PROTEIN BIOSYNTHESIS

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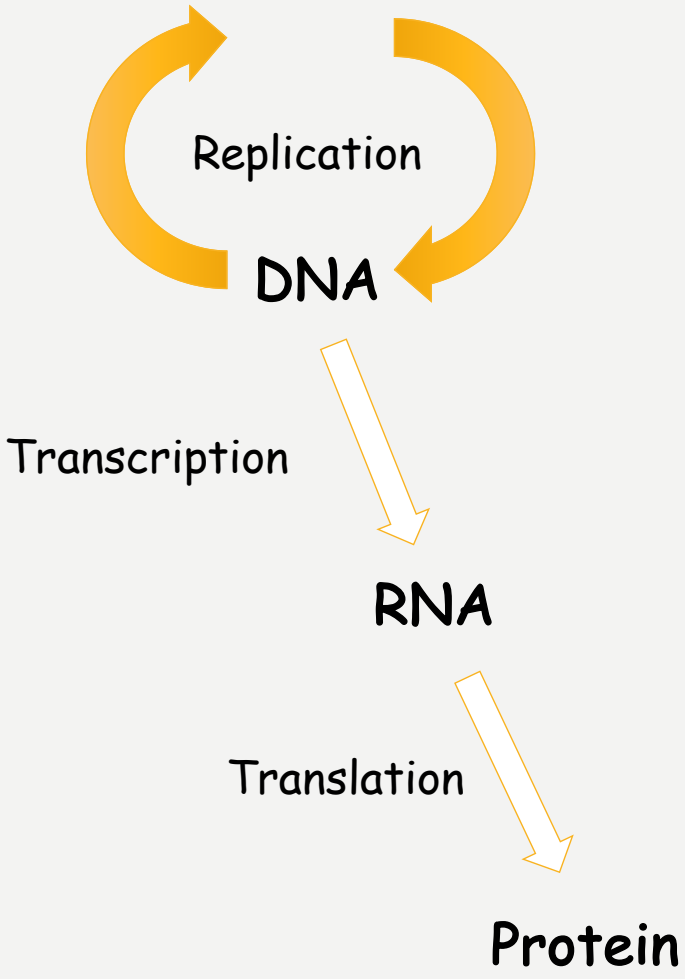
- Nucleic acids;

➢ required for the storage and expression of genetic information.

1. DNA (deoxyribonucleic acid)
2. RNA (ribonucleic acid)

Genome, Chromosom, DNA, Gene

CENTRAL DOGMA



DNA

-in chromosomes in the nucleus of eukaryotic organisms,

-in mitochondria and

-in the chloroplasts of plants.

DNA

Procaryotic cells are lack of nucleus

- ✓Have a single chromosome
- ✓They also contain nonchromosomal DNA in the form of "Plazmid"s

STRUCTURE OF DNA

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- a polymer of deoxyribonucleoside monophosphates covalently linked by 3'→ 5'-phosphodiester bonds.
- doublestranded (ds) molecule : forming a "double helix".
- In eukaryotic cells, DNA is associated with nucleoprotein
- In prokaryotes, the protein-DNA complex is present in a region called nucleoid.

1. 3′-5′ PHOSPHODIESTER BONDS

- join the 3′-OH^- group of the deoxy pentose of one nucleotide to the 5′-OH^- group of the deoxy pentose of an adjacent nucleotide through a phosphate group
- 5'-end of the chain to the 3'-end.
- The backbone of DNA: Deoxyribose-phosphate cycleton

HYDROLYSIS OF PHOSPHODIESTER BONDS

a) Chemical hydrolysis
b) Enzymatic hydrolysis: Nucleases
    -Ribonuclease
    -Deoxyribonuclease

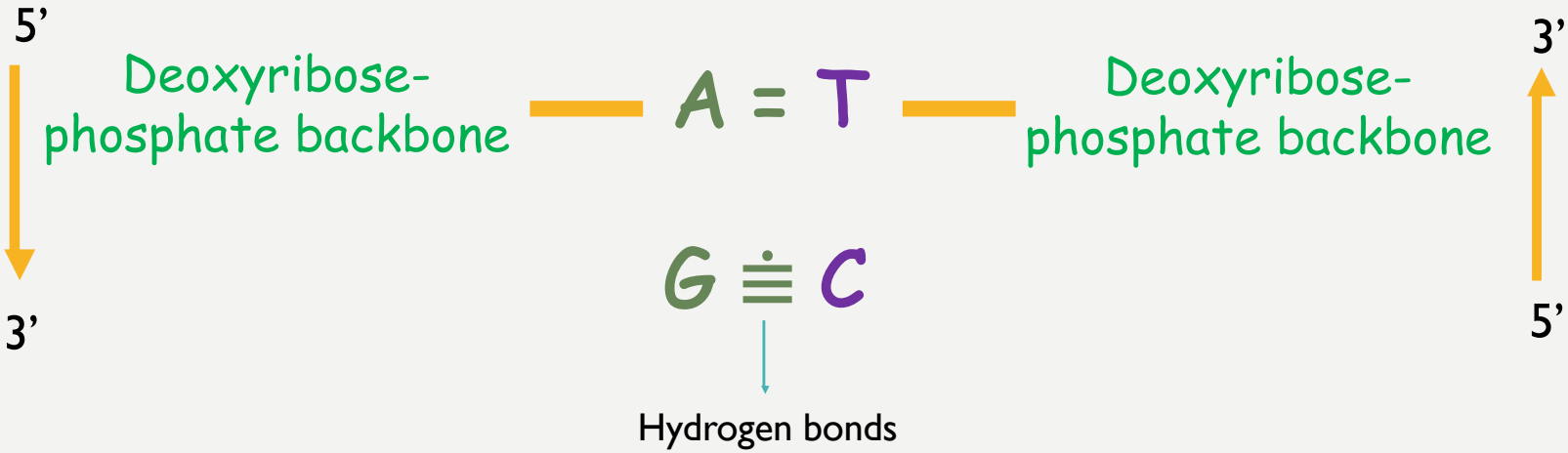
Endonucleases (cleaves to the bonds of the DNA from the molecule)

Exonucleases (cleaves the nucleotides at the end of the DNA molecule)

2. DOUBLE HELIX

- The chains are paired in an antiparallel manner,
- the 5'-end of one strand is paired with the 3'-end of the other strand
- Major and minor grooves

➢ BASE PAIRING



- One polynucleotide chain of the DNA double helix is always the complement of the other !!!!

➢ SEPARATION OF THE TWO DNA STRANDS IN THE DOUBLE HELIX

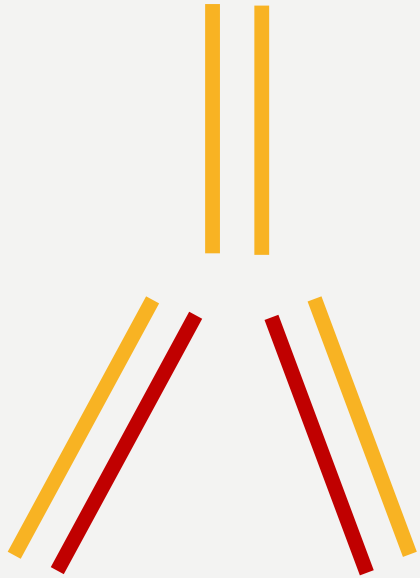
- The two strands of the double helix separate when hydrogen bonds between the paired bases are disrupted.
- The alteration of pH of the DNA solution or
- The heating of the solution may separate the double helix.

- Phosphodiester bonds are resistant to alteration of pH and temperature

➢ LINEAR AND CIRCULAR DNA MOLECULES

- Each chromosome in the nucleus of a eukaryote contains one long, linear molecule of dsDNA,
- Eukaryotes have closed, circular DNA molecules in their mitochondria, as do plant chloroplasts.
- A prokaryotic organism typically contains a single, double-stranded, supercoiled, circular chromosome.
- Plasmids ???

DNA SYNTHESIS : SEMICONSERVATIVE



1. SEPARATION OF THE TWO COMPLEMENTARY DNA STRANDS

- In order for start replication process, the two strands must be separated
- Why??
- The polymerases use only ssDNA as a template

1. SEPARATION OF THE TWO COMPLEMENTARY DNA STRANDS

- In prokaryotic organisms, there is only one "origin of replication"
- In eukaryotes, replication begins at multiple sites along the DNA helix (length !!!)

2. FORMATION OF THE REPLICATION FORK

- Replication fork ?
- It moves along the DNA molecule as synthesis occurs.
- Replication of dsDNA is bidirectional

PROTEINS REQUIRED FOR DNA STRAND SEPARATION

- ✓DnaA protein
- ✓DNA helicase
- ✓Single stranded DNA binding protein (SSB)

DNAa PROTEIN

- DnaA protein binds to specific nucleotide sequences at the origin of replication,
- AT-rich regions
- Melting is ATP-dependent,
- It results in strand separation with the formation of localized regions of ssDNA

DNA HELICASES

- Binds to ssDNA near the replication fork,
- Unwind the double helix.
- Helicases require energy provided by ATP

SINGLE STRANDED DNA BINDING (SSB) PROTEINS

- These proteins bind to the ssDNA generated by helicases
- They bind cooperatively
- **keep the two strands of DNA separated** in the area of the replication origin,
- **protect the DNA from nucleases** that degrade ssDNA.

3. "SUPERCOILING" PROBLEM !!!

- the appearance of positive supercoils (also called supertwists) in the region of DNA ahead of the replication fork.
- The accumulating positive supercoils interfere with further unwinding of the double helix.

THE ENZYMES REMOVING SUPERCOILS IN THE HELIX

DNA Topoisomerases

DNA TYPE I TOPOISOMERASES

- These enzymes reversibly cut one strand of the double helix.
- They have both nuclease (strand-cutting) and ligase (strand-resealing) activities.
- They do not require ATP, but rather appear to store the energy from the phosphodiester bond they cleave, reusing the energy to reseal the strand.

DNA TYPE I TOPOISOMERASES

- In E. coli, Type I topoisomerases relax negative supercoils
- In eukaryotic cells, Type I topoisomerases relax both negative and positive supercoils

DNA TYPE II TOPOISOMERASES

- Required for both prokaryotes ve eukaryotes !!!!!
- They also separate the interlocked molecules of DNA following chromosomal replication.

DNA GYRASE

- Present in bacteria (E.coli) and plants (a kind of type 2 topoisomerase)
- Has ability to introduce negative supercoils into relaxed circular DNA using energy from the hydrolysis of ATP.

- Why????

- This facilitates the future replication of DNA because the negative supercoils neutralize the positive supercoils introduced during opening of the double helix.

4. DIRECTION OF DNA REPLICATION

DNA Polimerases….

- responsible for copying the DNA templates
- They're only able to "read" the parental nucleotide sequences in the 3'→5' direction,

✓ and they synthesize the new DNA strands only in the 5'→3' (antiparallel) direction.

✓ DNA polimerases are not able to synthesized by 3'-5' direction !!!

4. DIRECTION OF DNA REPLICATION

- **Leading strand:** synthesized continuously.
- **Lagging strand:** synthesized discontinuously
- Okazaki fragments ???

RNA PRIMER

- DNA polymerases cannot initiate synthesis of a complementary strand of DNA on a totally single-stranded template.
- Rather, they require an RNA primer
- A short, double-stranded region consisting of RNA base-paired to the DNA template,

5. CHAIN ELONGATION

- Eucaryotic and prokaryotic RNA polymerase enzymes
- DNA polymerases elongate a new DNA strand by adding deoxyribonucleotides, one at a time, to the 3'- end of the growing chain

DNA POLIMERASE III

- DNA chain elongation is catalyzed by DNA polymerase III.
- Using the 3'-hydroxyl group of the RNA primer as the acceptor of the first deoxyribonucleotide, DNA polymerase III begins to add nucleotides along the single-stranded template that specifies the sequence of bases in the newly synthesized chain.
- The new strand grows in the 5'→3' direction, antiparallel to the parental strand

DNA POLIMERAZ III

- Four deoxyribonucleoside triphosphates must be present for DNA elongation !!!!!
- dATP, dGTP, dTTP, dCTP
- If one of the four is in short supply, DNA synthesis stops when that nucleotide is depleted.

PROOFREADING OF NEWLY SYNTHESIZED DNA

- The nucleotide sequence of DNA be replicated with as few errors as possible !!!
- Misreading of the template sequence could result in deleterious, perhaps lethal, mutations.

- Control mechanisms …

- DNA polymerase III :

-5'→3' polymerase activity,

-a "proofreading" activity (3'→5' exonuclease).

EXCISION OF RNA PRIMERS AND THEIR REPLACEMENT BY DNA

- DNA polymerase III continues to synthesize DNA on the lagging strand until it is blocked by proximity to an RNA primer.
- When this occurs, the RNA is excised and the gap filled by DNA polymerase I.

5′-3′ EXONUCLEASE ACTIVITY

- DNA polimerase III

a) 5′-3′ polimerase activity

b) 3′-5′ exonuclease activity

DNA Polimeraz I (in addition to activities above)

- 5′-3′ exonuclease activity: The RNA primer is hydrolytically removed by this way

- DNA polimerase I
  - Exonuclease activity in the direction of 5′-3′ and 3′-5′

- DNA polimerase III
  - Exonuclease activity, only in the direction of 3′-5′

- 5′-3′ exonuclease activity, removes from one to ten nucleotides at a time.
- Important in DNA Repair !!!!!

DNA LIGASE

- The final phosphodiester linkage between the 5'-phosphate group on the DNA chain synthesized by DNA polymerase III
- and the 3'- hydroxyl group on the chain made by DNA polymerase I is catalyzed by DNA ligase.
- Requires energy, by the cleavage of ATP to AMP + PPi.

EUKARYOTIC DNA REPLICATION

- The process of eukaryotic DNA replication closely follows that of prokaryotic DNA synthesis.
- Differences:
- The multiple origins of replication in eukaryotic cells versus single origins of replication in prokaryotes
- The functions of eukaryotic single-stranded DNA-binding proteins and ATP-dependent DNA helicases are different
- RNA primers are removed by RNase H and FEN1 rather than by a DNA polymerase I.

EUKARYOTIC DNA POLYMERASES

- On the basis of molecular weight, cellular location, sensitivity to inhibitors, and the templates or substrates on which they act, eukaryotic DNA polymerases are separated at least into five classes.

EUKARYOTIC DNA POLYMERASES

- **Pol** α

-a multisubunit enzyme

-One subunit has primase activity, which initiates strand synthesis on the leading strand and at the beginning of each Okazaki fragment on the lagging strand.

-The primase subunit synthesizes a short RNA primer that is extended by the pol α 5'→3' polymerase activity, generating a short piece of DNA.

- Pol ε and pol δ

-Pol ε is thought to be recruited to complete DNA synthesis on the leading strand

-Pol δ elongates the Okazaki fragments of the lagging strand,

-They both use 3'→5' exonuclease activity to proofread the newly synthesized DNA.

- Pol β

-Pol β is involved in "gap filling" in DNA repair

-Pol γ replicates mitochondrial DNA.

ORGANISATION OF EUKARYOTIC DNA

- A typical human cell contains 46 chromosomes,
- Total DNA is approximately 1m long.

!!! It is difficult to imagine how such a large amount of genetic material can be effectively packaged into a volume the size of a cell nucleus ???

- Eukaryotic DNA is associated with tightly bound basic proteins, called **histones**.
- The DNA strand binds to histon proteins and **Nucleosome** structure is formed

HISTONES AND THE FORMATION OF NUCLEOSOMES

- 5 types of histon proteins: H1, H2A, H2B, H3, H4

HISTONES AND THE FORMATION OF NUCLEOSOMES

- Histones;
- are positively charged at physiologic pH as a result of their high content of lysine and arginine.
- -Because of their positive charge, they form ionic bonds with negatively charged DNA.

Histones, neutralize the negatively charged DNA phosphate groups.

NUCLEOSOMES

- Two molecules each of H2A, H2B, H3, and H4 form the structural core of the individual nucleosome "beads."
- Around this core, a segment of the DNA double helix is wound nearly twice, forming a negatively supertwisted helix

FORMATION OF POLYNUCLEOSOMES

- Neighboring nucleosomes are joined by "linker" DNA approximately 50 base pairs long.
- Nucleosomes can be packed more tightly to form a polynucleosome (also called a nucleofilament)
- This structure assumes the shape of a coil, often referred to as a 30-nm fiber.
- Additional levels of organization lead to the final chromosomal structure

REFERENCES

- Lippincott's Biochemistry, 5th Edition
- Harper's Illustrated Biochemistry, 28th Edition