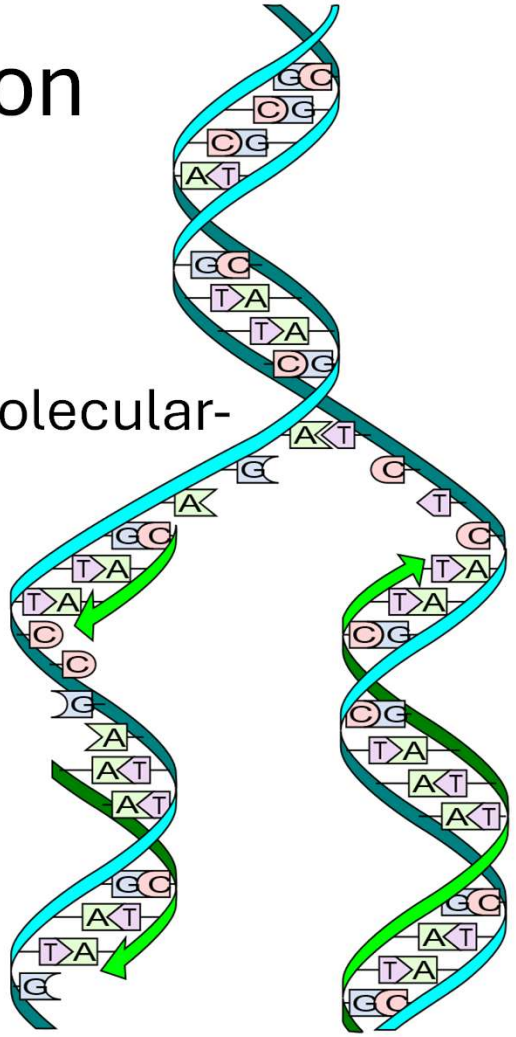
Jerusalem Science Contest

Molecular Biology

Lecture 2: DNA Replication

Outside reading:

https://www.nature.com/scitable/topicpage/major-molecular-events-of-dna-replication-413/



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DNA Replication – The Essence of Heredity

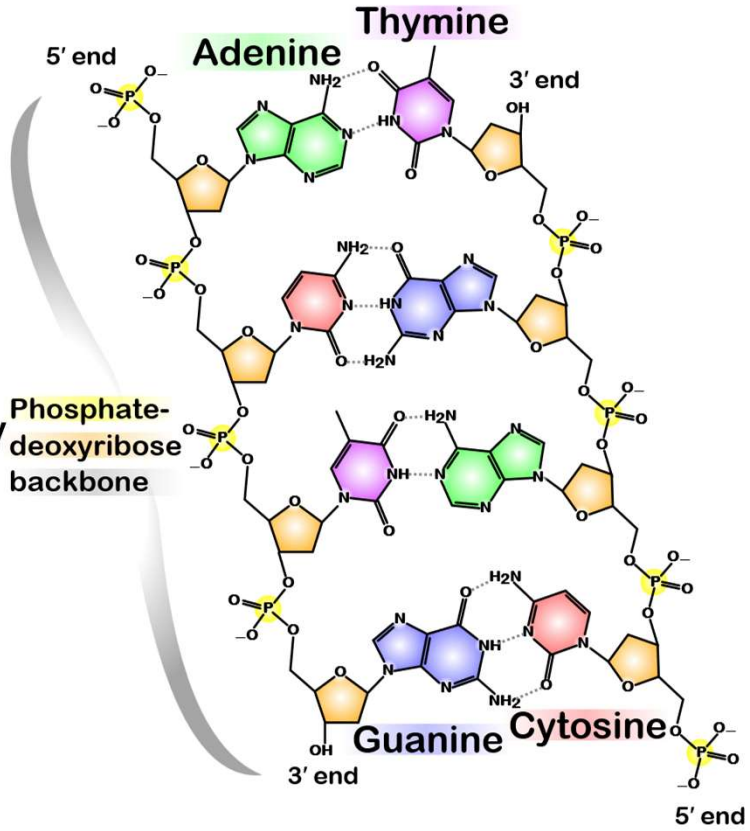
- In previous lecture we learned how DNA was identified as molecule of inheritance
- Every time a cell divides it must accurately copy the DNA
- One copy of the complete **genome** must go into each “daughter” cell

The DNA Molecule

- As a reminder, DNA is an antiparallel double helix
- Each nucleotide base on one strand pairs with a complementary nucleotide base on the other strand: A with T, G with C
- This complementary pairing is the basis for exact replication of the DNA molecule: one strand serves as the template for copying the other strand

The DNA Molecule

- Each strand has two ends: the 5’ end and the 3’ end, which are chemically distinct
- This is what gives directionality to the molecule
- The 5’ end of one strand pairs with the 3’ end of the other strand, and vice versa



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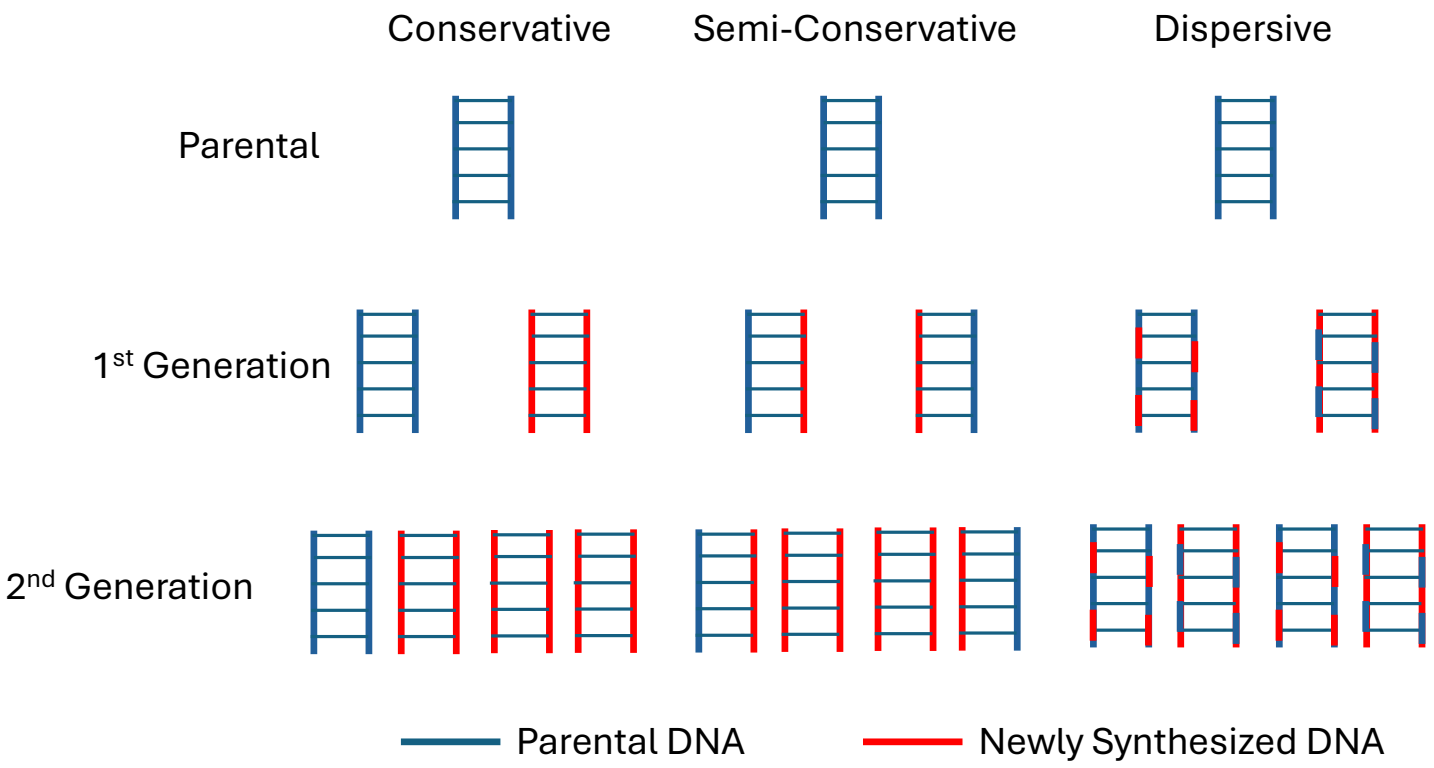
Three Models for Replication

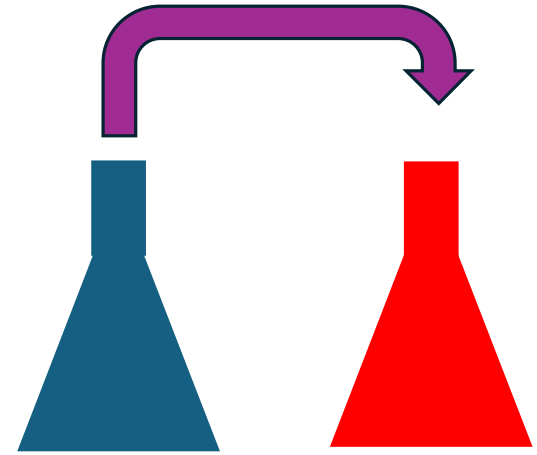
- Once Watson and Crick determined the DNA structure, 3 hypotheses arose to explain replication:
- **Conservative model**: The "parental" DNA molecule remains intact, and a completely new "daughter" molecule is synthesized
- **Semi-conservative model**: Replication generates two DNA molecules, each containing one "parental" strand and one newly synthesized strand
- **Dispersive model**: The two "daughter" DNA molecules contain both "parental" and newly synthesized sequences randomly distributed in both strands

Three Models for Replication



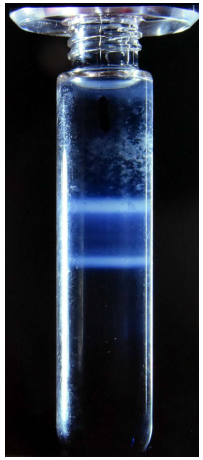
Meselson-Stahl Experiment (1958)

- Matthew Meselson and Franklin Stahl conducted an experiment to distinguish between the three models
- Grew *E. coli* bacteria in medium containing exclusively "heavy" nitrogen (^{15}N) for many generations so that all the nitrogen in the DNA would be ^{15}N
- Bacteria were switched to medium containing "light" nitrogen (^{14}N, the more common isotope) and allowed to replicate



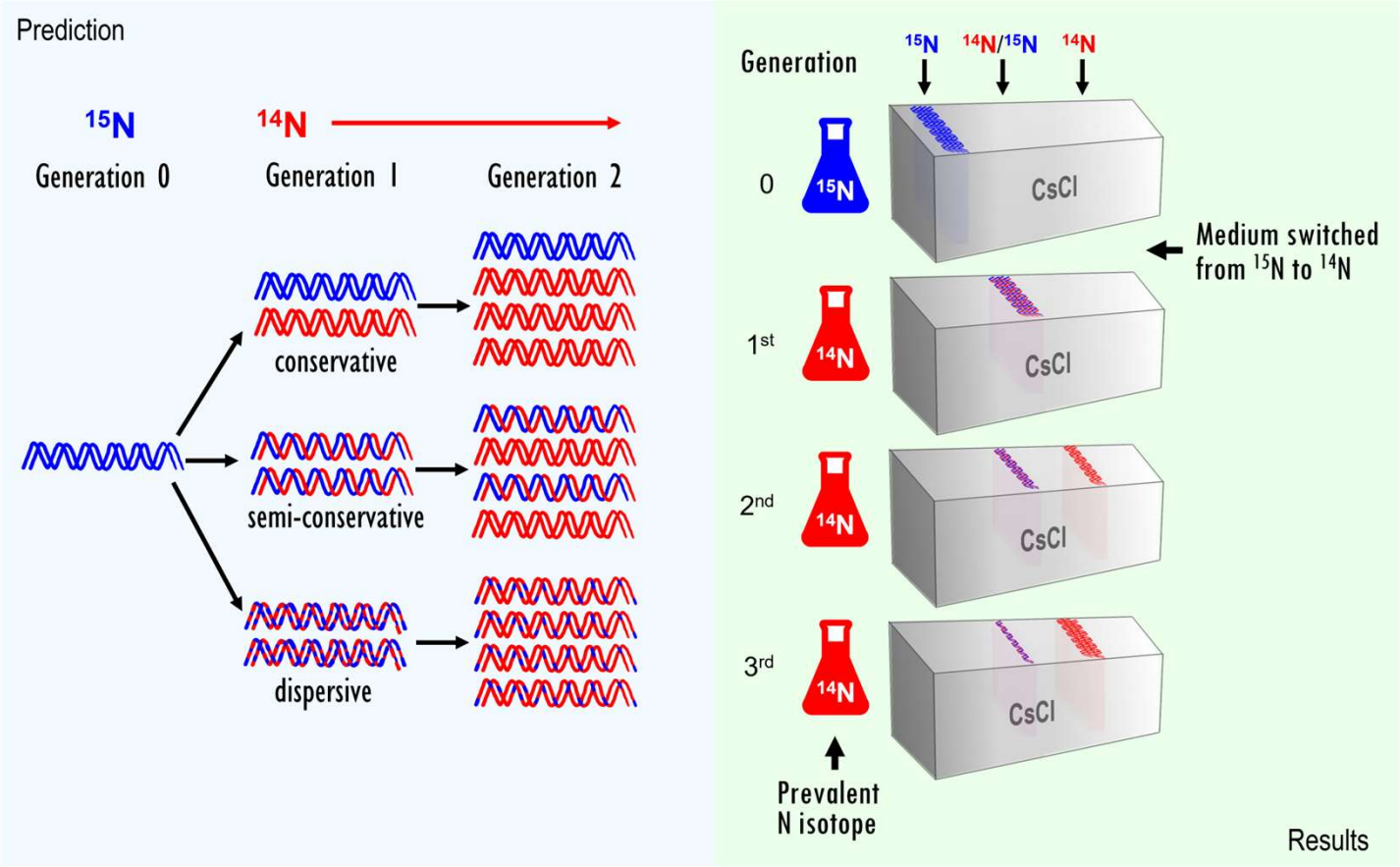
Meselson-Stahl Experiment (continued)

- After each generation, DNA was extracted from the bacteria and centrifuged over a *density gradient* – a solution of CsCl that was denser at the bottom and less dense at the top
- The DNA settles at a position in the tube that has equivalent density: heavier DNA sinks more, lighter DNA floats more
- The "parental" DNA (all ^{15}N) formed a single band deep in the tube
- After 1 generation, the DNA formed a single band that was less dense than the "parental" DNA, but denser than DNA containing only ^{14}N: intermediate density
- After 2 generations, the DNA formed two equal bands, one of "intermediate" density and one similar to ^{14}N-only density



Density Gradient

Meselson-Stahl Experiment



DNA Polymerase

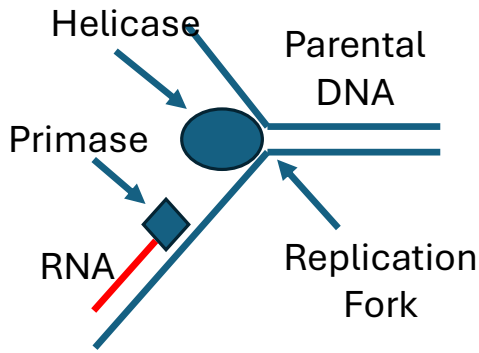
- The Meselson-Stahl experiment definitively showed that DNA replicates by a semi-conservative mechanism.
- We now know that DNA is copied by a complex molecular machine, the core of which is an enzyme called DNA polymerase
- This enzyme uses one strand of DNA as a template to build a *complementary* strand
- The template strand remains intact, and the complementary strand is synthesized completely *de novo*

DNA Polymerase

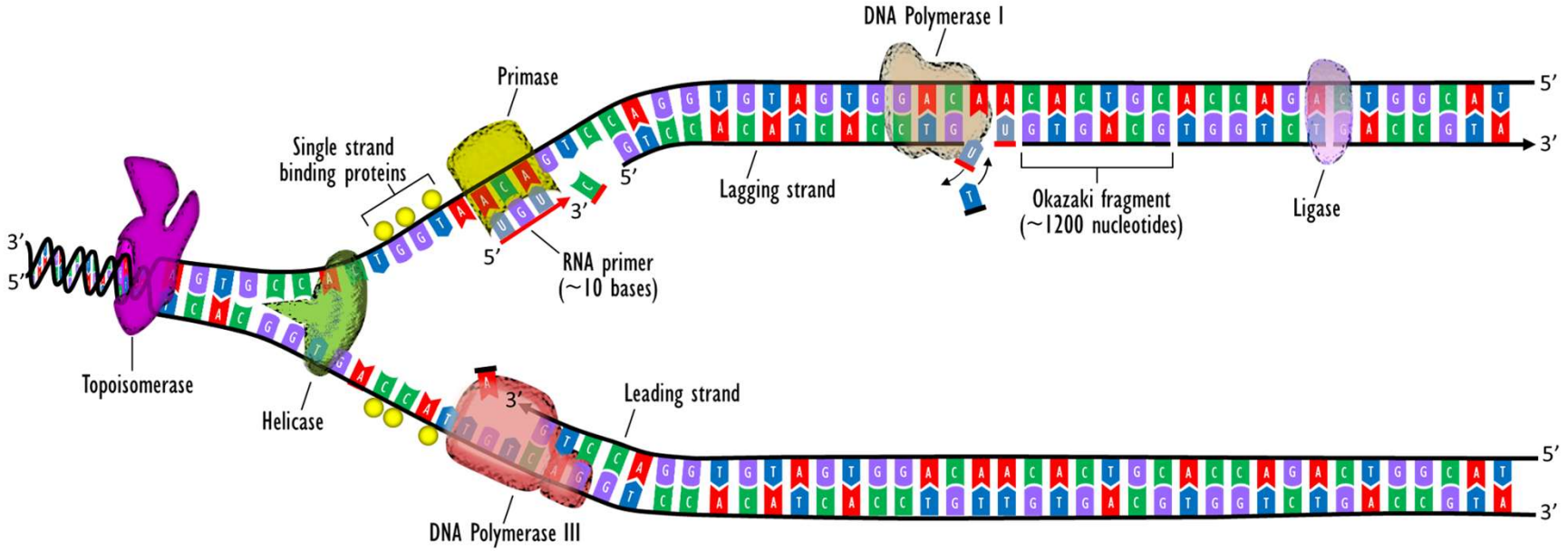
- DNA polymerase synthesizes a new strand of DNA by adding DNA nucleotides, one at a time, to the 3’ end of the growing strand
- It ***always*** builds in the 5’->3’ direction (and since DNA is antiparallel, it always reads the template in the 3’->5’ direction)
- However, DNA polymerase cannot start a new strand by itself:
- It cannot unwind the double helix to be able to read the template
- It also cannot start a new strand, but can only add nucleotides to an existing strand

The Replication Complex

- A complex of 3 enzymes assemble together to form a "replication complex" at a special sequence of DNA: the "origin of replication".
- DNA helicase unwinds the DNA to form a "replication fork"
- DNA primase synthesizes a short (10-20 nucleotides long) stretch of RNA that is complementary to the DNA template strand (a "primer")
- DNA polymerase then extends the primer by attaching DNA nucleotides, instead of RNA nucleotides
- Eventually, the primer is removed and replaced with DNA



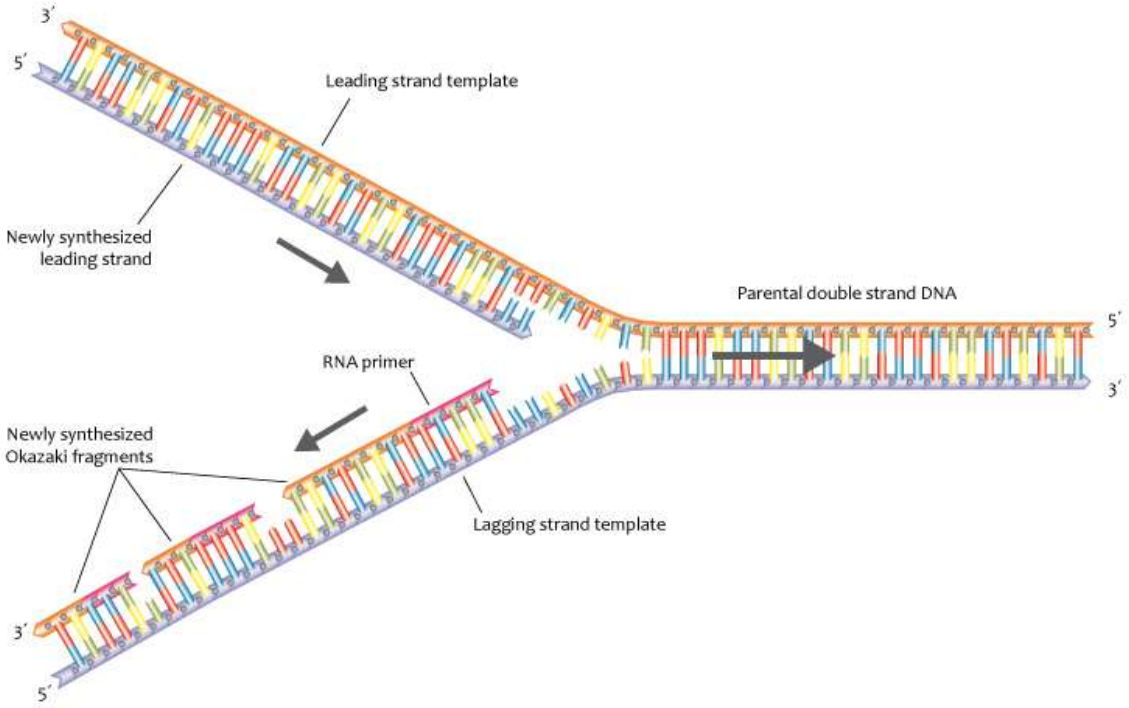
Replication Fork



One Last Quirk: “Leading” and “Lagging” Strands

- Once helicase has unwound the DNA, both strands are copied at the same time by the DNA polymerase complex
- However, since DNA polymerase can only synthesize in the 5’->3’ directions, the process is different for both (antiparallel) strands
- The “leading strand”, which can be built in the same direction as helicase is moving, is made as a single, long, continuous strand
- The “lagging strand” must be built in the opposite direction, and is made as a series of short segments (“Okazaki fragments”) that eventually get linked together by DNA ligase

“Leading” and “Lagging” Strands



DNA Replication in Action

Video from HHMI BioInteractive (https://www.biointeractive.org/classroom-resources/dna-replication-advanced-detail)

Next Lecture: RNA Transcription