INTRODUCTION OF MOLECULER BIOLOGY

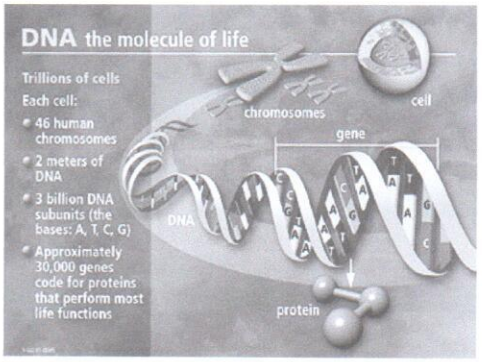
NI WAYAN TIANING
DEPARTEMENT OF PHYSIOTERAPI
FACULTY OF MEDICINE
UDAYANA UNIVERSITY

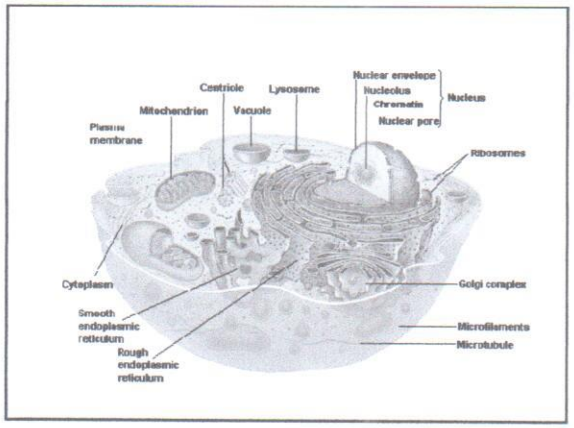
❖ OUTLINE:

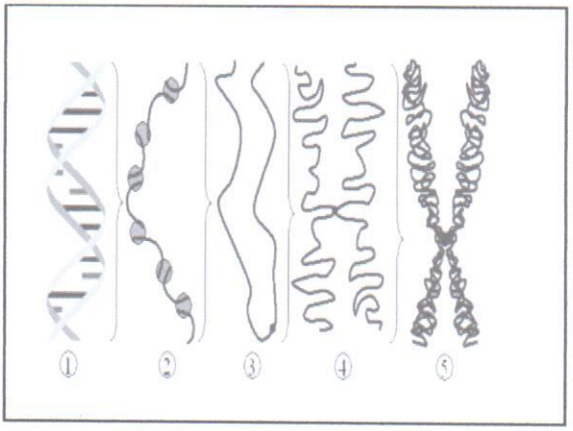
1. STRUCTURE OF **DNA** and **RNA**
2. **FUNCTION OF DNA and RNA**
3. EXPRESION OF DNA (CENTRAL DOGMA): DNA → DNA (**DNA Replication**), DNA → **PROTEIN** (transcription and translation)
4. DNA MUTATION
5. DNA REPAIR

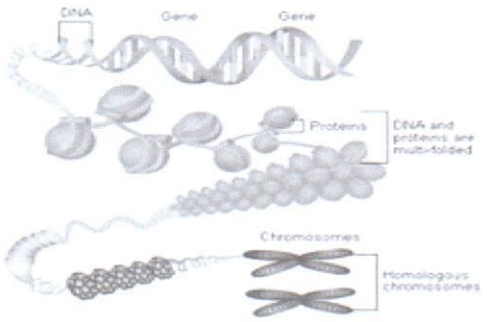
HISTORY OF DNA

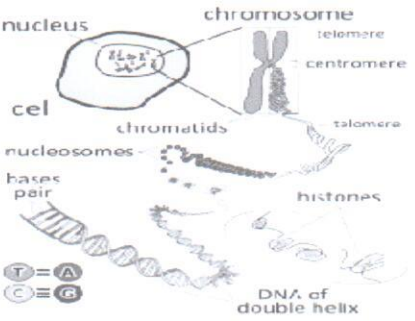
- **Freidrich Miescher** 1868-1890: Nuclear material → **DNA**.
- **Walter Sutton** 1902: **DNA in chromosomes.**
- **Fredrick Mischer** 1928: **Transformation experiments**
- **Levene** 1920: found the nucleotide.
- **Hershey and Chase** 1952: DNA, not protein, hereditary material.
- **Rosalind Franklin** 1950 used **x-rays to photograph DNA** crystals.
- **Erwin Chargaff** 1950 determined of A=T and C=G in DNA.
- **Watson and Crick 1953:** double helix DNA →**The 1st model.**
- **RECOMBINAN OF DNA**











NUCLEIC ACIDS (DNA AND RNA)

DEOXYRIBO **N**UCLEIC **A**CID **(DNA)**

- Polimer of nucleotide (polynucleotides)
- Nucleic → found in nucleus **and also in mitochondria (mtDNA).**
- Acid: phosphate group

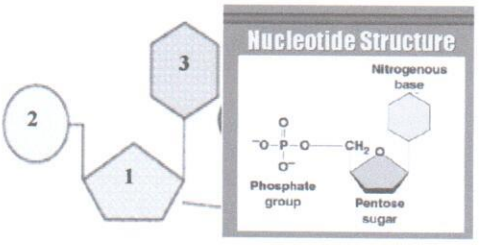
DNA → Phosphate + sugar (pentosa) + nitrogenous bases.

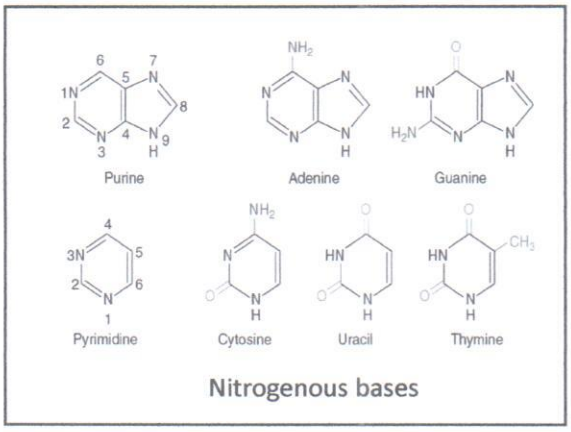
DNA

- Contain genetic information
- **Double helix** (nucleotide chain)
- Nucleotide purine (A & G) and pyrimidin (C & T) → **complementary**
  - A - T → 2 hydrogen bond
  - G - C → 3 hydrogen bond
- **Antiparalel**
- Sugar: **deoxyribose**

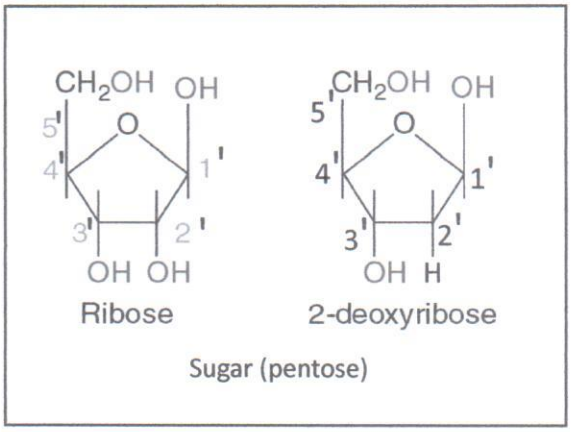
DNA

- Encodes all the proteins in our body (enzymes, hormone, etc)
- Can be denatured and renatured
- Can be digested by endonuclease restriction enzyme
- Template for
  - Replication
  - Transcription





Nitrogenous bases



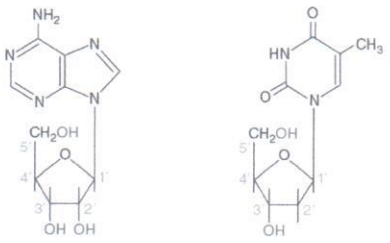
Sugar (pentose)

BASA - RIBOSA - FOSFAT

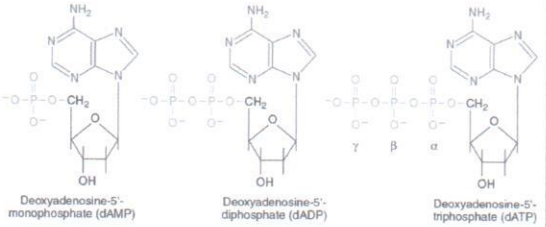
NUKLEOSIDA

NUKLEOTIDA

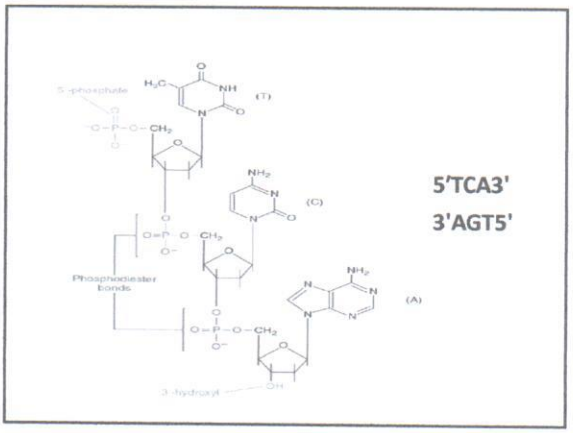
NUCLEOSIDE

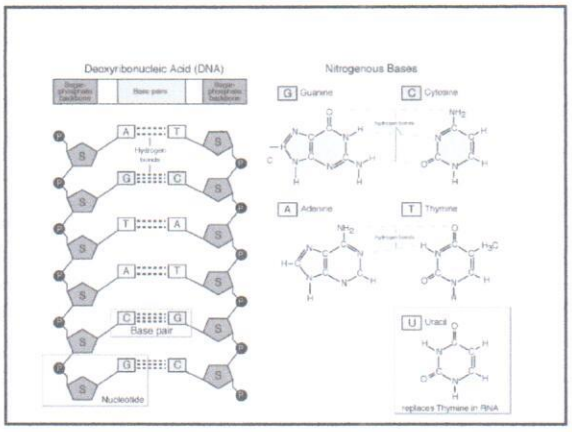


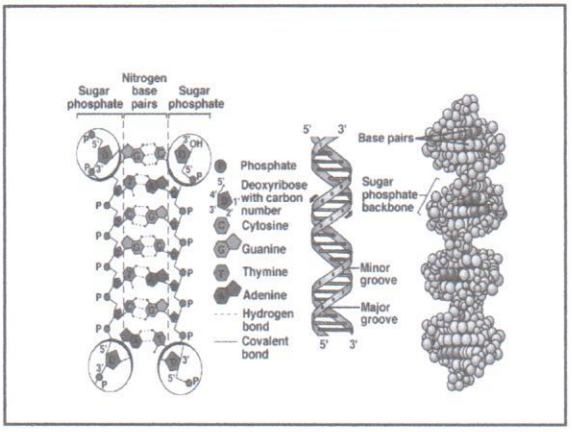
NUCLEOTIDE



AMP **ADP** **ATP**



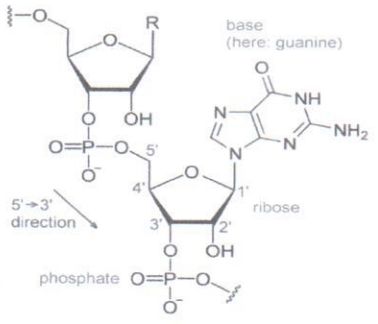


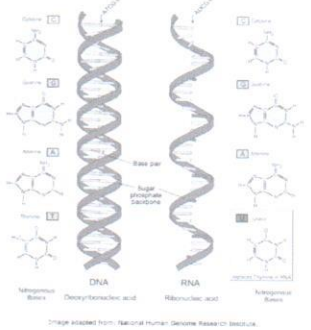


RIBO NUCLEIC ACID (RNA)

- Single strand molecul
- Found: in **nucleus** and **cytoplasm**
- Sugar: **ribose**
- Nitrogen base **uracil (U)** instead of **thymine (A-U)**
- Three types of RNA (mRNA, tRNA, and rRNA)

- **rRNA** and **tRNA** are molecules with same types in every type of protein synthesis).
- Information coded in **DNA** directs the synthesis of different **RNA** molecules, involve in protein synthesis.
- Three consecutive bases on **mRNA** called a codon (**UAA, CGC, AGU**)





Second letter

First letter	U	C	A	G	Third base
U	UUU Phenylalanine UUC Phenylalanine	UCU Serine UCC Serine UCA Serine UCG Serine	UAU Tyrosine UAC Tyrosine	UGU Cysteine UGC Cysteine	U C A G
	UUA Leucine UUG Leucine		UAA Stop codon UAG Stop codon	UGA Stop codon UGG Tryptophan	
C	CUU Leucine CUC Leucine CUA Leucine CUG Leucine	CCU Proline CCC Proline CCA Proline CCG Proline	CAU Histidine CAC Histidine CAA Glutamine CAG Glutamine	CGU Arginine CGC Arginine CGA Arginine CGG Arginine	U C A G
A	AUU Isoleucine AUC Isoleucine AUA Isoleucine AUG Methionine; initiation codon	ACU Threonine ACC Threonine ACA Threonine ACG Threonine	AAU Asparagine AAC Asparagine AAA Lysine AAG Lysine	AGU Serine AGC Serine AGA Arginine AGG Arginine	U C A G
G	GUU Valine GUC Valine GUA Valine GUG Valine	GCU Alanine GCC Alanine GCA Alanine GCG Alanine	GAU Aspartic acid GAC Aspartic acid GAA Glutamic acid GAG Glutamic acid	GGU Glycine GGC Glycine GGA Glycine GGG Glycine	U C A G

CHROMOSOME, GENE, GENOM, DNA AND RNA

CHROMOSOME ⇨ CHROMATIN: DNA,RNA,PROTEIN

⇩

GENE ⇨ **DNA** : SUGAR, ACID, BASE

⇩

RNA ⇨ **PROTEIN**

DNA : contain the nucleotides sequence

Gene : unit of inheritance sequence of nucleotides coding for polypeptide or for an RNA molecule.

Genome: entire DNA content of a cell, including all of the genes and all of the intergenic region. Human genome ± 80.000 genes, but coding region only 3% (nuclear genome: 3 billion of DNA)

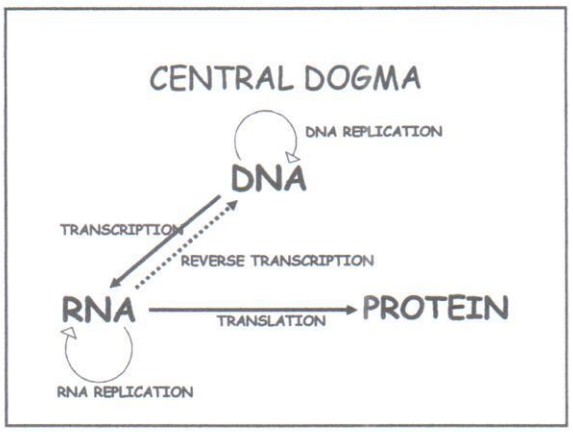
Chromosome: is a chromatin in the cell.

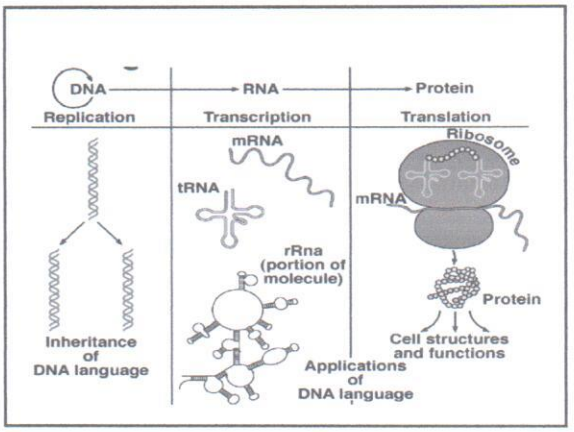
DNA REPLICATION

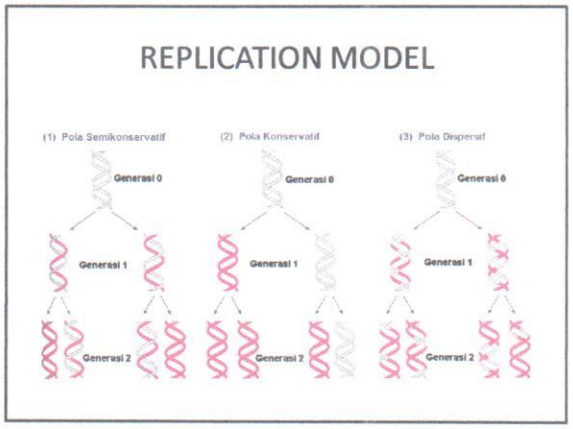
- **Definition:** The process **to synthesis** DNA from DNA → **Replicon.**
- **Purpose: To sustain** the genetic information when cell division.
- **Occurs:** In the cell during **S phase** before cell division
- Rapid and accurate .
- **SEMICONSERVATIVE MODEL.**

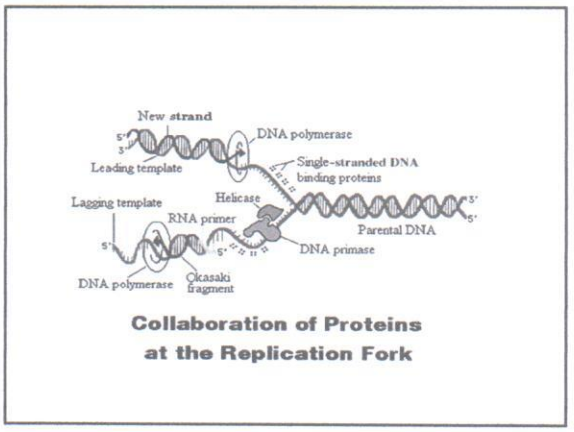
COMPONENT REQUIRES

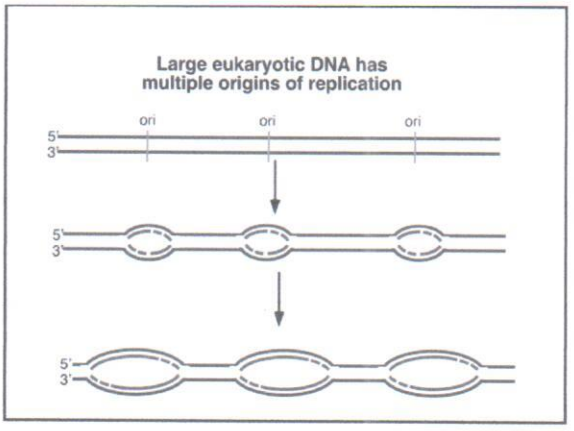
- DNA **(TEMPLATE)**
- Origins of replication **(ORI)**
- **Nucleotides (A,G,C,T)**
- **Enzymes and protein:**
  - **DNA Polymerase III:** to adds new nucleotide DNA 5'→3'
  - **Primase:** to synthesis RNA primer
  - **Helicase:** to oven double helix of DNA
  - **Ligase** to conjunct Okazaki fragment
  - **Topoisomerase** (girase) to unwind the supercoil
  - **Protein SSBP** to avoid renaturation of DNA

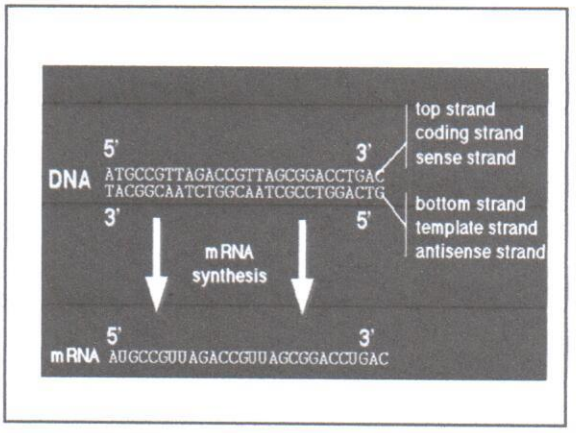

CENTRAL DOGMA
DNA REPLICATION
DNA
TRANSCRIPTION
REVERSE TRANSCRIPTION
RNA
TRANSLATION
PROTEIN
RNA REPLICATION

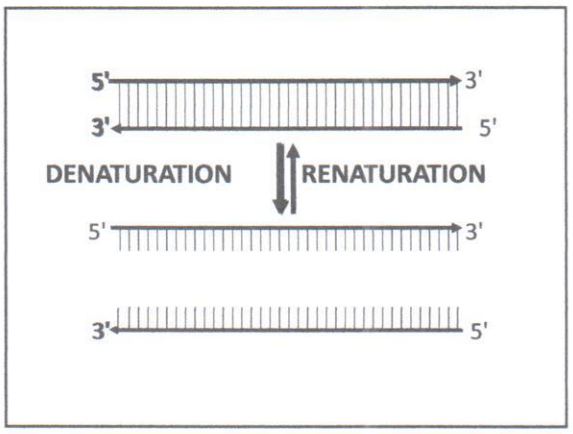

DNA
RNA
Protein
Replication
Transcription
Translation
mRNA
Ribosome
tRNA
mRNA
rRna
(portion of
molecule)
Protein
Inheritance
of
DNA language
Cell structures
and functions
Applications
of
DNA language

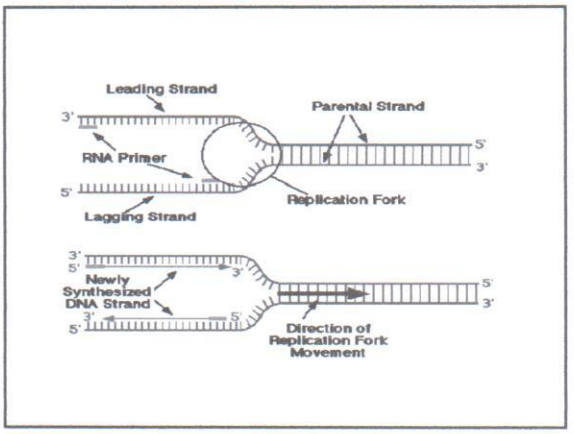

REPLICATION MODEL
(1) Pola Semikonservatif
(2) Pola Konservatif
(3) Pola Dispersif
Generasi 0
Generasi 1
Generasi 2

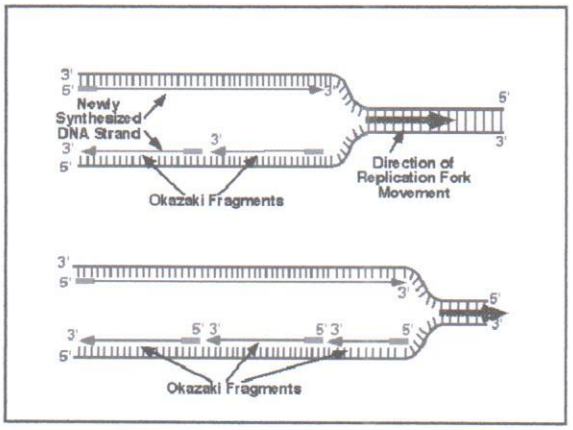

New strand
DNA polymerase
Leading template
Single-stranded DNA
binding proteins
Lagging template
Helicase
RNA primer
Parental DNA
DNA primase
DNA polymerase
Okasaki
fragment
Collaboration of Proteins
at the Replication Fork


Large eukaryotic DNA has
multiple origins of replication
ori
ori
ori


top strand
coding strand
sense strand
DNA
ATGCCGTTAGACCGTTAGCGGACCTGAC
TACGGCAATCTGGCAATCGCCTGGACTG
bottom strand
template strand
antisense strand
mRNA
synthesis
mRNA
AUGCCGUUAGACCGUUAGCGGACCUGAC







DNA MUTATION

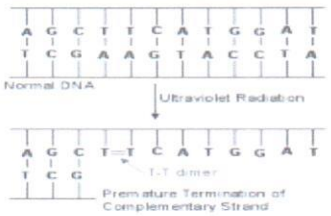
MUTATION

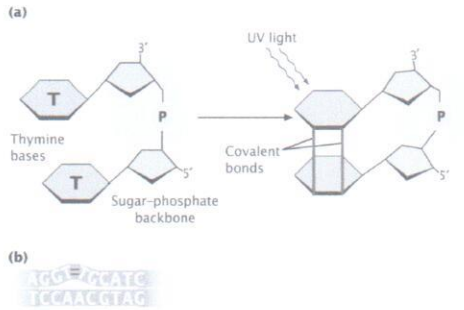
- DEFINITION: ANY CHANGE IN THE NUCLEOTIDE/BASE SEQUENCE.
- CAN BE DETECTED BY COMPARING THE MUTATED ORGANISM (MUTANT) WITH ANOTHER NONMUTATED ORGANISM OF THE SAME TYPE (WILD TYPE)
- THE EFFECT IS DEPEND ON SIZE (SINGLE TO MILLIONS BASES) AND LOCATION OF MUTATION

Mutation

- **SPONTANEUS**
  Occurs every one million - one bilion per one cell division
- **INDUCIBLE** (exogenus and endogenus)
  Exogenus: chemist, ultraviolet
  Endogenus: OXPHOS reaction →
  Reactive Oxygen Species (ROS)

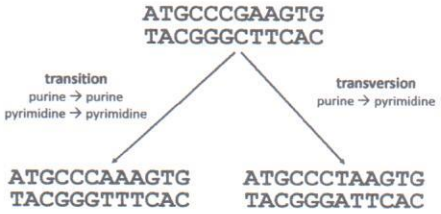
Mutation: Exposure to Ultraviolet Radiation



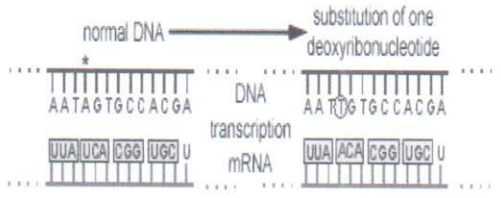


Mutation

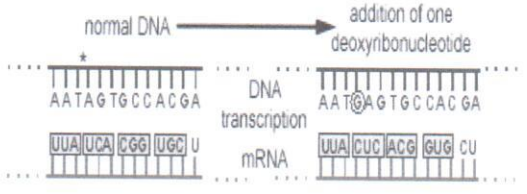
1. Base Substitution (point mutation) :
   - Transition
   - Transvertion
2. Non Base Substitution :
   - Deletion
   - Insertion/addition



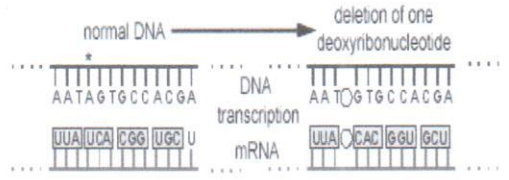
Mutation: Substitution of a Nucleotide



Mutation: Addition of a Nucleotide



Mutation: Deletion of a Nucleotide

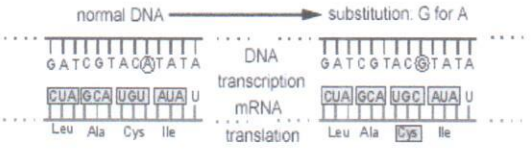


EFFECT OF MUTATION

- **Molecular effect:**
  - Silent = sense (**the same amino acid**)
  - Missense (**one wrong amino acid**)
  - Nonsense (**terminated**)
  - Frameshift
- **Clinical effect**
  - Acceptable
  - Partially acceptable
  - Unacceptable (fatal/lethal)

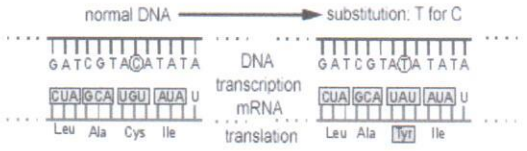
Sense Mutation:

Results for the same amino acid.



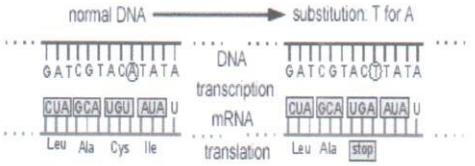
Missense Mutation:

Results in one wrong codon and one wrong amino acid.



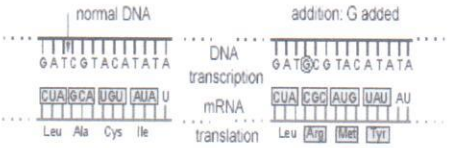
Nonsense Mutation:

Results in a "stop" codon and premature termination of the protein.



Frameshift Mutation:

Results in a reading frame shift. All codons and all amino acid after the shift are usually wrong.



DNA REPAIR

DNA REPAIR

- DNA IS A DOUBLE-STRANDED → EASIER TO BE REPAIRED
- RNA (HIV VIRUS) → SINGLE STRAND, HAVE A VERY HIGH RATE OF MUTATION

DNA REPAIR

	Type of Defect	Repair Mechanism	Biochemical Mechanism
Spontaneous			
	Wrong base incorporated during DNA replication	Proofreading by DNA polymerase	Removal of mispaired base by DNA polymerase
		Mismatch repair	Excision of short stretch of unmethylated single-stranded DNA and synthesis of new strand by DNA polymerase
		Excision (dark) repair	Excision of short stretch of DNA and synthesis of new strand by DNA polymerase
Mutagens			
Chemical	Wrong base incorporated during DNA replication	Same as for spontaneous mutations	Same as for spontaneous mutations
UV light	Thymine dimer formation	Photoreactivation (light repair)	Breaking of covalent bond forming thymine molecules
		Excision repair (dark repair)	Excision of a short stretch of single-stranded DNA containing thymine dimer and synthesis of a new strand by DNA polymerase
		SOS repair	DNA synthesis by a modified DNA polymerase bypasses site of damaged DNA

MISMATCH REPAIR

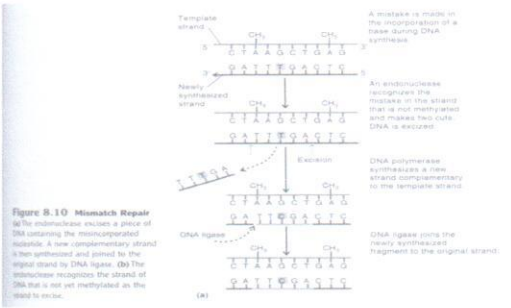


Figure 8.10 Mismatch Repair (a) The endonuclease excises a piece of DNA containing the misincorporated nucleotide. A new complementary strand is then synthesized and joined to the original strand by DNA ligase. (b) The endonuclease recognizes the strand of DNA that is not yet methylated as the strand to excise.

PHOTOREACTIVATION

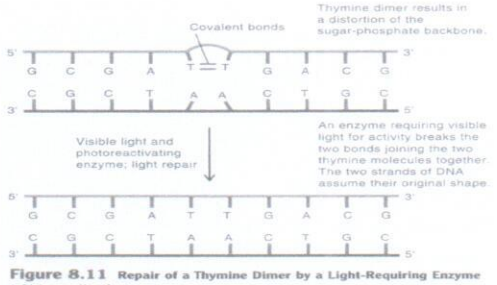


Figure 8.11 Repair of a Thymine Dimer by a Light-Requiring Enzyme (photoreactivation)

EXCISION REPAIR

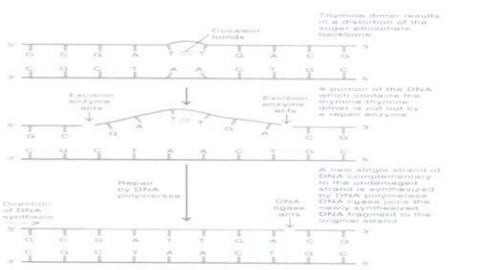


Figure 8.12 Dark, or Excision, Repair of a Thymine Dimer The single strand of DNA containing the thymine dimer is removed and destroyed. The newly synthesized strand is joined to the end of the original strand by the enzyme DNA ligase.

CONCLUSIONS

- TO UNDERSTAND:
  - STRUCTURE and FUNCTION OF DNA, RNA
  - GENE EXPRESSION (CENTRAL DOGMA/REPLICATION)
  - DNA MUTATION AND DNA REPAIR.

THANK YOU