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Deptt. of Home Science [CCS University campus]
Food and Nutrition - Sem II nd
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Subject :- Nutritional Biochemistry
Instructor:- Ms. Sheeba Khan

Unit - IV [c] Nucleic Acid

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DNA

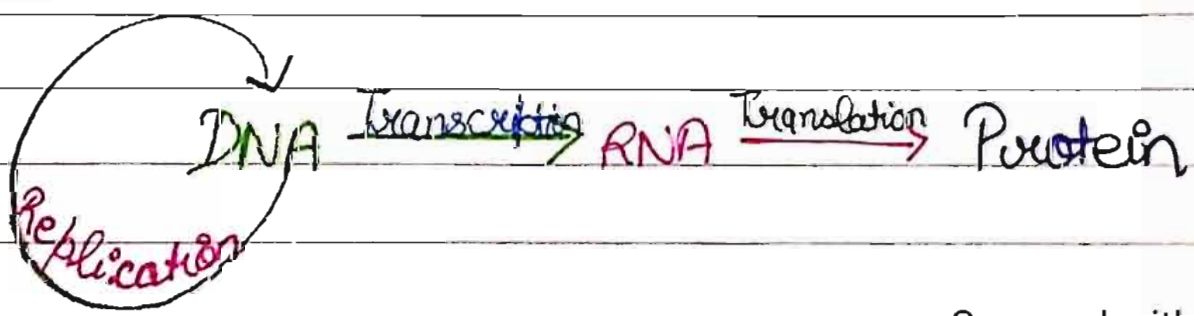
- INTRODUCTION

Deoxyribonucleic acid (DNA) is a macromolecule that carries genetic information from generation to generation.
It is responsible to preserve the identity of the species over millions of years.
DNA may be regarded as a reserve bank of genetic information or memory bank.

A single mammalian fetal cell contains only a few picograms (10^{-12} g) of DNA.
It is surprising that this little quantity of DNA stores information that will determine the differentiation & every function of an adult animal.

- The central dogma of Life

The biological information flows from DNA to RNA & from there to proteins. This is the central dogma of life. Its the DNA only that controls every function of cell through Protein Synthesis.



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DNA in a cell must be duplicated (replicated), maintained and passed down accurately to the daughter cells.
Three distinct processes are designed for this purpose. The 'three Rs' of DNA are- Replication, Recombination and Repair.

• DNA REPLICATION

- Definition

Replication is a process in which DNA copies itself to produce identical daughter molecules of DNA.
Replication is carried out with high fidelity which is essential for the survival of the species. Synthesis of a new DNA molecule is a complex process involving a series of steps

- Replication Enzymes

DNA replication would not occur without enzymes that catalyze various steps in the process.
Enzymes that participate in the Eukaryotic DNA replication processes include :-

a. DNA Helicase

It unwinds & separates double stranded DNA as it moves along the DNA.
It forms the replication fork by breaking hydrogen bonds between nucleotide pairs in DNA

b. DNA Primase
A type of RNA polymerase that generates RNA primers. Primers are short RNA molecules that act as templates for the starting point of DNA replication.

c. DNA Polymerases
It synthesize new DNA molecules by adding nucleotides to leading & lagging DNA strands.

d. Topoisomerase or DNA Gyrase
It unwinds and rewinds DNA strands to prevent the DNA from becoming tangled or supercoiled.

e. Exonucleases
Groups of Enzymes that remove nucleotide bases from the end of a DNA chain.

f. DNA Ligase
Joins DNA fragments together by forming phosphodiester bonds between nucleotides.

→ Preparation for Replication

This involves four main steps in the whole replication process:-

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Step 1 : Replication Fork Formation

- Before DNA can be replicated, the double stranded molecule must be 'unzipped' into two single strands. DNA has four bases :-
- Adenine (A), Thymine (T), Cytosine (C), Guanine (G) that forms pair between the two strands.
  ADENINE + THYMINE & CYTOSINE + GUANINE
- In order to unwind DNA, these interactions between base pairs must be broken.
- This is performed by an enzyme known as DNA Helicase. It disrupts the Hydrogen Bonding between base pairs to separate the strands into a Y shape known as the Replication Fork.
- This area will be the template for Replication to begin.
  Replication Begins.

Step 2 : Primer Binding

- The leading strand is the simplest to replicate
- Once the DNA strands have been separated, a short piece of RNA called a Primer binds to 3' end of the strand.
- The primer always binds as the starting point for Replication.
- Primers are generated by the enzyme DNA-Primase.

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Step 3: Elongation

- Enzymes known as DNA Polymerases are responsible for creating the new strand by a process called ELONGATION.
- There are five different known types of DNA Polymerases in Bacteria & Human Cells.
- In Bacteria: E. Coli, Polymerase III is the main replication enzyme.
- In Eukaryotic Cells:- Polymerase Alpha, Delta, & Epsilon are the Primary Polymerases involved in DNA Replication.
- Because replication proceeds in the 5' to 3' direction on the leading strand, the newly formed strand is continuous.
- The Lagging Strand begins replication by binding with multiple primers.
- Each primer is only several bases apart. DNA polymerase then adds pieces of DNA, called Okazaki Fragments, to the strand between primers.

This process of Replication is discontinuous as the newly created fragments are disjointed.

Step 4: Termination

Once both the continuous and discontinuous strands are formed, an enzyme called

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Exonuclease removes all RNA primers from the original strands.

- These primers are then replaced with appropriate bases.
- Another exonucleus removes all 'proofreads' the newly formed DNA to check, remove & replace any errors.

Summary

DNA replication is the production of identical DNA helices from a single double-stranded DNA molecule. Each molecule consists of a strand from the original molecule & a newly formed strand. Prior to Replication the DNA uncoils & strands separate. A replication fork is formed which serves as a template for replication. Primers bind to the DNA and DNA Polymerases, add new nucleotide sequences in the 5' to 3' direction.

This addition is continuous in the leading strand and fragmented in the lagging strand.

Once elongation of the DNA strands is complete, the strands are checked for errors, repairs are made, and Telomere sequences are added to the ends of the DNA.

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DNA TRANSCRIPTION

→ INTRODUCTION

- Transcription is the first step in Gene Expression. It involves copying a gene's DNA sequence to make an RNA molecule.

- Transcription is performed by enzymes called RNA Polymerases, which link nucleotides to form an RNA strand (using a DNA strand as a template)

- Transcription has three stages:- Initiation, Elongation and Termination.

- In Eukaryotes, RNA molecules must be processed after transcription: they are spliced and have 5' cap and a poly-A tail put on their ends.

- Transcription is controlled separately for each gene in your genome.

→ STAGES OF TRANSCRIPTION

Stage: 1 Initiation

RNA polymerase binds to a sequence of DNA called Promotere, found near the begining

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of a gene. Each gene has its own promoter. Once bound, RNA polymerase separates the DNA strands, providing the single-stranded template needed for transcription.

Stage 2: Elongation

One strand of DNA, the template strand, acts as a template for RNA polymerase. As it "reads" this template one base at a time, the polymerase builds an RNA molecule out of complementary Nucleotides, making a chain that grows 5' to 3'. The RNA transcript carries the same information as the non-template (coding) strand of DNA, but it contains the Base Uracil (U) instead of Thymine (T).

Stage 3: Termination

Sequences called terminators signal that the RNA transcript is complete. Once they are transcribed, they cause the transcript to be released from the RNA Polymerase.
Eg:- Formation of a hairpin in the RNA.

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DNA Repair Systems

→ Introduction

Cells have a variety of mechanisms to prevent mutations, are permanent changes in DNA sequence.

During DNA synthesis, most DNA polymerases "check their work", fixing the majority of misPaired bases in a process called Proofreading.

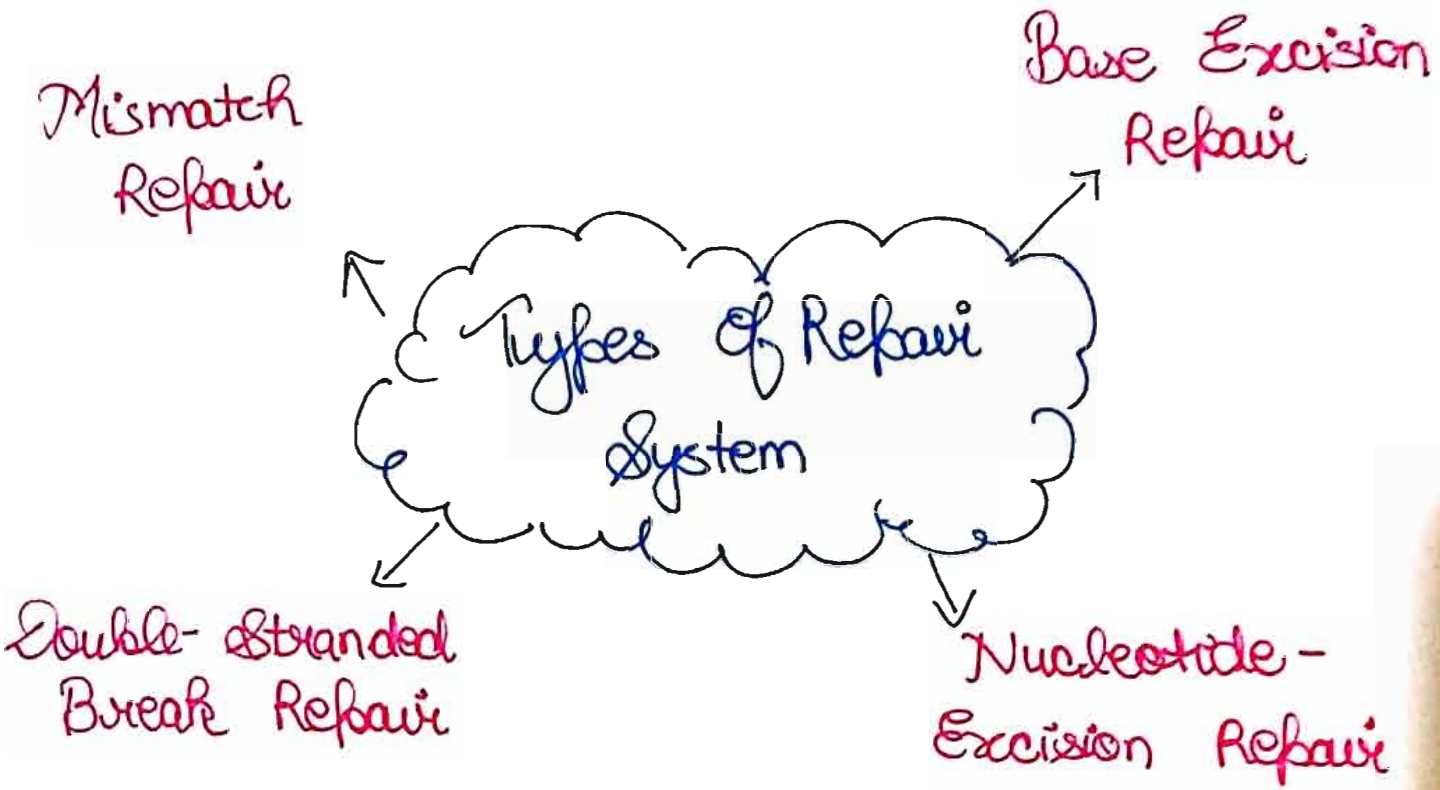
Immediately after DNA synthesis, any remaining misPaired bases can be detected and replaced in a process called Mismatch Repair.

If DNA gets damaged, it can be repaired by various mechanisms including chemical reversal, excision repair and double-stranded break repair

→ DNA damage repair mechanisms

Bad things can happen to DNA at almost any point in a cell's lifetime, not just during replication. Infact, your DNA is getting damaged all the time by outside factors like U.V light, chemicals and X-rays.

Fortunately, your cells have repair mechanisms to detect and correct many



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types of DNA damage. Repair processes that help fix damaged DNA include:-

- **Direct Reversal:**

Some DNA-damaging chemical reactions can be directly "undone" by enzymes in the cell.

- **Excision Repair**

Damage to one or a few bases of DNA is often fixed by removal (excision) and replacement of the damaged region.

- **Double Stranded Break Repair**

Two major pathways, non-homologous end-joining & homologous recombination are used to repair double-stranded breaks in DNA

→ **TYPES**

1. **Base Excision Repair**

- It is a mechanism used to detect and remove certain types of damaged bases.
- A group of enzymes called Glycosylases play a key role in base excision repair.

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- Each glycosylase detects and removes a specific kind of damaged base.
Eg:- A chemical reaction called Deamination can convert a cytosine base into Uracil a base typically found only in RNA.
During DNA Replication, uracil will pair with Adenine rather than Guanine, so uncorrected cytosine-to-uracil change can lead to Mutation.

To prevent such mutations, a glycosylase from the base excision repair pathway detects and removes deaminated cytosines. Once the base has been removed, the "empty" piece of DNA backbone is also removed, and the gap is filled and sealed by other enzymes.

2. Nucleotide Excision Repair

It is another pathway used to remove & replace damaged bases. Nucleotide excision repair detects and corrects types of damage that distort the DNA double helix.

For instance, this pathway detects bases that have been modified with bulky chemical groups, like the ones that get attached to your DNA when it's exposed to chemicals in cigarette smoke.

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This excision Repair is also used to fix some types of damage caused by U.V radiation for instance, when you get a Sunburn. U.V radiation can make cytosine and thymine bases reacts with neighbouring bases that are Cs or Ts forming bonds that distort the double helix and cause errors in DNA Replication.

3. Double - Stranded Break Repair

Some types of environmental factors, such as high - energy radiation, can cause double - stranded breaks in DNA (splitting a chromosome in two).
This is the kind of DNA damage linked with Superhero origin stories in Comic books & with disasters like Chernobyl in Real life.

Double - stranded breaks are dangerous because large segments of chromosomes, and the hundreds of gene they contain, may be lost if the break is not repaired.
Two pathways involved in the repair of double-stranded DNA breaks are the non - homologous end joining & homologous recombination pathways.

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4° Mismatch Repair

Despite high accuracy in Replication, defects do occure when the DNA is copied. For instance cytosine (instead of thymine) could be incorporated opposite to Adenine. Mismatch repair corrects a single mismatch base pair. Eg:- C to A, instead of T to A.

Hereditary Nonpolyposis Colon Cancer (HNPCC) is one of the most common inherited cancers. This cancer is now linked with family mismatch repair of Defective DNA.

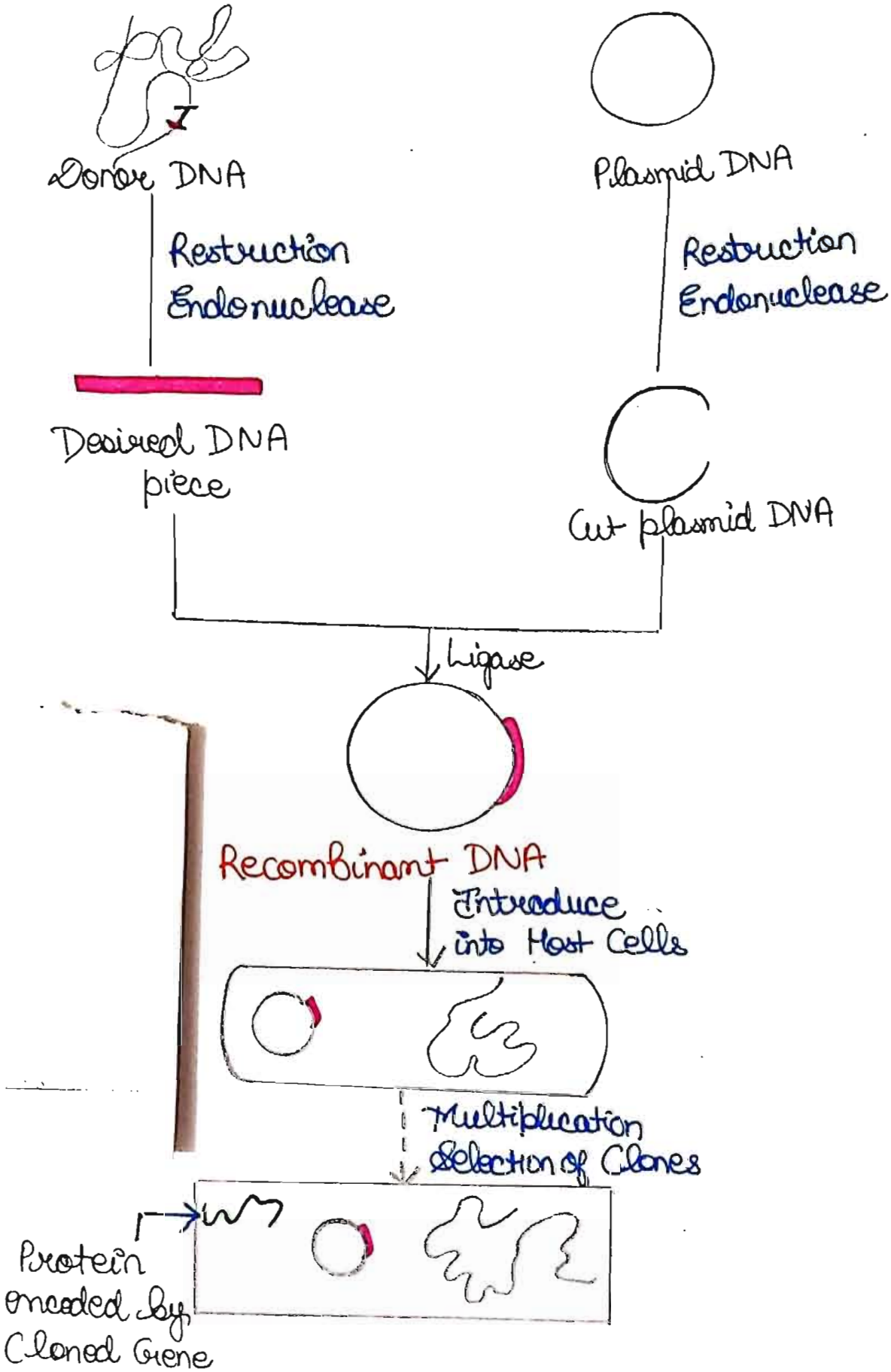

Fig:- Basic Principle of Recombinant DNA Technology

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DNA RECOMBINANT

→ INTRODUCTION

The new or modern biotechnology embraces all the genetic manipulations, cell fusion techniques and improvements made in the old biotechnological processes. The biotechnology with particular reference to recombinant DNA in human health and disease are discussed below.

Genetic Engineering primarily involves the manipulation of genetic material (DNA) to achieve the desired goal in a pre-determined way.

Some other terms are also in common use to describe genetic engineering :-

- Gene Manipulation
- Recombinant DNA (rDNA) technology
- Gene cloning (molecular cloning)
- Genetic modifications
- New Genetics

→ HISTORY OF RECOMBINANT DNA Technology

The present day DNA technology has its roots in the experiments performed by Boyer and Cohen in 1973.

In their experiments, they successfully recombined two plasmids and cloned the new plasmid in E. Coli.

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In later experiments the genes of a frog could be successfully transplanted, and expressed in E.Coli.
This made the real beginning of modern rDNA technology and laid foundations for the molecular biotechnology.

- Some biotechnologists who admire Boyer-Cohen experiments divide the subject in two chronological categories -

BBC - biotechnology - Before Boyer and Cohen.

ABC - biotechnology - After Boyer and Cohen.

→ Recombinant DNA

Recombinant DNA or rDNA, is the term used to describe the combination of two DNA strands that are constructed artificially.
Genetic scientists can do this to create unique DNA strand for different purposes, using several types of techniques.

Like naturally occuring DNA, recombinant DNA has the ability to produce recombinant proteins. It is often these proteins that play the key role in the application of recombinant DNA.

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→ BASIC PRINCIPLES OF rDNA Technology

There are many diverse and complex techniques involved in gene manipulation.
However, the basic principles of recombinant DNA technology are reasonably simple, and broadly involve the following stages:-

1. Generation of DNA fragments and selection of the desired piece of DNA (E.g: a human gene)

2. Insertion of the selected DNA into a cloning vector (E.g: a Plasmid) to create a recombinant DNA or chimeric DNA.

3. Introduction of the recombinant vectors into host cells. (E.g: bacteria)

4. Multiplication and selection of clones containing the recombinant molecules.

5. Expression of the gene to produce the desired product.

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GENETIC MUTATIONS

→ **Introduction**

Mutations refers to a change in the DNA structure of a gene.
The substances (chemicals) which can induce mutations are collectively known as **Mutagens**.
The changes that occure in DNA an mutation are reflected in Replication, Transcription and Translation.

→ **Types of Mutations**

1. Spontaneous vs Induced Mutation

- A mutation which occured without any known cause is called as Spontaneous mutation.
- It arises due to metabolic errors, replication errors are during development errors.
- Spontaneous mutations are rare and occured without any reason.
- Spontaneous mutations are generally occured by Birth.

- Induced Mutation is from exposure of an organism to mutagenic agents.
- The general mutagenic agent are radiation, U.V light and chemicals.

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- The U.V light is responsible for Xeroderma pigmentation & skin cancer by penetrating into the skin.

2. Somatic vs Germline Mutations

- Mutations which are occured in the somatic cells of an organism is called as a somatic mutation.
- Somatic mutations are non-inherited because only germ cells can only undergo fertilization.
- Though it is non inherited, it can still cause some life-threatening phenotypes.
- The commonest type of somatic mutation results in cancer.

- Germline Mutation inherits from one generation to other generation.
- Two types of germ cells sperm and egg, have haploid numbers of a genome and undergo fertilization.
- Hence, if it occures in germ cell it is inherited into the next generation.
- Germline mutations may or may not affect the parental organism but it will surely affect the off-springs.
- These are non-curable.

Types of Mutations

- SPONTANEOUS VS INDUCED
- MORPHOLOGICAL
- LETHAL
- CONDITIONAL
- BIOCHEMICAL
- TEMPERATURE SENSITIVE
- GENE

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5. Morphological Mutations

The genetic mutations which affects the outer characteristic or physical characteristic of an organism is called as Morphological Mutation.

This type of mutation alters the physical properties like shape, size and colour of an organism.

4. Lethal Mutation

A mutation which causes the death of an organism or affects the survival of an organism is called a Lethal mutation.

If a mutation causes death in a certain environment that than the mutation is called as a conditional lethal mutation.

5. Conditional Mutation

In this type of mutation the mutant allel causes mutant phenotype in a certain specific environment while remaining wild type in some other environment.

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Eg:- Bacteria; the conditions which favour the growth of mutant colonies are called Restrictive conditions.
In contrast, conditions which cause the growth of wild-type phenotype are called permissive conditions.

6. Biochemical Mutations

Each cell required energy and nutrients for differentiation and survival.
Different biochemical pathway inside the cell provides that essential requirement.

7. Temperature Sensitive Mutant

Mutant that is growing at one temperature and remain suppressed at another temperature are called as a Temperature Sensitive Mutation

8. Gene Mutations

a. Forward mutation
Genetic mutation from wild type to mutant or evolution of new mutation from wild-type is called a Forward Mutation.

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6. Backward Mutation

Mutation is a unidirectional process, but sometimes some mutation gives original traits back to population, such as the Backward or back mutation.

c. Point Mutation

Mutation in which a single base is altered, termed as a point mutation.

d. Silent Mutation

It is non-expressive. In this, the new codon is created from the mutation but it codes for the same amino acid as wild type.

e. Frameshift Mutation

Alternation in a base pair which results in an abnormal reading frame, and leads to abnormal protein.

In frameshift mutation, alternation in DNA leads to shifting of this reading frame hence the position of start or stop codon altered which results in an abnormal protein.

GENE EXPRESSION

→ Introduction

Gene expression refers to a complex series of processes in which the information encoded in a gene is used to produce a functional product such as protein that dictates cell function.
It involves several different steps through which DNA is converted to an RNA which in turn is converted to an protein or in some cases an RNA.
Eg:- Genes encoding the necessary information for transfer RNA's and Ribosomal RNA's

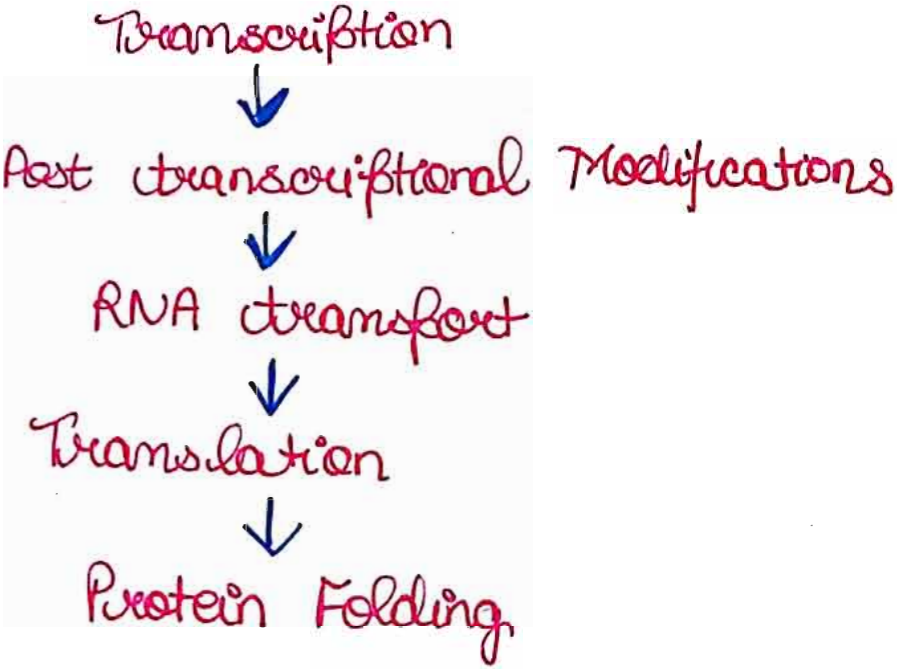
→ Key Phases in Gene Expression

Gene expression constitutes several complex processes that finally produce a functional biomolecule. Key steps involved in gene-expression include the following

1. Transcription - conversion of DNA to RNA

This is the first step in gene expression in which DNA molecules are transcribed into their corresponding RNA copy.

Stages of Gene Expression



This process is aided by an enzyme called DNA - RNA dependent RNA polymerase.

2. Post - transcriptional modifications

In this process, the primary RNA obtained after transcription is modified to produce a mature messenger RNA or mRNA. This involves many different ways wherein the pre-mRNA is processed to make it fully functional for the next step in gene expression.

3. RNA transport

Most of the mature mRNAs produced after modifications are transported from the nucleus to the cytoplasm where the next step in gene expression takes place. This is achieved by moving the mRNAs through tiny pores in the nucleus to reach the cytosol.

4. Translation or Protein Synthesis

Upon successful transport of mRNAs to the cytoplasm, the sequence in the mRNA is

translated into a protein with the help of several components such as ribosomes, tRNAs or transfer RNAs, and enzymes called Aminoacyl tRNA synthetases.
Translation of mRNA involves 3 important steps- Initiation, Elongation & termination, leading to the formation of Polypeptide chains.

5. ~~Translation or Protein synthesis~~

5. Protein Folding
In this final step the polypeptide chains or random coils formed during translation fold into a 3D structure giving rise to a functional protein.
Failure to fold leads to protein inactivity & misfolded proteins have abnormal functionalities compared to correctly folded ones.

→ Comparison of Gene Expression in Prokaryotes & Eukaryotes :-

Gene expression is different and less complex in prokaryotes as compared to that in Eukaryotes. This is due to the following reasons:-

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- In Prokaryotic cells, the product of initial transcription is a mature messenger RNA. Therefore prokaryotic gene expression does not invole post-transcriptional processing unlike eukaryotes where post-transcriptional processing of pre-mRNA is crucial to the rest of the steps in gene expression.

- Although in Eukaryotic gene expression is more complex compared to prokaryotic gene expression, the complexity provides several opportunities for regulation of gene expression in Eukaryotes.

Prokaryotic gene expression is controlled primarily during Transcription.

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Protein Biosynthesis

Introduction

The protein synthesis which involves the translation of nucleotide base sequence of mRNA into the language of amino acid sequence may be divided into the following stages for the convenience of understanding.

Stage 1 :- Activation of Amino Acid

This reaction is brought about by the binding of an amino acid with ATP.
The step requires enzymes called Amino Acyl RNA Synthetases. Due to this reaction amino acid (AA) and Adenosine triphosphate (ATP), mediated by above enzyme, amino acyl - AMP enzyme complex is formed.

Stage - 2 :- Transfer of Amino acid to tRNA.

The AA - AMP - enzyme complex formed reacts with specific tRNA. Thus amino acid is transferred to tRNA. As a result the enzyme & AMP are liberated.

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AA - AMP - enzyme complex + tRNA - AA - tRNA + AMP enzyme.

3. Stage 3 :- Initiation of Polypeptide Chain

Charged tRNA shifts to Ribosome. The ribosome consists of structural RNAs and 80 different proteins. Ribosomes is the site where the protein synthesis occures.
The mRNA binds to 30S sub-unit of Ribosomes of 70S type.

4. Stage 4 :- Chain Termination

The termination of Polypeptide is signally by one of the three terminal triplets (codons) in the mRNA.
The three terminal codons are UAG (Amber), UAA (ochre) & UGA (opal). They are also called stop signals.

At the time of Termination the terminal codon immediately follows the last amino acid codon.
After this the Polypeptide chain, tRNA and mRNA are released. The subunits of ribosomes gets dissociated.

5. Stage 5:- Protein Translocation.

Two classes of Polyribosomes have been identified.

(a) Free Polyribosomes
(b) Membrane bound Polyribosomes

For free ribosomes, termination of protein synthesis leads to the release of completed protein into cytoplasm.
Some of these specific proteins are translocated to mitochondria & nucleus by special type of mechanisms.

On the other hand, in membrane bound Polyribosomes, Polypeptide chain which grows on mRNA is inserted into the lumen of ER membrane.

Stages of Protein Biosynthesis

Activation of Amino Acid

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Transfer of Amino Acid to tRNA

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Initiation of Polypeptide Chain

↓

Chain Termination

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Protein Translocation

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