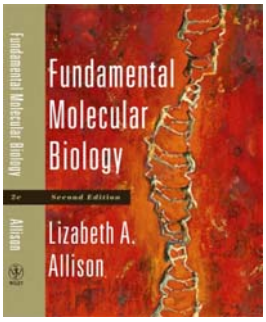
Terry Brown

Genomes
Third Edition

Chapter 15:
Genome Replication

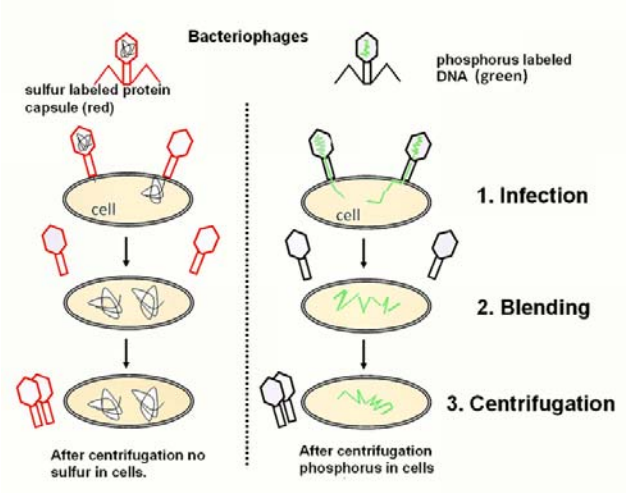


Fundamental Molecular Biology
Second Edition

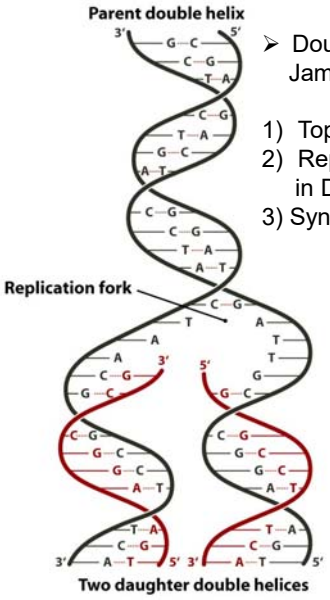
Lisabeth A. Allison

Chapter 6
DNA Replication and Telomere Maintenance

- Alfred Hershey and Matha Chase confirmed that DNA is the hereditary material using bacteriophage in 1952.



http://en.wikipedia.org/wiki/File:Hershey_Chase_experiment.png



- Double helix structure of DNA:
  James D. Watson and Francis Crick, Nature (1953), 171:737

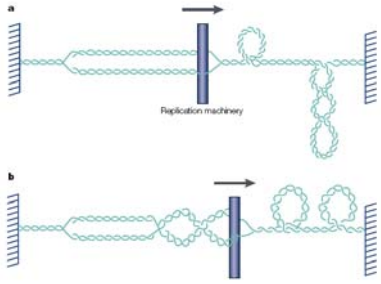
1) Topological problem (unwinding of DNA double helix)
2) Replication mechanism (finding enzymes involved in DNA replication)
3) Synchronize replication and cell division

Figure 15.1 *Genomes 3* (© Garland Science 2007)

15.1 The Topological Problem

15.1.2 DNA topoisomerases provide a solution to the topological problem

- Topological problem arises from unwinding of DNA double helix during replication.
- Human Chromosome 1 is 250Mbp in length, and the DNA double helix makes one turn every 10 bp.
- Human Chromosome 1 requires 25 million rotation for one replication event.
- Bacterial genomes are circular double helix molecules.
- To solve the problem, scientists proposed
  1) balanced equal length of right-handed and left-handed structure
  2) linear ribbon structure.



- The first real progress is “breakage-and-reunion” model proposed by Delbruck in 1954
- The DNA strands are separated not by unwinding the DNA helix but by breaking one strand, passing the second strand through the gap, and rejoining the first strand.

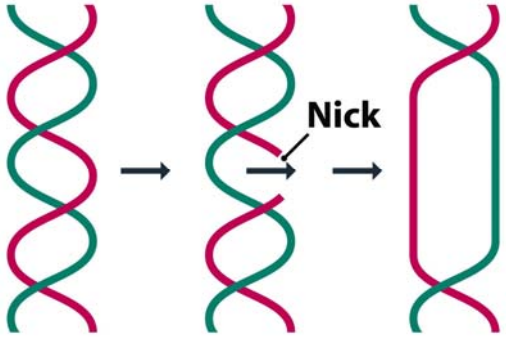
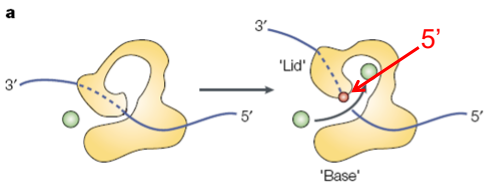


Table 1 | **Subfamilies of DNA topoisomerases**

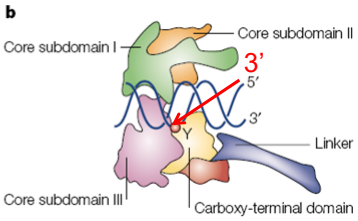
Subfamily	Representative members
IA	Bacterial DNA topoisomerases I and III Yeast DNA topoisomerase III *Drosophila melanogaster* DNA topoisomerases IIIα and IIIβ Mammalian DNA topoisomerases IIIα and IIIβ
IB	Eukaryotic DNA topoisomerase I Mammalian mitochondrial DNA topoisomerase I Pox virus topoisomerase
IIA	Bacterial gyrase, DNA topoisomerase IV Phage T4 DNA topoisomerase Yeast DNA topoisomerase II *Drosophila* DNA topoisomerase II Mammalian DNA topoisomerases IIα and IIβ
IIB	*Sulfolobus shibatae* DNA topoisomerase VI (subunit A homologous to yeast Spo11)

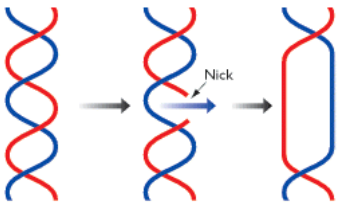
Table 15.1 *Genomes 3* (© Garland Science 2007)

Enzyme-bridging mechanism : Type IA



DNA rotation: Type IB



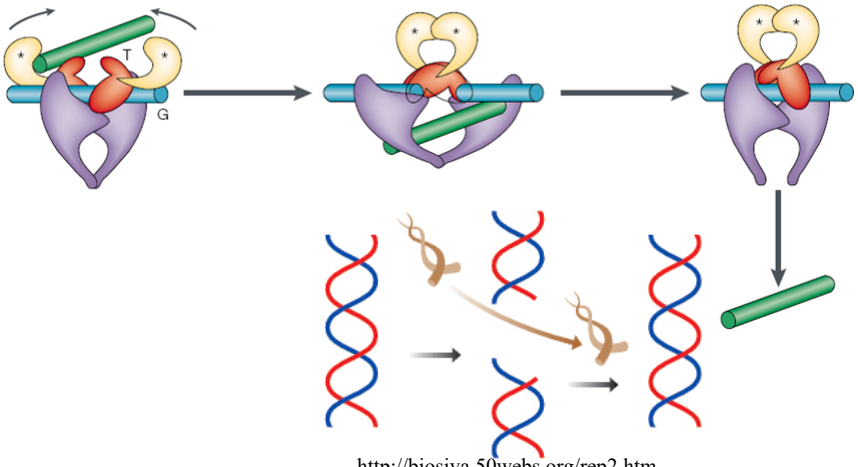


http://biosiva.50webs.org/rep2.htm

- Type I topoisomerase:
  - substrate: single-strand DNA
  - make a nick in one strand, pass the second strand through the nick, and religate the gap.
  - do not require ATP.

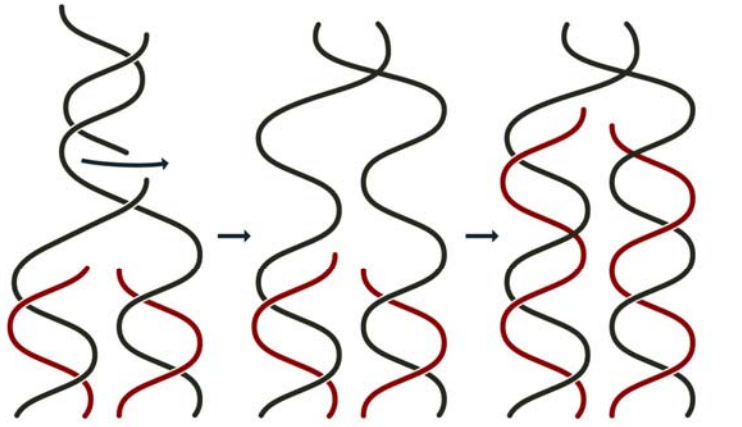
ATP-dependent reaction: Type II

c



http://biosiva.50webs.org/rep2.htm

- Type II topoisomerases
  - substrate: double-strand DNA
  - break both strands of the double helix, creating a gate.
  - through the gate, a second segment of the helix is passed.
  - usually ATP-dependent

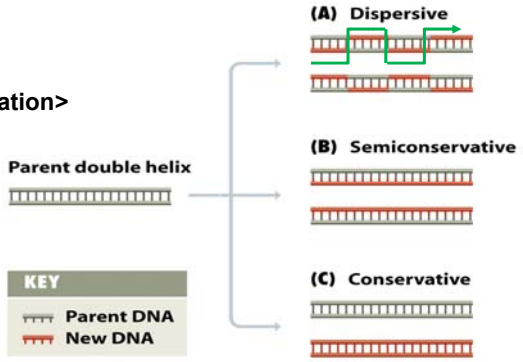


- During replication, the double helix is unwinded as a result of the action of DNA topoisomerases.

Figure 15.5 *Genomes 3* (© Garland Science 2007)

15.1.1 Experimental proof for the Watson-Crick scheme for DNA replication

<Possible Models for DNA replication>



- Dispersive model: the daughter helix is made up partly of parental DNA and partly of newly synthesized DNA.
- Semiconservative model: the parental DNA and newly synthesized DNA compose the daughter helix.
- Conservative model: one daughter helix is made of the two parental strands and the other is made of newly synthesized strands.

Figure 15.2 *Genomes 3* (© Garland Science 2007)

1. The Meselson-Stahl experiment (1958)

- "the most beautiful experiment in biology".
- a way to distinguish original DNA from newly synthesized DNA

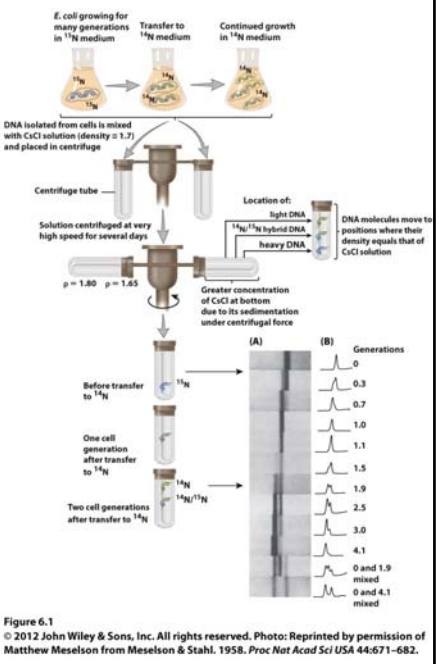


Figure 6.1

Figure 15.3a *Genomes 3* (© Garland Science 2007)

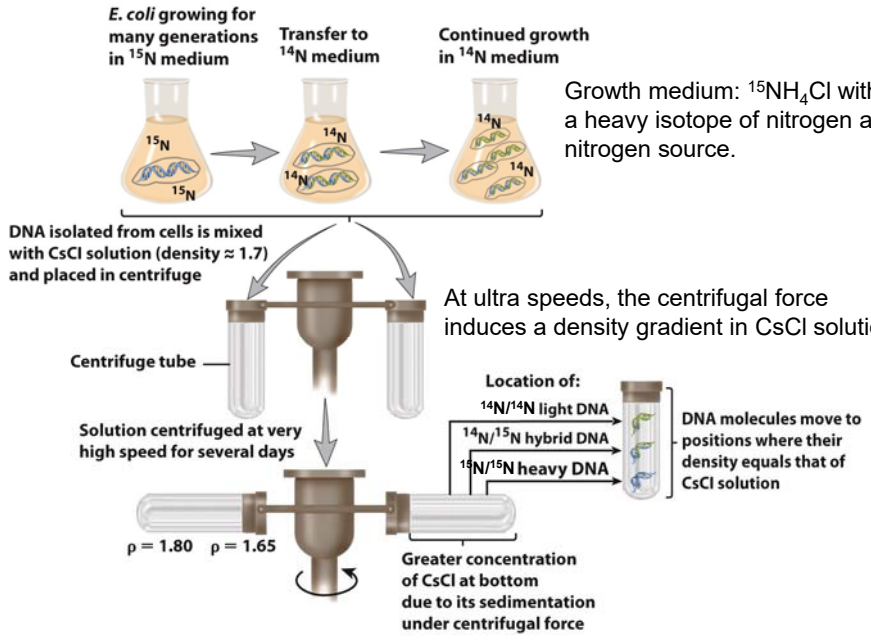
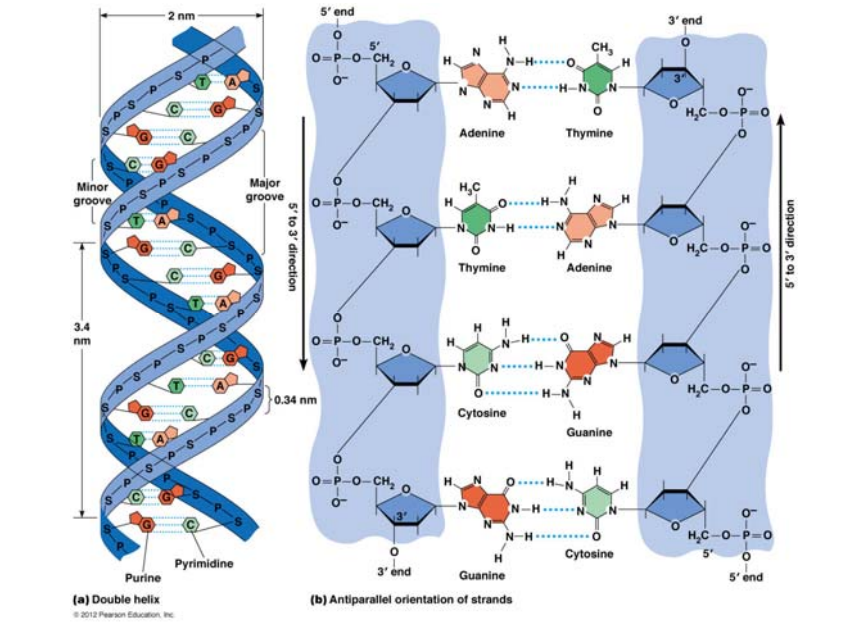
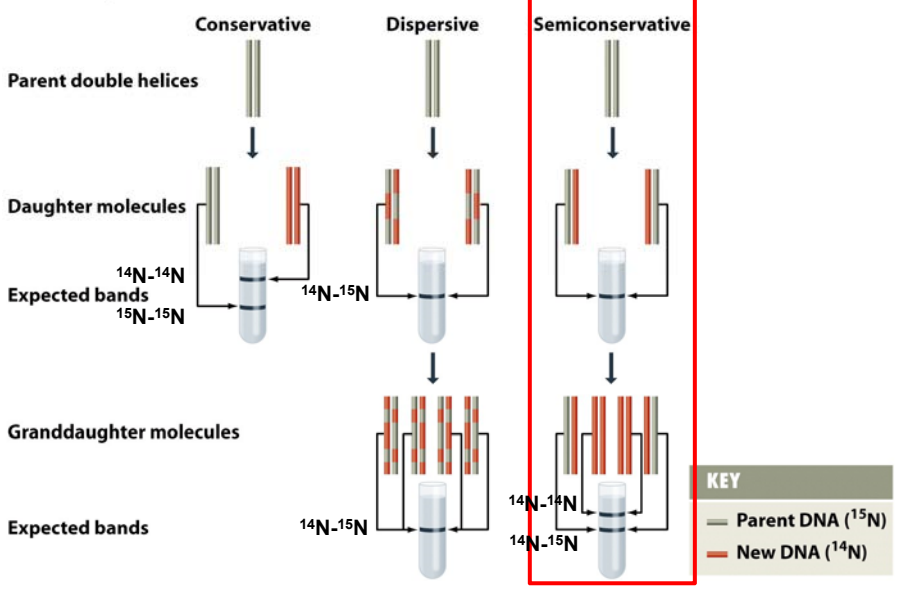


Figure 6.1 part 1





- DNA replication follows the semi-conservative model.

Figure 15.3b *Genomes 3* (© Garland Science 2007)

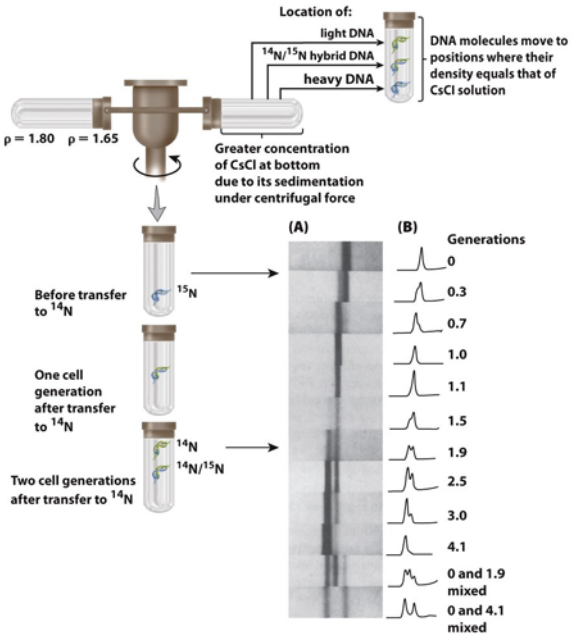
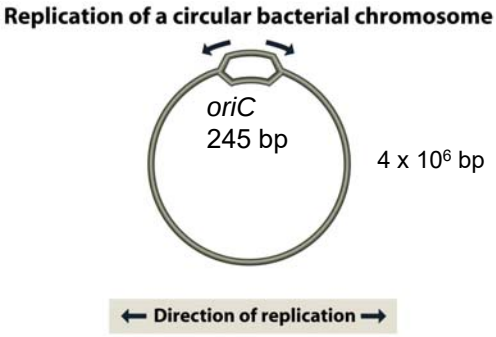


Figure 6.1 part 2

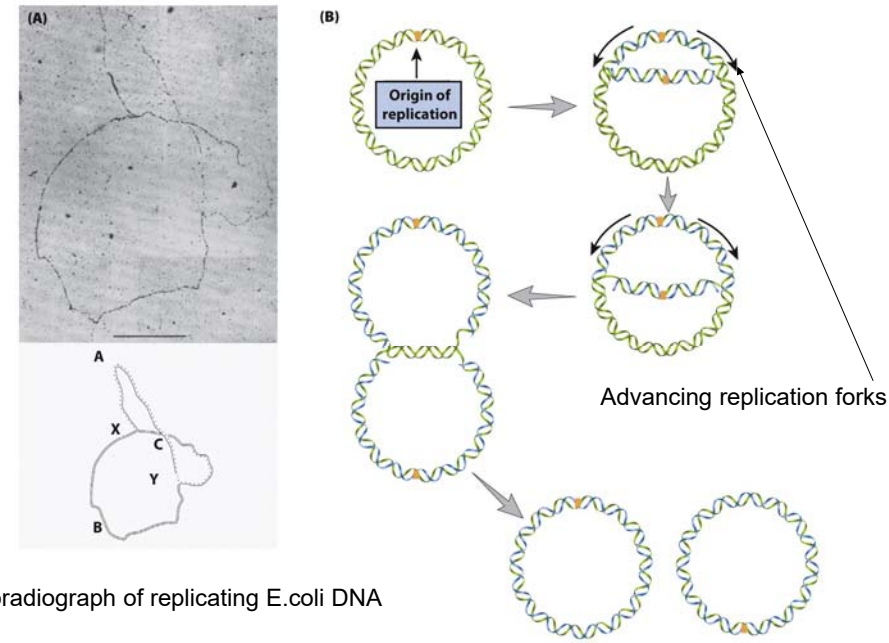
15.2 The Replication Process

15.2.1 Initiation of genome replication



- Initiation of replication is not a random process.
- It begins at the same position called the “origin of replication” of a circular bacterial genome.
- From this site, two replication forks progress in opposite directions along the DNA helix.
- E. coli origin of replication (oriC) is 245 bp in length.

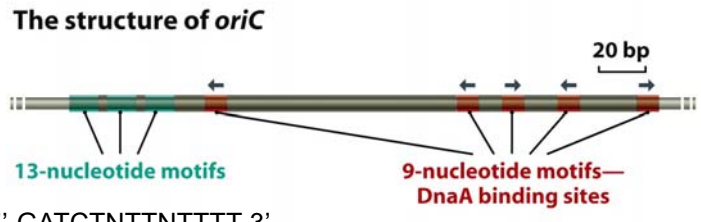
Figure 15.8a *Genomes 3* (© Garland Science 2007)



Autoradiograph of replicating E.coli DNA

Figure 6.2

1. Initiation at the E. coli origin of replication



5'-GATCTNTTNTTTT-3'

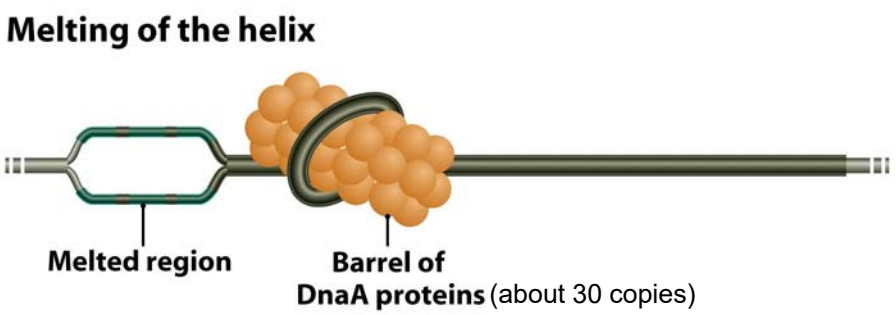
5'-TT(A/T)T(A/C)CA(A/C)A-3'

Melted region

DnaA binding

- OriC contains two short repeat motifs.
- Five copies of 9-nucleotide motifs, which are DnaA binding sites.
- Three copies of 13-nucleotide motif.

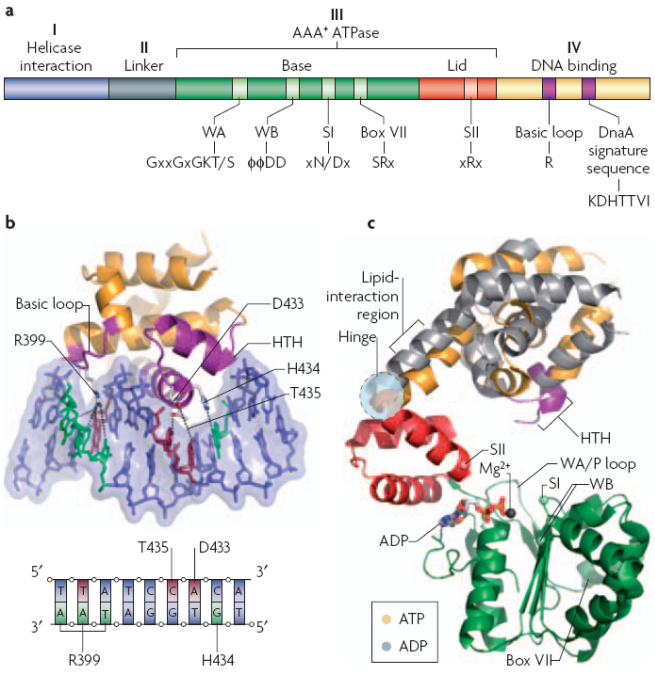
Figure 15.9a *Genomes 3* (© Garland Science 2007)

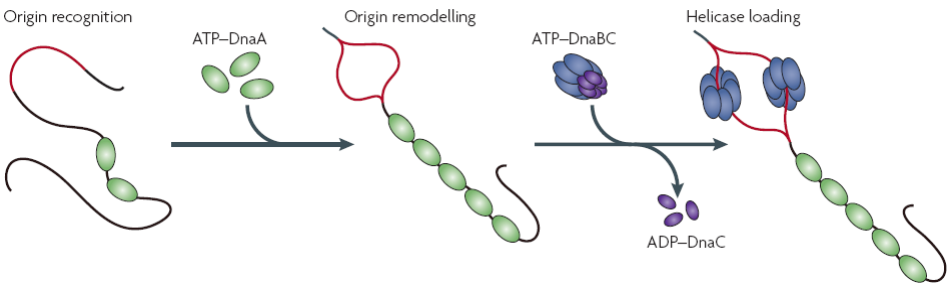


13-nucleotide motifs
5'-GATCTNTTNTTTT-3'

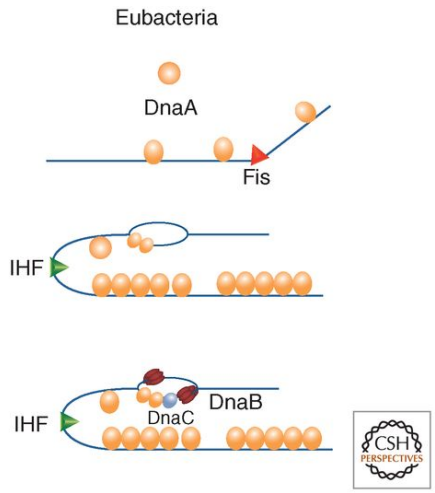
- About 30 DnaA proteins are associated with the origin.
- This DnaA association results in melting the double helix within the AT-rich 13-nucleotide motifs.

Figure 15.9b *Genomes 3* (© Garland Science 2007)





- The DNA meting initiates a series of events.
- The first step is the attachment of a prepriming complex.
- Prepriming complx
  - consists of 6 DnaB proteins and 6 DnaC proteins.
  - DnaB: helicase
  - DnaC: helicase loader.
- After loading DnaB, DnaC is released.
- DnaB can break base-pairs so increase the single-strand region within the origin, and enabling the enzymes involved in the elongation phase of replication to attach.



DNA-bending proteins, such as Fis or IHF

15.2.2 The elongation phase of replication

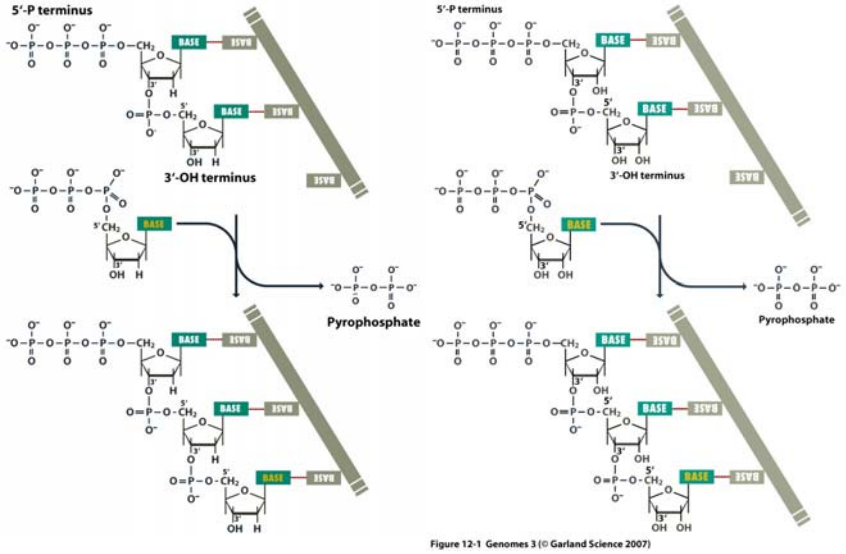


Figure 12-1 Genomes 3 (© Garland Science 2007)

- Chemically, template-dependent synthesis of DNA is very similar to template-dependent synthesis of RNA.

Figure 15.11 *Genomes 3* (© Garland Science 2007)

2. Discontinuous strand synthesis

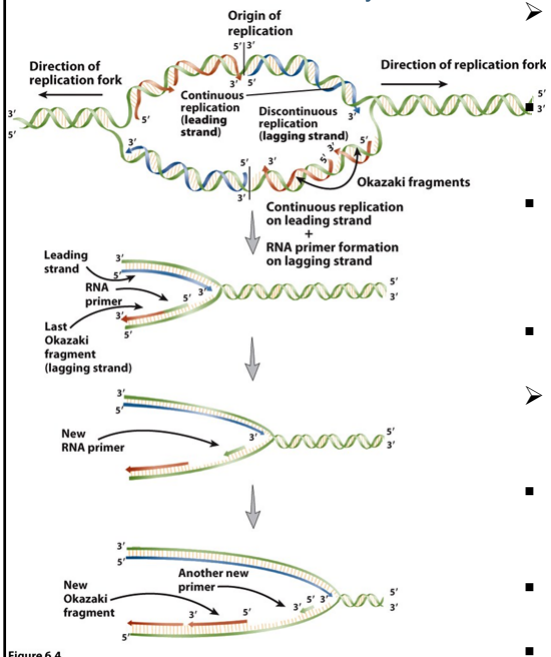


Figure 6.4

- During DNA replication both strands of DNA helix must be copied.
- DNA polymerases are only be able to synthesize DNA in the 5' to 3' direction.
  - The leading strand can be copied in a continuous manner, but the lagging strand should be copied in a discontinuous manor.
  - The lagging strand consists of a series of short segments.
- In 1969, Reiji Okazaki and Tsuneko Okazaki confirmed the hypothesis.
  - They added 3[H] Thymidine to E.coli and synthesize DNA for only ten seconds
  - Okazaki fragment: 1,000-2,000 nts (E. coli)
  - Eukaryotes: 100~200 nts

1. The DNA polymerases of bacteria and eukaryotes

Table 15.2 DNA polymerases involved in replication of bacterial and eukaryotic genomes

Enzyme	Subunits	Exonuclease activities 3′→5′	Exonuclease activities 5′→3′	Function
Bacterial DNA polymerases				
DNA polymerase I	1	Yes	Yes	DNA repair, replication
DNA polymerase III	At least 10	Yes	No	Main replicating enzyme
Eukaryotic DNA polymerases				
DNA polymerase α	4	No	No	Priming during replication
DNA polymerase γ	2	Yes	No	Mitochondrial DNA replication
DNA polymerase δ	2 or 3	Yes	No	Main replicative enzyme
DNA polymerase κ	1	?	?	Required for attachment of cohesin proteins which hold sister chromatids together until the anaphase stage of nuclear division (Section 15.2.3)

Bacteria and eukaryotes possess other DNA polymerases involved primarily in repair of damaged DNA. These enzymes include DNA polymerases II, IV, and V of *Escherichia coli* and the eukaryotic DNA polymerases β, ε, ζ, η, θ, and ι. DNA repair processes are described in Section 16.2.

- 3'→5' exonuclease activity, called proofreading activity is important to reduce the occasional base-paring error.
- 5'→3' exonuclease is less common and the activity requires lagging strand synthesis.

Table 15.2 *Genomes 3* (© Garland Science 2007)

Bacterial DNA polymerases

1) DNA polymerase I (Arthur Kornberg, 1956)
- Primer removal, gap filling between Okazaki fragments, and nucleotide excision repair pathway.

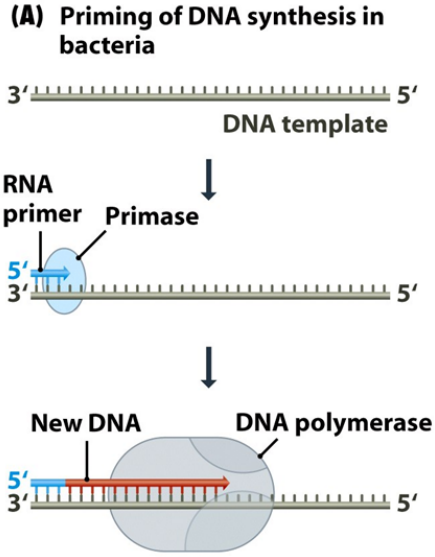
- DNA polymerase I is enzymatically cleaved by the protease subtilisin: Klenow fragment has 5′→3′ polymerase activity and 3′→5′ exonuclease activity; the other fragment has 5′→3′ exonuclease activity.

- Unique ability to start replication at a nick in the DNA sugar-phosphate backbone.

2) DNA polymerase III
- Main replicative polymerase.

3) DNA polymerase II
- Involved in DNA repair mechanisms.

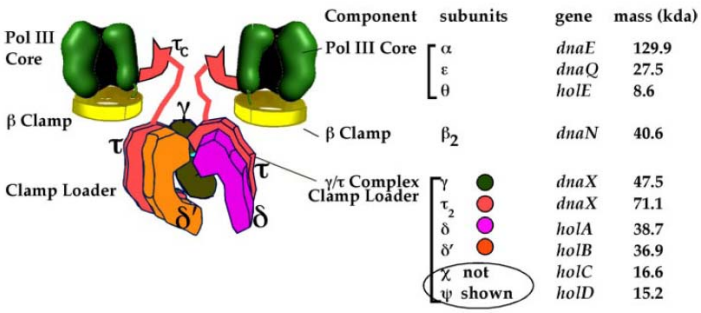
4) DNA polymerases IV and V
- Mediate translesion synthesis.



- DNA polymerase enzymes cannot initiate DNA synthesis on a single-strand DNA.
  - They need short double-strand region of parental strand and primer.
- Primase (DnaG) generates RNA primer (4 to 15 nts).
- DNA polymerase III extends the new DNA strand.

Figure 15.13 *Genomes 3* (© Garland Science 2007)

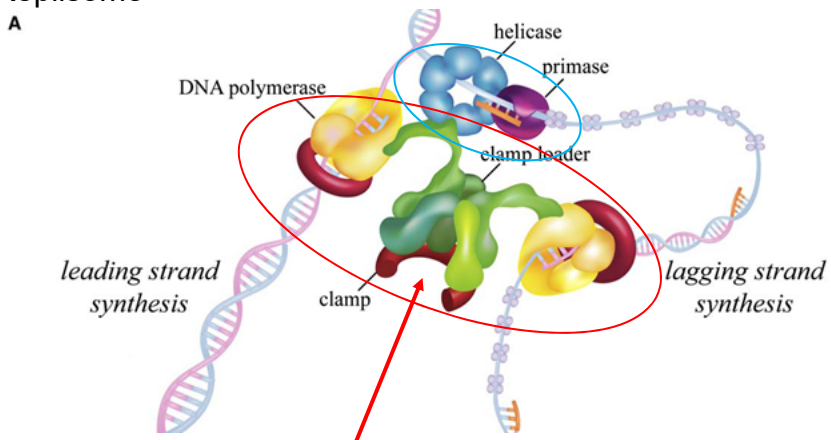
A. Pol III Holoenzyme (~900 kDa multisubunit protein)



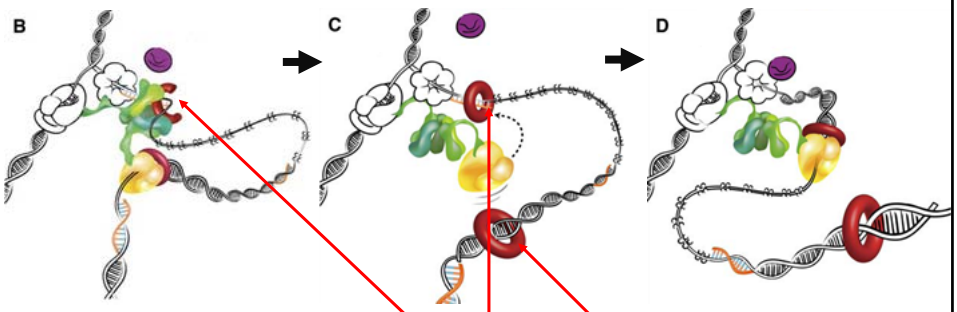
- 2 DNA Pol III cores made up of α, ε and θ subunits.
  - α subunit: polymerization activity.
  - ε subunit: 3' to 5' proofreading activity.
  - θ subunit: stimulates the ε subunit's proofreading activity.
- 2 β units act as sliding DNA clamps, they keep the polymerase bound to the DNA.
- 2 τ units connect the 2 DNA Pol III cores.
- 1 γ unit acts as a clamp loader for the lagging strand Okazaki fragments, helping the two β subunits to form a unit and bind to DNA.

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 281, NO. 16, pp. 10653–10656, April 21, 2006

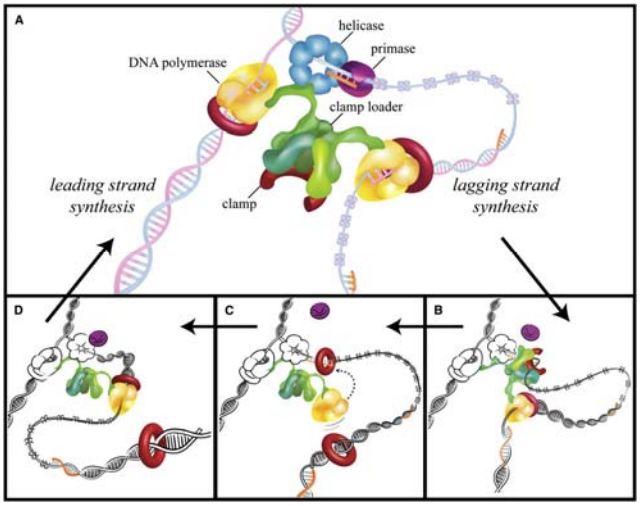
Replisome



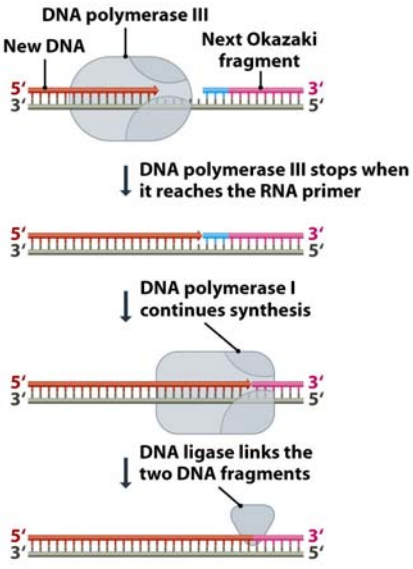
- The DNA pol III holoenzyme and primosome consisting of helicase and primase make a complex called replisome.
- During the lagging strand polymerase synthesizes an Okazaki fragment, the clamp loader opens a new clamp.
- The helicase recruits primase to the replication fork to initiate primer production for the next Okazaki fragment.



- After synthesis of the RNA primer, the clamp loader displaces primase and loads the clamp onto the new primer/template junction.
- Completion of Okazaki fragment synthesis triggers recycling of the lagging strand polymerase to the newly loaded clamp, leaving the old clamp behind.
- The lagging strand polymerase synthesizes the new Okazaki fragment.



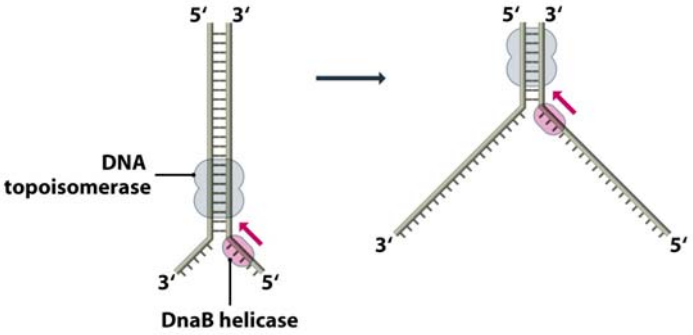
- Fork unwinding and leading strand synthesis continue throughout the cycle



- Okazaki fragments are short polynucleotides including RNA primer.
- The RNA primer must be removed and Okazaki fragment should be ligated.
- Main DNA pol III doesn't have 5' to 3' exonuclease activity so the enzyme cannot remove the RNA primer.
- DNA pol I and RNase H remove the RNA primer and replace it with DNA.
- DNA ligase links the two DNA fragments.

Figure 15.18 *Genomes 3* (© Garland Science 2007)

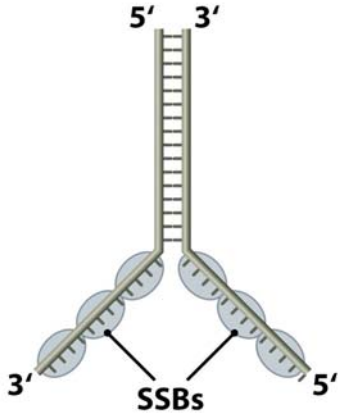
3. Events at the bacterial replication fork



- DnaB helicase binds to melting region and recruits primase.
- DnaB is a 5’ to 3’ helicase so migrates along the lagging strand.
- DnaB unzip the DNA helix and generates replication fork.
- The torsional stress generated by the unwinding is relieved by DNA topoisomerase.

Figure 15.14 *Genomes 3* (© Garland Science 2007)

SSBs attach to the unpaired polynucleotides

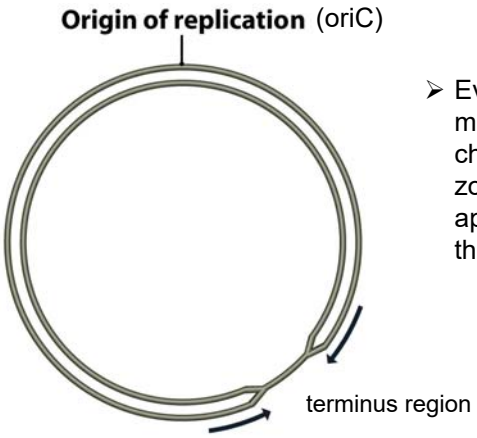


- Single-strand binding proteins attach to the single-strand DNA and prevent the single-strand DNA from reassociation or degradation.

Figure 15.15a *Genomes 3* (© Garland Science 2007)

15.2.3 Termination of replication

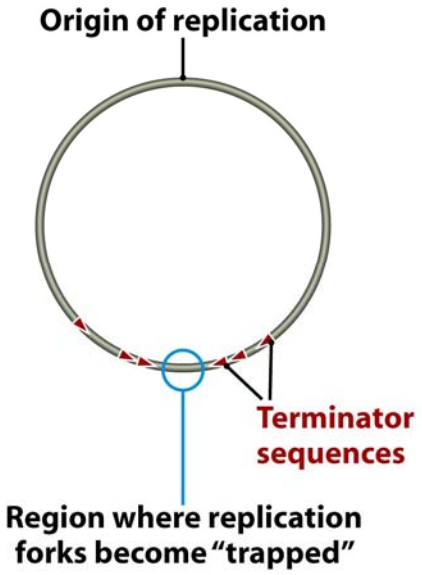
1. Replication of the E. coli genome terminates within a defined region



- Eventually, the two replication forks moving around the circular chromosome meet in a specific zone of the chromosome, approximately opposite oriC, called the terminus region.

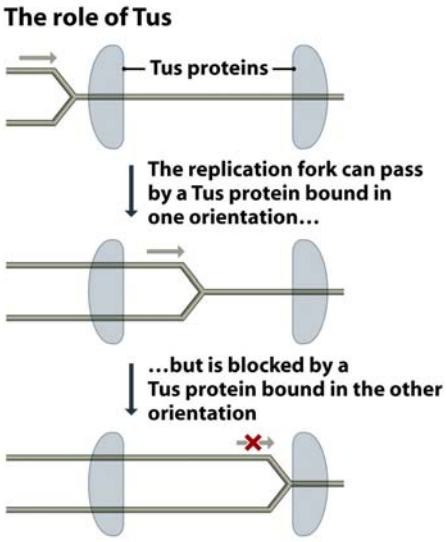
Figure 15.21 *Genomes 3* (© Garland Science 2007)

Terminator sequences in the *E.coli* genome



- The terminus region has several DNA replication terminator sequences.
- A special "replication terminator" protein called Tus protein is bound at a terminator sequence to pause replication.
- Each terminator sequence has polarity of action.
- The arrangement of the terminator sequences forms two opposed groups.

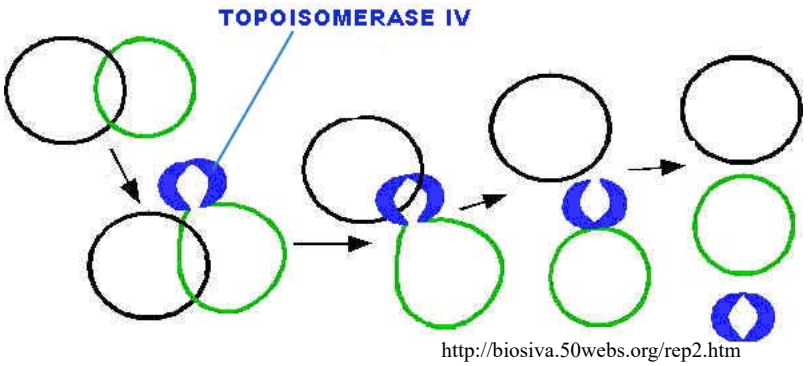
Figure 15.22a *Genomes 3* (© Garland Science 2007)



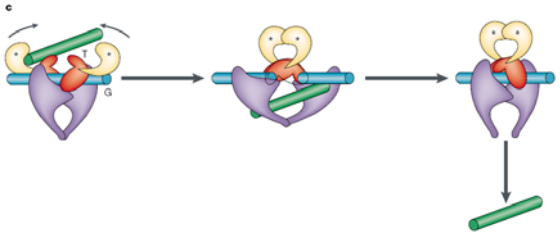
- A Tus protein allows a replication fork to pass if the fork is moving in one direction, but blocks if the fork is moving in the opposite direction.
- The two forks meet each other within the terminus region.

Figure 15.22b *Genomes 3* (© Garland Science 2007)

- Topoisomerase IV separates two daughter molecules.



http://biosiva.50webs.org/rep2.htm



Replication of a linear eukaryotic chromosome

36 kb (yeast) ~ 332 origins
150 kb (mouse) ~ 25,000 origins

- Eukaryotic cells also have origin of replication, but they have multiple origins.
- Yeast has about 332 origins and mouse has 25,000 origins.
- Average length between origins in yeast is 36 kb and 150 kb in mouse.
- Humans have 30,000 to 50,000 origins.

Figure 15.8b *Genomes 3* (© Garland Science 2007)

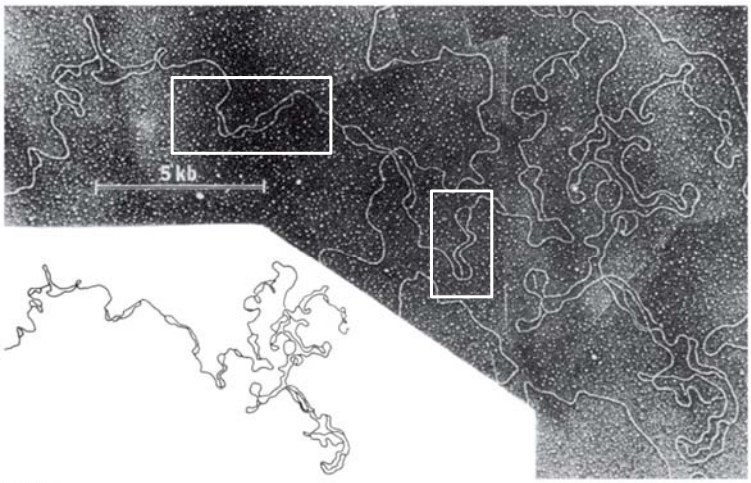


Figure 6.10b
Reprinted by permission of David S. Hogness from Kriegstein & Hogness. 1974. *Proc Nat Acad Sci USA* 71:135–139.

- Electron micrograph of replicating Drosophila DNA

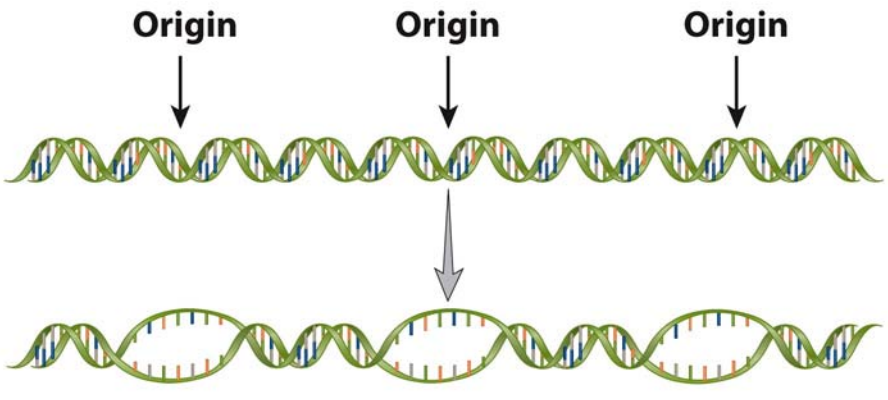
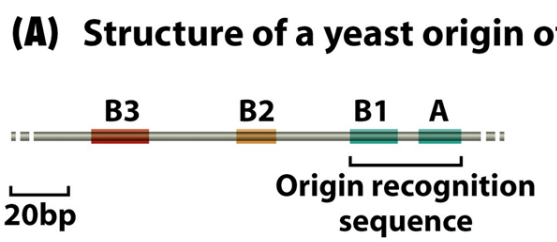


Figure 6.10a

2. Origins of replication in yeast have been clearly defined



Autonomously replicating sequences (ARSs)
:~ 200 bp

- Yeast origin of replication is "Autonomously replicating sequences (ARSs)"
- It's length is about 200 bp.

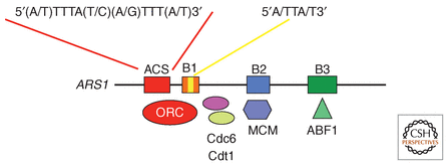
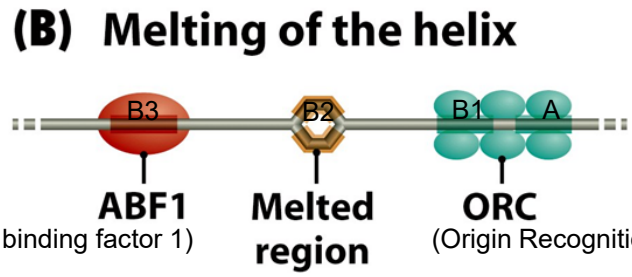


Figure 15.10 *Genomes 3* (© Garland Science 2007)

2. Origins of replication in yeast have been clearly defined



- Origin Recognition Complex (ORC) is a set of six proteins and binds to origin recognition sequence, subdomain B1 and A.
- ORCs act as mediators between replication origins and the regulatory signals coordinating the initiation of DNA replication with the cell cycle.
- Subdomain B2 appears to correspond to 13-nucleotide repeats in E.coli.
- ARS binding factor 1 (ABF1) biding to subdomain B3 induces melting in the subdomain B2.
- Helicase and other replication enzymes attach to the origin and initiate elongation of replication.

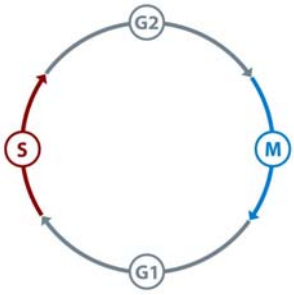
Figure 15.10 *Genomes 3* (© Garland Science 2007)

3. Replication origins in higher eukaryotes have been less easy to identify

- Mammalian origin sequences are usually AT rich but lack a consensus sequence.

4. Prereplication complex formation and replication licensing

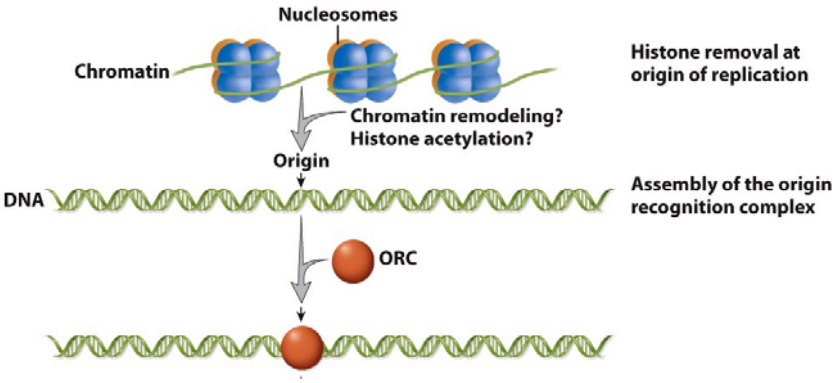
- DNA replication is restricted to S phase of the cell cycle.
- Unlike bacterial replication, eukaryotic cells separate origin selection from initiation.
- Separation of these two events prevents overreplication of the genome.



5. Selective activation of origins of replication

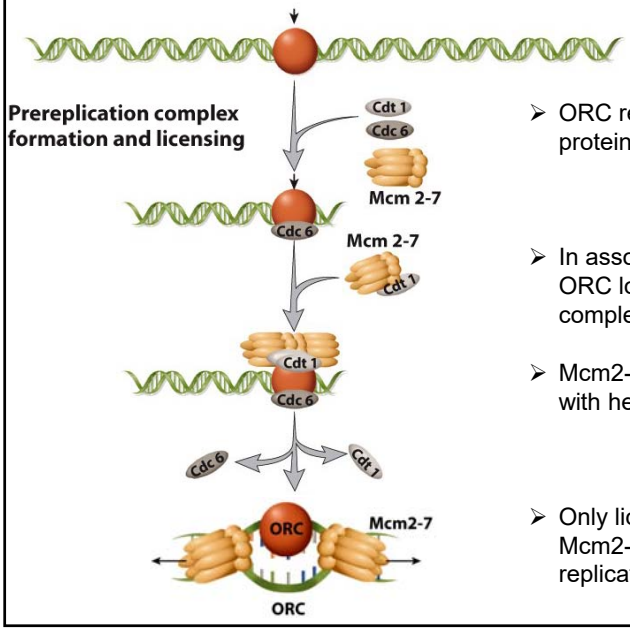
- The overall rate of replication is largely determined by the number of origins used and the rate at which they initiate.
- During early embryogenesis, origins are uniformly activated.
- At the mid-blastula transition, replication becomes restricted to specific origin sites.
- The parameters regulating the transition are not clear but may include changes in the level of nucleotide pools, changes in chromatin structure, and the ratio of origin recognition proteins to DNA.

Assembly of the origin recognition complex



- Histone modification and chromatin remodeling factors may loosen the chromatin to allow disassembly of the nucleosome and access to the template DNA.
- Once eukaryotic chromosome has been opened up, the ATP-dependent origin recognition complex (ORC) binds origin sequences.

Assembly of the origin recognition complex



- ORC recruits Cdc6 and Cdt1 proteins.
- In association with Cdc6 and Cdt1, ORC loads the licensing protein complex, Mcm2-7.
- Mcm2-7 is a hexameric complex with helicase activity.
- Only licensed origins containing Mcm2-7 can initiate a pair of replication forks.

Eukaryotic DNA polymerases

Table 6.2 The eukaryotic DNA polymerases.*

Name	Function
High-fidelity replicases	
Pol α (alpha)	Priming DNA synthesis during replication and repair
Pol δ (delta)	DNA replication of lagging strand during replication and repair (BER, DSBR, MMR, NER)
Pol ε (epsilon)	DNA replication of leading strand during replication and repair (BER, DSBR, NER)
Pol γ (gamma)	Mitochondrial DNA replication and repair
High-fidelity repair	
Pol β (beta)	BER, DSBR
Pol η (eta)	Translesion DNA synthesis (relatively accurate replication past thymine–thymine dimers)
Error-prone repair	
Pol ζ (zeta)	Translesion DNA synthesis (thymine dimer bypass)
Pol θ (theta)	Repair of DNA interstrand cross-links
Pol ι (iota)	Translesion DNA synthesis (required during meiosis)
Pol κ (kappa)	Translesion DNA synthesis (deletion and base substitution), DSBR (nonhomologous end joining)
Pol λ (lambda)	Translesion DNA synthesis
Pol μ (mu)	DSBR (nonhomologous end joining)
Pol ν (nu)	DNA cross-link repair?
Rev1	Abasic site synthesis (deoxycytidyl transferase activity inserts C across from a nucleotide lacking a base)

* Terminal deoxynucleotidyl transferase (TdT) is sometimes included in the list of DNA polymerases. This enzyme is a lymphoid, cell-specific, template-independent polymerase that adds nucleotides nearly randomly to coding ends during V(D)J recombination (see Fig. 12.25).

BER, base excision repair; DSBR, double-stranded break repair; MMR, mismatch repair; NER, nucleotide excision repair.

Table 6.2

Eukaryotic DNA polymerases

- At least 14 different eukaryotic DNA polymerases

1) Chromosomal DNA replication

   DNA pol α, pol δ, pol ε

2) Mitochondrial DNA replication

   DNA pol γ

3) Repair processes

   All the rest

4. The eukaryotic replication fork: variations on the bacterial theme

Table 1. Key Components of the Replisome

Function	*E. coli* Complex	Subunit Organization	Eukaryotic Complex	Subunit Organization
Fork unwinding	DnaB	Homohexamer	Mcm2-7	Heterohexamer
Primase	DnaG	Monomer	Pol α/Primase	Heterotetramer
DNA polymerase	Pol III core	Heterotrimer	Pol δ and Pol ε	Heterotetramers[a]
Sliding clamp	β	Homodimer	PCNA	Homotrimer
Clamp loader	γ complex	$\gamma\tau_2\delta\delta'\chi\psi$[b]	RFC	Heteropentamer
SSB	SSB	Homotetramer	RPA	Heterotrimer
Uncertain			GINS, Cdc45, Dpb11, Mcm10, Sld2, Sld3, others	

[a] Pol δ is a heterotrimer in *S. cerevisiae*.
[b] In *E. coli*, the clamp loader consists of five subunits ($\gamma\tau_2\delta\delta'$) plus χ and ψ, which have auxiliary roles.

Duplex unwinding and RNA priming

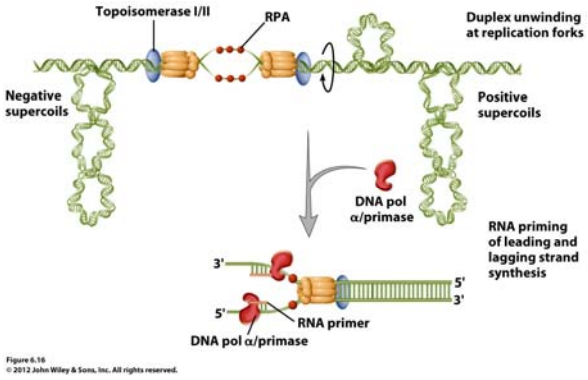


Figure 6.16

- Mcm2-7 helicase is bound to the leading strand template and moves 3′→5′.
- In eukaryotes, the RNA primer is synthesized by DNA polymerase (pol) α and its associated primase.
- The pol α/primase enzyme synthesizes a short strand of 10 bases of RNA, followed by 20-30 bases of initiator DNA (iDNA).

Polymerase switching and strand elongation

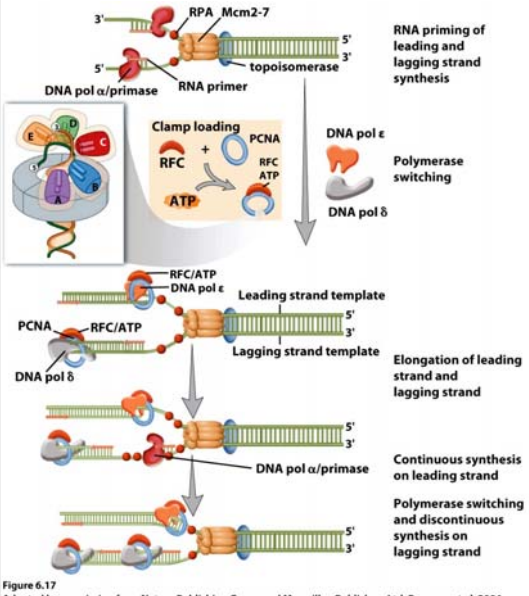


Figure 6.17
Adapted by permission from Nature Publishing Group and Macmillan Publishers Ltd: Bowman et al. 2004. *Nature* 429:724–730. Copyright © 2004

- Leading strand: switch from DNA polymerase α to pol ε.
- Lagging strand: switch from pol α to pol δ.
- Once DNA pol ε is recruited to the leading strand, synthesis is continuous.
- Lagging strand synthesis requires repeated cycles of polymerase switching from DNA pol α to DNA pol δ.

Proofreading

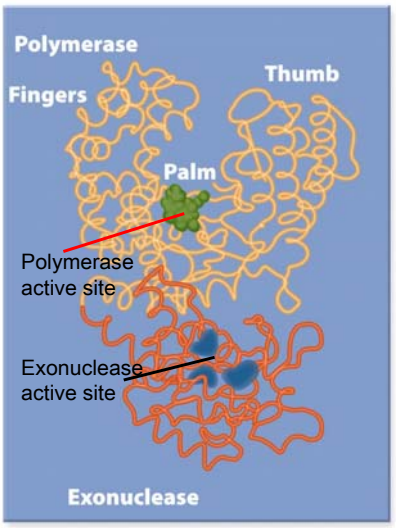
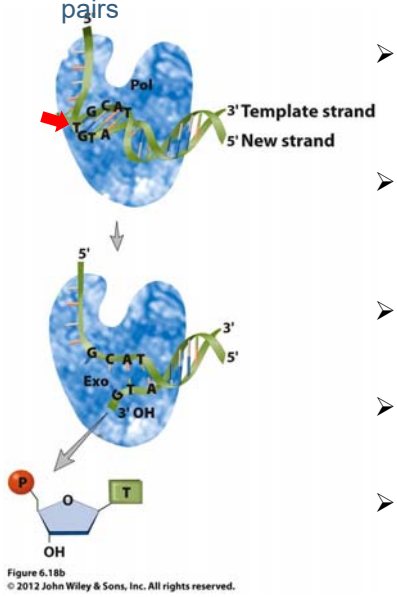


Figure 6.18a
Adapted from Kunkel & Bebenek. 2000. *Ann Rev Biochem* 69:497–529. Copyright © 2000, with permission from Annual Reviews

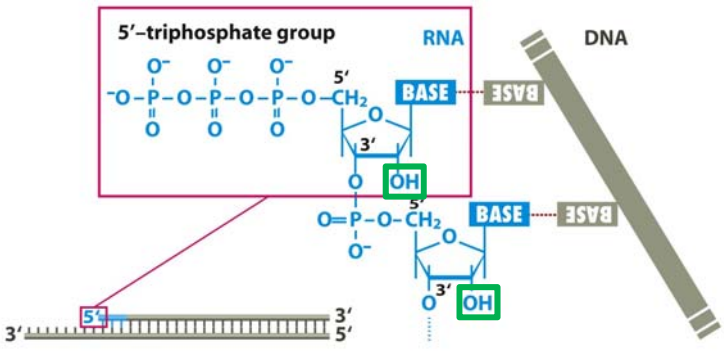
- Replicative polymerases are high fidelity but not perfect: 10^{-4} to 10^{-5} errors per base pair.
- Proofreading exonuclease activity reduces the error rate to 10^{-7} to 10^{-8} errors per base pair.
- DNA polymerase has a hand-shaped structure.
- 5′→3′ polymerase activity is within the fingers and thumb.
- 3′→5′ exonuclease activity is at the base of the palm.

Nucleotide selectivity largely depends on the geometry of Watson-Crick base pairs



- Incorporation of a mismatched nucleotide causes a melting of the newly formed double-stranded DNA.
- As a result, the polymerase pauses, and the 3' end of the new strand is transferred to the exonuclease domain.
- The mismatched base is excised and released as a dNMP.
- After the mispaired base is removed, elongation resumes.
- The abnormal geometry of mismatched base pairs results in steric hindrance at the active site.

RNA primer removal



- 5'-end of Okazaki fragment is RNA.
- The RNA is removed by DNA pol I and RNase H in E. coli.
- There is no eukaryotic DNA pol with 5' to 3' exonuclease activity.
- In eukaryotes flap endonuclease (FEN1), which is associated DNA pol δ complex degrades the RNA primer.
- FEN1 cannot initiates primer degradation because 5'-end of the primer carries 5'-triphosphate, which blocks FEN1 activity.

Figure 15.19 *Genomes 3* (© Garland Science 2007)

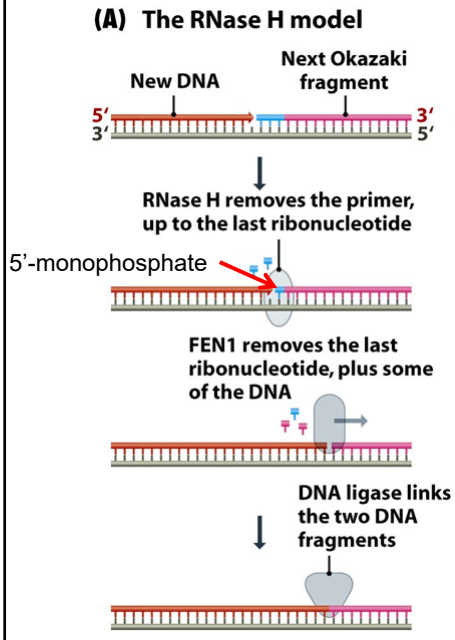


Figure 15.20 *Genomes 3* (© Garland Science 2007)

- RNase H can degrade the RNA of the RNA-DNA helix.
- It cannot cleave the phosphodiester bond between the last ribonucleotide and the first deoxyribonucleotide.
- RNase H removes the primer up to the last ribonucleotide, which has a 5'-monophosphate.
- Then, FEN1 (exonuclease activity) removes the last ribonucleotide, plus some deoxyribonucleotides.
- The last step is that DNA ligase links the two DNA fragments.
- Cells lacking RNase H are still able to do lagging strand replication.

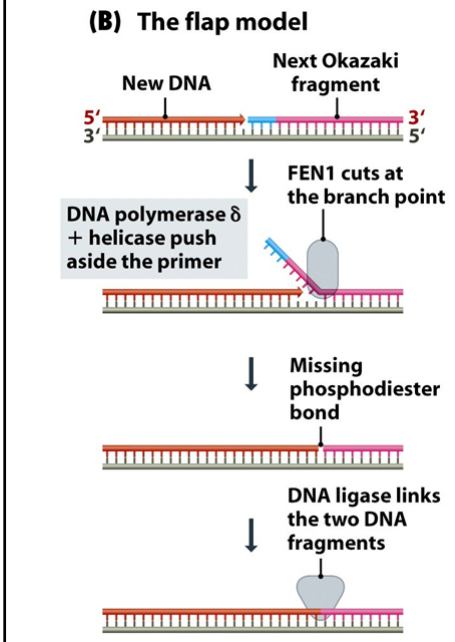
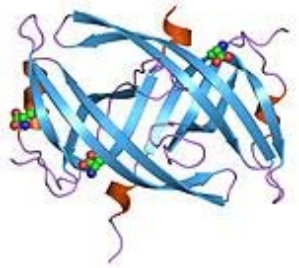
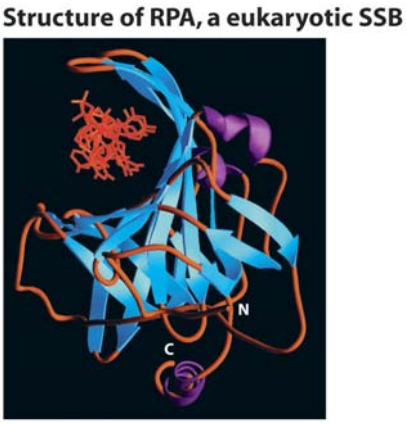


Figure 15.20 *Genomes 3* (© Garland Science 2007)

- DNA pol δ and helicase push aside the primer and FEN1 (endonuclease activity) cuts at the branch point.
- DNA ligase links the two DNA fragments.

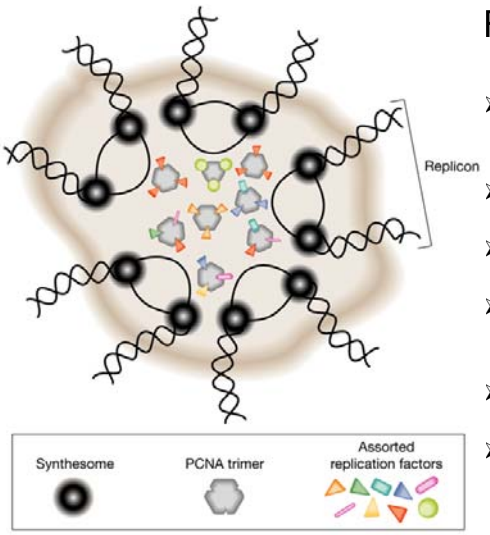


➢ *E.coli* SSB



➢ Replication protein A (RPA).

Figure 15.15b *Genomes 3* (© Garland Science 2007)



Replication factories

- Bacteria have repliosome including DNA pol III holoenzyme and primosome for replication.
- Eukaryotes do not have equivalent of the replisome.
- Proteins involved in replication form specific structures within the nucleus.
- Each structure contains 40 to several hundreds of individual replication complexes.
- These structures attach to the nuclear matrix (immobile).
- In case of replication factory, DNA molecules move through the complexes as they are replicated.

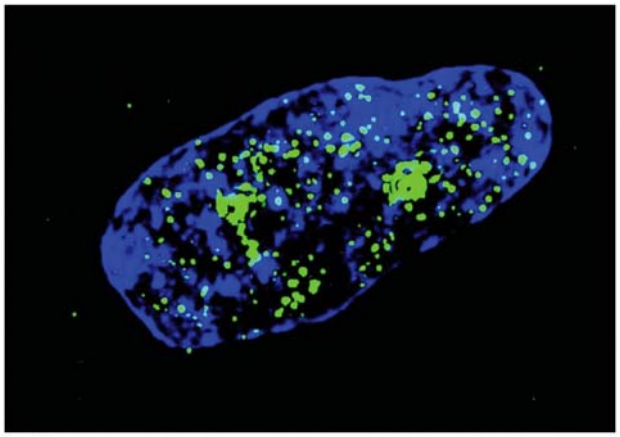


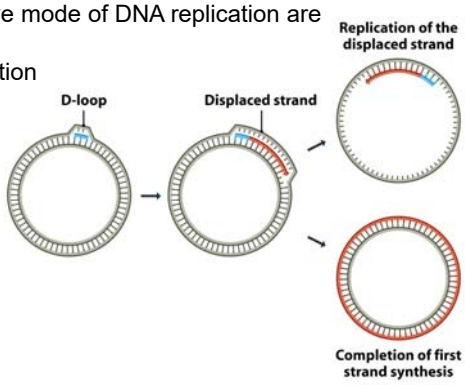
Figure 6.12
Reprinted from Barbie et al. 2004. *Mol Cell Biol* 24:595–607. Copyright © 2004, with permission from the American Society for Microbiology. Photograph courtesy of Brian K. Kennedy, University of Washington.

- Eukaryotic DNA replication factories
- Pulse-chase BrdU labeling of cells in S phase and detection with anti-BrdU antibody (Green)
- DNA: DAPI staining (Blue)
- If replication forks distributed diffusely throughout the nucleus, the entire nucleus would have appeared faint green.

15.1.2 Variations on the semiconservative theme

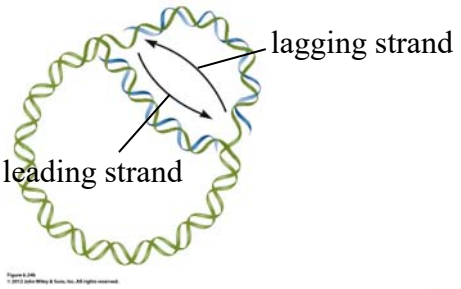
- No exceptions to the semiconservative mode of DNA replication are known.
- Models for mitochondrial DNA replication

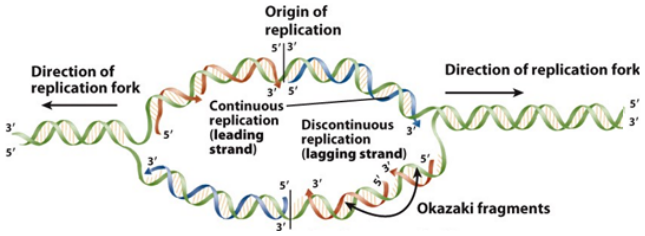
1) Strand displacement model



- The most widely accepted model.
- Replication is unidirectional round the circle and there is one replication fork for each strand.
- 500 bp-length D-loop initiates replication (DNA polymerase γ).
- An RNA molecule base-paired to one of DNA strands and acts as a primer for DNA synthesis.
- The new strand copies one strand of the helix and the other strand is displaced and subsequently copied using a second RNA primer.
- The RNA primers are removed by the multifunctional endonuclease RNase MRP.

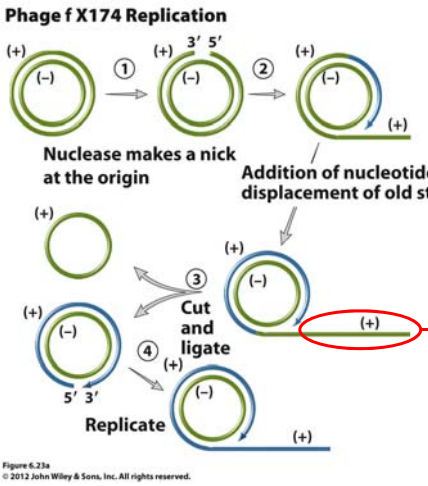
2) Strand coupled model





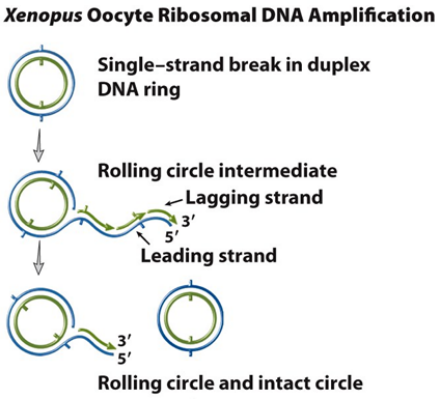
- Recently proposed model.
- Researchers found Okazaki fragments.
- Semidiscontinous and bidirectional replication.

3) Rolling circle replication



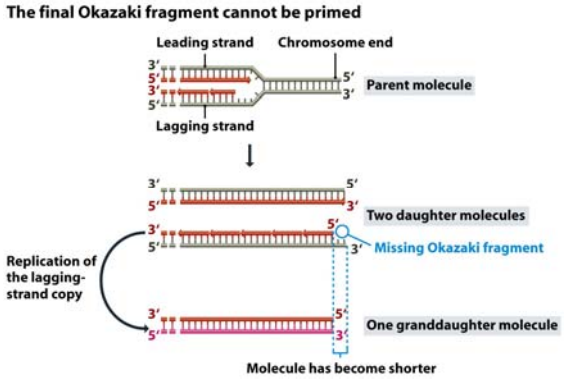
- An efficient mechanism for the rapid synthesis of multiple copies of a circular genome.
- A nuclease makes a nick in the (+) strand of the parent strand.
- Continued DNA synthesis rolls off a linear copy of the circular genome.
- The unit length of ss (+) DNA that has been displaced is cleaved off by an endonuclease and the ends are ligated to form a ss circular DNA.
- Replication continues.

Figure 15.7 *Genomes 3* (© Garland Science 2007)



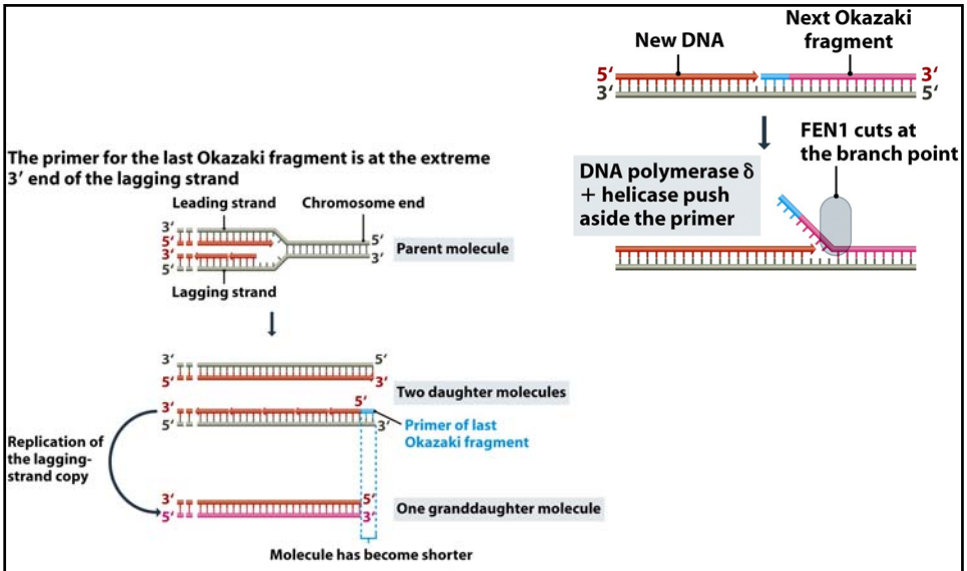
- Single-strand break in duplex DNA ring initiates replication
- The leading strand elongates continuously.
- It rolls off a linear copy of the circular genome.
- The lagging strand elongates discontinuously.
- The ds DNA grows to form many rDNA repeat units in length before one rDNA repeat's worth is cleaved off by a nuclease.
- DNA ligase joins the ends to form a ds circular DNA molecule.

15.2.4 Maintaining the ends of a linear DNA molecule



- The final Okazaki fragment cannot be primed, so the extreme 3'-end of the lagging strand might not be copied.
- This causes chromosome shortening.

Figure 15.24a *Genomes 3* (© Garland Science 2007)



- If the priming is possible, chromosome shortening will still occur.
- The terminal RNA primer cannot be converted into DNA by the standard procedures for primer removal.

Figure 15.24b *Genomes 3* (© Garland Science 2007)

1. Telomeric DNA is synthesized by the telomerase enzyme

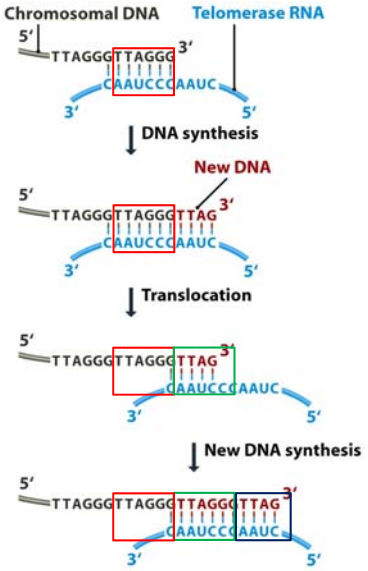
Table 15.3 Sequences of telomere repeats and telomerase RNAs in various organisms

Species	Telomere repeat sequence	Telomerase RNA template sequence
Human	5′–TTAGGG–3′	5′–CUAACCCUAAC–3′
Oxytricha	5′–TTTTGGGG–3′	5′–CAAAACCCCAAAACC–3′
Tetrahymena	5′–TTGGGG–3′	5′–CAACCCCAA–3′

Oxytricha and *Tetrahymena* are protozoans which are particularly useful for telomere studies because at certain developmental stages their chromosomes break into small fragments, all of which have telomeres: they therefore have many telomeres per cell.

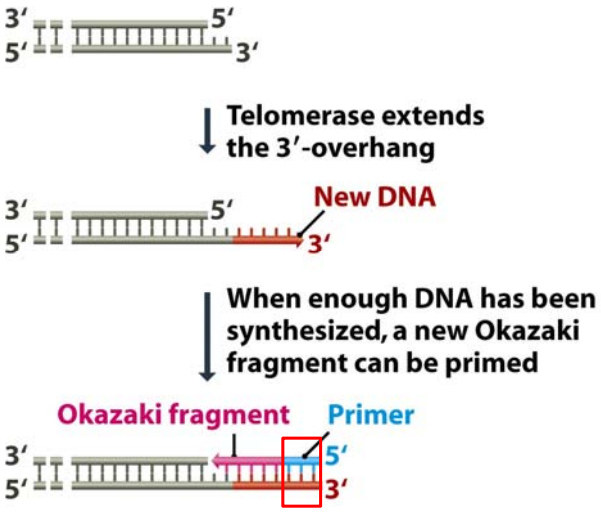
- Telomeres are unusual DNA sequences at the ends of eukaryotic chromosomes.
- Telomeric DNA is made of a few hundred copies of a short repeat motif, TTAGGG in most higher eukaryotes.
- There are telomere repeats in various organisms.
- Telomere repeats are G-rich sequences.
- Most of telomeric DNA is synthesized by normal DNA replication but at the very end region telomeric DNA is extended by the telomerase enzyme.

1. Telomeric DNA is synthesized by the telomerase enzyme



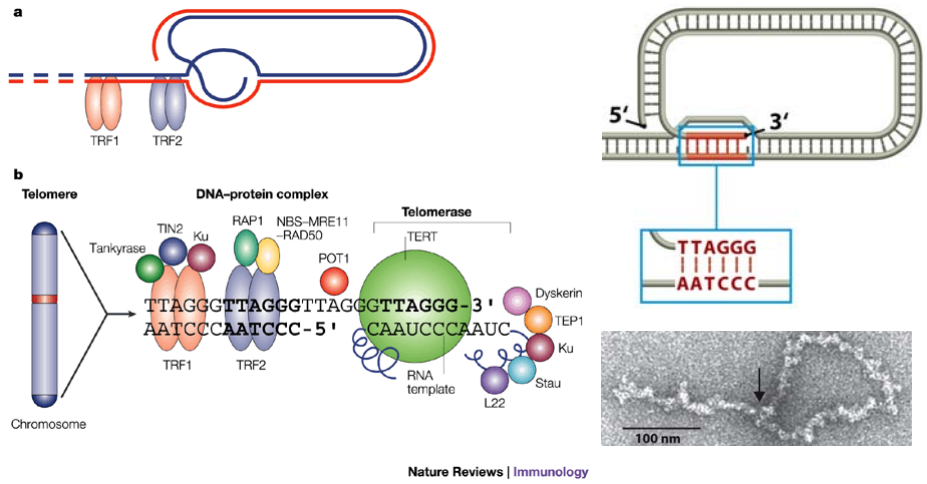
- The enzyme consists of protein and RNA parts.
- The RNA contains the reverse complementary sequence of the telomere repeat sequence.
- The telomerase RNA base-paired to the end of the chromosomal DNA.
- Reverse transcriptase activity of Telomerase extends the telomeric DNA using the telomerase RNA as a template.
- The telomerase RNA translocates to a new position.
- The process can be repeated until the choromosome end has been extended by a sufficient amount.

Figure 15.25 *Genomes 3* (© Garland Science 2007)



- Over all length of the chromosomal DNA is not reduced.

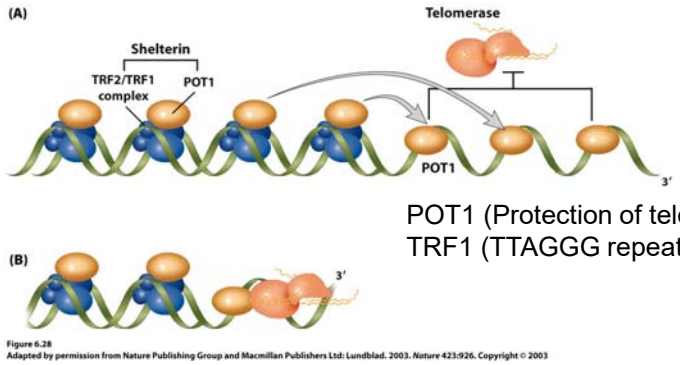
Figure 15.26 *Genomes 3* (© Garland Science 2007)



- T-loop structure
- The loop in association with TRF1 and TRF2 may aid in preventing telomerase access, since telomerase requires an unpaired 3’ end for function.

Figure 15.27 *Genomes 3* (© Garland Science 2007)

Model for telomerase length control



POT1 (Protection of telomeres)
TRF1 (TTAGGG repeat binding factor 1)

Figure 6.28
Adapted by permission from Nature Publishing Group and Macmillan Publishers Ltd: Lundblad. 2003. *Nature* 423:926. Copyright © 2003

- POT1 binds to the TRF2/TRF1 complex on the dsDNA of telomeres.
- The complexes sense the length of the telomere and transmit this information to telomerase by transferring POT1 to the 3’-overhang at the telomere tip.
- (A) High level of POT1 at the 3’-end inhibits telomerase access to telomeres.
- (B) Little or no POT1 at the 3’-end allows telomerase access to telomeres.

2. Telomerase, aging, and cancer

- In most unicellular organisms, telomerase has a “housekeeping function.”
- In the somatic cells of invertebrates, fish, amphibians, and reptiles, telomerase activity persists.
- In most human somatic cells, not enough telomerase is expressed to maintain a constant telomere length: Progressive shortening of telomeres.
- High levels of telomerase activity in ovaries, testes, rapidly dividing somatic cells, and cancer cells.
- Adult stem cells have weak telomerase activity.

1) Telomerase and aging: the Hayflick limit

- In 1962, Leonard Hayflick discovered, contrary to long-standing dogma, that cultured normal human and animal cells have a limited capacity for replication.
- The Hayflick limit is the point at which cultured cells stop dividing and enter an irreversible state of cellular aging (senescence).
- Proposed to be a consequence of telomere shortening.

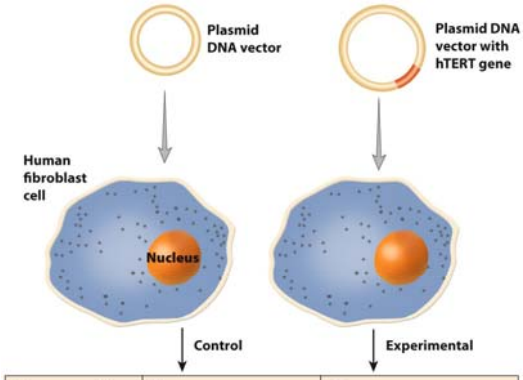
2) Telomere shortening: a molecular clock for aging?

- Telomerase: A target for anti-aging therapy or anti-cancer therapy?
- Cellular senescence may be a mechanism to protect multicellular organisms from cancer.
- Cancer cells become immortalized and thus can grow uncontrolled.
- In most cancer cells, telomerase has been reactivated.

3) Direct evidence for a relationship between telomere shortening and aging

- Evidence from experiments in human cells in culture and in transgenic mice.
- However, there are reports of instances where short telomere length does not correlate with entry into cellular senescence.

Ex. 1. Effect of experimental activation of telomerase on normal human somatic cells



Telomerase activity	No	Yes
Telomere loss	Yes	No
Cell proliferation	20 divisions	Immortal
Senescence (cells stop dividing)	Yes	No

Figure 6.29

- Experiment carried out in telomerase-negative normal human cell types.
- Demonstrated a link between telomerase activity and cellular immortality.

Ex. 2. Insights from telomerase-deficient mice

- Cells from mice engineered to lack a telomerase RNA component:

1) Cells were still proliferating after 20 divisions.

2) Progressive telomere shortening after 300 cell divisions.

3) After 450 divisions, cell growth stopped.

➔ Mice have very long telomeres, more than three times as long as human telomeres.

- Sixth-generation mice lacking telomerase RNA component

1) Defects in spermatogenesis.

2) Impaired proliferation of hematopoietic cells.

3) Premature graying and hair loss.

Dyskeratosis congenita: loss of telomerase activity

- Premature aging syndrome.
- Problems in tissues where cells multiply rapidly and where telomerase is normally expressed.
- Two forms of dyskeratosis congenita:

1) X-linked recessive
   - Mutations in dyskerin gene.
   - Dyskerin is a pseudouridine synthase that binds to small nucleolar RNAs (snoRNAs) and to telomerase RNA.
   - Patients with dyskerin mutations have 5-fold less telomerase activity than unaffected siblings.

2) Autosomal dominant
   - Mutations in telomerase RNA gene.
   - Partial loss of function of telomerase RNA.

Ex. 3. Gene therapy for liver cirrhosis

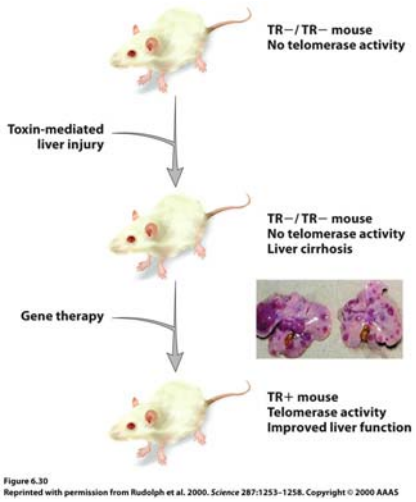


Figure 6.30
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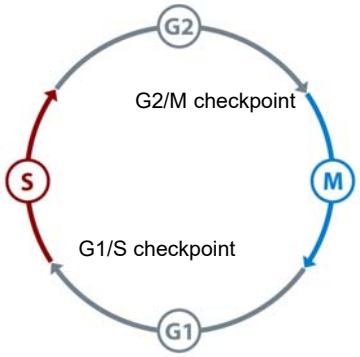
- In humans, chronic alcoholism leads to cirrhosis-heavy scarring of the liver.
- Excessive telomere shortening has been shown in patients with liver cirrhosis.
- One possible treatment is liver transplant.
- Telomerase (RNA component)-deficient mice: liver cirrhosis.
- Upon injection of an adenovirus vector carrying the telomerase RNA gene, there was restored telomerase activity, a reduction in scarring of the liver, and improved liver function.
- Why hasn't this gene therapy strategy progressed to human trials?

15.3 Regulation of Eukaryotic Genome Replication

15.3.1 Coordination of genome replication and cell division



- Genome replication in eukaryotic cells is regulated at two levels.

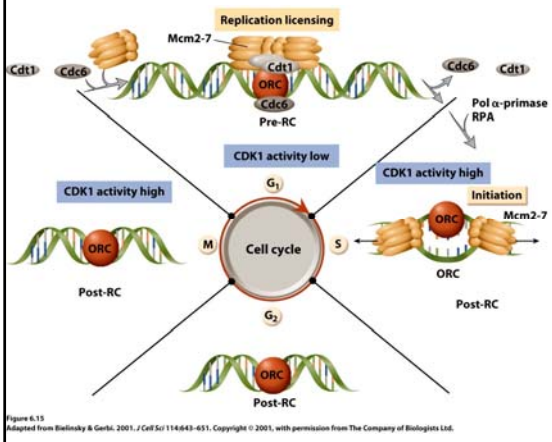
1) Replication is coordinated with the cell cycle so that two copies of the genome are available when the cell divides.
2) The replication process should be arrested until damaged DNA is repaired.

- Replication machinery needs sensors for the cell cycle and DNA damage.

Mitosis (M phase): the period when the nucleus and cell divide.
Gap1 (G1 phase): an interval when transcription, translation, and other general cellular activities occur.
Synthesis (S phase): when genome is replicated.
Gap2 (G2 phase): a second interval period preparing the cell for mitosis.

Figure 15.29 *Genomes 3* (© Garland Science 2007)

1. Establishment of the prereplication complex enables genome replication to commence



- Only licensed origins with Mcm2-7 can initiate a pair of replication forks.
- Replication licensing is regulated by the activity levels of cyclin-dependent kinases (CDKs).
- For catalysis, CDKs must associate with a regulatory subunit called a cyclin.
- When CDK activity is low in the G1 phase, Mcm2-7 can be loaded onto origins to prepare for replication (pre-RC).
- When CDK activity rises at the G1/S transition, the pre-RC is disassembled.

- ORC, Cdc6, Cdt1, and Mcm2-7 are downregulated by high CDK activity.
- No further Mcm2-7 can be loaded onto origins in S phase, G2, and early mitosis when CDK activity is high.

2. Checkpoints within S phase

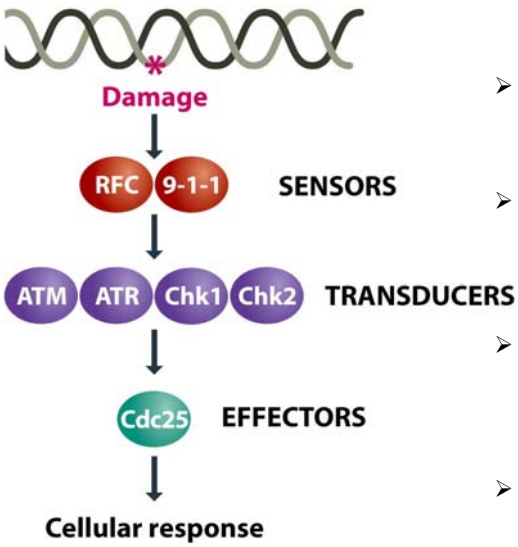


Figure 15.34 *Genomes 3* (© Garland Science 2007)

- DNA damage causes a slowing down and possibly a complete halting of the genome replication.
- Damage sensing proteins transduce the signal through transducer proteins to effector proteins, such as Cdc25.
- Cdc25 interacts with cyclin-dependent kinases to initiate the appropriate cellular response (DNA repair or apoptosis).
- 9-1-1 (Rad9-Rad1-Hus1) complex exhibits structural similarity with PCNA and is loaded onto DNA by Rad17–RFC.
- The 9-1-1 complex is directly associated with many DNA repair proteins.

https://www.youtube.com/watch?v=yqESR7E4b_8