

BCH 4054 Fall 2000 Chapter 30 Lecture Notes

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Meselson Stahl Experiment

- DNA labeled with ¹⁵NH₄⁺
- Culture diluted with excess ${}^{14}NH_4^+$
- DNA isolated and analyzed by ultracentrifugation (See Fig 30.4)
 - Original DNA bands at 1.724 g/mL
 - First generation bands at 1.717 g/mL
 - Second generation bands at 1.710 g/mL

These density labeling experiments are most consistent with the **semiconservative** mechanism of replication. Subsequent analysis of the single strands of the **hybrid** after the first generation ruled out the **dispersive** mechanism.

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Problems with Semiconservative Model

- Strands run in opposite directions
- Twisted strands must come apart
- Error rate must be very low
 - (< 1 per 10⁹-10¹⁰ bases copied)

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Chemical Components of Replication

- Template
- DNA strand determining order of new bases
- Primer
 - Strand grows at 3' end
- Deoxynucleotide triphosphates
 - "monomer" unit is activated, adding to free 3'-OH of the primer
 See Fig 30.8

The alternative would be for the terminal end of the primer to carry the triphosphate group, with linkage made to OH of the monomer unit.

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Some Features of Replication

- Bidirectional
 - Two replication "forks" which move in opposite direction.
 - Fig 30.5
- Semidiscontinuous
 - "Leading" strand copied continuously
 - "Lagging" strand made in segments (Okasaki fragments) which must be joined.
 - Fig 30.6

Slide 7 Enzymology: DNA Polymerase • Three different polymerases in E. coli • Pol I, Pol II, and Pol III (order of discovery) • See Table 30.1 • Pol I—monomer of 103 kD • 400 molecules/cell; turnover 600/min • Pol II—monomer of 90 kD • ? Molecules/cell; turnover 30/min • Pol III—complex ("core" contains 3 subunits) • 40 molecules/cell; turnover 1200/min

Pol III, the third disovered, does the bulk of new DNA synthesis. Pol I has a role in normal replication, but is also involved in DNA repair. Pol II is probably involved only in repair.

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DNA Polymerase I

- First discovered
 - Arthur Kornberg, 1957
- Catalyzes incorporation of radioactive nucleotides into polymeric form
 - Requires primer, template, and all dNTP's
- Two additional enzymatic activities
 - 3'-5' exonuclease
 - 5'-3' exonuclease

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Klenow Fragment of Pol I

- Contains the 5'-3' polymerase and 3'-5' exonuclease activity
 - Fig 30.10 diagram shows protein-DNA interaction and two enzymatic sites
 - Fig 30.9 shows model of fragment
- 3'-5' Exonuclease has proofreading function
 - Polymerase is slowed if there is a mismatch
 - Mismatch corrected by exonuclease
 See Fig 30.11

Klenow fragment is separated from the 5'-3' exonuclease component by proteolytic action (subtilisin or trypsin)

5'-3' Exonuclease Activity of Pol I

- Removes polymer in front of polymerase
- Allows "nick translation" of DNA with single strand cut
 - See Fig 30.12
- Functions in lagging strand synthesis to remove RNA primers

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DNA Polymerase III

The "real" polymerase in E. coli

- At least 10 different subunits
- "Core" enzyme has three subunits α , ϵ , and θ
- Alpha subunit is polymerase
- Epsilon subunit is 3'-exonuclease
- Theta function is unknown
- The beta subunit dimer forms a ring around DNA
 Fig 30.13
- Enormous processivity 5 million bases!

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Features of Replication

Mostly in E. coli, but many features are general

- Replication is bidirectional
- The double helix must be unwound by helicases
- Supercoiling must be compensated by DNA gyrase
- Replication is semidiscontinuous
- Leading strand is formed continuously
- Lagging strand is formed from Okazaki fragments

More Features of Replication

- DNA Pol III uses an RNA primer
- A special primase forms the required primer
- DNA Pol I excises the primer
- DNA ligase seals the "nicks" between Okazaki fragments (See Figure 30.14 for mechanism)
- See Figure 30.15 for a view of replication fork

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Mechanism of Replication Initiation

- Occurs at oriC site
 - (See Fig's 30.16 and 30.17)
 - Recognized by DnaA; requires ATP
 - DnaB (a helicase) binds, delivered by DnaC and DnaT
 - Strands separate and SSB binds
 - Primase (an RNA polymerase) binds, forming the **primosome**

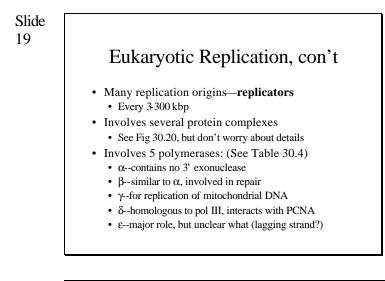
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Mechanism of Replication Elongation

- DNA Pol III complex binds at replication fork (Fig 30.17)
- DnaB serves as helicase to unwind strands
- SSB binds to keep strands separated

SSB stands for single strand binding protein. A helicase unwinds DNA

Slide 16	Mechanism of Replication
	Elongation, con't.
	Pol III forms an "asymmetric dimer"
	Lagging strand template forms a loop
	 Okasaki fragments made on the lagging strand
	• See Fig 30.18
	 "Gyrase", a topoisomerase II needed to introduce negative supercoiling ahead of replication fork
	• Topoisomerase I needed behind replication site to relax supercoiling
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17	Mechanism of Replication
	Termination
	• Occurs at the Ter locus
	 Replication forks meet here
	 Ter sequence impedes replication fork progression
	• Requires the Tus protein, a "contrahelicase"
	 Daughter strands often concatenated and
	require topoisomerase II to separate.
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10	Eukaryotic Replication
	Human cell has 6 billion base pairs to copy
	• DNA is linear, not circular
	 DNA is complexed with histones to form a "nucleosome"
	• Cell cycle more complex (See Fig 30.19)
	 G₁ phase—growth and metabolic activity S phase—DNA replication and growth
	• G ₂ phase—preparation for division
	 M phase—mitosis and cell division



Eukaryotic Replication, con't

- Replication fork, leading strand and lagging strand synthesis, similar to prokaryotes but more complex. (See Fig 30.22)
- Regulatory proteins probably controlled by phosphorylation/dephosphorylation
 - For example, the T antigen of SV40 virus
- Therefore complex second messenger signaling pathways are involved in the control

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Eukaryotic Replication, con't

- Linear DNA has ends
- Lagging strand synthesis cannot occur at the 3' end of the lagging strand, leaving a dangling 3' single strand end
- Every round of replication leads to a shorter piece of DNA
- Problem is overcome by repeating units at ends of DNA called **telomeres**

PCNA is proliferating cell nuclear antigen. Forms a ring structure similar to ₂ clamp in E. coli that makes the reaction highly processive. (See Fig 30.21)

Slide 22 Telomeres and Telomerase • Telomerase adds repeated oligomers at the 3' end. • Vertebrate telomers have TTAGGG consensus sequence • An RNA dependent DNA polymerase • A ribonucleoprotein containing 9-30 base RNA that serves as template • DNA Polymerase can then copy the extended 3' end (See Fig 30.23 and Page 382)

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Telomeres and Telomerase, con't.

- Most somatic cells lack telomerase
- Germ line cells contain 1000 to 1700 copies
- Every cell division leads to some loss
 (~ 50 bp?)
- Therefore there are a limited number of cell divisions before a cell dies
- Cancer cells have regained telomerase and are therefore "immortalized"

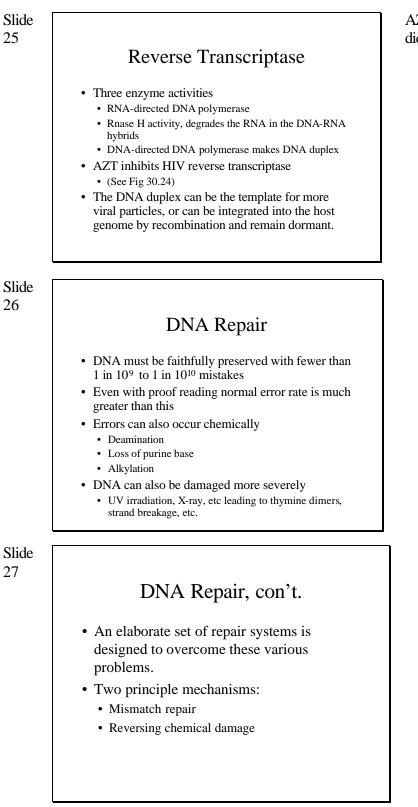
Telomerase research is important on two fronts. One is to find ways to turn off telomerase activity in cancer cells. Another is to find a way to turn on telomerase activity in somatic cells with the possibility of increasing normal life span.

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Reverse Transcriptase

- Found in some RNA viruses, especially tumor viruses and AIDS virus
- Primer is a tRNA molecule captured by the virus
- RNA is the template to make a complementary DNA (cDNA) to form a DNA-RNA hybrid

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AZT is 3'-azido-2',3'dideoxythymidine

Mismatch Repair

- Recognize and remove mismatched bases
- Must distinguish the "correct" base from the "incorrect" base in the mismatch
- In E. coli, methylation of DNA helps keep track of old strand.
 - Methylation occurs shortly after replication, but there is a short period when the "old" strand is methylated, and the "new" one is not.

Methylation occurs at the Adenine of a GATC palindromic site. Recall the way methylation also plays a role in the restriction phenomenon, helping bacteria to distinguish their own DNA from foreign DNA.

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Mismatch Repair

- One protein (Mut S) binds to mismatch site
- Another (Mut L) binds to MutS
- Another (Mut H) finds hemimethylated site
- Mut L bridges connection between Mut S and Mut H
- New strand is removed and replaced (maybe by Pol I?)
- Sometimes called "long patch" repair because long stretch of DNA is removed

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Reversing Chemical Damage

- Pyrimidine dimers can be corrected by a **photolyase** using energy of visible light to break the dimer bonds
- Base Excision (See Fig 30.27)
 - Glycosylase removes base, creates AP site
 - An endonuclease breaks the backbone, and an exonuclease removes several bases
 - Gap is filled by Pol I and DNA ligase

Photolyase is also called photoreactivating enzyme. An AP site is an *apurinic* or *apyrimidinic* site where the sugar-phosphate backbone is intact.

Reversing Chemical Damage, con't.

- Nucleotide excision
 - Removes larger sections of damaged DNA. An alternative way to correct thymine dimers.
- SOS Response
 - Activation of a number of proteins in E. coli in response to UV damage.
 - These include the recombination proteins involved in recombination (RecA, etc)
 - "Error prone" repair occurs
 - Recombination is enhanced to recapture stretches of DNA from other daughter strand when extensive damage is done.