The Replication of DNA in Escherichia Coli

By Matthew Meselson and Franklin W. Stahl

1958

Background

In 1952, Hershey and Chase developed experiments that proved that DNA was hereditary material

In 1953, Watson and Crick proposed DNA was double helix structure and suggested that it replicated semi-conservatively

But How Does DNA Replicate?
- Hypotheses of this DNA replication differed concerning the distribution of parental material among the progeny molecules

Three Prominent Theories of DNA Replication Emerged:
1. Conservative
2. Semi-conservative
3. Dispersive

Meselson and Stahl used this process of density-gradient centrifugation to determine the molecular weight of DNA to conclude that DNA replicates semi-conservatively

- Previous experiments (1956-1958) used radioisotopic labels to determine the distribution of parental atoms among progeny molecules in several organisms
  - DNA was labelled with N\textsuperscript{15} and N\textsuperscript{14}
    - N\textsuperscript{15} is more dense
  - A concentrated sample of DNA was then mixed with a solution of cesium chloride (CsCl) and was then centrifuged until equilibrium was approached which produced a stable concentration gradient of CsCl
  - Each species of DNA will form a band at the position where the density of CsCl solution is equal to the buoyant density of that DNA species

Experiment

1. E. Coli were grown in a medium of glucose salts with ammonium chloride as the sole source of nitrogen
population growth was monitored by cell count and colony assays

2. N\textsuperscript{15} bacteria were prepared by washing cells for 14 generations
   - N\textsuperscript{15} was incorporated into cells DNA
   - DNA containing N\textsuperscript{15} was distinguishable due to the higher density

3. Cells with DNA containing N\textsuperscript{15} was transferred to a medium that contained N\textsuperscript{14}
   - N\textsuperscript{14} was incorporated into any new DNA that was synthesized

4. Sample of bacteria was sampled from the culture just before the addition of N\textsuperscript{14} and at intervals for several generations after
   - Each sample was chilled and centrifuged in the cold for 5 minutes
   - Resuspended in a cold solution of NaCl and EDTA, which prevented further growth/deterioration
   - Cells were lysed by the addition sodium dodecyl sulfate and stored in the cold

5. Each sample then underwent density-gradient centrifugation
   - Centrifuged for 20 hours
   - Ultraviolet absorption photographs were taken during the course of each centrifugation

**Bouyant density of a DNA molecule will vary directly in relation to the fraction of the N\textsuperscript{15} label it contains.**

- The density gradient is constant in the region between fully labelled N\textsuperscript{15} and the unlabelled DNA bands
- The degree of labelling can be determined by the relative position of the DNA within this gradient
  - Estimated 2\% error

**Video**

**Results**

Meselson and Stahl found 3 main points:

1. *The nitrogen of a DNA molecule is divided equally between two subunits which remain intact through many generations*
   - After the second generation, it can be seen that DNA is comprised of two equal subunits of nitrogen. One subunit is labelled with N15, and the other a newly synthesized, unlabelled subunit

2. *After replication each daughter molecule contains one parental subunit*
   - If DNA was replicated any other way than semiconservatively then some daughter cells would by fully labelled and some would be fully unlabelled, meaning that some cells received two or no parental subunits respectively

3. *The replicative act results in a molecular doubling*
   - Each daughter molecule receives one of the two parental subunits

**Discussion**

The Meselson and Stahl experiment supported the *Watson and Crick model of DNA duplication.*
They proved that DNA replication is semi-conservative and DNA strands are conserved throughout generations but not throughout cells.

- two complimentary strands of DNA bonded together by hydrogen bonds
- Proposed the idea that the two chains separate, and synthesis of the complimentary chain begins according to the base pairing restrictions
- Accordingly each daughter molecule contains one parental chain, paired with a newly synthesized chain

**Heat Denaturation**

- The DNA molecules derived from E. coli by detergent-induced lysis have a bouyant density in CsCl similar to that of T2 an T4 bacteriophage DNA and purified calf-thymus and salmon-sperm DNA
- DNA heated under certain conditions react as follows:
  - Salmon-sperm and E. coli DNA collapses and undergo a similar density increase
  - Salmon DNA retains original weight
  - Bacterial DNA disassociates into the two subunits which are conserved during replication.
- These findings can be interpreted 2 ways:
  - assuming salmon DNA contains subunits analogous to those found in E. Coli DNA, then the subunits of salmon DNA are bound together more tightly than those of bacterial DNA.
  - assuming the molecules of salmon DNA do not contain these subunits, then bacterial DNA molecules are more complex than salmon DNA. However, this challenges the Watson-Crick DNA model to explain the observed distribution of parental nitrogen atoms among progeny molecules.

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