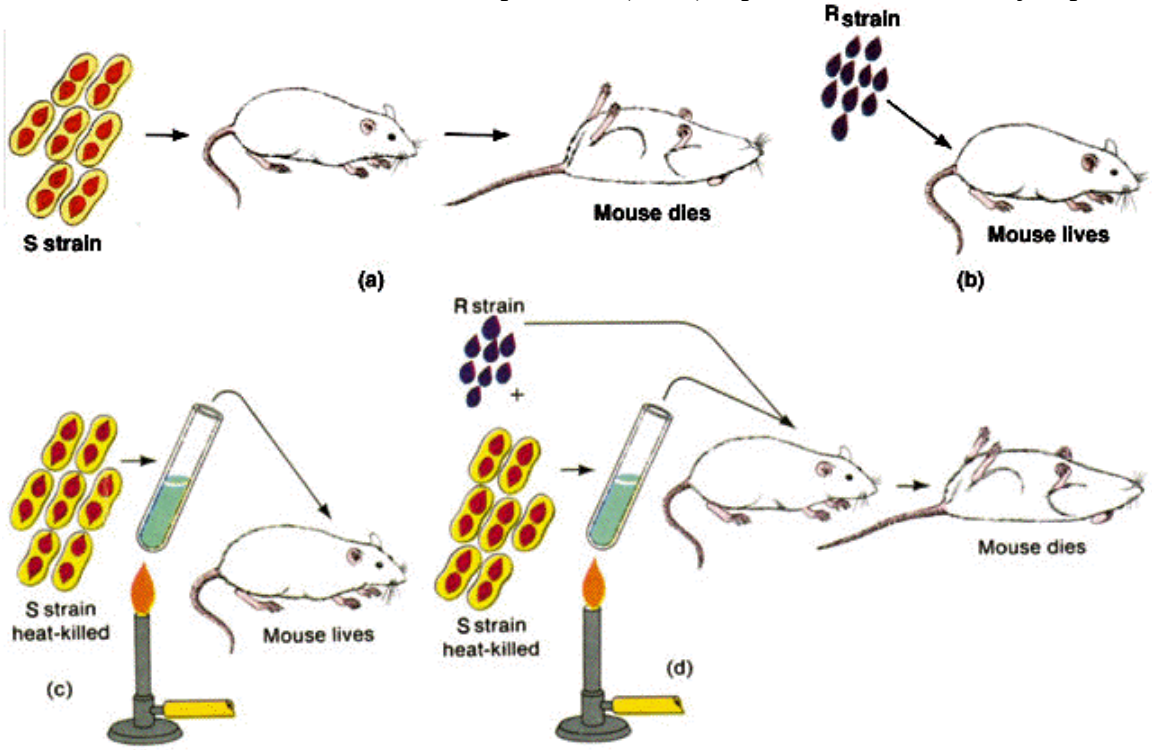
Chapter 11 Lecture Notes: The Structure of DNA

I. Prelude to the discovery of DNA as the genetic material
   A. Genes were known to be associated with specific character traits but their physical nature was unknown.
   B. Genes were known to be carried on chromosomes.
   C. Chromosomes were known to contain DNA and protein.
   D. The composition of DNA was known, and it was thought to be too "simple" to carry the genetic information.
   E. Criteria needed for a molecule to be the carrier of genetic information.
      1. Ability to store genetic information and transmit it to the cell as needed.
      2. Ability to transfer the information to daughter cell with minimal error.
      3. Physical and chemical stability so that information is not lost.
      4. Capacity for genetic change.

II. The discovery that DNA was the genetic material
   A. Griffith's transformation experiment (1928) – precursor to the Avery experiment



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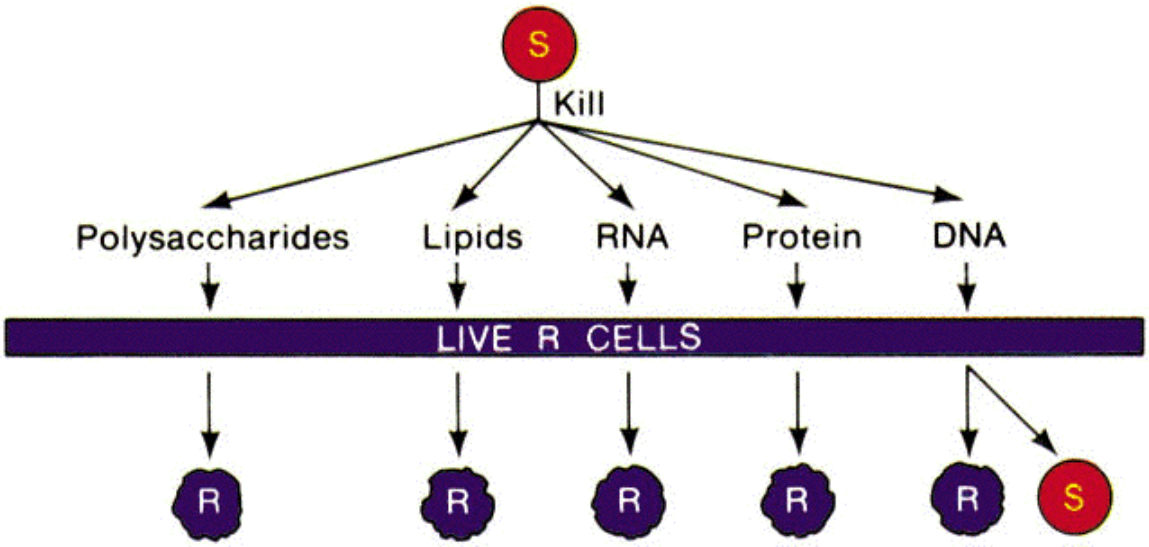
B. Avery, MacLeod, and McCarty experiment (1944)

Showed that transforming material in Griffith's experiment was DNA



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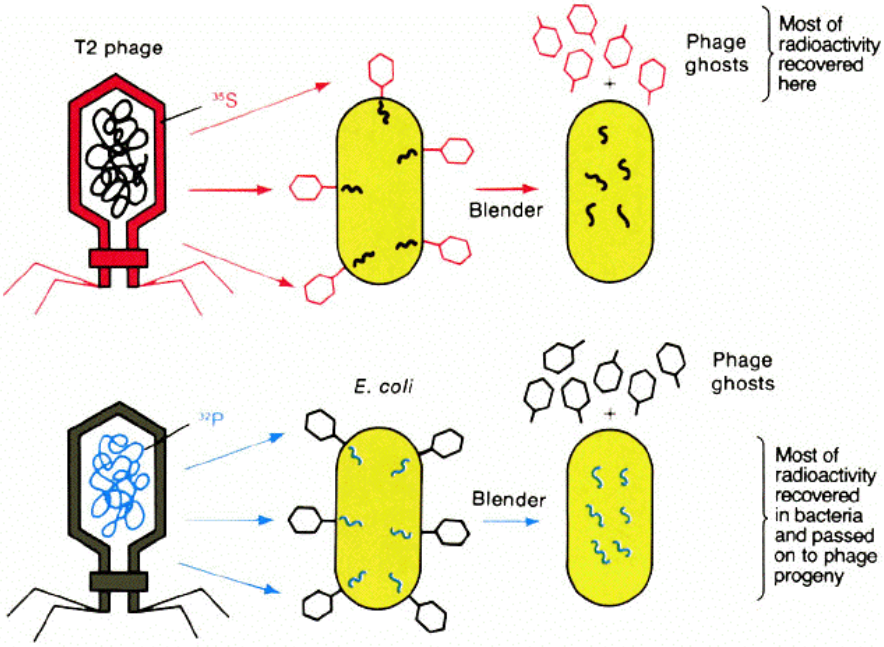
C. Hershey-Chase experiment (1952)

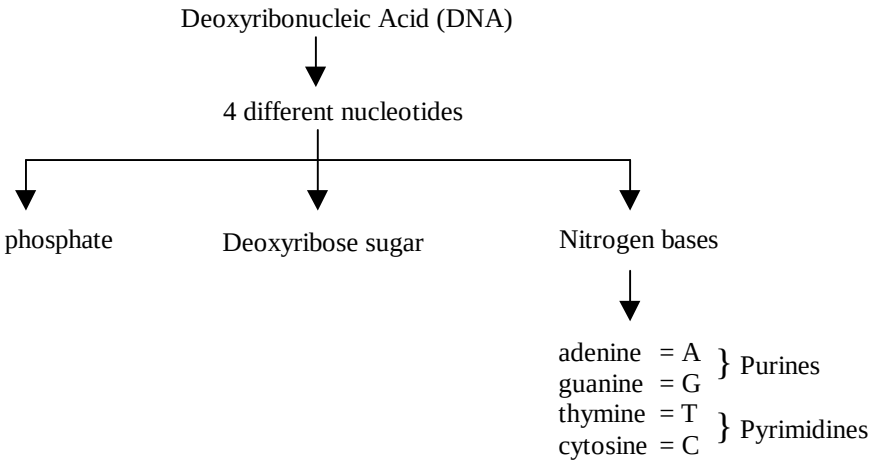
Hershey and Chase knew that phage infection of *E. coli* required introduction of viral genetic information. Selectively labeled the phage protein with ^{35}S and the phage DNA with ^{32}P → Infected *E. coli* with labeled phage → After infection, removed empty phage heads → Looked to see whether the labeled DNA or the protein was in *E. coli* → Found ^{32}P in bacteria so DNA carried the genetic info



III. The discovery of the structure of DNA

A. The composition of DNA was known.

1. Composed of 4 different nucleotides. A nucleotide is composed of a phosphate group, a deoyribose sugar, and a nitrogen base.



B. Chargraff's rules (in each DNA molecule: T+C =A+G and T=A and C=G)

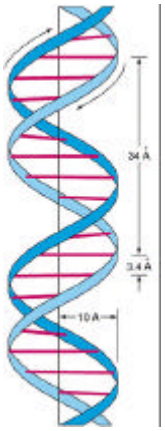
C. Franklin and Wilkins x-ray diffraction data suggested that the molecule was long and skinny, had two parallel components, and was helical

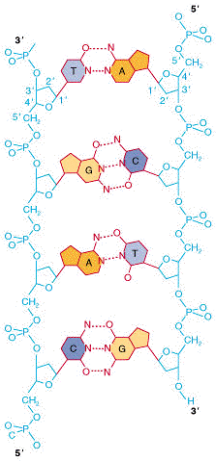
D. Watson and Crick put it all together to solve the structure of DNA in 1953

IV. The structure of DNA

A. Important features of the DNA structure:

1. Right-handed double helix
2. The helices are <u>antiparallel</u>
3. Each helix has a series of nucleotides held together with phosphodiester bonds between the OH groups in two adjacent sugar residues.
4. The helices themselves are held together by hydrogen bond between the nitrogen bases (G pairs with C; A pairs with T).
5. 10.5 basepairs per turn of the helix.





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B. DNA molecule has major and minor grooves (Figure 11-9)

C. DNA molecule usually depicted as an inflexible rod, but it can be bent or curved sometimes.

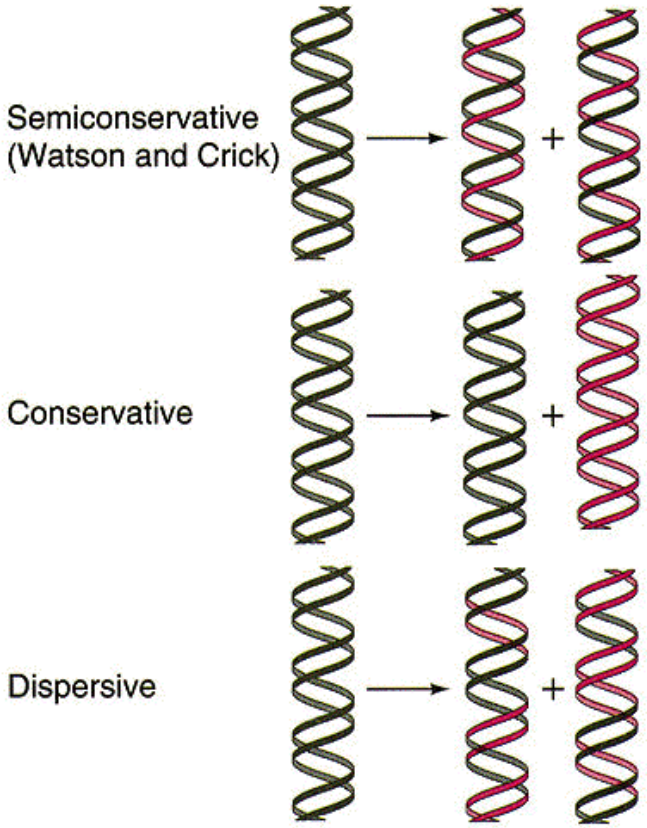
D. Stabilization forces in DNA are from the hydrogen bonding between bases (GC base pair provides more stability because 3 H bonds vs. 2 H bonds for AT basepair) and the "stacking" of the bases which excludes water molecules.

E. Several alternative structural forms of DNA have been found:

1. B form DNA is the typical form.
2. A form DNA is formed in less hydrated solutions.
3. Z form DNA is formed when the DNA molecules have alternating G's and C's on the same strand.

V. Discovery of semiconservative DNA replication in prokaryotes (no nucleus, circular chromosome)

A. Original 3 hypothesis for the mechanism of DNA replication



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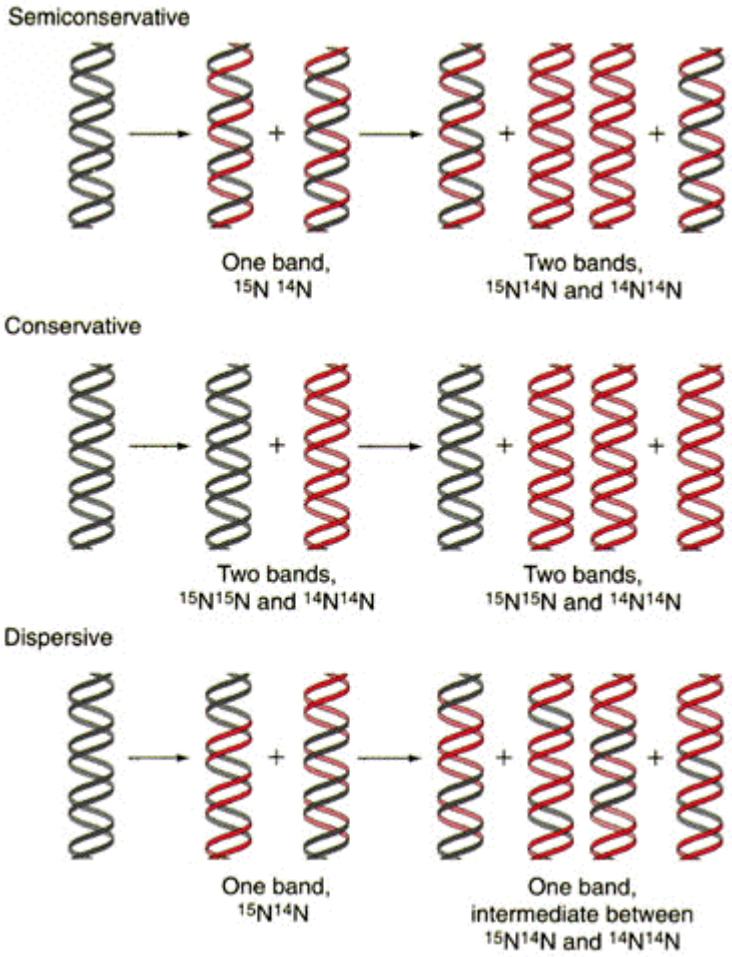
B. The Meselson-Stahl experiment

Meselson and Stahl used nitrogen isotopes that had different densities to follow DNA replication in *E. coli.*

Grew *E. coli* in ^{15}N for several generations so that all the DNA was labeled → Shifted cells to ^{14}N media and allowed them to replicate their DNA 1 time → Sample of DNA was taken → Cells were allowed to replicate their DNA again (total of 2 times) → Sample of DNA was taken → Used CsCl gradient centrifugation of DNA samples to determine the isotope composition and pattern of labeling in the DNA → found pattern matched semiconservative

Expected results if…..



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VI. Mechanism of DNA replication in prokaryotes

A. Requirements for DNA replication in prokaryotes

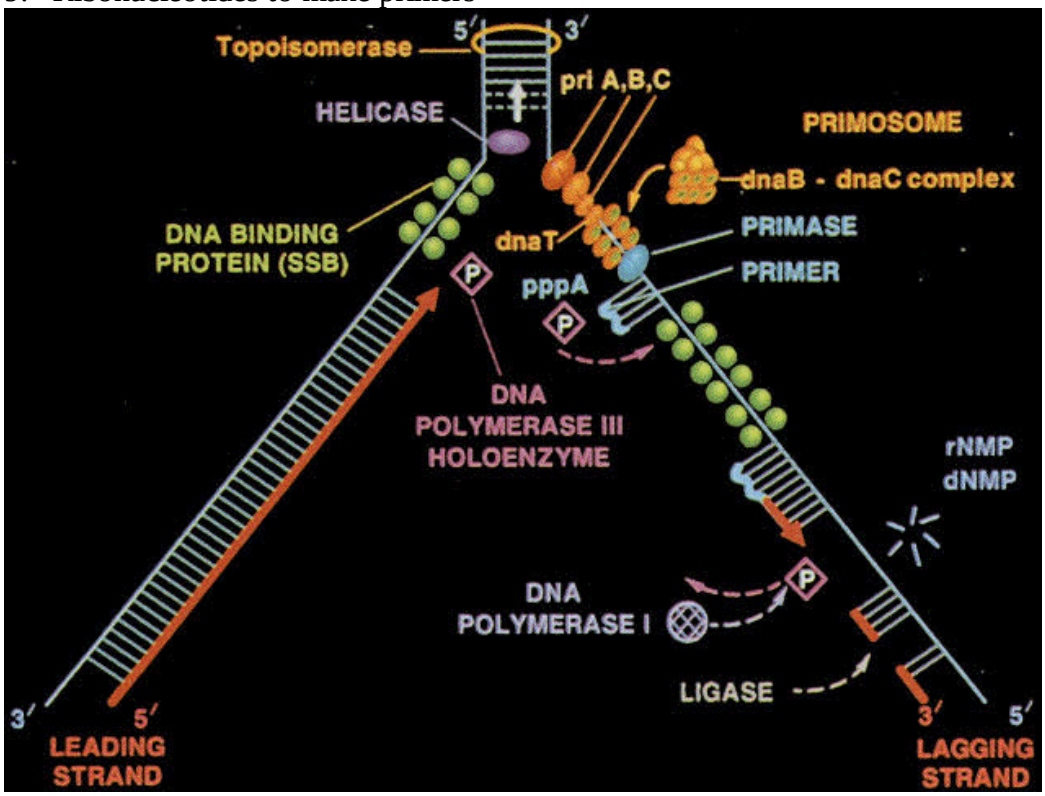
1. origin of replication (*oriC*) which is a 245 basepair site that contains multiple direct repeats where DNA replication begins
2. DnaA (unwinds the DNA strands at *oriC*)

3. SSB (single stranded binding protein to keep the DNA strands apart)
4. Primosome
    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 Mg^{2+})

	Main function	5'→3' polymerase	3'→5' exonuclease	5'→3' exonuclease	# per cell	genes
DNA pol I	Removing RNA primers & gap fill in	Y	Y	Y	400	polA
DNA pol II	DNA repair	Y	Y	N	?	polB
DNA pol III	Main polymerase	Y	Y	N	10-20	**dnaE dnaQ holB

** For DNA pol III, alpha, epsilon, and theta subunits constitute the core enzyme and 6 other subunits are important for loading the enzyme on the DNA and for holding the enzyme together; holoenzyme (complete enzyme with all its subunits) is a dimer

7. Deoxyribonucleotides to incorporate into the new DNA
8. Template DNA
9. Ribonucleotides to make primers



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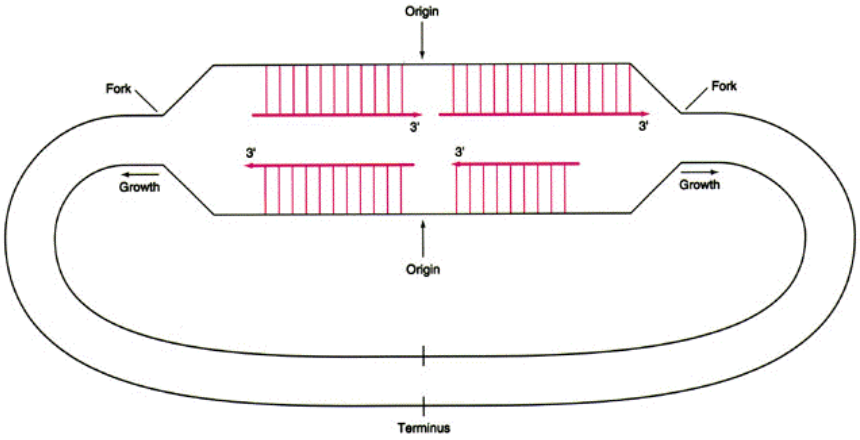
B. Direction of synthesis of each new strand is 5'→3'
C. Replication is bidirectional



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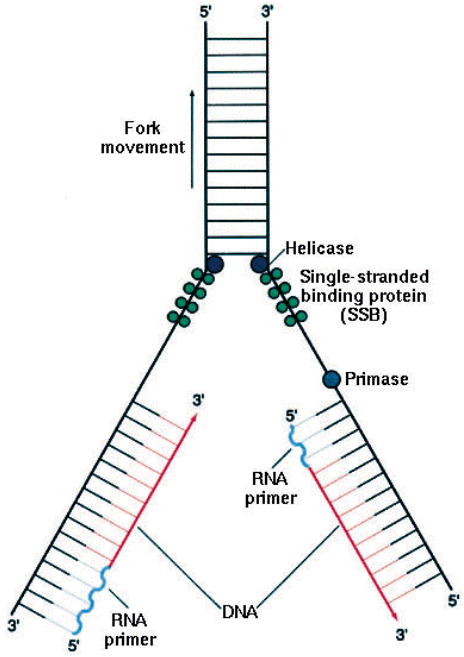
D. Speed 100 kilobasepairs/minute
E. Steps in DNA replication
    1. Binding of DnaA to *oriC* and initial unwinding of the helix
    2. RepA helicase "melts" the DNA at the replication fork
    3. Priming DNA synthesis – Primase synthesizes RNA primers.



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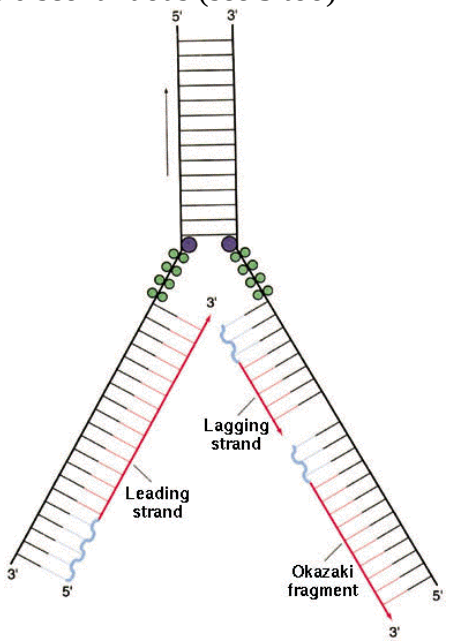
4. Leading and lagging strand DNA synthesis: DNA pol III synthesizes new chains in the 5' to 3' direction. Since the DNA helices are antiparallel, the direction of movement relative to the template DNA strand is 3' to 5'. Thus, for the two new strands made, leading stand synthesis is continuous and lagging strand DNA synthesis is discontinuous (see 5 too)



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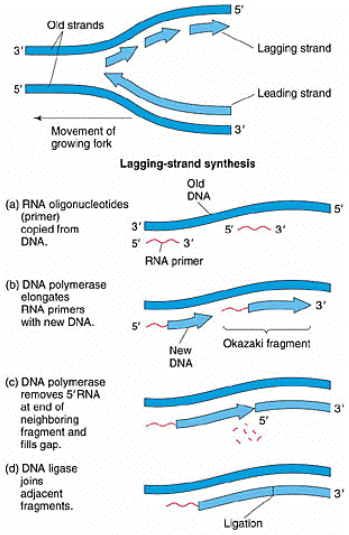
5. Lagging strand is synthesized discontinuously as short fragments (Okasaki fragments). The RNA primers in these fragments are later removed by DNA pol I and the fragments are joined together by DNA ligase.



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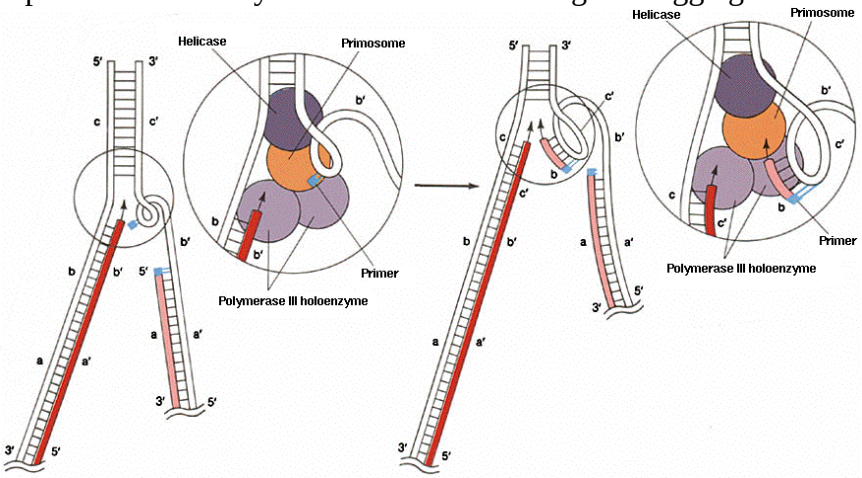
6. If leading strand synthesis is going in 1 direction and lagging strand synthesis is going in the other direction, how does 1 DNA pol III dimer synthesize both strands at once?????
Looping of the template strand for lagging strand synthesis allows DNA pol III at replication fork to synthesize both the leading and lagging strands simultaneously.



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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)

VII. DNA replication in eukaryotes (nucleus, linear chromosome)

A. Additional considerations
   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 (Figures 11-16 and 11-17).

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