



PITHAPUR RAJAH'S GOVERNMENT COLLEGE

Autonomous and NAAC Re-accredited with 'A' Grade (3.17/4.00 CGPA)

Affiliated to Adikavi Nannaya University

Opp. Mc Laurin School, Raja Ram Mohan Roy Road, Kakinada 533001, Andhra Pradesh, India

E-mail: kakinada.jkc@gmail.com, Tel: 0884-2379480



DR. SUDHAKAR cheedi Msc.MPhil PHD

LECTURER IN MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY

P.R.G.C (A), KAKINADA

molecular biology and
microbial genetics

Unit-1 : DNA/RNA as genetic material ; replication of DNA

DNA (Deoxyribose nucleic acid)

- * It is a genetic material
- * Nitrogen bases are separated to electrophoresis then are get purines of pyrimidines

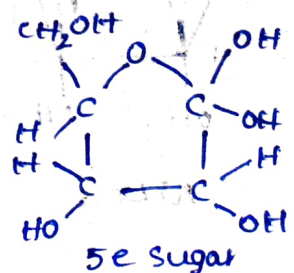
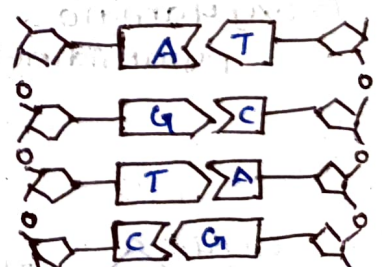
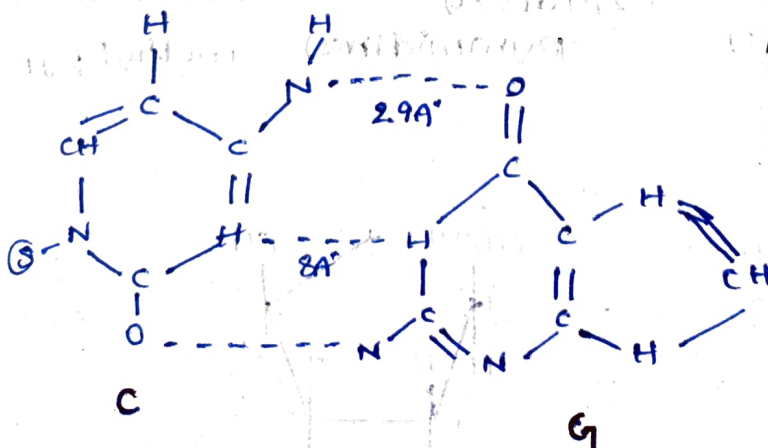
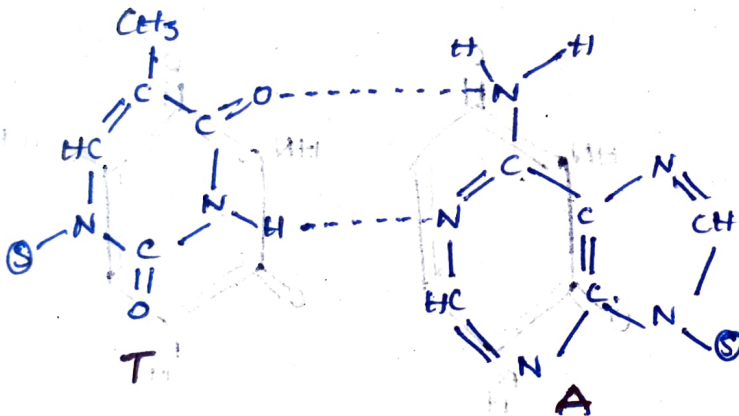
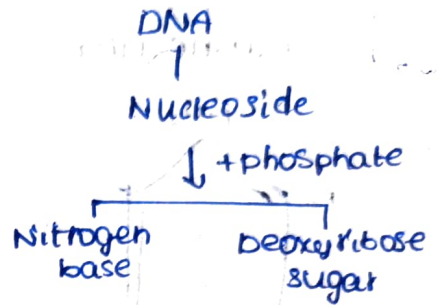
→ Purine →  adenine
guanine

→ Pyrimidines -  Thymine
Cytosine

→ $A \equiv T$ $G \equiv C$

→ Diameter of DNA is - $20 \text{ \AA}$

Each helix - $34 \text{ \AA}$



DNA as genetic material

(1) Deoxyribo nucleic acid (DNA):

J. Watson and F. Crick proposed a model for explaining the structure of DNA molecule.

structure of DNA:

- 1) DNA is the chemical basis of heredity.
- 2) DNA is composed of four deoxyribonucleotides: deoxyadenylate, deoxyguanylate, deoxycytidylate, and deoxythymidylate.
- 3) these units are combined through 3' — 5' phosphodiester bonds to polymerase into a long chain.
- 4) the nucleotide is formed by a combination of base + sugar + phosphoric acid.
- 5) the 3' - hydroxyl of one sugar is combined to the 5' - hydroxyl of another sugar through a phosphate group.
- 6) In DNA molecules the concentration of adenosine (A) nucleotides equal that of thymidine (T) nucleotides ($A = T$).
- 7) the concentration of thymine (G) nucleotides equals that of cytosine (C) nucleotides ($G = C$).

RNA as genetic material

(2) Ribonucleic acid (RNA)

Ribonucleic acid is a polymer of ribonucleotide of adenine, uracil, guanine and cytosine joined together by 3'-5' phosphodiester bonds. thymine is absent in RNA. RNA is found in the nucleolus, Nissi granules, ribosomes,

mitochondria and cytoplasm. the pentose sugar of the nucleotide is D-ribose.

Structure of RNA:

- 1) RNA is single strand and having ribose sugar, nitrogenous bases and phosphoric acid.
- 2) there are three forms of RNA in the cell.

(i) m-RNA

(ii) t-RNA

(iii) r-RNA

Experimental evidences that established DNA and RNA as genetic material:

Exp-1: Frederick Griffith - Bacterial transformation

In 1928, British bacteriologist Frederick Griffith conducted a series of experiments using *Streptococcus pneumoniae* bacteria and mice.

Griffith was not trying to identify the genetic material, but rather, trying to develop a vaccine against pneumonia. they are used two related strains of bacteria.

R-strain: When grown in a petri dish, the R bacteria formed colonies or clumps of related bacteria, that had well-defined edges and a rough appearance. the R bacteria, that had were non virulent, meaning that they did not cause sickness when injected into a mouse.

S Strain: S bacteria formed colonies that were rounded and smooth, the smooth appearance was due to a polysaccharide, or sugar-based, coat produced by the bacteria. this coat protected the S bacteria from the mouse immune system, making them virulent (capable of causing disease).

In this experiment.

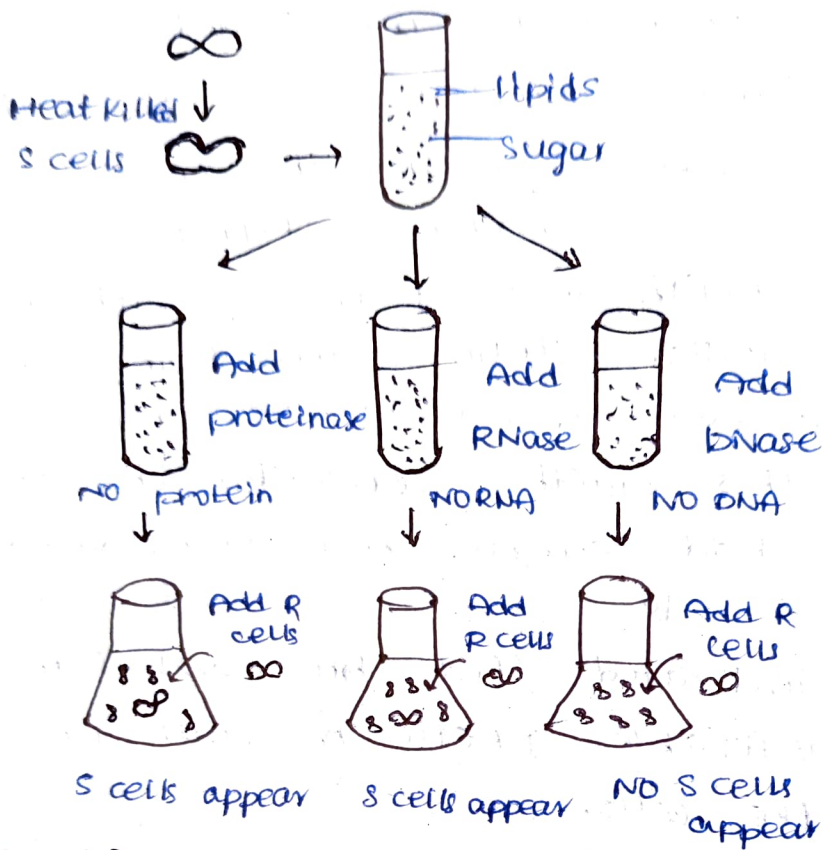
- * When Griffith infected mice with the non-virulent bacteria (strain R), the mice survived.
- * When Griffith infected mice with the virulent bacteria (strain S) the mice died.
- * When Griffith infected mice with heat-killed virulent bacteria (strain S) the mice survived as the bacteria had been killed.
- * When Griffith infected mice with a mix of heat-killed strain S and living strain R, the mice were found to have died.
- * From this Griffith concluded that the living R cells had somehow been transformed into virulent S cells.
- * This indicated that there was some form of transferable genetic material present within the cells.

Exp-2: Avery, McCarty and McCleod: identifying the transforming principle

Later Oswald Avery, Colin Macleod and Maclyn McCarty in 1944 repeated Griffith's experiments in an 'in vitro' system in order to identify the nature of the transforming substance responsible for converting a non-virulent strain into virulent strain.

They observed that DNA, RNA and proteins isolated from the heat killed S-strain when added to R-strain changed their surface character from rough to smooth and also made them pathogenic. But when the extract was treated with DNase the transforming ability

was lost. RNase and protease did not affect the transformation. this phenomenon of R strain changing into S strain is referred to as transformation.



Exp-3: the Hershey and chase experiment

The scientists believed that the genetic material was protein in 1952, hersey & chase were the ones to conclusively prove that DNA is the genetic material. Hershey & chase used bacteriophage to experiment.

labelling: some viruses were grown on a medium containing radioactive phosphorus.

Viruses: Grown on radioactive phosphorus have radioactive DNA but not protein since DNA contains phosphorus but protein does not

Infection: Each batch of phage was used to infect a different culture of bacteria. the were centrifuged by

Proof of semiconservative replication Meselson and Stahl cracked experiment

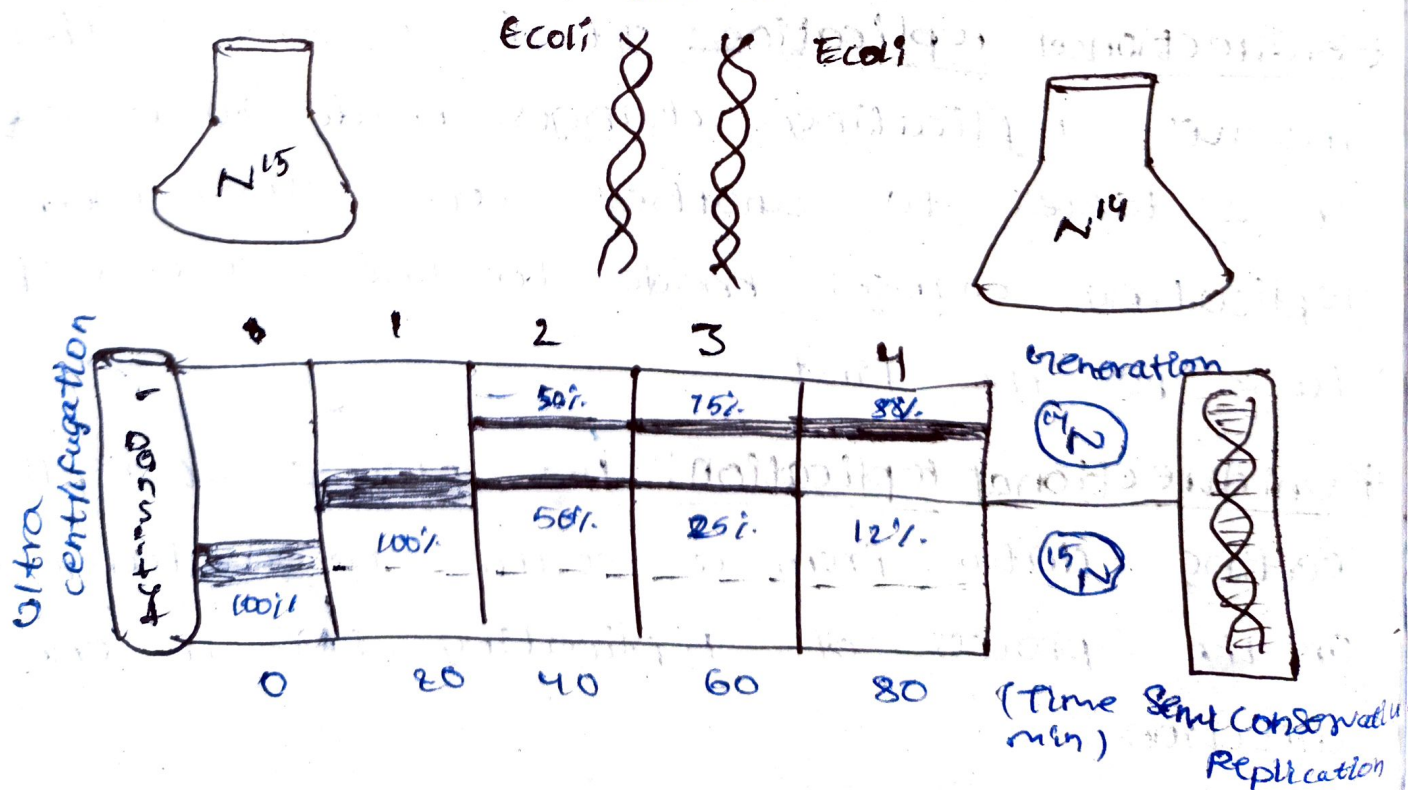
DNA replication using *E. coli* bacteria model system

In the year 1957 gave experimental evidence

the experiment done by Meselson and Stahl demonstrated that DNA replicated semi-conservatively, meaning that each strand in a DNA molecule serves as a template for synthesis of a new, complementary strand.

Although Meselson and Stahl their experiment in the bacterium *E. coli* we know that semi-conservative DNA replication is a universal mechanism shared by all organisms

14 generations of growth



Enzymes involved in DNA replication:

DNA polymerase

this is

DNA Replication:

DNA replication is the process of producing two identical copies of DNA from one original DNA molecule.

Enzymes used in DNA replication

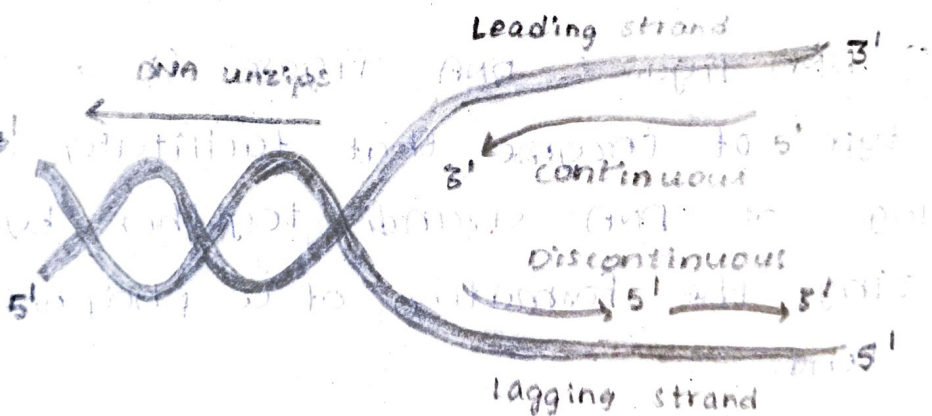
- (1) Nucleases: A nuclease is an enzyme that can cleave the phosphodiester bonds present in between the nucleotides.
- (2) DNA polymerase: DNA polymerase are the enzyme that is responsible for adding new nucleotides and synthesizing a new strand of DNA by taking the old fragmented strand as a template.
- (3) DNA ligase: DNA ligase is a specific type of enzyme that facilitates the joining of DNA strands together by catalyzing the formation of a phosphodiester bond.
- (4) DNA helicase: DNA helicase is a motor protein that move directionally along a nucleic acid phosphodiester backbone separating two nucleotides of DNA molecule.
- (5) Single strand binding proteins: the single strand binding (SSB) protein are DNA binding proteins, that binds to single stranded DNA to facilitate DNA replication.

steps in DNA Replication

(B) formation of replication fork.

(i) Leading strand: the leading strand is the strand of nascent DNA which is synthesized in the same direction as the growing replication fork. this sort of DNA replication is continuous.

(ii) Lagging strand: the lagging strand is the strand of nascent DNA whose direction of synthesis is opposite to the direction of the growing replication fork. Because of its orientation, replication of the lagging strand is more complicated as compared to that of the leading strand.



Initiation:

For the replication to begin there is a particular region called the origin of replication. This is the point where the replication begins with the spotting of this origin followed by the unwinding of the two DNA strands.

unzipping of DNA strand in their entire length is not feasible due to high energy

input. Hence, first a replication fork is created catalysed by the helicase enzyme, which unzips the DNA strand.

Elongation:

Strands are separated, the polymerase enzymes start synthesising the complementary sequence in each of the strands. The parental strands will act as a template for newly synthesising daughter strands.

Elongation is unidirectional, DNA is always polymerised only in the $5'$ to $3'$ direction. Therefore, one strand ($3' \rightarrow 5'$) it is continuous, hence called continuous replication. Other strand ($5' \rightarrow 3'$) it is discontinuous replication. They occur as fragments called Okazaki fragments - the enzyme called DNA ligase joins them later.

Termination:

Termination of replication occurs in different ways in different organisms.

In E. coli like organisms, chromosomes are circular. and this happens when the two replication forks between the two terminals meet each other. Forms the synthesised new DNA strands.

Applications

Medical applications.

* Genetic testing

* Gene therapy

Biotechnology applications

* DNA cloning

* Genetic engineering

* Forensic Analysis

Extra chromosomal genetic elements

they are plasmids and transposons

Plasmids

- plasmids are small circular DNA fragments double-stranded, self replicating extra chromosomal DNA molecules that are maintained in a stable and characteristic number of copies Joshua Lederberg in 1952.

* physical nature of plasmids is quite simple.

* they are small double stranded DNA molecules.

eg: *Streptomyces* spp and *Borrelia* spp.

* Most of the plasmid DNA isolated from bacterial exist in the supercoil configuration.

* Some plasmids are present in cell in only 1-3 copies by interaction between the host and the plasmid.

* *Borrelia burgdorferi* that causes Lyme disease.

* plasmid is not accommodated and is lost during subsequent replication.

Structure of plasmids:

* Origin of replication in plasmids the specific location at A=T rich region

* selectable marker site: of antibiotic resistance genes.

* Promoter region the transcriptional machinery is loaded.

* primer binding site: single strand DNA which is useful in DNA amplification or DNA sequencing.

Unit-11 : Concept of Gene , Transcription

- (1) The term 'gene' was first coined by Johansson in 1907.
- (2) Hugo Devries postulated one gene one character hypothesis which holds that a particular character of an individual is controlled by a specific chromosome which appears as series of granules on the chromosome might be the genes.

Presently, the genes are regarded as specific DNA segments capable of producing a definite cell product, consisting a mutable sites which can exist in several forms and capable of self duplication.

- (3) The concept of gene was proposed by Sutton in 1902, the term gene was first used by Johannsen in 1909, earlier, it was referred to as Mendelian factors.

Genes are located in the chromosome where these are arranged in a single linear order genes are ~~ordinarily~~ ^{originarily} transmitted from one generation to the next one. Each gene has a specific position in a specific chromosome. this position is called locus.

Classical Definition of Gene: Gene is the unit of function (one gene specifies one characters), recombination and mutation.

Modern concept of gene:

Gene is the unit of genetic information the sequence of DNA that specifies one polypeptide. It includes coding as well as non-coding regulatory sequences.

Year	Scientist	Gene concept
1866	G. J. Mendel	A unit factor that controls specific phenotypic trait.
1902	Sir A. E. H. H. H. H.	One gene - one metabolic block theory
1940	Beadle & Tatum	One gene - one enzyme theory
1957	U. H. Ingram	One gene one polypeptide theory
1960	C. Yanofsky & co-workers	Gene is a unit of recombination
Early 1970's	E. B. Lewis	Complementation test in Drosophila

Units of a gene:

A gene can be defined as a polynucleotide chain that consists of segments each controlling a particular trait.

Genes are considered as a unit of function (Lison) a unit of mutation (mutant) and a unit of recombination (recon).

Cistron:

* one gene - one - enzyme hypothesis of Beadle and Tatum was defined by several works in coming years. A single mRNA is transcribed by a single gene.

* A single mRNA is also transcribed by more than one gene and it is said to be polycistronic.

* the concept has been given as one - gene - one protein hypothesis. the proteins are the polypeptide chain of aminoacids translated by mRNA.

* cenes are present within the chromosome and their cis-trans effect govern the function.

* S-Benzer termed the functional gene as cistron.

Recon:

* the smallest unit capable of undergoing recombination is known as recon.

* Benzer (1955) found that the cultures of T₄ bacteriophage formed plaques on agar plates of E. coli. Normally T₄ found small plaques of smooth edges, whereas the mutant phage formed the larger plaques of rough edges.

* The smallest segment of DNA capable of being separated and exchange with other chromosome is called recon.

MUTON:

- * The smallest unit of chromosome that undergoes mutational change. the small unit of DNA which may be changed is the nucleotide.
- * If the size of a recon is equal to muton.
- * Usually a single-nucleotide pair, but can contain 3 to 4 nucleotides.
- * It is the smallest unit of DNA which can undergo Mutation.
- * the mutation and leads to production of mutant phenotype.

One gene one enzyme hypothesis:

The one gene one enzyme hypothesis, proposed by Beadle and Tatum, states that each gene is responsible for producing a single enzyme. this concept was later refined to "one gene one polypeptide" as it was discovered that some proteins are composed of multiple polypeptide chains, each coded by a separate gene.

One gene one polypeptide hypothesis:

The one gene one polypeptide hypothesis refines the earlier "one gene one enzyme concept". It states that each gene directs the synthesis of a single polypeptide chain, a component of a protein. This is more accurate.

of multiple polypeptide chains, each from a different gene.

one gene one product hypothesis:

The hypothesis is a modern extension of the earlier one gene - one enzyme / polypeptide concepts. It states that one gene codes for one final functional product. This product can be a polypeptide chain (which may or may not be an enzyme), an RNA molecule (like tRNA or rRNA) or any other functional molecule directly transcribed from the gene. It acknowledges that not all genes code for proteins.

Open Reading Frame (ORF):

It is a continuous stretch of nucleotides in a DNA or RNA sequence that has the potential to be translated into a protein. It begins with a start codon, typically "AUG" in RNA (which codes for the amino-acid methionine), and ends with a stop codon, such as "UAA", "UAG" or "UGA". These codons signal the beginning and termination of protein synthesis, respectively.

ORF's are important for gene expression because they indicate the regions of the genome that can be translated into functional proteins. The sequence between the start and stop codon is considered the coding

dictate the structure and properties of the protein.

(1) Structural genes

* Structural genes are those that code for proteins or RNA molecules that have a direct role in the organism's structure or function.

* They provide the blueprint for the synthesis of enzymes, structural proteins and other molecules necessary for the cell's structure and functioning.

Example: Genes coding for enzymes, cytoskeletal proteins etc.

(2) Constitutive genes:

* These are the genes that are always expressed at a constant level, regardless of external signals or environmental conditions.

* These genes are essential for basic cellular functions and are constantly active.

Example: Genes encoding for house keeping proteins like Ribosomal RNA (rRNA) or enzymes involved in basic metabolic processes [eg: glycolysis].

(3) Regulatory genes:

* Regulatory genes are involved in controlling

the expression of other genes. They produce regulatory proteins (eg: transcription factors) that can either promote or inhibit the transcription of specific target genes.

* They regulate the timing, location and amount of gene expression, thus controlling various cellular processes like differentiation, development and responses to stimuli.

Example: genes that produce transcription factors, repressors, or activators that regulate the expression of structural genes.

(4) Uninterrupted genes:

* These genes are genes that consist of a continuous sequence of coding regions (exons) without interruptions like introns.

* The entire gene is translated into protein without the need for splicing, making the gene simpler in terms of its expression.

Example: Found in prokaryotes (eg: bacterial genes) where genes typically do not contain introns.

(5) Split genes:

* These genes that have both coding (exons) and non-coding (introns) regions.

* The exons are separated by introns and the introns are removed during RNA processing before the gene can be translated into proteins.

* This structure allows for alternative splicing, where different combinations of exons

Transcription:

To produce a single-stranded RNA copy (mRNA) of a gene to carry genetic information for protein synthesis.

Replication:

It is to create an exact copy of the entire genome (DNA) to ensure genetic material is passed to the next generation.

Promoter:

It is a specific sequence of DNA located upstream of a gene that signals the start of transcription. It is recognized by RNA polymerase and its associated sigma factor.

RNA polymerase:

It is an enzyme responsible for synthesizing RNA from a DNA template during transcription.

In prokaryotes, RNA polymerase consists of a core enzyme and a sigma factor, which is responsible for recognizing the Promoter region.

It reads the DNA template strand and synthesizes a complementary RNA strand in the 5' to 3' direction.

Mechanism of transcription:

(1) Initiation

(2) Elongation

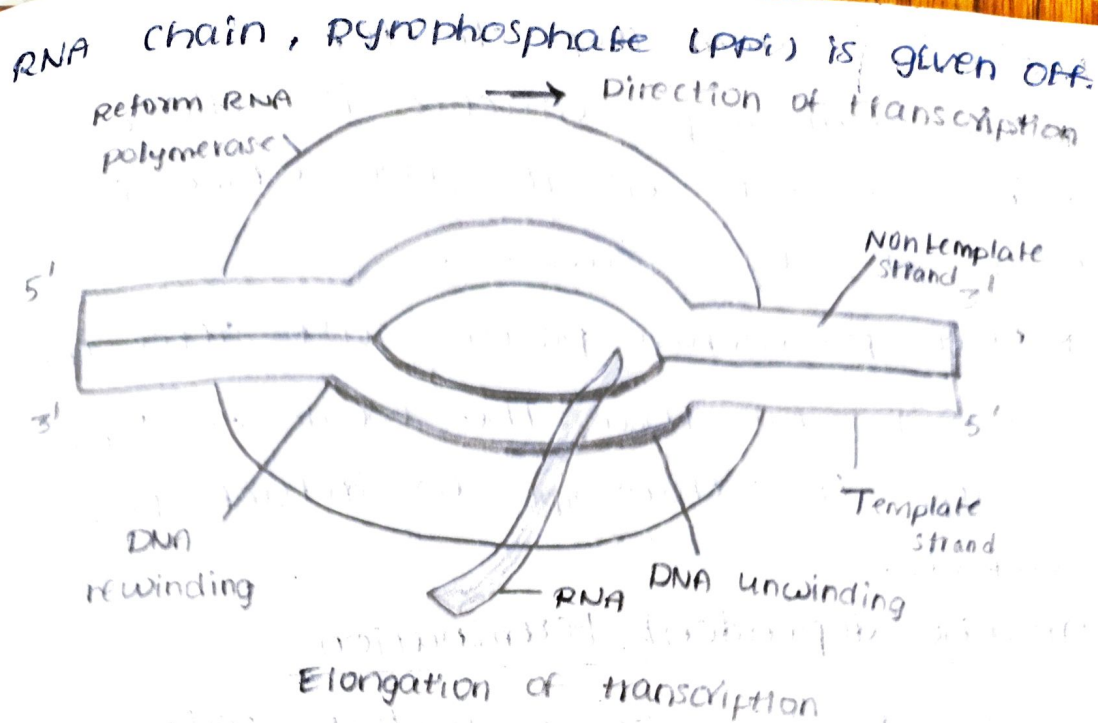
13) Termination

Initiation:

- 1) The RNA polymerase binds to the promoter region of the DNA
- 2) The enzyme covers about 60 base pairs of the promoter including the -10 and -35 boxes.
- 3) The σ subunit then dissociates from the open promoter complex leaving the core enzyme.

Elongation:

- 1) RNA polymerase moves along the DNA molecule.
- 2) The enzyme adds ribonucleotides to the 3' end of the growing RNA molecule.
- 3) During transcription only a small portion of the double helix is unwound at any point of time.
- 4) It extends over 12-17 base. this imposes strain on the DNA molecule.
- 5) The RNA is released from the template DNA as it is synthesized allowing the DNA double helix to reform RNA
- 6) It takes place by addition of activated ribonucleoside triphosphates (ATP, UTP, GTP and CTP) to one strand of the DNA template.



Termination:

- 1) Transcription terminates when RNA polymerase reaches a terminator sequence on the DNA, signalling the end of the gene.
- 2) the RNA polymerase dissociates from the DNA and the newly synthesized mRNA is released.

Termination can be of two types.

- i, Rho independent termination
- ii, Rho dependent termination

(i) Rho independent termination:

- * In E. coli, termination occurs at sequence known as palindromes.
- * The first half of the sequence is followed by its exact complement in the second half.
- * In single-stranded RNA molecule, this allows the feature of first half of the sequence to base pair with second half to form it.

Unit - III : Translation and regulation of gene expression

Genetic code: Genetic code is the sequence of nucleotide in a gene coded in triplets. it determines the sequences of aminoacids in protein synthesis.

The genetic code is proposed by F.H.Crick. It holds the existence of a triplet code i.e. the sequence of three nitrogenous bases, codes for one aminoacid.

characteristics of genetic code:

- (1) Genetic code is a triplet.
- (2) The code is degenerate i.e. more than one code for an aminoacid.
- (3) The code is non-overlapping i.e. the same letter is not used for 2 different codons.
- (4) The code is non-ambiguous i.e. a particular codon will always code for the same aminoacid.
- (5) The code is commaless - No punctuations are needed between any 2 codons i.e. after one aminoacid is coded, the 2nd aminoacid will be automatically coded by the 3 letters.
- (6) The code is universal - In all living organisms the same genetic code is used.
Eg: UUU codes for phenylalanine in bacteria it will code for the same aminoacid in man or higher plants

(7) Codons are carried by messenger RNA to the ribosomes.

(8) Out of 64 codons, 61 of which code for amino acids and three for chain termination.

(9) The anticodons are the complementary to the bases in a transfer RNA that are complementary to the bases of a specific code in a messenger RNA.

(10) Genetic code was cracked by Khorana & Nirenberg.

START CODON :- (AUG)

The codon AUG (adenine - uracil - guanine) serves as the start codon, signaling the beginning of protein synthesis. It codes for the amino acid methionine in eukaryotes (formylmethionine in prokaryotes).

Triplet codon:

A codon is made up of three nucleotides each, derived from the four nucleotides in RNA (adenine, cytosine, guanine, uracil). This triplet is the basic unit that encodes genetic information.

Initiation codon:

The codon AUG codes for amino acid methionine. This is positioned first in a cistron & initiates the synthesis of a polypeptide chain initiation codon.

Non-sense
The codons UAA, UAG and UGA do not code for any amino acid. Hence there are known as non-sense codons. These are positioned at the end of a cistron and are utilized to stop synthesis, hence these are also known as termination codons.

Degenerate codes:

These are 64 possible codons in a triplet code of which 61 have shown to code for amino acids. Since only 20 amino acids take part in protein synthesis. It is obvious that there are many more codons than amino acid types except for tryptophan and methionine, which have a single code each.

Wobble Hypothesis:

There are more than one codon for one amino acid. This is called degeneracy of genetic code. To explain the possible cause of degeneracy of codons, in 1966, Francis Crick proposed the wobble hypothesis.

According to the wobble hypothesis, only the first two bases of the codon have a precise pairing with the bases of the anticodon of tRNA, while the pairing between the third bases of codon and anticodon may wobble.

correct amino acid is linked to its matching tRNA.

Mechanism of initiation of polypeptide synthesis:

- (1) The small ribosomal subunit binds to the mRNA, facilitated by initiation factors.
- (2) The initiator tRNA, carrying methionine (in eukaryotes) (or) formylmethionine (in prokaryotes) binds to the start codon (AUG) of the mRNA.
- (3) The large ribosomal subunit then joins the small subunit, forming the complete ribosome.
- (4) This results in the initiation complex, where the tRNA is positioned in the P site of the ribosome.

Mechanism of elongation of polypeptide chains:

The elongation of the polypeptide chain involves three main steps:

Codon recognition:

The aminoacyl tRNA, carrying the correct amino acid, binds to the A site of the ribosome, matching its anticodon with the mRNA codon.

Peptide bond formation:

The enzyme peptidyl transferase in the ribosome catalyzes the formation of a peptide bond between the amino acid in the P site and the one in the A site.

Translocation

The ribosome moves along the mRNA, shifting the tRNA from the A site to the P site, and the empty tRNA moves to the E site, where it is released.

Mechanism of termination of polypeptide synthesis:

- * Termination occurs when the ribosome reaches a stop codon (UAA, UAG or UGA) on the mRNA.
- * Release factors bind to the stop codon, prompting the release of the polypeptide chain from the tRNA in the P site.
- * The ribosome dissociates, releasing the newly synthesized polypeptide and the mRNA.

Inhibitors of protein synthesis:

Several substances can inhibit protein synthesis at various stages, these include:

1) Chloramphenicol:

Inhibits peptidyl transferase activity in prokaryotic and eukaryotic ribosomes.

2) Tetracycline:

Blocks the attachment of aminoacyl tRNA to the ribosome's A site, inhibiting elongation.

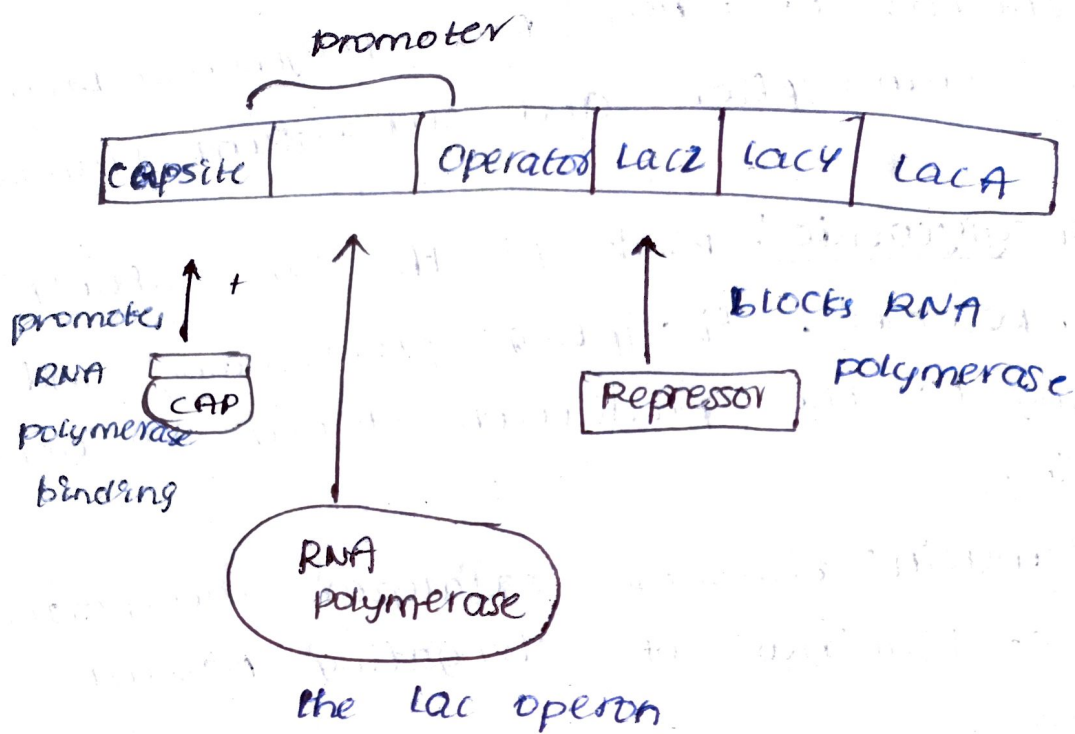
3) Erythromycin:

Prevents translocation by binding to the ribosome.

4) Streptomycin:

causes misreading of the mRNA in prokaryotes, leading to incorrect amino acid

Control the expression of these genes



(1) Structural Genes

"Lac Z": Encodes "B-galactosidase" an enzyme that breaks down lactose into glucose and galactose.

"Lac Y": Encodes "Lactose permease" a protein that transports lactose into the cell.

"Lac A": Encodes "thiogalactoside transacetylase", which is involved in detoxifying certain by products of lactose metabolism.

(2) Regulatory elements

Promoter (P)

A region where RNA polymerase binds to initiate transcription of the lac operon.

Operator (O)

A regulatory DNA sequence located between the promoter and the lac genes.

Unit - IV : Mutations and DNA repair

Mutations:

- (1) Mutation refer to sudden heritable change in the phenotype of an individual.
- (2) In molecular terms, mutations is defined as the permanent and relatively rare change in the number or sequence of nucleotides.
- (3) Mutation was first discovered by wright in 1791. Later mutations was reported (or) coined by Hugode wies in 1900, in *Drosophila*.

Importance:

- (1) Mutations are responsible for sustenance of life.
- (2) It is the source of all genetic variation, thus the raw material of evolution.
- (3) Mutations have detrimental effects and mutation is the source of many human diseases and disorders.

classification of mutations:

- (1) In multicellular organisms, mutations categorizes, into
 - (1) somatic mutations
 - (2) germline mutations

Somatic mutations:

Mutations that are in the somatic tissues of the body. Mutations are not transmitted to progeny. the extent of the

phenotypic effect depends upon whether the mutation is dominant or recessive.

Germline mutation:

These mutations arise in cells that ultimately produce gametes. A germline mutation can be passed to future generations, producing individual organisms that carry the mutation in all their somatic and germline cells.

Spontaneous mutation:

The spontaneous mutations occur suddenly in the nature and their origin is unknown. They are also called "background mutation" and have been reported in many organisms such as maize, bread molds, microorganisms (bacteria and viruses), *Drosophila*, mice, man, etc.

Induced mutations:

The mutations can be induced artificially in the living organisms by exposing them to abnormal environment such as radiation, certain physical conditions (i.e. temperature) and chemicals.

Physical mutagens:

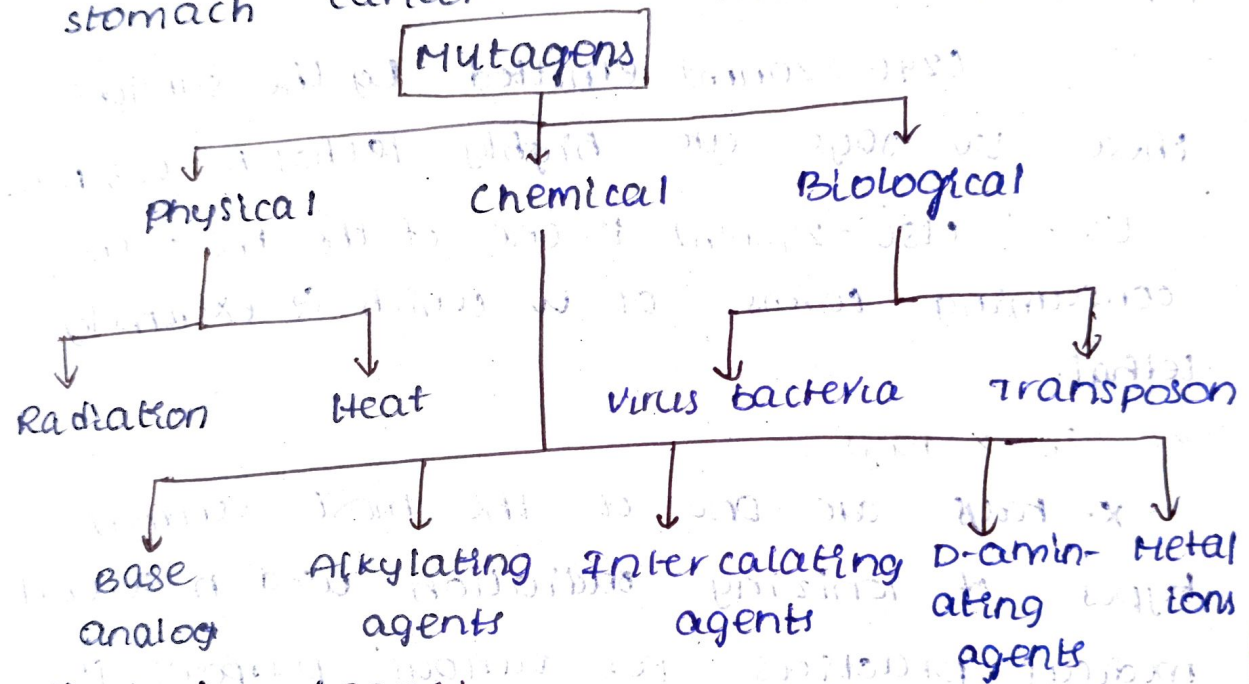
These include ionizing radiation, such as x-rays, gamma rays and alpha particles. Ultra violet radiation can also behave as potential mutagens.

chemical mutagens:

Elements such as arsenic, nickel and chromium are considered to be mutagens. Some organic compounds like benzene are also considered to be mutagenic in nature.

Biological mutagens:

It involves transposons and viruses further. more, certain bacteria such as *Helicobacter pylori* can increase the risk of developing stomach cancer.



Physical mutagens:

(1) Radiations as mutagens:

Radiation is the most important among the physical mutagens. Radiations damaging the DNA molecules fall in the wavelength range below 340nm and photon energy above 1 electron-volt (eV). The destructive radiation consists of ultraviolet (UV) rays, X rays, γ-rays, alpha (α) rays, beta (β) rays, neutrons, etc.

(2) Ultraviolet (UV) Radiation:

The UV light is a non-ionizing type of radiation having less energy. It is used in the sterilization and decontamination process during the cell culture and microbiological experiments. The DNA and protein absorb UV light of 260 and 280 nm, respectively.

(1) UV-A: Nearly visible range (320nm) causes pyrimidine dimers.

(2) UV-B: (290-320nm) emitted by the sunlight. These UV rays are highly lethal to our DNA.

(3) UV-C: (180-290nm) is one of the most energy consuming forms of UV which is extremely lethal.

(3) The X-rays:

X-rays are one of the most common types of ionizing radiation used in several medical practices for various purposes. It is even used in sterilizing practice for destroying microorganisms.

At the molecular breaks levels, the lethal dose of X-ray (350-500 rads) breaks the phosphodiester bonds between the DNA and thus results in strand breakages.

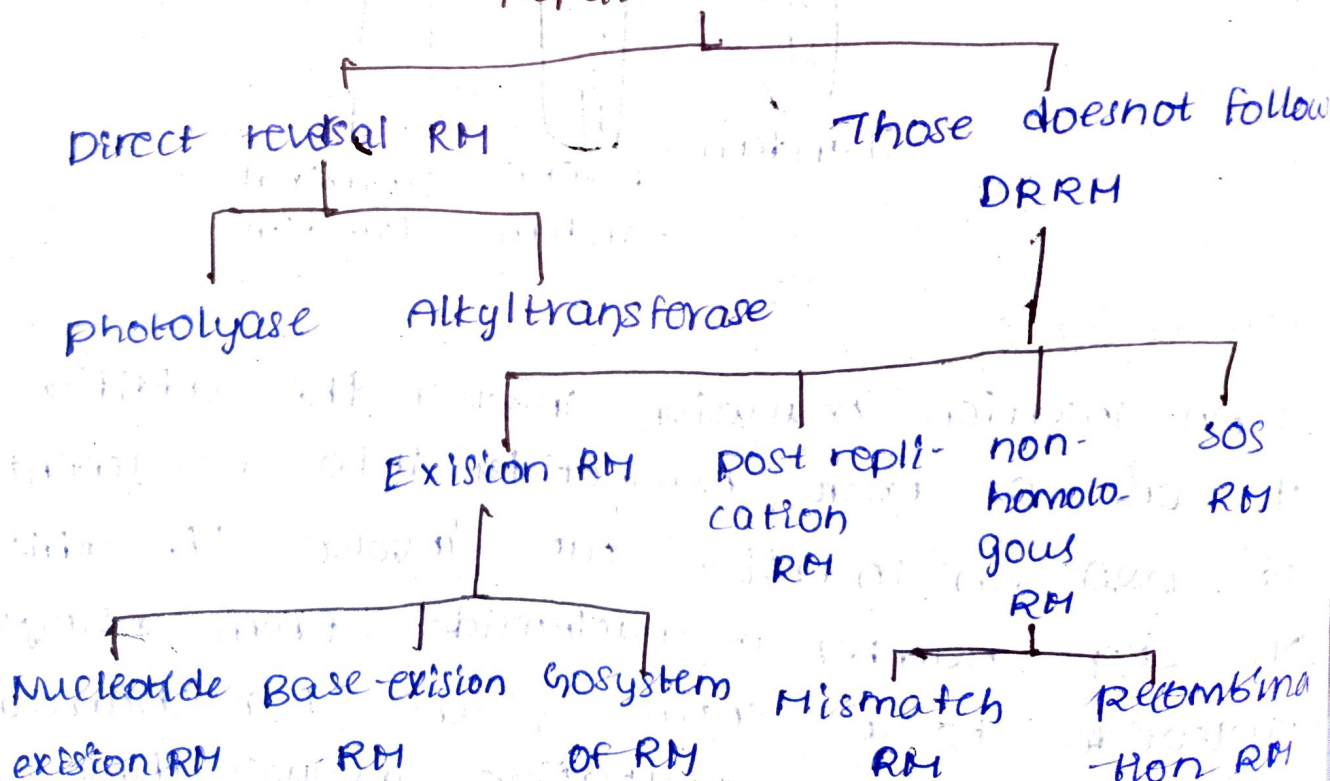
a microinsertion and

Repair Mechanism:

(When DNA is damaged or when mistakes occur in its synthesis it is important that it is repaired rapidly. Some damage can be directly reversed, whereas other types of damage requires the the faculty. stretch of DNA is removed and replaced.) In DNA replication interventions between damage and repair the consequences are often more extreme.

there are different types of repair mechanisms.

Repair mechanism



Direct Reversal Repair mechanism (DRRM)

RR is done in the same pathway in reversal direction to the mutation occurs.

Photolyase: When bacteria damaged by UV light they are exposed to an intense source of visible light (320-370nm). A large portion of the damaged cells recover. This process is known as photoreactivation, and it is due to reactivation by visible light of an enzyme which cleaves the pyrimidine dimers and restores the two bases to their original form.

Alkyl transferases:

Enzyme covalently attached to alkyl groups of N₂ bases are effected. so the enzyme confirmation is changed and its activity is altered so this type of category is said to be as a suicidal enzyme.

Those does not follow DRRM

Excision RR :-

This kind of DNA repair involves a sequence of enzyme catalysed steps in which the thiamine dimers are removed from DNA molecules and a new DNA segment of DNA synthesized. this is not light dependent.

(a) Nucleotide excision Repair mechanism:-

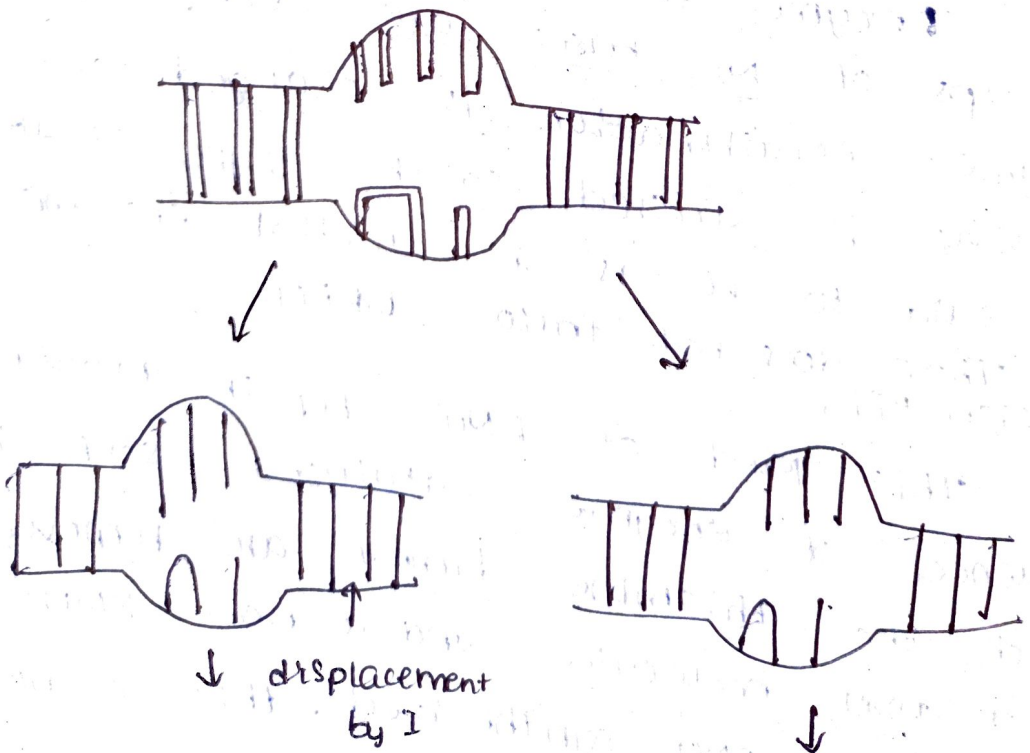
If the dimer distortion is more severe the nucleotide repair mechanism comes in actions.

In this UVR-A, B, C genes are involved

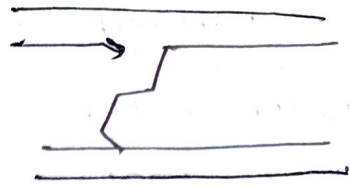
* UVR-A product recognise the distortion site and makes UVR-B protein to bind to the distorted area and then UVR-C protein act as endonucleases and make a cut in the distorted area is called "exonuclease".

* DNA ligase close the gap and joins the resynthesizing segment to the polynucleotide chain.

(b) Base excision.

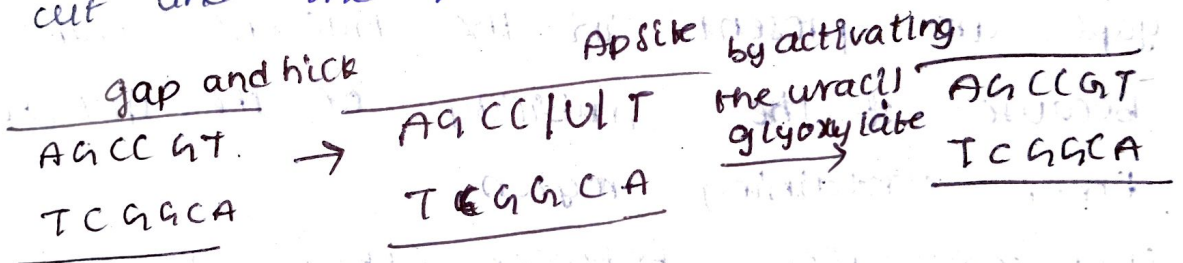


2 modes of excision replication



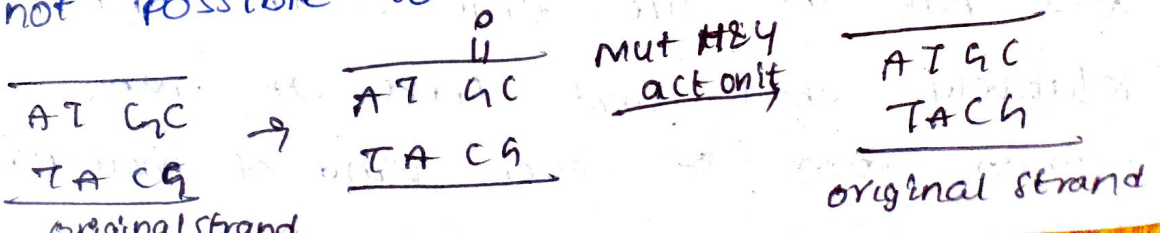
Base Excision repair mechanism:-

- 1) The bases are repaired
- 2) The undesired bases in the DNA are removed, with the help of the enzyme uracyl of DNA glycosylase. This makes a cut in the N-glycosidic bond & forms a AP site (a purine (or) apuramidine). The removed base is to be replaced by C. This capacity has only one enzyme AP endonuclease. This recognizes the site, cuts, and the portion of DNA is lost.



(3) GO system:-

Guanine on oxidation gives 8-Oxy 7-hydro guanine (or) Guanidine oxide (G-O). Two proteins Mut-H & Mut-Y both cooperatively with one another to eliminate this G-O. Mut-H recognizes the G-O, it is not possible to remove the GO completely.



post transcription repair mechanisms

Another type of dark repair of UV-damage that occurs in the G-coil involves both replication and recombination and is called post replication recombination. Each detail is unknown but the process clearly involves the replication of the damaged chromosomes.

- (a) Segment of a DNA molecule containing adjacent thymine bases.
- (b) Formation of thymine bases during UV radiation.
- (c) Replication of dimer containing DNA gaps are present in the nascent strand because of the inability of the distorted dimer containing region.
- (d) Recombination between the sister chromosomes producing one undamaged DNA molecule the net effect is the production of one undamaged chromosome from two damaged one or quite possibly, one viable product from two inviable ones.

These errors probably do occur in RNA also but the life time of the RNA is relatively short and there are many copies form of each protein.

Non-homologous RM:

Recombinant RM:

This occurs mainly in the homologous chromosomes. Due to the presence of the thymine dimer in the template strand that particular portion is not accessible to the replication. So the gapped DNA daughter strands are produced during replication with the help of the recombination mechanism called - sister strand exchange, proper - double strand molecule can be made.

This essential idea in sister strand exchange is that a single stranded segment free of any defect, is excised from a 'good' strand on the homologous DNA segment at the replication fork and some how inserted into the gap created by excision of a thymine dimer. The combined action of polymerase I and DNA ligase joins the inserted piece to adjacent regions thus filling in the gap. The gap formed in the donor molecule I and ligase. The system pairs it two dimers in opposite strands are very nearer to one another because then no undamaged sister strand segments are unavailable to be excised.

(1) Spontaneous mutations

(2) Induced mutations

(3) Base pair changes

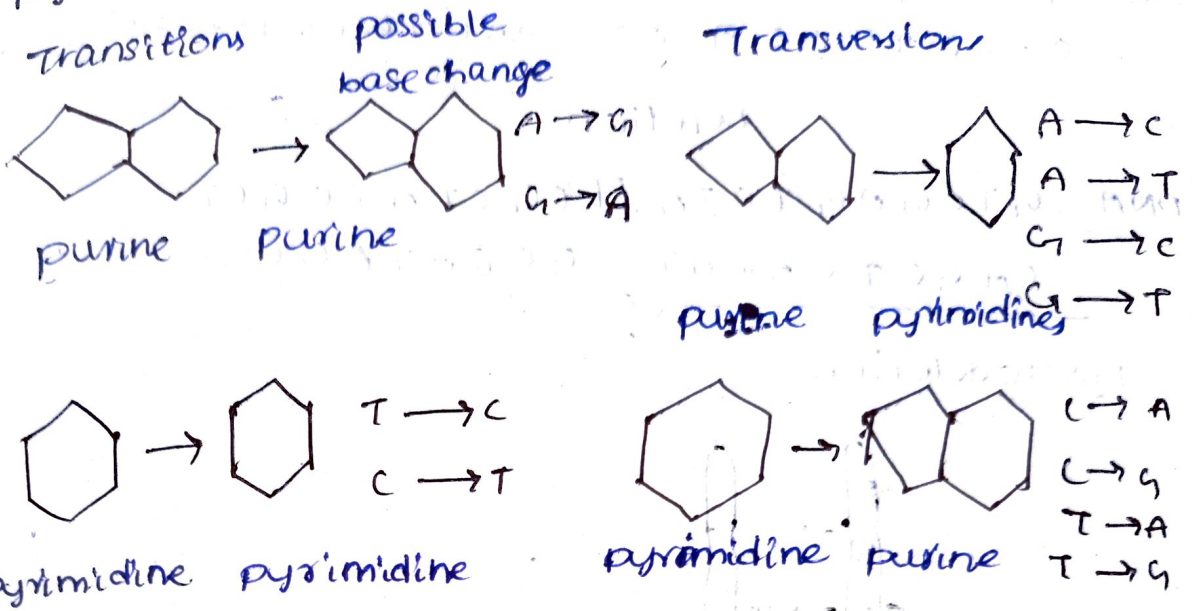
The simplest type of gene mutation is a base substitution, the alteration of a single nucleotide in the DNA. Because of the complementary nature of the two DNA strands, the base of the corresponding nucleotide on the opposite strand also will be altered in the next round of replication. A base substitution therefore usually leads to base pair substitution.

→ It is of two types:

(1) Transition: Here a purine is replaced by a different purine or a pyrimidine is replaced

by a different pyrimidine.

(2) Transversion: Here a purine is replaced by a pyrimidine and vice versa.



(4) Frame shift mutations:

A frame shift mutation in a gene refers to the insertion or deletion of nucleotide bases in number that are not multiples of three. This is important because a cell reads a gene's code in groups of three bases when making a protein. Each of these "triplet codons" corresponds to one of 20 different amino acids, used to build a protein. If a mutation disrupts this normal reading frame,

(5) Deletion:

A deletion is a type of mutation that involves the loss of one or more nucleotides from a segment of nucleotide DNA. It can involve the loss of any number of nucleotides from a single nucleotide to an entire piece of an chromosome. It causes a large number

Conjugation in Bacteria

Bacterial conjugation 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 pilus (Pili in plural).

During conjugation, the *donor* cell provides a conjugative genetic element that is most often a plasmid or transposon. Most conjugative plasmids have systems ensuring that the *recipient* cell does not already contain a similar element.

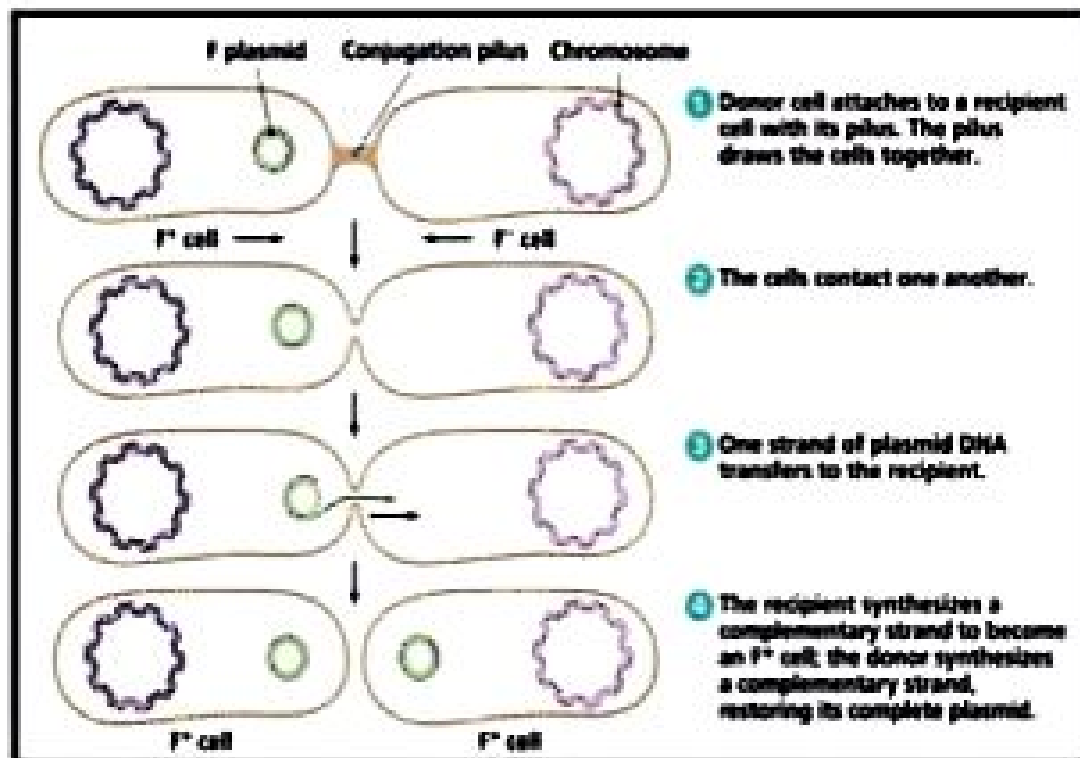


Figure: Mechanism of conjugation.

Conjugation has the following steps:

1. Donor cell produces pilus.
2. Pilus attaches to the recipient cell and brings the two cells together.
3. The mobile plasmid is nicked and a single strand of DNA is then transferred to the recipient cell.
4. Both cells synthesize a complementary strand to produce a double-stranded circular plasmid and also reproduce pili; both cells are now viable donors for the plasmid, for example, F-factor.

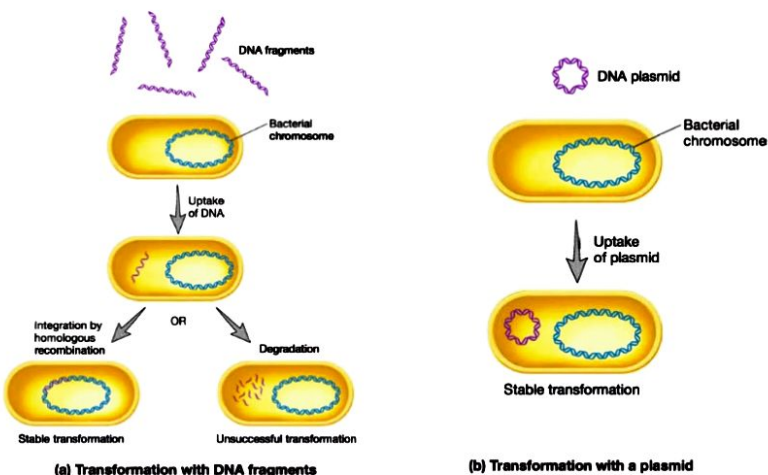
Bacterial Transformation

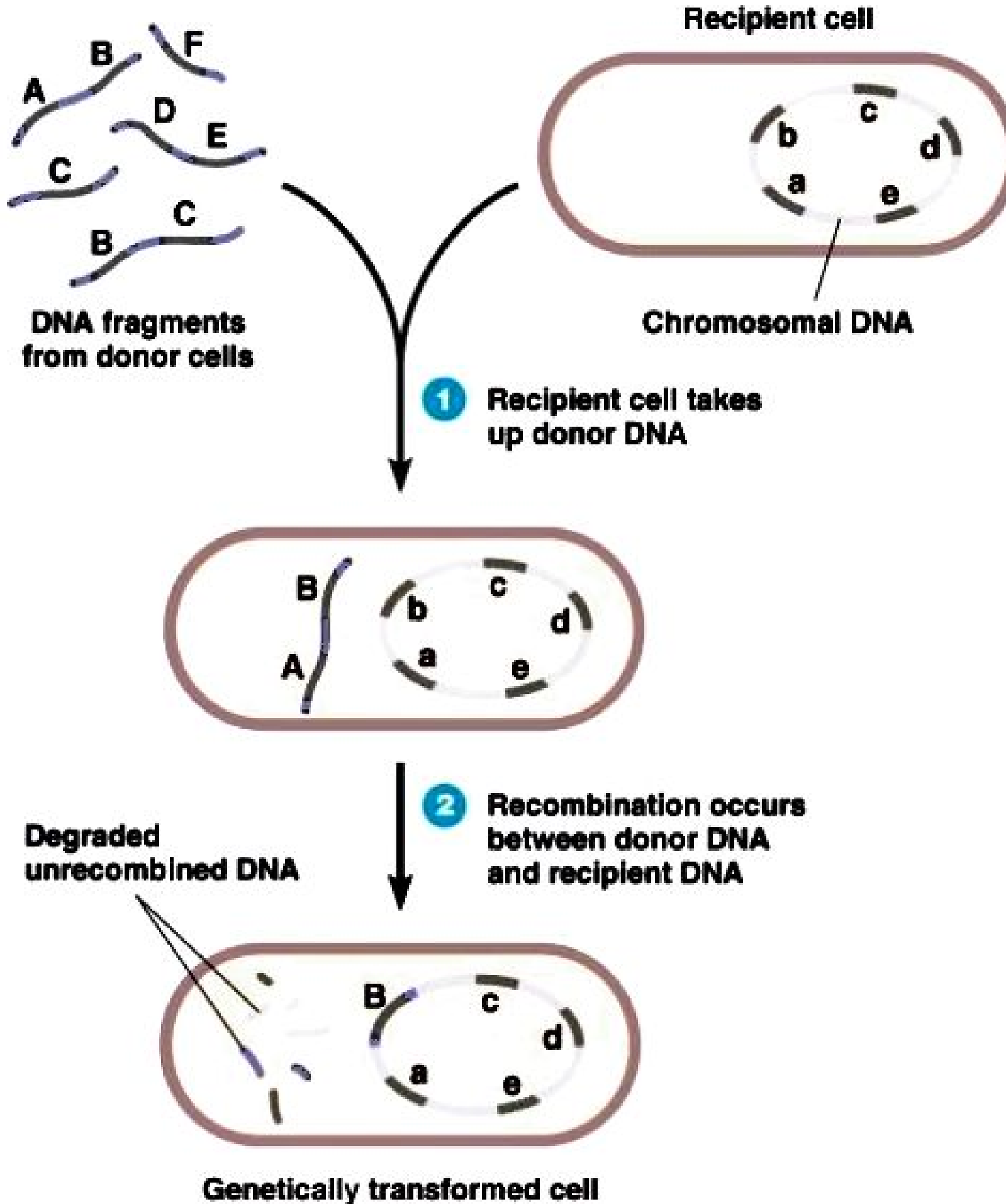
It is another way, DNA can move between bacteria is through transformation, discovered by **Fred Griffith in 1928**.

Transformation is the uptake by a cell of DNA, either a plasmid or a fragment of linear DNA, from the surroundings and maintenance of the DNA in the recipient in a heritable form.

In natural transformation, the DNA comes from a **donor** bacterium. The process is random, and any portion of the donor's genome may be transferred. When bacteria lyse, they release considerable amounts of DNA into the surrounding environment. **These fragments may be relatively large and contain several genes.**

If a fragment contacts a competent cell (a cell that is able to take up DNA and be transformed) the DNA can be bound to the cell and taken inside. The transformation frequency of very competent cells is around 10^{-3} for most genera when an excess of DNA is used. That is, about one cell in every thousand will take up and integrate the gene.





Generalized transduction

- ❖ During generalized transduction virtually any bacterial gene can be transferred.
 - ❖ The genetic transfer is mediated by virulent or lytic bacteriophage. Lytic bacteriophages are bacterial viruses that upon the infection of a host bacterial cell destroys the DNA of the host and ultimately lyse the cell, releasing numerous viral progeny.
 - ❖ Upon infection of a host cell by a lytic bacteriophage, viral enzyme degrade the host's DNA into fragments.
 - ❖ Viral DNA is not degraded because some of the bases into the bacteriophage genome are modified so that they are not recognized by viral enzymes.
 - ❖ The viral DNA is then replicated and viral proteins are synthesized.
 - ❖ The newly replicated DNA is packaged into the coat proteins, and then infectious viral particles are assembled. When the viruses are fully assembled, viral enzymes degrade the cell's envelopes, lysing the cell and releasing the viral progeny.
-

❖ Infrequently, however, some of that host's DNA is packaged into the virus along with an incomplete viral genome.

❖ When this happens, a generalized transducing phage is formed, which, although capable of initiating an infection, is unable to replicate itself or lyse the host cell.

❖ Some phage genes must be given up to accommodate the bacterial genes within the confines of the virus head.

❖ These defective transducing phages serves as vehicle for transfer of the host DNA (incorporated during viral assembly) from one cell to another.

❖ Because the packaging of host DNA into the viral particle is a random event, any given bacterial gene has an equal chance of being packaged and transferred to a recipient cell.

❖ Upon infection of a bacterial host cell by the transducing phage, the transducing DNA is introduced into the host's cytoplasm and becomes incorporated into the bacterial genome by homologous recombination.

❖ The infected cell is not destroyed because the transducing phage is defective in that it does not have a full complement of genes.

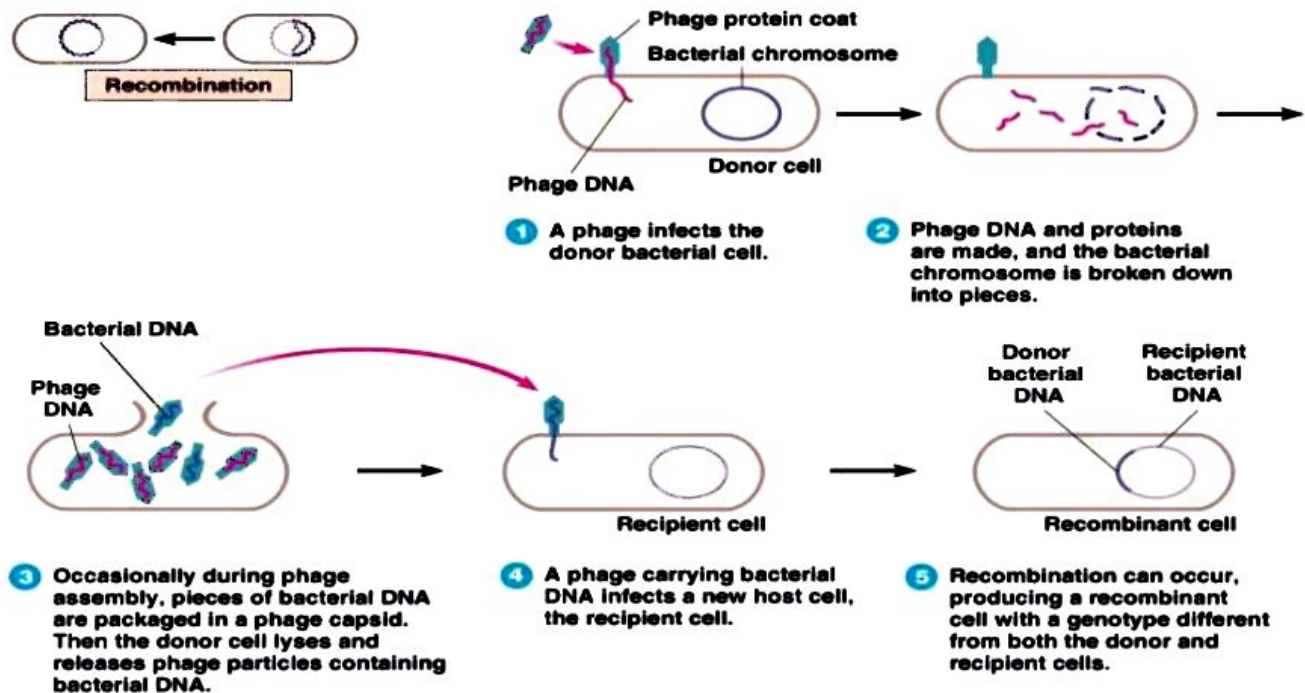


Figure : Schematic of generalized transduction

Specialized transduction

Specialized or restricted transduction is a process whereby a lysogenic bacteriophage serve to transfer a specific gene at a high frequency. When lysogenic bacteriophages infect host cells, their DNA is incorporated into the host's genome by site-specific recombination. Specialized transduction is carried only by temperate bacteriophage (ability to adapt lysogenic lifecycles) which undergoes lysogenic cycle in donor cell.

At first temperate bacteriophage enter into donor bacteria and then its genome gets integrated with host cell's DNA at certain location and remains dormant and pass generation to generation into daughter cell during cell division. The bacteriophage which follows lysogenic cycle is known as temperate phage.

When such lysogenic cell is exposed to certain stimulus such as some chemicals or UV lights, it causes induction of virus genome from host cell genome and begins lytic cycle.