"The Most Beautiful Experiment in Biology..."

The Replication of DNA in *Escherichia Coli*

By Matthew Meselson and Franklin Stahl, 1958

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HISTORICAL BACKGROUND

Matthew Meselson

- **born May 24th, 1930 in Denver, Colorado.**
- **graduated with aPh.D in liberal acts from the University of Chicago**
- **California Institute of Technology as a research fellow under Linus Pauling**
- **associate professor of chemistry**
- **very accredited person, winning awards such as the National Academy of Science Prize (1963), the Eli Lilly Award for Microbiology and Immunology (1964), the Presidential Award from the New York Academy of Science (1983), the Science Freedom and Responsibility Award (1990) and the Albert Lasker Achievement Award (2004).**
- **1957 developed the technique of density gradient centrifugation with Frank Stahl**
- **1961, along with Frank Stahl, Sidney Brenner and Francois Jacob he later demonstrated that ribosomal RNA molecules are stable, which later proved the existence of mRNA.**
- **demonstrated that genetic recombination results from the splicing of DNA molecules, with the help of Charles Radding.**
- **demonstrated the enzymatic basis of a process by which cells recognize and destroy foreign DNA, and discovered methyl-directed mismatch repair, which enables cells to repair mistakes in DNA.**
- **concerned about chemical and biological weapons in warfare:**
  - **participated in many scientific studies pertaining to the accidental and misuse of biological weapons**
  - **acted as a consultant for numerous government agencies.**
  - **anthrax outbreak of 1979 in Sverdlovsk, USSR.**
  - **"yellow rain" in southeast Asia**

Franklin Stahl

- **Born on October 8th, 1929 in Boston, Massachusetts.**
- **Attended Harvard University where he received a B.A,**
- **Graduate studies at the University of Rochester**
- **Molecular biology course at Woods Hole, taught be James Watson and Francis Crick.**
- **Met Matthew Meselson in 1954.**

*To find more information on the various awards, see the Historical Timelines of Meselson and Stahl

In 1957 he, along with Matthew Meselson, developed the technique of density gradient centrifugation, which led to the realization that DNA replication is semi-conservative through their experiment with *E.coli* DNA.

In 1959, he accepted a position at the University of Oregon where he is now a distinguished professor of Molecular Biology, with his current research interest focusing on the mechanics of genetic recombination.

The Meselson-Stahl experiment was an experiment which demonstrated that DNA replication was semiconservative. This was realized by using *E.coli* DNA which had N^{15} nitrogen isotope (heavier than common nitrogen) and then placing it into N^{14} media.

Thus semi-conservative replication would be seen within the first generation in with the replicated DNA contained one N^{15} strand and one N^{14} strand.

PAPER SUMMARY

Introduction:

- **DNA is the genetic material needed for replication and to understand more, there was studies done on living organisms.**
- **From doing studies with bacteria, and more particularly *E. coli* it could be determined that DNA can transmit hereditary information as well as self replicate.**
- **Use radioisotopic labels in DNA which would increase density and cause distinguishing features to be recognized based on sedimentation.**
    - **Nitrogen isotopes were used: N^{14} (light) and N^{15} (heavy)**
- **By using the isotopes the experiment could be used to test Watson and Cricks model of replication**
- **Until this time (1958) certain equipment wasn't available**
    - **Zonal Ultracentrifuge never used in this manner before**

Procedure

Colonies of *Escherichia coli* were grown at 36 degrees C in bulk cultures

- **Have Ammonium chloride as sole nitrogen source on a salt medium**
- **Analysis of the population growth by using the methods of cell count and colony assays.**

Colonies were then labelled with an N^{15} isotope

N15 became incorporated into DNA

DNA that was isolated from these cells would be easily recognizable from normal DNA because it would have a higher density.

Preparation of N^{15} isotope DNA made possible by introducing these colonies to a differing medium

This medium contained the N^{14} isotope for one round of replication.

- The N^{14} isotope incorporates into any new DNA that is made.

Before cultivation in N^{14} medium some samples were taken to be centrifuged in the cold for 5 minutes

- **Then placed in NaCl to suspend and EDTA to prevent any further deterioration or growth**
- **Cells lysed by sodium dodecyl sulphate and stored in the cold**

- used as an anionic detergent to solubilize proteins

This procedure of centrifuging, lysing and cooling was done also for the colonies transferred and grown in the N^{14} medium at intervals of time

At these intervals of time a sample was taken and placed in an Ultracentrifuge with a density gradient medium

To view the sedimentary bands of this process a special photographing technique had to be applied

Once this technique was used the results from the analysis were found and interpretations of such could be made

Further tests were done on E.coli using heat denaturing processes. To get the DNA to go from double stranded to single stranded this process needed to be completed.

Another test conducted using salmon sperm and *E.coli* DNA was done for a broad comparison.

SUMMARY VIDEO

LOGIC

E. coli was used in the experiment because:

- easily grown in test tubes, agar plates or petri-dishes in/on simple media
- can grow in either aerobic or anaerobic conditions
- relatively quick generation times
  - thought to be only about 12 hours in natural conditions (i.e. gastrointestinal tract)
  - can be reduced to approximately 40 minutes within a laboratory setting
  - this varies based on the different strains of the bacterium
- simple DNA structure.
- contains a single, circular DNA molecule

Originally, Three Hypotheses were proposed for the possible methods of DNA replication

The results of the Meselson-Stahl experiment provided evidence for the Watson-Crick method:

- that DNA replication is Semi-Conservative
- DNA strands are conserved throught generations but not throughout all cells

Vertebrate or more complex DNA structures often appear to replicate DNA in a dispersive mode due to the high number of replications for reproduction and the occurrence of spontaneous crossovers/reciprocal exchanges between sister chromatids within one chromosome

- Harlequin chromosomes are used to demonstrate Semi-Conservative replication in more complex DNA structures found in higher animals.

CRITICAL REVIEW

Misinterpretation

- Often the results are misinterpreted by readers
  - mode of centrifugation for separating the DNA of different densities is often assumed to be swinging bucket
    - this method was only used initially in order to isolate the original DNA
  - many believe the 'bands' of DNA seen in the photographs would actually be seen by the naked eye in the test tubes but infact are the result of UV radiation absorption and reflection

Organization

- Heat Denaturation
  - misplaced within the paper
  - introduced in the discussion - did not provide any introduction, materials and methods, or references for this experiment
  - not clearly explained; needs more explanation and refinement within the paper such as how it relates to the semi-conservative replication in DNA
- Explanation of Figures and Graphs
  - figures and graphs should be more clearly define for what they represent or what they actually are.
- Use of CsCl
  - not clearly explained why this substance was used
  - quantity - how did they know how much to use?
- Noted in paper that authors were unsure whether the DNA in *E. coli,* that was extracted in this experiment and assumed to be the original parental strand, was a single polynucleotide chains
  - justified in still assuming the DNA replication follows the Watson-Crick molecule?
  - Watson-Crick model is based on two polynucleotide chains wound together helically

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REFERENCES