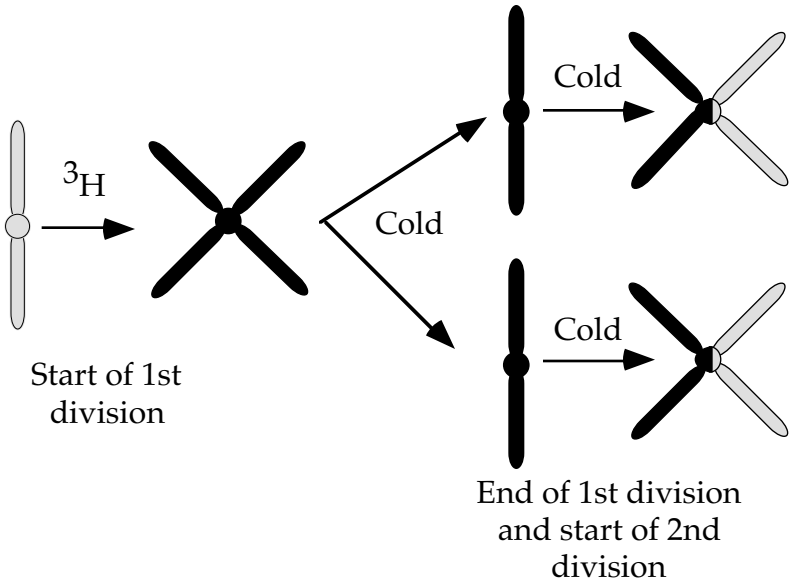
These results indicate that bacterial DNA is replicated by a semiconservative mechanism.

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.

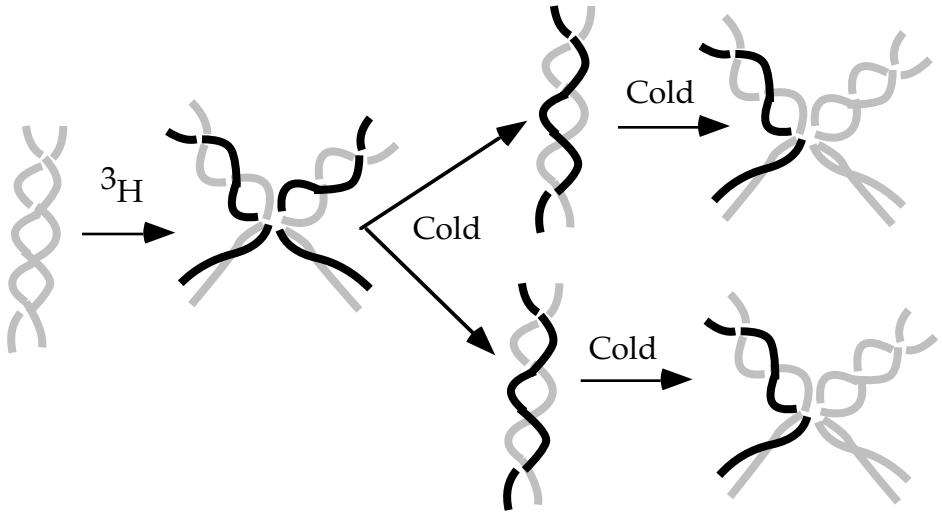
Experimental Results:



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.