

BIO 1140 Unit 2-1: Replication

□ **Reading**

- Chapter 12, p264-276
- Other sections referred to in the Figures
- Material from the scientific literature

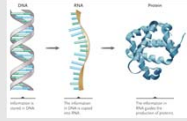
□ **Objectives**

- DNA Replication in eucaryotes
- What is a gene?
- Transcription in eucaryotes
- Translation in eucaryotes
- Regulation

The Central Dogma

DNA → RNA → Protein

Replication Transcription Translation



Unit 2 - What explains the variety of systems and their regulation?

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BIO 1140 Unit 2-1: Replication

DNA Replication in Eucaryotes

Key Points to Remember (you likely know them already)

- The DNA structure is antiparallel. DNA strands go from 5' to 3'.
- Two strands of parental DNA molecule unwind aided by helicases.
- Helicases unwind DNA to expose template strands for DNA synthesis.
- Each is a template for the synthesis of a complementary copy.
- Synthesis follows the base-pairing rules, A-T, G-C.
- DNA replication is semiconservative.
- One new DNA strand is synthesized continuously; the other, discontinuously.

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BIO 1140 Unit 2-1: Replication

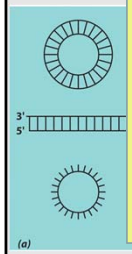
DNA Replication in Eucaryotes

Key Points to Remember (continued)

- Synthesis of DNA (or RNA) is ALWAYS 5' to 3'
- DNA polymerases are the primary enzymes of DNA replication.
- Multiple enzymes coordinate their activities in DNA replication.
- DNA replication begins at replication origins (*ori*, *ars*).
- RNA primers provide the starting point for DNA polymerase to begin synthesizing a new DNA chain
- Telomerases solve a specialized replication problem at the ends of linear DNA molecules

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BASIC PROBLEM:
DNA polymerase cannot start synthesis *de novo*



Understanding these examples allows you to **predict** what will happen when a new replicon is encountered

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relate to how the cell deals with these
short RNAs.

DNA Replication in Eucaryotes

Key Points to PONDER

- Why do you need 3 types of DNA synthesis for a chromosome? (and multiple DNA polymerases in the cell?)
- What happens when you need more or faster DNA synthesis?
- Do all DNAs have ends? What happens to them?
- When DNA replicates what happens to the chromosome? (think, what is a chromosome?)
- What signals the cell to replicate the DNA?

Enzymes of DNA Replication

- Helicase:** unwinds the DNA
- Primase:** synthesizes RNA primer (starting point for nucleotide assembly by DNA polymerases)
- DNA polymerases:** assemble nucleotides into a chain, remove primers, and fill resulting gaps
- DNA ligase:** closes remaining single-chain nicks

These enzymes work as multisubunit complexes

Assembling Antiparallel Strands

**Leading strand=continuous
Lagging strand=discontinuous
(notice the orientation)**

Figure 12.14
Replication of antiparallel template strands at a replication fork. Synthesis of the new DNA strand on the top template strand is continuous. Synthesis on the new DNA strand on the bottom template strand is discontinuous—short lengths of DNA are made, which are then joined into a continuous chain. The overall effect is synthesis of both strands in the direction of replication fork movement.

BIO 1140 – SLIDE #7 Fig. 12-14, p. 271

Two Antiparallel Strands

- As the DNA helix unwinds, one template strand runs in a direction allowing new DNA strand to be made continuously in the direction of unwinding
- Other template strand is copied in short lengths that run in the direction opposite to unwinding
Discontinuous replication produces short lengths, that are then ligated into a continuous strand

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Enzyme Activities 1

These short (~1000 nts or less) segments of RNA plus DNA are called "Okazaki fragments" in honour of their discoverer Reiji Okazaki

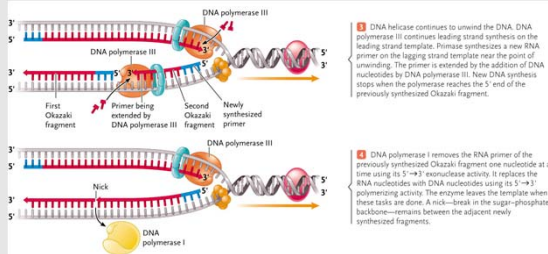
Okazaki fragment

Helicase unwinds the DNA. Primases synthesize primers in the 5' → 3' direction—in the direction of the leading strand template, and in the opposite direction on the lagging strand template. RNAase prevents twisting ahead of the replication fork.

DNA polymerase III adds DNA nucleotides to the RNA primers in the 5' → 3' direction of synthesis.

BIO 1140 – SLIDE #9 Fig. 12.13, p. 272

Enzyme Activities 2



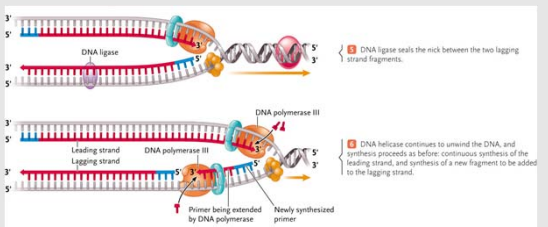
1 DNA helicase continues to unwind the DNA. DNA polymerase III continues leading strand synthesis on the leading strand template. Primase synthesizes a new RNA primer on the lagging strand template near the point of unwinding. The primer is extended by the addition of DNA nucleotides by DNA polymerase III. New DNA synthesis stops when the polymerase reaches the 3' end of the previously synthesized Okazaki fragment.

2 DNA polymerase I removes the RNA primer of the previously synthesized Okazaki fragment one nucleotide at a time using its 5' → 3' exonuclease activity. It replaces the RNA nucleotides with DNA nucleotides using its 5' → 3' polymerizing activity. The enzyme leaves the template when these tasks are done. A nick—break in the sugar-phosphate backbone—remains between the adjacent newly synthesized fragments.

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Fig. 12.13, p. 272

Enzyme Activities 3



3 DNA ligase seals the nick between the two lagging strand fragments.

4 DNA helicase continues to unwind the DNA, and synthesis proceeds as before: continuous synthesis of the leading strand, and synthesis of a new fragment to be added to the lagging strand.

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Fig. 12.13, p. 272

Major Enzymes of DNA Replication

Enzyme	Activity
Helicase	Unwinds DNA helix
Single-stranded binding proteins	Stabilize single strands; prevent the two strands of replication fork from re-annealing
Topoisomerase	Prevents overwinding of the replication fork by cutting the DNA, relieving tension, and rejoining the two strands
Primase	Assembles RNA primer in the 5' to 3' direction to initiate a strand
DNA polymerase III	Main replication enzyme; extends the RNA primer with DNA nucleotides to it
DNA polymerase I	Enzyme that uses its 5' → 3' exonuclease activity to remove the RNA of the previously synthesized Okazaki fragment, and uses its 5' → 3' polymerization activity to replace the RNA nucleotides with DNA nucleotides
DNA ligase	Seals nick left between adjacent bases after RNA primers replaced with DNA

Refer to Slide 5

Many of these enzymes are actually multiprotein complexes

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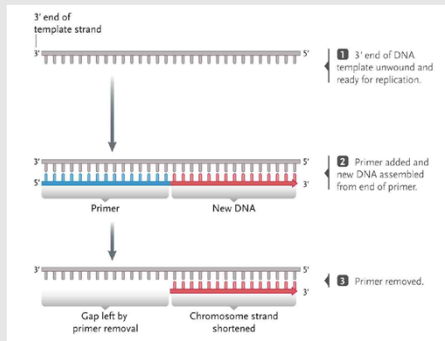
Table 12.1, p. 273

Telomeres

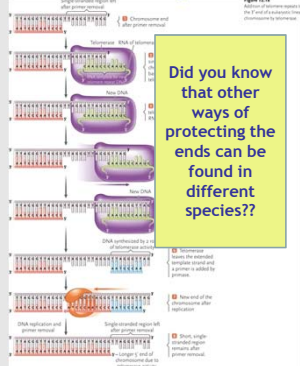
Q? Ends of eukaryotic chromosomes. What happens to them?

- Short sequences repeated hundreds to thousands of times (Humans have (TTAGGG)_n)
- Repeats protect against chromosome shortening during replication
- Chromosome shortening is prevented in some cell types which have a telomerase enzyme (adds telomere repeats to chromosome ends)

DNA Ends, the Problem



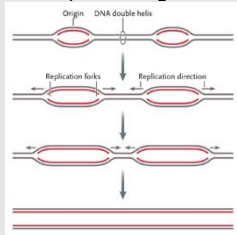
DNA Ends, the Solution: Adding Telomere Repeats



DNA Synthesis

Q? What happens when you need more or faster DNA synthesis?

- Begins at sites that act as replication origins (*ori*, *ars*)
- Proceeds from the origins as two replication forks moving in opposite directions
- Control the number of replication origins.



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Fig. 12.16, p. 274

DNA REPLICATION IN BACTERIA: Overview

The bacterial genome is a circular replicon. Replication initiates at a unique site, called the origin of Replication (*oriC*) and proceeds in a bidirectional manner until the terminus is reached (*ter*). (these can be cloned and sequenced!!)

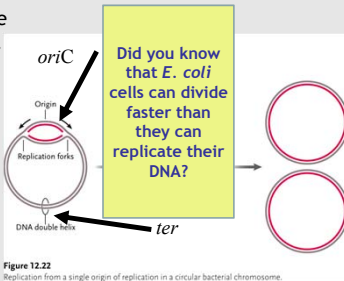


Figure 12.22 Replication from a single origin of replication in a circular bacterial chromosome.

BIO 1140 – SLIDE #17

Fig. 12.22 p. 280

How to synthesize a chromosome in a shorter time: Bacterial style

Consider:

- Bacterial growth is assessed by the **doubling time**. *E. coli* can grow at rates ranging from 18 minutes to 180 minutes.
- The bacterial chromosome is a single replicon and the frequency of replication cycles is controlled by the number of initiation events at the single origin.
- Time to replicate a chromosome is fixed at about ~40 minutes.

The solution is to reinitiate before termination!



From Genes VII: <http://flylib.com/books/en/2.643.1.154/1/>

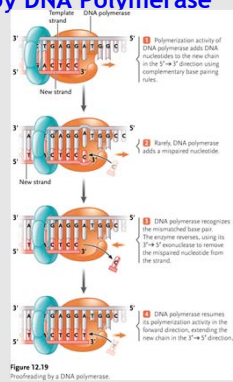
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DNA Repair Mechanisms

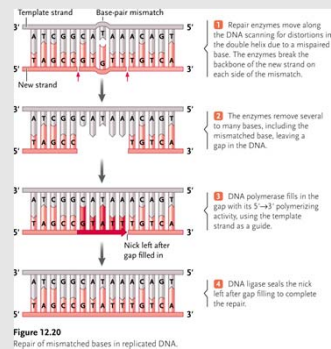
- Many agents such as alkylating agents, X-rays, oxygen radicals, etc. cause DNA damage (see supplemental info).
- Repair complexes with DNA polymerase enzymes attempt to repair the DNA.
 - Recognize distorted regions caused by mispaired base pairs (**Proofreading**) ;
 - Remove DNA section with mispaired base from the newly synthesized nucleotide chain;
 - Resynthesize the section correctly, using original template chain as a guide.

Proofreading by DNA Polymerase

- If a replication error causes a base to be mispaired, **DNA polymerase** reverses and removes the most recently added bases.
- The enzyme then resumes DNA synthesis in the forward direction



Mismatch Repair: general model for post-replication damage



DNA Repair Mechanisms

In spite of proofreading and mismatch repair, mutations arise!

Crude estimates of the number of DNA-damage events in a **single human cell range from 10^4 - 10^6 per day, requiring therefore in an adult human (10^{12} cells) about 10^{16} - 10^{18} repair events per day** ... it is clear that DNA-repair systems effectively counteract this threat. (from Schärer (2003) *Angewandte Chemie Int. Ed.* 2003, 42: 2946-2974).

Do mutations still occur? Yes, they are the "stuff" of evolution. Here's a quick calculation. In most plants the error rate per base pair is 7 per 10^9 . How many **SNPs** (*single-nucleotide polymorphisms*) in a field of soybeans if planted at a density of 240,000/hectare? And there are other types of mutations that are occurring at the same time.

DNA Replication in Eucaryotes

□ Key Points to CONSIDER (Summary)

- Why do you need 3 types of DNA synthesis for a chromosome?
- What happens when you need more or faster DNA synthesis?
- Do all DNAs have ends? What happens to them?
- When DNA replicates what happens to the chromosome? (think, what is a **chromosome**?)
- What signals the cell to replicate the DNA?
- Organelles and replication (**more DNA polymerases**)
