DNA Replication

(CHAPTER 11- Brooker Text)

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Sequence Complexity in the Genome

60-70% of human DNA fragments are unique DNA sequences
What are the structural features of DNA that enable its function?

- complementarity of DNA strands (AT/GC)
- The two DNA strands can come apart
- Each serves as a template strand for the synthesis of new strands
- Template strand also encodes for RNA

Figure 11.1

(a) The mechanism of DNA replication
(b) The products of replication
Which Model of DNA Replication is Correct?

- In the late 1950s, three different mechanisms were proposed for the replication of DNA
  - Conservative model
    - Both parental strands stay together after DNA replication
  - Semiconservative model
    - The double-stranded DNA contains one parental and one daughter strand following replication
  - Dispersive model
    - Parental and daughter DNA are interspersed in both strands following replication

Figure 11.2
Dispersive hypothesis

Meselson and Stahl Experiment (1958)

- Differentiated between the 3 different replication mechanisms by experimentally distinguishing daughter from parental strands

- Method
  - Grow *E. coli* in the presence of $^{15}$N (a heavy isotope of Nitrogen) for many generations
    - The population of cells had heavy-labeled DNA
  - Switch *E. coli* to medium containing only $^{14}$N (a light isotope of Nitrogen)
  - Collect sample of cells after various times
  - Analyze the density of the DNA by centrifugation using a CsCl gradient
Figure 11.3
Interpreting the Data

After ~ two generations, DNA is of two types: “light” and “half-heavy”
This is consistent with only the semi-conservative model

After one generation, DNA is “half-heavy”
This is consistent with both semi-conservative and dispersive models
• Overview
  – DNA synthesis begins at a site termed the **origin of replication**
    • Each bacterial chromosome has only one
  – Synthesis of DNA proceeds **bidirectionally** around the bacterial chromosome
  – The replication forks eventually meet at the opposite side of the bacterial chromosome
    • This ends replication
**Figure 11.6**

- DNA helicase separates the two DNA strands by breaking the hydrogen bonds between them.
- This generates positive supercoiling ahead of each replication fork.
  - DNA gyrase travels ahead of the helicase and alleviates these supercoils.
- Single-strand binding proteins bind to the separated DNA strands to keep them apart.
- Then short (10 to 12 nucleotides) RNA primers are synthesized by DNA primase.
  - These short RNA strands start, or prime, DNA synthesis.
    - They are later removed and replaced with DNA.
DNA Polymerases

- DNA polymerases are the enzymes that catalyze the attachment of nucleotides to make new DNA

- DNA pol I
  - Composed of a single polypeptide
  - Removes the RNA primers and replaces them with DNA

- DNA pol III
  - Composed of 10 different subunits
  - The complex of all 10 is referred to as the DNA pol III holoenzyme
  - It is the workhorse of replication
The Reaction of DNA Polymerase

- DNA polymerases catalyzes a phosphodiester bond between the
  - Innermost phosphate group of the incoming deoxynucleoside triphosphate
  - AND
  - 3'-OH of the sugar of the previous deoxynucleotide
- In the process, the last two phosphates of the incoming nucleotide are released
  - In the form of pyrophosphate (PPi)
The two new daughter strands are synthesized in different ways

- **Leading strand**
  - One RNA primer is made at the origin
  - DNA pol III attaches nucleotides in a 5' to 3' direction as it slides toward the opening of the replication fork

- **Lagging strand**
  - Synthesis is also in the 5' to 3' direction
    - However it occurs away from the replication fork
  - Many RNA primers are required
  - DNA pol III uses the RNA primers to synthesize small DNA fragments (1000 to 2000 nucleotides each)
    - These are termed Okazaki fragments after their discoverers
• **DNA pol I** removes the RNA primers and fills the resulting gap with DNA
  – It uses its 5’ to 3’ exonuclease activity to digest the RNA and its 5’ to 3’ polymerase activity to replace it with DNA

• After the gap is filled a covalent bond is still missing

• **DNA ligase** catalyzes a phosphodiester bond
  – Thereby connecting the DNA fragments

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**Figure 11.7**

- Breaks the hydrogen bonds between the two strands
- Alleviates supercoiling
- Synthesizes an RNA primer
- Covalently links DNA fragments together
- Keeps the parental strands apart
- Synthesizes daughter DNA strands
- Replication fork
-Leading strand
(Logging strand)
- DNA ligase
- Direction of fork movement
Termination of Replication

- Opposite to oriC is a pair of termination sequences called ter sequences.
- A termination protein binds to these sequences.
  - It can then stop the movement of the replication forks.
- DNA replication ends when oppositely advancing forks meet (usually at T1 or T2).
- DNA replication often results in two intertwined molecules.
  - Intertwined circular molecules are termed catenanes.
  - These are separated by the action of topoisomerases.
Figure 11.12

Proofreading Mechanisms

• DNA replication exhibits a high degree of **fidelity**
  – Mistakes during the process are extremely rare
    • DNA pol III makes only one mistake per $10^8$ bases made

• There are several reasons why fidelity is high
  – 1. Instability of mismatched pairs
  – 2. Configuration of the DNA polymerase active site
  – 3. Proofreading function of DNA polymerase
Proofreading Mechanisms

1. Instability of mismatched pairs
   – Complementary base pairs have much higher stability than mismatched pairs
   – This feature only accounts for part of the fidelity
     • It has an error rate of 1 per 1,000 nucleotides

2. Configuration of the DNA polymerase active site
   – DNA polymerase is unlikely to catalyze bond formation between mismatched pairs
   – This induced-fit phenomenon decreases the error rate to a range of 1 in 100,000 to 1 million

Proofreading Mechanisms

3. Proofreading function of DNA polymerase
   – DNA polymerases can identify a mismatched nucleotide and remove it from the daughter strand
   – The enzyme uses its 3’ to 5’ exonuclease activity to remove the incorrect nucleotide
   – It then changes direction and resumes DNA synthesis in the 5’ to 3’ direction
Bacterial DNA Replication is Coordinated with Cell Division

• Bacterial cells can divide into two daughter cells at an amazing rate
  – *E. coli* → 20 to 30 minutes
  – Therefore it is critical that DNA replication take place only when a cell is about to divide

• Bacterial cells regulate the DNA replication process by controlling the initiation of replication at *oriC*

Eukaryotic DNA Replication

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EUKARYOTIC DNA REPLICATION

• Eukaryotic DNA replication is not as well understood as bacterial replication
  – The two processes do have extensive similarities,
    • The bacterial enzymes discussed have also been found in eukaryotes
  – Nevertheless, DNA replication in eukaryotes is more complex
    • Large linear chromosomes
    • Tight packaging within nucleosomes
    • More complicated cell cycle regulation

Multiple Origins of Replication

• Eukaryotes have long linear chromosomes
  – They therefore require multiple origins of replication
    • To ensure that the DNA can be replicated in a reasonable time

• DNA replication proceeds bidirectionally from many origins of replication
Telomeres and DNA Replication

- Linear eukaryotic chromosomes have telomeres at both ends
- The term telomere refers to the complex of telomeric DNA sequences and bound proteins
- Telomeric sequences consist of
  - Moderately repetitive tandem arrays
  - 3’ overhang that is 12-16 nucleotides long
Figure 11.23

- Telomeric sequences typically consist of
  - Several guanine nucleotides
  - Often many thymine nucleotides
  - Differ between species

Figure 11.24

- DNA polymerases possess two unusual features
  - 1. They synthesize DNA only in the 5’ to 3’ direction
  - 2. They cannot initiate DNA synthesis
- These two features pose a problem at the 3’ end of linear chromosomes
• The linear chromosome becomes progressively shorter with each round of DNA replication if not solved

• Solution= adding DNA sequences to the ends of telomeres

• Requires a specialized mechanism catalyzed by the enzyme telomerase (e.g. stem cells, cancer)

• Telomerase contains protein and RNA
  – The RNA is complementary to the DNA sequence found in the telomeric repeat (binds to the 3’ overhang)