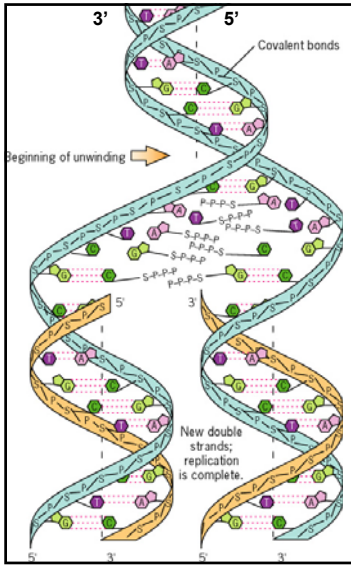
DNA Replication Chapter 11

What you need to know:

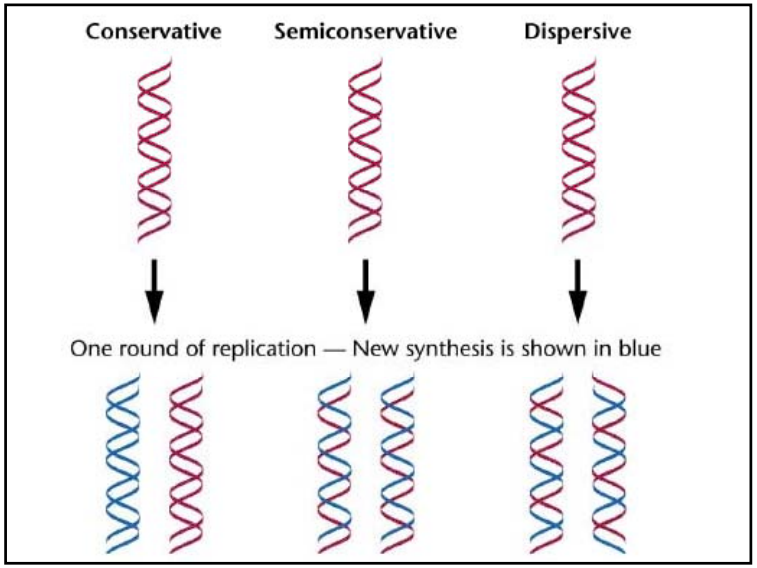
Sections 11.1- 11.7

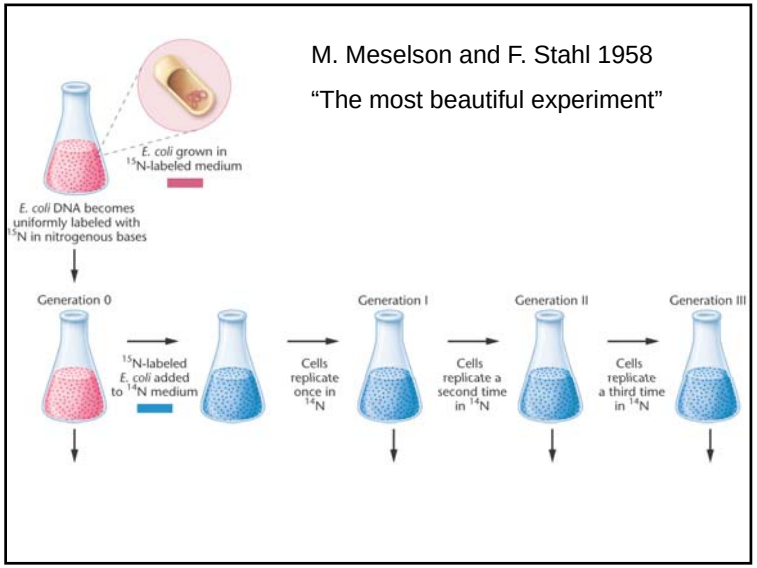
You do not need to DNA recombination and Holiday model and Gene conversion sections (last two sections of Chapter 11).



You already know that replication is in a semi-conservative manner.

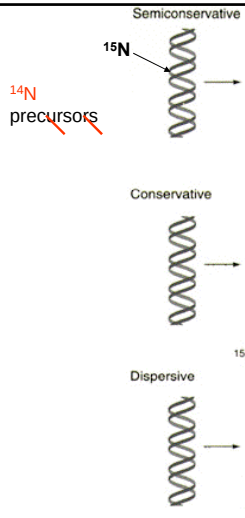
What were the other possible mechanisms and how were they refuted?



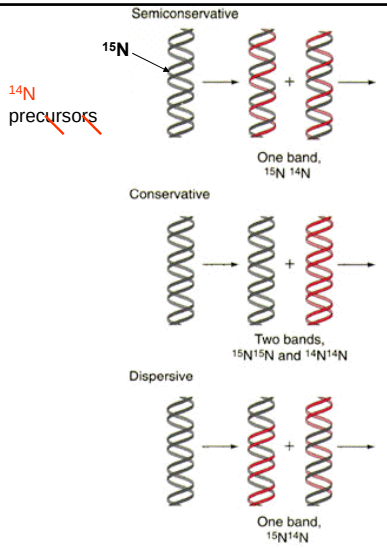


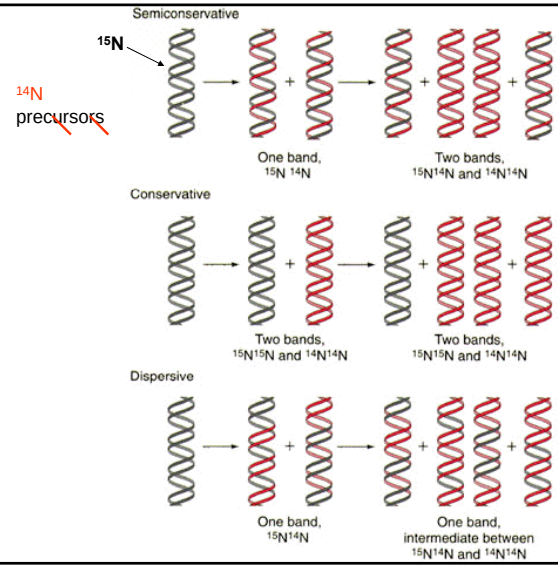
M. Meselson and F. Stahl 1958

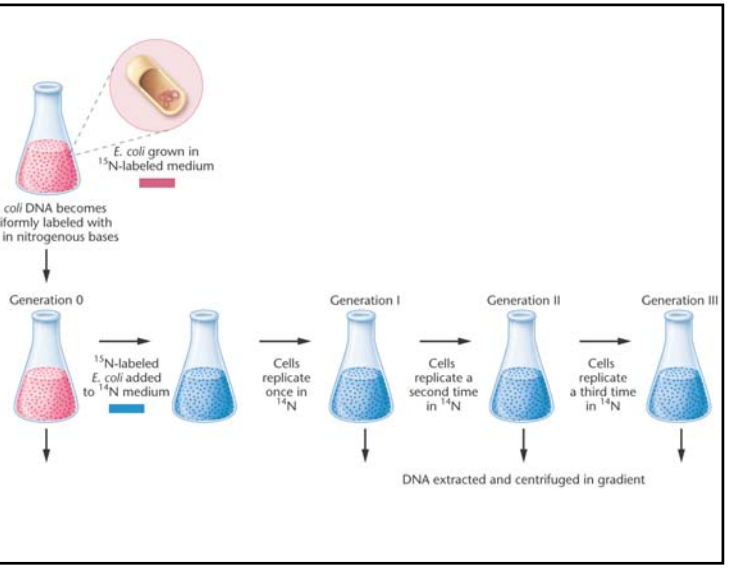
"The most beautiful experiment"

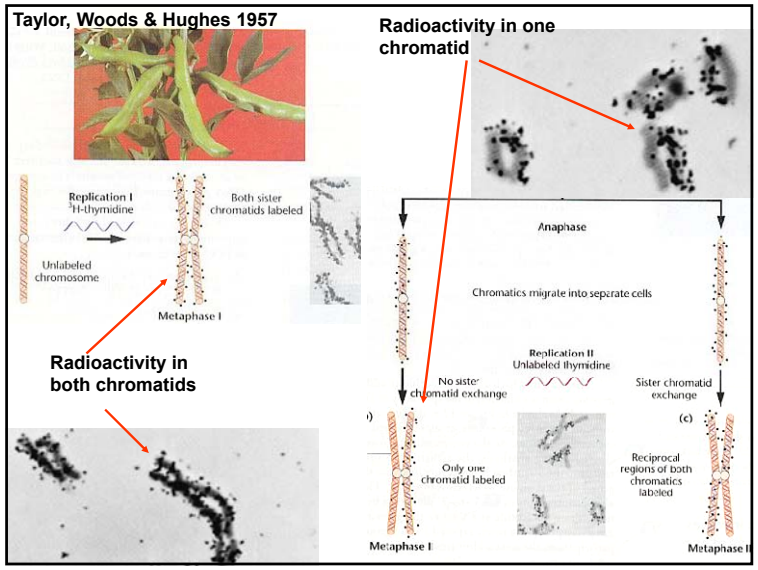


Could you tell me the expected banding patterns for each model after generation 1 and 2?









How does the cell replicate its DNA?

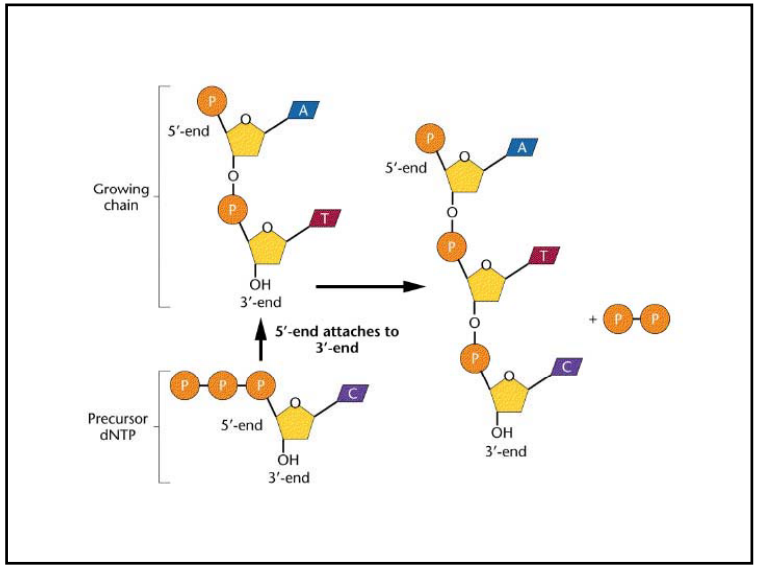
What are the enzymes involved?

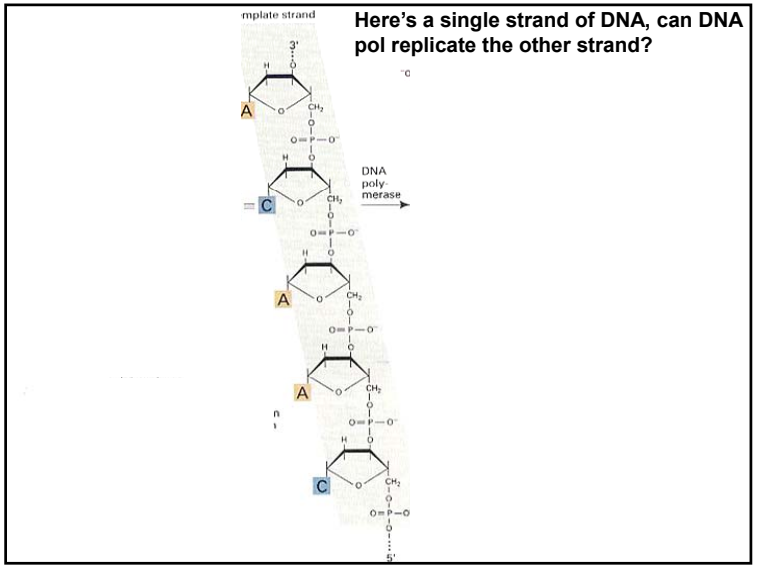
Kornberg 1958 isolated DNA polymerase

Replication in vitro of ϕX174 (5386 bases) bacteria phage.

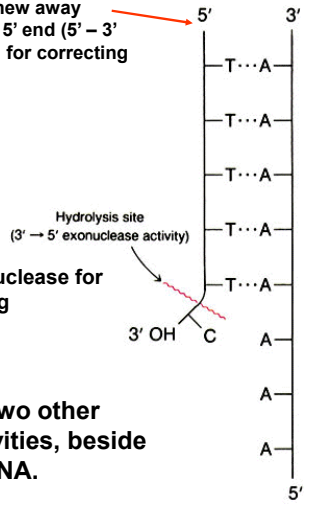
- DNA template
- dNTPs (dATP, dGTP, dCTP, dTTP)
- Mg++
- DNA polymerase

Reaction was inefficient but made biologically active product (infectious). **What is missing?**



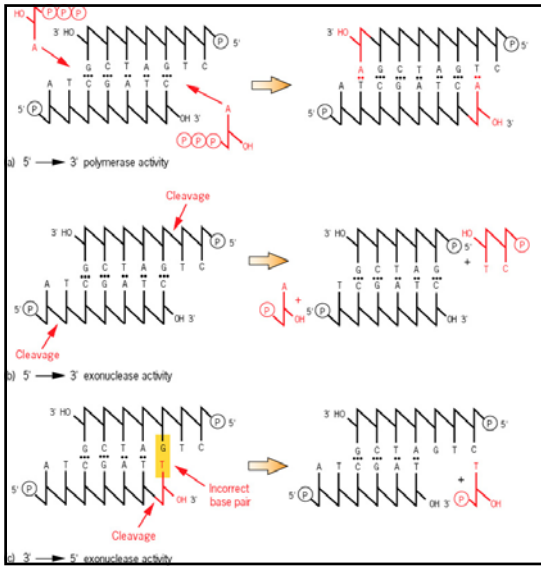


DNA Pol I also can chew away nucleotides from the 5' end (5' – 3' exonuclease activity) for correcting mismatches.



3' to 5' exonuclease for proof reading

DNA pol I has two other enzymatic activities, beside synthesizing DNA.



Summary of the three activities of DNA polymerase I of *E. coli*.

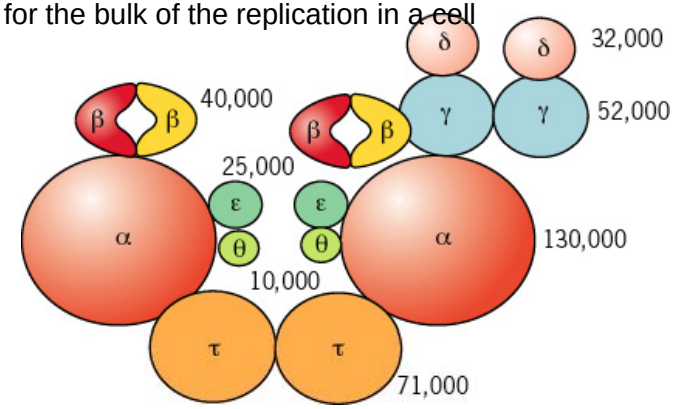
Problem:
DeLucia & Cairns (1969) found a mutant deficient in DNA Pol I that still can replicate.

Conclusion:
There must be additional DNA polymerases in the cell!!

Properties of Three Bacterial (E. coli) DNA polymerases

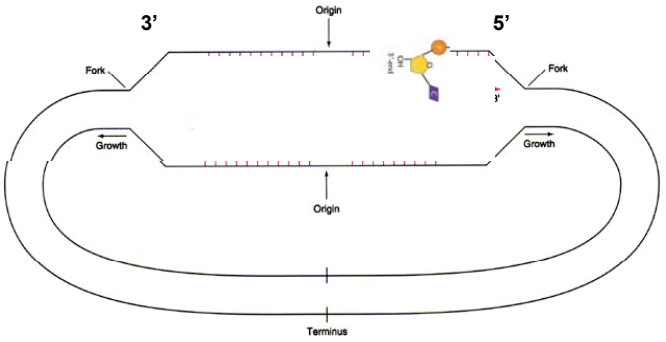
DNApol	I	II	III
Initiation of synthesis	No	No	No
5' to 3' polymerization	Yes	Yes	Yes
3' to 5' exonuclease activity (proof reading)	Yes	Yes	Yes
5' to 3' exonuclease activity (repair, primer removal)	Yes	No	No
Molecules/cell	400	varies	15
Number of subunits (genes)	1	1	10
Size of Molecule (kDaltons)	103	90	430

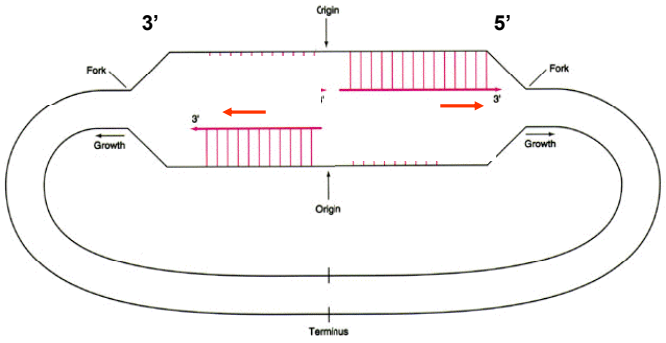
E. coli DNA pol III is a complex molecule responsible for the bulk of the replication in a cell

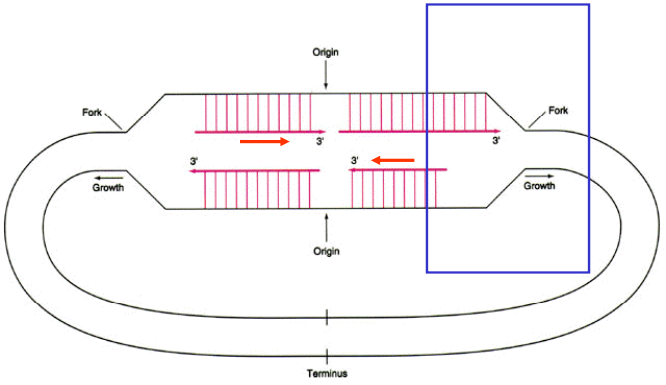


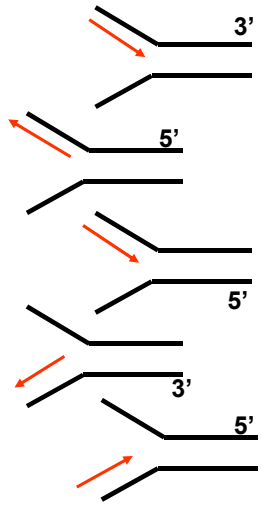
Structure of the *E. coli* DNA polymerase III holoenzyme. The numbers give mass in daltons.

How does replication work?









A

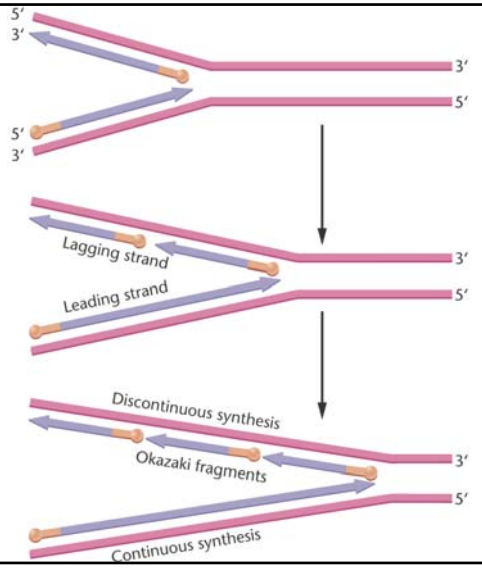
B

C

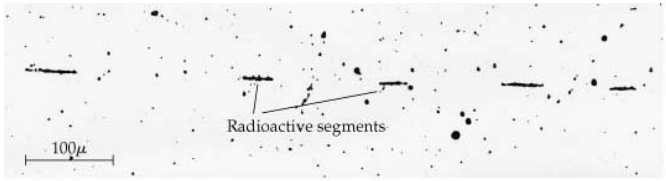
D

E

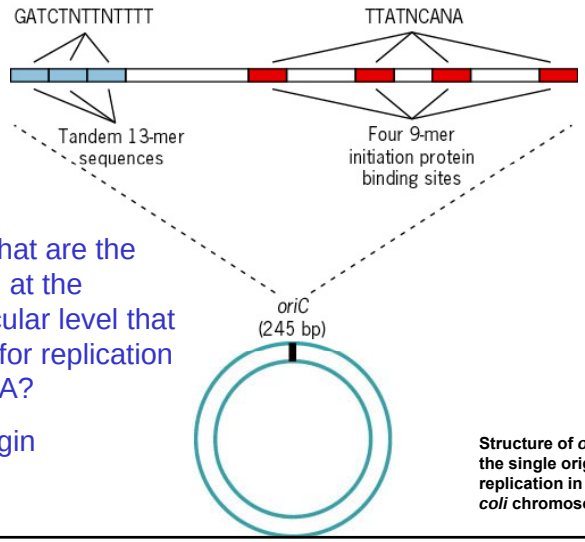
Which shows a correct orientation?



Pulse-chase experiments show Okazaki Fragments on lagging strand.



(a) Autoradiograph of a portion of a DNA molecule from a Chinese hamster cell that had been pulse-labeled with ^{3}H-thymidine.

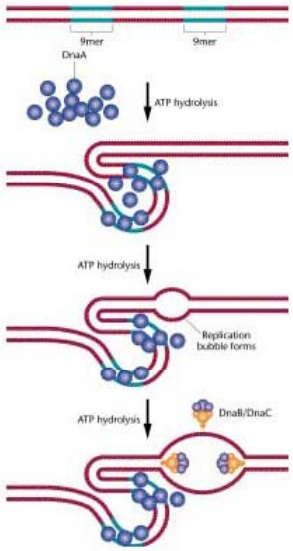


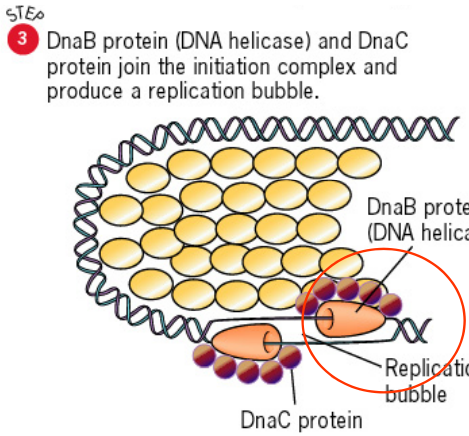
So, what are the steps, at the molecular level that allow for replication of DNA?

1) Origin

Structure of *oriC*, the single origin of replication in the *E. coli* chromosomes.

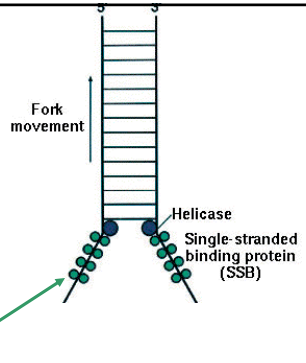
2) The protein DnaA binds to oriC





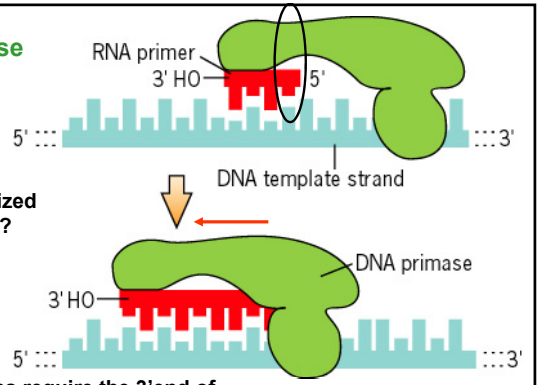
3) Additional proteins, DnaB & DnaC combine to form a Helicase

Let's focus on one fork to follow the next several steps in the replication process.



4) After Helicase opens up the double helix, Single Stranded Binding proteins (SSBP) prevent helix from snapping back.

5) RNA polymerase (Primase) lays down an RNA primer

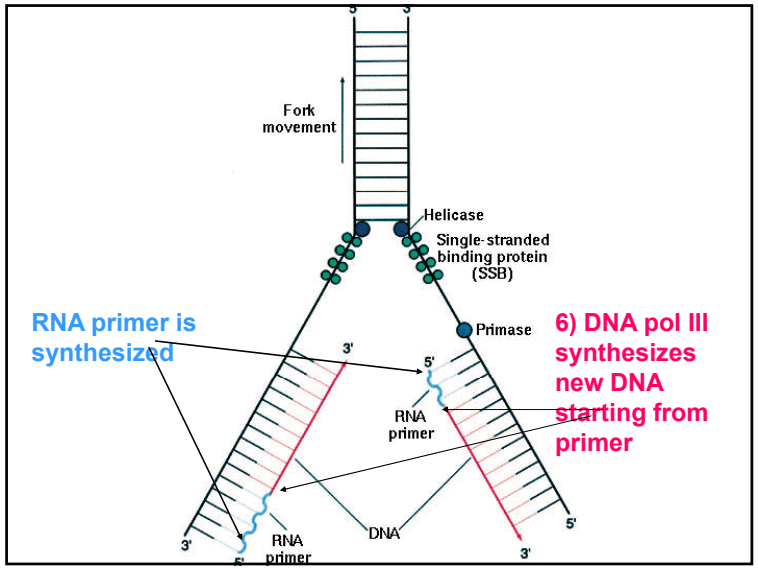


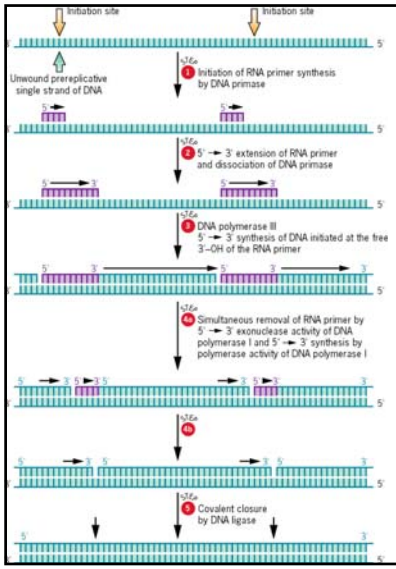
Why is RNA synthesized on the DNA template?

All DNA polymerases require the 3'end of an existing piece of DNA to synthesize more DNA.

RNA polymerase (Primase) can synthesize nucleic acid without a starting primer!!

The initiation of DNA strands with RNA primers.





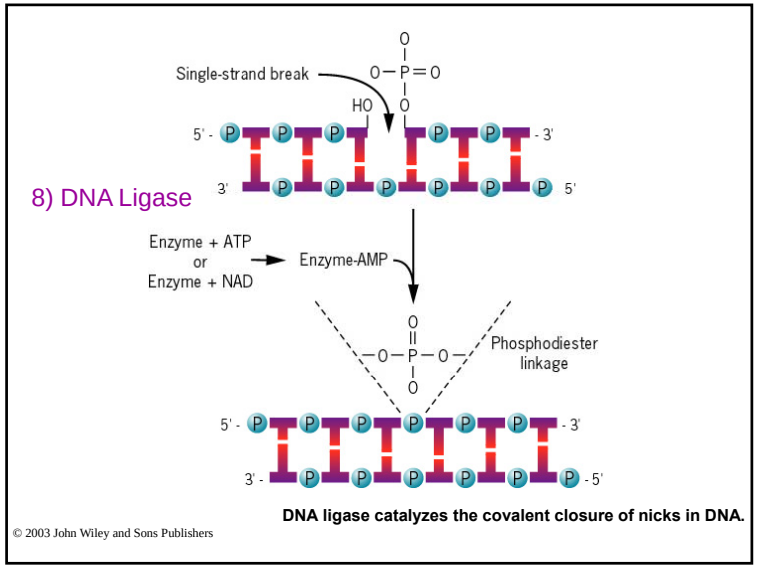
Problem: DNA Pol III uses the 3' end of the primer to begin synthesizing DNA, but RNA primers remain!

7) Solution: Another enzyme (old friend DNA pol I) chews away primer (5' – 3') and can replace space with DNA

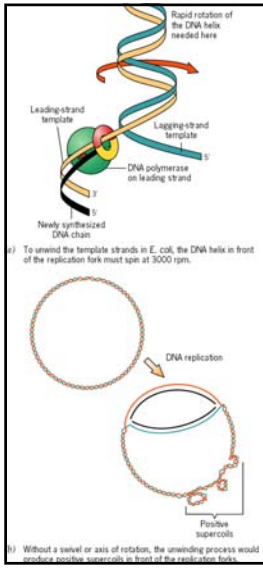
Another problem: Once DNA Pol III replaces RNA primer, this piece is not attached to the piece in front of it.

8) Solution: Another enzyme, DNA ligase "clips" the pieces together.

Synthesis and replacement of RNA primers during replication of the lagging strand of DNA.



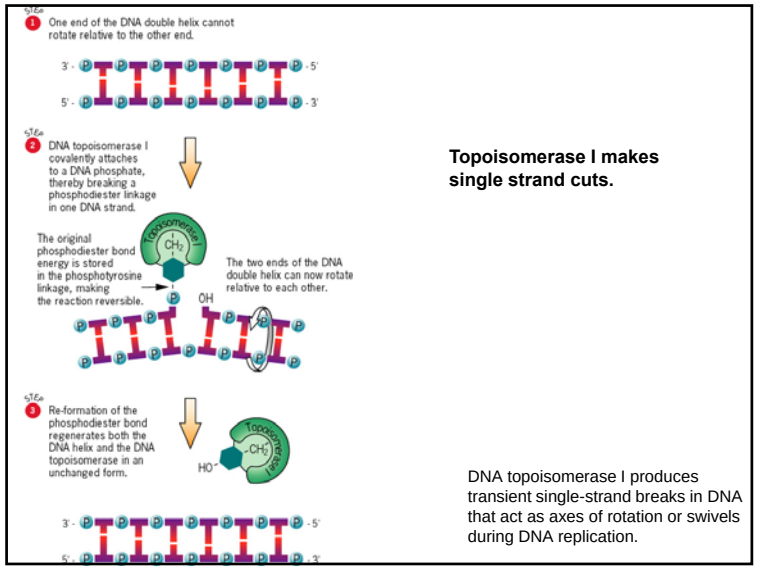
DNA ligase catalyzes the covalent closure of nicks in DNA.



Another problem: As the replicating fork unwinds and expands the remainder of the DNA will become more super-coiled!

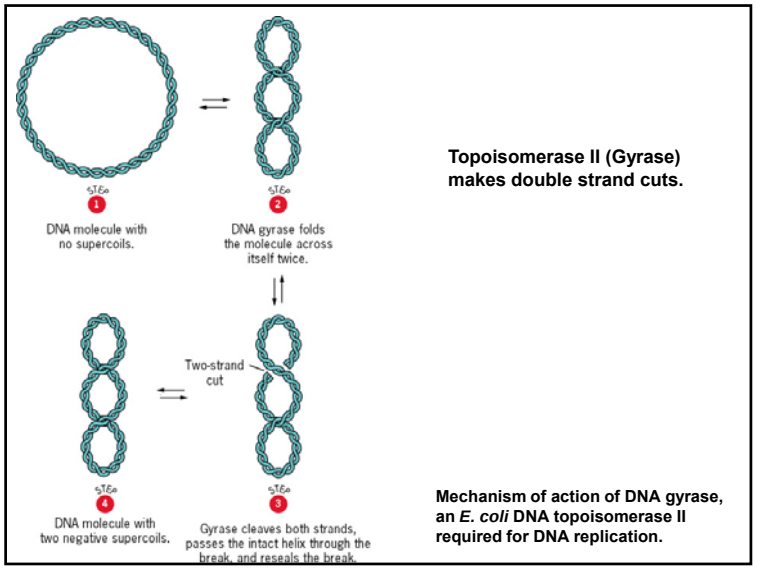
9) Solution: Other enzymes (gyrases/topoisomerases) are needed to relieve the tension.

A swivel or axis of rotation is required during the replication of circular molecules of DNA like those in the *E. coli* or phage λ chromosomes.



Topoisomerase I makes single strand cuts.

DNA topoisomerase I produces transient single-strand breaks in DNA that act as axes of rotation or swivels during DNA replication.



Topoisomerase II (Gyrase) makes double strand cuts.

Mechanism of action of DNA gyrase, an *E. coli* DNA topoisomerase II required for DNA replication.

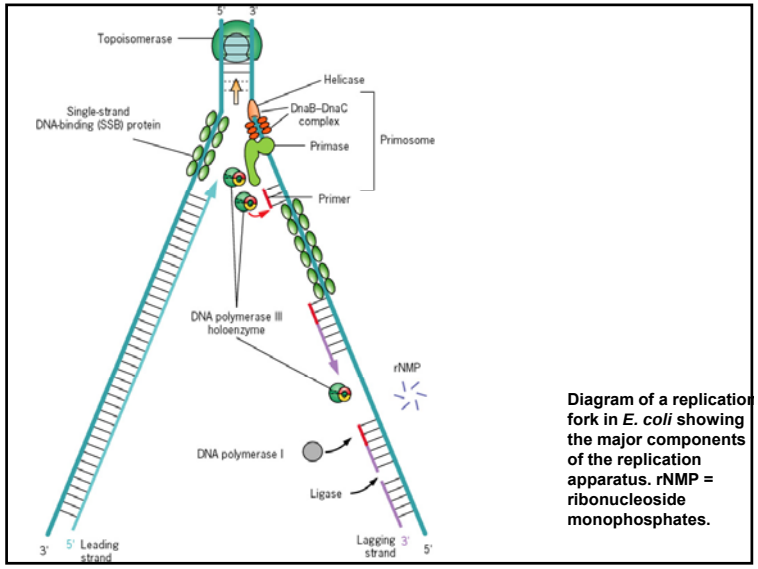


Diagram of a replication fork in *E. coli* showing the major components of the replication apparatus. rNMP = ribonucleoside monophosphates.

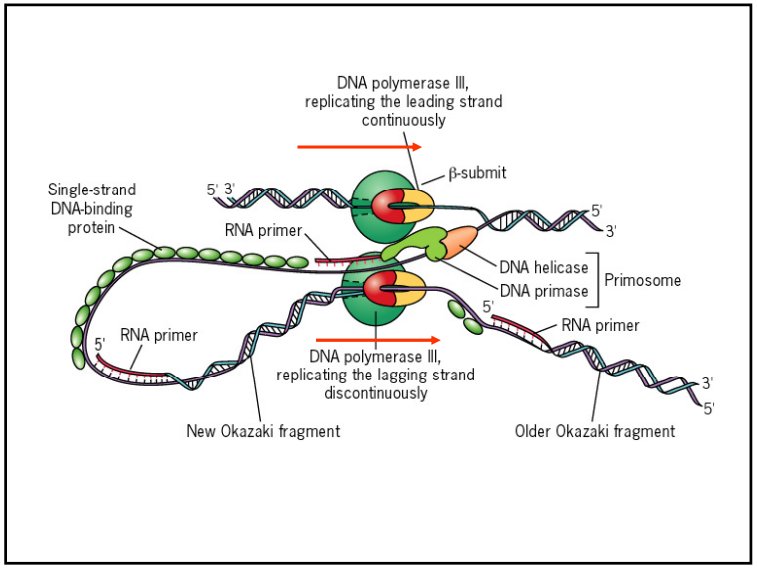


TABLE 11.4 SOME OF THE VARIOUS *E. COLI* MUTANT GENES AND THEIR PRODUCTS OR ROLE IN REPLICATION

Mutant Gene	Enzyme or Role
polA	DNA polymerase I
polB	DNA polymerase II
dnaE, N, Q, X, Z	DNA polymerase III subunits
dnaG	Primase
dnaA, I, P	Initiation
dnaB, C	Helicase at *oriC*
oriC	Origin of replication
gyrA, B	Gyrase subunits
lig	Ligase
rep	Helicase
ssb	Single-stranded binding proteins
rpoB	RNA polymerase subunit

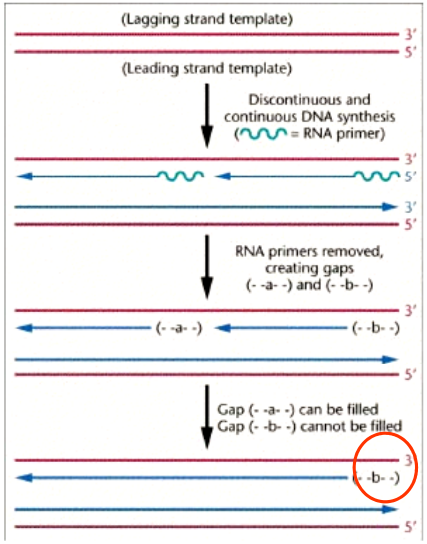
Comparison of Prokaryotic and Eukaryotic DNA Synthesis

	E. coli	Eukaryotes
No. of DNA polymerases	**DNApol I, II, III**	**α δ ε, β ζ, γ**
No. of DNAPol/Cell	**15 DNA PolIII**	**50,000 Alpha**
No. of Origins	**1**	**3500 (Drosophila) 25,000(Mammals)**
Speed of Synthesis	**100 kb/min.**	**0.5-5 kb/min.**
Genome Replication Time	**20-40 min.**	**3 min. (Drosophila) to hours**

Problem at the Ends!!!

How do you fill in the last 30 base on the lagging strand?

Telomeres!



<u>Telomerase</u> (reverse transcriptase) ribonucleoprotein

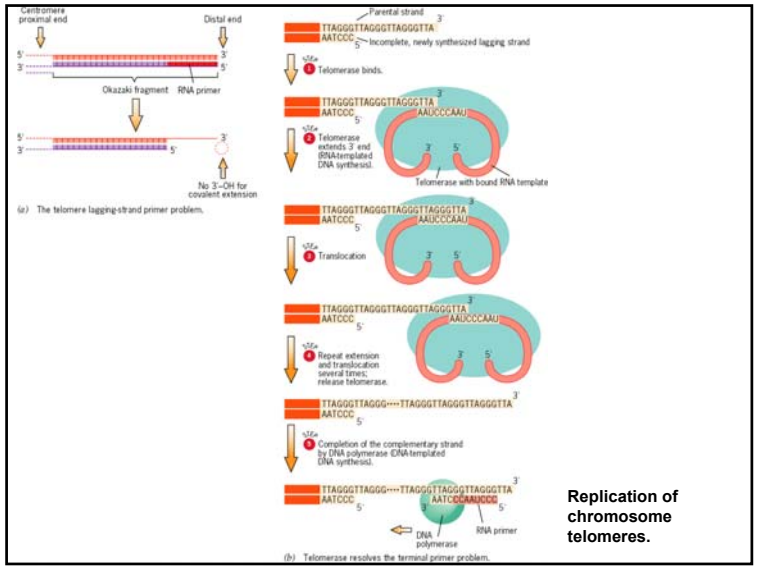
Enzyme extends 3' end and new synthesis occurs from this

Telomerase adds copies with RNA template having AAUCCC sequence

Centromere-- ---**5'**TTAGGG-'TTAGGG-'TTAGGG-'TTAGGG**3'-end**
Centromere-- ---**3'**AATCCC-**5'**

-----few kb-150 kb--- ---few 100s---

To "save" your chromosomes, from slowly being lost, a dynamic equilibrium becomes established:
Ends are **Lost** after every round of replication,
Ends are **Gained** by telomerase adding back the repeat!!



Replication of chromosome telomeres.

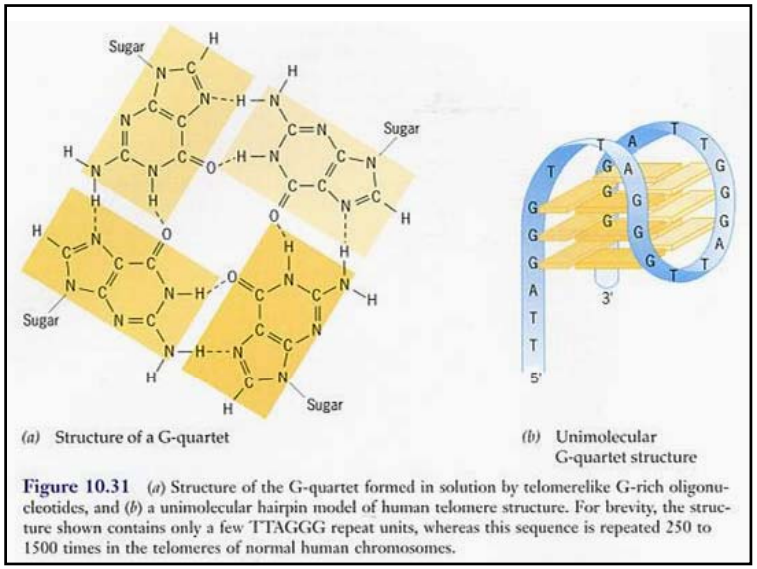


Figure 10.31 (*a*) Structure of the G-quartet formed in solution by telomerelike G-rich oligonucleotides, and (*b*) a unimolecular hairpin model of human telomere structure. For brevity, the structure shown contains only a few TTAGGG repeat units, whereas this sequence is repeated 250 to 1500 times in the telomeres of normal human chromosomes.