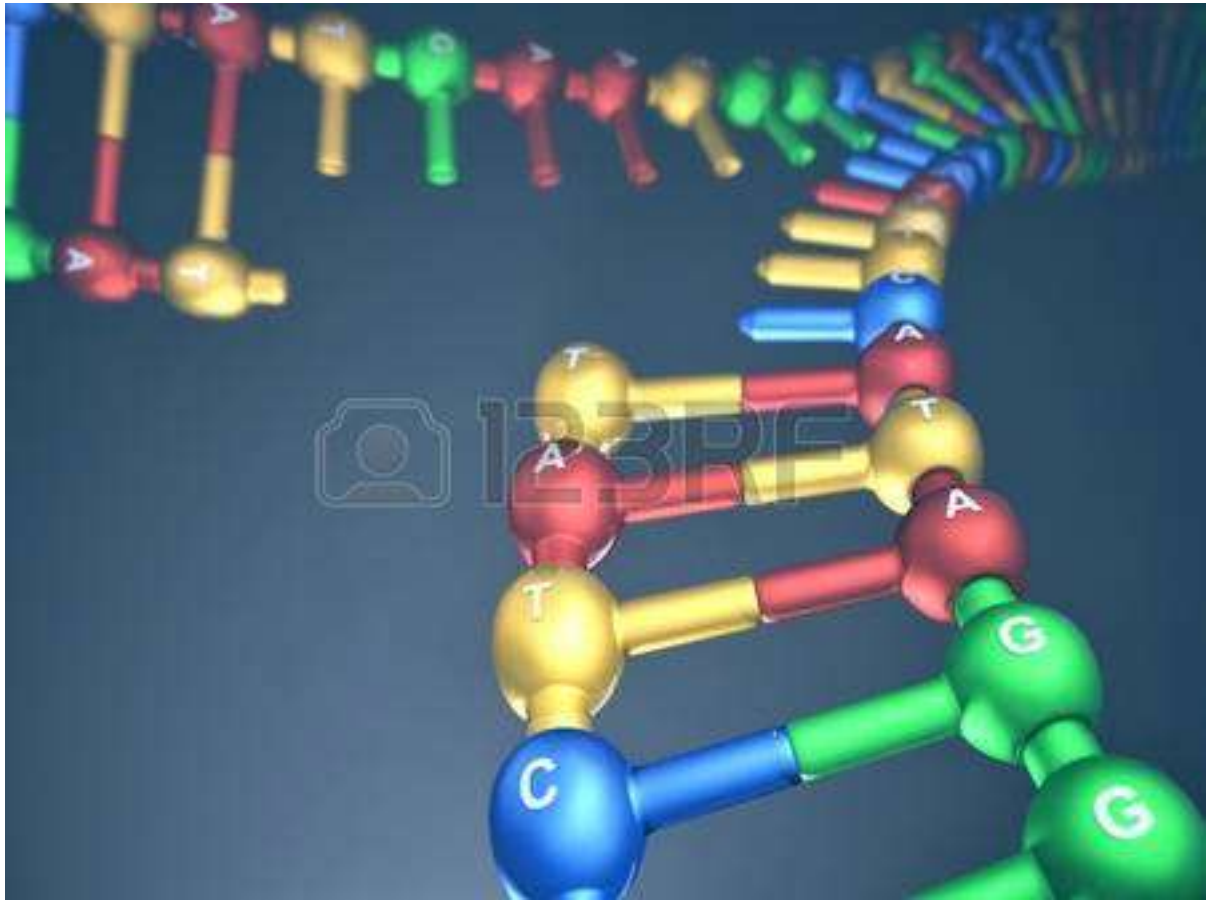
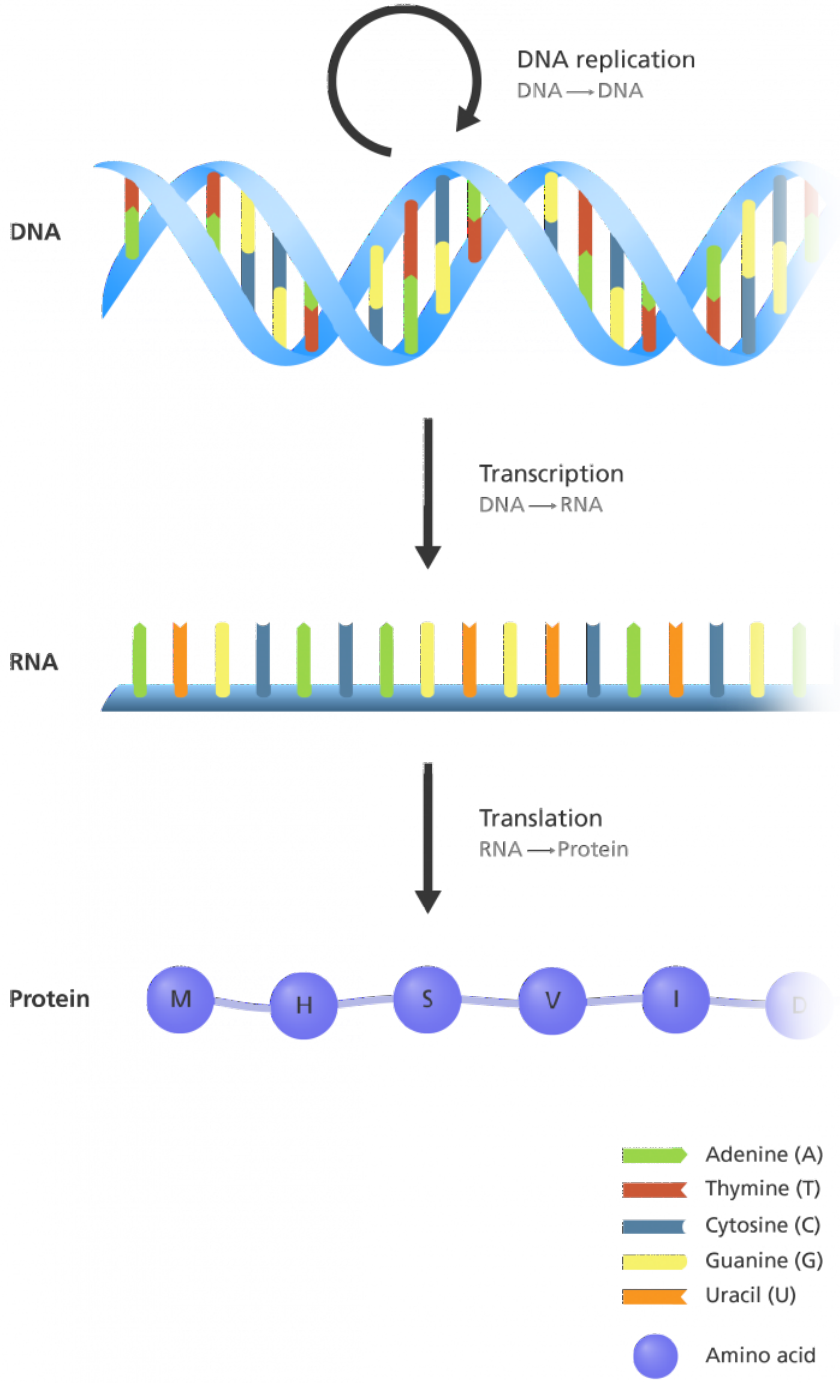
L5: Replication



Dr. P. Satpati,
BSBE, IIT Guwahati

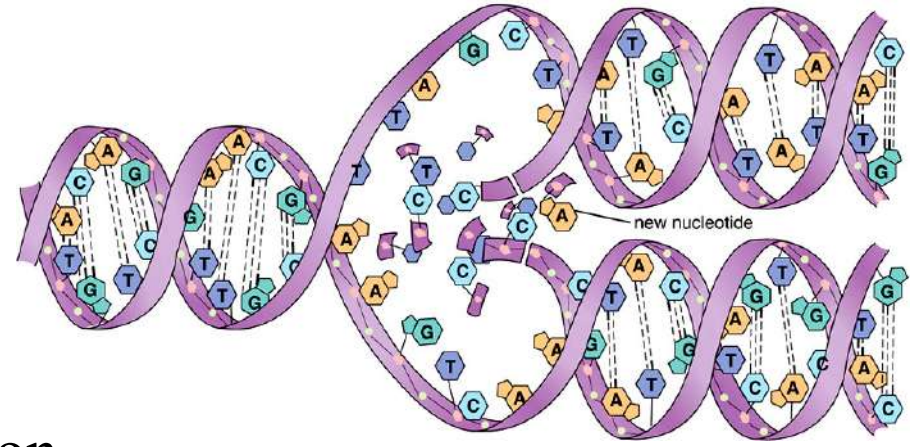
Central Dogma of Molecular Biology

- Flow of information.
- What an organism can do with the information in DNA ?



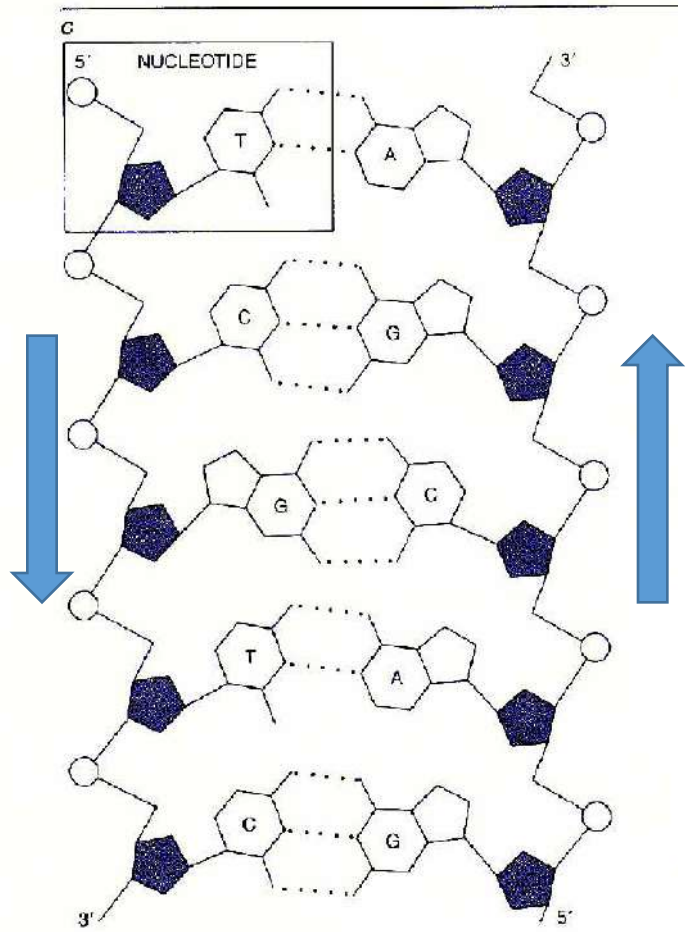
Replication (DNA → DNA)

DNA Replication



- Most accurate process (Probability of error = 10^{-9})
- Principle: Complementarity (A:T, G:C) = Information

- Involves unwinding the double helix and synthesizing two new strands.
- More than a dozen enzymes and other proteins participate in DNA replication
- The replication of a DNA molecule begins at special sites called origins of replication, where the two strands are separated
- Each strand acts as a template for synthesizing new strand.



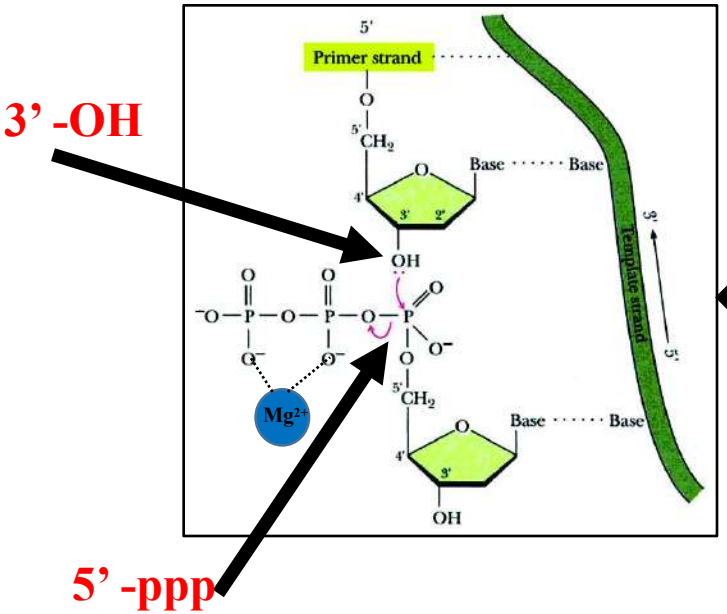
http://tigger.uic.edu/classes/phys/phys461/phys450/ANJUM04/

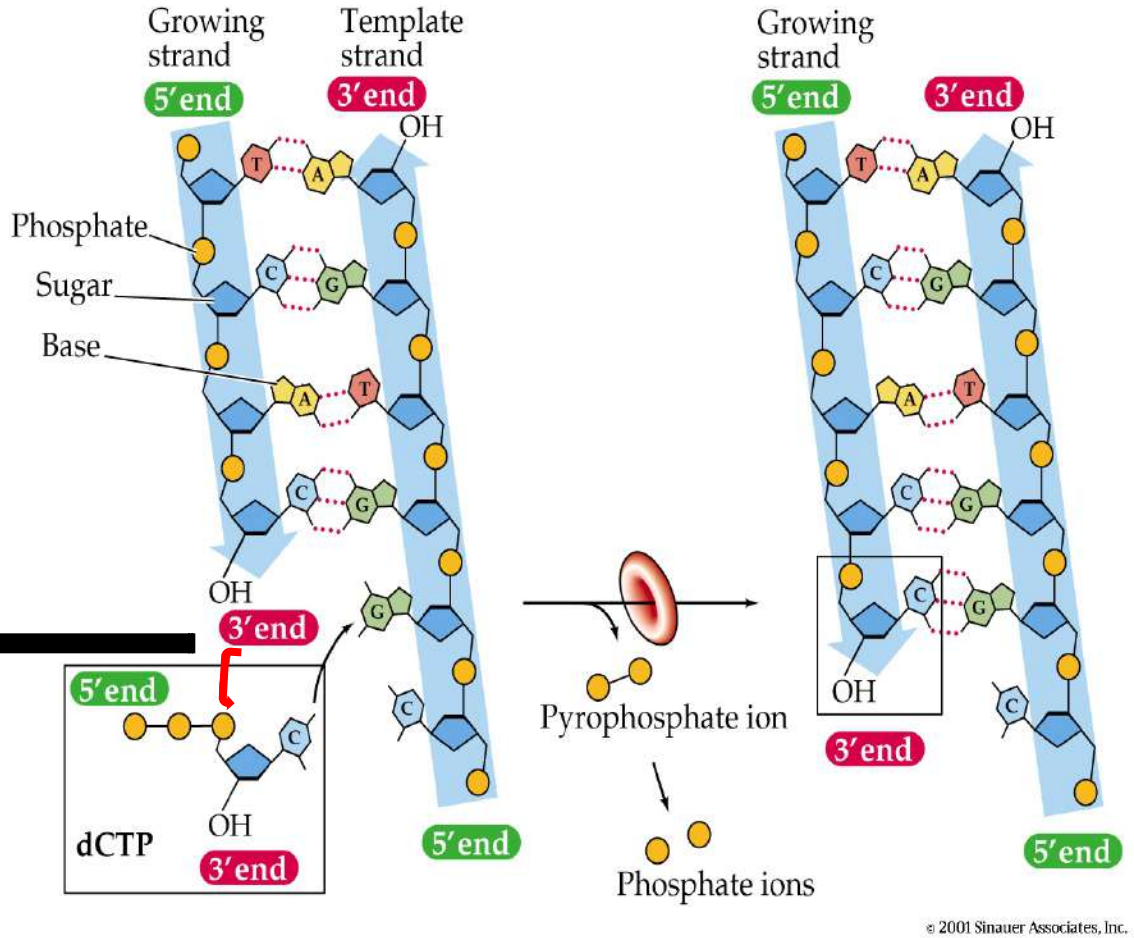
- **DNA is a double-stranded helix with antiparallel strands [Watson and Crick].**

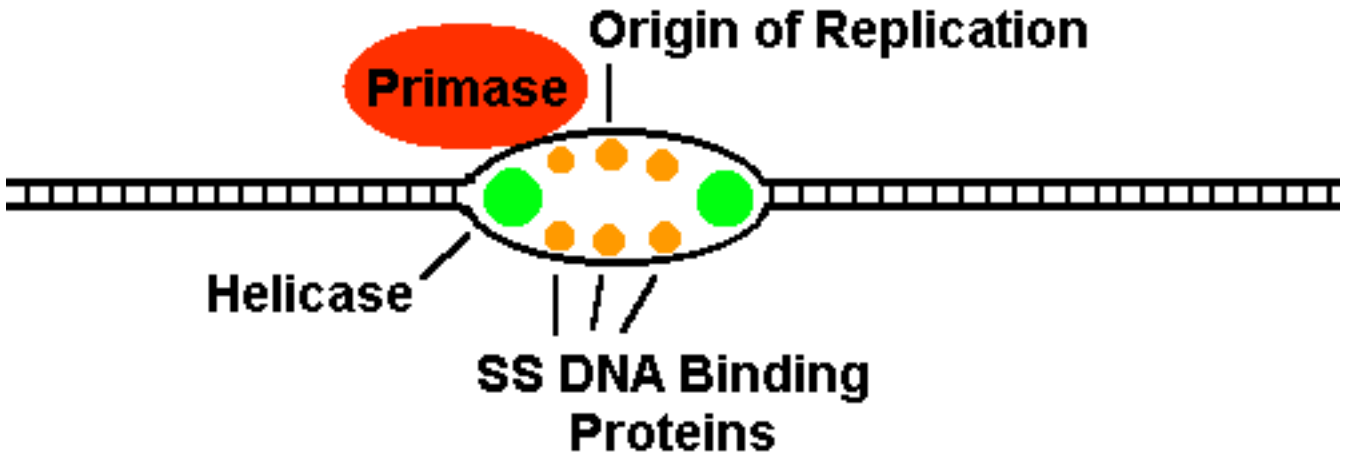
Chemistry of DNA Replication

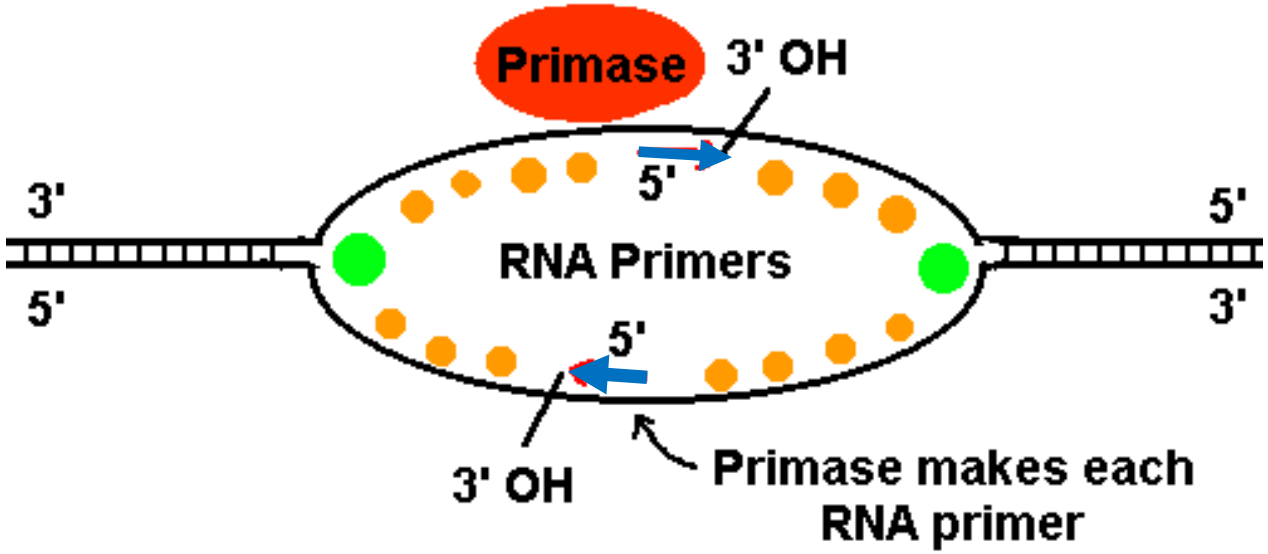
- Nucleotides are added by complementary base pairing with the template strand
- The substrates, deoxyribonucleoside triphosphates **(dNTP),** are hydrolyzed as added, releasing energy for DNA synthesis.

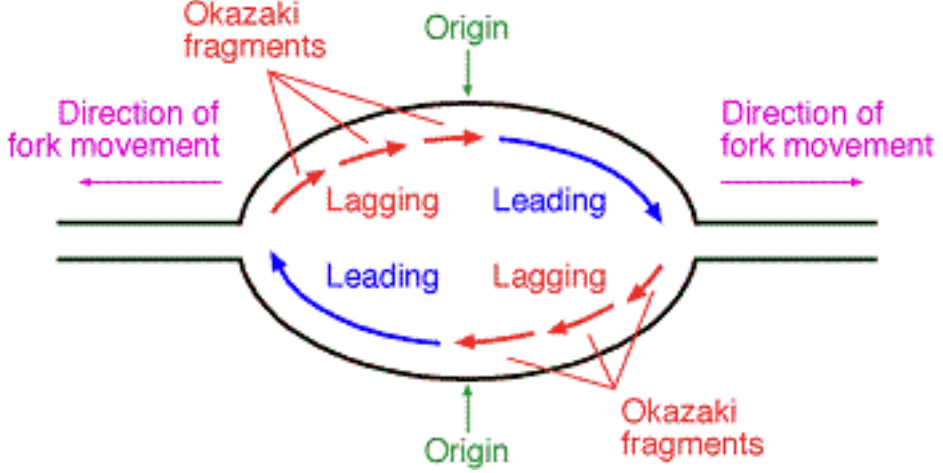
Direction of replication 5' → 3'





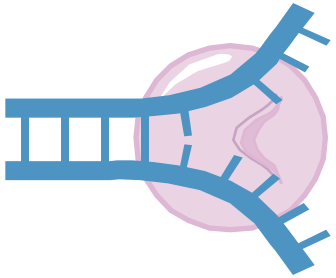

Origin of Replication
Primase
Helicase
SS DNA Binding
Proteins


Primase
3' OH
5'
3'
5'
RNA Primers
5'
5'
3'
3' OH
Primase makes each
RNA primer

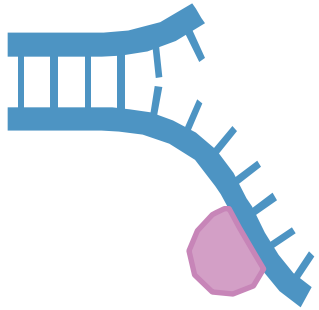


- DNA synthesis on the leading strand is continuous.
- The lagging strand grows the same *general* direction as the leading strand (in the same direction as the Replication Fork). Therefore, DNA synthesis on the lagging strand is discontinuous
- DNA is added as short fragments (Okasaki fragments) that are subsequently ligated together

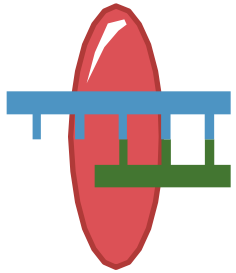
Enzymes in DNA replication



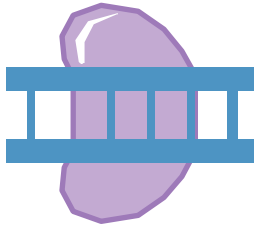
Helicase unwinds parental double helix



Binding proteins stabilize separate strands

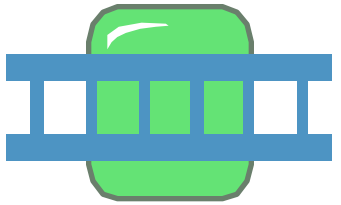


Primase adds short primer to template strand



DNA polymerase III binds nucleotides to form new strands

DNA polymerase I (Exonuclease) removes RNA primer and inserts the correct bases



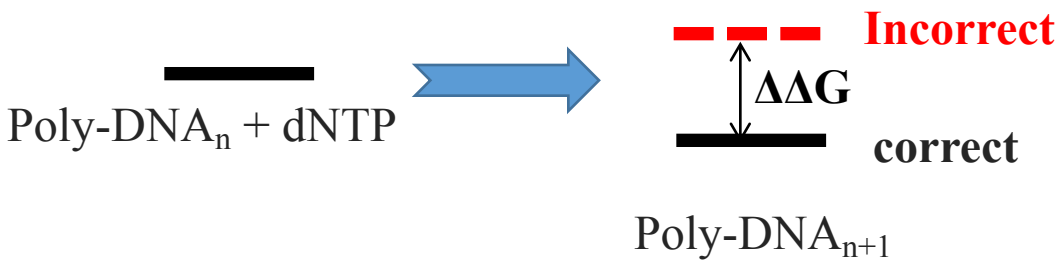
Ligase joins Okazaki fragments and seals other nicks in sugar-phosphate backbone

https://www.youtube.com/watch?v=TNKWgcFPHqw

Binding proteins stabilize separate strands : NOT SHOWN

How good is DNA replication ?

- Accuracy of DNA polymerase = 1 out of 10^7 nucleotides added **??**



Accuracy = P_{error} = $e^{-\Delta\Delta G/kT}$

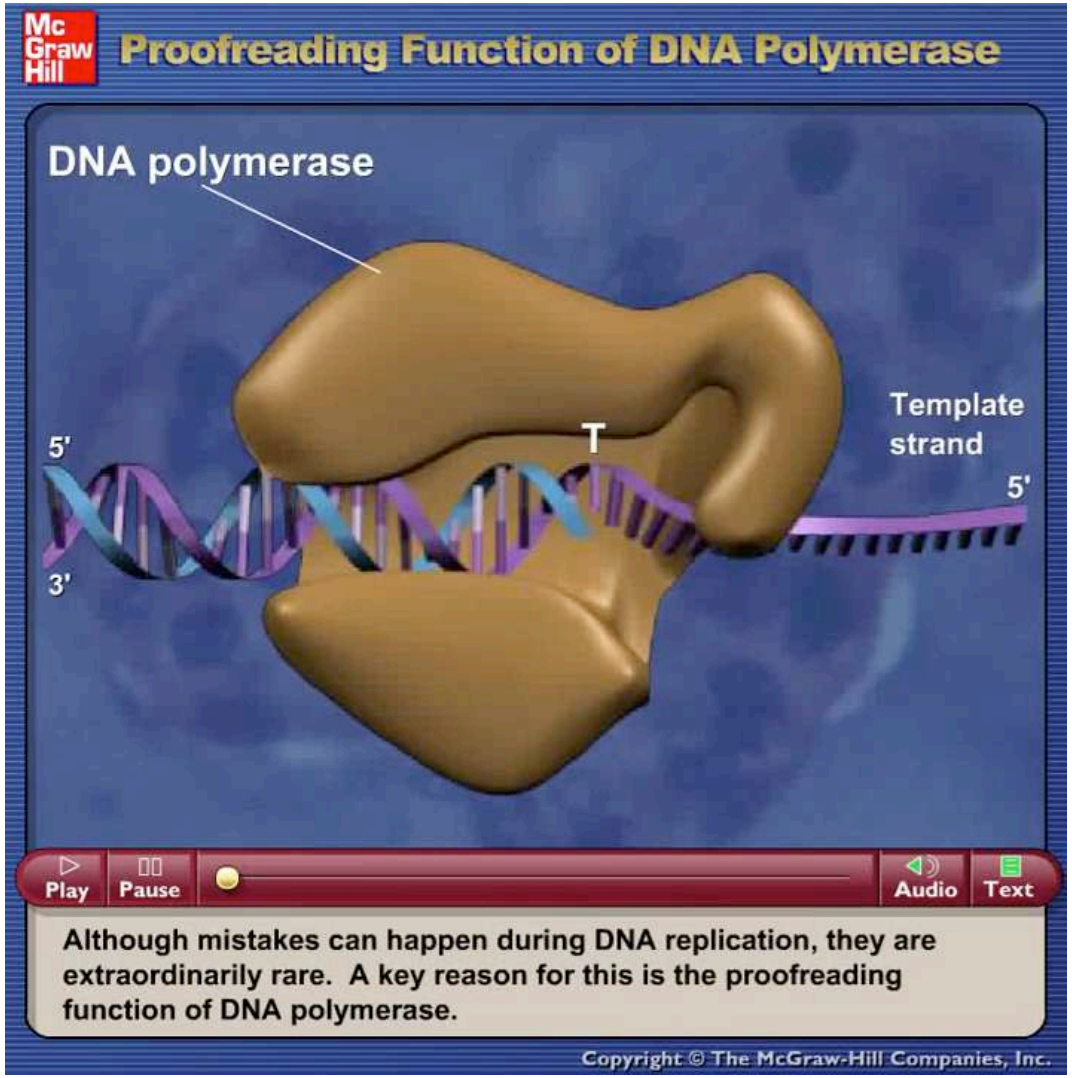
k = Boltzmann Const

T = Temperature

- **In addition, DNA polymerases Mismatch repair ability: 'wrong' inserted base can be removed (proofreading)**

- Overall accuracy = 1 out of 10^9 nucleotides added.

Proof Reading activity of DNA polymerase



https://www.youtube.com/watch?v=42boKYMontE

5’ → 3’ DNA replication ?

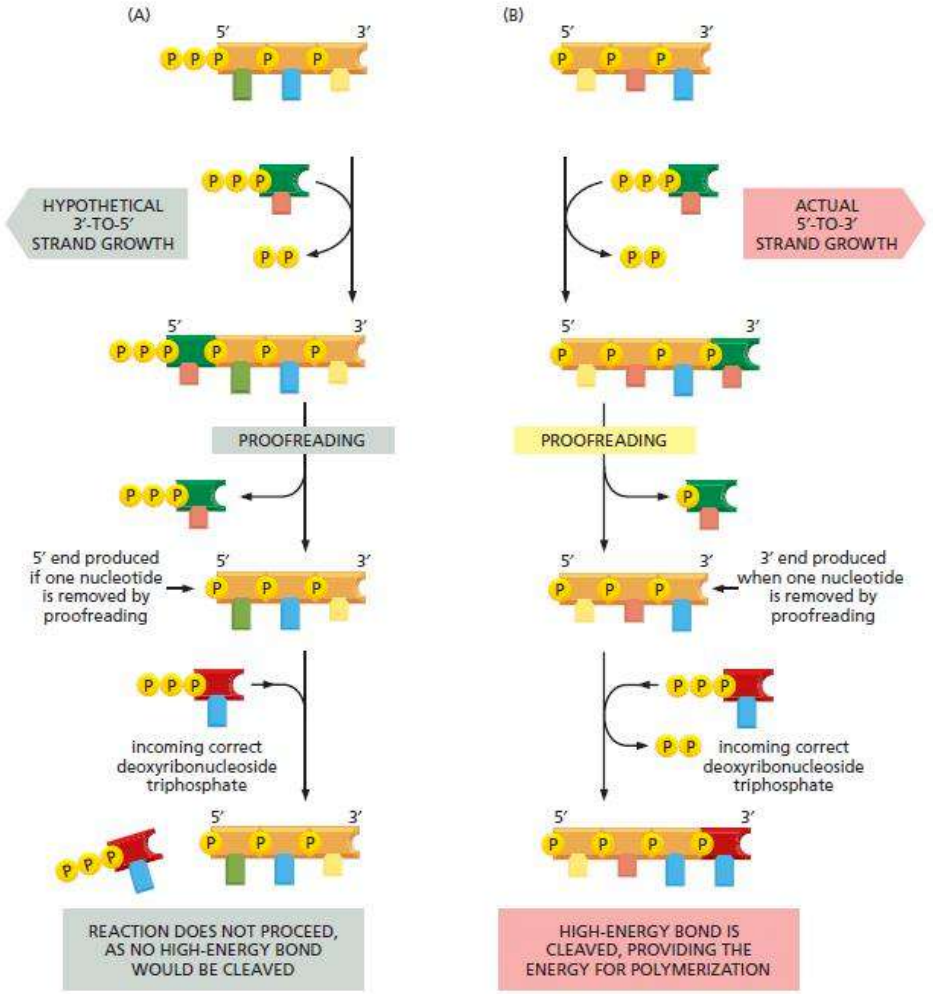


Figure 6–15 **A need for proofreading explains why DNA chains are synthesized only in the 5′ to 3′ direction.** (A) In the hypothetical 3′-to-5′ polymerization scheme, proofreading would remove an incorrect nucleotide (*dark green*), which would then block addition of the correct nucleotide (*red*) and thereby prevent further chain elongation. (B) Growth in the 5′-to-3′ direction allows the chain to continue to be elongated when an incorrect nucleotide has been added and then removed by proofreading (see Figure 6–14).

from *Essential Cell Biology*

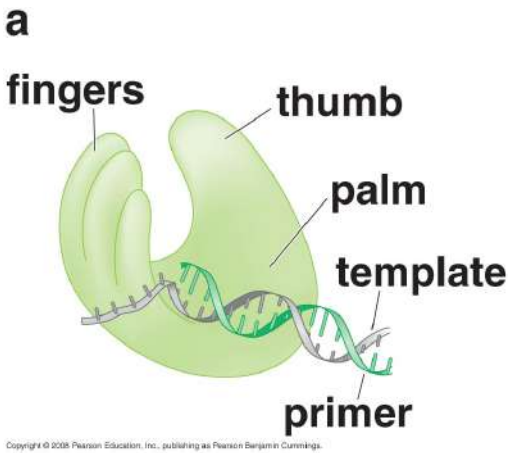
DNA replication = Order from chaos.
What about 2nd Law ?

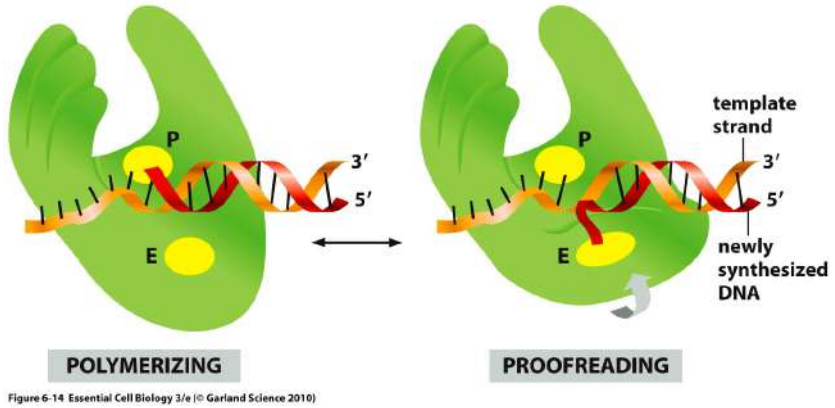
$$\text{Poly-DNA}_n + \text{dNTP} = \text{Poly-DNA}_{n+1} + \text{P-P} = \text{DNA}_{n+1} + \text{P} + \text{P}$$

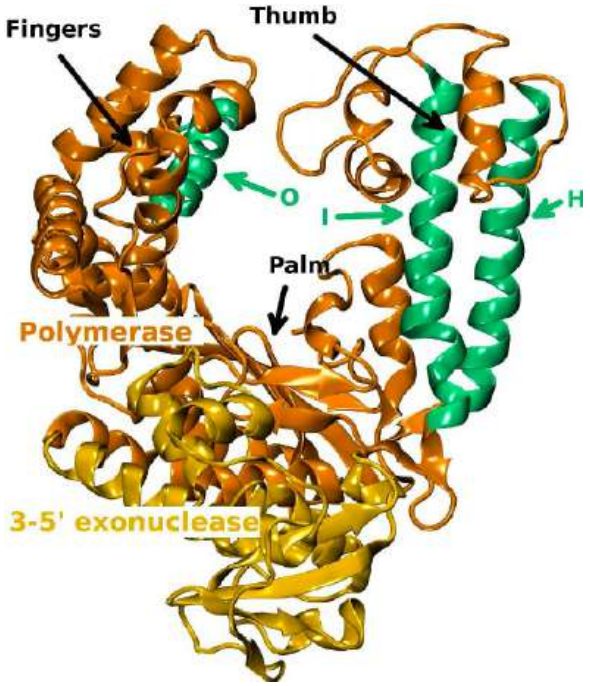
Start: 2 molecules → End: 3 molecules, $\Delta S_{Universe} > 0$

Hope you have understood why dNTP NOT dNMP is a substrate ?

DNA polymerase looks like a hand: Thumb + Palm+ Fingers

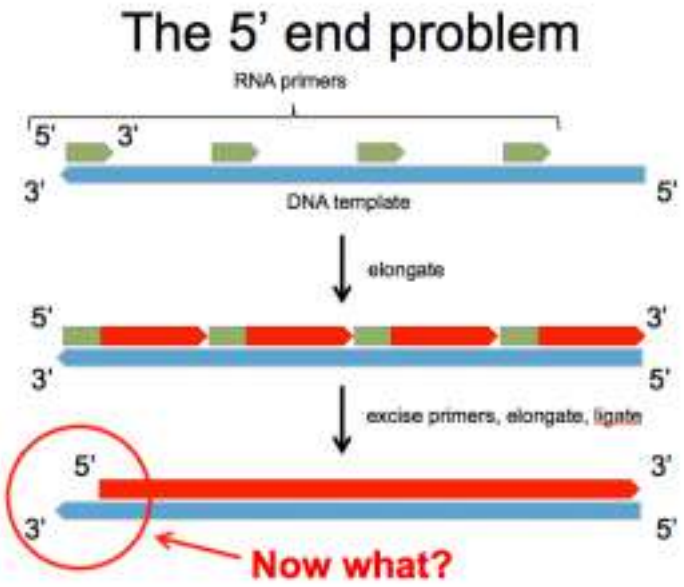


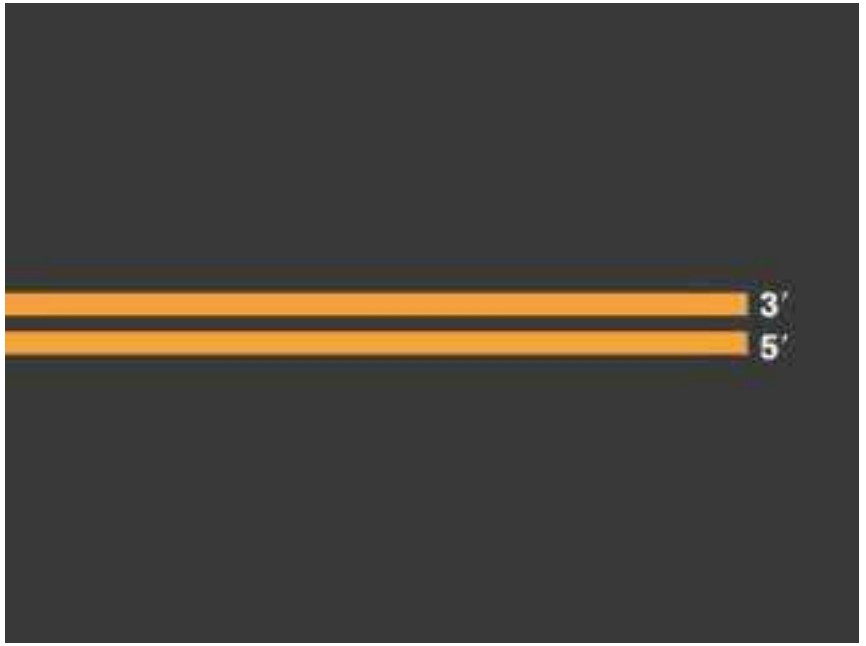




The Journal of Physical Chemistry B 116(35):10676-83 · August 2012

What about ends of chromosomes?



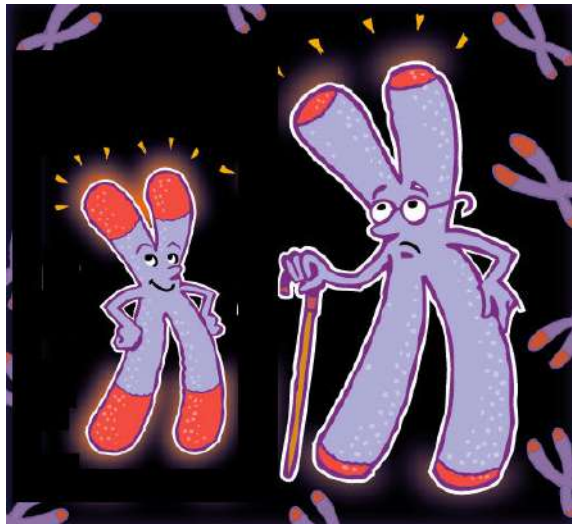


https://www.youtube.com/watch?v=AJNoTmWsE0s

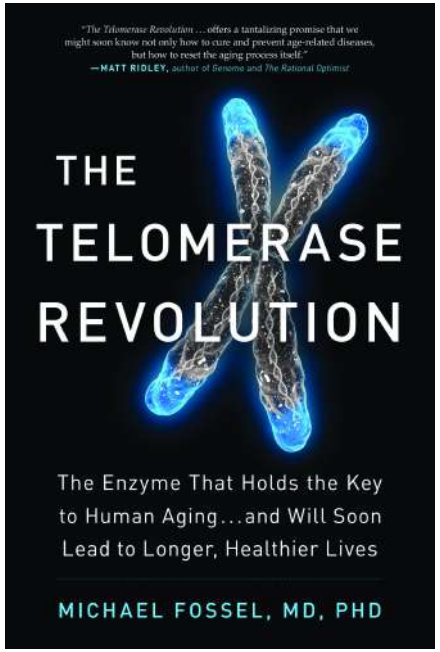
End replication problem..

End replication problem for both leading and lagging strand

telomeres dictate a cell's life span

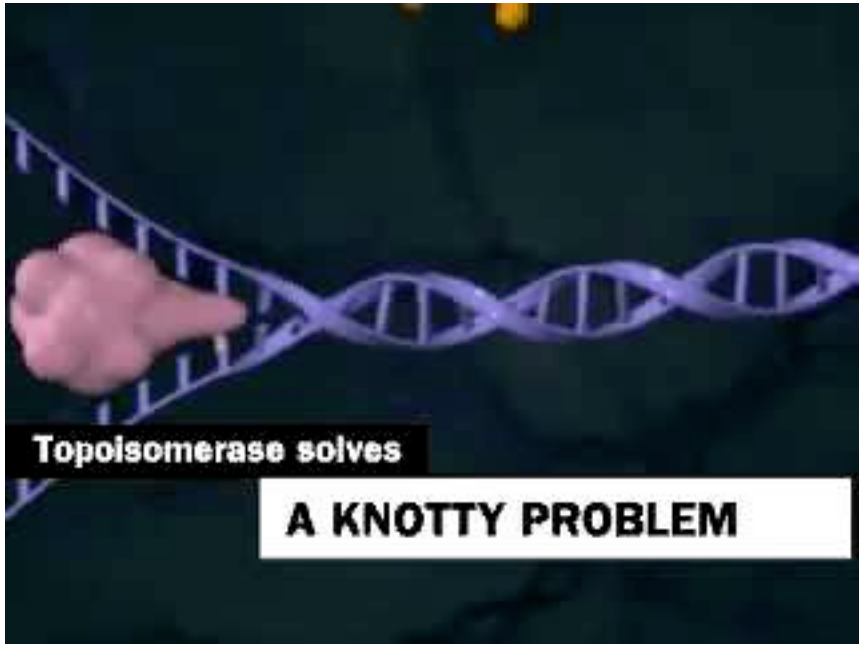


http://www.freegrab.net/telomere.htm



Bernardes de Jesus, Bruno et al. "Telomerase Gene Therapy in Adult and Old Mice Delays Aging and Increases Longevity without Increasing Cancer." *EMBO Molecular Medicine* 4.8 (2012): 691–704.

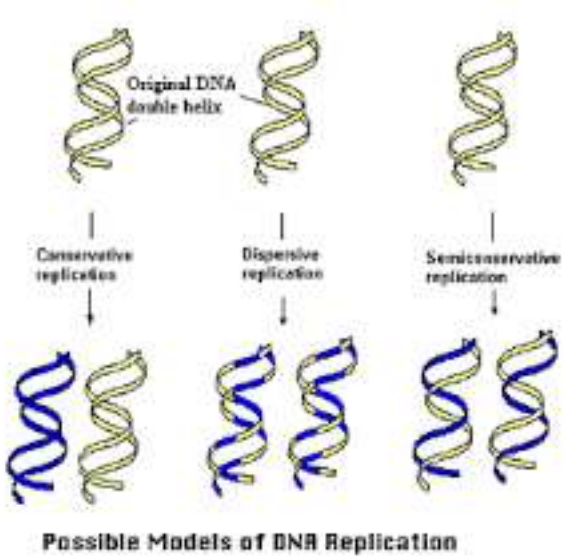
DNA unwinding during replication introduces strain. What to solve this problem ?



https://www.youtube.com/watch?v=EYGrElVyHnU

Topoisomerase not shown in the video (slide 10) ?

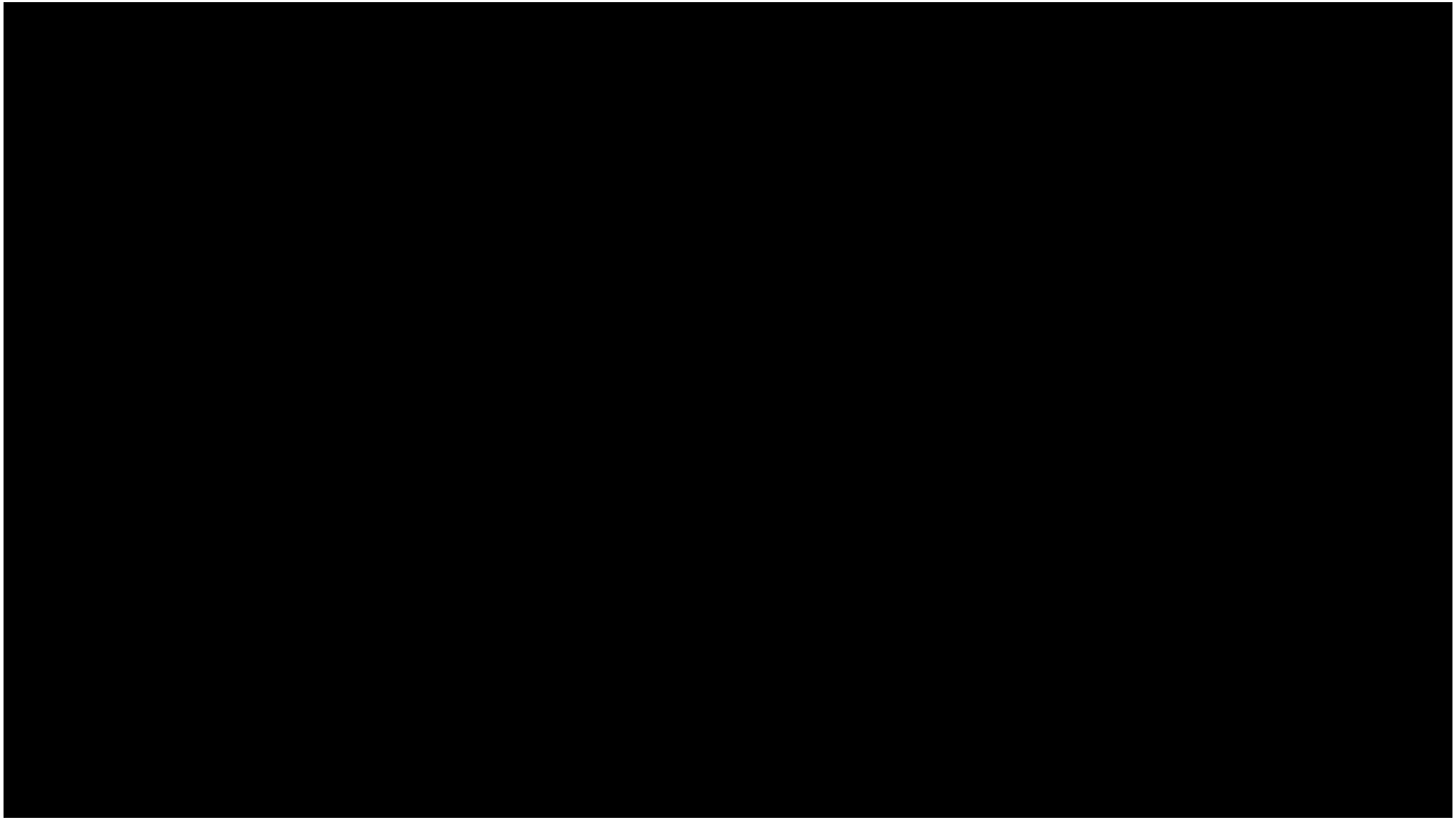
Which of the following is the mechanism of DNA replication ?



Possible Models of DNA Replication

The Most Beautiful Experiment in Biology: Meselson & Stahl …

Meselson & Stahl experiment **: DNA replication is semiconservative**



https://www.youtube.com/watch?v=3RQfSdTrIIk