Molecular Biology

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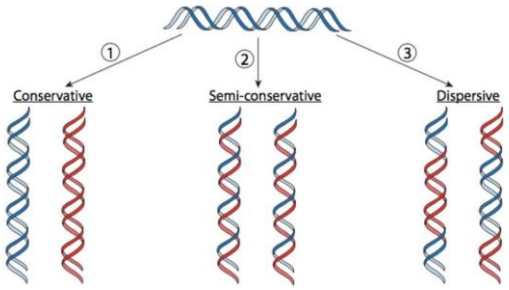
ARKAN ACADEMY

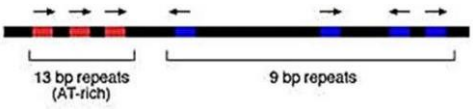
DNA Replication

- It is the process of making an accurate identical copy of a DNA molecule, using ***DNA polymerase*** enzyme and deoxyribonucleotides (***deoxyribonucleoside triphosphate***) as substrates for the reaction
- There are 3 hypotheses for DNA replication:
  - ***Conservative:*** Production of 2 DNA molecules, the first having the 2 paternal (original) strands and the second having the 2 newly synthesized strands
  - ***Semi-conservative:*** Production of 2 DNA molecules, each one of them has *one original* and *one newly* synthesized strand
    - ✓ The **approved** model is the semi-conservative
  - ***Dispersive:*** Each strands formed of a mixture of original and new segments



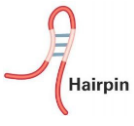
- Bacterial cell has a single circular chromosome
- Human cell has multiple linear chromosomes complexed with histones forming chromatin
  - In order to perform replication and transcription, genetic material must ***decondenses*** into *euchromatin* (***loosen*** the interaction with histone) instead of heterochromatin

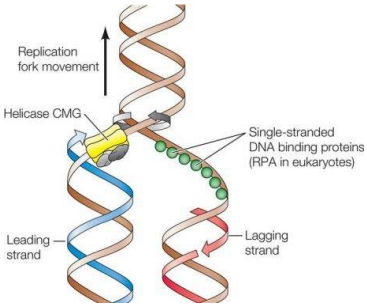
- ***Origin of replication (OriC):*** It is the sequence where replication starts
  - Bacteria: ***Only one*** OriC, contain a *consensus* sequence of:
    - ✓ *13-mers:* ***Tandemly*** repetitive 13 bp sequence, and they are *AT rich* where it facilitates the *separation of the double stranded* structure of DNA to start copying



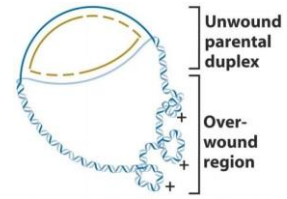
    - ✓ *9-mers:* Repetitive 9 bp sequences, represent a *binding site for the DnaA* protein
      - DnaA protein binds 9-mers and applies stress on the 13-mer region causing it to open up
  - Human: ***Many*** origins of replication (30,000) with somewhat different sequences (less consensus)
    - ✓ Before replication, ***chromatin must decondense*** for gene activation which occurs by:
      - *Histone modification*
      - *G-quadraplex* secondary structures, which can be formed in G rich sequences
    - ✓ Gene activation is important in transcription and it is cell specific

- ***DNA Helicase:*** It is the enzyme that binds the OriC and *separates the double stranded* structure of DNA
  - The separated DNA becomes single stranded where the DNA polymerase can start replication
  - The ssDNA must be bound with single stranded DNA-binding protein (***SSB***) which is also called ***replication protein A (RPA)***, which is important to:
    - Prevent ***Degradation*** of ssDNA
    - Prevent ***renaturation*** of ssDNA
    - Prevent formation of short ***hairpin*** structures

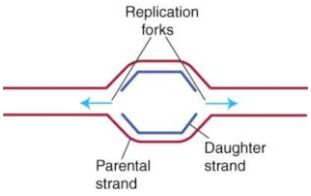




- Unwinding (opening) of dsDNA in a certain region causes the overwinding in the upstream region which can damage DNA and impede replication process
- ***Topoisomerase:*** Enzymes that form a cut (swivel) in the dsDNA (break a phosphodiester bond) to *relieve the strain* caused by helicase, then reforms it
  - *Topoisomerase I:* Form a break in *one strand only*
  - *Topoisomerase II:* Form breaks in *both strands*, it is also required for <u>mitotic chromosome condensation</u> and <u>separation of daughter chromatids</u> in mitosis
    - ✓ These processes are important during ***cell division***
    - ✓ Some cancer drugs are '*anti-neoplastic anti-topoisomerase II inhibitors*' such as ***anthracyclines***, ***doxorubicin*** and ***mitoxantrone***



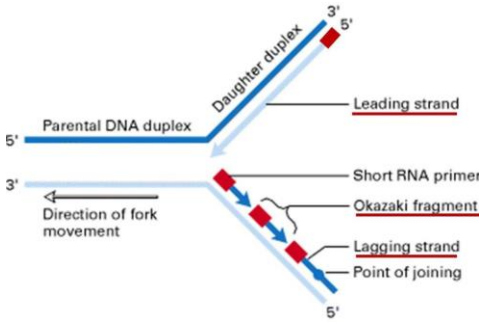
- DNA replication is ***bidirectional***
  - *Replication bubble:* The separated region during replication
  - *Replication fork:* The region where replication is active

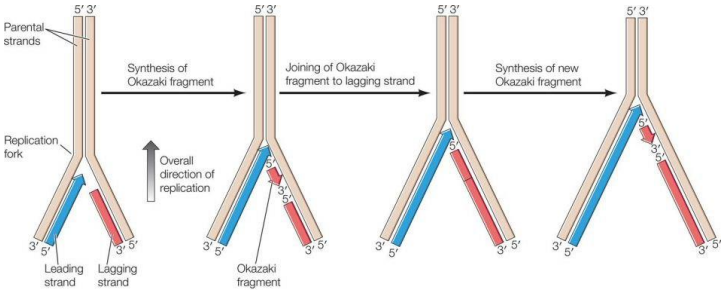


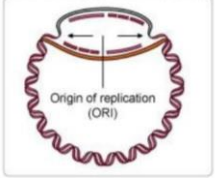
- DNA polymerases ***can't*** initiate replication ***de novo*** (from scratch), where they require a primer
- ***Primer:*** 3-10 nucleotide long *RNA* molecule that is complementary to the DNA template
  - It is synthesized by a **primase**
  - Bacteria: A *primase* adds the primer then *DNA polymerase III* starts addition of deoxynucleotides
  - Human: *DNA polymerase α* has a dual activity (primase and polymerase) where it adds the primer then adds some deoxyribonucleotides then *DNA polymerases ε and δ* continues the replication

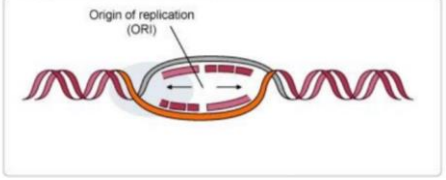
- DNA synthesis occurs from ***5' to 3'*** on the newly synthesized strand
  - ***Nucleotides are added on the 3' end***
  - Nucleotides are in the form of deoxyribonucleoside *mono*-phosphate instead of triphosphate where the remaining <u>2 phosphate groups are broken to provide energy</u> to drive the reaction

- DNA replication produces 2 forms of strands:
  - ***Leading strand:*** Synthesized *continuously*, *in* the direction of the replication fork
  - ***Lagging strand:*** Synthesized *discontinuously* in the form of many *Okazaki fragments*, and in the *opposite* direction of the replication fork



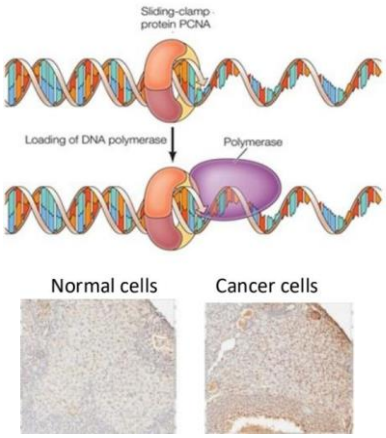






- During the synthesis of the new strand
  - Synthesis form 5' to 3' appears on the template (parental, original) strand in ***3' to 5'*** direction

- Bacteria: They have DNA polymerase **I, II, III**
  - *DNA polymerase III* is the major one and the responsible for *synthesis of leading and lagging strands*
- Human: Having DNA polymerase α, ε, δ with different functions
  - ***Pol α:*** It has a dual function (*primase*, polymerase), to start synthesis (add the *initial part*)
  - ***Pol ε:*** Synthesis the *leading strand*
  - ***Pol δ:*** Synthesis the *lagging strand*
- In human: ***Sliding clamp*** or Proliferating cell nuclear antigen (***PCNA***) *load the major polymerases* onto the primer and stabilizes their interaction with the DNA template
  - ✓ PCNA is a diagnostic marker of **<u>cancer</u>**



- The leading strand requires **only one** primer, but the lagging strand requires **many** primers (one for each Okazaki fragment)
- In the lagging strand, the primers must be removed and replaced by a DNA fragment the joined together
  - Bacteria: Primer is removed by *DNA polymerase I* which has 2 activities:
    - ✓ *5' to 3' exonuclease* activity: *hydrolyze* (*remove*) primer from 5' to 3'
    - ✓ *DNA polymerase* activity: *Fill the gaps* with a DNA fragment after removing the primer
  - Human: Primer is removed and replaced using 3 enzymes:
    - ✓ *RNase H:* is a *5' to 3' exonuclease* that removes the primer
    - ✓ *DNA polymerase δ fills the gaps*
    - ✓ *DNA ligase: Join* the fragments together
- DNA replication is an accurate process where only one incorrect nucleotide per 10^9 nucleotides
- High accuracy is due to:
  - *DNA pol* can catalyze the formation of phosphodiester bond if only the nucleotide is complementary to the one on the template strand (accuracy 10^{-5})
  - *Proofreading mechanism* (***3' to 5'*** *exonuclease* activity) increases the accuracy to 10^{-8}
  - *Repair mechanisms*
- In the human genome, there are <u>4 genetic clusters</u> containing a total of 65 histone-coding genes
  - During replication, histones are disassembled and then reassembled
  - The added histones can be either old ones that are recycled and reused or newly synthesized ones
  - All histones must be folded and recycled by ***histone chaperon***
- ***Telomeres:*** Ends of chromosomes, having repeated sequence of GGGTTA extending 10,000 nucleotides
  - The lagging strand can cause *shortening* in the length of the telomere because when the primer is removed, DNA polymerase has no space to sit and fill the gaps
  - This problem doesn't appear in the leading strand

- ***Telomerase:*** A *reverse transcriptase*, that *prevents the shortening* of the telomeres
  - It elongates the parental strand (5' to 3') using an ***intrinsic RNA template*** where the DNA polymerase can bind and fill the gaps of the primer
  - It prevents the shortening of the chromosomes and telomeres and prevents the lose of genetic material and coding regions

- ***Most somatic*** cells ***do not have*** a high level of telomerase which makes their ***division limited (finite)***
  - The level of telomerase decreases with age
  - ***Older*** individual = ***Shorter*** chromosomes and telomere
  - The gradual shortening of the chromosome ends leads to senescence and cell death

- ***Germline (stem cells)*** and ***cancer cells*** express ***high levels*** and activity of telomerase, so they are ***immortal*** (having ***unlimited*** number of divisions, ***infinite***)
  - Chromosomes ***do not get shorter*** as they divide

- The length of the chromosomes, indicates the age
  - An experiment to approve that was done on a sheep called Dolly

Past Papers

1. Replication fork is the junction between the two:
   A. Newly separated DNA strands and the unreplicated DNA
   B. Newly synthesized DNA
   C. Newly separated DNA strands and newly synthesized DNA strands
   D. Unreplicated DNA

2. The high accuracy of DNA replication PRIMARILY comes from :
   A. The DNA repair system
   B. Replication protein A
   C. The specific hydrogen bonding of nucleotides
   D. The enzymatic specificity of the DNA polymerases themselves
   E. The proofreading activity of the DNA polymerases

3. Which of the following is correct?
   A. Two phosphate groups are removed from nucleoside triphosphate that are added to the growing RNA sequence because they are too long
   B. Transcription requires a primer
   C. DNA replication is more accurate than RNA transcription
   D. All of the above are correct

4. Telomerase is considered a reverse transcriptase because :

   A. It can act as both a DNA polymerase and a RNA polymerase
   B. It acts in 3'- to 5' direction rather than 5'-to-3’
   C. It uses RNA as a template to synthesize DNA
   D. It uses a RNA primer
   E. A RNA molecule is pair of it

5. RNase H is important in this process :

   A. Termination of translation in eukaryotic cells
   B. Degradation of noncoding RNA
   C. DNA repair in prokaryotic cells
   D. Removal of primers from a replicating DNA in eukaryotic cells
   E. RNA processing in eukaryotic cells

6. When replicating DNA in bacteria, the number of leading strands is expected to be :

   A. Unlimited
   B. 1
   C. 2
   D. 4
   E. cannot be determined

7. As far as we know, the origins of replication in human cells are determined by:

   A. Three-dimensional structures of DNA
   B. Noncoding RNA molecules
   C. Localization of nucleosomes
   D. Automatically replicating sequences
   E. DnaA protein

8. DNA topoisomerase function through:

   A. Removing stem-loop structures in DNA
   B. Assembling nucleosome structural of DNA
   C. Breaking phosphodiester bonds in DNA
   D. Separating the 2 strands of DNA
   E. Altering DNA structures from heterochromatin to euchromatin

9. Which of the following is NOT correct about DNA replication?

   A. The direction of synthesis is from 5'-to-3’
   B. DNA polymerases require 5'-OH provided by the RNA primer
   C. Single-stranded DNA-binding proteins help in protecting the ssDNA from degradation
   D. DNA replication is semiconservative
   E. Lagging strand results from joining Okazaki fragments by ligase enzyme

10. Inhibition of Topoisomerase results in:

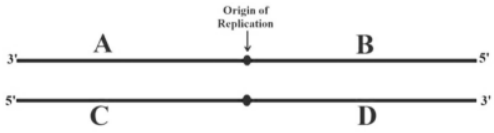
A. Interrupting the fidelity of DNA replication
B. Suppressing the synthesis of primers during DNA replication
C. Inability to remove the winding of DNA that arises during replication
D. Breakage of chromosomes
E. Inhibiting the formation of the replication fork

11. Which of the following is NOT correct about prokaryotic DNA polymerase I

A. It has 5'- to -3' exonuclease activity
B. It plays roles in repairing damaged DNA
C. It fills in the gaps between the leading strand fragments
D. It removes RNA primers of each Okazaki fragment
E. It participates in the prokaryotic DNA replication

12. in the following figure which regions will serve as template for leading strands ?

A. A and B
B. A and C
C. A and D
D. B and C
E. B and D



13. Which of the following enzymes play a role in the condensation of DNA ?

A. Ligase
B. DNA polymerase
C. topoisomerase 1
D. topoisomerase 2

14. A template of DNA is 5'-ATCGGCTACAATGTA-3'; what is the complementary DNA sequence that is created during DNA replication?

A. 5'-TAGCCGATGTTACAT-3’
B. 5'-TACAAAGTAGCCGAT-3’
C. 5'-ATCGGCTACAATGTA-3’
D. 5'-TACATTGTAGCCGAT-3’
E. 5'-UACAUUGUAGCCGAU-3'

15. Experiments proved the right mode of DNA replication, which is :

A. Conservative
B. Dispersive
C. Semiconservative
D. Semi dispersive
E. None of the above

16. Which enzyme is crucial for synthesizing DNA from an RNA template?

A. DNA polymerase
B. Restriction endonuclease
C. Reverse transcriptase
D. Ligase

17. Primase in eukaryotes that initiates DNA synthesis:

A. Polymerase alpha
B. Polymerase Beta
C. Polymerase gamma
D. Polymerase delta

18. Which of the following is incorrect?

A. Single-strand DNA binding proteins prevent the formation of hairpin structures
B. SSB proteins stabilize single strand DNA
C. SSB proteins cover the nitrogenous bases
D. All of the above are true

19. In the case of a circular DNA synthesis how many replication forks are observed?

A. 1
B. 2
C. 3
D. 4

20. A select mutation is causing a cell lineage to be unable to replicate DNA successfully. When observed under a microscope, researchers observe that the DNA is able to be separated, but the template strands keep coming back together before the new strands can be replicated. Based on this observation, which protein involved in DNA replication is most likely mutated?

A. Single-stranded binding protein
B. DNA primase
C. DNA helicase
D. DNA polymerase

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