
بسم الله الرحمن الرحيم

Bacterial Genetics

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(PhD)

bacterial genetics

Genetics - Medical Microbiology - NCBI Bookshelf

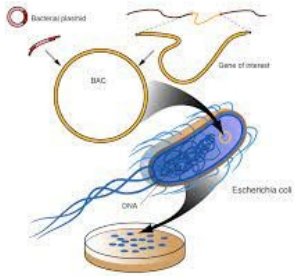
Lecture Objectives

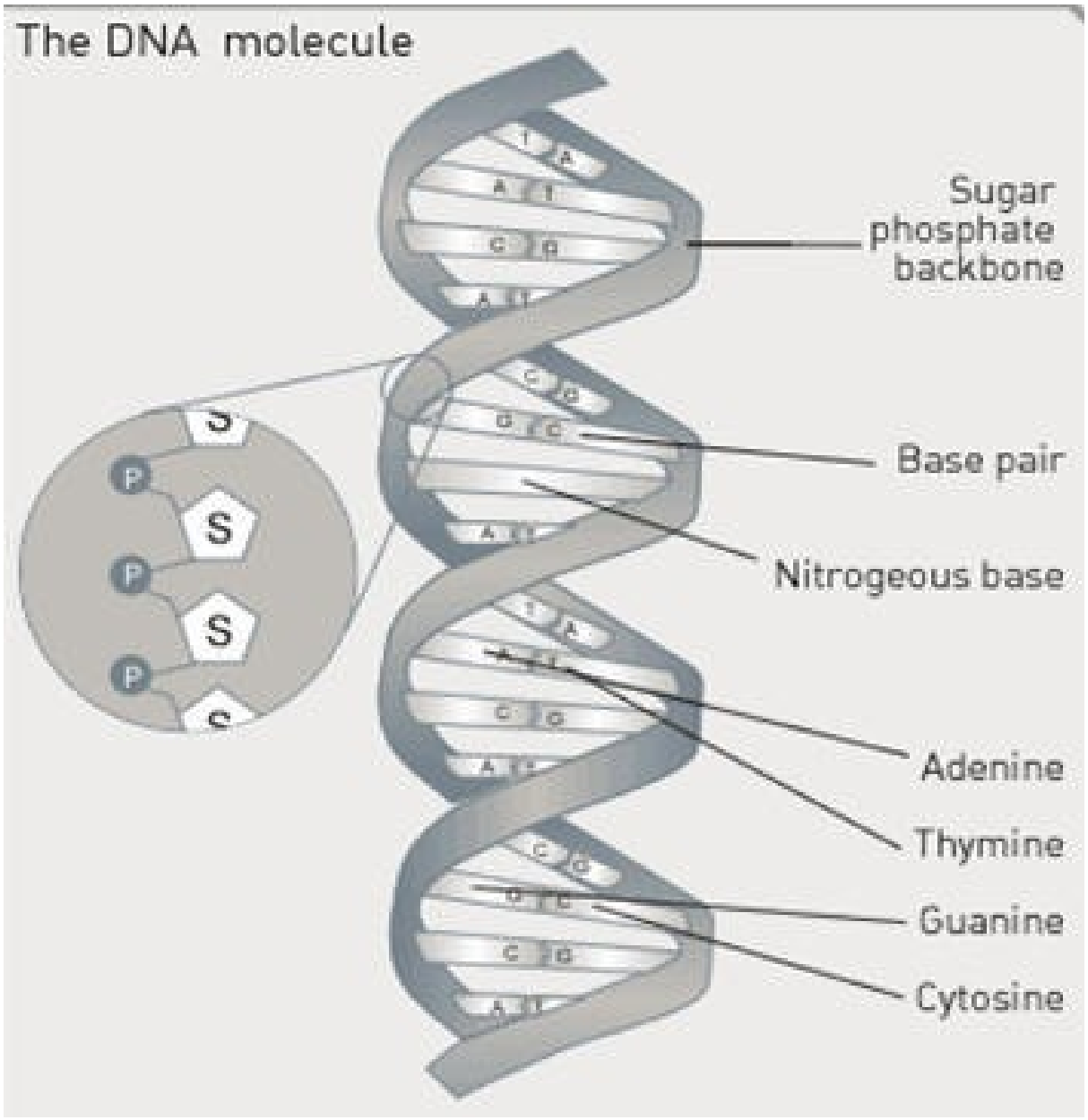
At the end of this session, students of 3rd Year MBBS should be able to

1. Define mutation (# 93)
2. Describe various types of mutations and their common causes (# 94)
3. Describe methods of transfer of DNA within bacterial cells including process of conjugation, transduction, transformation and recombination (# 95).

Bacterial Genes

- Majority of Bacterial genes are haploid (single, circular chromosome; double stranded DNA)
- Molecular weight 2×10^9 (*E coli)*
- Composed of approx. 5×10^6 base pairs,
- Code for about 2000 proteins with average weight of 50,000 Daltons.
- Elements like plasmids, transposons, integrons, or gene cassettes are shorter sequences that mainly contribute to recombination events or drug resistance.

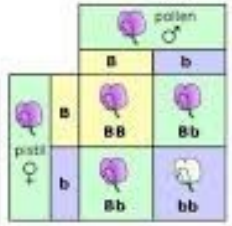

Bacterial plasmid
BAC
Gene of interest
Escherichia coli
DNA


The DNA molecule
Sugar phosphate backbone
Base pair
Nitrogeous base
Adenine
Thymine
Guanine
Cytosine
P
S

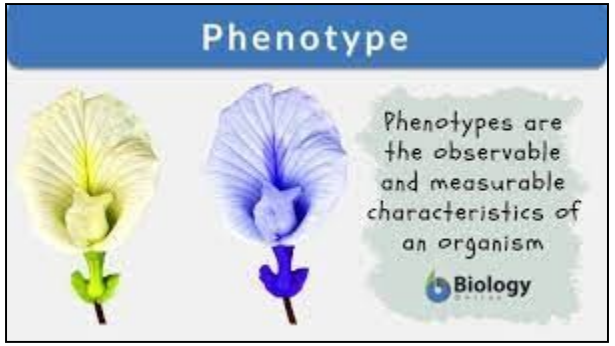
genotype

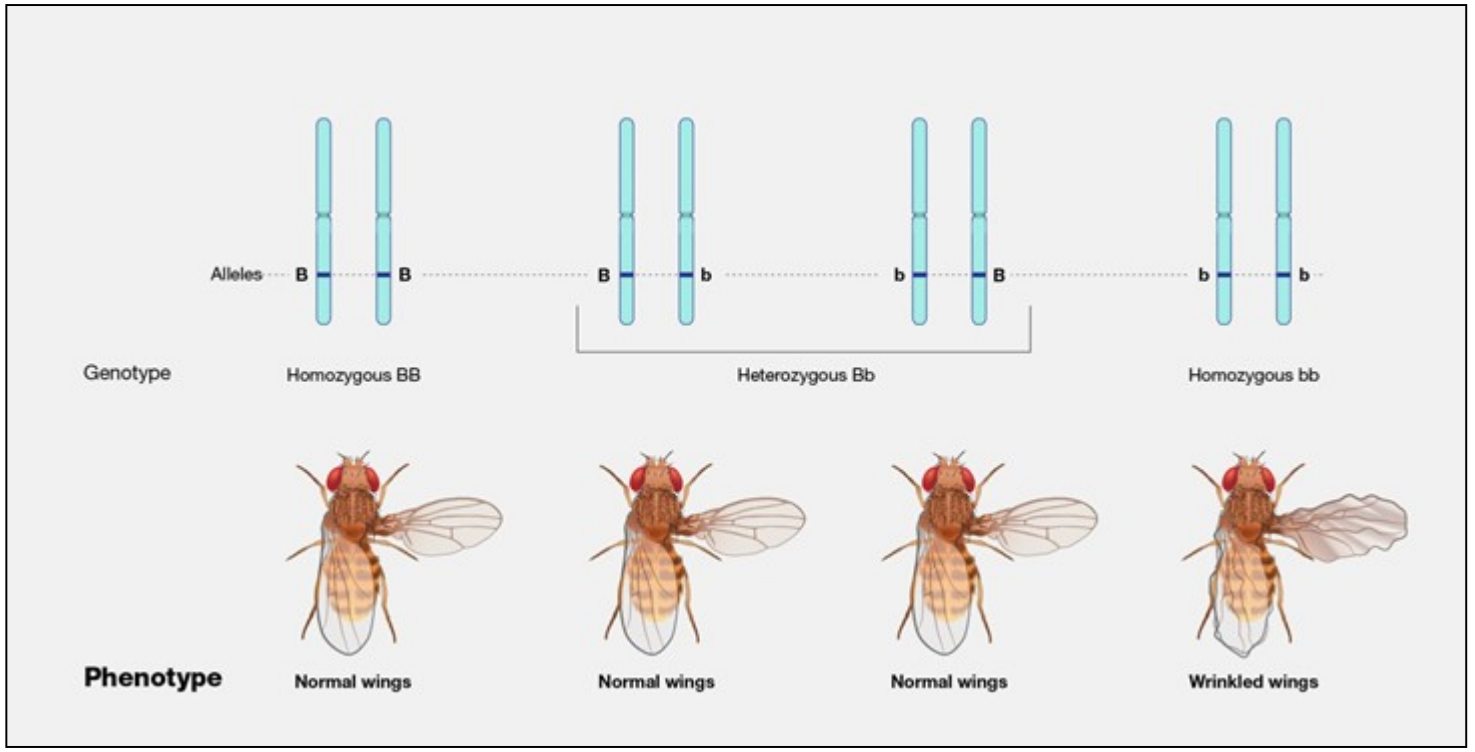
n., ˈdʒiːnəʊtaɪp

The genetic makeup of a cell, an organism, or an individual that contributes to its phenotype



Biology





MUTATION

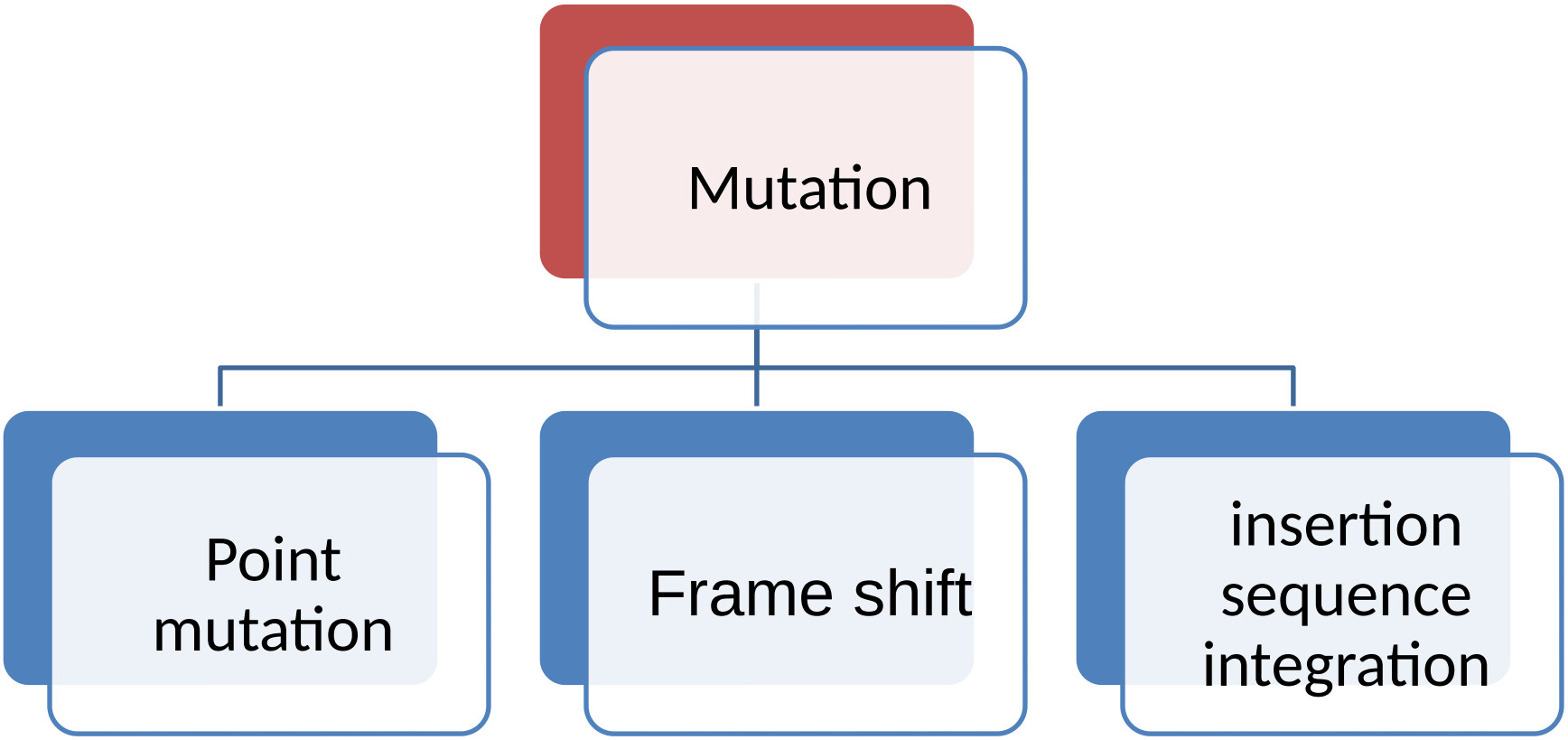
- A mutation is a change in the base sequence of DNA that usually results in insertion of a different amino acid into a protein and the appearance of an altered phenotype.
- phenotypic results may vary on the severity and location of the mutation
- Mutations can occur spontaneously by error during replication or induced by exposure to mutation-inducing agents (like chemicals, UV light or radiation).

Mutation

- **Spontaneous mutation** occurs naturally, about one in every million ($1:10^6$) to one in every billion ($1:10^9$) divisions, and is probably due to low level natural mutagens present in the environment.
- **Induced mutation** is caused by mutagens that cause a much higher rate of mutation; induced by chemicals or radiations
- Other mutations are caused by transposable genetic elements.

Spontaneous Mutations

- Spontaneous mutations occur as a result of errors during DNA replication.
- When DNA Polymerase III synthesizes a new strand of DNA, occasionally a nucleotide will be
  - **Mispaired, base substitution (**one nucleotide substitutes for another leading to one mutated grand-daughter DNA strand),
  - a nucleotide will be **added**, or
  - a nucleotide will be **Omitted / deleted**.

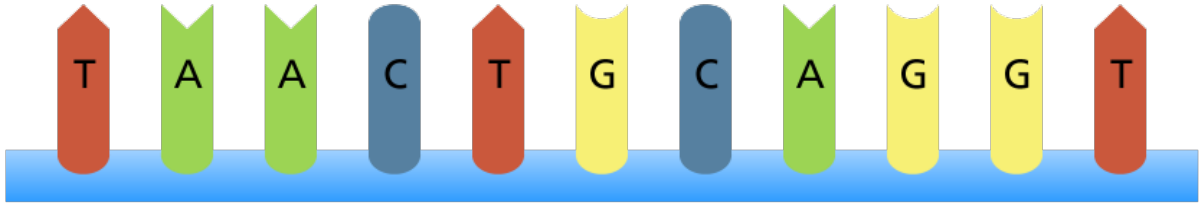

Mutation
Point mutation
Base substitution
Frame shift
insertion sequence integration

Point mutation

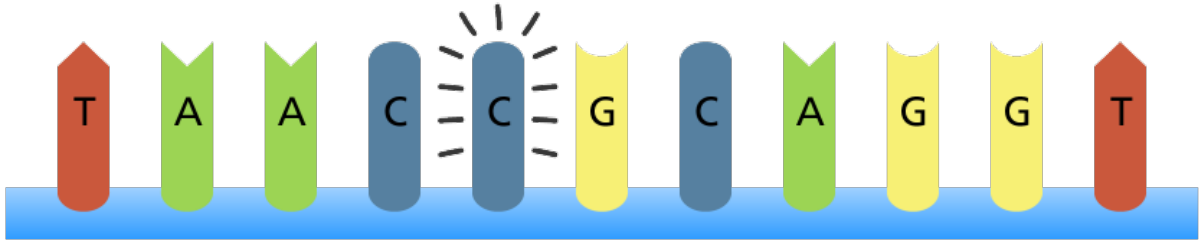
Base substitution

- most common mechanism of mutation;
- It is a substitution of one base for another during DNA replication;
- for example, a shift in the hydrogen atom of adenine enables it to form hydrogen bonds with cytosine rather than thymine.

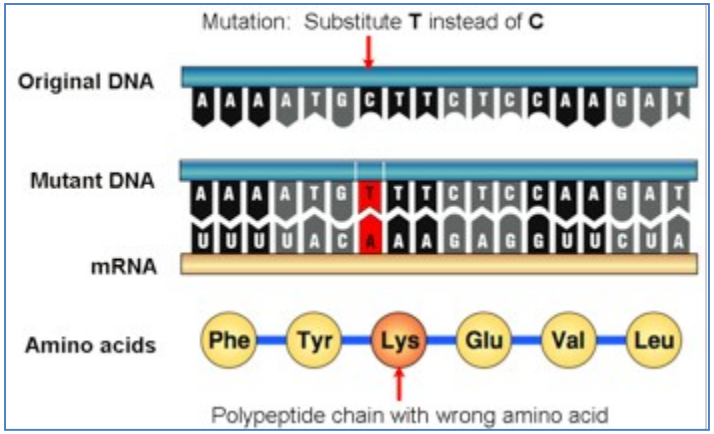
Original sequence



Point mutation

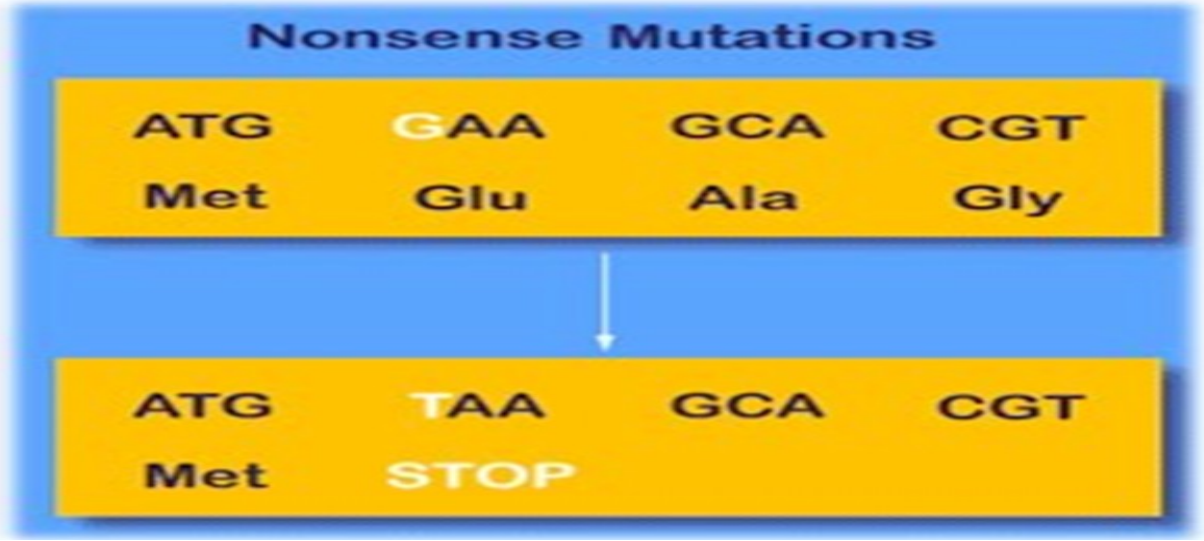


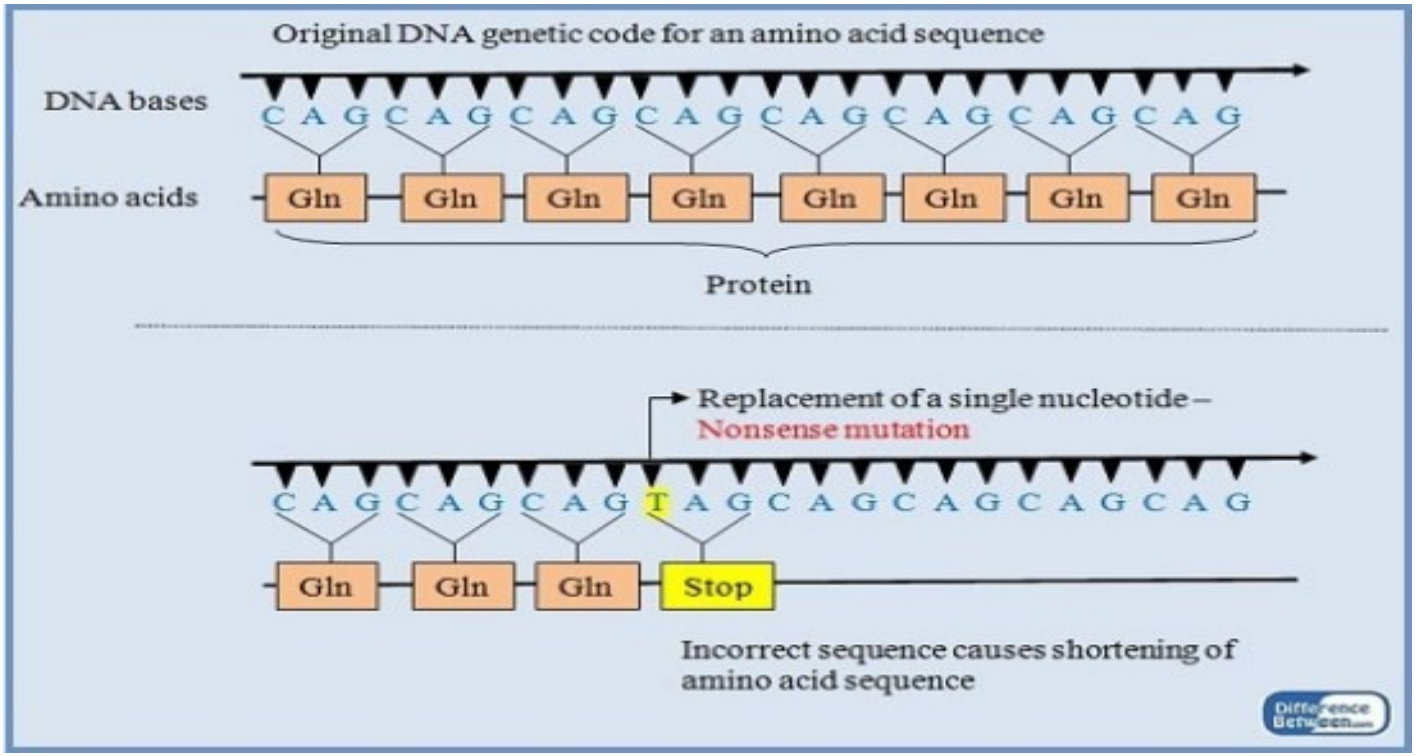
Adenine form hydrogen bonds with cytosine rather than thymine



Base substitution

- When an addition or deletion of amino acid occurs, the potential genomic outcomes are as follows:
- **Silent mutation**: The mutation changes the original codon into another codon that codes for the same amino acid
- **Missense mutation**: When a mutation in the sequence causes a codon to code for a different amino acid
- **Nonsense mutation**: A mutant “stop” codon replaces a wild-type codon, terminating translation resulting in a shortened protein.


Nonsense Mutations
ATG GAA GCA CGT
Met Glu Ala Gly
ATG TAA GCA CGT
Met STOP


Original DNA genetic code for an amino acid sequence
DNA bases
C A G C A G C A G C A G C A G C A G C A G C A G
Amino acids
Gln Gln Gln Gln Gln Gln Gln Gln
Protein
Replacement of a single nucleotide –
Nonsense mutation
C A G C A G C A G T A G C A G C A G C A G C A G
Gln Gln Gln Stop
Incorrect sequence causes shortening of amino acid sequence

II: **Frame shift mutation**

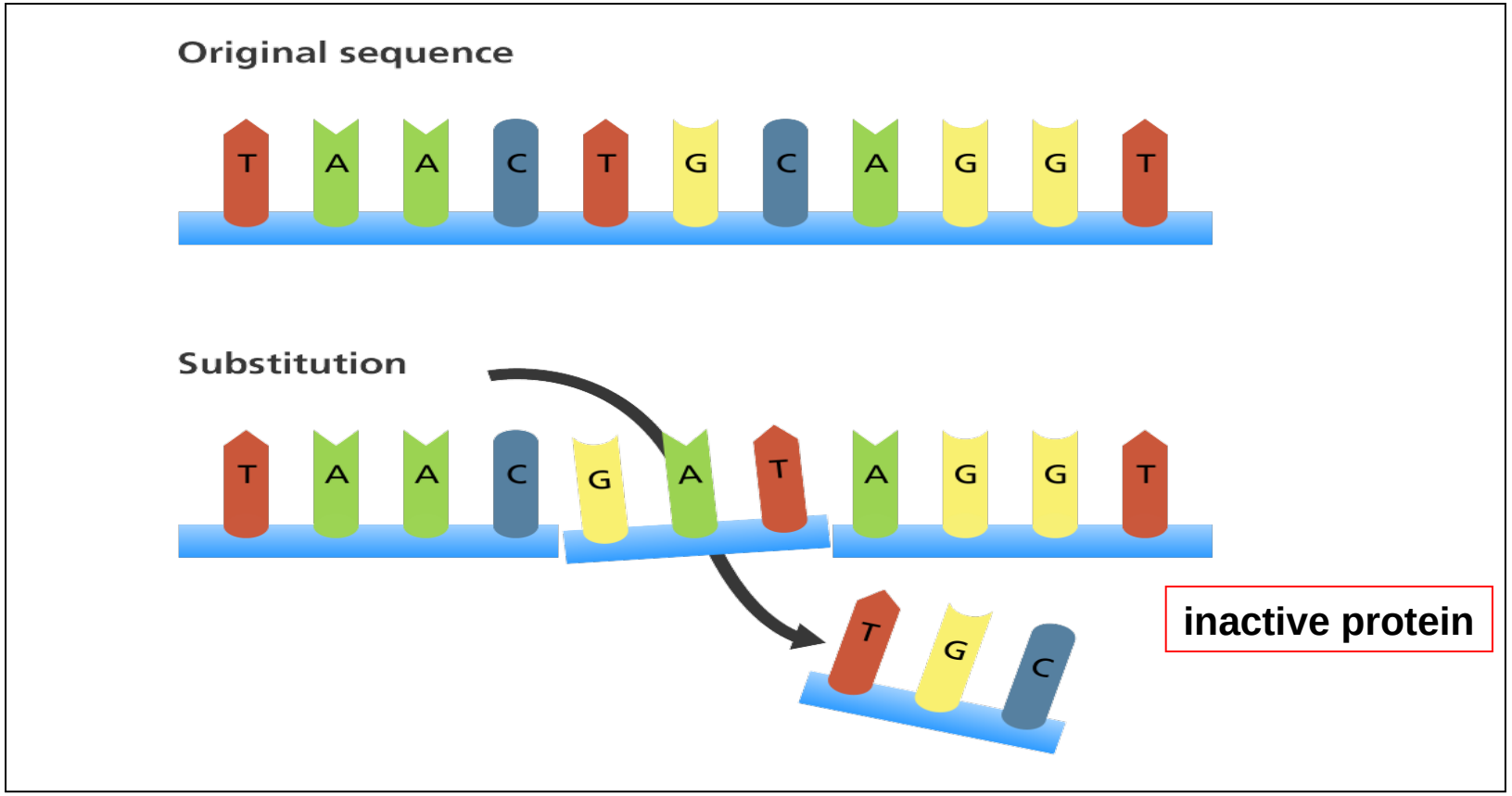
- In this one or more base pairs are added or deleted,
- It results in incorporation of wrong amino acids downstream (on ribosome) that leads to **inactive protein**.

1. **Substitution**
2. **Inversion**
3. **Insertion**
4. **Deletion**

Substitution

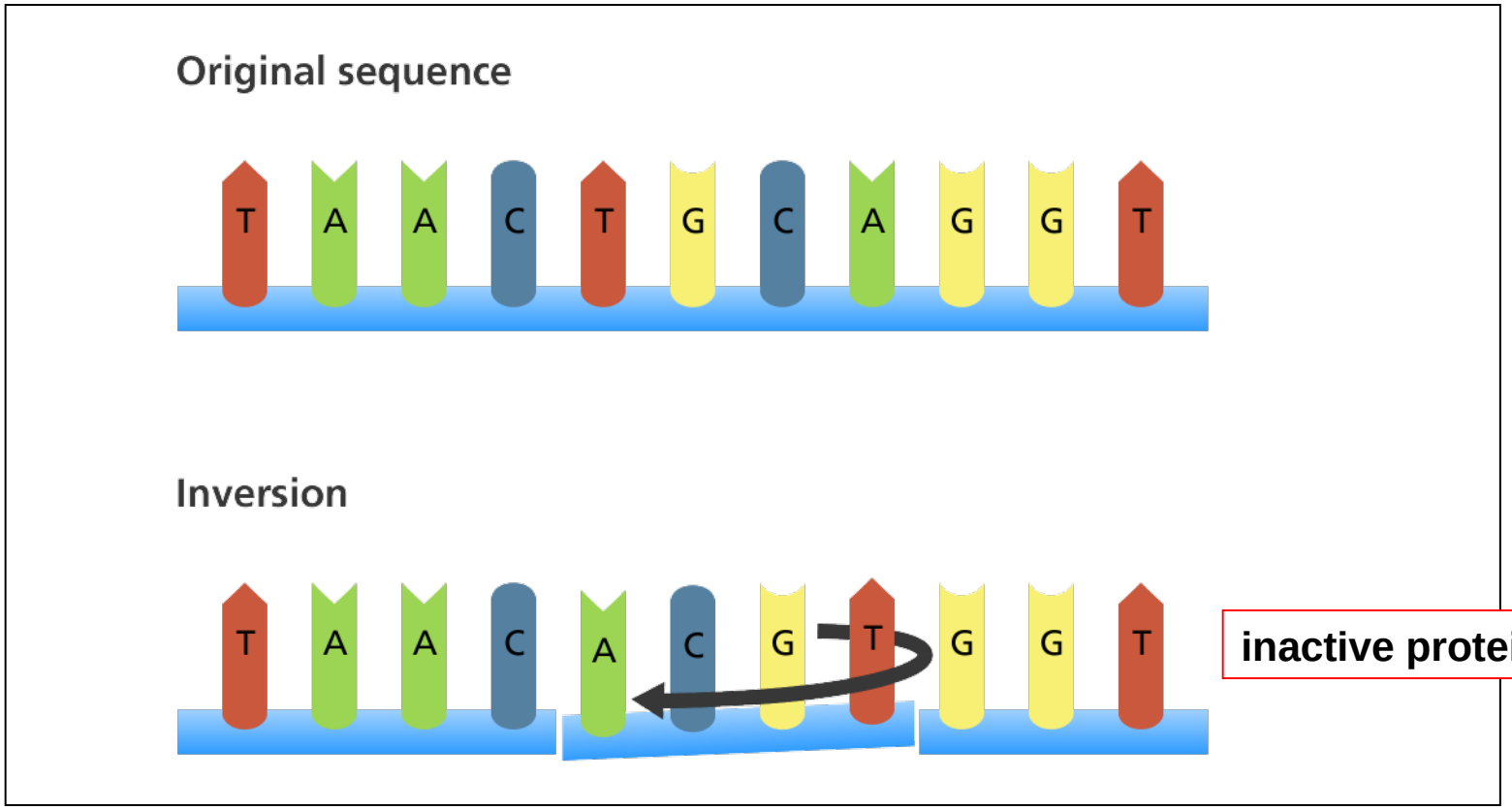
- when one or more bases in the sequence is replaced by the same number of bases

(for example, a cytosine substituted for an adenine).



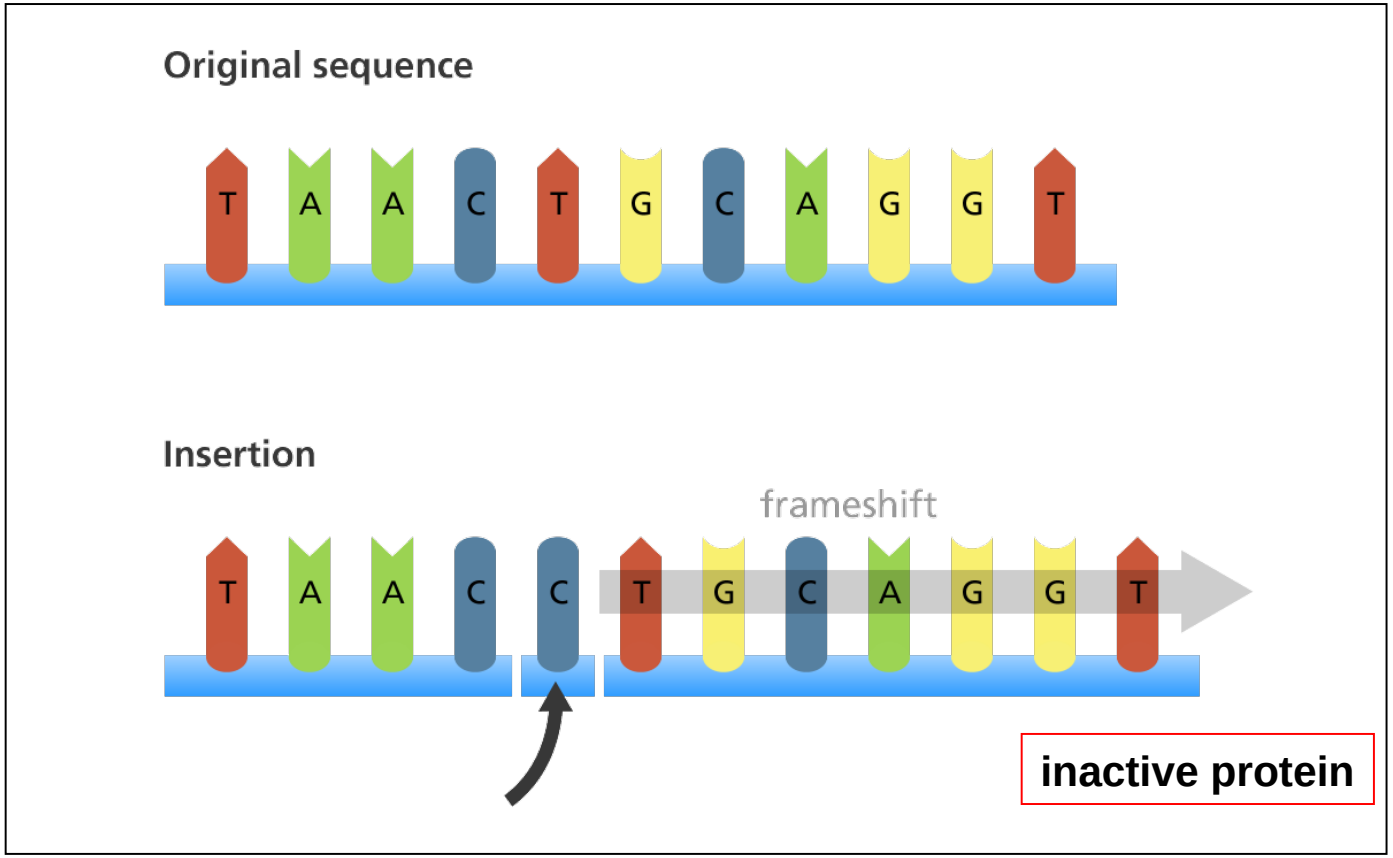
Inversion

- when a segment of a chromosome is reversed end to end.



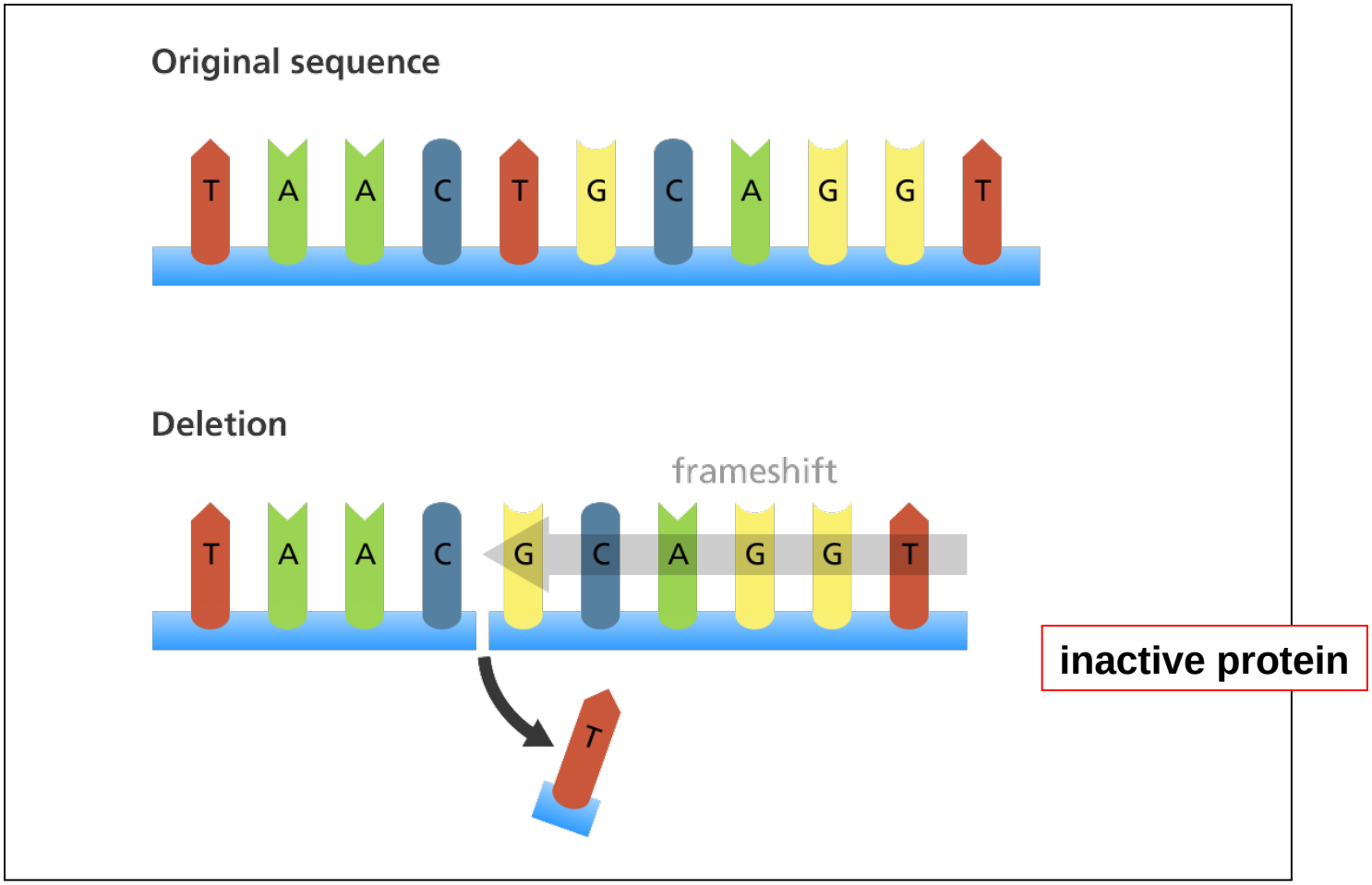
Insertion

- when a base is added to the sequence.



Deletion

- when a base is deleted from the sequence.



III: **Transposons or insertion sequence integration in DNA**

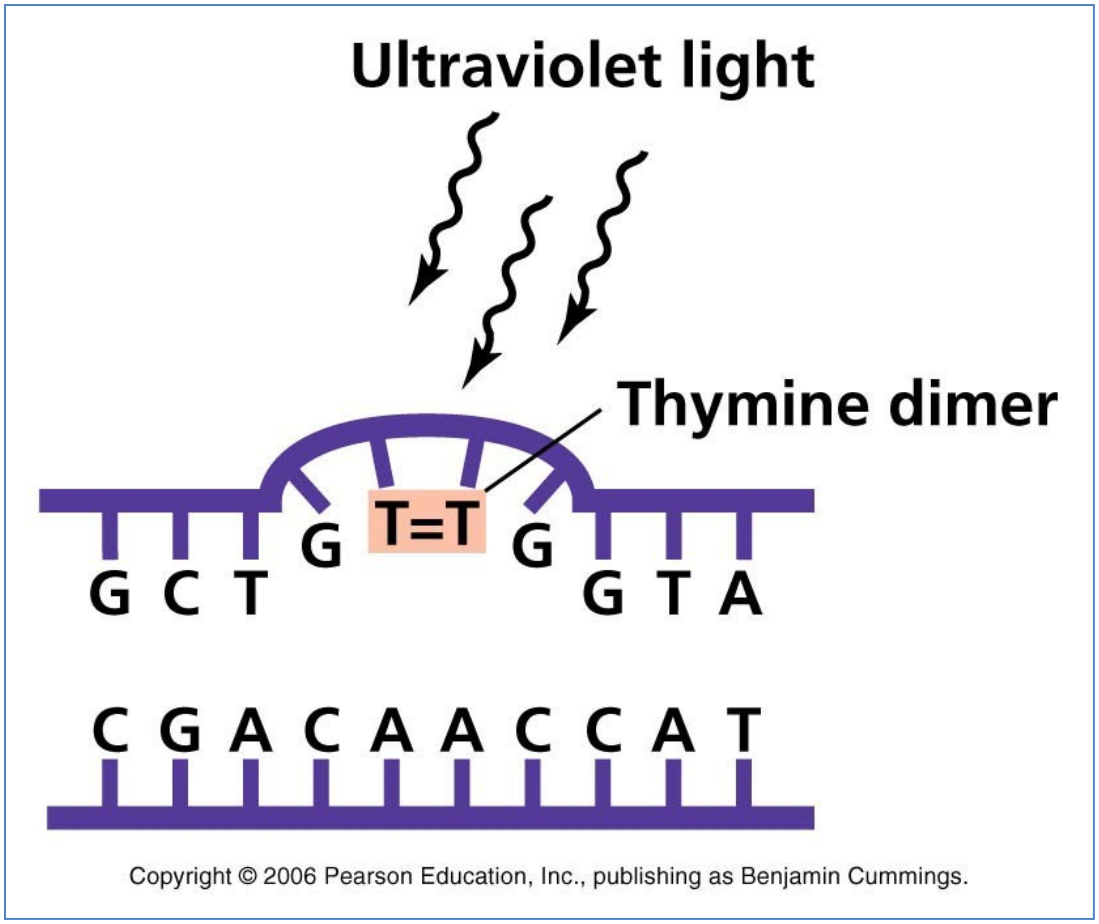
- It can cause profound changes in the genes.

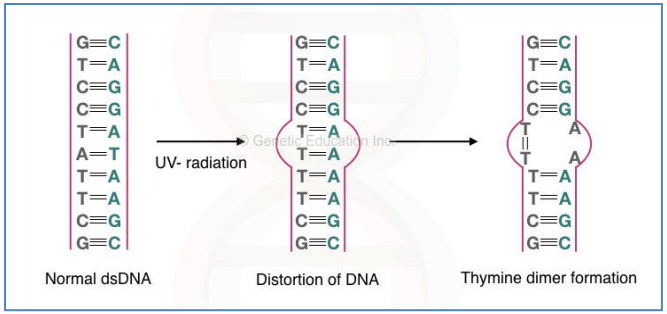
Induction of mutation

- Mutagens may be of physical, chemical, or biological origin.
- Mostly they act on the DNA directly, causing damage, which may result in errors during replication.

Physical Mutagens

- Physical mutagens include radiation or UV exposure.
- Non-ionizing radiations i.e. UV radiation damages DNA by creating covalent linkages between adjacent pyrimidine bases. This pyrimidine **dimer** inhibits replication and translation.
- Ionizing Radiations (e.g. X-rays & γ-rays) have much more energy and penetrating power than ultraviolet radiations; they ionize water and other molecules to form free radicals that can break DNA strands and alter purine and pyrimidine bases.





Chemical Mutagens

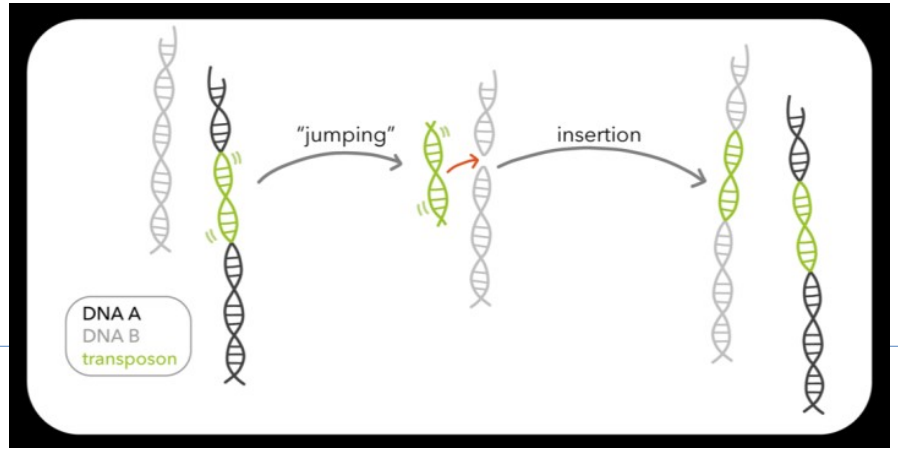
- Chemical mutagens directly or indirectly induce mutations by
  - replacing a base in DNA,
  - alter a base's composition and pairing behavior, or
  - damage the base so that it can no longer pair.

Chemical Mutagens

- **5-bromouracil**, causes point mutations.
- **Hydroxyl radicals** attack guanine, thereby producing 8-hydroxy-deoxyguanosine (8-OhdG), which mispairs with adenine instead of cytosine, which results in a (G -> T) transversion during replication.
- **Deaminating agents** produce an adenine species that pairs with cytosine and a cytosine species (uracil) that pairs with adenine.
- **Acridines like ethidium bromide** disrupts the reading frame during translation and can cause insertions or deletions.
- **Alkylating Agents** like ethyl methanesulfonate and dimethyl nitrosoguanidine lead to point mutations through base mispairing. However, alkylation can cause crosslink formation, which inhibits replication.

Biological Mutagens

- Biological agents of mutation are sources of DNA from elements like transposons and viruses.
- Transposons are sequences of DNA that can relocate and replicate autonomously. Insertion of a transposon into a DNA sequence can disrupt gene functionality.



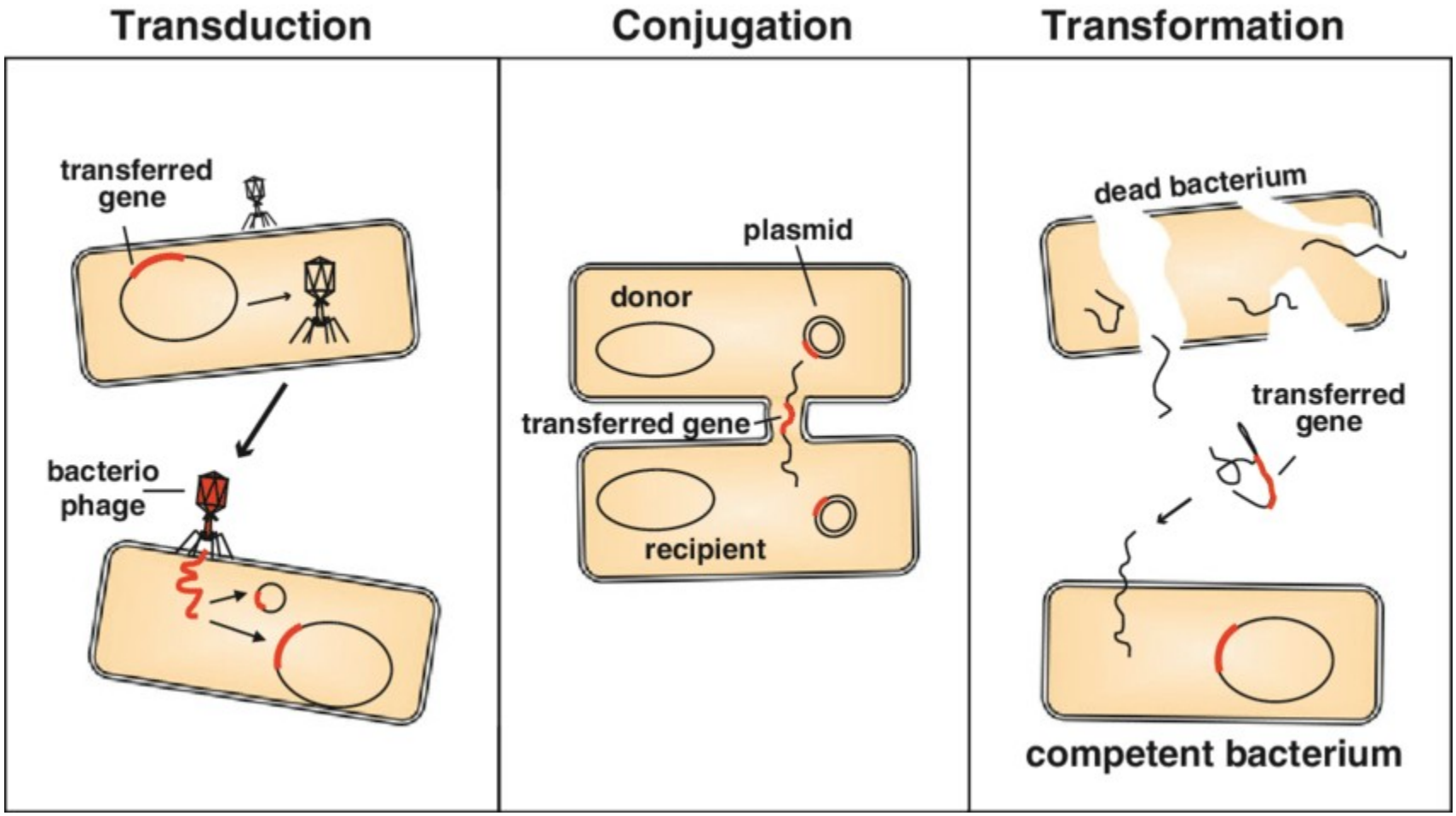
Results of mutations

- can produce changes in structural or colony characteristics or induce drug resistance.
- Some potential consequences of mutations are as follows:
  - Auxotrophs: mutation that dysfunctions an essential nutrient process.
  - Resistant mutants: can withstand the stress of exposure to inhibitory molecules or antibiotics secondary to acquired mutation.
  - Regulatory mutants have disruptions on regulatory sequences like promotor regions.
  - Constitutive mutants: continuously express genes that usually switch on and off as in operons.

Clinical Significance

- Resistance to antibiotic
  - Through genetic mutation
  - Acquisition of resistance from other bacteria
- Antibiotic resistance can also occur by incorporating resistance genes into plasmids, transposons, and integrons.
- These genes spread through horizontal transfer by conjugation, transformation, or transduction mechanisms.

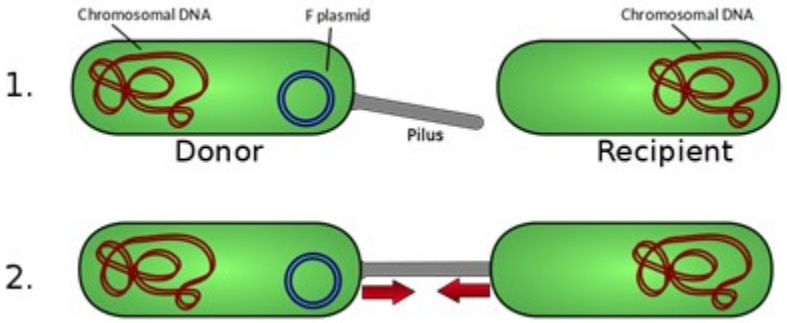
Methods of transfer of DNA

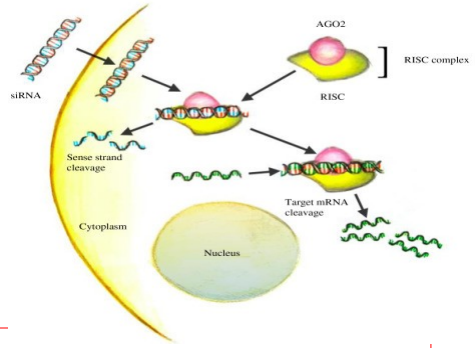


- **Transduction** is **the process by which a virus transfers genetic material from one bacterium to another**. When gene-carrying bacteriophages infect a new host cell, the DNA may be incorporated into the genome of the new host.

- **Transformation** is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material from its surroundings through the cell membrane(s).

- **<u>Bacterial conjugation</u>** is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells. This takes place through a pilus. The mating process is controlled by an **F (fertility) plasmid** (F factor), which carries the genes for the proteins required for conjugation. One of the most important proteins is pilin, which forms the **sex pilus** (conjugation tube).





- **Transfection** is a modern and powerful method used to artificially insert foreign nucleic acids (DNA or RNA) into eukaryotic cells utilizing non-viral means.
- The ability to modify host cells' genetic content enables the broad application of this process in studying normal cellular processes, molecular mechanism of disease and gene therapeutic effect.

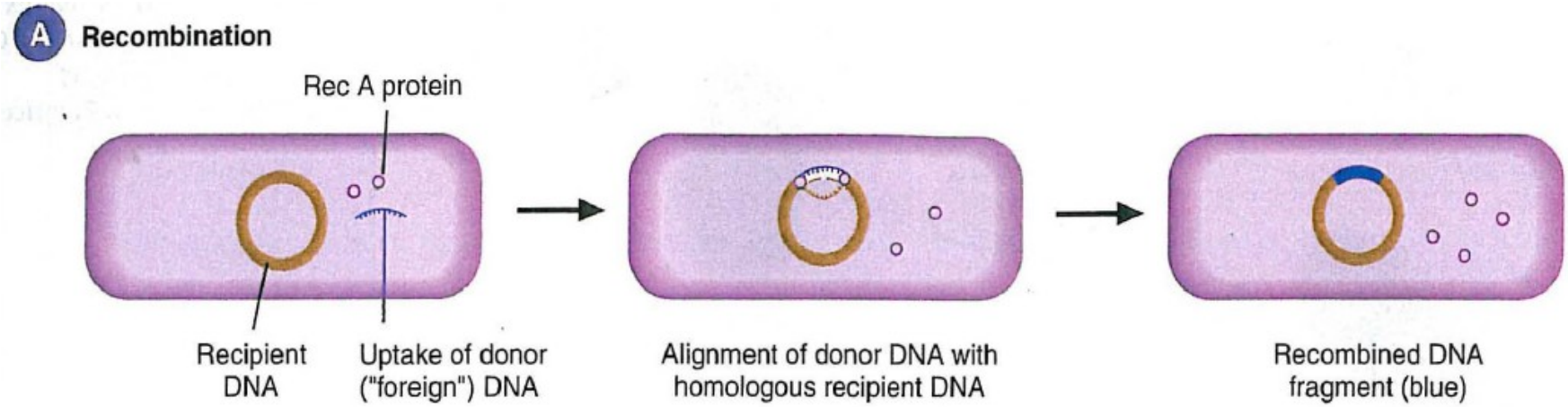
https://www.sciencedirect.com/topics/neuroscience/transfection

RECOMBINATION

- Once the DNA is transferred from the donor to the recipient cell by one of the above processes, it can integrate into the host cell chromosome by recombination.
- There are two types of recombination

  (1) **Homologous recombination,** in which two pieces of DNA that have extensive homologous regions pair up and exchange pieces by the processes of breakage and reunion.

  (2) **Nonhomologous recombination,** in which little, if any, homology is necessary.
- It is presumed that different enzymes are involved.


A Recombination
Rec A protein
Recipient DNA
Uptake of donor ("foreign") DNA
Alignment of donor DNA with homologous recipient DNA
Recombined DNA fragment (blue)

Homologous Recombination vs Site-Specific Recombination

More Information Online WWW.DIFFERENCEBETWEEN.COM

	Homologous Recombination	Site-Specific Recombination
DEFINITION	Homologous recombination is a type of genetic recombination in which genetic material is exchanged between two identical molecules of double-stranded or single-stranded nucleic acids such as DNA or RNA	Site-specific recombination is a type of genetic recombination in which DNA strand exchange takes place between DNA segments that possess at least a certain degree of sequence homology but no extensive homology
LOCATION OF RECOMBINATION	Occurs anywhere within the homology	Occurs only at specific sites
RECOMBINATION TAKES PLACE BETWEEN	Occurs between long DNA strands	Occurs between short DNA sequences
ENZYMES AND ENZYMATIC PATHWAYS	One or a small number of common enzymatic pathways are involved	Special enzymatic machinery such as one specific enzyme or enzyme system is involved
EXAMPLES	General recombination of eukaryotes in meiosis	Site specific recombination system that involves when bacteriophage integrates into a bacterial chromosome and rearrangement of immunoglobulin genes in vertebrate animals

Thank you