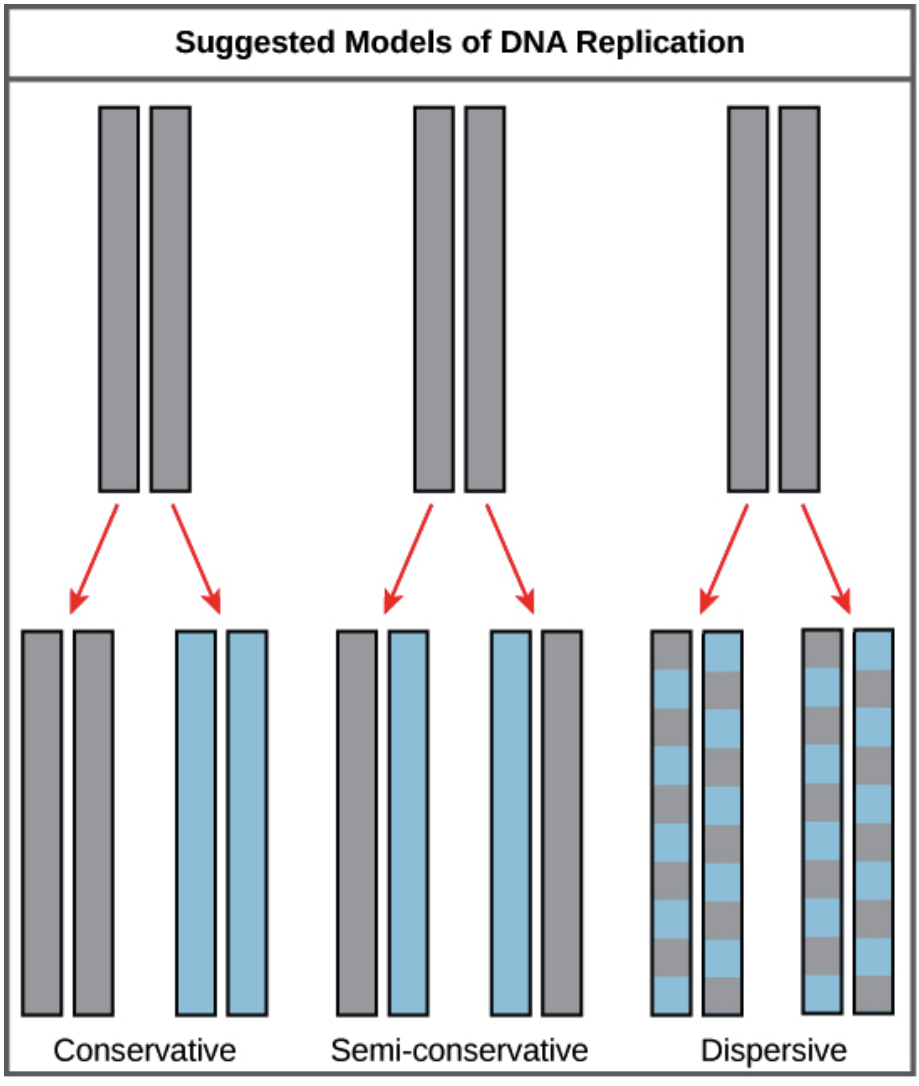
ST.PHILOMENA'S COLLEGE
B.Sc V SEM
Department of Biochemistry
Unit: 1 MOLECULAR BIOLOGY
Section 1.2 (Part I)
Topics Included: Modes/Models of DNA Replication, Meselson and Stahl Experiment

The three models for DNA replication

There were three basic models for DNA replication that had been proposed by the scientific community after the discovery of DNA's structure. These models are illustrated in the diagram below:



- **Semi-conservative replication.** In this model, the two strands of DNA unwind from each other, and each acts as a template for synthesis of a new, complementary strand. This results in two DNA molecules with one original strand and one new strand.
- **Conservative replication.** In this model, DNA replication results in one molecule that consists of both original DNA strands (identical to the original DNA molecule) and another molecule that consists of two new strands (with exactly the same sequences as the original molecule).
- **Dispersive replication.** In the dispersive model, DNA replication results in two DNA molecules that are mixtures, or "hybrids," of parental and daughter DNA. In this model, each individual strand is a patchwork of original and new DNA.

Matt Meselson and Franklin Stahl originally met in the summer of 1954, the year after Watson and Crick published their paper on the structure of DNA. Although the two researchers had different research interests, they became intrigued by the question of DNA replication and decided to team up and take a crack at determining the replication mechanism

The Meselson-Stahl experiment

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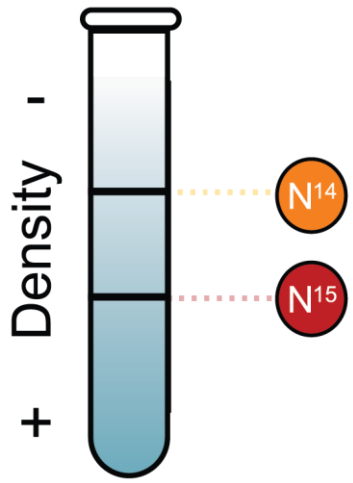
They began by growing *E. coli* in medium, or nutrient broth, containing a "heavy" isotope of nitrogen, ^{15}N. (An **isotope** is just a version of an element that differs from other versions by the number of neutrons in its nucleus.) When grown on medium containing heavy ^{15}N, the bacteria took up the nitrogen and used it to synthesize new biological molecules, including DNA.

After many generations growing in the ^{15}N medium, the nitrogenous bases of the bacteria's DNA were all labeled with heavy ^{15}N. Then, the bacteria were switched to medium containing a "light" ^{14}N isotope and allowed to grow for several generations. DNA made after the switch would have to be made up of ^{14}N, as this would have been the only nitrogen available for DNA synthesis.

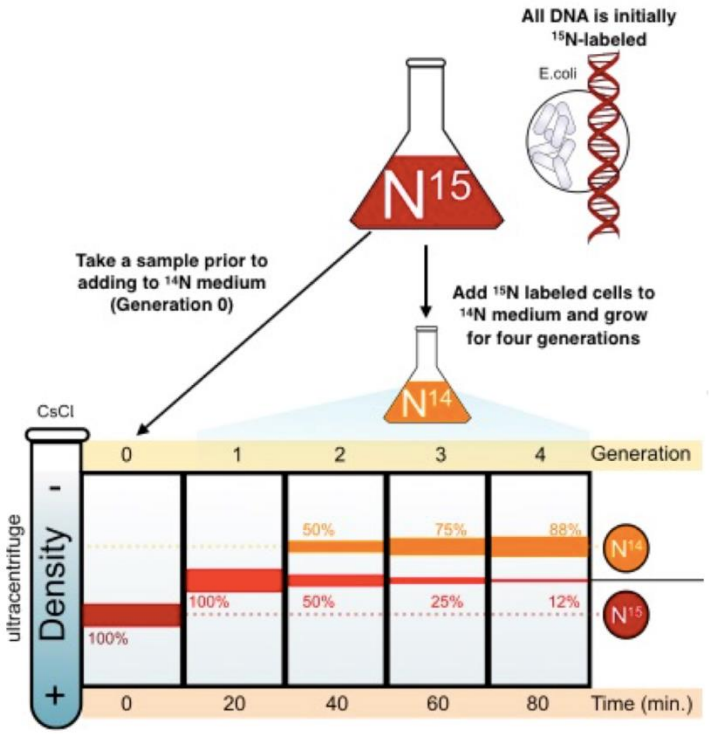
Meselson and Stahl knew how often *E. coli* cells divided, so they were able to collect small samples in each generation and extract and purify the DNA. They then measured the density of the DNA (and, indirectly, its ^{15}N and ^{14}N content) using **density gradient centrifugation**.

This method separates molecules such as DNA into bands by spinning them at high speeds in the presence of another molecule, such as cesium chloride, that forms a density gradient from the top to the bottom of the spinning tube. Density gradient centrifugation allows very small differences—like those between ^{15}N - and ^{14}N -labeled DNA—to be detected.



Results of the experiment

When DNA from the first four generations of *E. coli* was analyzed, it produced the pattern of bands shown in the figure below:



Generation 0

DNA isolated from cells at the start of the experiment ("generation 0," just before the switch to ^{14}N medium) produced a single band after centrifugation. This result made sense because the DNA should have contained only heavy ^{15}N at that time.

Generation 1

DNA isolated after one generation (one round of DNA replication) also produced a single band when centrifuged. However, this band was higher, intermediate in density between the heavy ^{15}N DNA and the light ^{14}N DNA.

The intermediate band told Meselson and Stahl that the DNA molecules made in the first round of replication was a hybrid of light and heavy DNA. This result fit with the dispersive and semi-conservative models, but not with the conservative model.

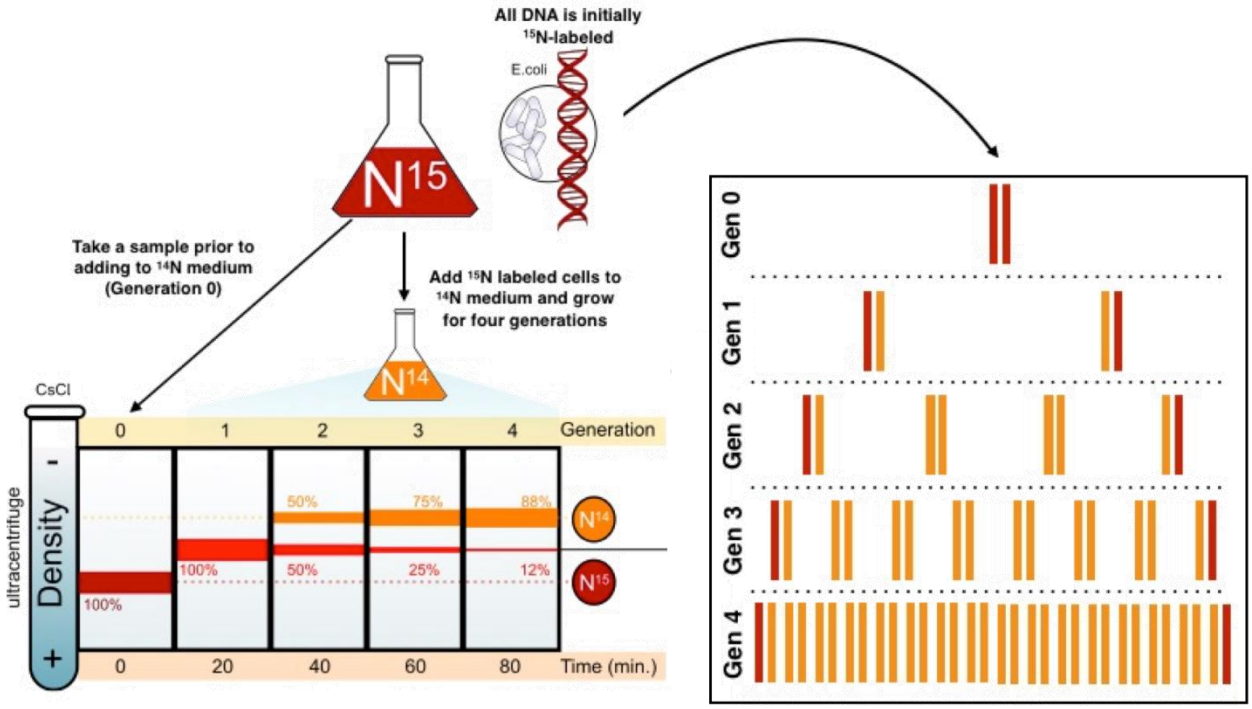
The conservative model would have predicted two distinct bands in this generation (a band for the heavy original molecule and a band for the light, newly made molecule).

Generation 2

Information from the second generation let Meselson and Stahl determine which of the remaining models (semi-conservative or dispersive) was actually correct.

When second-generation DNA was centrifuged, it produced two bands. One was in the same position as the intermediate band from the first generation, while the second was higher (appeared to be labeled only with ^{14}N).

This result told Meselson and Stahl that the DNA was being replicated semi-conservatively. The pattern of two distinct bands—one at the position of a hybrid molecule and one at the position of a light molecule—is just what we'd expect for semi-conservative replication (as illustrated in the diagram below). In contrast, in dispersive replication, all the molecules should have bits of old and new DNA, making it impossible to get a "purely light" molecule.



Generations 3 and 4

In the semi-conservative model, each hybrid DNA molecule from the second generation would be expected to give rise to a hybrid molecule and a light molecule in the third generation, while each light DNA molecule would only yield more light molecules.

Thus, over the third and fourth generations, we'd expect the hybrid band to become progressively fainter (because it would represent a smaller fraction of the total DNA) and the light band to become progressively stronger (because it would represent a larger fraction).

As we can see in the figure, Meselson and Stahl saw just this pattern in their results, confirming a semi-conservative replication model.

Conclusion

The experiment done by Meselson and Stahl demonstrated that DNA replicated semi-conservatively, meaning that each strand in a DNA molecule serves as a template for synthesis of a new, complementary strand.

Although Meselson and Stahl did their experiments in the bacterium *E. coli*, we know today that semi-conservative DNA replication is a universal mechanism shared by all organisms on planet Earth. Some of your cells are replicating their DNA semi-conservatively right now!