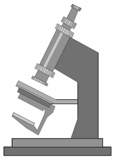
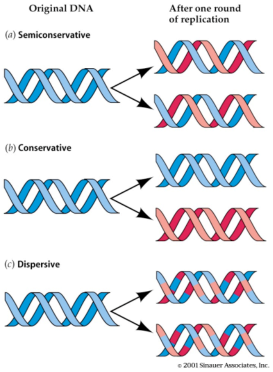
DNA Replication and Recombination

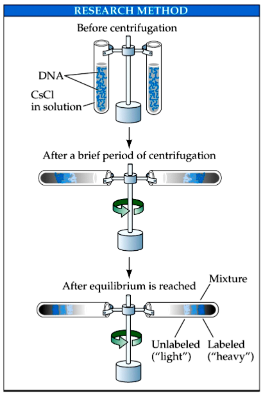


Three possible models for DNA replication

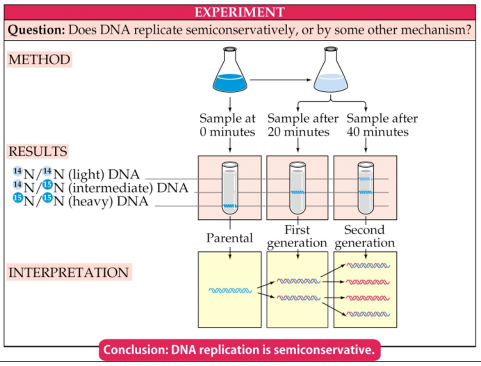


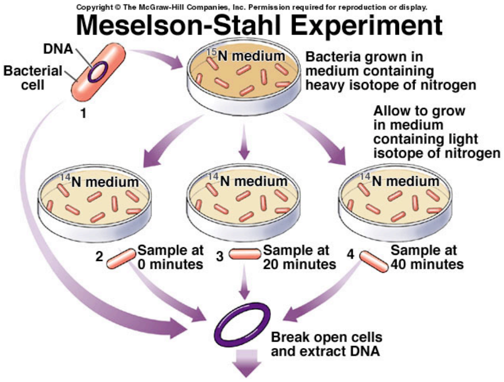
Centrifuging DNA

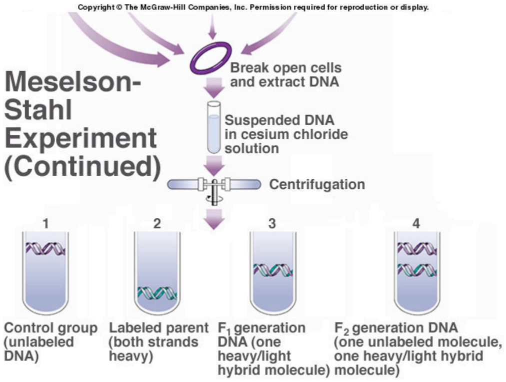
Svedberg coefficient (S)



Meselson and Stahl

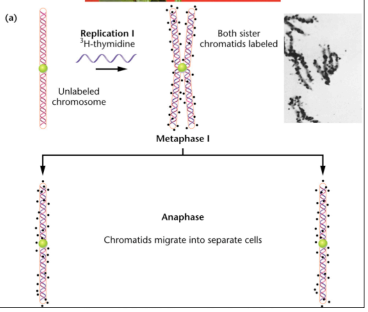


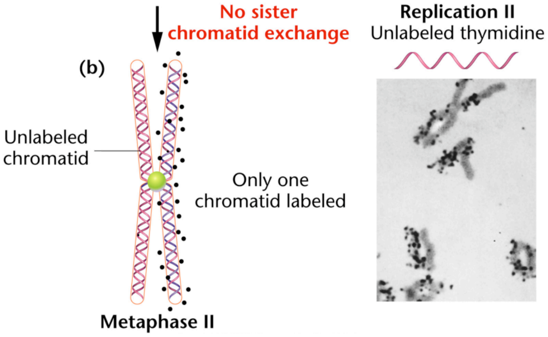


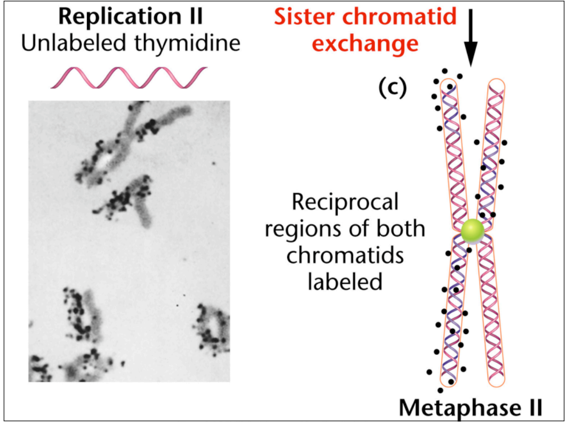


Taylor, Wood and Hughes

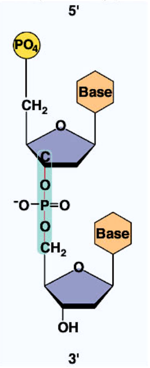
DNA replication is semiconservative in eukaryotes







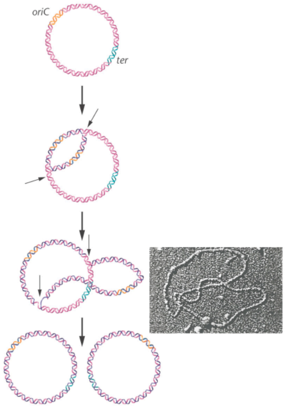
Phosphodiester Bond



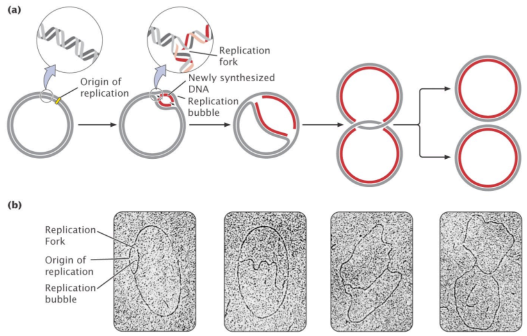
E. coli Replication

DNA replication begins at the origin of replication and is bidirectional rather than unidirectional

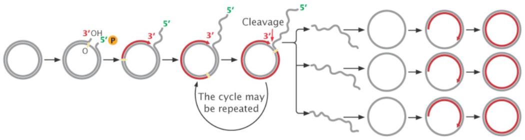
A replicon include the DNA replicated from on ori site



Theta replication

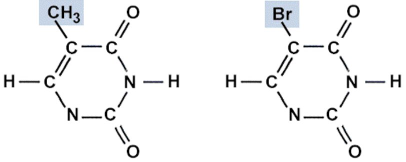


Rolling Circle replication



Base analog

Tracking synthesis of DNA using base analogs



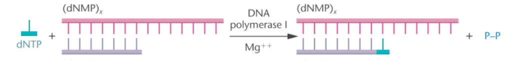
Thymine

5-bromouracil

Kornberg's approach

DNA Polymerase I

- 4 dNTPs
- DNA template



Deoxyribose nucleoside triphosphate (A,T,C, or G)

DNA template $(dNMP)_x$ and its partial complement $(dNMP)_n$

Complement to template strand is extended by one nucleotide (n + 1)

Inorganic pyrophosphate

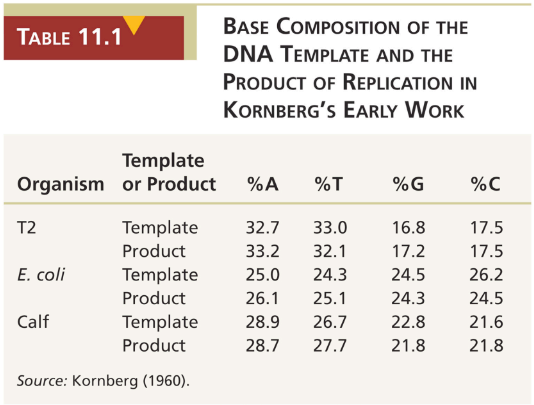
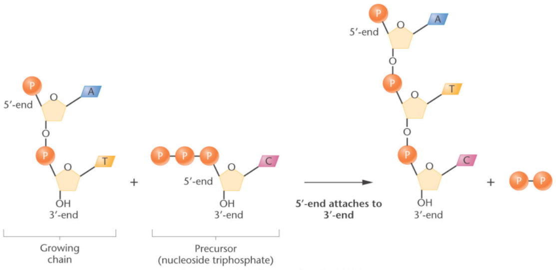


Table 11.1 **Base Composition of the DNA Template and the Product of Replication in Kornberg's Early Work**

Organism	Template or Product	%A	%T	%G	%C
T2	Template	32.7	33.0	16.8	17.5
	Product	33.2	32.1	17.2	17.5
E. coli	Template	25.0	24.3	24.5	26.2
	Product	26.1	25.1	24.3	24.5
Calf	Template	28.9	26.7	22.8	21.6
	Product	28.7	27.7	21.8	21.8

Source: Kornberg (1960).

Polymerization of nucleotides



Elogation occurs in the 5' to 3' direction with the release of a pyrrophosphate

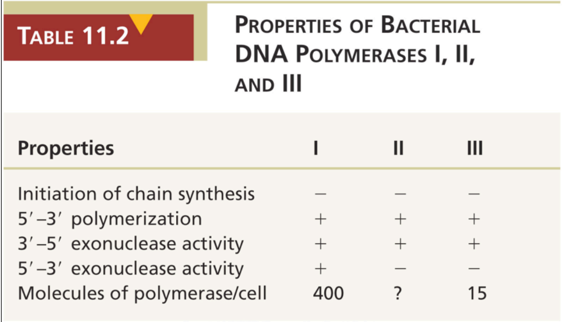


Table 11.2 **Properties of Bacterial DNA Polymerases I, II, and III**

Properties	I	II	III
Initiation of chain synthesis	−	−	−
5′–3′ polymerization	+	+	+
3′–5′ exonuclease activity	+	+	+
5′–3′ exonuclease activity	+	−	−
Molecules of polymerase/cell	400	?	15

✓DNA polymerases I, II, and III can elongate an existing DNA/RNA strand (called a primer) but cannot initiate DNA synthesis
✓All three possess 3' to 5' exonuclease activity (Proofreading)
✓only DNA polymerase I demonstrates 5' to 3' exonuclease activity (primer removal)

Prokaryotic polymerases

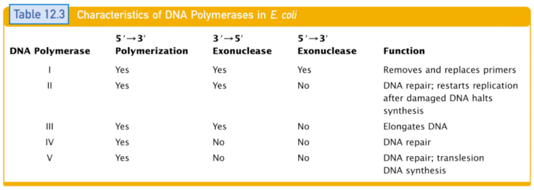


Table 12.3 Characteristics of DNA Polymerases in *E. coli*

DNA Polymerase	5′→3′ Polymerization	3′→5′ Exonuclease	5′→3′ Exonuclease	Function
I	Yes	Yes	Yes	Removes and replaces primers
II	Yes	Yes	No	DNA repair; restarts replication after damaged DNA halts synthesis
III	Yes	Yes	No	Elongates DNA
IV	Yes	No	No	DNA repair
V	Yes	No	No	DNA repair; translesion DNA synthesis

✓ DNA polymerases I, II, IV, and V are involved in various aspects of repair of damaged DNA.

Prokaryotic polymerases

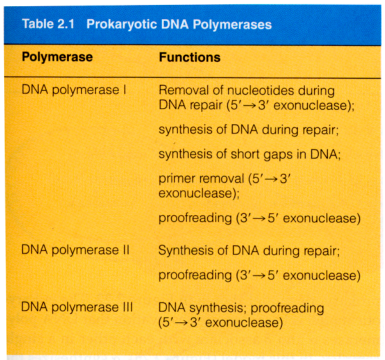


Table 2.1 Prokaryotic DNA Polymerases

Polymerase	Functions
DNA polymerase I	Removal of nucleotides during DNA repair (5′→3′ exonuclease); synthesis of DNA during repair; synthesis of short gaps in DNA; primer removal (5′→3′ exonuclease); proofreading (3′→5′ exonuclease)
DNA polymerase II	Synthesis of DNA during repair; proofreading (3′→5′ exonuclease)
DNA polymerase III	DNA synthesis; proofreading (5′→3′ exonuclease)

DNA Polymerase III Molecule

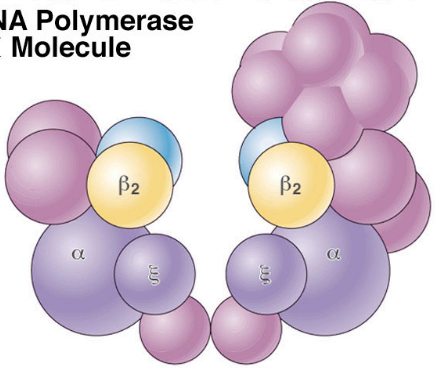
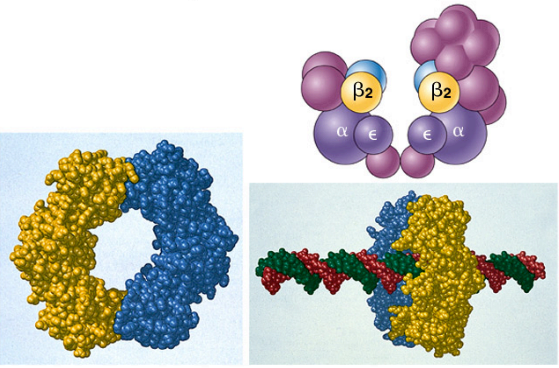


Fig. 14.15

DNA Polymerase III Complex



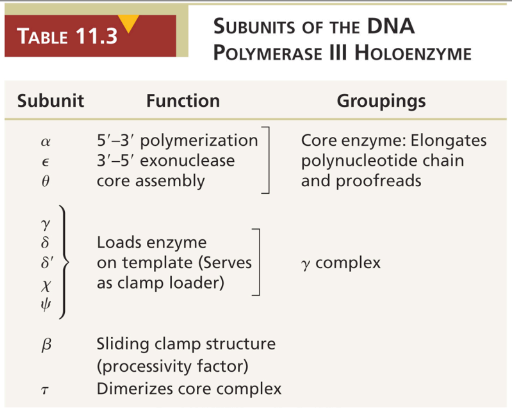


TABLE 11.3 SUBUNITS OF THE DNA POLYMERASE III HOLOENZYME

Subunit	Function	Groupings
α ϵ θ	5′–3′ polymerization 3′–5′ exonuclease core assembly	Core enzyme: Elongates polynucleotide chain and proofreads
γ δ δ' χ ψ	Loads enzyme on template (Serves as clamp loader)	γ complex
β	Sliding clamp structure (processivity factor)	
τ	Dimerizes core complex	

Three models of replication

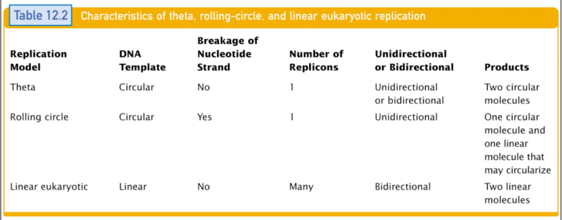
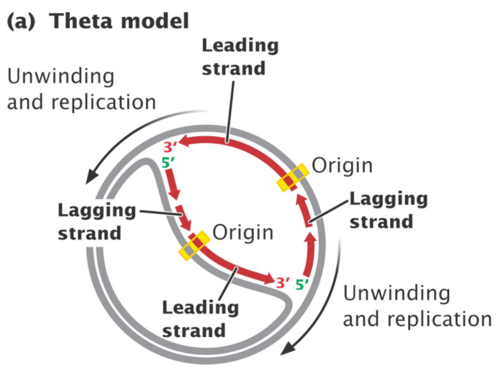
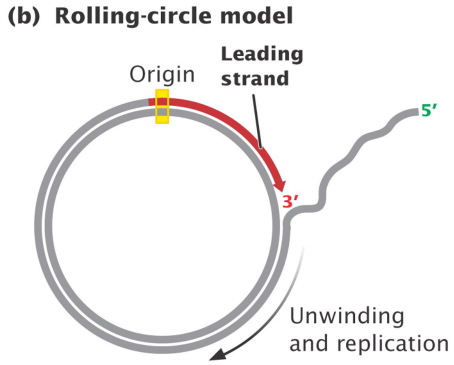
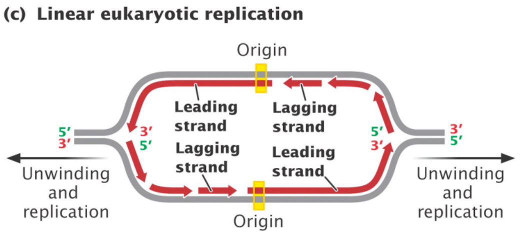


Table 12.2 Characteristics of theta, rolling-circle, and linear eukaryotic replication

Replication Model	DNA Template	Breakage of Nucleotide Strand	Number of Replicons	Unidirectional or Bidirectional	Products
Theta	Circular	No	1	Unidirectional or bidirectional	Two circular molecules
Rolling circle	Circular	Yes	1	Unidirectional	One circular molecule and one linear molecule that may circularize
Linear eukaryotic	Linear	No	Many	Bidirectional	Two linear molecules







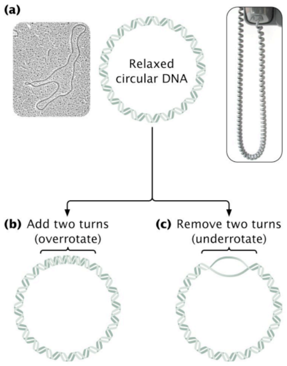
Key issues during DNA Replication

- unwinding of the helix
- reducing increased coiling generated during unwinding
- synthesis of a primer for initiation
- discontinuous synthesis of the second strand
- removal of the RNA primers
- joining of the gap-filling DNA to the adjacent strand
- proofreading

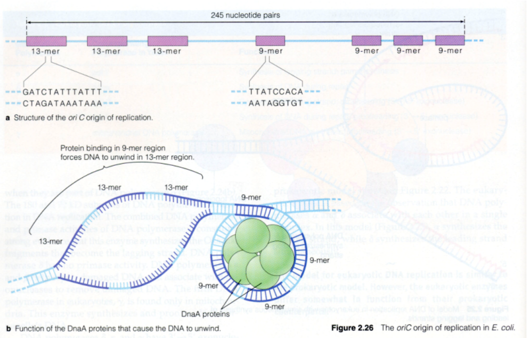
Table 12.1 Number and length of replicons

Organism	Number of Replication Origins	Average Length of Replicon (bp)
Escherichia coli (bacterium)	1	4,200,000
Saccharomyces cerevisiae (yeast)	500	40,000
Drosophila melanogaster (fruit fly)	3,500	40,000
Xenopus laevis (toad)	15,000	200,000
Mus musculus (mouse)	25,000	150,000

Source: Data from B. L. Lewin, *Genes V* (Oxford: Oxford University Press, 1994), p. 536.



ORI site

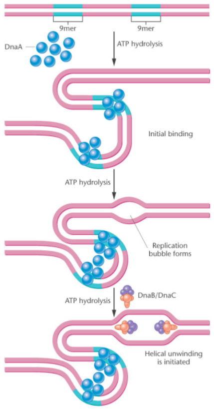


a Structure of the *ori C* origin of replication.

b Function of the DnaA proteins that cause the DNA to unwind.

Figure 2.26 The *oriC* origin of replication in *E. coli.*

Opening the ori site



- ✓ binding of DnaB and DnaC further opens and destabilizes the helix.
  - ATP is required to break hydrogen bonds and denature the double helix, aka helicases
- ✓ Single-stranded binding proteins (SSBPs) stabilize the open conformation
- ✓ Unwinding produces supercoiling
  - relieved by DNA gyrase
    - aka DNA topoisomerases

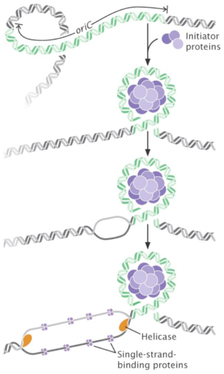
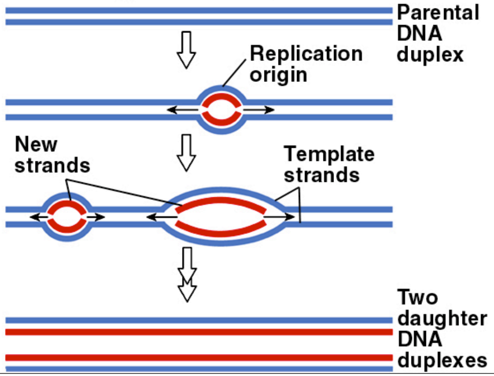


Fig. 14.13

Origins of Replication



Parental DNA duplex

Replication origin

New strands

Template strands

Two daughter DNA duplexes

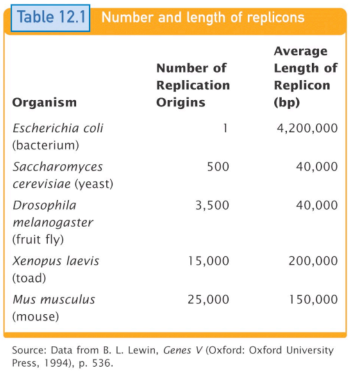


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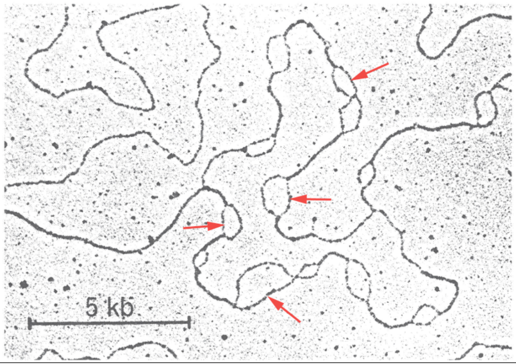
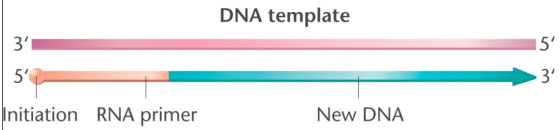
Table 12.1 Number and length of replicons

Source: Data from B. L. Lewin, *Genes V* (Oxford: Oxford University Press, 1994), p. 536.

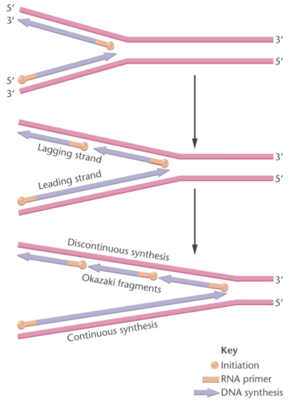
Eukaryotic ori sites



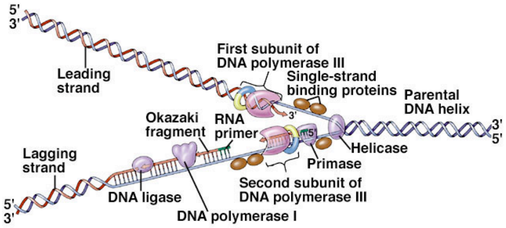
DNA Pol III Primer extension



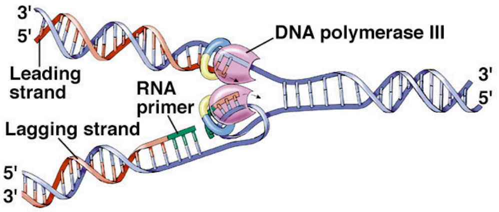
Continuous and Discontinuous extension



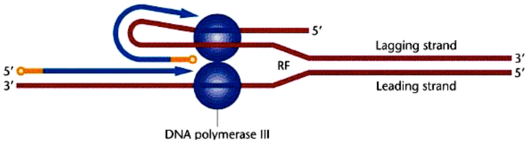
DNA Replication Fork



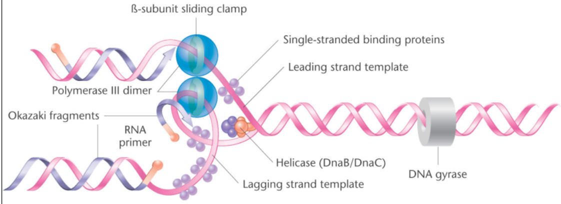
Mechanism of DNA Polymerase III



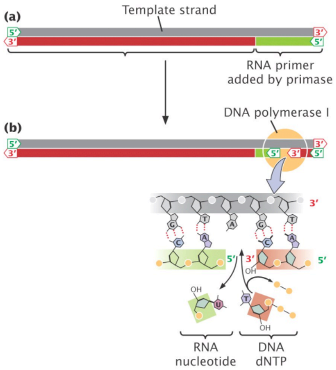
Holoenzyme



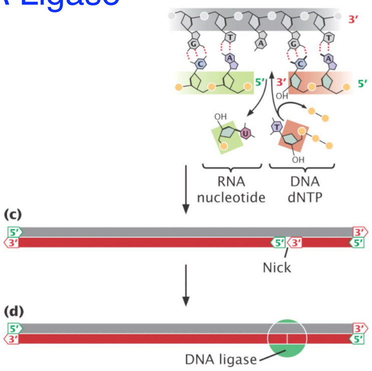
Holoenzyme



DNA Polymerase I



DNA Ligase



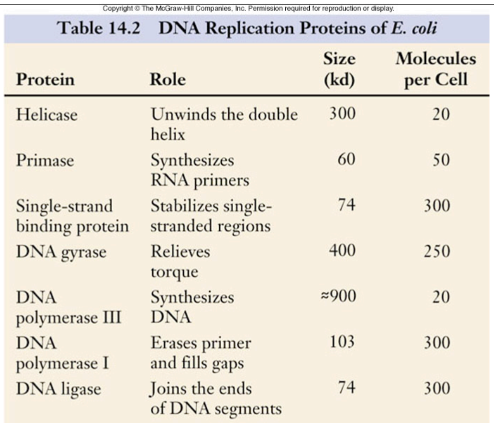


Table 14.2 DNA Replication Proteins of *E. coli*

Protein	Role	Size (kd)	Molecules per Cell
Helicase	Unwinds the double helix	300	20
Primase	Synthesizes RNA primers	60	50
Single-strand binding protein	Stabilizes single-stranded regions	74	300
DNA gyrase	Relieves torque	400	250
DNA polymerase III	Synthesizes DNA	≈900	20
DNA polymerase I	Erases primer and fills gaps	103	300
DNA ligase	Joins the ends of DNA segments	74	300

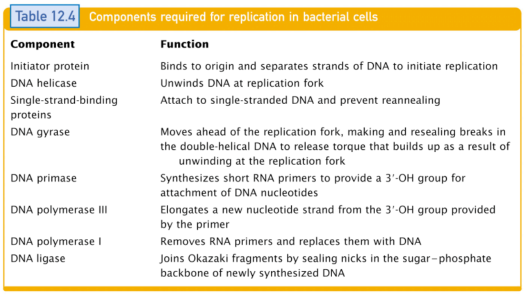


Table 12.4 Components required for replication in bacterial cells

Component	Function
Initiator protein	Binds to origin and separates strands of DNA to initiate replication
DNA helicase	Unwinds DNA at replication fork
Single-strand-binding proteins	Attach to single-stranded DNA and prevent reannealing
DNA gyrase	Moves ahead of the replication fork, making and resealing breaks in the double-helical DNA to release torque that builds up as a result of unwinding at the replication fork
DNA primase	Synthesizes short RNA primers to provide a 3′-OH group for attachment of DNA nucleotides
DNA polymerase III	Elongates a new nucleotide strand from the 3′-OH group provided by the primer
DNA polymerase I	Removes RNA primers and replaces them with DNA
DNA ligase	Joins Okazaki fragments by sealing nicks in the sugar–phosphate backbone of newly synthesized DNA

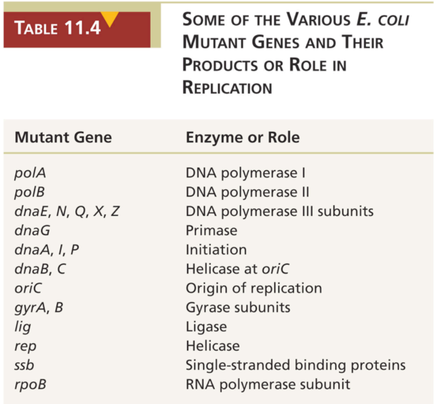


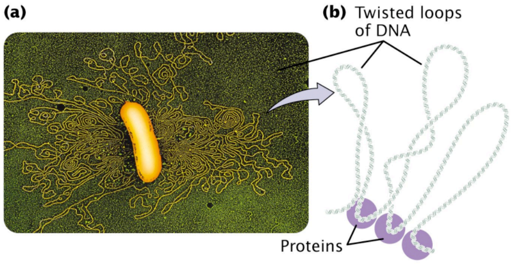
TABLE 11.4 SOME OF THE VARIOUS *E. COLI* MUTANT GENES AND THEIR PRODUCTS OR ROLE IN REPLICATION

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

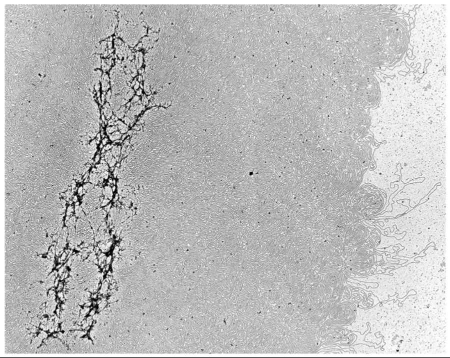
Conditional mutation

✓ A temperature-sensitive mutation is an example of a conditional mutation. It may not be expressed at a particular permissive temperature, but when mutant cells are grown at a restrictive temperature, the mutant phenotype is expressed and can be studied.

Prokaryotic DNA



Eukaryotic DNA



Prokaryotic holoenzyme

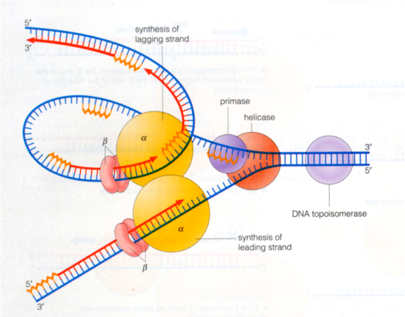
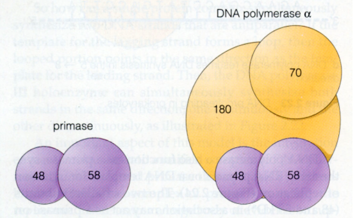


Figure 2.22 Model of DNA replication in prokaryotes with simultaneous synthesis of leading and lagging strands.

Eukaryotic Holoenzyme



a The 48 and 58 kD subunits of DNA polymerase α may function on their own as a primase.

b They may also function as a primase when part of the entire enzyme.

Figure 2.24 DNA polymerase α in mammals.

Eukaryotic replicon

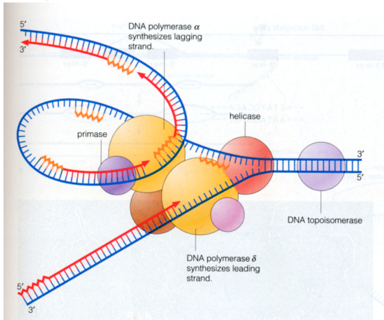


Figure 2.25 Model of DNA replication in eukaryotes with simultaneous synthesis of leading and lagging strands.

Eukaryotic verses prokaryotic

- ✓ Eukaryotic cells contain more DNA
- ✓ chromosomes are linear
- ✓ DNA is complexed with proteins
- ✓ more complex in eukaryotes than in bacteria
- ✓ eukaryotic contain multiple origins of replication

Yeast ARS

- ✓ Yeast autonomously replicating sequences (ARSs)
  - ✑contain an 11-bp consensus sequence
  - ✑ flanked by other short sequences
- ✓The ARSs are initially bound by a group of proteins to form the origin recognition complex (ORC)

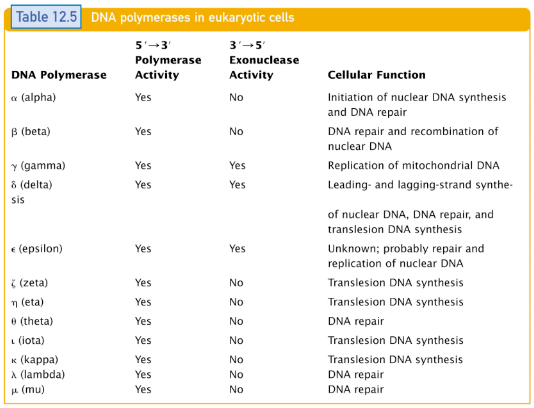


Table 12.5 DNA polymerases in eukaryotic cells

DNA Polymerase	5′→3′ Polymerase Activity	3′→5′ Exonuclease Activity	Cellular Function
α (alpha)	Yes	No	Initiation of nuclear DNA synthesis and DNA repair
β (beta)	Yes	No	DNA repair and recombination of nuclear DNA
γ (gamma)	Yes	Yes	Replication of mitochondrial DNA
δ (delta)	Yes	Yes	Leading- and lagging-strand synthesis of nuclear DNA, DNA repair, and translesion DNA synthesis
ϵ (epsilon)	Yes	Yes	Unknown; probably repair and replication of nuclear DNA
ζ (zeta)	Yes	No	Translesion DNA synthesis
η (eta)	Yes	No	Translesion DNA synthesis
θ (theta)	Yes	No	DNA repair
ι (iota)	Yes	No	Translesion DNA synthesis
κ (kappa)	Yes	No	Translesion DNA synthesis
λ (lambda)	Yes	No	DNA repair
μ (mu)	Yes	No	DNA repair

- ✓ Pol α and δ are the major forms of the enzyme involved in initiation and elongation.
  - ✑Pol α possesses low processivity
    - ☞Two of 4 subunits of Pol α functions in synthesis of the RNA primers during initiation on the leading and lagging strands
    - ☞After 10 ribonucleotides, the other subunits combine to synthesize 20-30 deoxyribonucleotides
  - ✑Polymerase switching occurs, and Pol α is replaced by Pol δ for elongation
    - ☞A high processivity enzyme that elongates the leading and lagging strand
- ✓Pol ε is essential and may help with the lagging strand

Eukaryotic replication fork

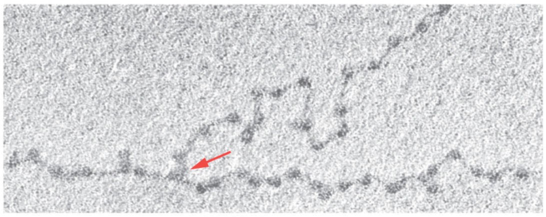
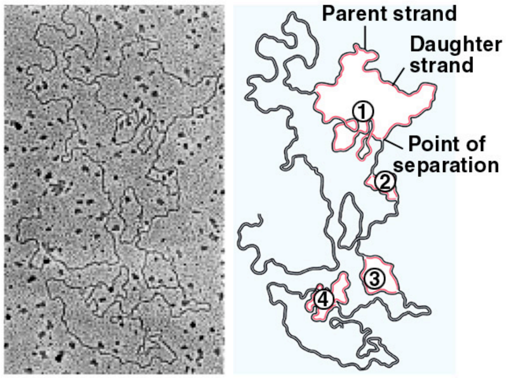


Fig. 14.19

Replication Units



E. coli replication fork

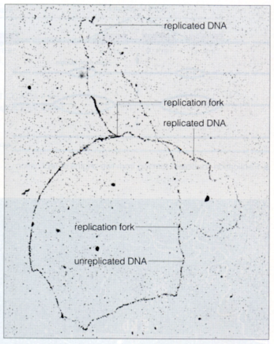
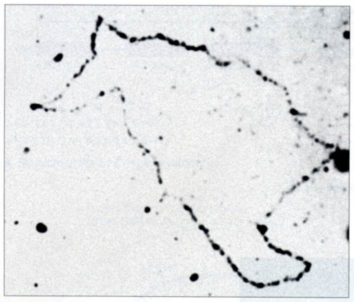


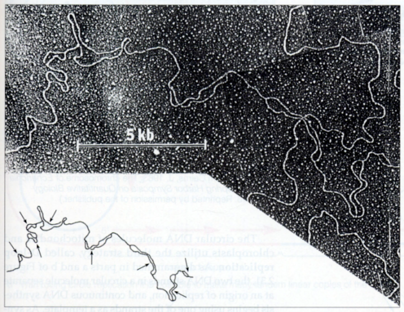
Figure 2.29 Electron micrograph of θ-mode replication in *E. coli*. (Photo from Cairns, J. 1963. The chromosome of *Echerichia coli*. *Cold Spring Harbor Symposia on Quantitative Biology* 28:43–46. Reprinted by permission of the publisher.)

E. coli replicon



a The bacterial DNA labeled with high-level radioactivity appears more dense on the developed film than the DNA labeled with low-level radioactivity.

Drosophila replicons



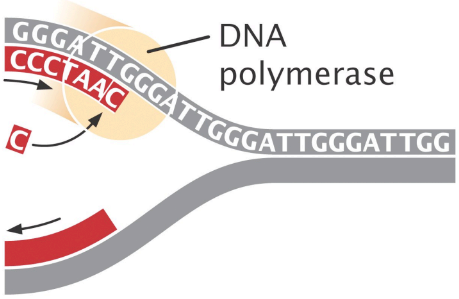
b Replication bubbles (indicated by arrows) in *Drosophila melanogaster* DNA.

Figure 2.27 Bidirectional replication. (Photo courtesy of D. S. Hogness.)

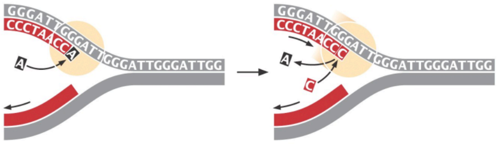
Fidelity of DNA Replication

- ✓ DNA Polymerases
  - ✎ 1 error in 10^5 nucleotides
- ✓ Proofreading
  - ✎ Epsilon subunit increases fidelity from 1 in 10^5 to 10^6 nuceotides
- ✓ Mismatch repair
  - ✎ Increases fidelity from 1 in 10^6 to 10^9 nucleotides

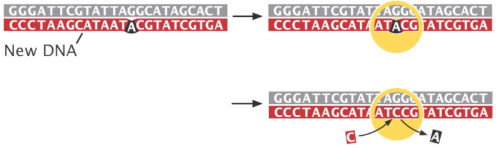
Nucleotide selection



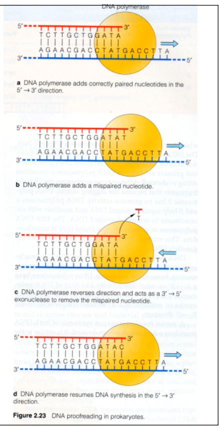
DNA proofreading



Mismatch repair



Proofreading



a DNA polymerase adds correctly paired nucleotides in the 5′ → 3′ direction.

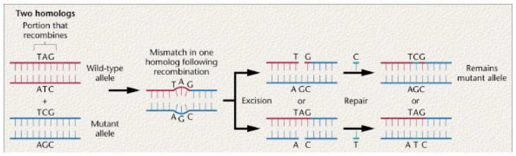
b DNA polymerase adds a mispaired nucleotide.

c DNA polymerase reverses direction and acts as a 3′ → 5′ exonuclease to remove the mispaired nucleotide.

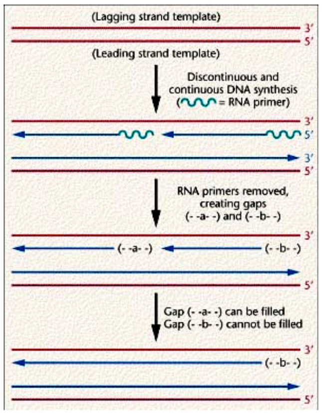
d DNA polymerase resumes DNA synthesis in the 5′ → 3′ direction.

Figure 2.23 DNA proofreading in prokaryotes.

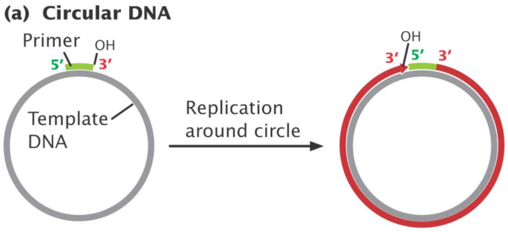
Mismatch Repair

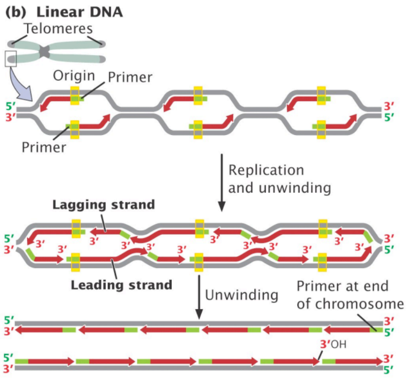


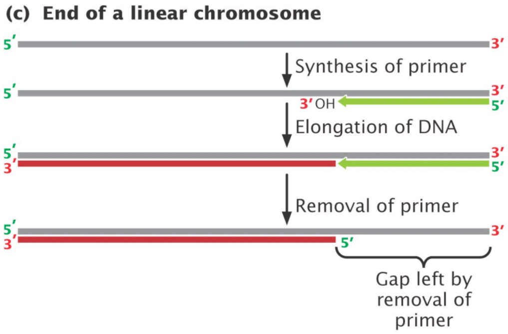
Telomer problem (Gaps at the ends)



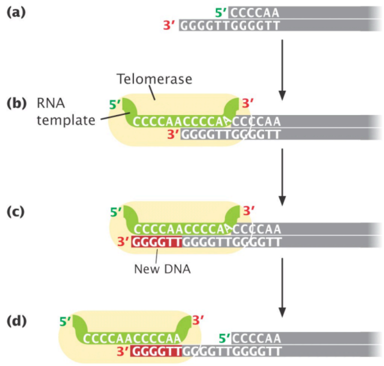
Circular DNA is no problem

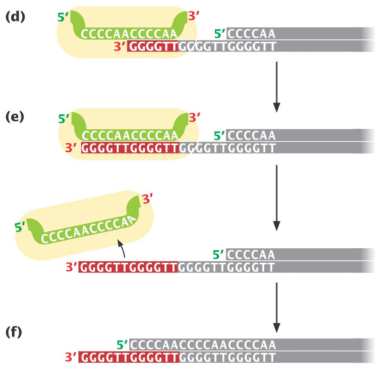


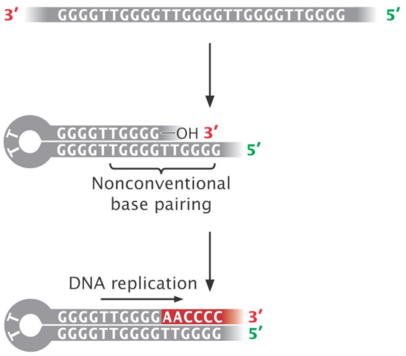




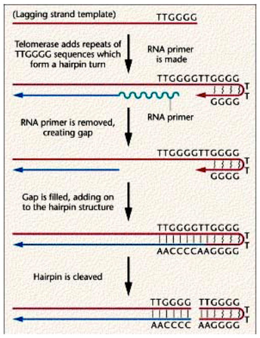
Telomerase







Telomers replicated with loop



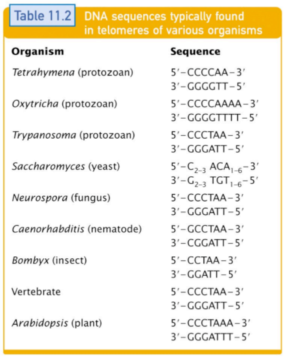


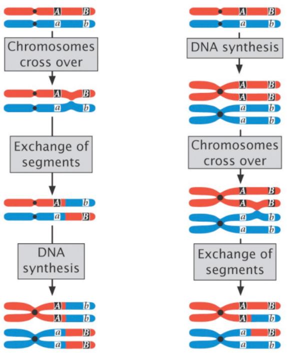
Table 11.2 DNA sequences typically found in telomeres of various organisms

Organism	Sequence
Tetrahymena (protozoan)	5′–CCCCAA–3′ 3′–GGGGTT–5′
Oxytricha (protozoan)	5′–CCCCAAAA–3′ 3′–GGGGTTTT–5′
Trypanosoma (protozoan)	5′–CCCTAA–3′ 3′–GGGATT–5′
Saccharomyces (yeast)	5′–C_{2-3} ACA_{1-6}–3′ 3′–G_{2-3} TGT_{1-6}–5′
Neurospora (fungus)	5′–CCCTAA–3′ 3′–GGGATT–5′
Caenorhabditis (nematode)	5′–GCCTAA–3′ 3′–CGGATT–5′
Bombyx (insect)	5′–CCTAA–3′ 3′–GGATT–5′
Vertebrate	5′–CCCTAA–3′ 3′–GGGATT–5′
Arabidopsis (plant)	5′–CCCTAAA–3′ 3′–GGGATTT–5′

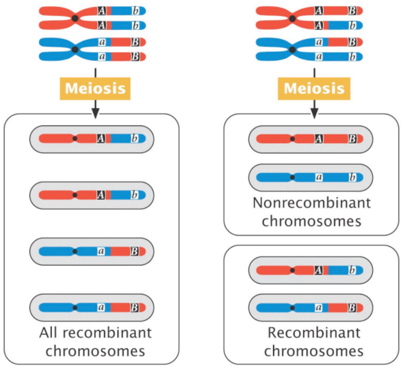
Source: V. A. Zakian, *Science* 270(1995): 1602.

Homologous recombination

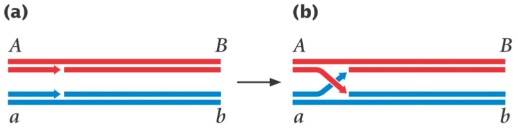
X-over
Increased genetic variation
Mapping
DNA repair



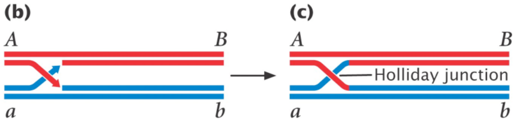
Homologous Recombination



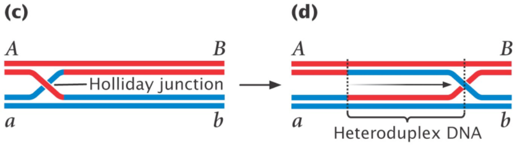
Endonuclease nicking/Strand displacement



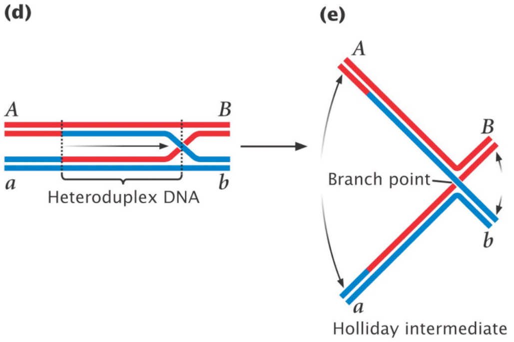
Ligation



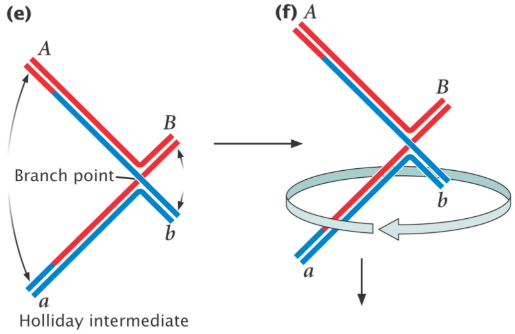
Holiday structure (chi form)/Heteroduplex



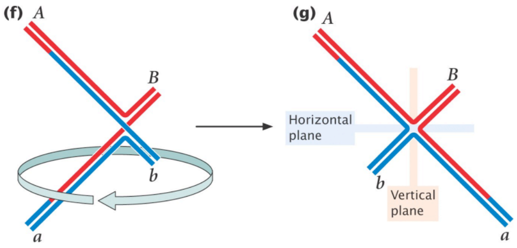
Migration branch



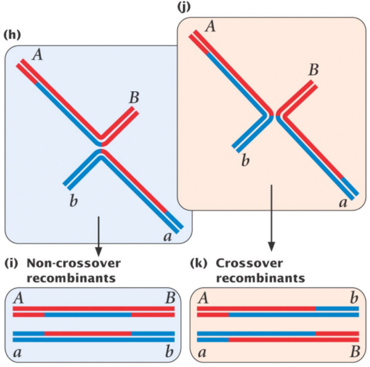
Rotation



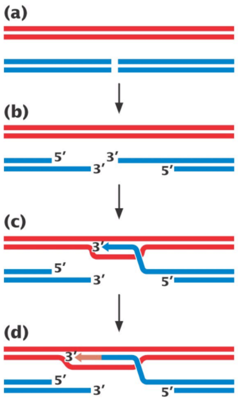
Two planes of cuting



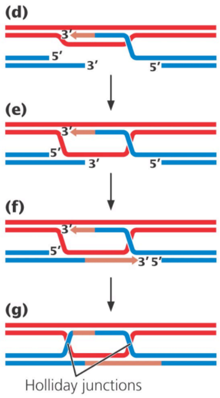
Opposite strands are nicked/ligated



Double strand break model



Double strand break model



Gene Conversion