DNA REPLICATION

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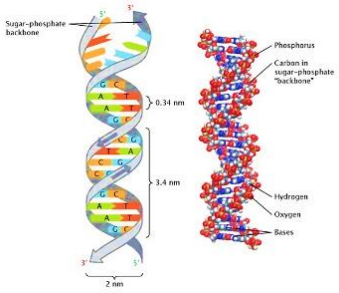
INTRODUCTION

DNA Replication

A. Watson & Crick (1952)

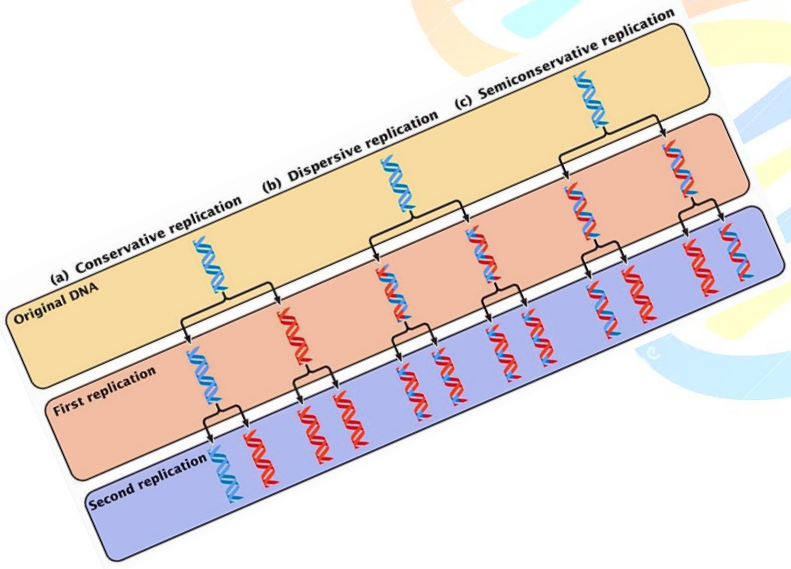


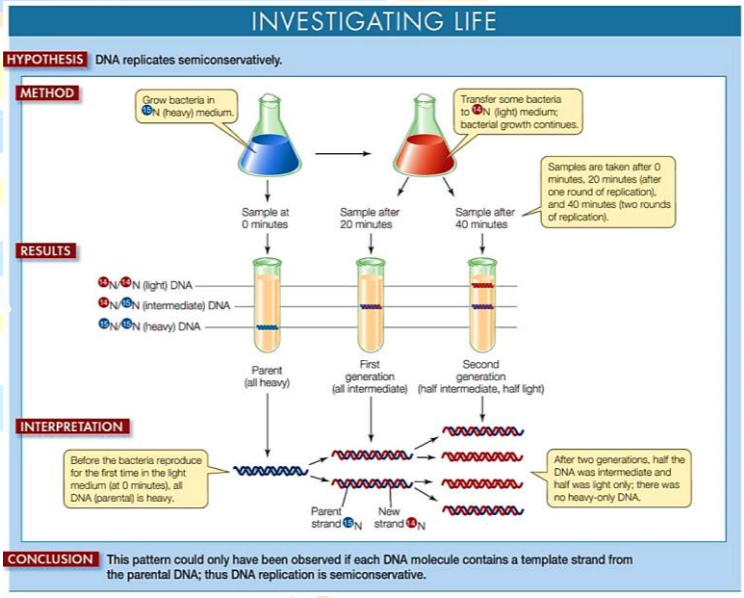
Proposed a model where hydrogen bonds break, the two strands separate, and DNA synthesis occurs semi-conservatively in the same net direction. While a straightforward and simple model, subsequent studies revealed that the polarity requirements of DNA polymerizing enzymes would not permit replication to occur in the same direction on both strands.

B. Meselsohn-Stahl (1958)

Demonstrated in a classic CsCl density-gradient experiment involving light (N^{14}) and heavy (N^{15}) nitrogen that DNA replication in E. coli was semi-conservative.

I. Grew cells for several generations on N^{15} so that both DNA strands would be N^{15}/N^{15}.
II. Transferred the cells to N14 medium. This is Generation 0.
III. Extracted DNA in every generation and centrifuge in CsCl density gradient.
IV. The results were consistent with semi-conservative, not conservative replication.
V. Semi-conservative replication in eukaryotes was demonstrated some time later.





C. Cairns (1963) autoradiographic experiment

I. Demonstrated that replication in E. coli was semi-conservative from a single origin of replication, and that DNA synthesis occurred in the same net direction. This again raises the "polarity" problem.

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II. Note: Cairns was correct that replication was initiated from a single origin, but incorrect initially in that he proposed that replication proceeded unidirectional. DNA replication in E. coli (and elsewhere as well) is bidirectional and proceeds is either direction (on both strands) from the origin of replication. This, too, raises the "polarity" problem.

III. The origin of replication in E. coli is called OriC and is well characterized molecularly.

a) OriC is 245 bp in length, containing two different repeat sequences.

b) One repeat (13 bp in length) is repeated three times in tandem. It is enriched in AT base pairs and serves as region where double strands separate, facilitating a structure called the replication bubble.

c) The other repeat (9 bp in length) is interspersed (not tandem) in OriC and serves as binding sites for a protein involved in formation of the replication bubble.

D. DNA polymerases (DNA dependent DNA polymerases)

1) A DNA polymerizing enzyme was first discovered in E. coli by Arthur Kornberg in1957. The enzyme historically has been called the "Kornberg enzyme" but also goes by the name DNAP-I.

a) Kornberg demonstrated that the enzyme could synthesize DNA in vitro (i.e., in a test tube). All that was needed were the four deoxyribonucleotide triphosphates (ATP, GTP, CTP, TTP), magnesium (Mg++), the Kornberg enzyme, and a pre-existing DNA that provided both a priming site and a template.

b) The priming site was a free OH (hydroxyl) group at the 3' end to which nucleotides were added. DNAP-I (in fact all polymerases) cannot initiate synthesis of DNA from "nothing" and require a pre-existing 3'-OH group. Enzymatically, DNAP-I catalyzes the formation of the phosphodiester link between the 3'-OH of the primer and the 5'phosphate of the incoming nucleotide tri-phosphate. This requirement for a pre-existing 3'-OH group is the "polarity" problem and the reason why DNA polymerases "read" a 3'⟶5' template and synthesize "new" DNA in a 5'⟶3' direction.

c) The template "function" of the pre-existing DNA provides the nucleotide sequence for the addition of complementary bases (A w/T, G w/C, etc.).

2) DNAP-I is a multifunctional enzyme that has both 5'⟶3' and 3'⟶5' exonuclease activity as well as polymerizing activity, i.e., can degrade DNA as well as synthesize (polymerize) DNA. DNAP-I, however, is not the replicator in E. coli.

a) Cairns' isolated a mutant (termed the polA mutant) in E. coli that had no Kornberg enzyme (DNAP-I) but replicated just fine, albeit a bit more slowly.

b) polA mutants were ultrasensitive to UV light, suggesting that DNAP-I might be involved in DNA repair. As it turned out, DNAP-I is involved in

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both DNA repair and DNA replication. Its role in DNA replication presumably is filled by other DNAPs in the polA mutant.

3) DNA polymerases in E. coli [at least three]

DNAP-I The Kornberg enzyme; DNA repair and DNA replication

DNAP-II DNA repair

DNAP-III DNA synthesis (replication)

Note: DNAP-II & DNAP-III have 5'⟶3' polymerase activity and 3'⟶5' exonuclease activity, but not 5'⟶3' exonuclease activity. The last is limited to DNAP-I.

4) DNA polymerases in eukaryotes [at least five]

DNAPα (I) replication of nuclear DNA [discontinuous strand]

DNAPβ DNA repair

DNAPγ replication of mitochondrial DNA

DNAPδ (III) replication of nuclear DNA [continuous strand]

DNAPε (II) DNA repair

a) All five polymerize by adding nucleotides to a 3'-OH group, i.e., the DNA polymerases "read" a 3'⟶5' template and synthesize "new" DNA in a 5'⟶3' direction.

b) Only DNAPγ, DNAPδ, and DNAPε have 3'⟶5' exonuclease activity. DNAPα and DNAPβ do not have nuclease activity at all.

c) None of the eukaryotic DNAPs have 5'⟶3' exonuclease activity.

5) 3'⟶5' exonuclease activities of DNA polymerases are involved in "proofreading" the growing DNA chain. When an improper base is added at the 3' end, the exonuclease activity of the enzyme "clips" off the unpaired base, and the 5'⟶3' polymerase activity fills in the correct base.

- All known DNA polymerases except DNAPα and DNAPβ (in eukaryotes) have this proofreading capability.

Principles for DNA replication

I. The DNA being replicated must be in a ready state for the start of replication, and there also has to be a clear start point from which replication proceeds. As each piece of DNA must only be copied one, there also has to be an end point to replication.

II. DNA replication must be carried out accurately, with an efficient proof reading and repair mechanism in place for any mismatches or errors. And finally, the system of replication must also be able to distinguish between the original DNA template and then newly copied DNA.

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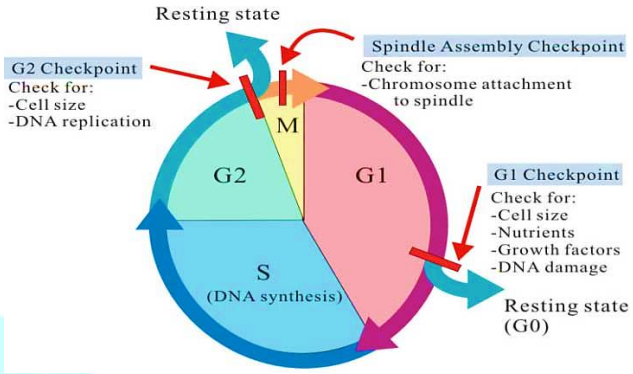
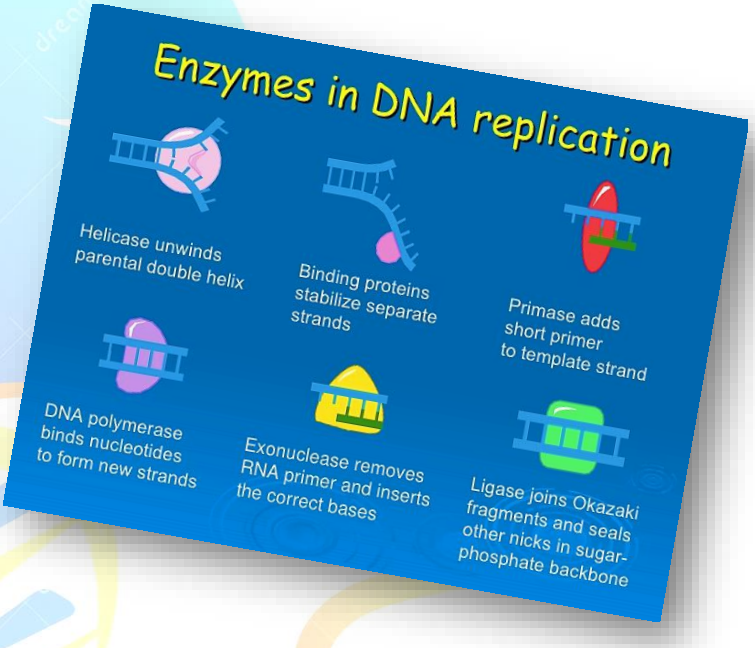
III. In order to be able to put these principles into context, it is helpful to look at the eukaryotic cell cycle to see where the main checkpoints are in the process.

IV. Actively dividing eukaryote cells pass through a series of stages known collectively as the **cell cycle**: two gap phases (G1 and G2); an S (for synthesis) phase, in which the genetic material is duplicated; and an M phase, in which mitosis partitions the genetic material and the cell divides.



Replication Enzymes:



I. **DNA Polymerase:**
Matches the correct nucleotide and then joins adjacent nucleotides together

II. **Primase:**
Provides and RNA primer to start polymerisation

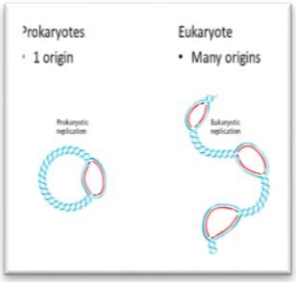
III. **Ligase:**
Joins adjacent DNA strands together

IV. **Helicase:**
Unwinds the DNA and melts it

V. **Single Strand Binding Proteins [SSBP]:**
Keep the DNA single stranded after it has been melted by helicase

VI. **Gyrase:**
A topoisomerase that relieves torsional strain in the DNA molecule
Telomerase: Finishes off the ends of the DNA strand

Mechanism of DNA Replication in Prokaryotes



A. Requirements for DNA replication in prokaryotes

1. Origin of replication (*oriC*) which is a 245 base pair site that contains multiple direct repeats where DNA replication begins
2. DnaA (unwinds the DNA strands at *oriC*)
3. SSB (single stranded binding protein to keep the DNA strands apart)
4. **Primosome**

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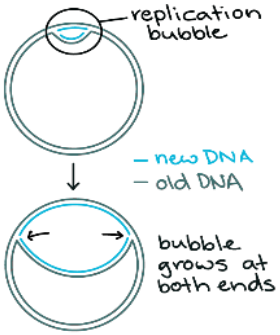
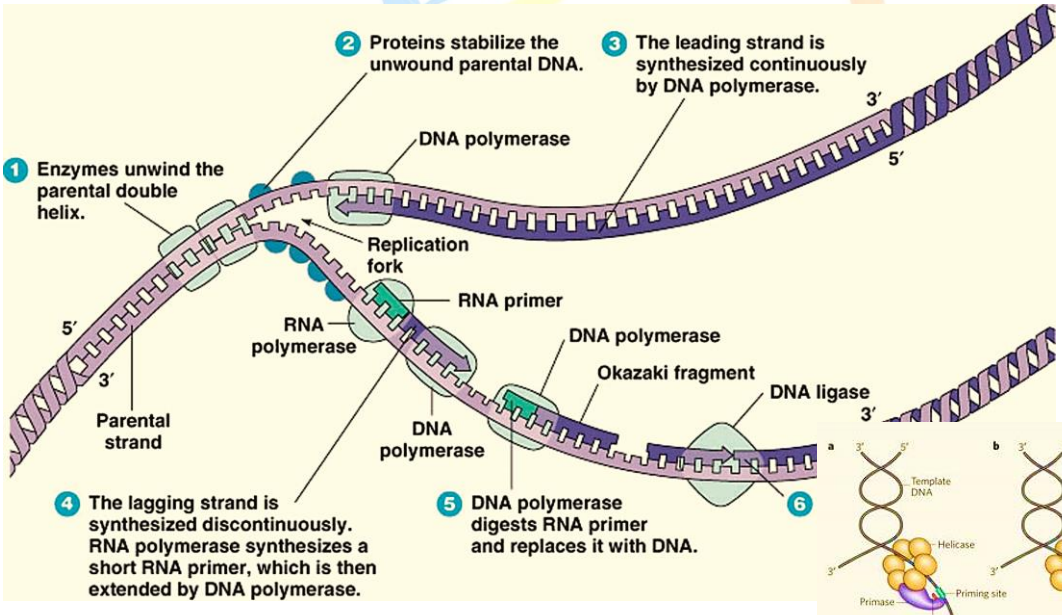
a) Primase (synthesizes RNA primers to start DNA replication)
b) DnaB, DnaT, PriA, PriB, PriC

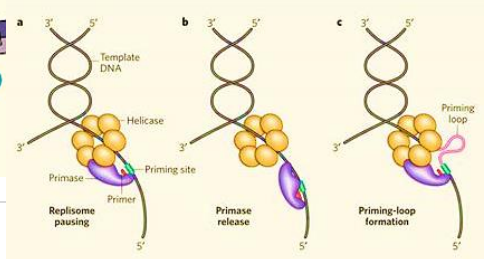
5. Rep is a helicase that disrupts ("melts" or "denatures") the H bonds at the replication fork.
6. DNA polymerase (there are 3 types of DNA polymerase that have been isolated from *E. coli* – all require Mg2+)
7. Deoxyribonucleotides to incorporate into the new DNA
8. Template DNA
9. Ribonucleotides to make primers



B. Direction of synthesis of each new strand is 5'–3'
C. Replication is bidirectional
D. Speed 100 kilobasepairs/minute
E. Steps in DNA replication

1. Binding of DnaA to oriC and initial unwinding of the helix
2. RepA helicase "melts" the DNA at the replication fork
3. Priming DNA synthesis - **Primase** synthesizes RNA primers.
4. **Leading** and **lagging** strand DNA synthesis: DNA pol III synthesizes new chains in the 5' to 3' direction. Since the DNA helices are antiparallel, the direction of movement relative to the template DNA strand is 3' to 5'. Thus, for the two new strands made, leading stand synthesis is continuous and lagging strand DNA synthesis is discontinuous (see 5 too).
5. Lagging strand is synthesized discontinuously as short fragments (**Okasaki fragments**). The RNA primers in these fragments are later removed by DNA pol I and the fragments are joined together by DNA ligase. If leading strand synthesis is going in 1 direction and lagging strand synthesis is going





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in the other direction, how does 1 DNA pol III dimer synthesize both strands at once?

6. Looping of the template strand for lagging strand synthesis allows DNA pol III at replication fork to synthesize both the leading and lagging strands simultaneously.
7. Rep helicase is continuously melting the DNA at the replication fork.
8. Exonuclease editing allows for proofreading of DNA synthesis: (epsilon subunit of DNA pol III and also DNA pol I itself)
9. Relaxing supercoils (DNA gyrase)

DNA replication in eukaryotes

1. More than one chromosome
2. Complex structure of the chromosomes
3. Much larger amount of DNA

B. Semiconservative replication shown in 1958 by Taylor and later using harlequin chromosome techniques.

C. Multiple origins (3500 in *Drosophila* and 25000 in mammals)

D. 4 DNA polymerases have been identified.

E. Enzymology of DNA replication similar to in prokaryotes

Eukaryotes vs. Prokaryotes

There is much conservation between the two systems, in as much as the enzymology, the replication fork geometry, the basic fundamental features and the use of multi-protein machinery are all very much the same in both.

However, there are more protein components in the Eukaryotic replication machinery. In prokaryotes, the replication form moves 10x faster than in eukaryotes.

Prokaryotic replication	Eukaryotic replication
semiconservative replication	semiconservative replication
single origin replication (oriC) primer synthesized by primase	multiple origins of replication (ARS)
primer synthesized by primase	primer synthesized by subunits of DNA polymerase α
processing enzyme: DNA polymerase III	processing enzymes: DNA polymerases α and δ
removal of primer: DNA polymerase I	removal of primer: DNA polymerase β
DNA free in cytoplasm as nucleoid	chromatin structure, chromosomes, histones
circular DNA	linear DNA: problem of replication of chromosome ends → telomerase