Genetics (4) - **Eukaryotes DNA replication .**

- Human genome is composed of 30,000-40,000 genes.
- Gene is segmented into 2 types of DNA pieces →
  - Exones (Coding regions) → DNA segment which after transcription to RNA codes directly to peptide units of a polypeptide .Each gene has on average between 5 and 8 exons .
    200 base-pairs on average, in human genome
  - Introns (Noncoding regions) → DNA segment which is not directly expressed for protein, involved in regulation,'splicing' and other functions.
    2000 bp's on average, in human genome

- Cell cycle in eukaryotes : (Interphase + Mitotic phases)
  - The interphase stage includes 3 phases represented by
    - two gap phases (G1 and G2) → preparative periods for cell division and an opportunity for the cell to make decision whether to go in to division or not.
    - S phase → in which the genetic material is duplicated .
      - without DNA replication there is no cell division.
      - In human the S period is carried out for about 8 hours.
  - M phase → nuclear division .
- Eukaryotic DNA synthesis is the same to prokaryotic , but here is some differences , due to eukaryotic DNA complexity :
  - There is more DNA than prokaryotic cells
  - The chromosomes are linear
  - The DNA complexes with proteins

Eukaryotic replication initiated at many points

- Many initiation points are found in each eukaryotic chromosome instead of one ,why ?

    - Eukaryotic genome is so large (about 100x the size of bacterial DNA).
    - It would take days to replicate the whole length of eukaryotic chromosome using the same single initiation point as in prokaryotes.
- How many initiation points are there in human DNA replication ?
    - about 10,000 .
- The initiation point is called a replicons which do not need specific termination sequences.
- For eukaryotic chromosomes, there are multiple replicons per chromosome.
- Not all replicons are activated simultaneously ,clusters of 20-80 adjacent replicons are activated throughout S phase until the whole chromosome is completely replicated .
- The rate of eukaryotic DNA replication is much slower than prokaryotes → only 100-200 nucleotides bases/sec are replicated in eukaryotic Okazaki fragments .
- The majority of replication forks results in the whole genome being replicated in only about 8 hours .
- Histones for packaging the DNA are synthesized simultaneously with DNA replication to bind the new DNA .

Enzymes involved in eukaryotic DNA replication

- DNA Polymerase α
    - Initiation the synthesis of RNA primer (about 20-30 ribonucleotides)
    - Adds DNA to the RNA primers
    - has low processivity (efficiency) of DNA synthesis.
    - no 3→ 5 exonuclease activity
- DNA Polymerase δ
    - The principal DNA polymerase in eukaryotic DNA replication .
    - has 3→ 5 exonuclease activity
    - Becomes highly processive , when it complexes with PCNA.
- DNA helicase

- carries out partial unwinding of double helix DNA at the initiation point before the starting of DNA replication.

- PCNA
  - Provides high processivity to DNA Polymerase δ

- RPA → Replication Protein A.
- ssDNA-binding protein
  - facilitates the unwinding of the helix to create two replication forks.

- RFC
  - binds PCNA at the end of the primer

- FEN1/RTH1 → Exonuclease complex .

Leading strand synthesis

I. Starts with → primase activity of DNA Pol α to put down RNA primer in 5' 3'-direction ,It also adds a piece of DNA to the primer .
II. RFC binds PCNA at the end of the primer
III. PCNA displaces DNA Pol α.
IV. DNA polymerase δ binds to PCNA at the 3' ends of the growing strand ,why ?to carry out polymerase switching to highly processive DNA synthesis activity.

- The RFC mediates the polymerase switching by helping in :
  - Assembly of PCNA
  - Removal of DNA Pol α
  - Addition of DNA Pol δ

Lagging strand synthesis

I. RNA primers synthesized by DNA polymerase α ,every 50 nucleotides and consist of 20-30 nucleotides RNA
II. DNA polymerase δ switching to extend the RNAprimers and generating Okazaki fragments

III. When the DNA Pol δ polymerizes the RNA primer of the downstream Okazaki fragment, RNase H1 removes all but the last RNA nucleotide of the RNA primer
IV. The FEN1/RTH1 exonuclease complex removes the last RNA nucleotide
V. DNA Pol δ fills in the gap as the RNA primer is being removed .
VI. DNA ligase joins the Okazaki fragment to the growing strand

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Telomeres problem during human DNA replication

- Telomeres present at the ends of linear chromosomal DNA and consist of long area of short repeating sequences TTAGGG ;to protect the integrity and stability of human chromosomes
- What is the problem ? during DNA synthesis these chromosome ends cannot be replicated with DNA polymerase.
- Sequence of TTAGGG is repeated approximately 2,500 times in humans
- In humans, average telomere length declines from about 11 kilobases at birth to less than 4 kilobases in old age (Aging process)
- Average rate of decline being greater in men than in women.
- The end of a telomere inserts back into the main body of the telomere to form a T-loop

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DNA can only be synthesized at the 3'-end of a preexisting DNA or RNA chain ; no available mechanism for achieving DNA synthesis all of the ways to the end of the lagging strand (يعني هاي مشكلة بالـ lagging) ، ليش بس فيها؟
Once the primer in the last Okasaki fragment is removed by a 5′ to 3′ exonuclease it is not possible to replace it with DNA, why is that ? because the 5 - ends of the lagging strands does not have enough space to put a new primer with free 3′-hydroxyl group and therefore it is not copied completely

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Repetition of this over many rounds of replication, will cause chromosomes to gradually develop major shortening in their ends.

Correction of the chromosome ends by the enzyme Telomerase

- progressive shortening of telomeres during successive cell divisions induces chromosomal instability .
- Telomerase enzyme → ribonucleo-protein complex containing RNA-dependent DNA polymerase activity and 450-nucleotide RNA. It can act as a reverse transcriptase enzyme by using its own repetitive RNA sequence (AAAACCCC) as a template to add a repeat complementary sequence of TTAGGG to the 3- OH end of leading strand in telomeres of human DNA.
- This addition step by telomerase is repeated several times until an extend 3- end of the DNA is formed.
- The role of telomerase enzyme is ended leaving gap in the 5- phosphate end of the opposite lagging DNA strand .
- This gap will be filled later by
    - primase (adding short RNA primer).
    - combined actions of DNA polymerase and ligase activities.
- somatic cells lack telomerase activity ,thus → their telomeres get shorter with each cell division (About 50 bases are lost from each telomere every time a cell divides)
- cancer cells have high activity of telomerase enzyme that increases their survivals.

Model for initiation of the DNA replication cycle in eukaryotes

ORC(Origin recognition complex) is present at the replicators throughout the cell cycle (Responsible for directing of DNA replication and is required for its initiation).

The pre-replication complex (pre-RC) → assembled through sequential addition of the RAP (replication activator protein) & RLFs (replication licensing factors)

- Phosphorylation of the RAP,ORC,and RLFs triggers replication, after initiation, a post-RC state (post replication)is established, and the RAP & RLFs are degraded→ preventing further initiation events .

Table 10.5

Differences in DNA Replication in Prokaryotes and Eukaryotes

Prokaryotes	Eukaryotes
Five polymerases (I, II, III, IV, V)	Five polymerases (α, β, γ, δ, ϵ)
Functions of polymerase:	Functions of polymerases:
I is involved in synthesis, proofreading, repair, and removal of RNA primers	α: a polymerizing enzyme
II is also a repair enzyme	β: is a repair enzyme
III is main polymerizing enzyme	γ: mitochondrial DNA synthesis
IV, V are repair enzymes under unusual conditions	δ: main polymerizing enzyme ϵ: function unknown
Polymerases are also exonucleases	Not all polymerases are exonucleases
One origin of replication	Several origins of replication
Okazaki fragments 1000–2000 residues long	Okazaki fragments 150–200 residues long
No proteins complexed to DNA	Histones complexed to DNA

This table is extremely imp , make sure to master it .