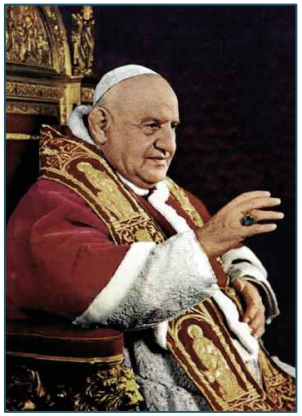
Investigation of DNA Replication Mechanisms

Meselson and Stahl

World Happenings of 1958

Politics

Alaska becomes the 49th State



Pope John XXII becomes Pope

War

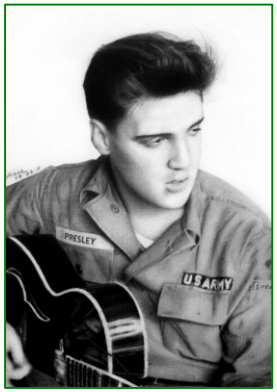
Cuban Revolution
Castro attacks Havana, Cuba

Icelandic Cod War
Iceland vs Great Britain over territorial waters

Social Aspects

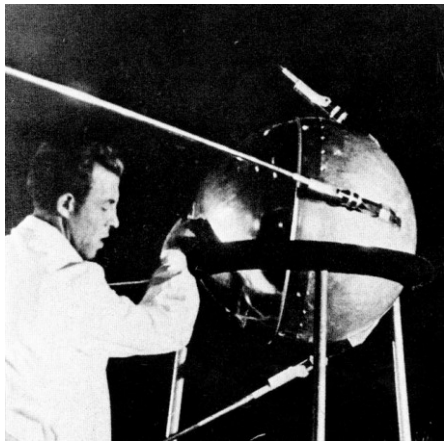
Beatles record first record

Pizza Hut Founded

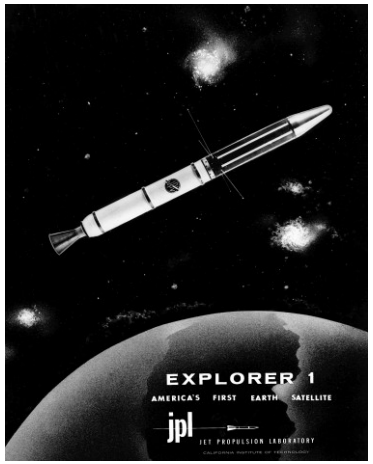


Elvis inducted into US Army

Science Happenings of 1958



Russian satellite Sputnik 1 crashes

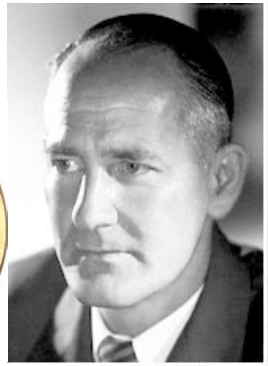


US launches our first satellite Explorer 1

NASA established

F104 star fighter sets world record flight speed. 1,404 mph.

US Project SCORE sends first communication satellite into orbit



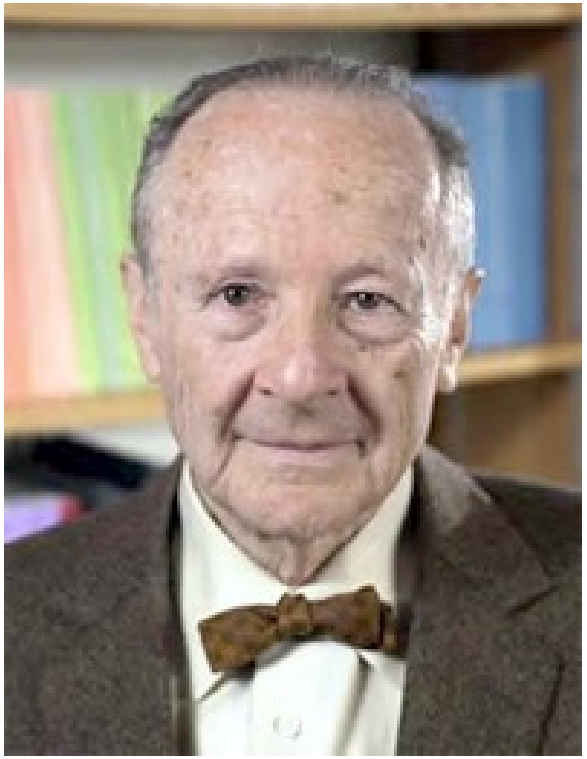
George Wells Beadle (1903 - 1989)

Edward Lawrie Tatum (1909 - 1975)

Nobel in Physiology or Medicine went to Beadle and Tatum – Role of genes in biochemical events

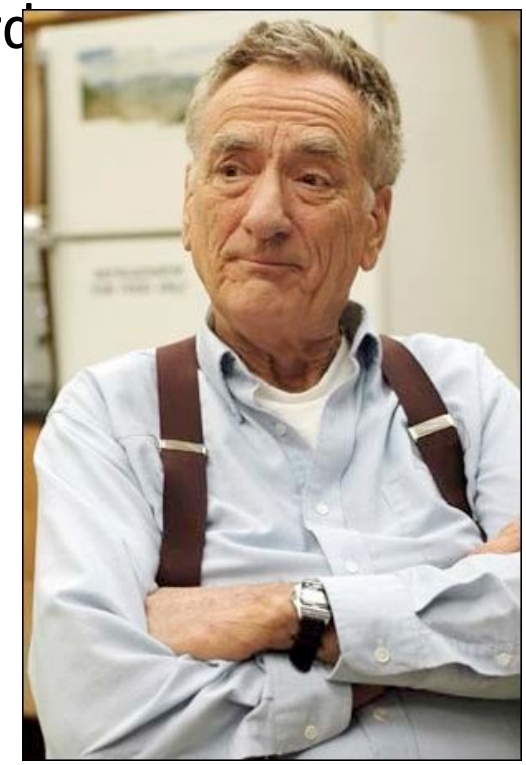
Matthew Stanley Meselson

- Assistant Professor and Senior research professor at CalTech
- Genetics and research professor at Harvard starting in 1960
- 1961 used density-gradient method to establish the existence of mRNA
- 1963 resident consultant in US Arms Control and Disarmament Agency
  - Chemical an biological weapons programs and policies
- 1980 served as CIA consultant investigating a major anthrax outbreak
- 1973-1983 developed a model for recombination between DNA duplexes



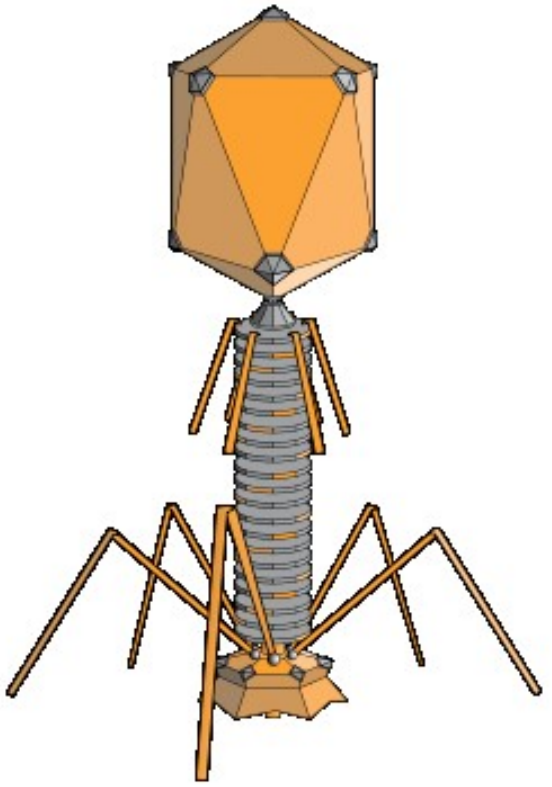
Frank William Stahl

- Born October 8, 1929 in Boston, Massachusetts
- 1951 A.B. degree in Biology from Harvard
- Began studying genetics after taking a course on phages at Spring Harbor
- 1956 Ph.D. or work on the T4 phage
- 1955 Post doc at Caltech in the Phage group
  - Bacterial genetics
  - Studied DNA replication with Meselson
- 1959 began working at University of Oregon as a Molecular biologist
  - Research involved T4 phage and budding yeast



The Phage Group

- Informal network of Biologist interested in both bacterial genetics as well as the origins of genes
- Lead by Max Delbruck
  - Max Delbruck
  - Salvador Luria
  - Alfred Hershey
  - Seymour Benzer
  - Gunther Stent
  - James Watson
  - Frank Stahl
  - Renato Dulbecco



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  - **Renato Dulbecco**

Matthew Meselson
Franklin W. Stahl

THE REPLICATION OF DNA IN ESCHERICHIA COLI

Biology
May 14, 1958

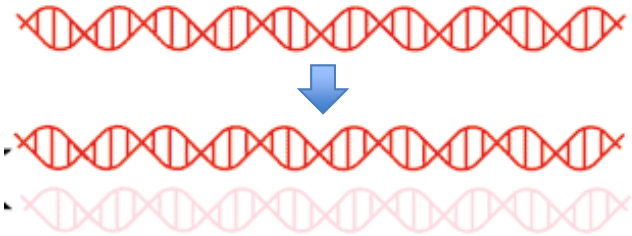
Purpose

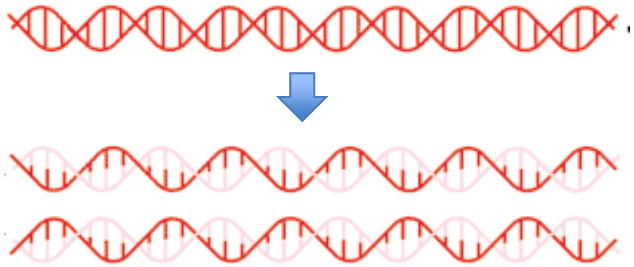
- Past studies show hat DNA can carry and transmit hereditary information
    - Chargoff
    - Hershey and Chase
    - Delbruck
- DNA has the capability to direct its own replication
    - Watson and Crick
    - Delbruck
- At this time there were a few hypothesis for how parental DNA was distributed among progeny molecules

Hypotheses for Distribution

Conservative

- Entire DNA acts as a template for new strands
- Parental DNA comes back together
- Progeny DNA comes together



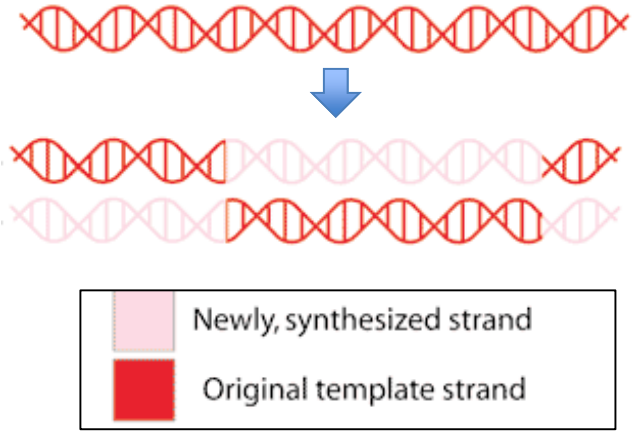


Semi-Conservative

- Watson and Crick Model
- Separation of two strands
- One strand serves as template for newly synthesized strand

Dispersive

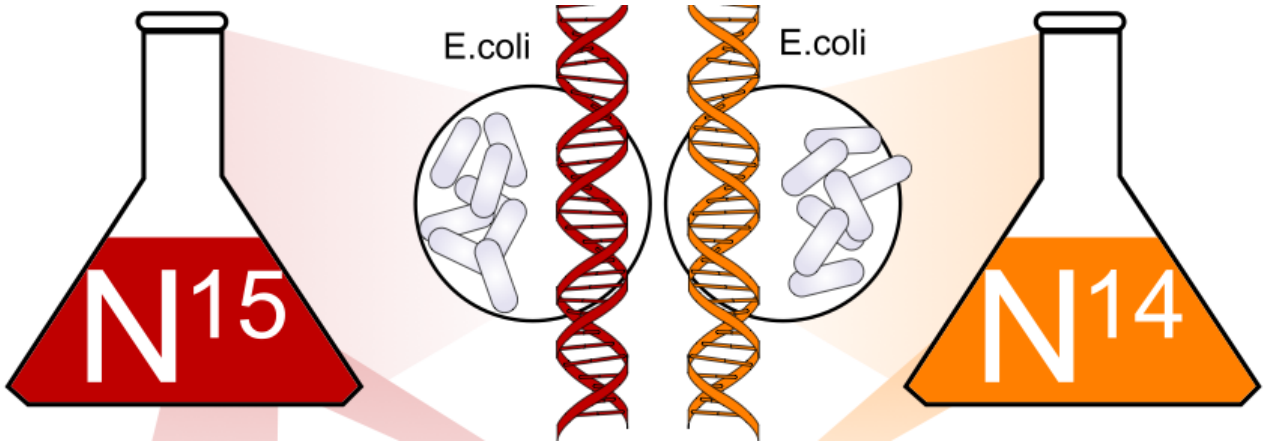
- Max Delbruck model
- Solves issues involved in unwinding
- Helix backbone broken along helix
- Short pieces can unwind and be copied
- Molecule is reformed by attaching alternating new pieces and old pieces



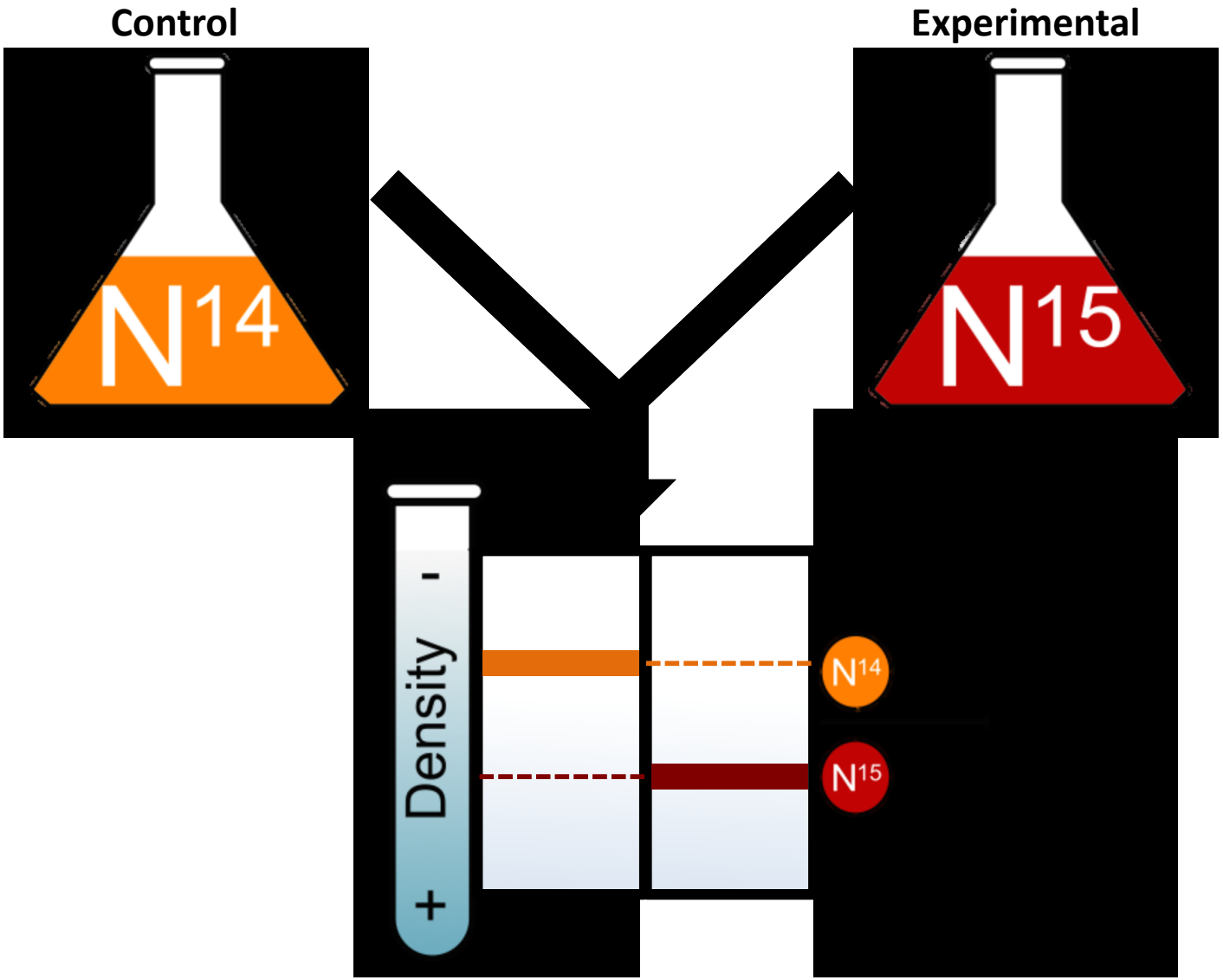
Following Molecules from Parental to Progeny

Radioisotopic labeling

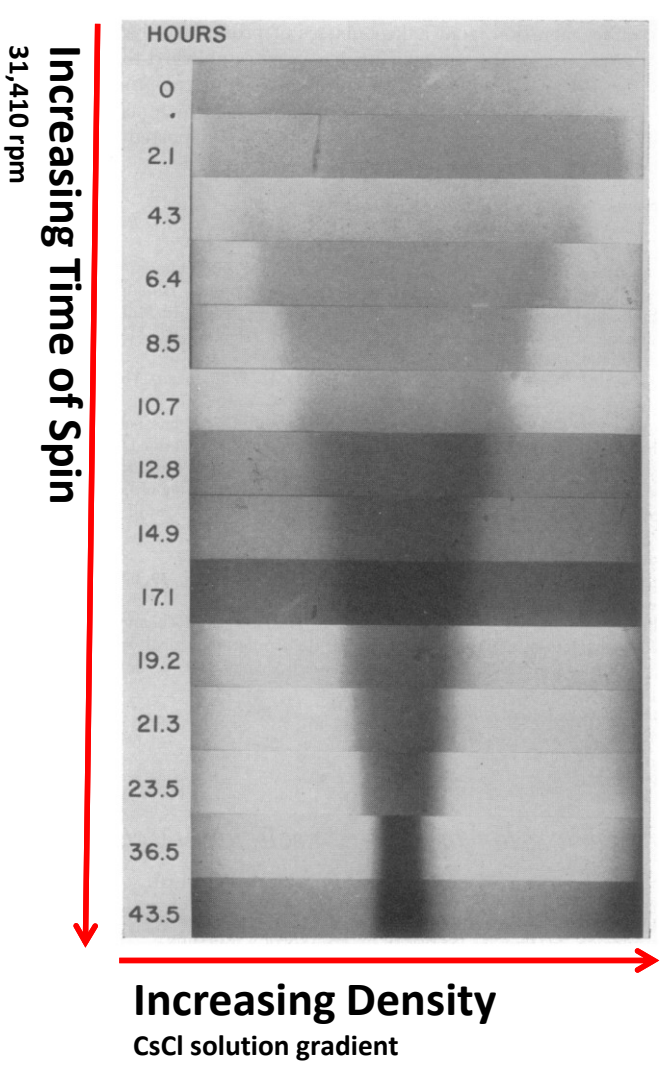
- Can be used to follow parental atoms into progeny
- Method concept
    - Increase density of nitrogen in parental molecules
    - Allow for replication to occur at lower nitrogen density
    - Examine the distribution by sedimentation analysis
        - Allows for detection of small density difference



Separation by Centrifugation



Banding of DNA by Centrifugation



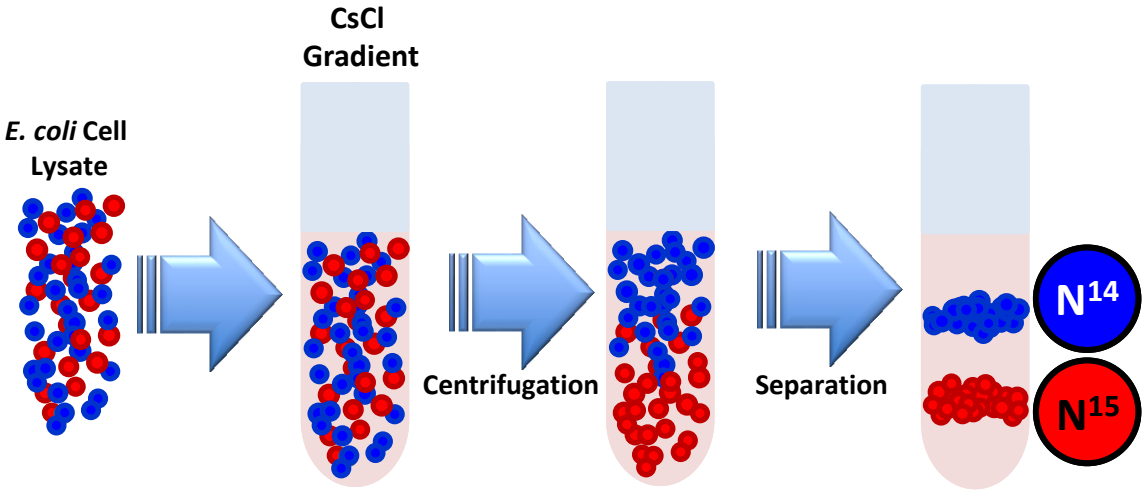
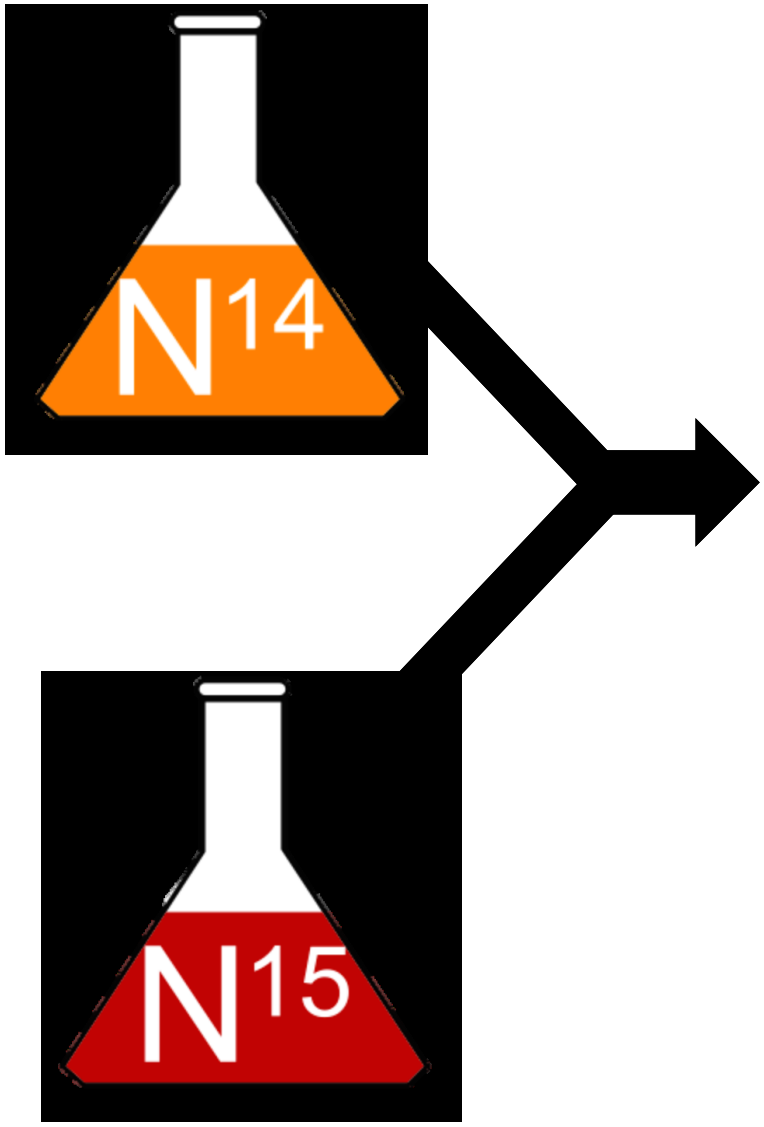
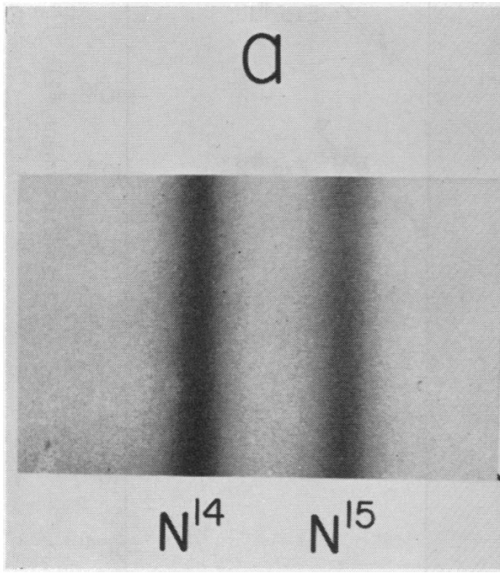


Figure 1

- Macromolecules of DNA are driven to the region of the gradient that they are "most like."
- Banding occurs in the density gradient that matches its own buoyant density

Determining Resolution of N^{14} vs N^{15}





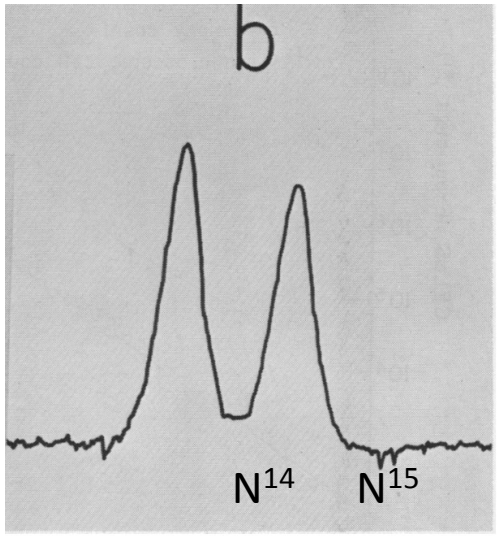
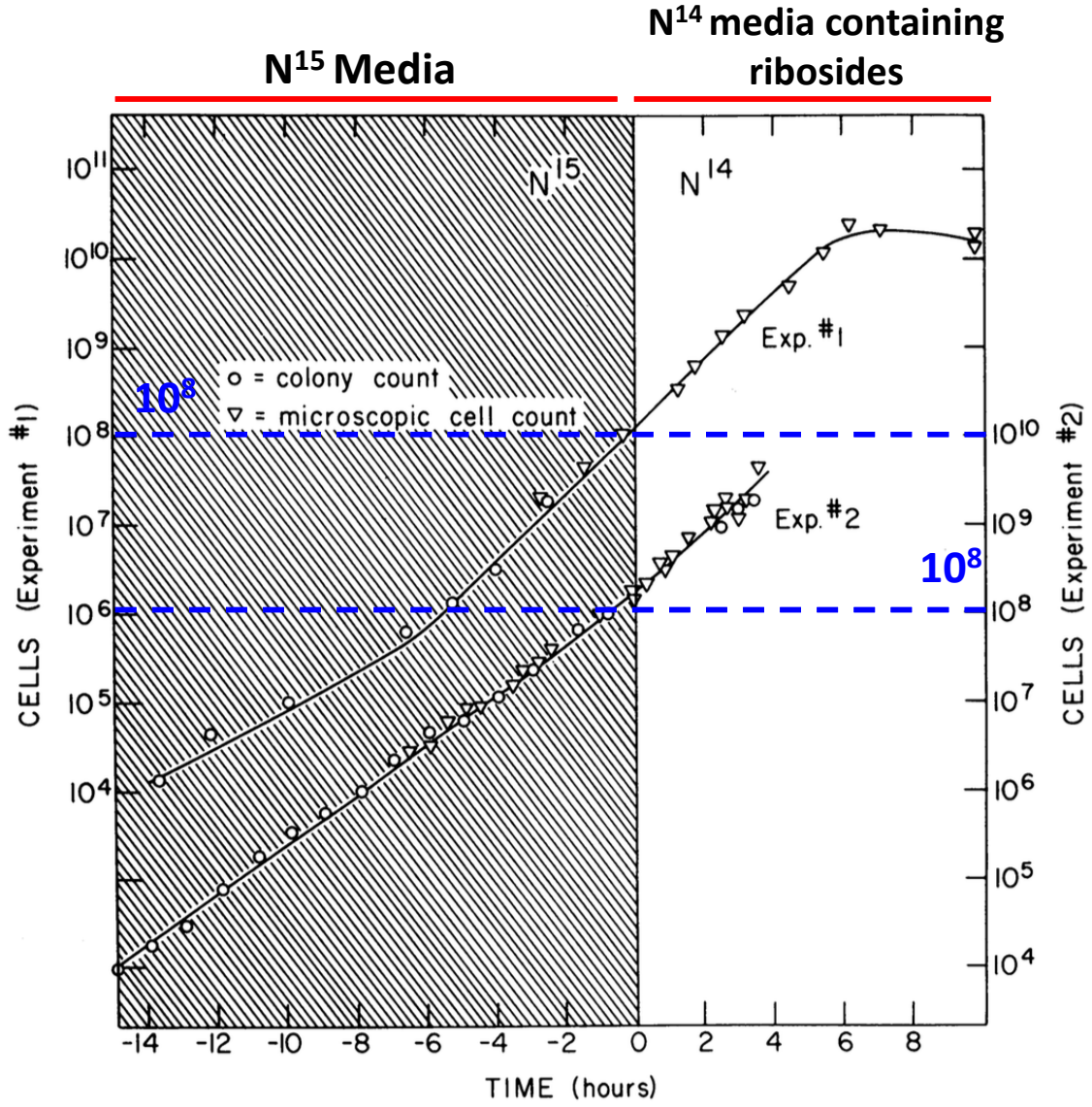


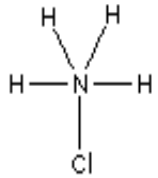
Figure 2:

- Mixture of equal amounts of N^{15} and N^{14} *E.coli* lysate were centrifuged
- Each band forms at the position most like its own buoyant density
- Labeled N^{15} can be discerned from unlabeled DNA

Microscopic Cell Counts and Colony Assays



Wildtype Growth

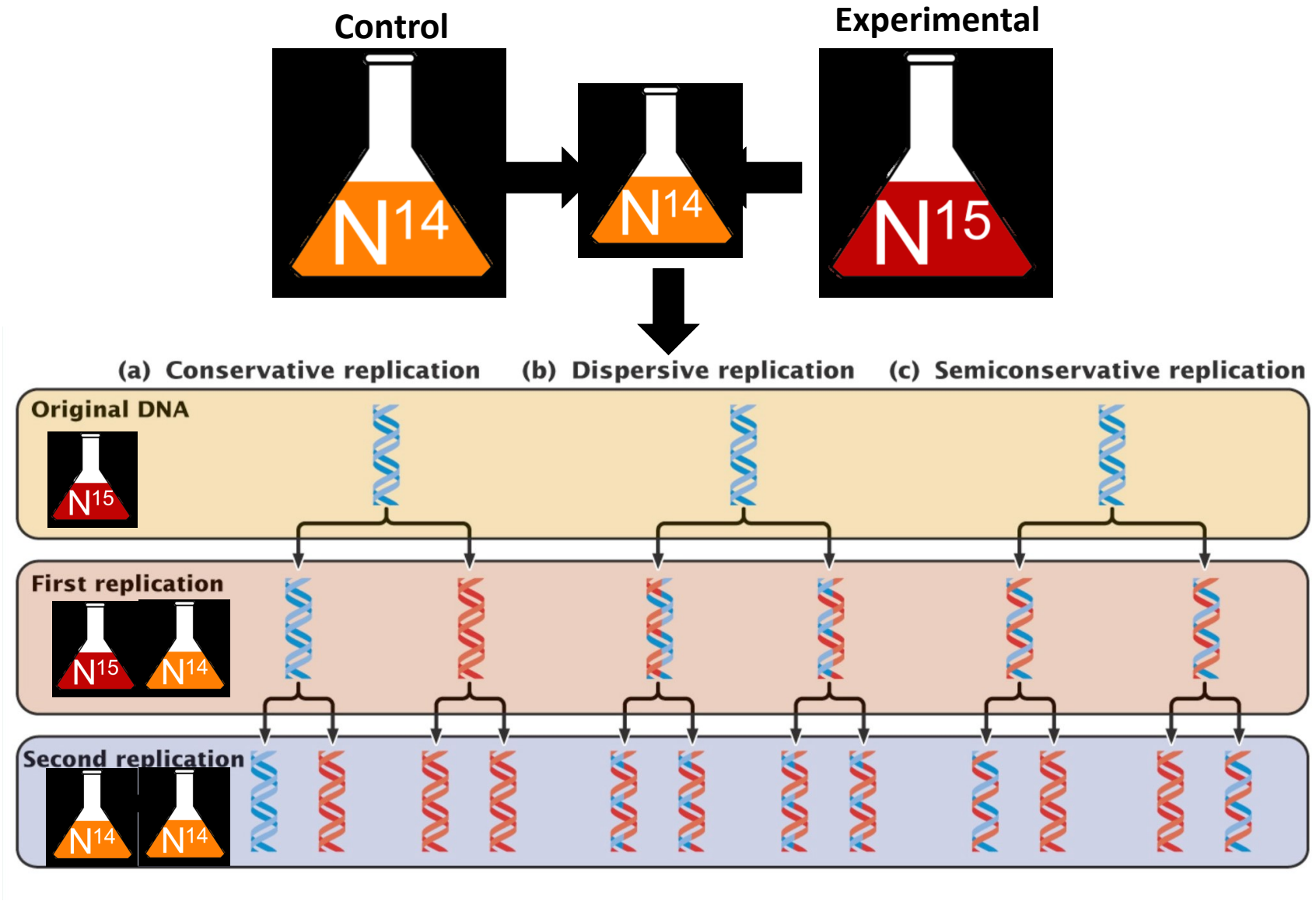


- Conditions
  - 36C
  - Aerated
  - Glucose with salts medium
  - Ammonium chloride as nitrogen source

N^{15} Growth

- Conditions
  - 100ug/ml of $N^{15}H_4Cl$ (14 generations)
  - 10 fold excess $N^{14}H_4Cl$ added
  - along with....
    - #1: adenine and uracil
    - #2: adenine, guanine, uracil, and cytosine

Determining Distribution



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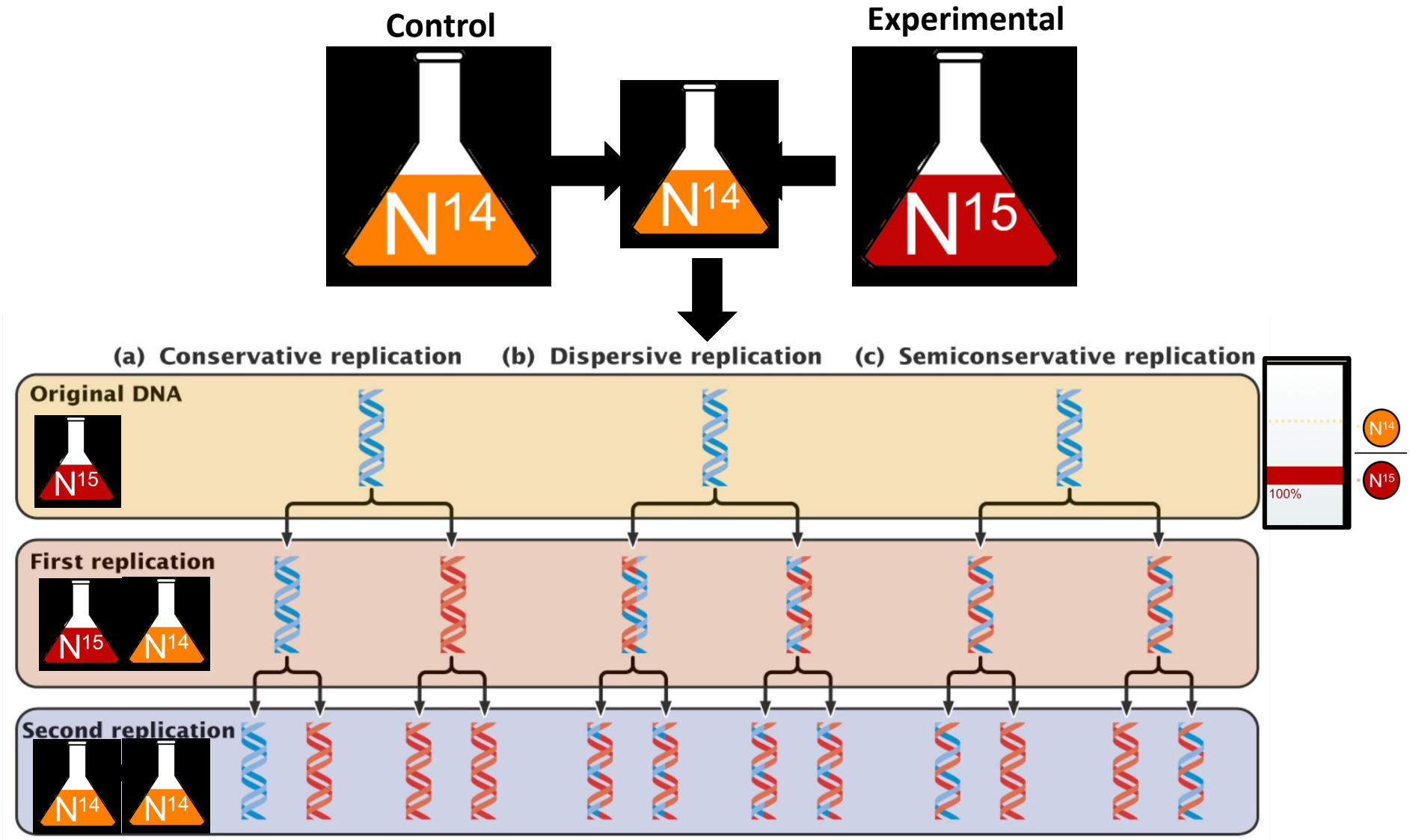
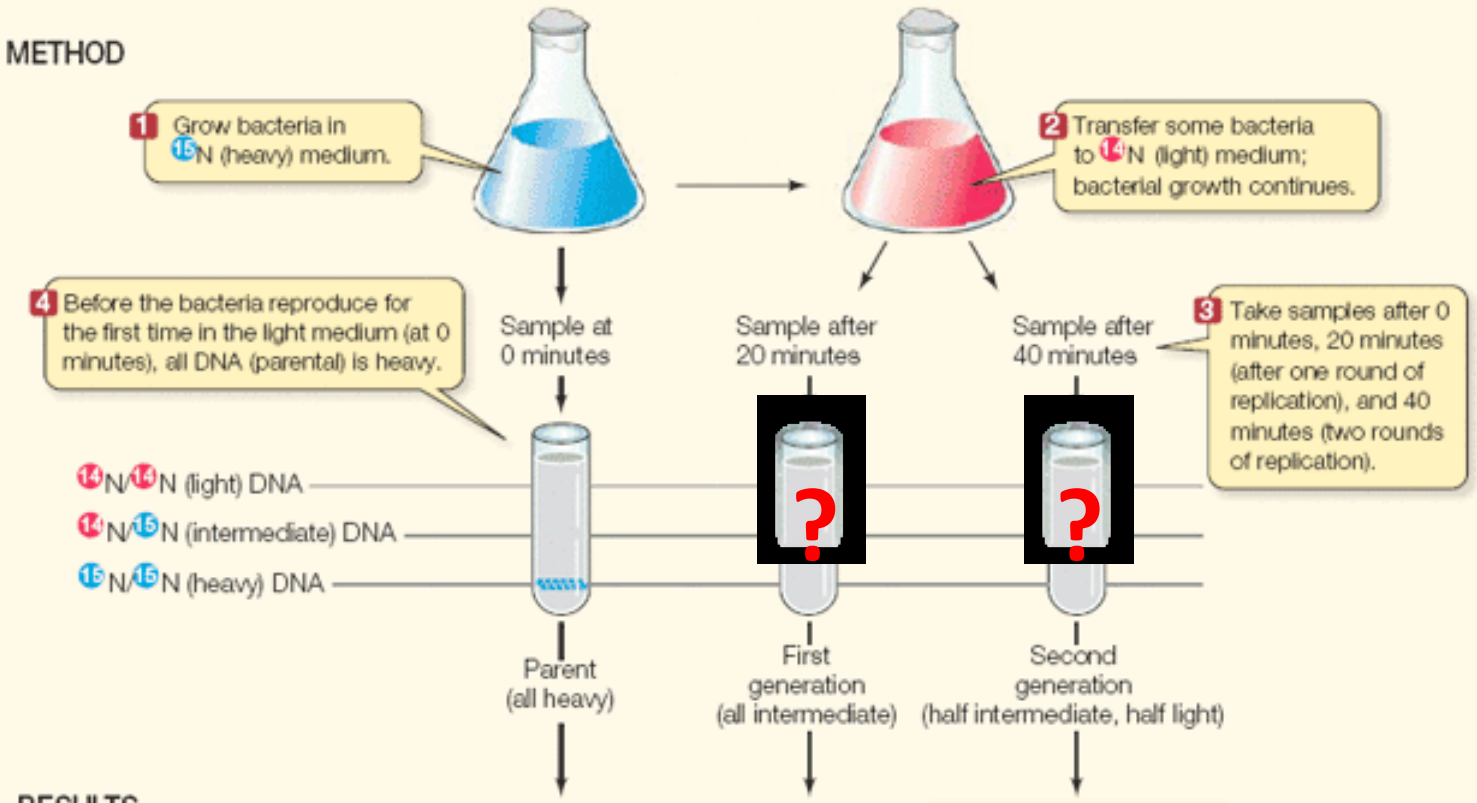


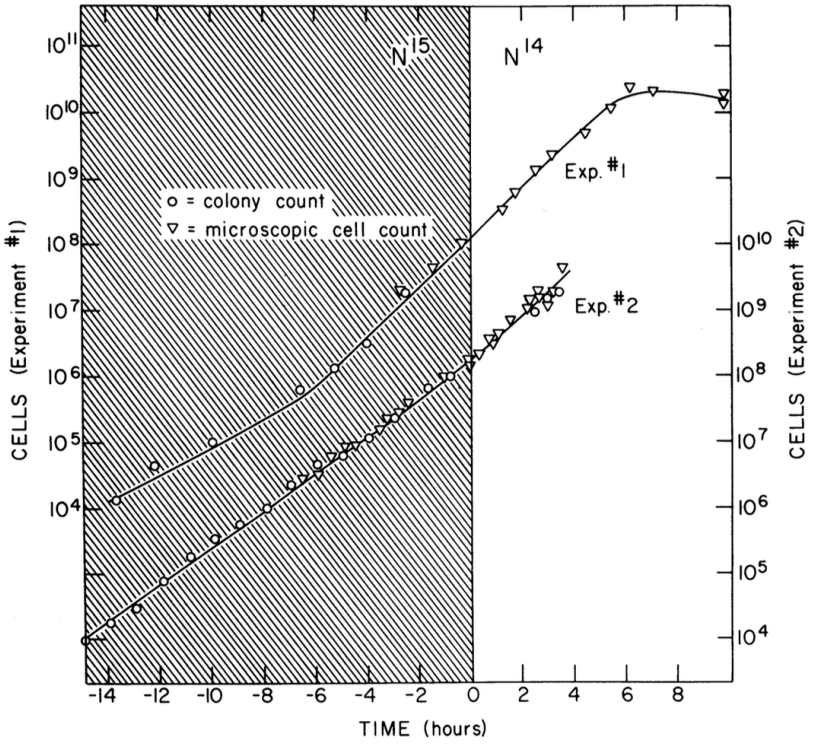
EXPERIMENT

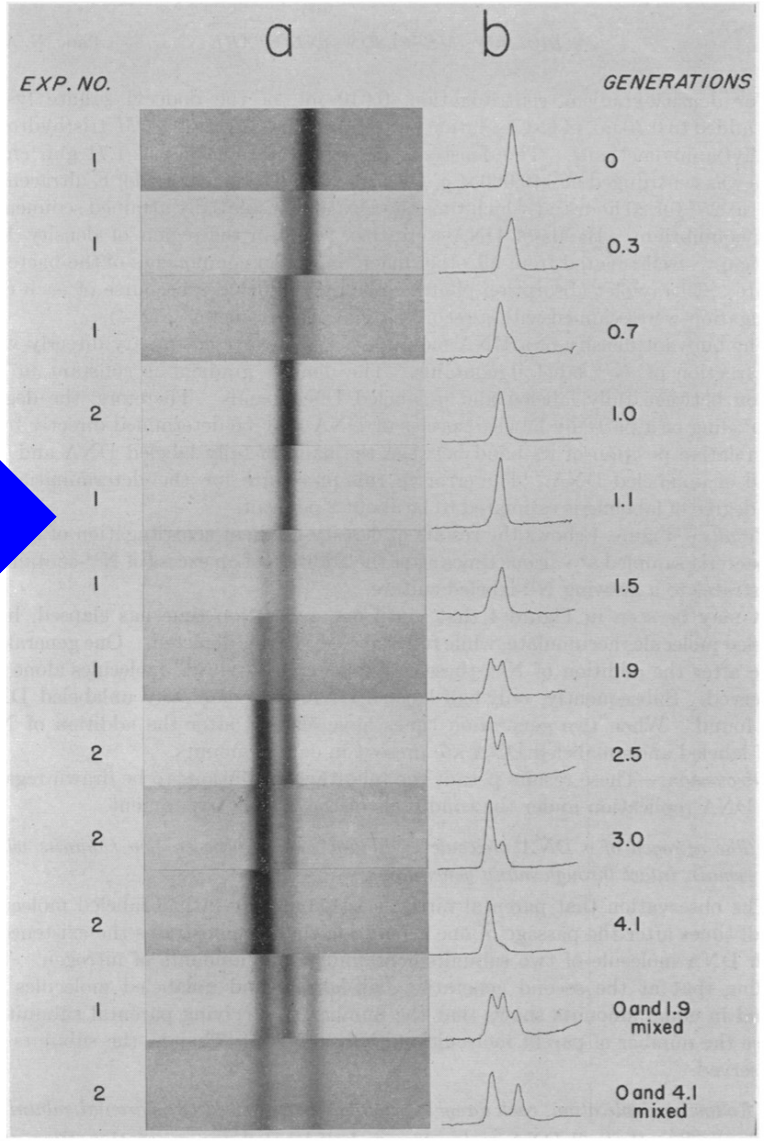
HYPOTHESIS: DNA replicates semiconservatively.



CONCLUSION: This pattern could only have been observed if each DNA molecule contains a template strand from the parental DNA; thus DNA replication is semiconservative.

Conservative, Semi, or Dispersive





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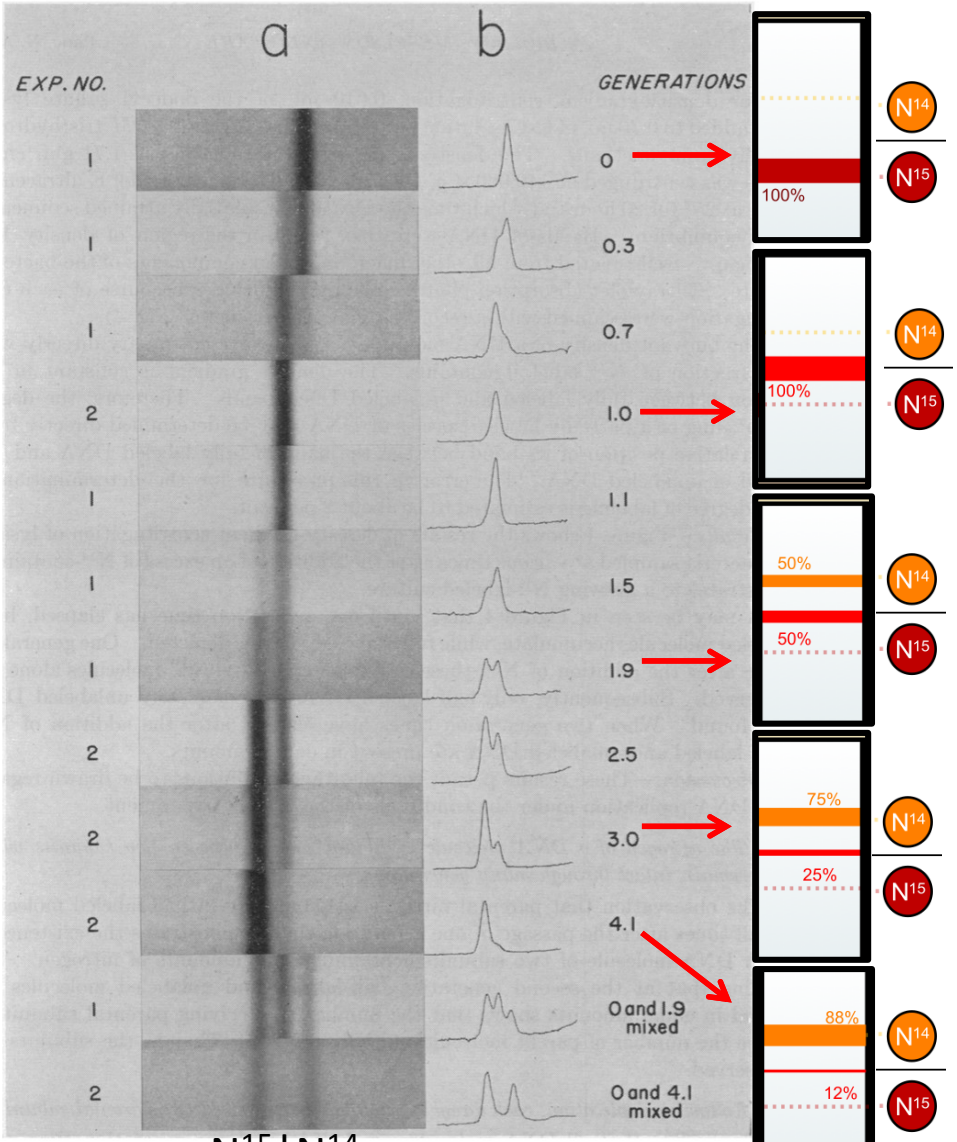
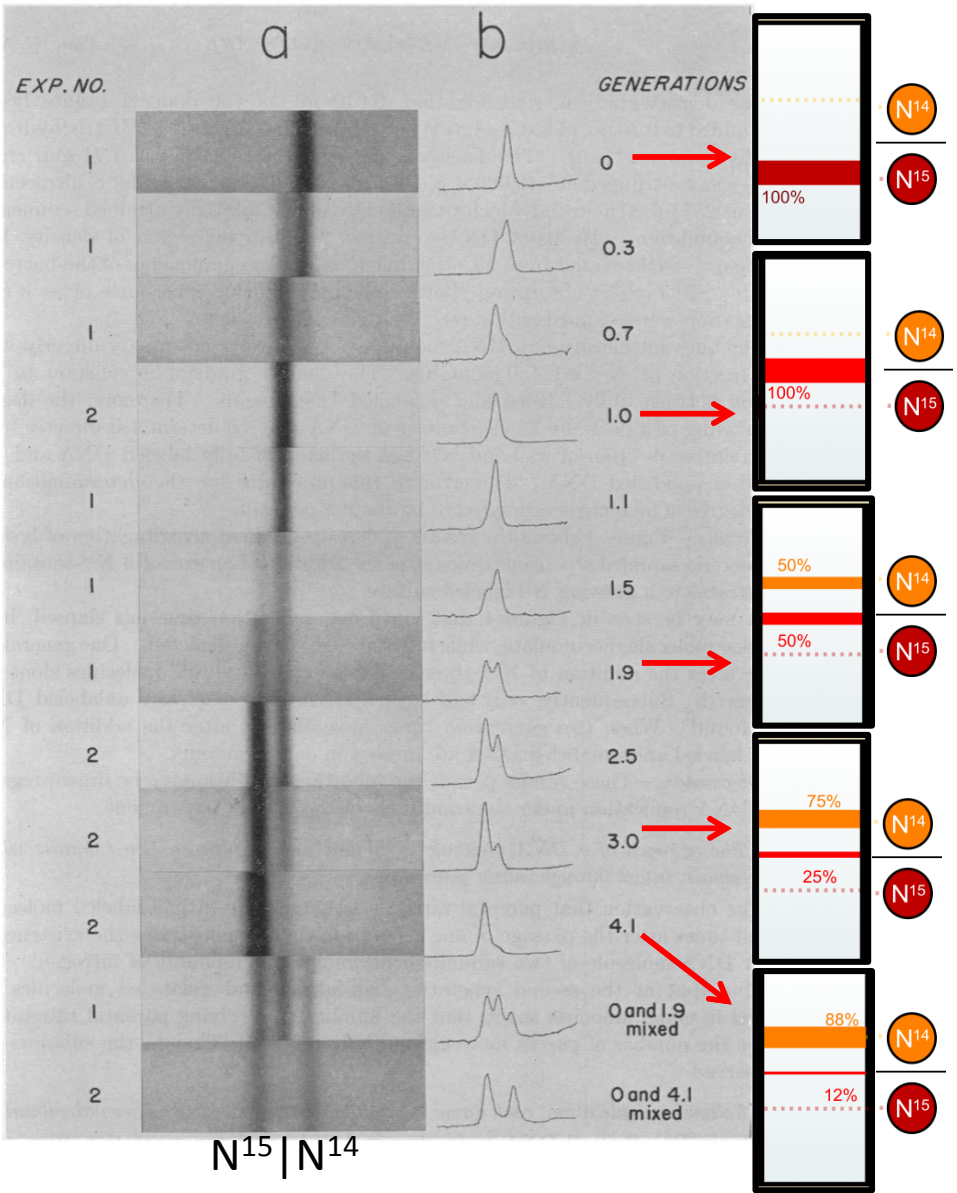


$N^{15} | N^{14}$

Conservative, Semi, or Dispersive



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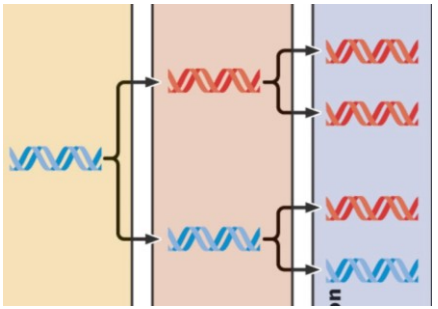
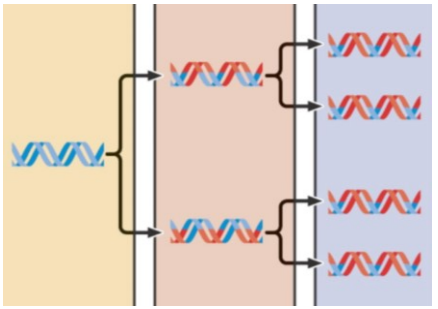
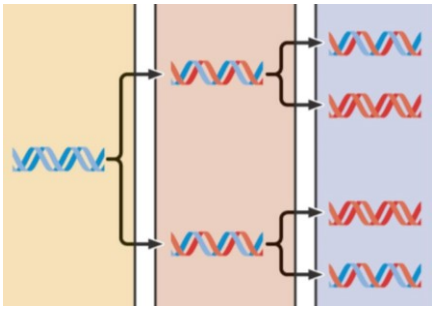
Conservative



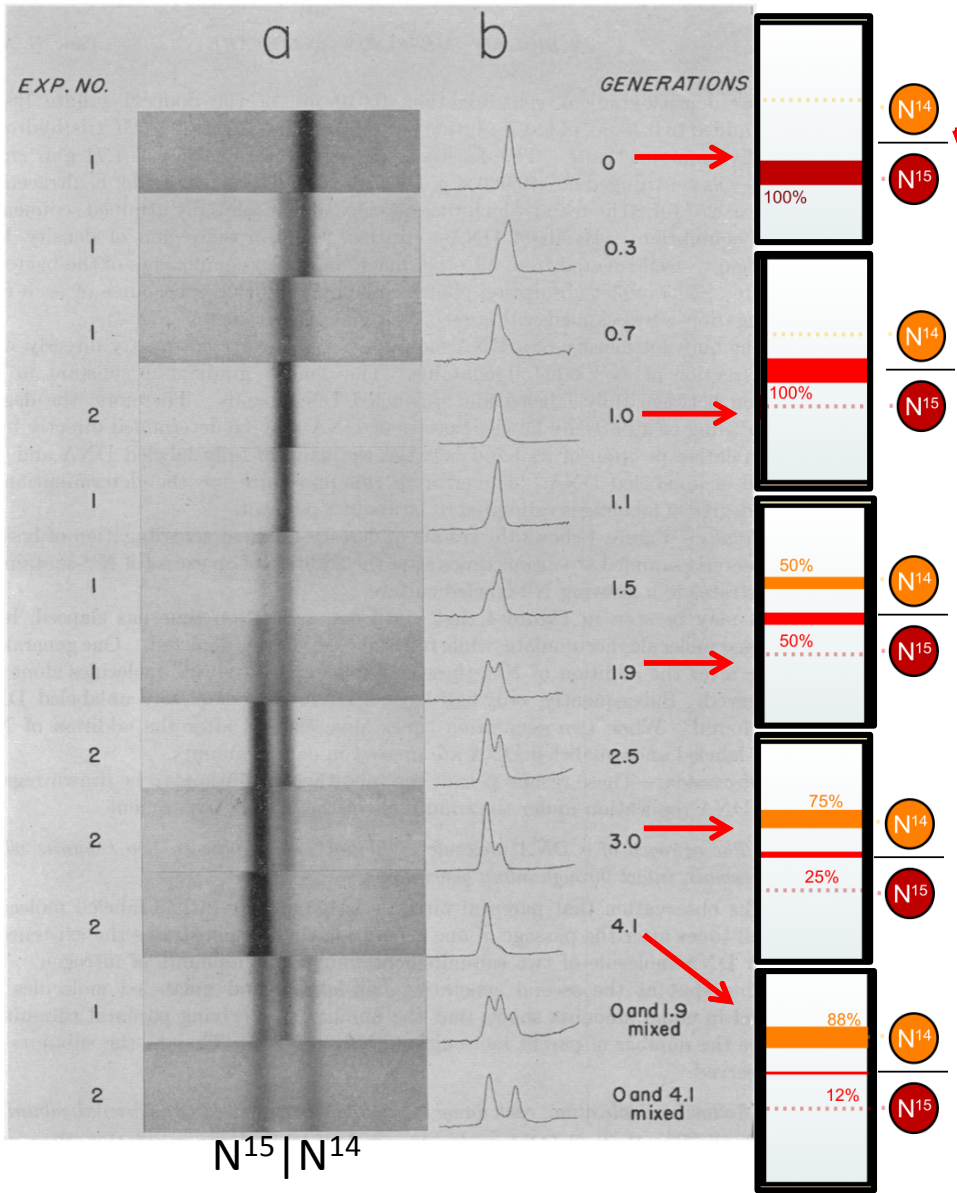
Dispersive

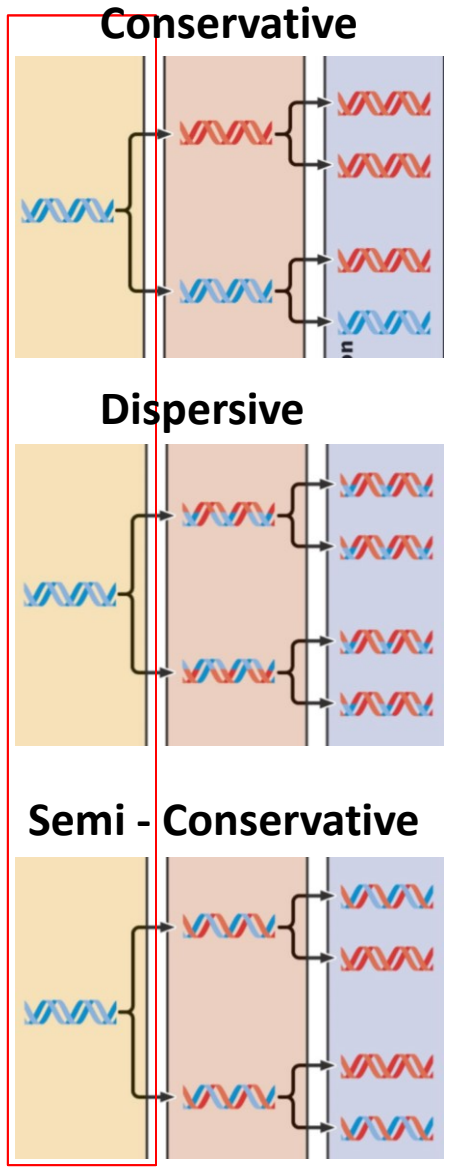


Semi - Conservative



Conservative, Semi, or Dispersive





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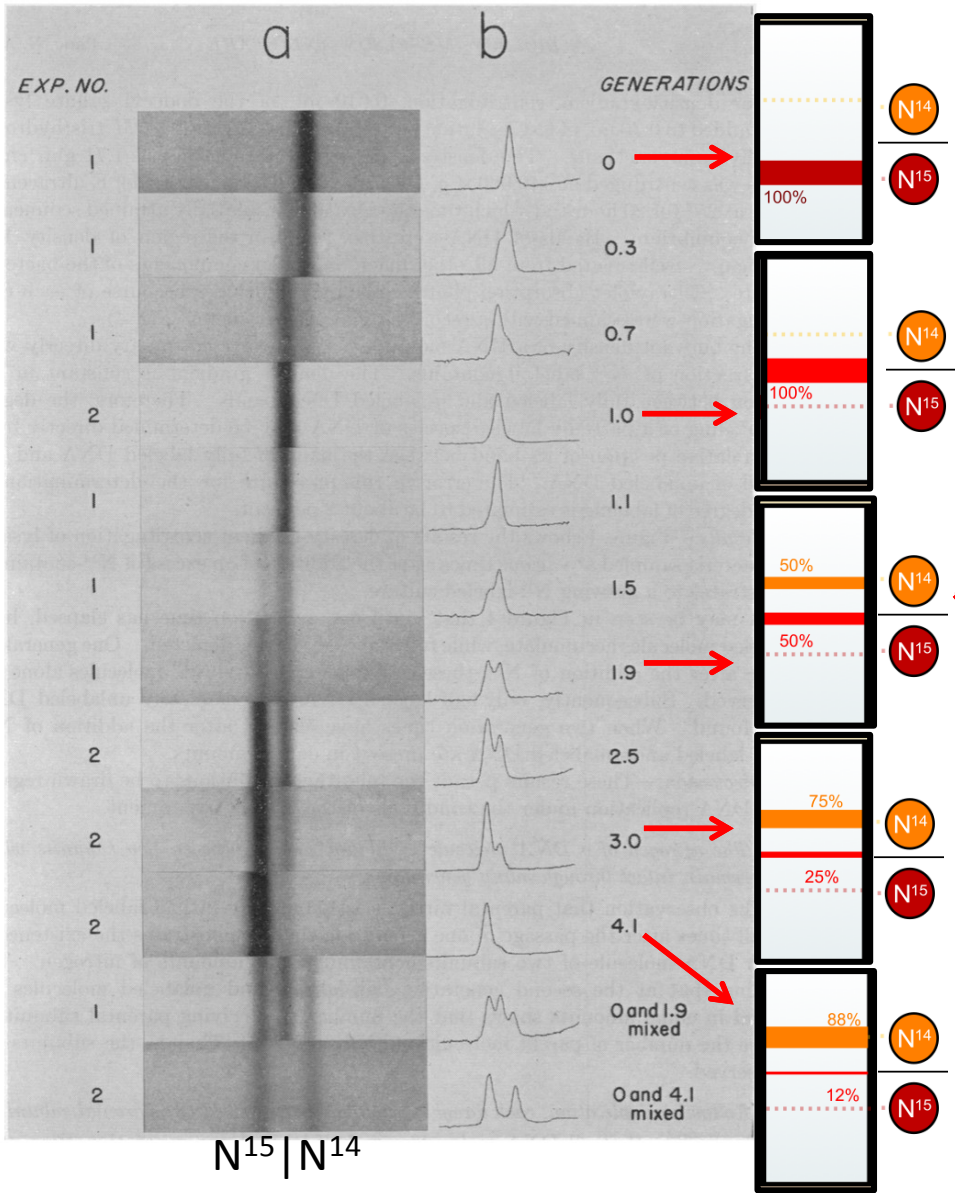
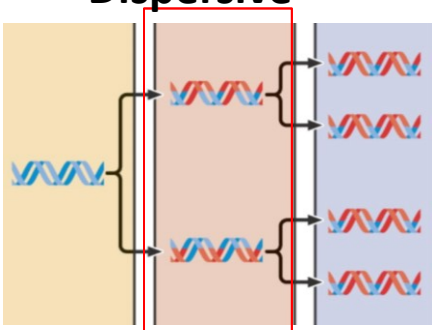


$N^{15} | N^{14}$

Conservative

Dispersive



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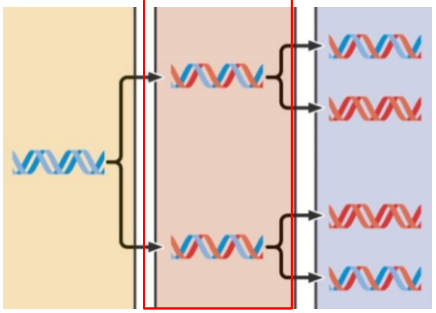

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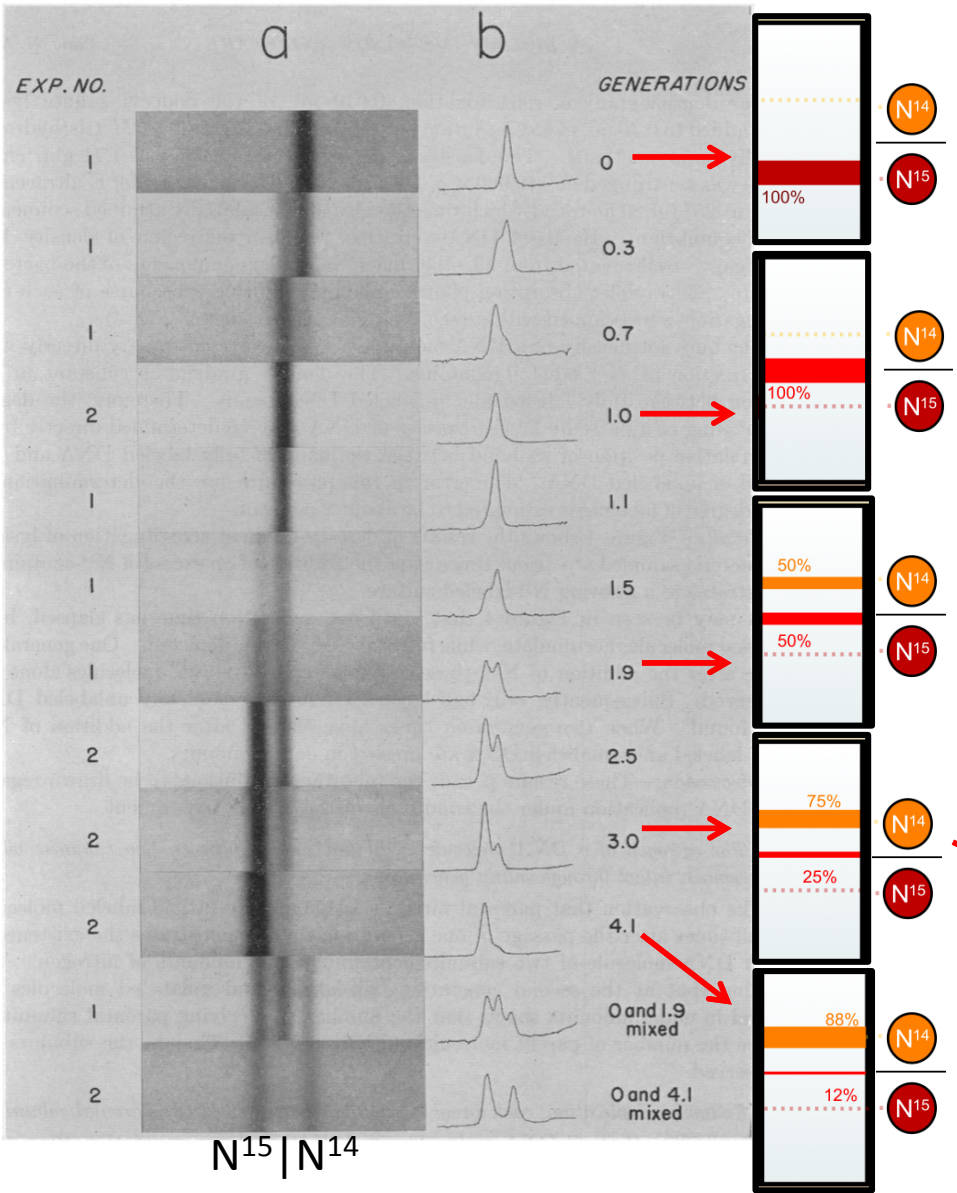
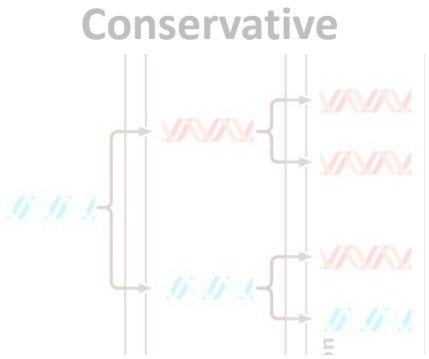
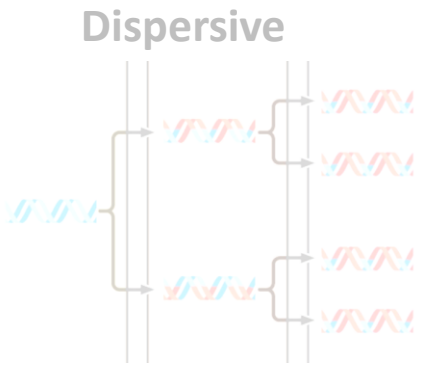
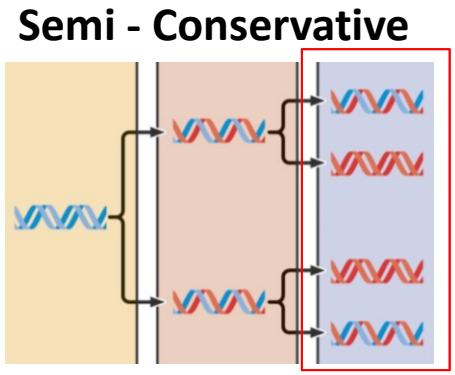


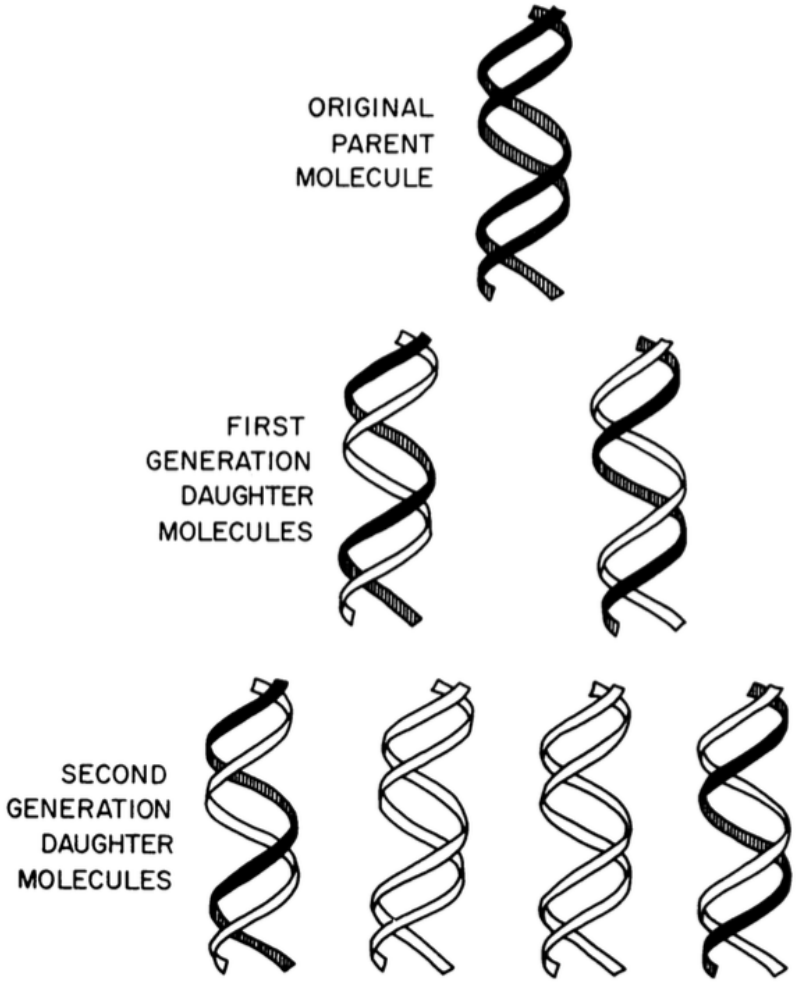




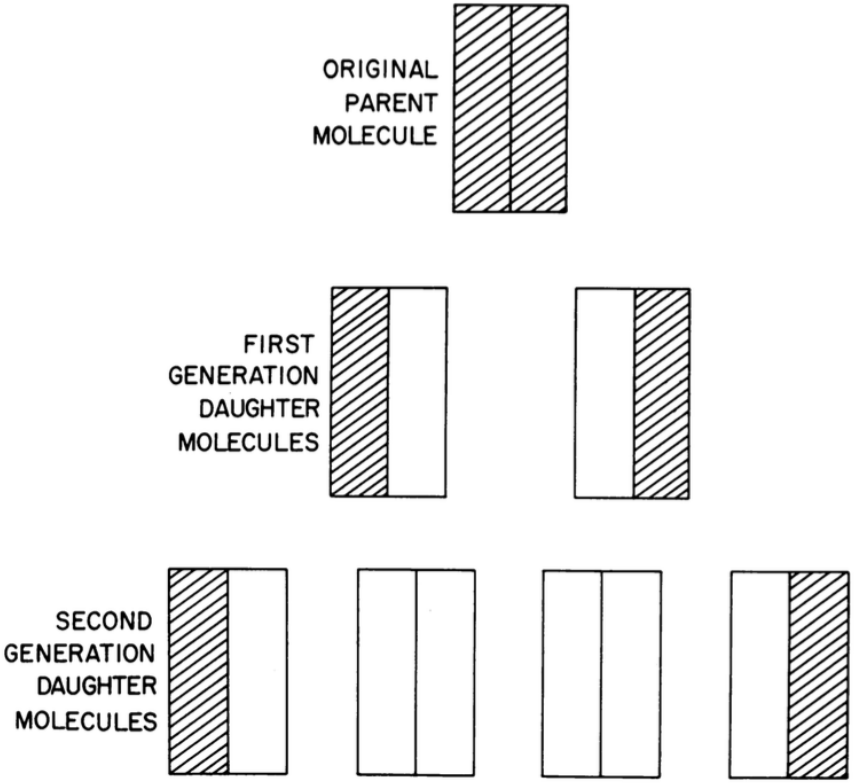


Models for DNA Duplication

Illustration of the DNA duplication mechanism proposed by Watson and Crick



Model established from Meselson-Stahl Centrifugation Experiments



Conclusions

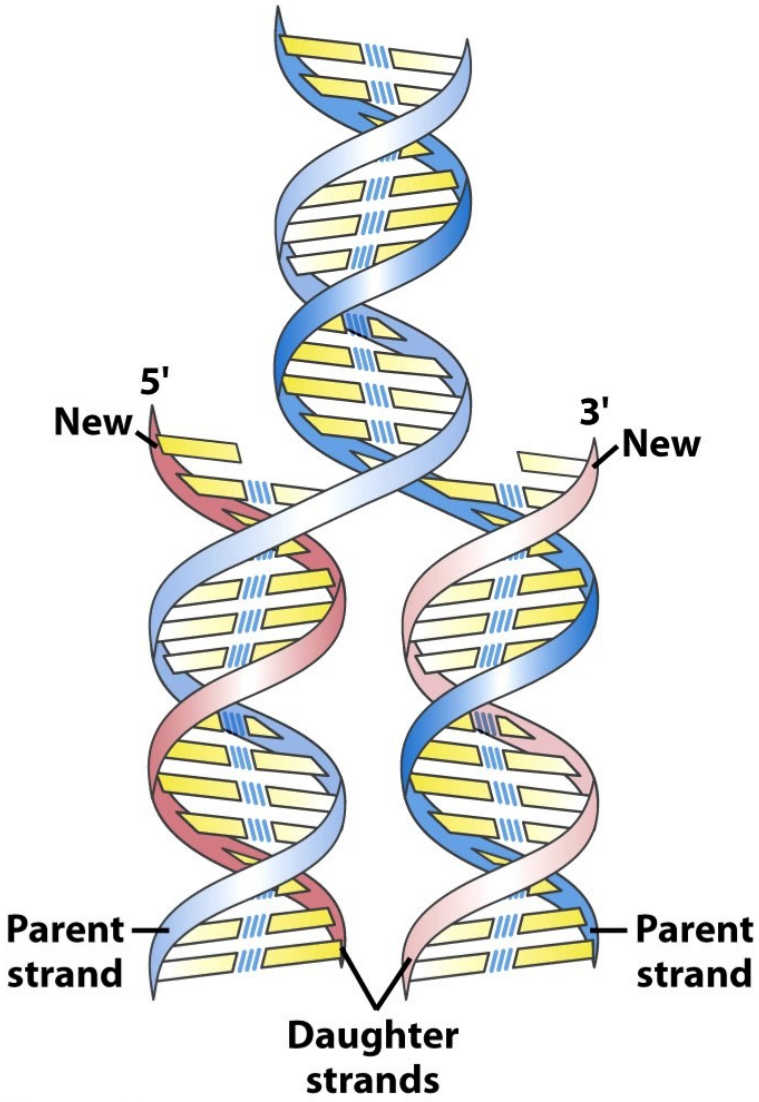


Figure 8-15
Lehninger Principles of Biochemistry, Fifth Edition

1. The nitrogen of a DNA molecule is divided equally between two subunits which remain intact through may generations

2. Following replication, each daughter molecule has received one parental subunit

3. The replicative act results in a molecular doubling

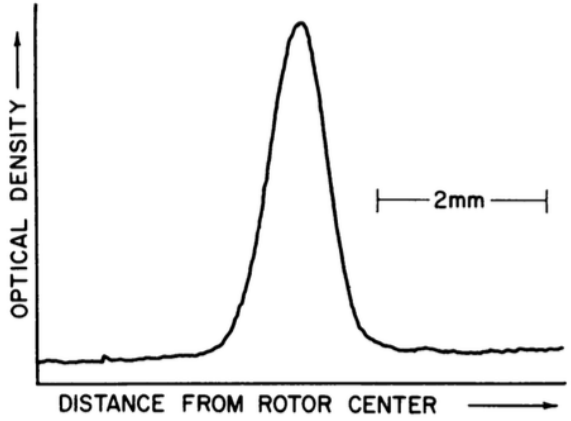
Mechanism for Replication

- Based on differences in DNA behavior from *E. coli* observed after heating
- Also on the ability of heat to cause collapse of rigid DNA structure
- Reported that collapse does not cause change in MW

Question:
If heat is changing the behavior and causing collapse of the structure but not changing the MW then what is happening to the DNA molecule?

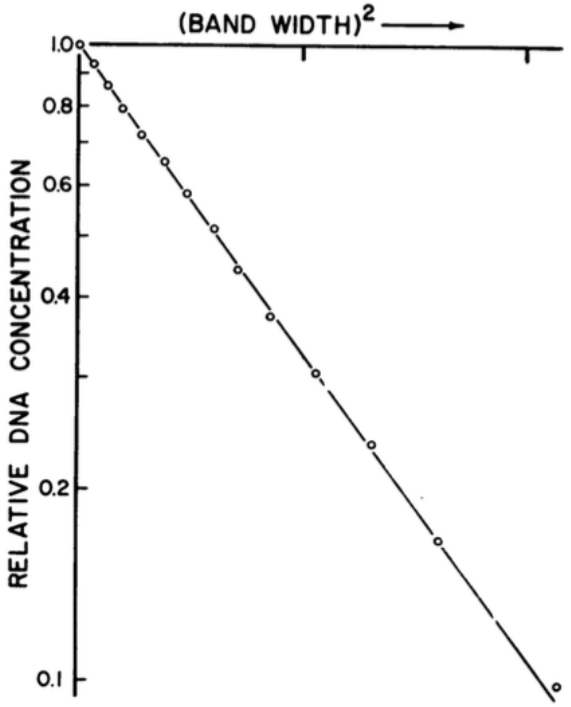
eated DNA Density

Density-gradient centrifugation of heated salmon sperm



Experiment

- Material heated to 100C for 30 min
- CsCl centrifugation



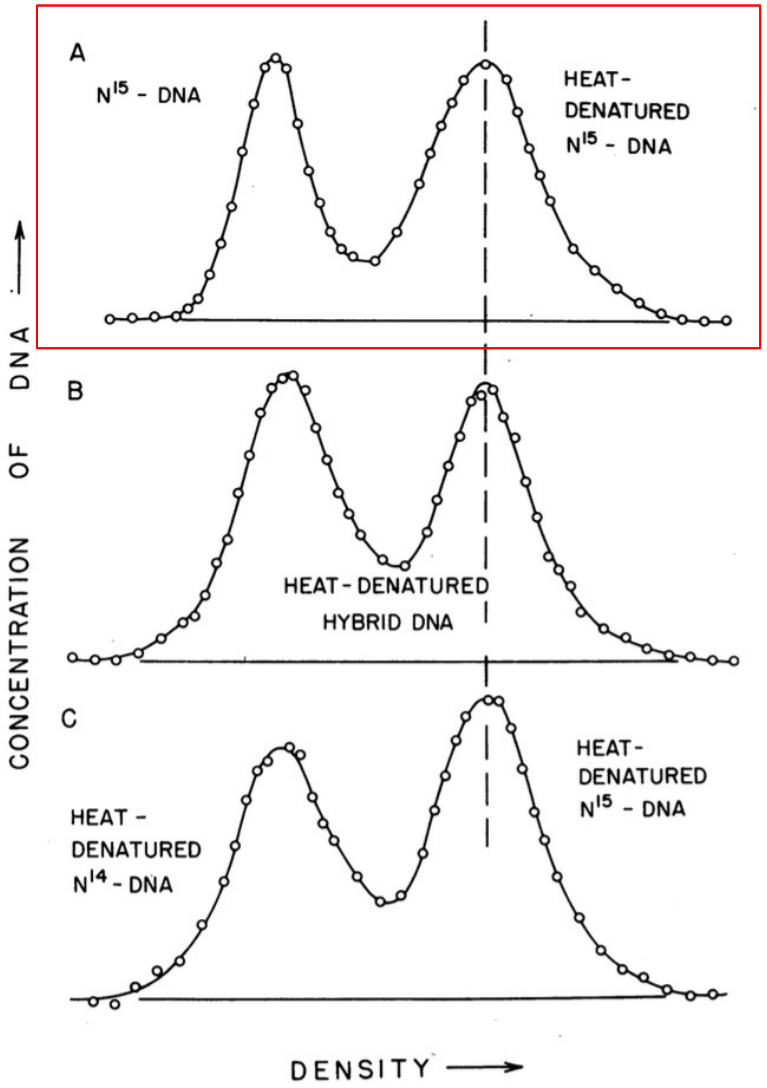
Results

- No change in apparent molecular weight
- Relative DNA concentration was not affected

Observations

- Shortened time required for band formation
  - increased diffusion indicated collapse of structure

Banding time of N^{14} vs N^{15}



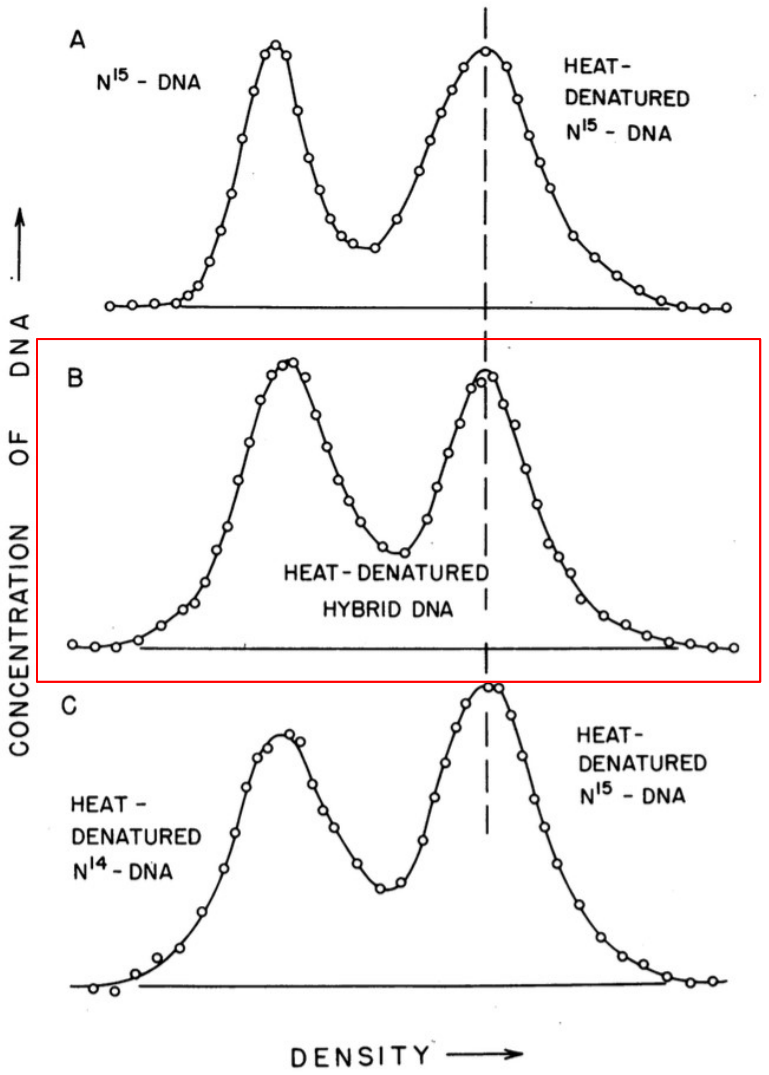
Experiment

- N^{15} material not heated
- N^{15} material heated to 100C for 30 min
- CsCl centrifugation of both DNA samples together

Results

- Apparent molecular weight of heated shifted to half unheated

Banding time of N^{14} vs N^{15}



Experiment

- Hybrid DNA molecule (N^{14} + N^{15})
- Heated to 100C for 30 min
- CsCl centrifugation

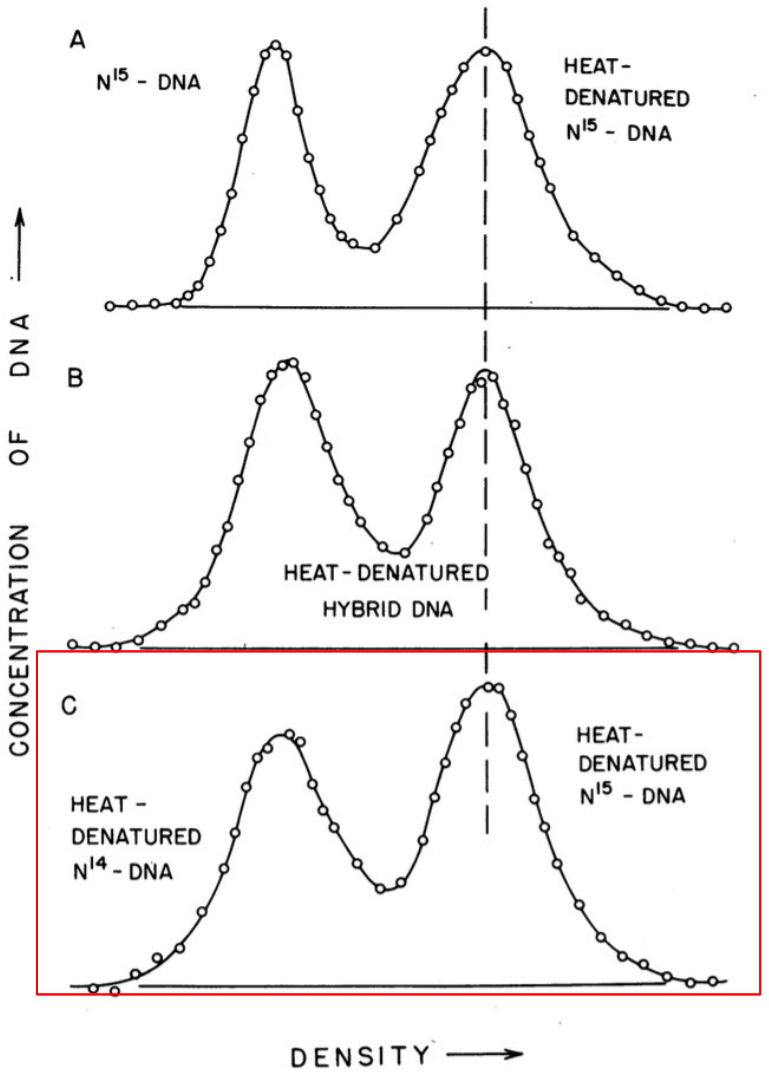
Results

- Loss of original half labeled material
- Formation of two new density species
- N^{14} separated from N^{15}

Conclusions

- Heating the hybrid molecule brings about the dissociation of the two subunits form one another

Banding time of N^{14} vs N^{15}



Experiment

- Mixture of N^{14} and N^{15}
- Heated to 100C for 30 min
- CsCl centrifugation

Results

- Resembled both heating DNA separately and the hybrid

Conclusion

- Clear evidence that the two molecular subunits have dissociated upon heating

Major Conclusions

- Apparent MW obtained for each subunit is half that of the intact molecule
  - Subuints of DNA molecule are single, continuous structure
    - Rules out Delbruck DNA replication scheme
- To replicate, DNA dissociates into two subunits which are conserved during duplications
- Duplication occurs in a semi-conservative manner