THE MOST BEAUTIFUL EXPERIMENT IN ALL OF BIOLOGY

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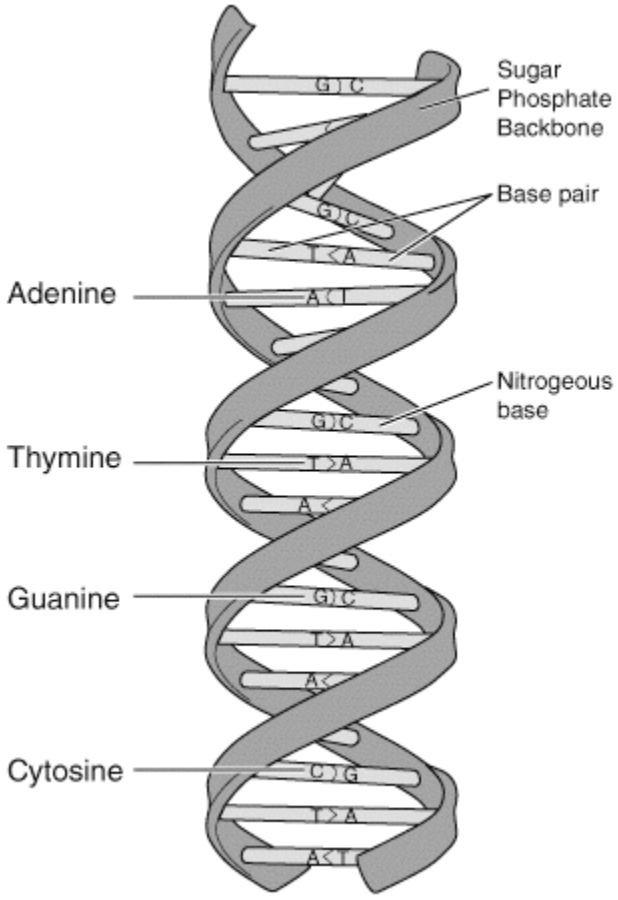
Abstract. A walkthrough of Meselson's and Stahl's 1958 experiment to establish the semi-conservative replication of DNA.

1. Introduction

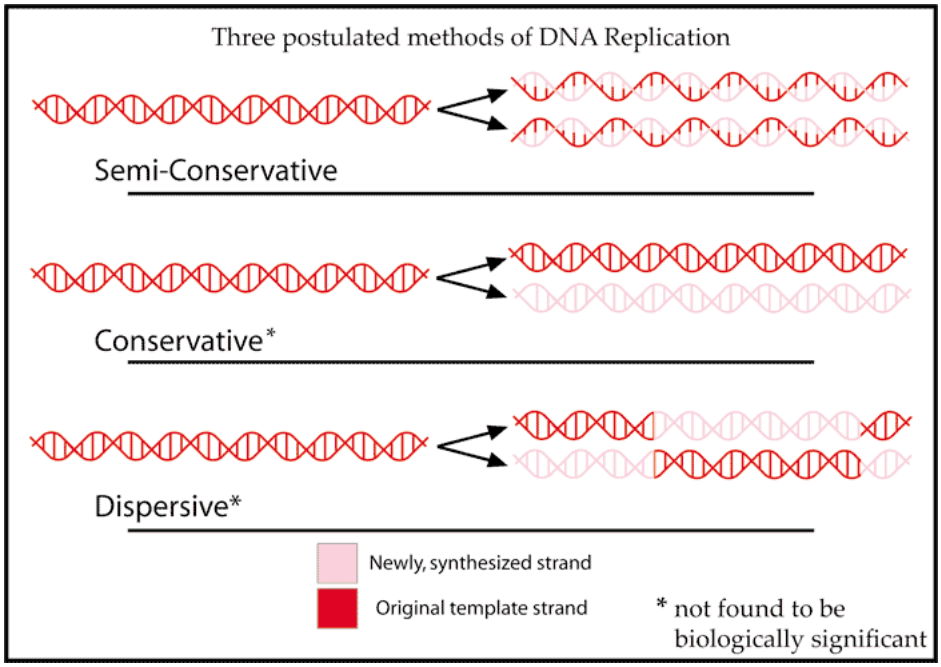
In 1953, Watson and Crick published their seminal paper "Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid," in which they described their discovery of the double helical structure of *DNA*.



DNA

With their presentation of the *double helix structure* of DNA they also proposed the *semi-conservative model of DNA replication.* To quote them directly, "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." [2]

The semi-conservative replication model is to be contrasted with both conservative and dispersive models of replication of genetic material.



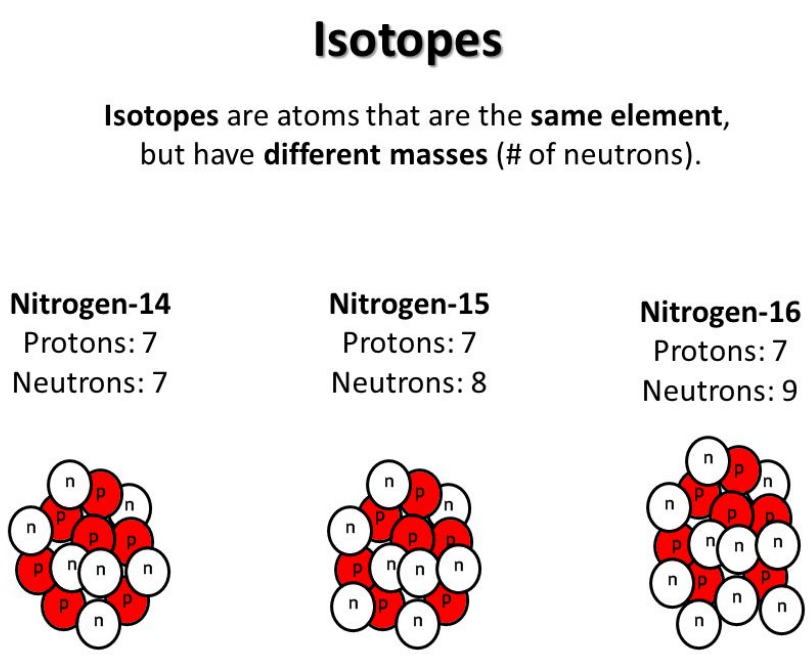
DNA Replication Modes.

In the semi-conservative model, the two strands of the double helix unwind, and each serve as a template for a new helical strand, the end result being two DNA molecules that consist of one strand from the old molecule and one newly synthesized strand.

Although Watson and Crick proposed this model in 1953, it wasn't until 1958 that Meselson and Stahl designed the experiment that would validate the semi-conservative model over the rival conservative and dispersive hypotheses.

2. The Experiment

In order to determine which replication hypotheses is correct, Meselson and Stahl would raise E. Coli for multiple generations in an environment containing the heavy nitrogen *isotope* N-15. Then, they would transfer the E. Coli to a medium containing regular nitrogen, N-14. As nitrogen is a major constituent of DNA, by observing the density of the DNA for a few replication cycles after the transfer, the scientists would be able to distinguish the competing hypotheses.



Isotopes of Nitrogen.

Problem:
If the dispersive hypothesis were correct, what would we expect of the DNA densities after a few replication cycles?

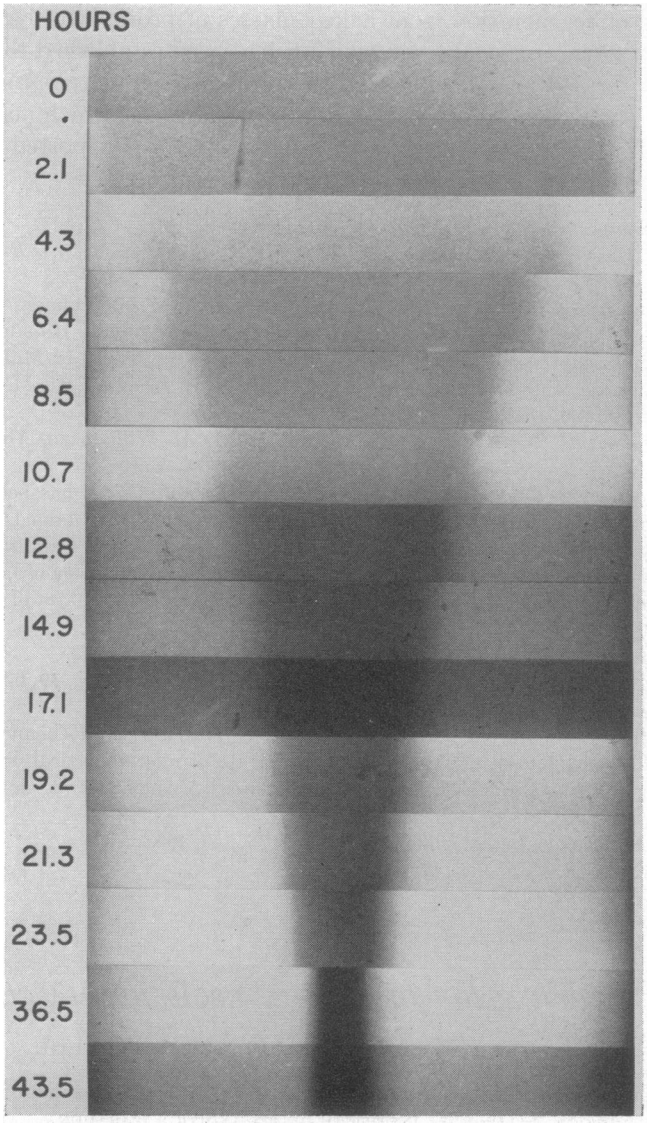
Solution:
The density of the DNA would be a gradient between that of N-14 and N-15 DNA.

3. Density-Gradient Centrifugation

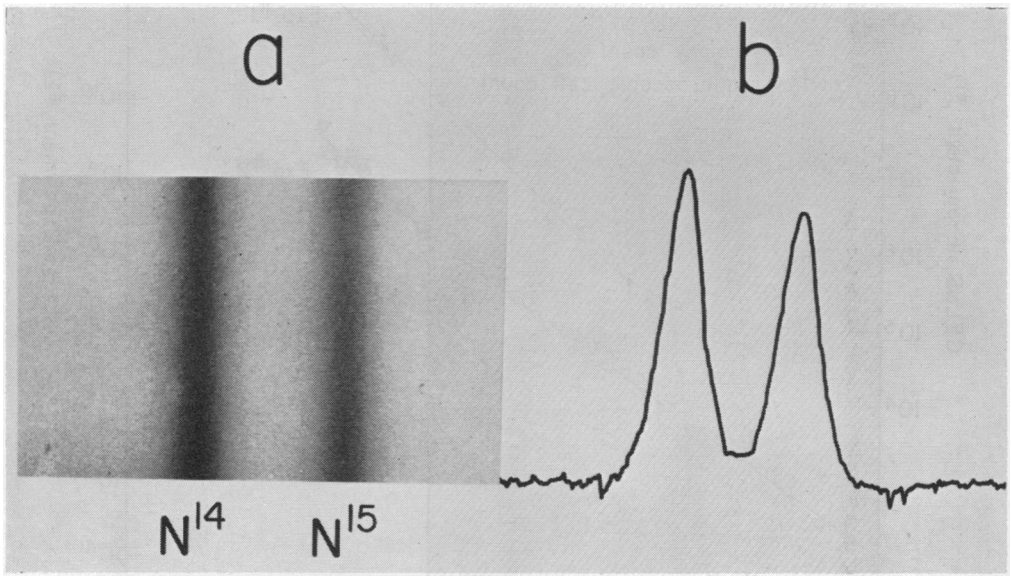
A critical component of this experiment was their development of a method for the detection of small density differences among macromolecules. A small amount of DNA in a concentrated solution of cesium chloride (CsCl) is *centrifuged* until equilibrium is closely approached. The centrifugal force drives the sedimentation of the CsCl to the bottom of the test tube, while diffusion creates a tendency for the CsCl to spread out, rising upward. The opposition of these two processes creates a *concentration gradient* of CsCl.

Centrifuge. A video of a centrifuge in operation can be watched at https://www.youtube.com/watch?v=8aLEK6SffD4

If DNA is added to the CsCl solution, then centrifugation will cause the DNA to settle at the place where the density of the solution matches the density of the DNA. Via these means, the minute differences in density between the DNA of E. Coli raised in N-14 and N-15 mediums can be visualized.



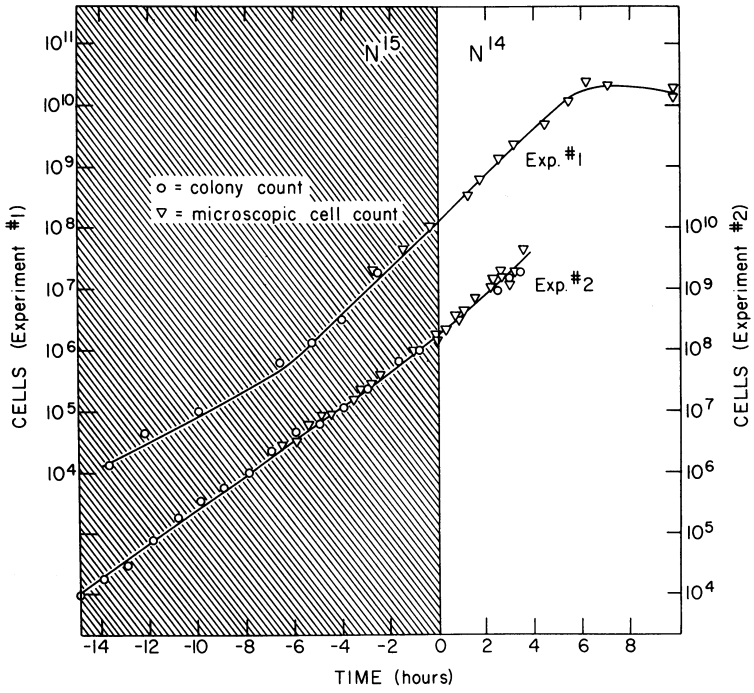
Meselson and Stahl, Figure 1 - Ultraviolet absorption photographs showing successive stages in the banding of DNA from E. coli. An aliquot of bacterial lysate containing approximately 108 lysed cells was centrifuged at 31,410 rpm in a CsCl solution as described in the text. Distance from the axis of rotation increases toward the right. The number beside each photograph gives the time elapsed after reaching 31,410 rpm.



Meselson and Stahl, Figure 2 - a: The resolution of N1-4 DNA from N-15 DNA by density-gradient centrifugation. A mixture of N-14 and N-15 bacterial lysates, each containing about 108 lysed cells, was centrifuged in CsCl solution as described in the text. The photograph was taken after 24 hours of centrifugation at 44,770 rpm. b: A microdensitometer tracing showing the DNA distribution in the region of the two bands of Fig. 2a. The separation between the peaks corresponds to a difference in buoyant density of 0.014 g * cmˆ-3.

4. Generation Time

Next Meselson and Stahl measured the growth of bacterial populations in both mediums, in order to find the *generation time* of the E. Coli under the experimental conditions. The generation time is the amount of time it takes for the E. Coli population to double.



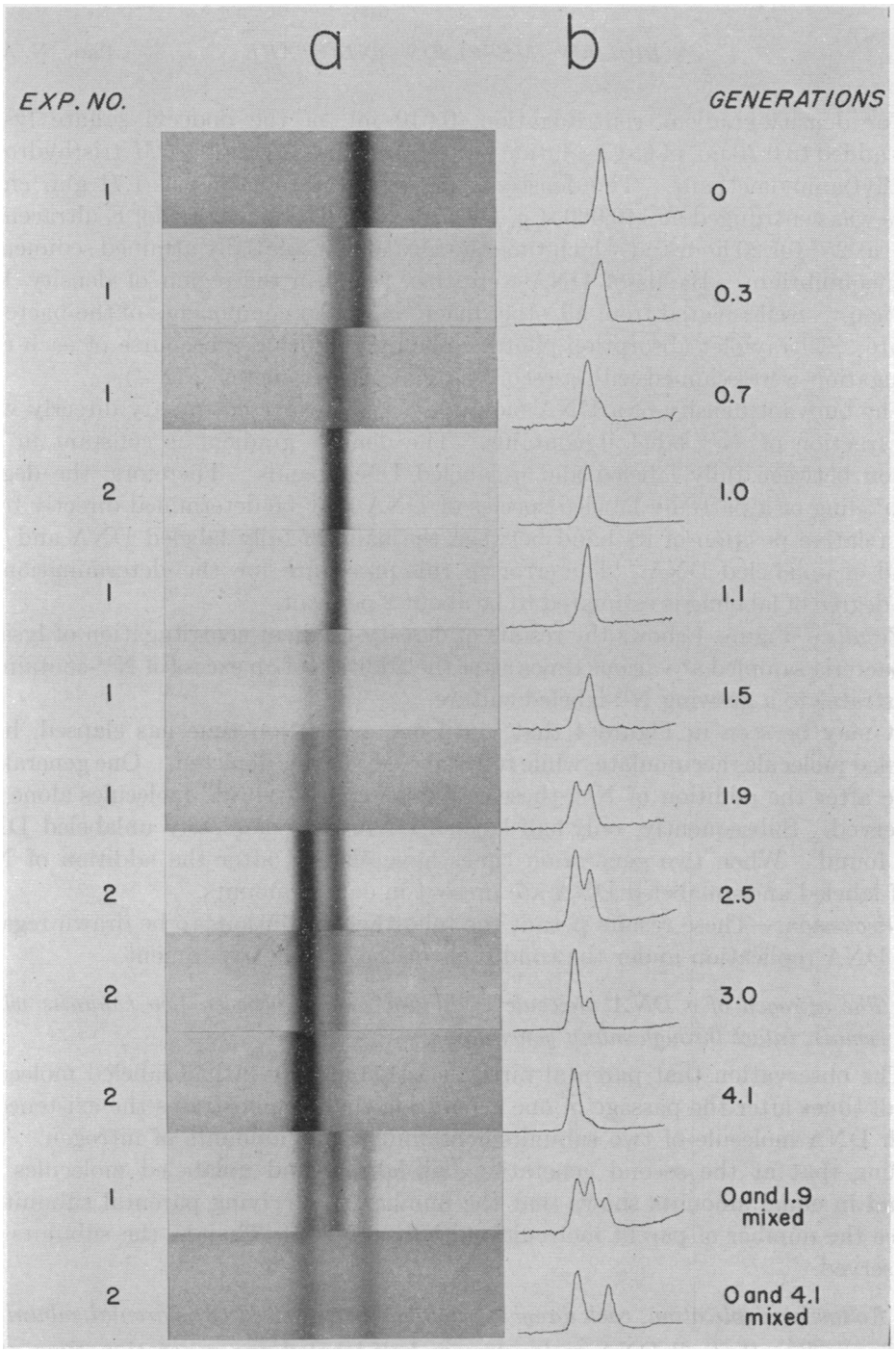
Meselson and Stahl, Figure 3 - Growth of bacterial populations first in N-15 and then in N-14 medium. The values on the ordinates give the actual titers of the cultures up to the time of addition of N-14. Thereafter, during the period when samples were being withdrawn for density-gradient centrifugation, the actual titer was kept between 1 and 2 X 108 by additions of fresh > medium. The values on the ordinates during this later period have been corrected for the withdrawals and additions. During the period of sampling for density-gradient centrifugation, the generation time was 0.81 hours in Experiment 1 and 0.85 hours in Experiment 2.

Problem:
How would you go about measuring the generation time based on this chart?

Solution:
The most critical piece of information while trying to construct the generation time from the graph above is that the scale of the graph is logarithmic. It's in base 10 and we want to find when the population doubles (base 2). Let's proceed by finding a power of 2 that's close to a power of 10, 2^{10} (=1024) is close to 10^3 (=1000). The time taken for the number of cells to increase by a factor of 10^3 corresponds to 10 generation times. For example, in experiment 2 the cell count hits 10^4 at nearly -7 hours and hits 10^7 at about +2 hours resulting in an a generation time of approximately 9/10 hours.

5. Snapshots after the Medium Transfer

Finally, Meselson and Stahl are able to produce these snapshots of the density of the DNA from the E. Coli after transfer to the N-14 medium.



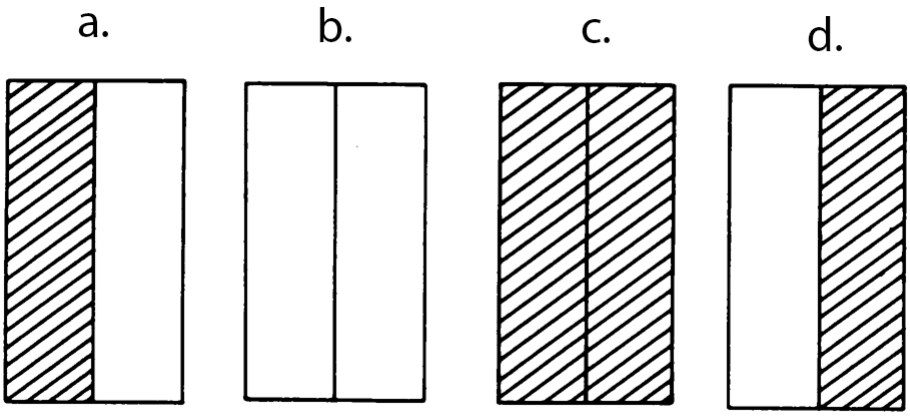
It is this image that proves the semi-conservative replication hypothesis, particularly the sub-image that shows generation time 0 and generation time 2 (1.9) mixed.

If replication were dispersive in nature, then we'd expect a smear, a gradient of densities from low to high, each DNA molecule a random mosaic of N-14 and N-15.

If replication was fully conservative, we'd expect to see two lines, one at the original CsCl density (horizontal position) at time zero, and one at a lighter density of CsCl corresponding to DNA consisting purely of N-14.

Instead, we see three bands in the mixed 0 and 2 generation picture. The far right band corresponds to the density of the original N-15 DNA molecules. The middle band corresponds to DNA that consists of one N-15 daughter strand and one N-14 daughter strand. And lastly, the far left band corresponds to DNA that consists of two N-14 daughter strands. Thus, Watson's and Crick's hypothesis for the method of DNA replication is vindicated by experiment.

Problem:
Which of these represent a valid schematic representation of the second generation daughter molecules from the Meselson-Stahl experiment? (The shaded regions represent isotropically labeled daughter strands of DNA)



Answer:
a, b, d

References

[1] Matthew Meselson and Franklin W. Stahl, *The Replication of DNA in Escherichia Coli* (1958).
[2] J. D. Watson and F. H. C. Crick, *Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid* (1953).

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