

Biotechnology and Gene Manipulation

Anandi R

Introduction

- Biotechnology involves the exploitation, genetic manipulation and alterations of micro-organisms or biological systems to make commercial valuable products and that also involves fermentation and various upstream and downstream processes.

Introduction

- Microorganisms produce an amazing array of valuable products such as macromolecules (e.g. proteins, nucleic acids, carbohydrate polymers, even cells) or smaller molecules and are usually divided into metabolites that are essential for vegetative growth (primary metabolites) and those which give advantages over adverse environment (secondary metabolites).
- They usually produce these compounds in small amounts that are needed for their own benefit.

In General, Biotechnology Techniques

Gene Manipulation

- Identify a gene from *another species* which controls
- A trait of interest
- Or modify an existing gene (create a new allele)

In General, Biotechnology Techniques

Gene Introduction

- Introduces that gene into an organism
- Technique called *transformation*
- Forms *transgenic organisms*

Recombinant DNA

The manipulation and combination of DNA from two sources.

- Bacterial DNA + human gene for insulin
- Plant DNA + bacterial DNA - *Agrobacterium tumefaciens*
- Mouse DNA + human DNA = transgenic

Recombination

- Insert a foreign gene into a host.
Plasmid (for example, exogenous DNA) into the bacterial cell – transformation or transfection-organism referred to as transgenic or recombinant
- Goal – To produce many copies (clones) of a particular gene
- Reporter gene – tags gene of interest – to identify the presence of a gene.

Genetic Manipulation for Biotechnology

- Molecular genetics can be used to manipulate genes in order to alter the expression and production of microbial products, including the expression of novel recombinant proteins.
- The compounds that are isolated from plants or animals can be synthesized by genetic manipulation of different micro-organisms to enhance the production and by environmental and other manipulations, even up to 1000-fold for small metabolites can be increased.

Genetic Manipulation for Biotechnology

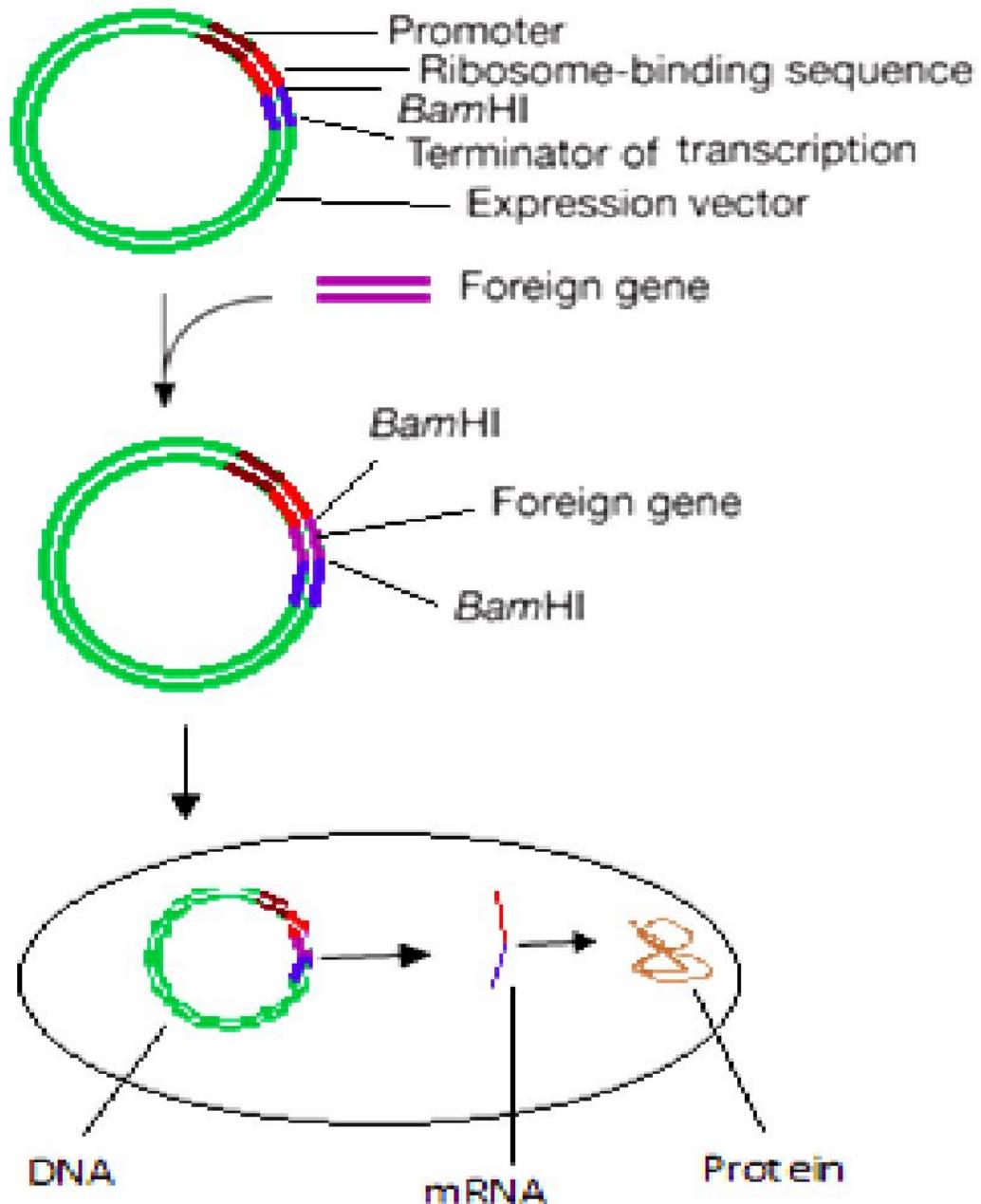
- The advent of recombinant DNA technology (also referred to as gene cloning or *in vitro genetic manipulation*) has dramatically broadened the spectrum of microbial genetic manipulations.
- With the advancement of recombinant DNA technology, many novel host systems have been **explored** to produce commercially important products like therapeutic proteins, antibiotics, small molecules etc.

Genetic Manipulation for Biotechnology

- The basis of this technology is the use of restriction endonucleases, polymerases and DNA ligases as a means to specifically cut and paste fragments of DNA.
- Similarly, foreign DNA fragments can be introduced into a vector molecule (a plasmid or a bacteriophage), which enables the DNA to replicate after introduction into a bacterial cell.
- The ability to modify and clone genes accelerated the rate of discovery and the development in biotech industries.

The basic steps in DNA cloning involves

- A fragment of DNA is inserted into a carrier DNA molecule, called a vector, to produce a recombinant DNA.
- The recombinant DNA is then introduced into a host cell, where it can multiply and produce numerous copies of itself within the host.
- The most commonly used host is the bacteria, although other hosts can also be used to propagate the recombinant DNA.



Expression of a foreign protein in a microbe

Potential applications of genetic manipulation

- Insulin for diabetics
- Factor VIII for males suffering from hemophilia A
- Factor IX for hemophilia B
- Human growth hormone (GH)
- Erythropoietin (EPO) for treating anemia
- Interferons
- Interleukins

Vectors

- Plasmids
- Viruses
- Particles (DNA coated bullets)
- Exogenous DNA

Characteristics of a Vector

- Can replicate independently in the host cell – contains an Ori
- Has restriction sites in the vector- Polylinker cloning region
- Has a reporter gene that will announce its presence in the host cell.
- Is a small size in comparison to the host chromosome for ease of isolation

Restriction Enzymes

- Cut Plasmid with restriction enzyme
- Cut gene of interest with restriction enzyme
- Splice together gene of interest

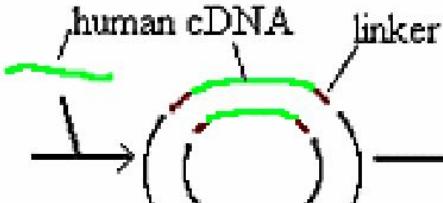
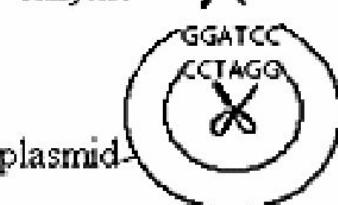
Production of recombinant enzymes:

1. Enzymes of industrial importance: Amylases, Proteases, Chymosin, Catalases, Isomerases recombinant Lipases.
2. Enzymes used for analytical purposes, such as glucose oxidase (GOs), alcohol dehydrogenase (ADH), hexokinase, cholesterol oxidases, horseradish peroxidase (HRP), alkaline phosphatase etc.
3. Enzymes of medicinal importance: Trypsin, Asparaginase, Proteases, Lipases etc.

Production of Insulin

Transfer of the Insulin gene

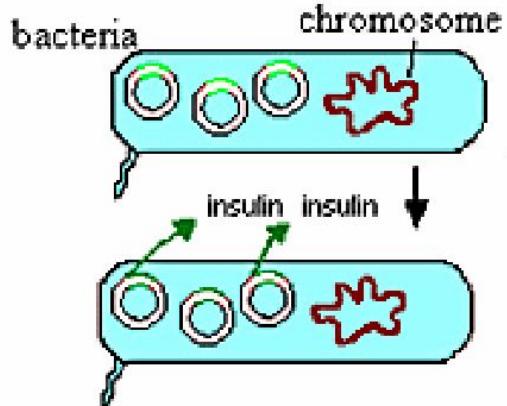
Restriction enzyme



Recombinant plasmid

Cloning the Insulin Gene

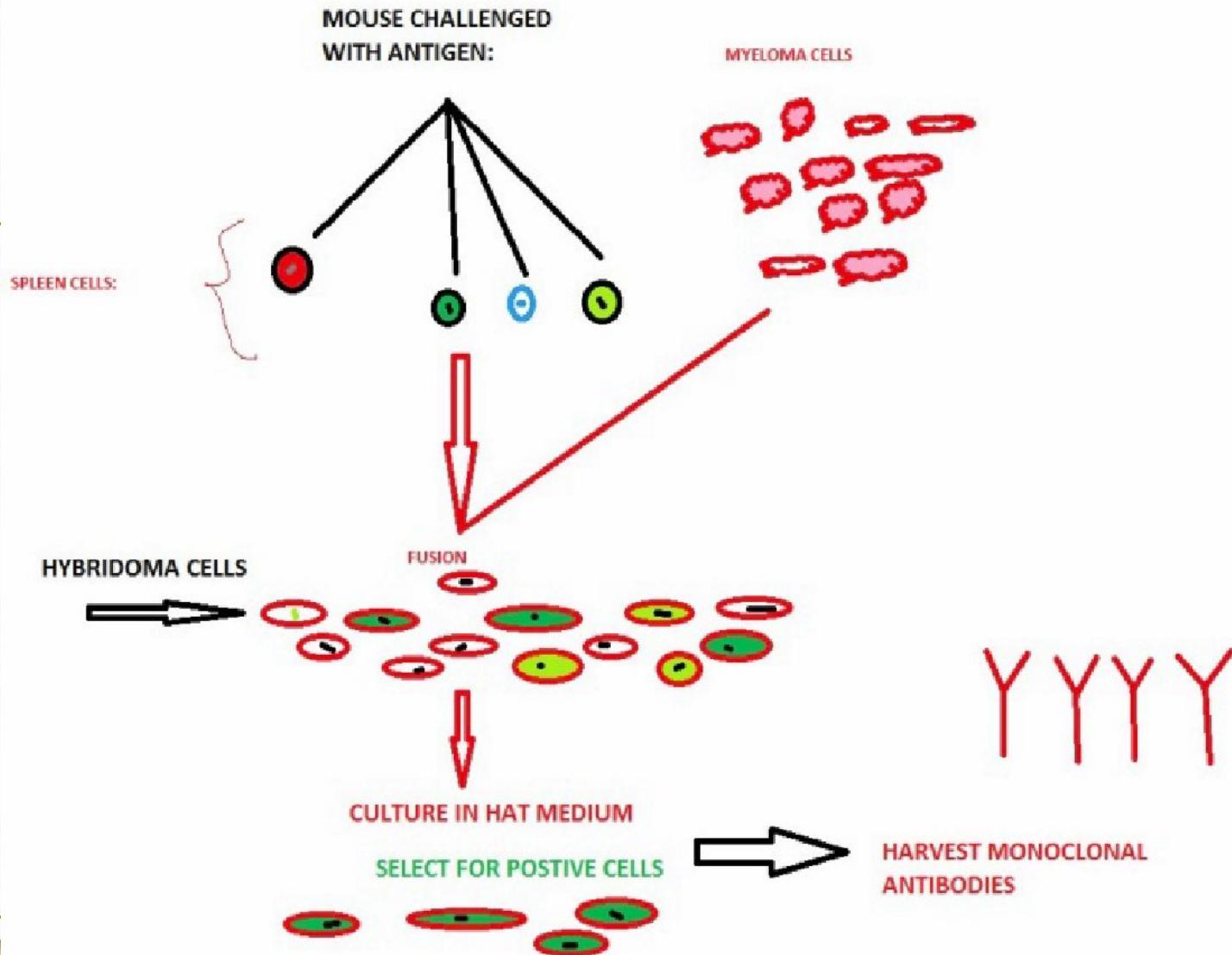
bacteria



Transfection

Transfer and cloning of the Insulin gene

Production of Monoclonal Antibodies



Comparative Eukaryotic genomics: Chloroplast, Mitochondrial and nuclear genome

- By Anandi Rebello

WHAT IS ORGANELLAR GENOME..?

- **ORGANELLE :**

Mitochondria , Chloroplast, Golgicomplex, Endoplasmic reticulum etc.,

- The genome present in the Chloroplast and Mitochondria are called as Organellar Genome.

ORGANELLAR GENOMES / EXTRANUCLEAR GENOMES:

- ✓ Mitochondria (animals and plants)
 - ✓ Chloroplasts (plants)
1. Mitochondria and chloroplasts occur outside the nucleus, in the cytoplasm of the cell.
 2. Contain genomes (mtDNA/cpDNA) and genes, i.e., extrachromosomal genes, cytoplasmic genes, organelle genes, or extranuclear genes.
 3. Inheritance is non-Mendelian (e.g., cytoplasm typically is inherited from the mother).

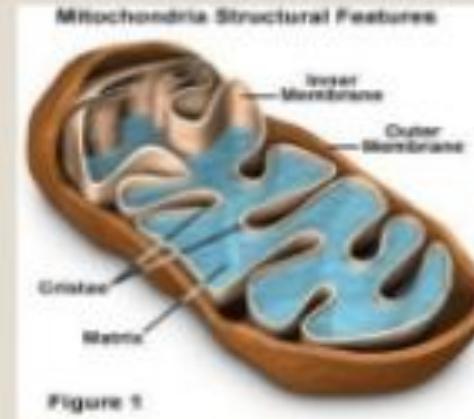
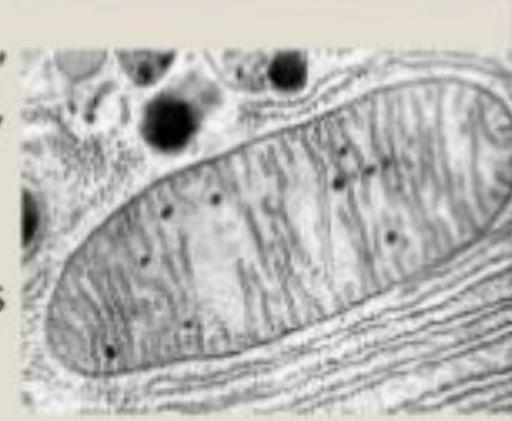


Figure 1



Organelle Genomes

- ❖ **Small but essential.**

- Mitochondria (site of respiration).

- Plastids (site of photosynthesis) .

- ❖ **Multiple organelles and organelle genomes per cell.**

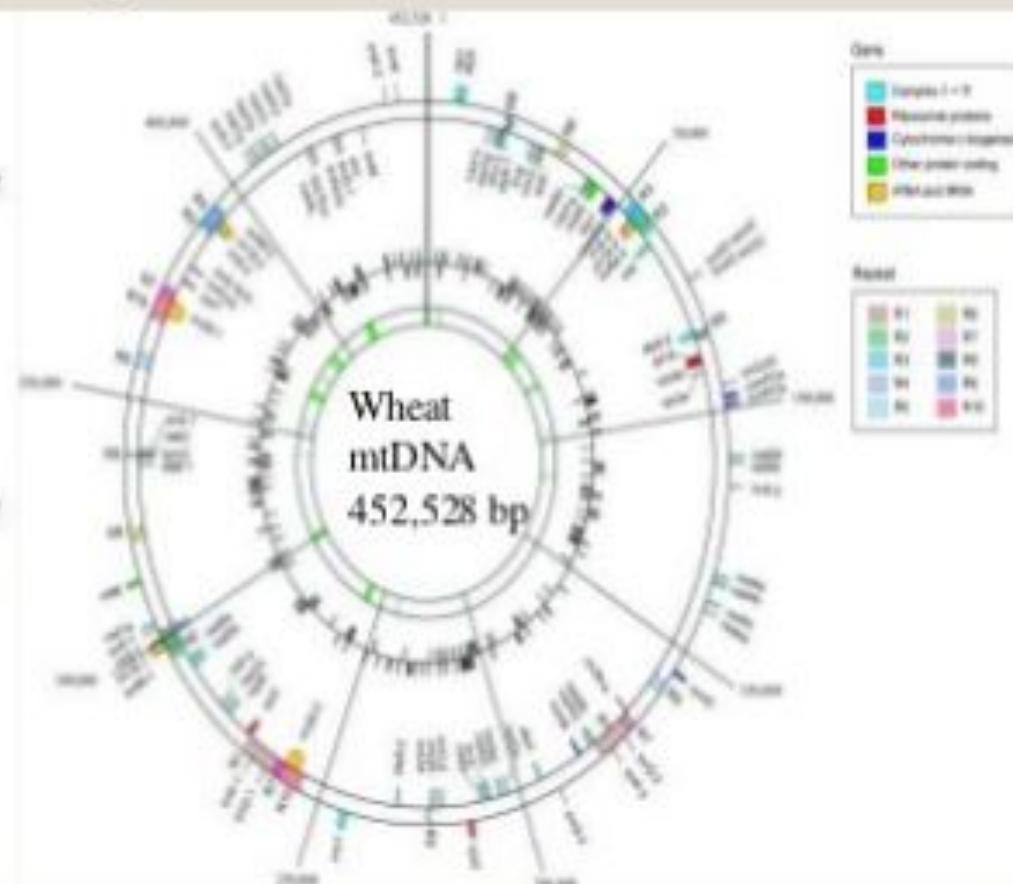
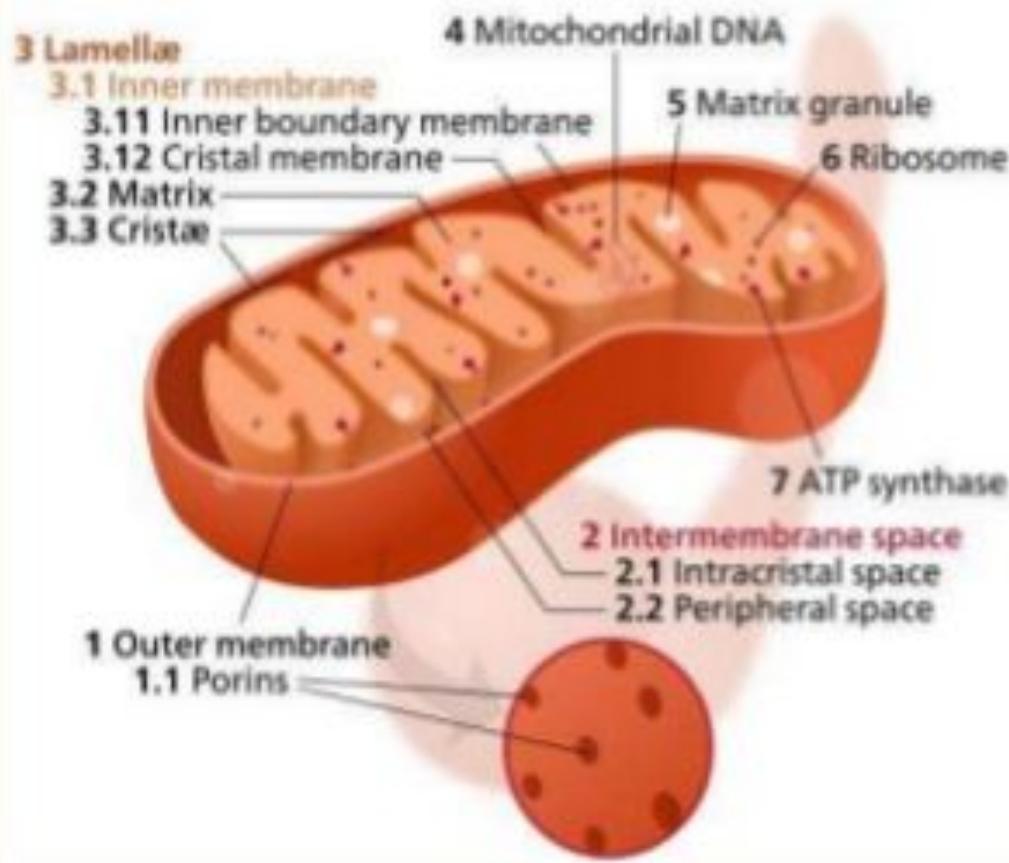
- 20 – 20,000 genomes per cell, depending on cell type.

- ❖ **Organized in nucleoids.**

- Nucleoprotein complexes containing multiple genome copies.

- Not to be confused with nucleosomes.

Mitochondrial genome



CONTENTS OF THE mtDNA GENOME

- ❖ mtDNA contains genes for:

- tRNAs
- rRNAs
- cytochrome oxidase, NADH-dehydrogenase, & ATPase subunits.
- mtDNA genes occur on both strands.

- Mitochondria's genetic information also occurs in the nuclear DNA:

- DNA polymerase, replication factors
- RNA polymerase, transcription factors
- ribosomal proteins, translation factors, aa-tRNA synthetase
- Additional cytochrome oxidase, NADH, ATPase subunits.

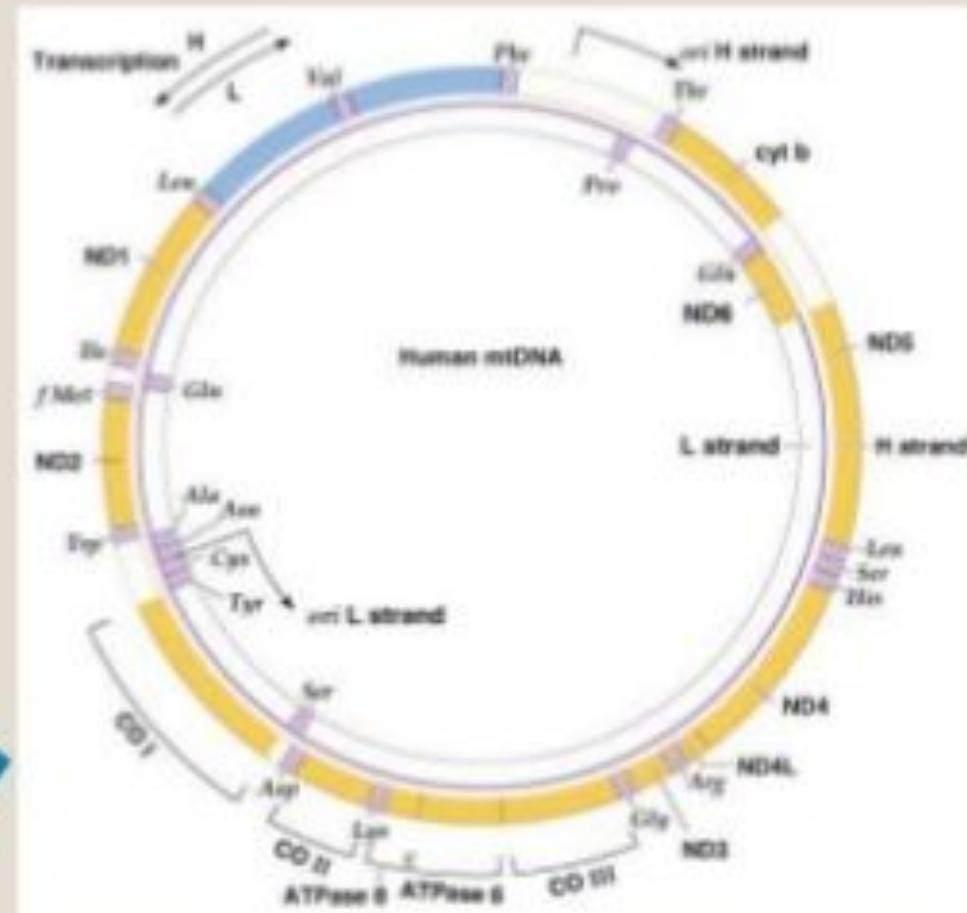
- Most required mitochondrial (and chloroplast) proteins are coded by nuclear genes in the nuclear genome.
 - Five mtDNA complexes with 13 mtDNA subunit genes are paired with 76 nuclear subunit genes to make the same proteins.

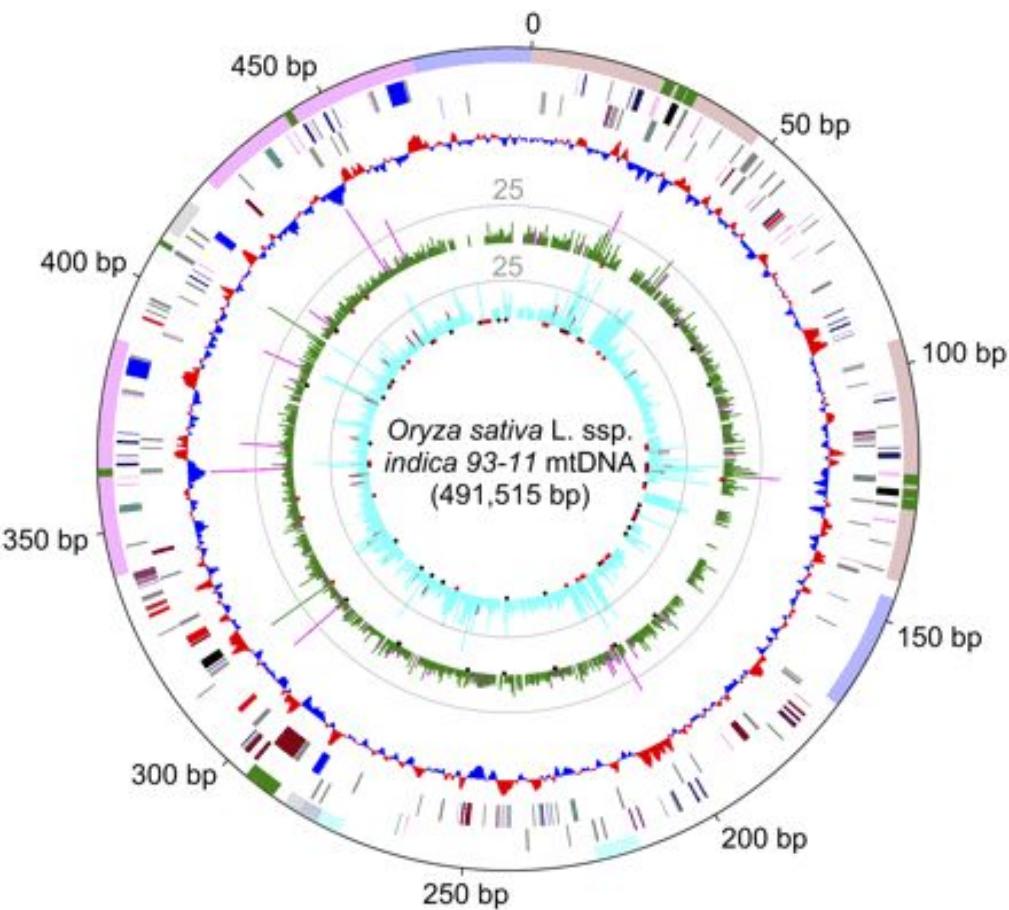
I - NADH; II - Succinate dehydrogenase; III - Cytochrome bc

IV - Cytochrome c oxidase; V - ATP synthase

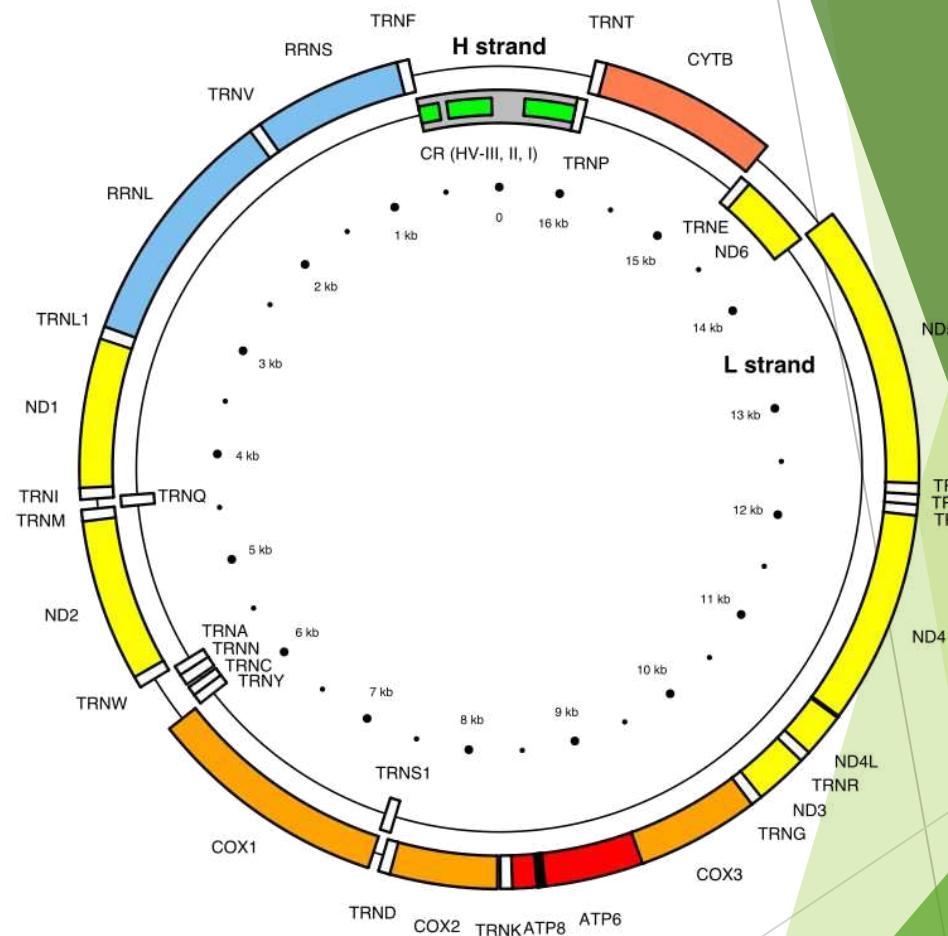
than mtDNA

Physical map of the human mtDNA

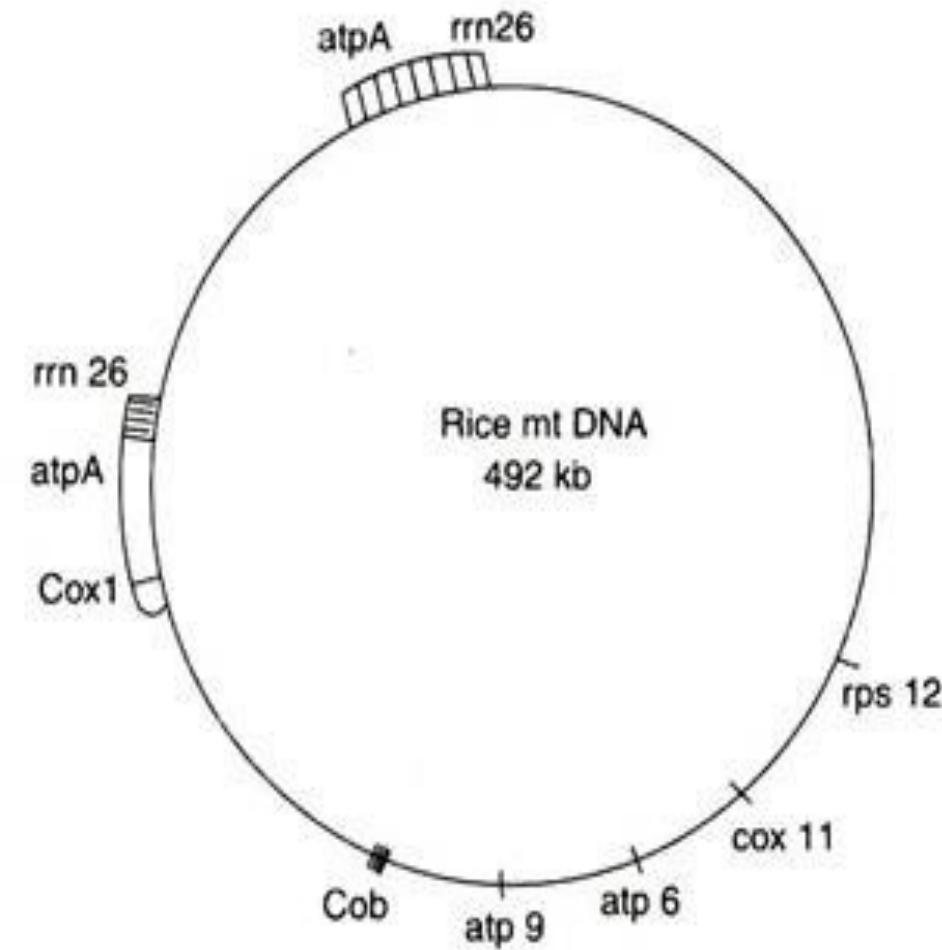




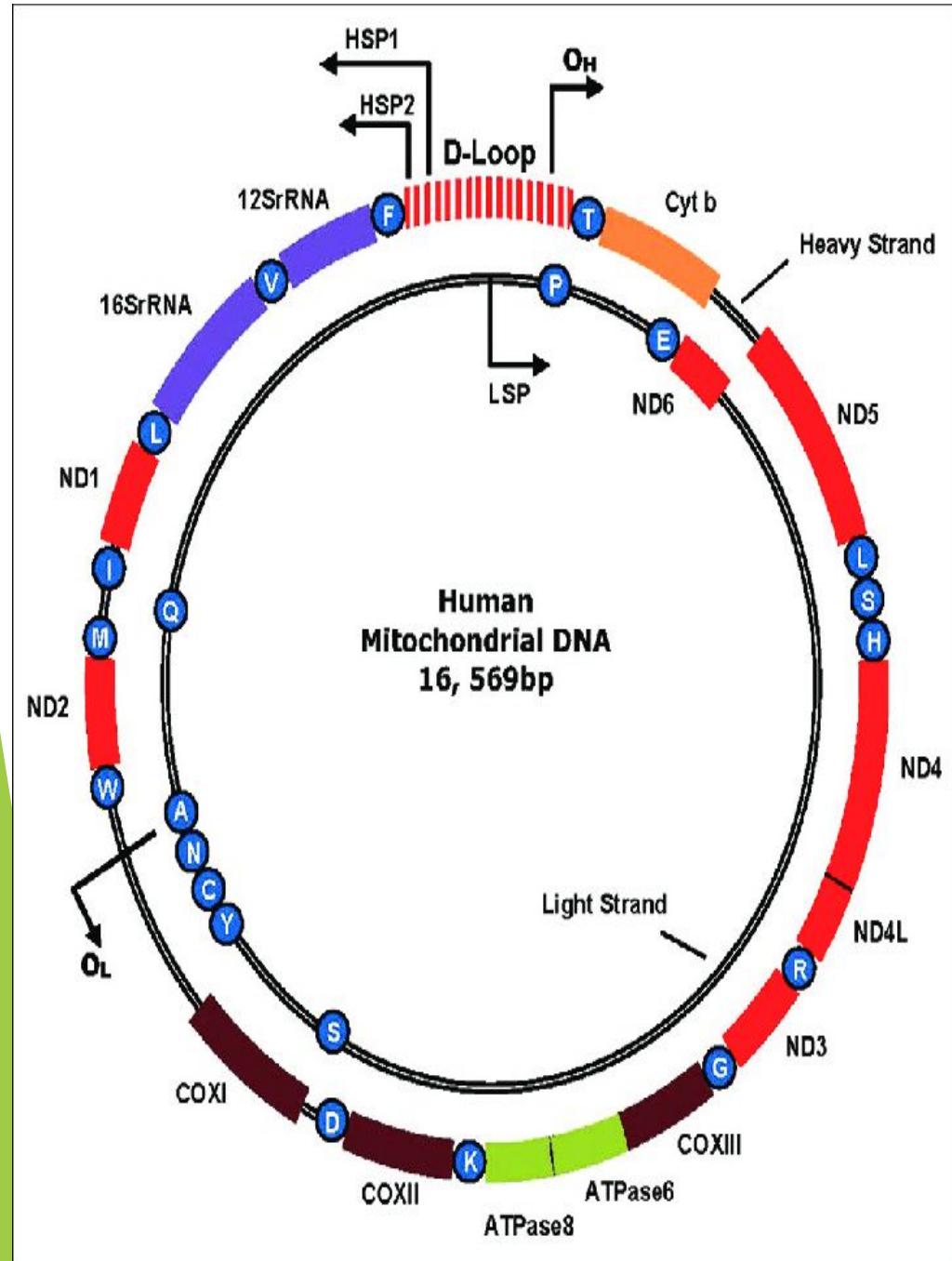
Rice mtDNA



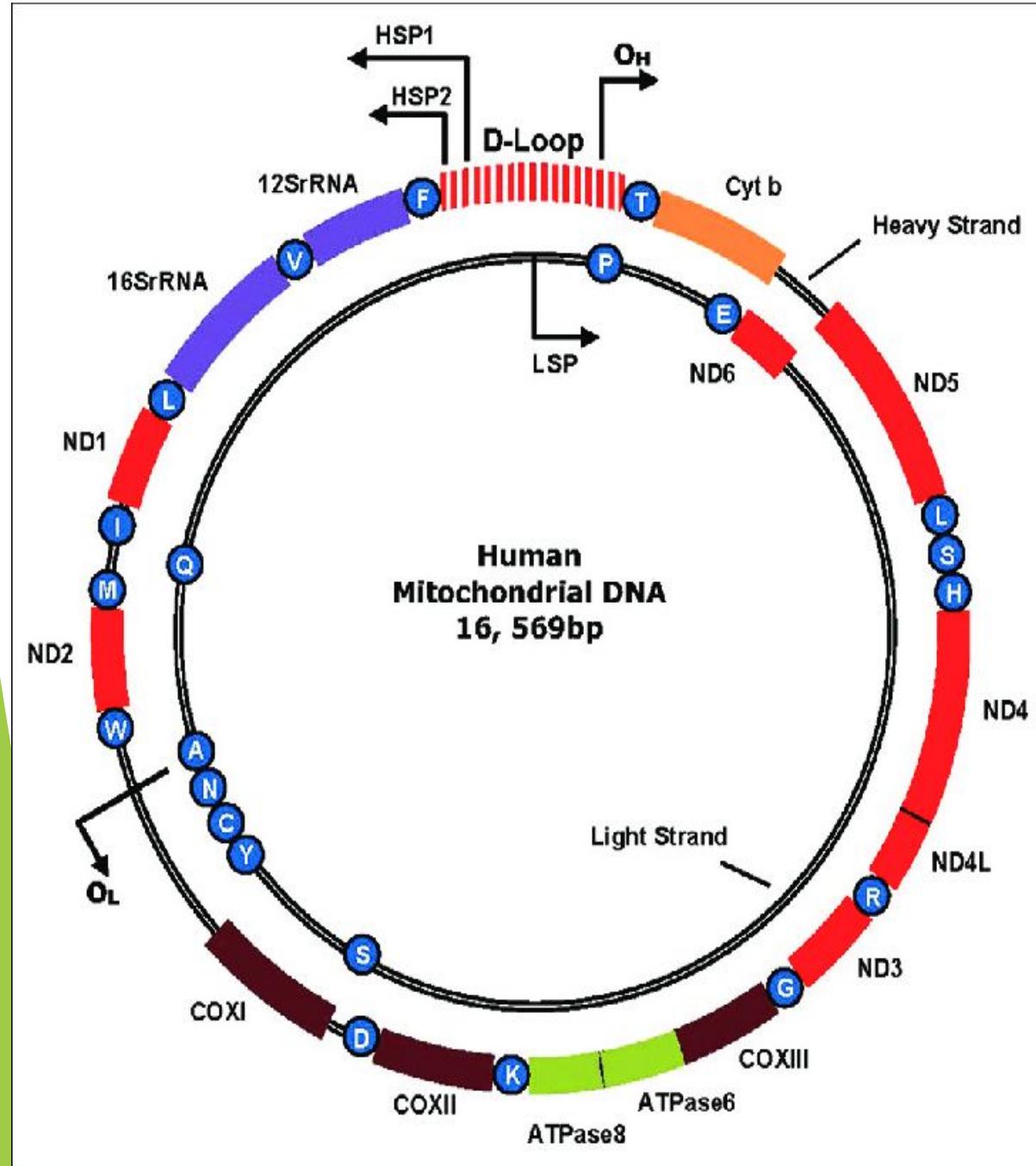
Human mtDNA



- Rice mitochondrial genome is made up of 492 kb nucleotides. The complexity of rice mitochondrial genome is due to the presence of chloroplast sequences. There are 16 chloroplast fragments in rice mtDNA ranging from 32 bp to about 6.8 kb in length. Thus, results show that about 6% (22 kb) of rice mtDNA is made up of chloroplast sequence.
- The mitochondrial genome of rice contains rearranged cluster of chloroplast genes, namely, *rpl2* and *yrpl23-rcL-atpB-atpE-trnM-trnV*.
- One of the palindromic repeated sequences (PRS) located in the intron of *rps3* in rice mtDNA, but not in maize mtDNA. Analysis of transferred sequences of ctDNA in mtDNA of rice was evidenced in that at least three repeated sequences about 60 bp in length are present in plant. It was also found that Rice mtDNA contained at least 10 copies of the small repeated sequence.



- ▶ Human mitochondrial DNA is a 16,569 base pair circle of double-stranded DNA that encodes 13 essential respiratory chain subunits.
- ▶ ND1-ND6 and ND4L encode seven complex I (NADH-ubiquinone oxidoreductase) subunits, CYT b encodes one subunit of complex III (ubiquinol: cytochrome c oxidoreductase), COX I-COX III encode the three major catalytic subunits of complex IV, and ATPase6 and ATPase8 encode two subunits of complex V (ATP synthase).
- ▶ There are the two ribosomal RNA (12S rRNA and 16S rRNA) genes and the 22 transfer RNA genes (blue spheres, depicted by single letter amino acid code abbreviation) required for mitochondrial protein synthesis. tRNAs are: F = Phenylalanine; V = Valine; L = Leucine; I = Isoleucine; Q = Glutamine; M = Methionine; W = Tryptophan; A = Alanine; N = Asparagine; C = Cysteine; Y = Tyrosine; S = Serine; D = Aspartic acid; K = Lysine; G = Glycine; R = Arginine; H = Histidine; E = Glutamic acid; T = Threonine; P = Proline.

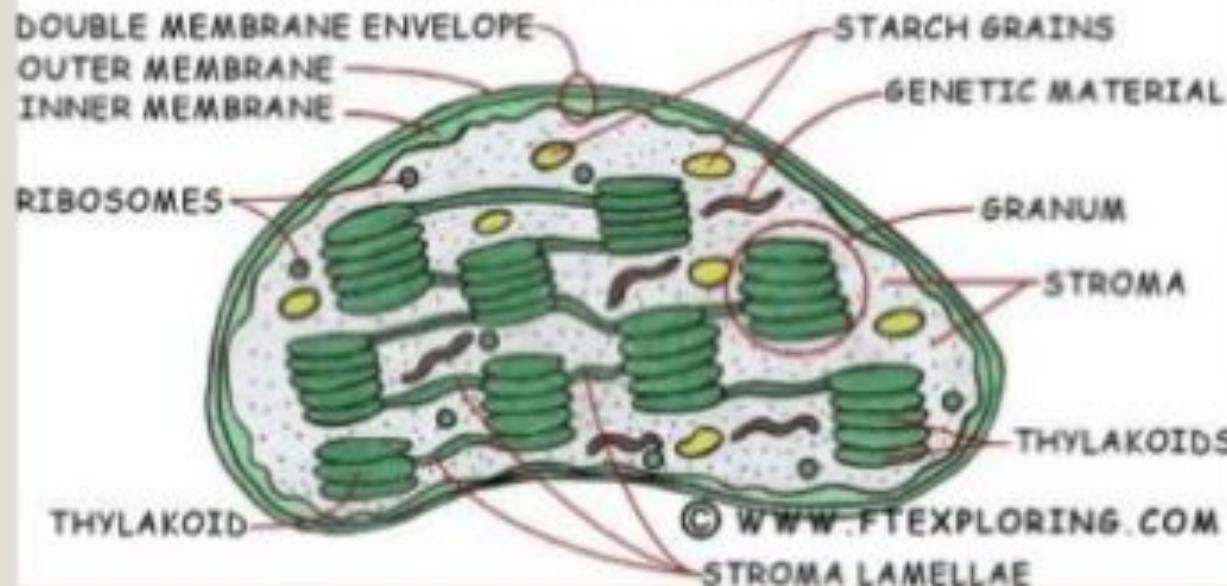


The displacement loop or noncoding control region contains the promoters for transcription of the L (LSP) and H strands (HSP1 and HS2) and the origin of replication of the H strand (O H).

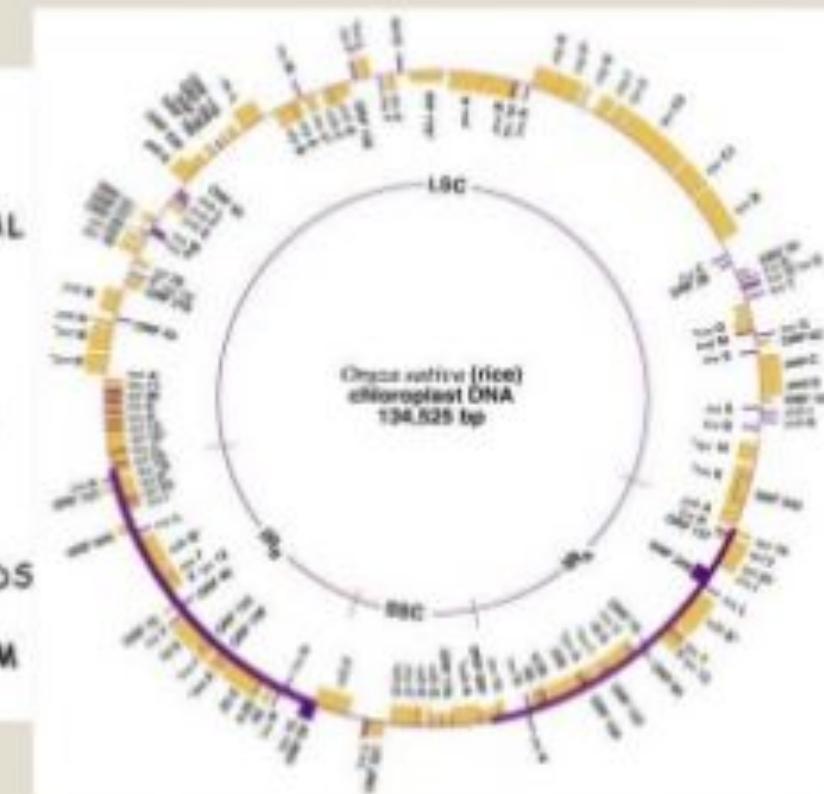
The origin of light strand replication is shown as O L

chloroplast genome

CHLOROPLAST



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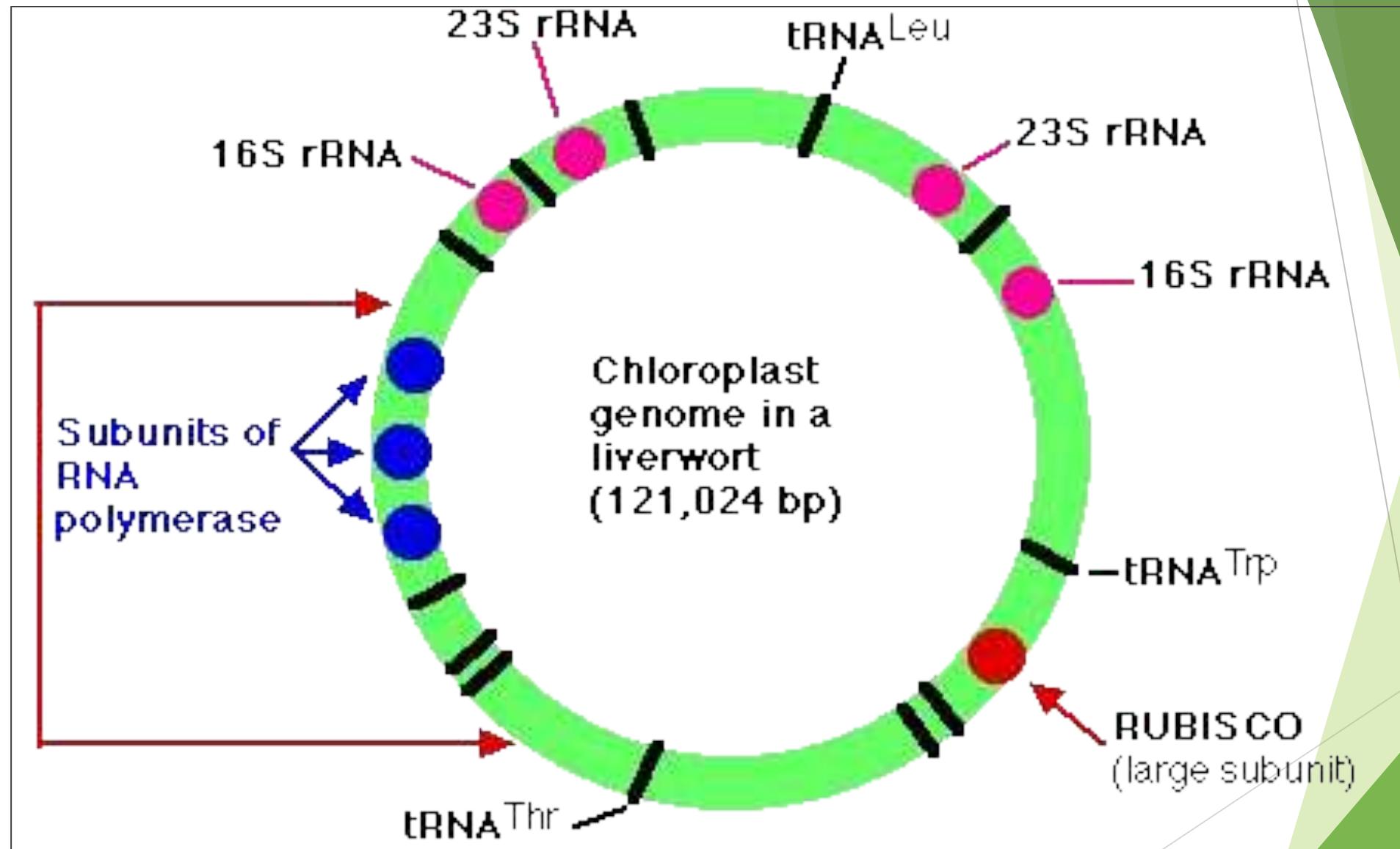


Chloroplast genomes (cpDNA)

- ❖ Chloroplast organelles are the site of photosynthesis and occur only in green plants and photosynthetic protists,
- ❖ Like mtDNA, chloroplast genome is:
 - Circular, double-stranded
 - Lacks structural proteins
 - %GC content differs
- ❖ Chloroplast genome is much larger than animal mtDNA, ~80-600 kb.
- ❖ Chloroplast genomes occur in multiple copies and carry lots of non-coding DNA.
- ❖ Complete chloroplast sequences have been determined for several organisms (tobacco 155,844 bp; rice 134,525 bp).

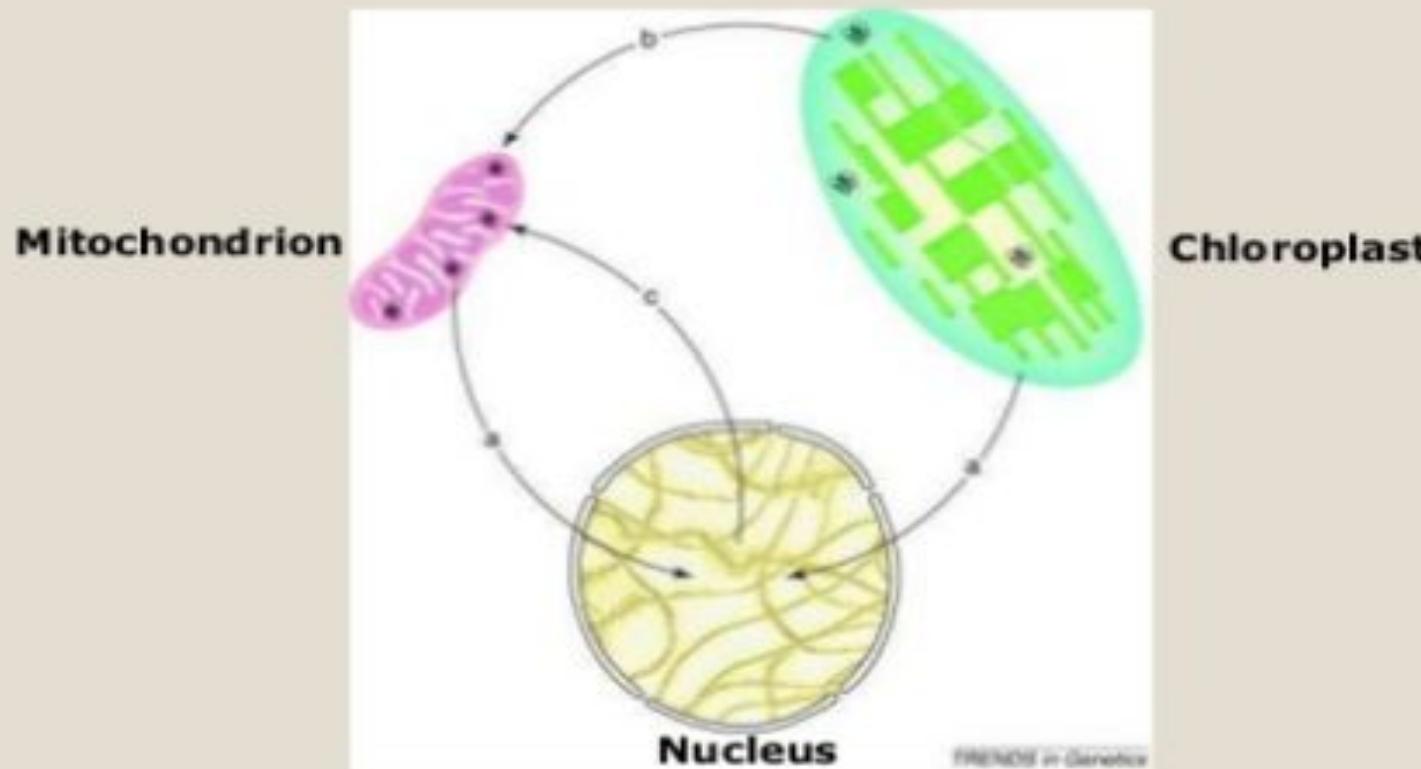
cpDNA organization

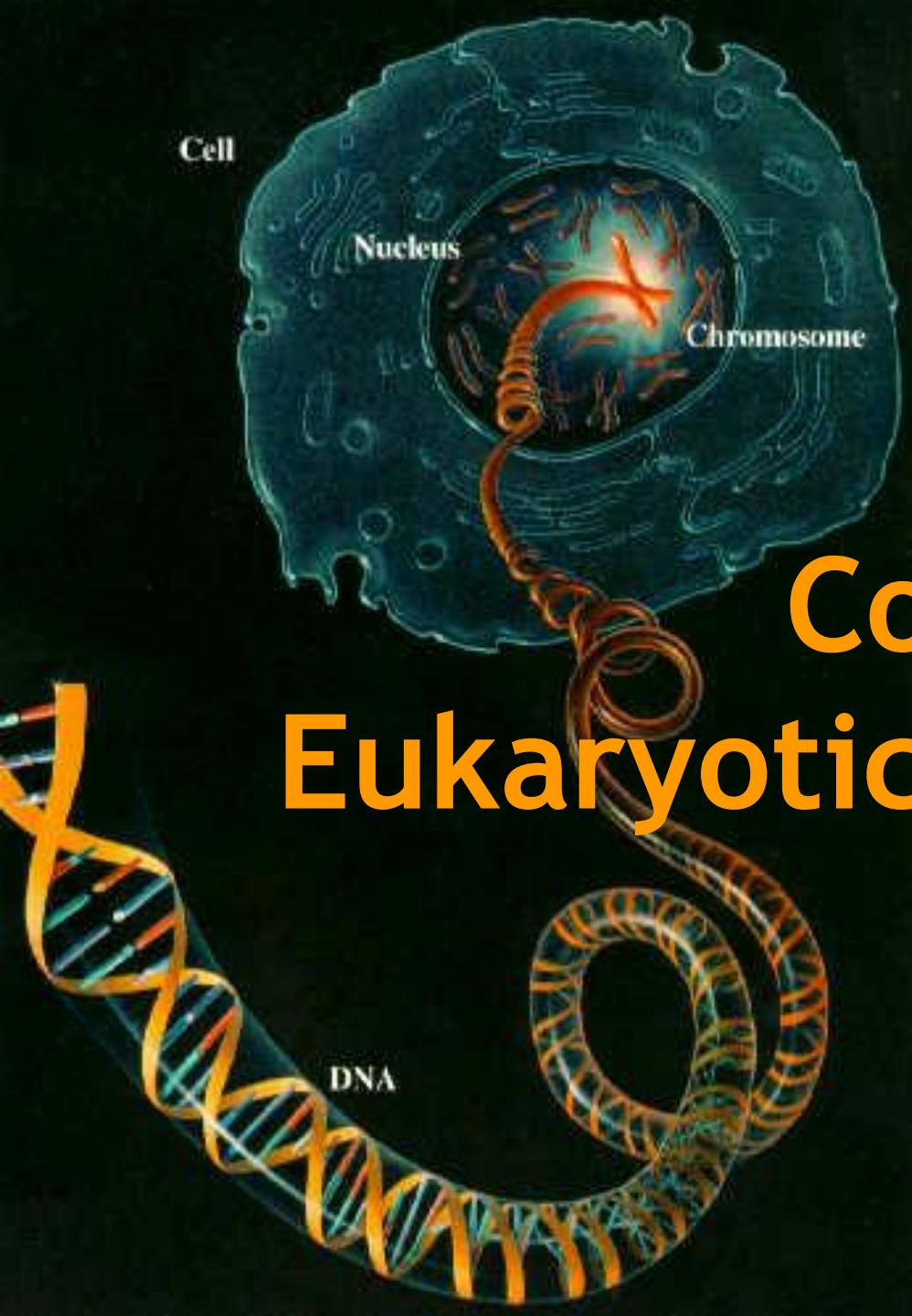
- ❖ Nuclear genome encodes some chloroplast components, and cpDNA codes the rest, including:
 - 2 copies of each chloroplast rRNA (16S, 23S, 4.5s, 5S)
 - tRNAs (30 in tobacco and rice, 32 in liverwort)
 - 100 highly conserved ORFs (~60 code for proteins required for transcription, translation, and photosynthesis).
- ❖ Genes are coded on both strands (like mtDNA).
- ❖ cpDNA translation- similar to prokaryotes:
 1. Initiation uses fMet-tRNA.
 2. Chloroplast specific IFs, EFs, and RFs.
 3. Universal genetic code.



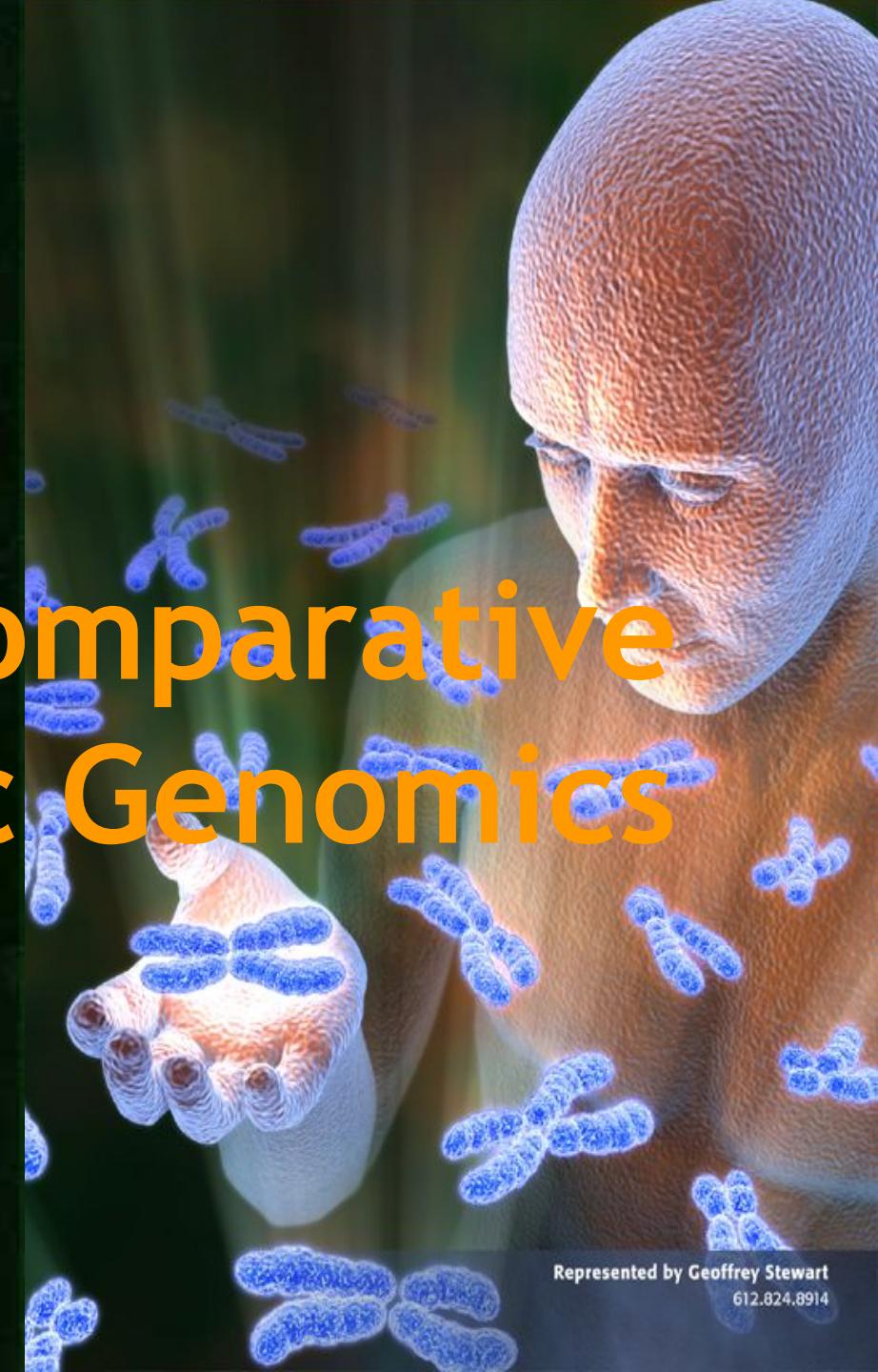
Relation between mt,cl and nuclear DNA.

Copies of **mtDNA** and **chloroplast** genes can be transposed to the nuclear genome and vice versa.





Comparative Eukaryotic Genomics



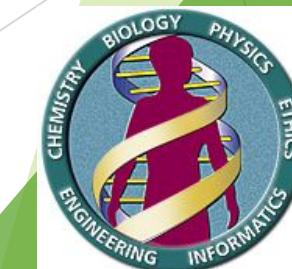
COMPARING THE GENOMES OF DIFFERENT ORGANISMS

- ▶ **Why Compare?**
 - ▶ Need to better understand the individual genomes
 - ▶ To understand the functioning of individual genes
 - ▶ To derive a comparative study of basic functions
 - ▶ To better understand evolutionary processes

HUMAN GENOME

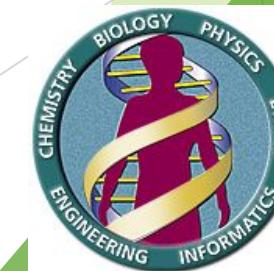
The Human Genome

- ▶ The human genome is by far **the most complex and largest genome**.
- ▶ Its size spans a length of about **6 feet of DNA, containing 30,000 to 40,000 genes**.



HUMAN GENOME PROJECT

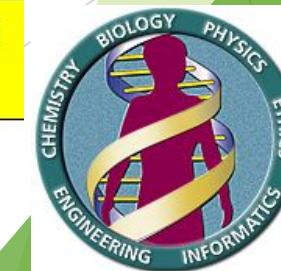
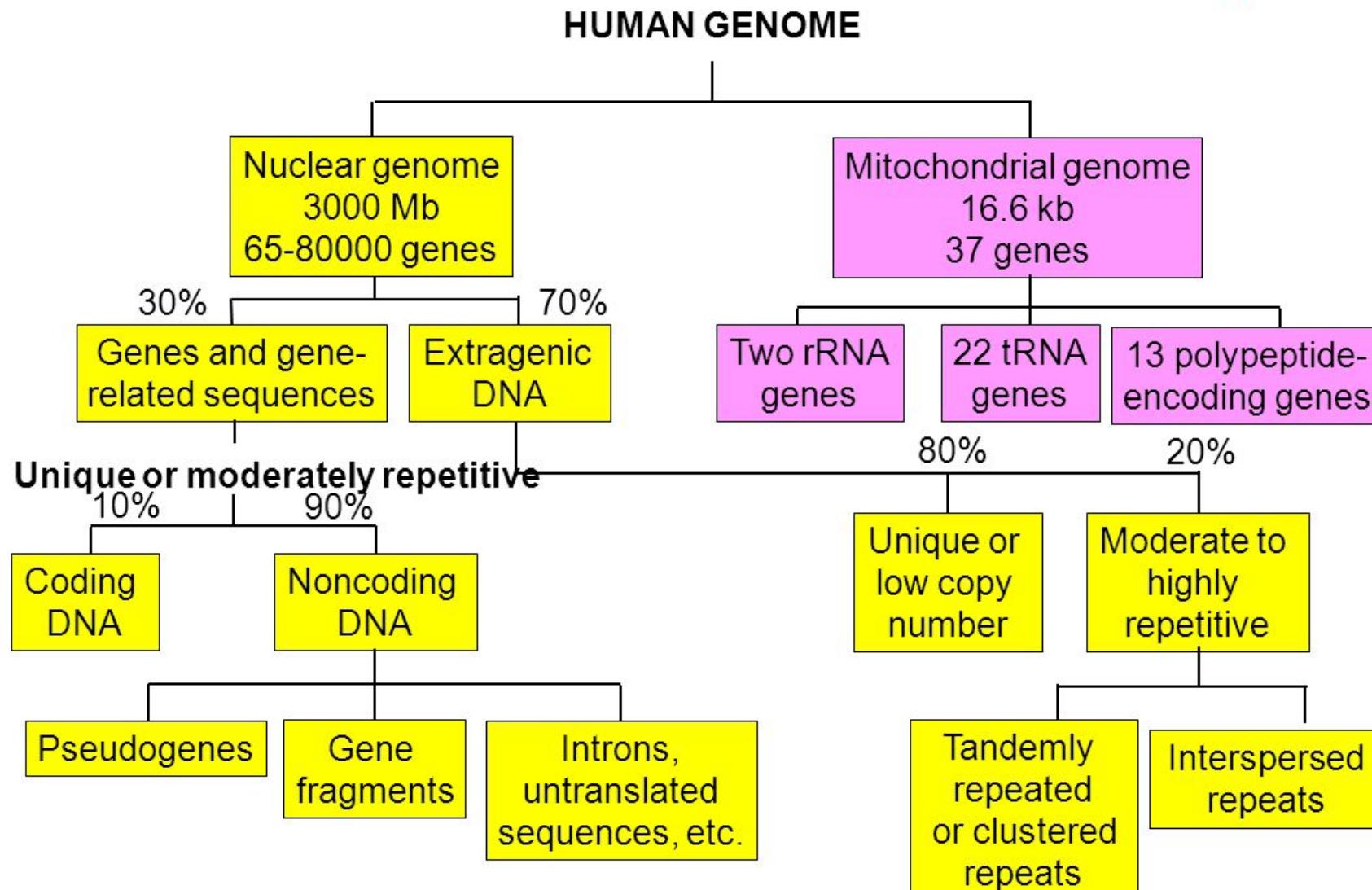
- ▶ Identify the approximate genes in human DNA.
- ▶ Determine the sequences of 3 billion chemical base pairs that make up human DNA.
- ▶ Store this information in databases.
- ▶ Improve tools for data analysis.



OVERVIEW OF HUMAN GENOME

- The human genome contains **3164.7 million nucleotide bases** (approx. 3 billion A,C,T and G).
- The average gene is made up of **3000 bases**, but sizes of genes vary greatly.

OVERVIEW OF HUMAN GENOME

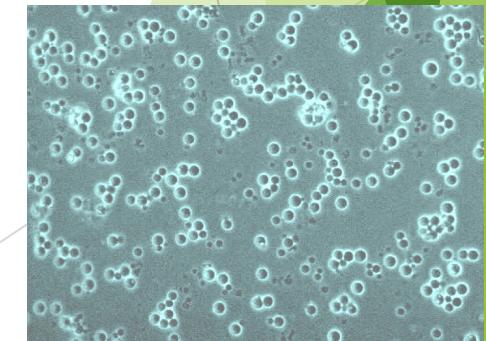


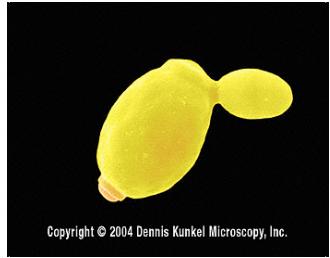


EUKARYOTIC GENOMES

Saccharomyces cerevisiae Genome

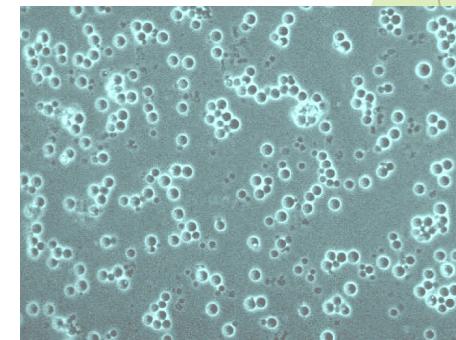
- ▶ One of the most important fungal organisms used in biotechnological processes.
- ▶ Considered as a **model eukaryotic organism**.
- ▶ The first eukaryotic organism to have its entire genome sequenced.





Saccharomyces cerevisiae Genome

- ▶ 16 chromosomes (2n)
- ▶ Approximate genome size - 15520 kb
- ▶ 5885 potential protein-coding genes.





Drosophila melanogaster (Fruit Fly) Genome

- ▶ Has been the most important tool for genetics studies in the twentieth century.
- ▶ Second multicellular organism to have its genome sequenced.
- ▶ Genome is about **180 Mb** in size.
- ▶ 4 chromosomes ($2n$)
- ▶ **13601** predicted genes.





Drosophila melanogaster (Fruit Fly) Genome

- ▶ Interestingly, the *Drosophila* genome contains genes that are similar to 177 of 289 human genes that are responsible for diseases.





Oryza sativa L. (rice) Genome

- ▶ One of the most important food crops in the world.
- ▶ Scientists use rice as a model plant in cereal genomics.
- ▶ 24 chromosomes (2n).





Mus musculus (Laboratory Mouse) Genome

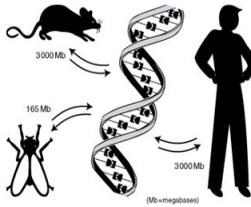
- ▶ The sequence of the mouse genome is important for understanding the contents of the human genome and it also serves as a key experimental tool for biomedical research.
- ▶ 20 chromosomes ($2n$)



Mus musculus (Laboratory Mouse) Genome

- ▶ The draft sequence was generated by assembling the sevenfold sequence coverage from female mice of the B6 strain.
- ▶ Genome size is 2.5 Gb.
- ▶ Seem to contain about 30000 protein-coding genes.





COMPARATIVE ANALYSIS OF THE HUMAN AND MOUSE GENOMES

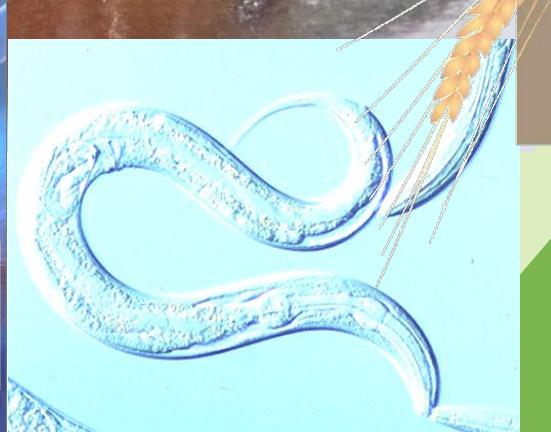
- ▶ The mouse genome is 14% smaller than the human genome.
- ▶ At the nucleotide level, approximately 40% of the human genome can be aligned to the mouse genome.

- ▶ The mouse and human genomes seem to contain about 30000 protein-coding genes.
- ▶ **Mouse-human sequence comparisons allow an estimate of the rate of protein evolution in mammals.**

GENERAL GENOMIC COMPARISONS

Organism	Genome Size (Bases)	Estimated Genes
Human (<i>Homo sapiens</i>)	3 billion	30,000
Laboratory mouse (<i>M. musculus</i>)	2.6 billion	30,000
Thale cress (<i>A. thaliana</i>)	100 million	25,000
Roundworm (<i>C. elegans</i>)	97 million	19,000
Fruit fly (<i>D. melanogaster</i>)	137 million	13,000
Yeast (<i>S. cerevisiae</i>)	12.1 million	6,000
Bacterium (<i>E. coli</i>)	4.6 million	3,200
Human immunodeficiency virus (HIV)	9700	9

Thank You !



Epigenetics

Overview and
concept

EPIGENETICS

- Epigenetics literally means "above" or "on top of" genetics. It refers to external modifications to DNA that turn genes "on" or "off." These modifications do not change the DNA sequence, but instead, *they affect how cells "read" genes.*
- The term epigenetics refers to heritable changes in gene expression that does not involve changes to the underlying DNA sequence; *a change in phenotype without a change in genotype.*

Conrad Waddington (1942) coined the term, “epigenetic”. He is known as father of epigenetics.



Conrad Hal
Waddington
(1905 to 1975)



➤ Epigenome

- An epigenome consists of a record of the chemical changes to the DNA and histone proteins of an organism.
- These changes can be passed down to an organism's offspring.
- Changes in the *epigenome* can result in changes to the structure of chromatin and changes to the function of the genome.
- The *epigenome* is a multitude of chemical compounds that can tell the genome what to do.

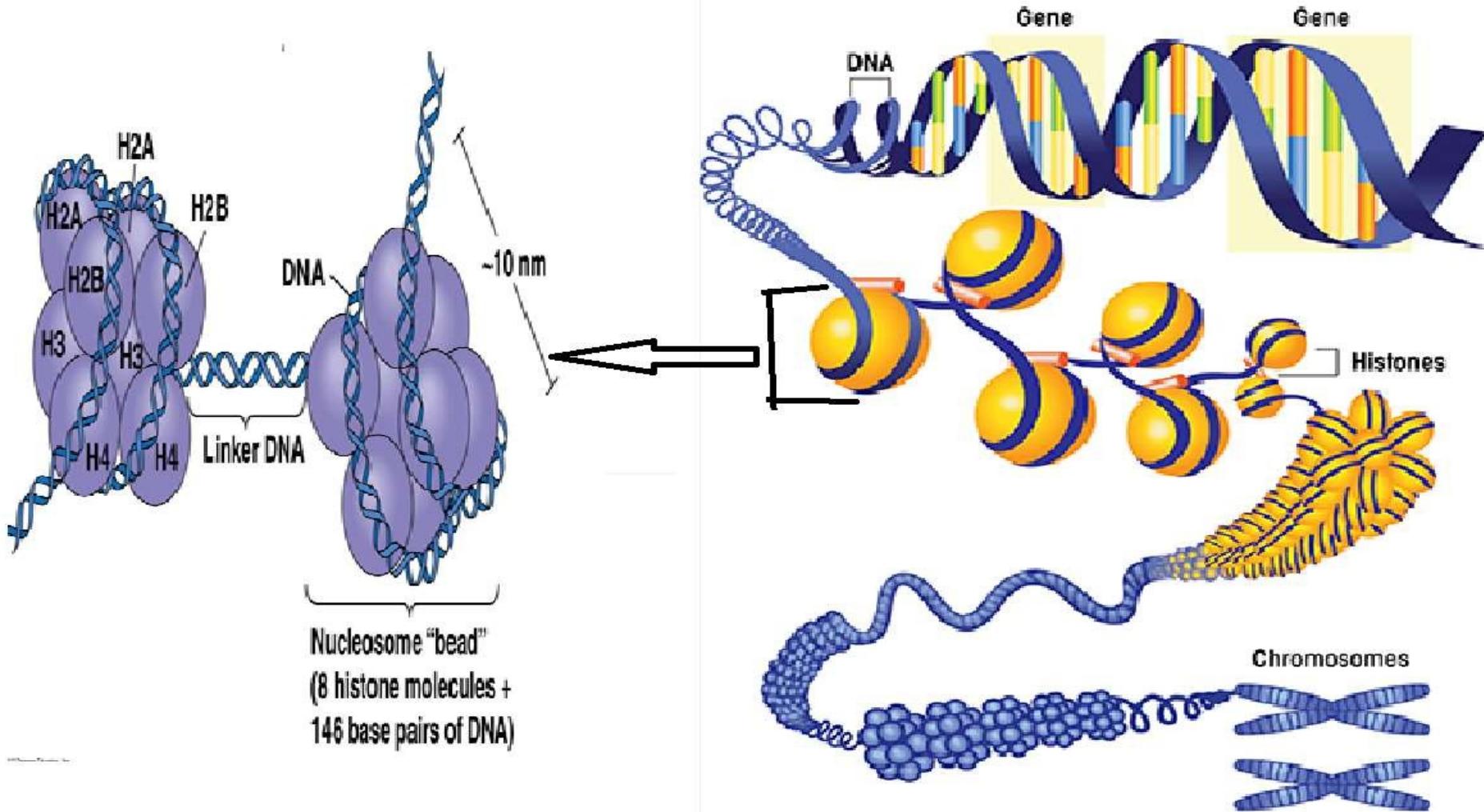
□ **Epialleles**

Alleles of a locus which have identical DNA sequences but display different epigenetic states and which have been proposed to influence a variety of phenotypes in plants and animals.



Molecular epigenetic mechanisms

DNA PACKAGING



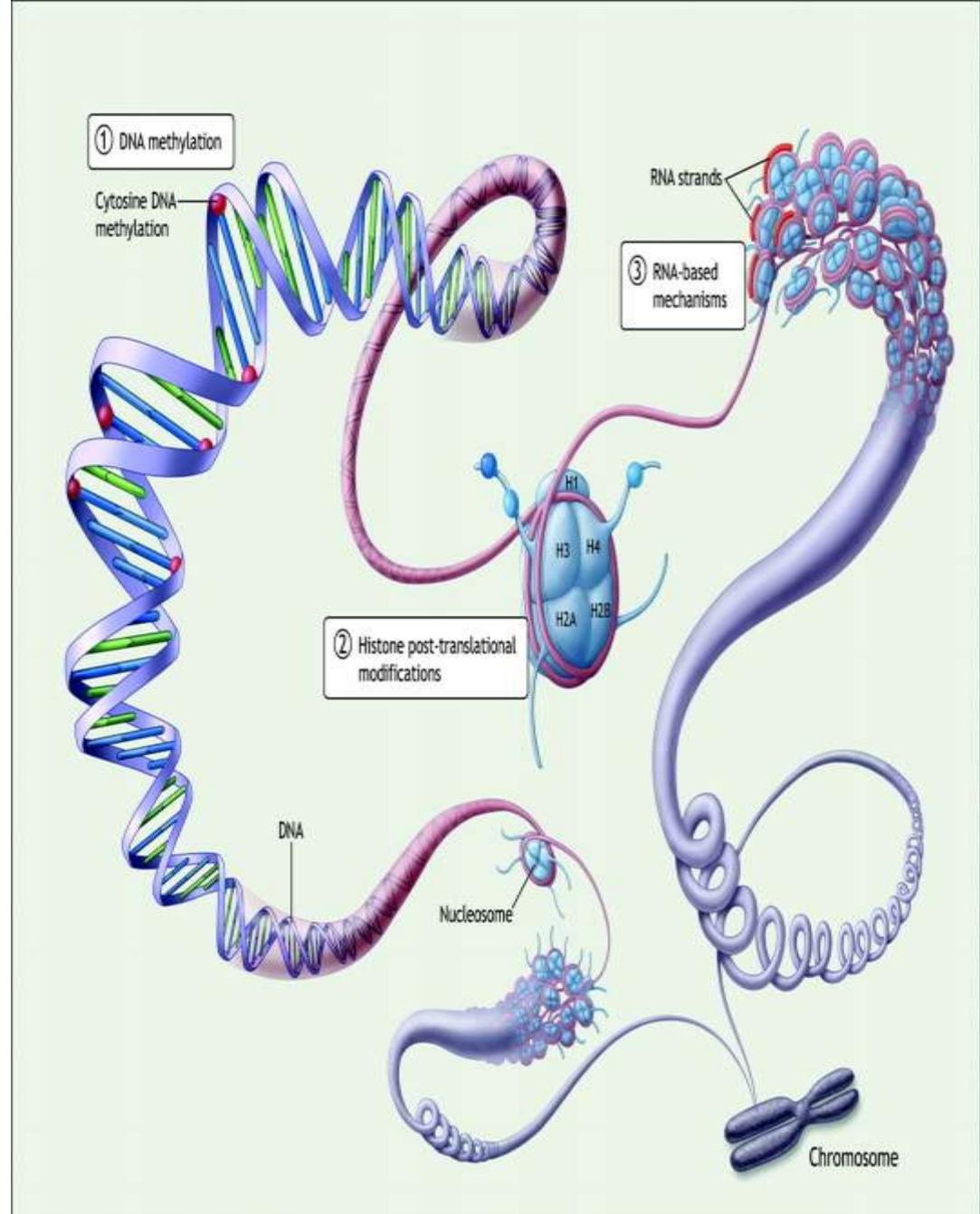
MECHANISMS

1. DNA methylation

2. Histone modifications

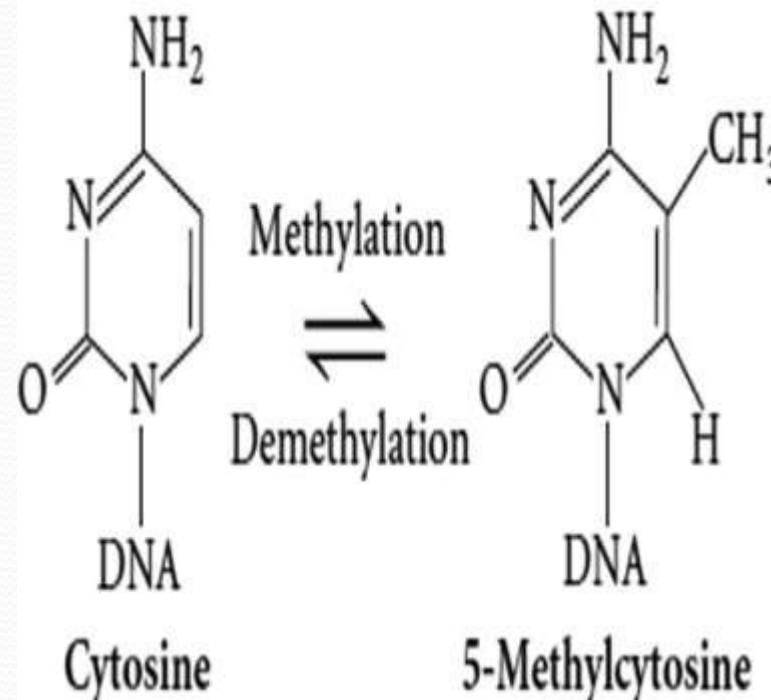
- Acetylation
- Methylation
- Phosphorylation,

3. RNA mediated interference



1.DNA METHYLATION

- DNA methylation is an epigenetic mechanism used by cells to control gene expression.
 - DNA methylation is the addition of a methyl group to the nitrogen atom of cytosine.
 - In eukaryotic cells, this process is catalysed out by a family of DNA methyltransferases enzymes, which transfers methyl groups from the methyl donor S-Adenosyl methionine (SAM) to the cytosine.
 - The resulting 5-methyl cytosine (5mC) is often repressive.

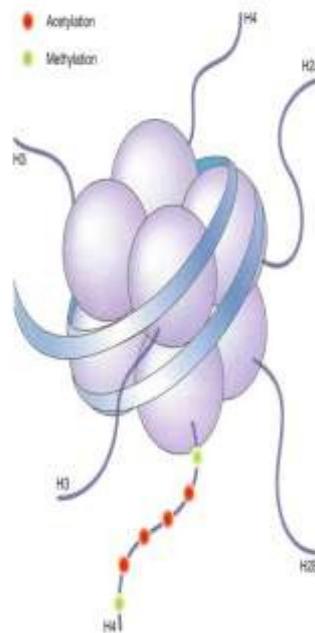


The repressive nature of 5mC is thought to inhibit the binding of DNA by transcription factors thus it is inaccessible for transcription

2. Histone modification

- They are the chief protein components of chromatin, acting as spools around which DNA winds, and playing a role in gene regulation.
- Histone modifications occur primarily on histone tails by three methods-
 - Methylation
 - Acetylation
 - Phosphorylation

Histone tails can be modified



a. Histone Methylation

- **Enzyme required**
 - Histone methytransferases (HMTs)
 - KMT- Lysine methyl transferase
-
- Methylation can result in activation or repression of genes.

b. Histone Acetylation &

□ **Histone acetylation**

- Histone acetyl transferases (HATs)

- Adds acetyl groups to histone tails.

- Reduces positive charge and weakens interaction of histones with DNA

- Facilitates transcription by making DNA more accessible to RNA polymerase II

□ **Histone deacetylation**

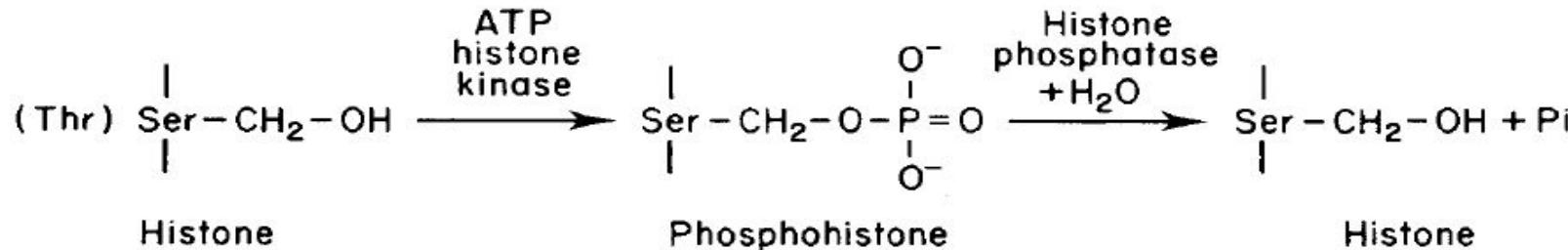
- Histone deacetylases (HDACs)

- Removes acetyl groups from histone tails

- Increases interaction of DNA and histones

- Represses transcription

C. Histone Phosphorylation



□ Phosphorylation

- Enzyme required – Protein kinase
- Phosphorylation increase the negative charge on Histone as a result less interaction between DNA and histones that leads to chromatin de-condensation.

□ Dephosphorylation

- Enzyme required - phosphatase
- increase positive charge followed by chromatin condensation.

3. RNA interference

- Also called post transcriptional gene silencing (PTGS)

CONCLUSION

- The field of epigenetics has rapidly developed into one of the most influential areas of scientific research.
- Recent advances in analytical methodology have allowed for a significant expansion of what is known about genome wide mapping of DNA methylation and histone modifications.
- Good knowledge of epigenetic mechanisms leads to better understanding of regulation of gene expression at transcriptional and post-transcriptional levels.
- Epigenetic mechanisms such as DNA methylation and histone modification play a key role in development and stress response.

THANK YOU
YOU

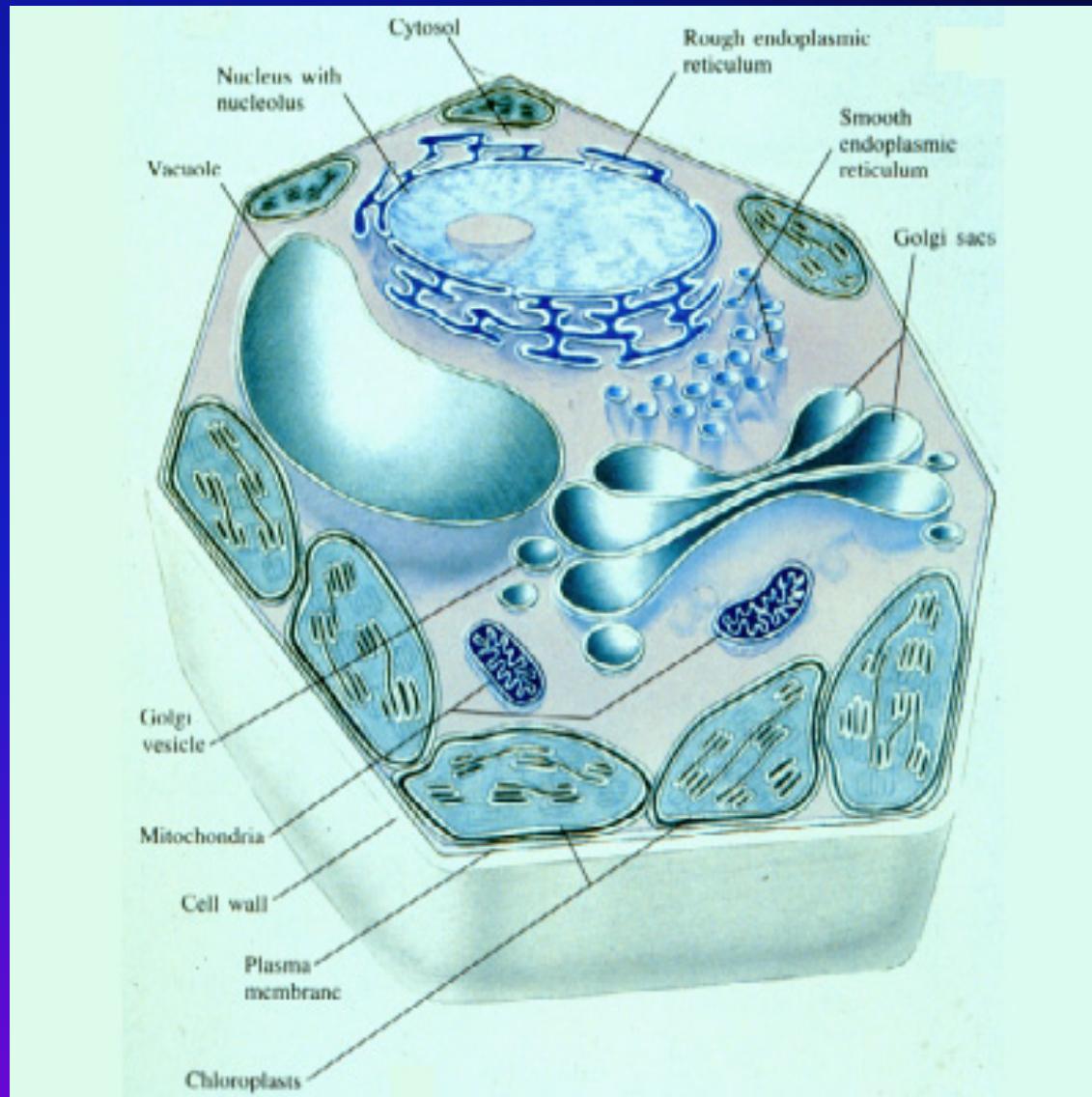
Eukaryotic and Prokaryotic Cells

Prokaryote = without a nucleus

Eukaryote = with a nucleus

Anandi R

Eukaryotic cells



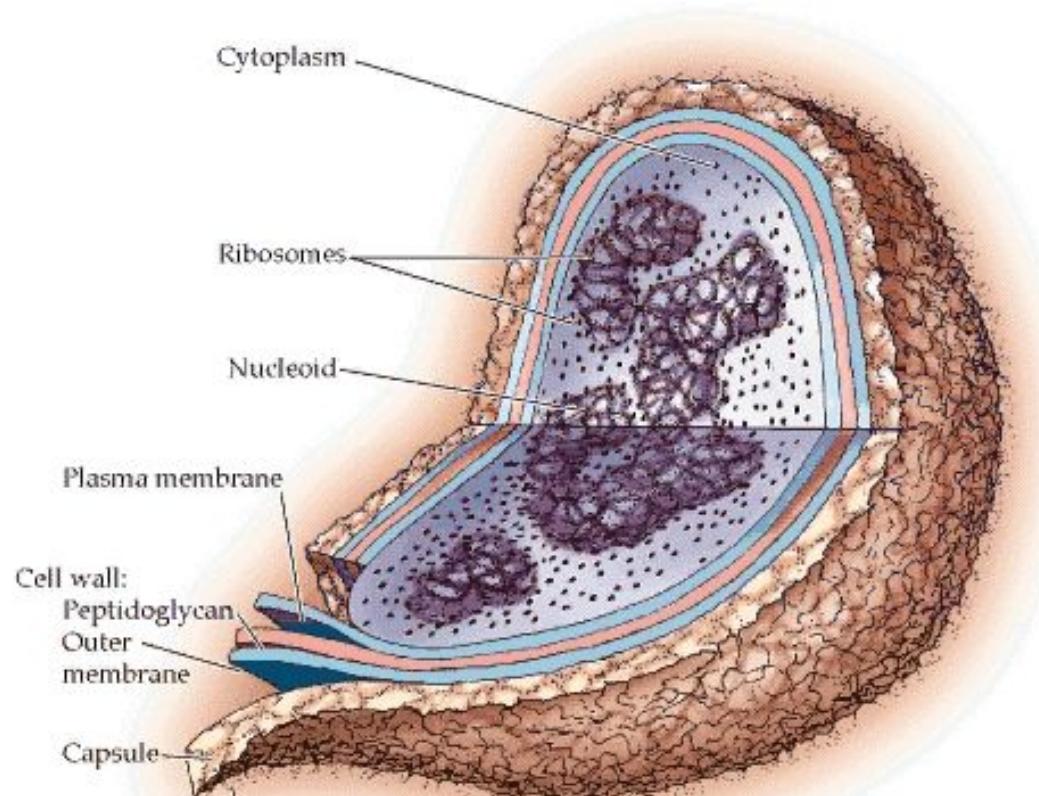
Components

- Cytoplasm
- Nucleus
- Mitochondria
- Chloroplast
- Ribosomes
- RER
- SER
- Golgi body
- Vacuoles

Components cont.

- Lysosomes
- Cytoskeleton
- Centriole
- Cilium and Flagellum
- Microvilli
- Cell membrane
- Cell Wall

Prokaryotic cells



Components

- Cytoplasm
- Ribosomes
- Nuclear Zone
- DNA
- Plasmid
- Cell Membrane
- Mesosome
- Cell Wall
- Capsule (or slime layer)
- Flagellum

Summary of differences!

Prokaryotic Cells	Eukaryotic cells
small cells (< 5 mm)	larger cells (> 10 mm)
always unicellular	often multicellular
no nucleus or any membrane-bound organelles	always have nucleus and other membrane-bound organelles
DNA is circular, without proteins	DNA is linear and associated with proteins to form chromatin
ribosomes are small (70S)	ribosomes are large (80S)
no cytoskeleton	always has a cytoskeleton
cell division is by binary fission	cell division is by mitosis or meiosis
reproduction is always asexual	reproduction is asexual or sexual

Basic terminology

- **Anticodon**

A sequence of three bases in tRNA that is complementary to a codon in mRNA.
Enables tRNA to sequence amino acids in the order specified by mRNA.

- **Autosome**

A non-sex chromosome. Synonymous with *somatic chromosomes* (chromosome pairs 1-22).

Basic terminology

- **Chromosome**

Rod-shaped structures within the cell nucleus that carry genes encoded by DNA.

- **Cis position**

Genes in the cis position are on the same chromosome of a pair of homologous chromosomes.

- **Cloned gene**

- A recombinant DNA molecule with the gene of interest.

Basic terminology

- **Co-dominant**

Genes are co-dominant if both alleles are expressed in the heterozygous state, e.g., K and k genes

- **Codon**

A sequence of three bases in DNA or RNA that codes for a single amino acid. Enables specific proteins to be made by specific genes.

Basic terminology

- **Crossing over**

The exchange of genetic material between members of a pair of homologous chromosomes.

- **Deletion**

An abnormality in which part of a chromosome (carrying genetic material) is lost.

Basic terminology

- **Diploid number of chromosomes**

The number of chromosomes found in somatic cells, which in humans is 46.

- **DNA**

Deoxyribonucleic acid. Composed of nucleic acids, these molecules encode the genes that allow genetic information to be passed to offsprings.

Basic terminology

- **DNA polymerases**

Enzymes that can synthesize new DNA strands using previously synthesized DNA (or RNA) as a template.

- **DNA probe**

A cloned DNA molecule labelled with a radioactive isotope (e.g., ^{32}P or ^{35}S) or a nonisotopic label (e.g. biotin). Used in molecular genetics to identify complementary DNA sequences by hybridizing to them.

Basic terminology

- **Dominant gene**

A gene is dominant if it is expressed when heterozygous but its allele is not.

- **Functional genes**

Genes that produce proteins, e.g., blood group genes that produce antigens.

- **Gene**

A segment of a DNA molecule that codes for the synthesis of a single polypeptide.

Basic terminology

- **Gene interaction**

The situation in which genes inherited at different loci.

- **Genome**

Term used to denote the entire DNA sequence (gene content) of a gamete, person, population, or species.

Basic terminology

- **Homologous chromosomes**

A matched pair of chromosomes, one from each parent.

- **Linkage**

Genes are linked if they are on the same chromosome within a measurable distance of each other and are normally inherited together.

- **Locus**

The location of allelic genes on the chromosome.
(*Plural = loci*)

Basic terminology

- **Messenger RNA (mRNA)**

Type of RNA polymerase using DNA as a template. Contains the codons that encompass the genetic codes to be translated into protein.

- **Nucleic acids**

Polymers of phosphorylated nucleosides, the building blocks of DNA and RNA.

Basic terminology

- **Nucleoside**

The building blocks of RNA and DNA.

Compounds consisting of a purine (adenine or guanine) or pyrimidine (thymine or cytosine) attached to ribose (in RNA) or deoxyribose (in DNA) at the 1^1 carbon.

Basic terminology

- **Operator**

A short sequence of nucleotides that controls the adjacent structural (functional) genes.

- **Operon**

A postulated unit of gene action that consists of an operator and the closely linked functional genes it controls.

Basic terminology

- **Plasmid**
- Extrachromosomal circular DNA in bacteria. Plasmids can independently replicate and encode a product for drug resistance or some other advantage. Used in molecular genetics as vectors for cloned segments of DNA.

Basic terminology

- **Reverse transcriptase**

An RNA-dependent DNA polymerase that synthesizes DNA from an RNA template. Used by retroviruses like the human immunodeficiency virus (HIV) to make proviral DNA from its RNA genome.

- **Transcription**

Synthesis of single-stranded RNA by RNA polymerase using DNA as a template.

Basic terminology

- **Restriction fragment length polymorphisms (RFLP)**

Regions of DNA of varying lengths that can be cut out of DNA by restriction endonucleases. Because the fragment lengths vary among individuals, they are polymorphic and can be used as genetic markers.

Basic terminology

- **Translation**
- The process of translating the codon sequence in mRNA into polypeptides with the help of tRNA and ribosomes.

Eukaryotic and prokaryotic genome

Genome

- The word “genome,” coined by German botanist Hans Winkler in 1920, was derived simply by combining *gene* and the final *chromosome*.
- An organism’s **genome** is defined as the complete haploid genetic complement of a typical cell.
- In diploid organisms, sequence variations exist between the two copies of each chromosome present in a cell.
- The genome is the ultimate source of information about an organism.

- The number of genomes sequenced in their entirety is now in the thousands and includes organisms ranging from bacteria to mammals.
- The first complete genome to be sequenced was that of the bacterium *Haemophilus influenzae*, in 1995.
- The first eukaryotic genome sequence, that of the yeast *Saccharomyces cerevisiae*, followed in 1996.
- The genome sequence for the bacterium *Escherichia coli* became available in 1997 .
- The much larger effort directed at the human genome was also accelerating

Prokaryotes and Eukaryotes genome

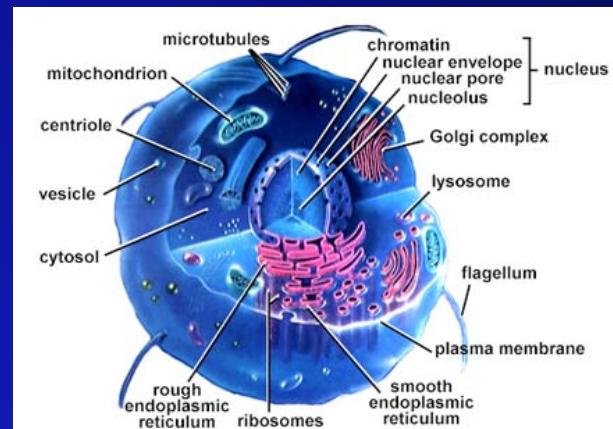
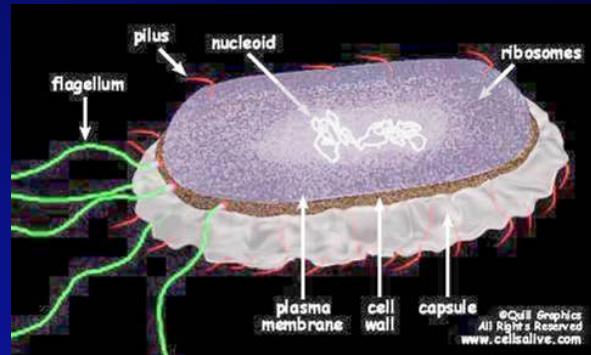
Prokaryotes	Eukaryotes
Single cell	Single or multi cell
No nucleus	Nucleus
One piece of circular DNA	Chromosomes
No mRNA post transcriptional modification	Exons/Introns splicing

Prokaryotic and Eukaryotic Cells

Chromosomal differences

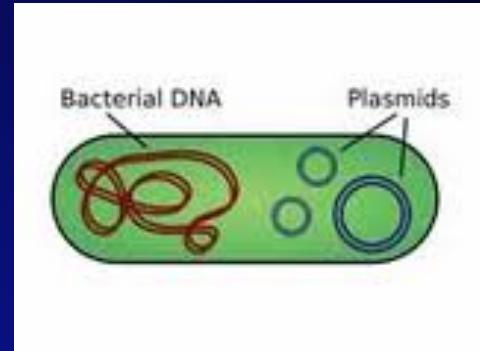
Prokaryotes

- The genome of E.coli contains amount of 4×10^6 base pairs
- > 90% of DNA encode protein
- Lacks a membrane-bound nucleus.
 - Circular DNA and supercoiled domain
- Histones not present



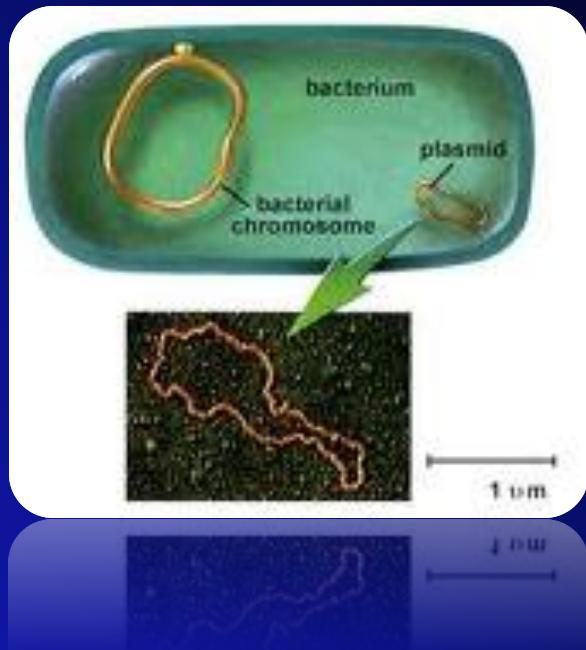
Continue...

- Prokaryotic genomes generally contain one large circular piece of DNA referred to as a "chromosome" (not a true chromosome in the eukaryotic sense).
- Some bacteria have linear "chromosomes".
- Many bacteria have small circular DNA structures called plasmids which can be swapped between neighbors and across bacterial species.



Plasmid

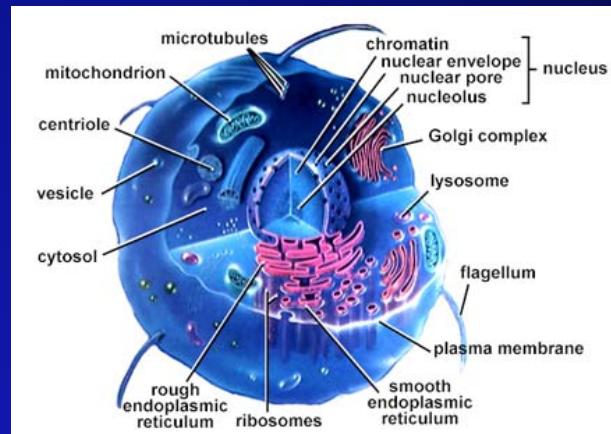
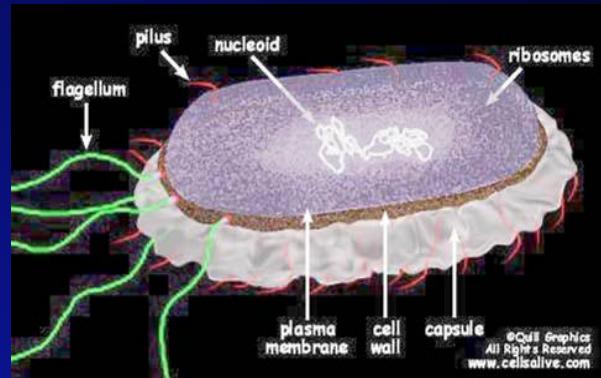
- The term *plasmid* was first introduced by the American molecular biologist Joshua Lederberg in 1952.
- A **plasmid** is separate from, and can replicate independently of, the chromosomal DNA.
- Plasmid size varies from 1 to over 1,000 (kbp).



Continue...

Eukaryotes

- The genome of yeast cells contains 1.35×10^7 base pairs
- A small fraction of the total DNA encodes protein.
 - Many repeats of non-coding sequences
- All chromosomes are contained in a membrane bound nucleus
 - DNA is divided between two or more chromosomes
- A set of five histones
 - DNA packaging and gene expression regulation



Karyotype

- The study of chromosomes, their structure and their inheritance is known as *Cytogenetics*.
- Each species has a characteristic number of chromosomes and this is known as *karyotype*.

• Bacteria	1
• Fruit fly	8
• Garden Pea	14
• Yeast	16
• Frog	26
• Cat	38

• Fox	34
• Mouse	40
• Rat	42
• Rabbit	44
• Human	46
• Chicken	78

Continue...

- Prior to 1950's it was believed that humans had 48 chromosomes but in 1956 it was confirmed that each human cell has *46 chromosomes* (Tjio and Levan, 1956).
- On the chromosomes the genes are situated in a linear order.
- Each gene has a precise position or *locus*.
- The size of bacterial chromosomes ranges from 0.6 -10 Mbp, and the size of Archaeal range from 0.5 - 5.8 Mbp, whereas Eukaryotic chromosomes range from 2.9 - 4,000 Mbp.

Bacterial genome

- Bacterial genomics can give us a broader understanding of how a bacteria functions, a bacteria's origins, and what bacteria live in our world that we can study by their DNA.
- Of medical interest, bacterial genomics is also anticipated to play a significant role in speeding up the development of better therapies and vaccines for controlling disease-causing bacteria.
- It will also be the cornerstone of anticipated DNA- based diagnostic tools that will hopefully enable doctors to make quicker, more accurate diagnoses of infectious disease.

Size of Bacterial genome

- The size of Bacterial chromosomes ranges from 0.6 Mbp to over 10 Mbp
- The smallest Bacterial genome identified thus far is from *Mycoplasma genitalium*, an obligate intracellular pathogen with a genome size of 0.58 Mbp (580 Kbp).
- *M. genitalium* is restricted to the intracellular niche because it lacks genes encoding enzymes required for amino acid biosynthesis and the peptidoglycan cell wall, genes encoding TCA cycle enzymes, and many other biosynthetic genes.

Size of Bacterial genome

- The smallest free-living organisms have a genome size over 1 Mbp.
- Currently largest sequenced prokaryotic genome is streptomyces, 8.7 Mbp.
- The average gene content is 3,100 genes per genome.

General features of Bacterial Chromosomes

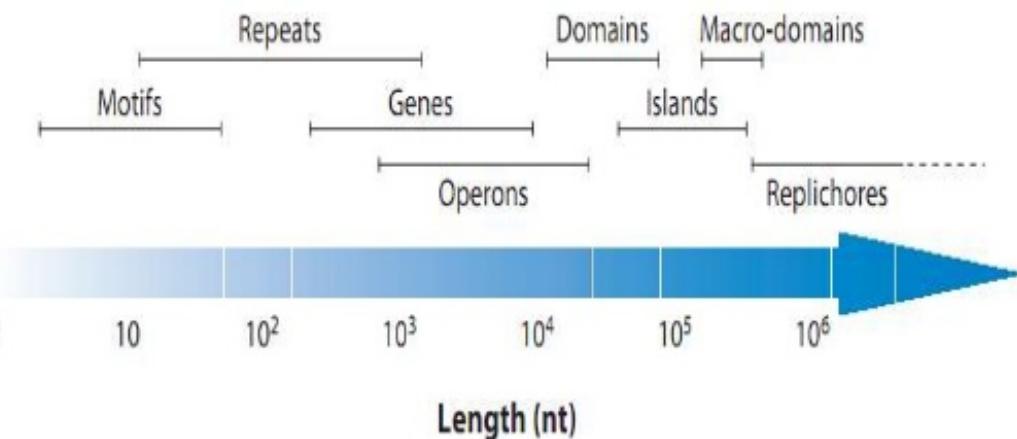
- Not all bacteria have a single circular chromosome.
- some bacteria have multiple circular chromosomes.
- Many bacteria have linear chromosomes and linear plasmids.
- In 1989, pulsed field gel electrophoresis had been developed, and this new technique provided convincing evidence that the chromosome of *Borrelia burgdorferi* was linear.
- *Agrobacterium tumefaciens*: One linear (2.1 Mb) +One circular (3.0 Mb) + Two circular plasmids (450 kb + 200 Kb)

Genome Packaging in Prokaryotes

- Prokaryotic cells do not contain nuclei or other membrane-bound organelles.
- In fact, the word "prokaryote" literally means "before the nucleus."
- The nucleoid is simply the area of a prokaryotic cell in which the chromosomal DNA is located.

The scales of Genome Organisation

The scales of Genome Organisation



The scales of genome organization.

The scales of Genome Organisation

- Replichores

Are the halves of the chromosome between the origin of replication and terminus region in the vicinity of the dif site.

- Sequence motifs

Is a nucleotide or amino acid sequence pattern

- Genomic islands

It's a code for symbiosis or pathogenesis.

DNA Supercoiling

- One way prokaryotes compress their DNA into smaller spaces is through supercoiling.
- Genomes can be negatively supercoiled, meaning that the DNA is twisted in the opposite direction of the double helix, or positively supercoiled, meaning that the DNA is twisted in the same direction as the double helix.
- Most bacterial genomes are negatively supercoiled during normal growth.

Proteins Involved in Supercoiling

- Multiple proteins act together to fold and condense prokaryotic DNA.
- In particular, one protein called HU, which is the most abundant protein in the nucleoid, works with an enzyme called topoisomerase I to bind DNA and introduce sharp bends in the chromosome, generating the tension necessary for negative supercoiling.
- Integration host factor (IHF), can bind to specific sequences within the genome and introduce additional bends.

Proteins Involved in Supercoiling

- The folded DNA is then organized into a variety of conformations that are supercoiled and wound around tetramers of the HU protein, much like eukaryotic chromosomes are wrapped around histones.
- Once the prokaryotic genome has been condensed, DNA topoisomerase I, DNA gyrase, and other proteins help maintain the supercoils.

Operons

- When different genes are to be expressed in exactly the same amount because they are part of a complex, transcription of all genes in a single transcript diminishes gene expression.
- Pairs of divergently oriented operons show correlated expression levels this is because sometimes they share bidirectional regulatory regions that allow co regulation of the two operons.

Table : Prokaryotic versus Eukaryotic Chromosomes

Prokaryotic Chromosomes	Eukaryotic Chromosomes
<ul style="list-style-type: none">• Many prokaryotes contain a single circular chromosome.• Prokaryotic chromosomes are condensed in the nucleoid via DNA supercoiling and the binding of various architectural proteins.• Because prokaryotic DNA can interact with the nucleus, and translation occurs in the cytoplasm, transcription and translation occur simultaneously.• Most prokaryotes contain only one copy of each gene (i.e., they are haploid).• Nonessential prokaryotic genes are commonly encoded on extrachromosomal plasmids.• Prokaryotic genomes are efficient and compact, containing little repetitive DNA.	<ul style="list-style-type: none">• Eukaryotes contain multiple linear chromosomes.• Eukaryotic chromosomes are condensed in a membrane-bound nucleus via histones.• In eukaryotes, transcription occurs in the nucleus, and translation occurs in the cytoplasm.• Most eukaryotes contain two copies of each gene (i.e., they are diploid).• Some eukaryotic genomes are organized into operons, but most are not.• Extrachromosomal plasmids are not commonly present in eukaryotes.• Eukaryotes contain large amounts of noncoding and repetitive DNA.

Regulation of Gene Expression in Eukaryotes

- By Anandi R

How does a eukaryotic organism
regulate the expression of gene
leading to the production of correct
protein?

Expression of Different Genes

- House keeping genes
- Genes required during cellular differentiation
- Genes which get triggered as a response to some external factors
- Genes which get triggered during apoptosis

Mechanism of Gene Regulation in Prokaryotes and Eukaryotes

- **In prokaryotes the primary control point is the process of transcription initiation**
- **In eukaryotes expression of gene into proteins can be controlled at various locations.**

Check Points for Gene Expression in Eukaryotes

- **Synthesis of proteins is controlled right from the chromatin stage.**
- **Expression of gene is controlled at many steps during the process of transcription and translation.**
- **Description of the control points is dealt in detail in the subsequent slides.**

1. Chromatin Structure

Two forms of chromatin

- **Euchromatin** – A less coiled transcriptionally active region which can be easily accessed by the RNA polymerases.
- **Heterochromatin** – A highly condensed transcriptionally inactive region. The genes in this region cannot be accessed by the RNA polymerases for active transcription.

1. Chromatin Structure

Mechanisms which affect the chromatin structure and hence the expression of gene are:

- **Histone modifications** – These modifications make a region of gene either transcriptionally active or inactive.
 - a) **Acetylation**
 - ↑ Acetylation ----↓ Condensation of DNA ----- ↑ Transcription of genes in that region

1. Chromatin Structure

Methylation

- **Methylation of histone H4 on R4 (arginine residue at the 4th position) →→ opens the chromatin structure →→ leading to transcriptional activation**
- **Methylation of histone H3 on K4 and K79 (lysines residues at the 4th and 79th position) →→ opens the chromatin structure →→ leading to transcriptional activation**
- **Methylation of histone H3 on K9 and K27 (lysines residues at the 9th and 27th position) →→ condenses the chromatin structure →→ leading to transcriptional inactivation**

1. Chromatin Structure

b) **Ubiquitination**

- **Ubiquitination of H2A – Transcriptional inactivation**
- **Ubiquitination of H2B - Transcriptional activation**

2) **Methylation of DNA**

- **Target sites of methylation are - The cytidine residues which exist as a dinucleotide, CG (written as CpG)**
- **↑ methylated cytidine -- ↓ Transcriptional activity**

2. Regulation of Transcription

- The differences in the mechanisms by which the transcription of gene is controlled in prokaryotes and eukaryotes are listed below:

Prokaryotes	Eukaryotes
The linked genes are organized into clusters known as operons which are under the control of a single promoter.	Eukaryotic genes are not organized into operons and each of these genes requires its own promoter.
These genes are primarily regulated by repressors.	Regulation by repressors is very occasional and the primary role of regulation is played by the transcriptional activators known as transcription factors.

2. Regulation of Transcription

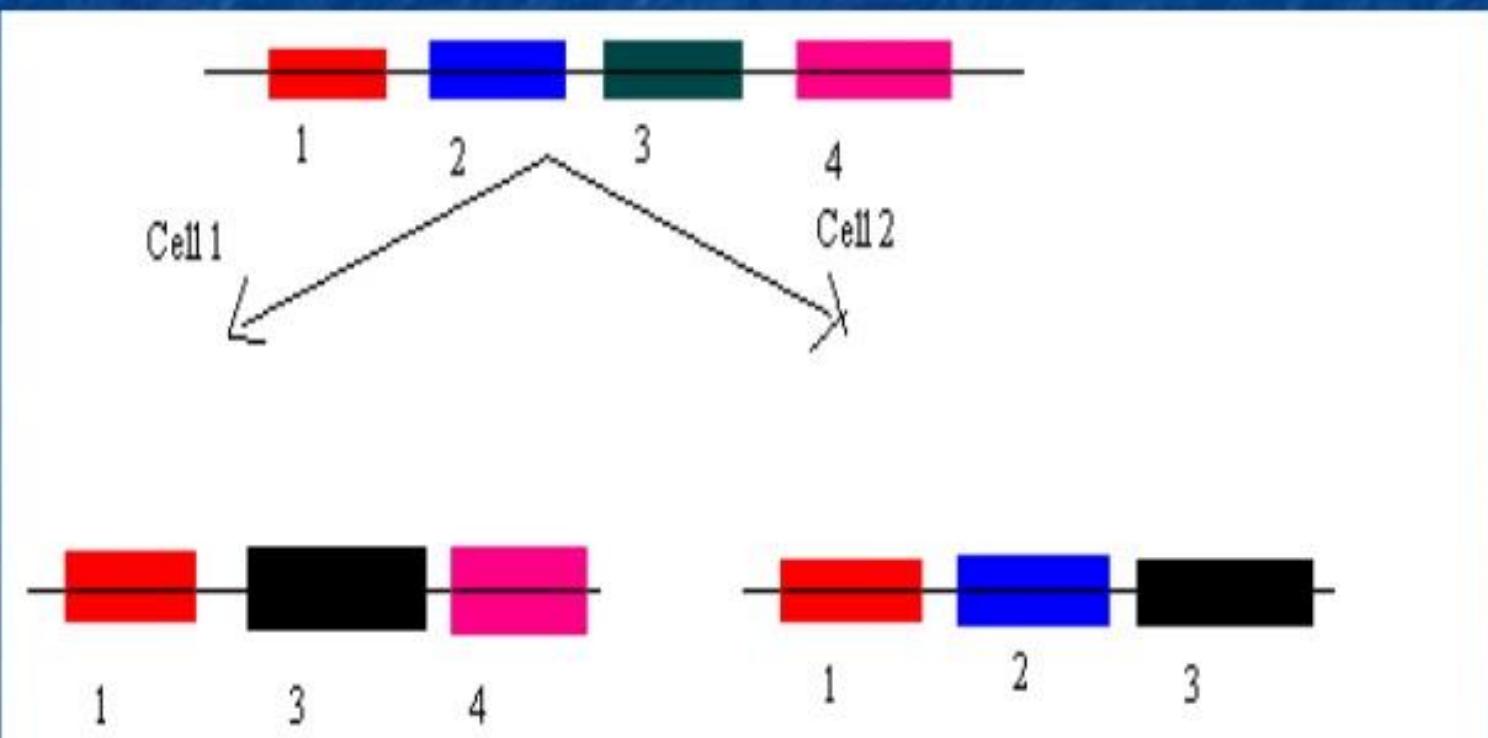
Prokaryotes	Eukaryotes
<p>A promoter sequence which controls an operon lies upstream of the operon.</p> <p>Accessory or the regulatory proteins control the recognition of the transcriptional initiation sites by RNA polymerases</p>	<p>Those genes which code for a protein have a basic structure consisting of:</p> <ul style="list-style-type: none">■ Exons – Gene sequences which encode for a polypeptide■ Introns – These sequences will get removed from the mRNA before it gets translated.■ A transcription initiation site■ Promoter sequences.
<p>A single operon gets transcribed into a polycistronic mRNA which can be translated into multiple proteins</p>	<p>Monocistronic mRNAs which can produce a single polypeptide are produced</p>

3. Regulation of RNA Processing

- RNA processing involves
 - Addition of 5' cap
 - Addition of a 3' poly (A) tail
 - Removal of introns
- The RNAs which get translated to proteins are transported out from the nucleus to cytoplasm.
- Depending on the final combination of exons after splicing different kinds of proteins are obtained which can perform different functions in the cell.

Exon Shuffling

- The functions of two proteins synthesized from the same mRNA are different in different cells as different combination of exons exist in different cells.



4. Regulation of RNA Transport

- Only some RNAs function within the nucleus whereas all other RNAs which are meant for protein synthesis have to be transported from the nucleus to the cytoplasm via nuclear pores.

5. Regulation of RNA Longevity

- mRNAs from different genes have different life spans.
- The information of the life span of mRNA is found in the 3' UTR.
- The sequence AUUUA within 3' UTR acts as a signal for early degradation.
- More the number of times the sequence is repeated → Shorter the lifespan of mRNA

6. Regulation of Translation

- **Translational initiation**
 - The expression of a gene product also depends on the ability of the ribosome to recognize the correct AUG codon out of the multiple methionine codons present in the mRNA.
- **Control of translational process**
 - In many animals large amounts of mRNAs are produced by the eggs but all of them do not get translated until the egg is fertilized.

7. Post Translational Control Points

- **Post translational modifications**
 - Functional state of protein depends on modifications like glycosylation, acetylation, fatty acylation, disulfide bond formations.
 - Chaperons
- **Protein transport**
 - Transportation to the site of action
- **Protein stability**
 - The lifespan of a protein depends on the specific amino acid sequence present within them

Summary of the Class

- The expression of genes is controlled at various levels in eukaryotes.
- At the chromatin stage the level of condensation determines whether the genes will remain transcriptionally active or not.
- The unique combination of the promoter sites, transcription factors and enhancers regulates the transcriptional rate of a gene.
- After transcription the gene expression is controlled by RNA processing.
- The expression of gene is also controlled at the level of translation and after translation.

Video link

<https://www.youtube.com/watch?v=gG7uCskUOrA>

Thank you

Genetic Manipulation of Chloroplast

Anandi R.

Chloroplast genetic system

- A 50-290 kb double stranded circular molecule
- A pair of 20-30 kb inverted repeat (IR) sequence

Chloroplast transformation

- Expression level of foreign genes is higher than nuclear transformation
- Multiple genes can be introduced as an operon

Stable transplastomic plants

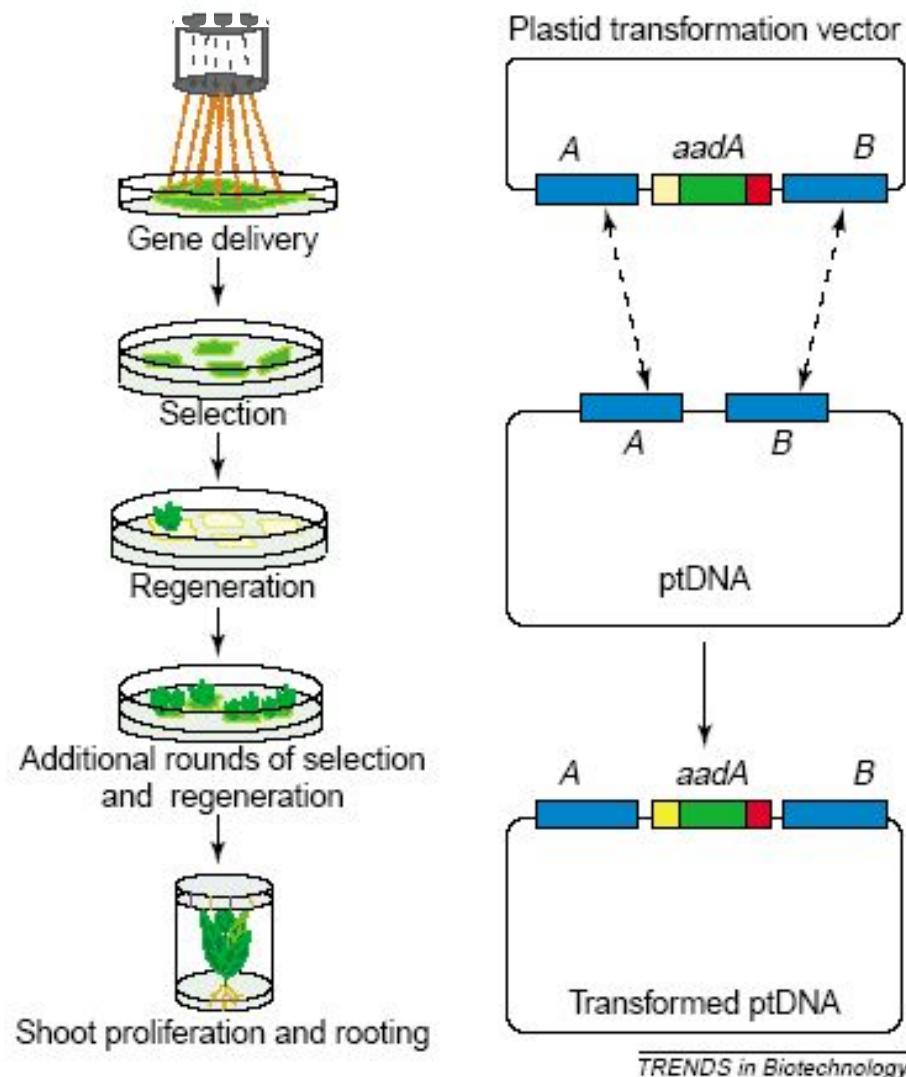
- Transformation of plastids has already been achieved for tobacco , *Arabidopsis*, soybean , cotton, lettuce, cauliflower and potato

Plastid transformed plants

Table I. Plant species for which DNA delivery to plastids

<i>Species</i>	<i>Delivery method</i>
Arabidopsis	Biolistic
Carrot	Biolistic
<i>Nicotiana plumbaginifolia</i>	Protoplast
Tobacco (NT1 suspension cells)	Biolistic
Marigold	Biolistic
Potato	Biolistic
Red pepper	Biolistic
Rice	Biolistic
Tobacco (Petit Havana, Xanthi)	Biolistic, protoplast

Basics of Chloroplast Transformation



TRENDS in Biotechnology

Chloroplast Transgenic Production

Chloroplast transformation techniques

- ❑ Biolistic delivery systems
- ❑ Polyethylene glycol (PEG) treatment of protoplast
 - The technique has a lower success rate than biolistic bombardment
 - long selection times required after initial DNA delivery
 - Technically demanding and requires specialized tissue culture skills
- ❑ Femtoinjection technique: injection of DNA material into chloroplasts using syringes with extremely narrow tips
- ❑ *Agrobacterium-mediated plastid* transformation

Particle Delivery System



Advantages and disadvantages of biolistic method

- Relatively high efficiency
- Technical simplicity

Advantages of injection technique

- Cells survive the injection
- Transformed cell can be spotted easily
- Cellular context remains intact

<https://www.youtube.com/watch?v=dX3jmX7qBlw>

Thank You



GENETICALLY MODIFIED ORGANISMS

What are GM's?

- Organism one that has been altered through recombinant DNA technology
- Involves either the combining of DNA from different genomes or the insertion of foreign DNA into a genome
- The most common genetically modified (GM) organisms are crop plants
- Microbes are the first organisms to be genetically modified

What is not a GMO?

- Does not include
 - Mutants.
 - Fusion of animal cells unless the product can form an animal.
 - Organisms formed by natural DNA transfer.

How transgenic organisms work...

Three Main Methods...

- **DNA Microinjection**

- A foreign gene is directly injected into a fertilized egg that is put into a female animal that acts as a surrogate mother for the egg.

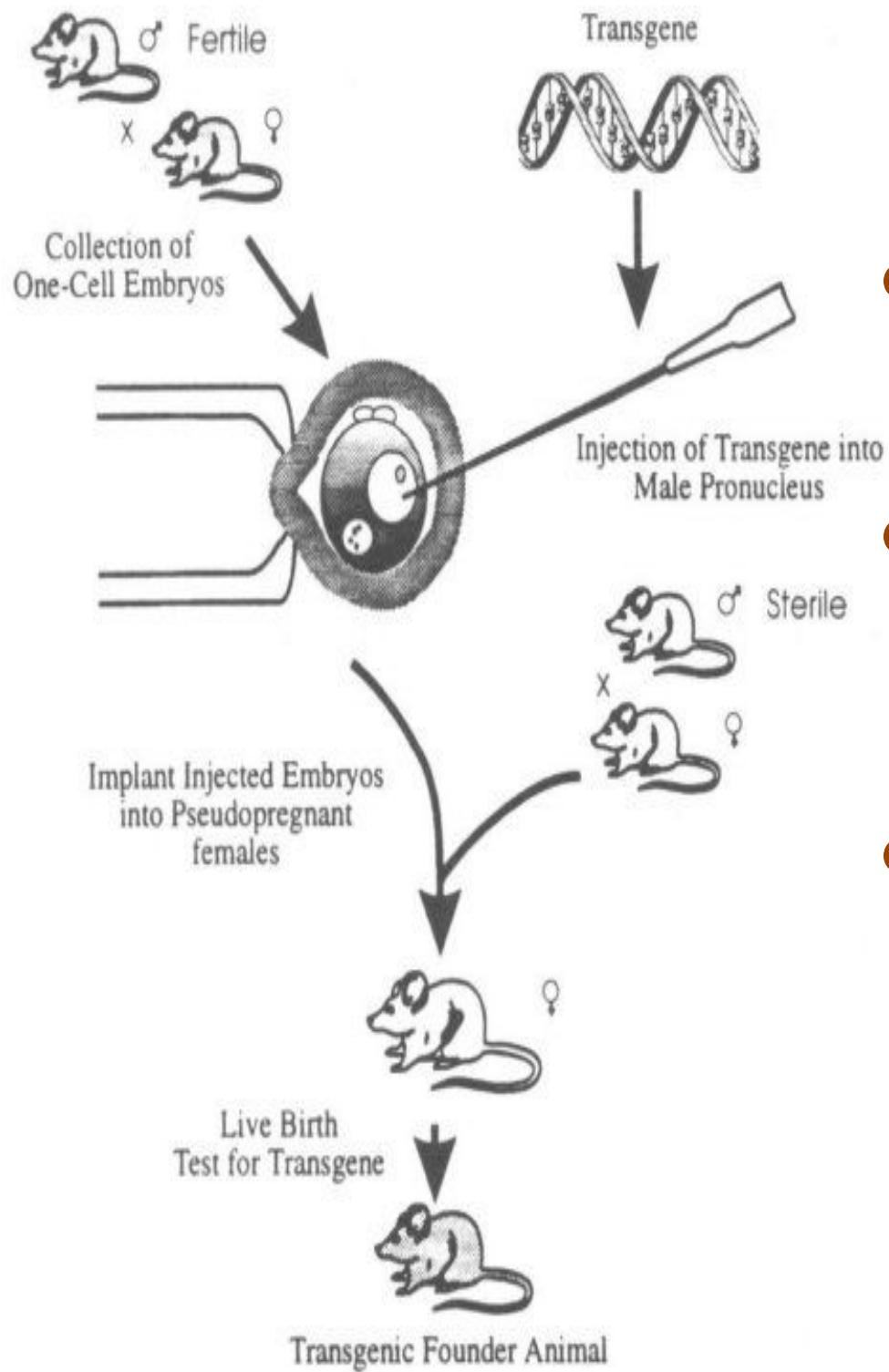
- **Retrovirus-Mediated Gene Transfer**

- A retrovirus is a virus that attaches to an organism's DNA and changes it to include a new characteristic. Scientists expose ordinary cells to a retrovirus when they are trying to create transgenic animals.

- **Embryonic Stem Cell-Mediated Gene Transfer**

- Stem cells are blank cells that can turn into any type of cell. Scientists modify these cells, and then add them to an embryo, which is a fertilized egg that develops and grows until it hatches or is born.

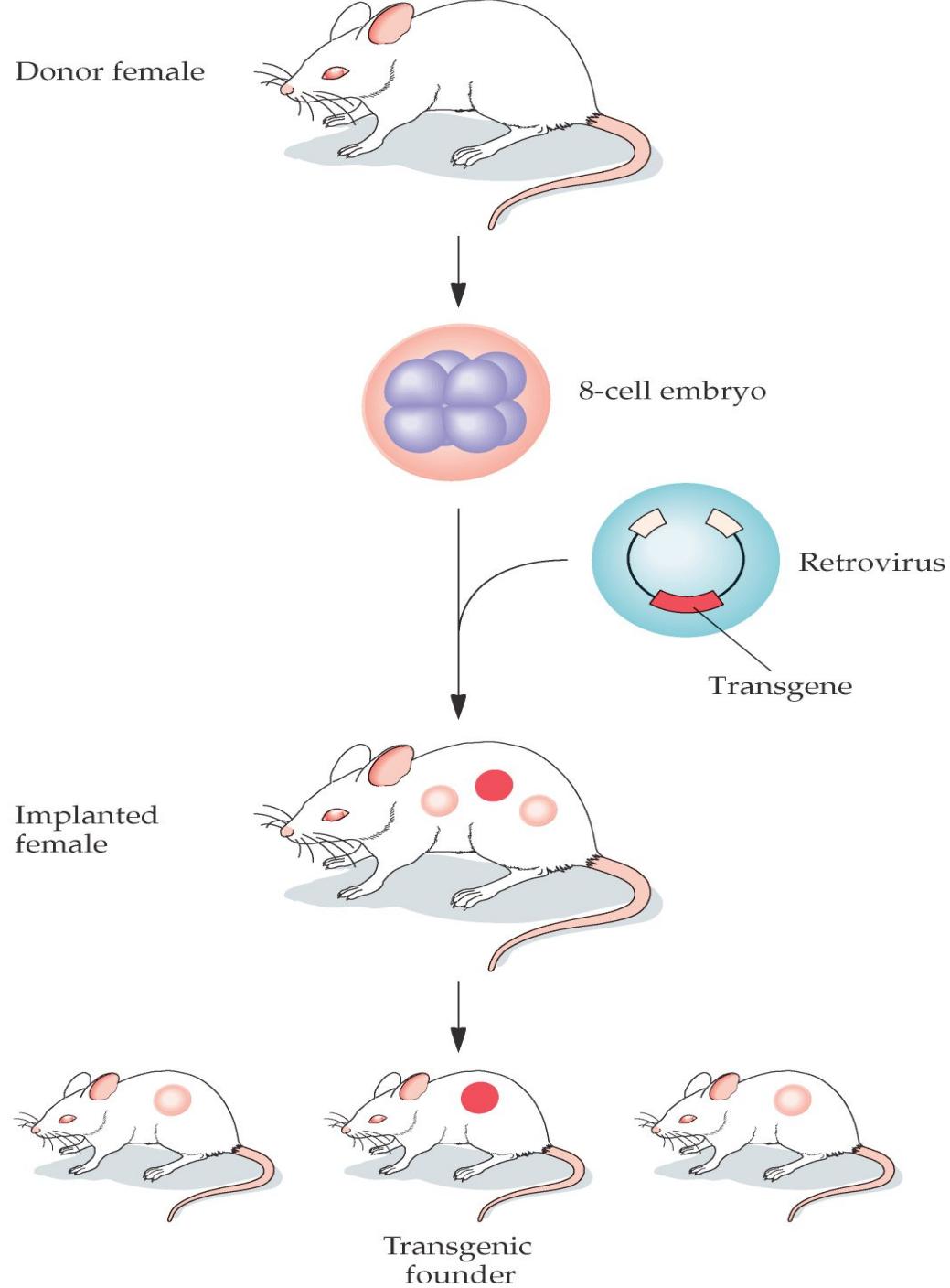
DNA MICROINJECTION



- Most commonly used method
- Need to check mouse for **DNA, RNA and protein** (by some specific assay method)
- Expression will vary in transgenic offspring: due to **position effect and copy number**

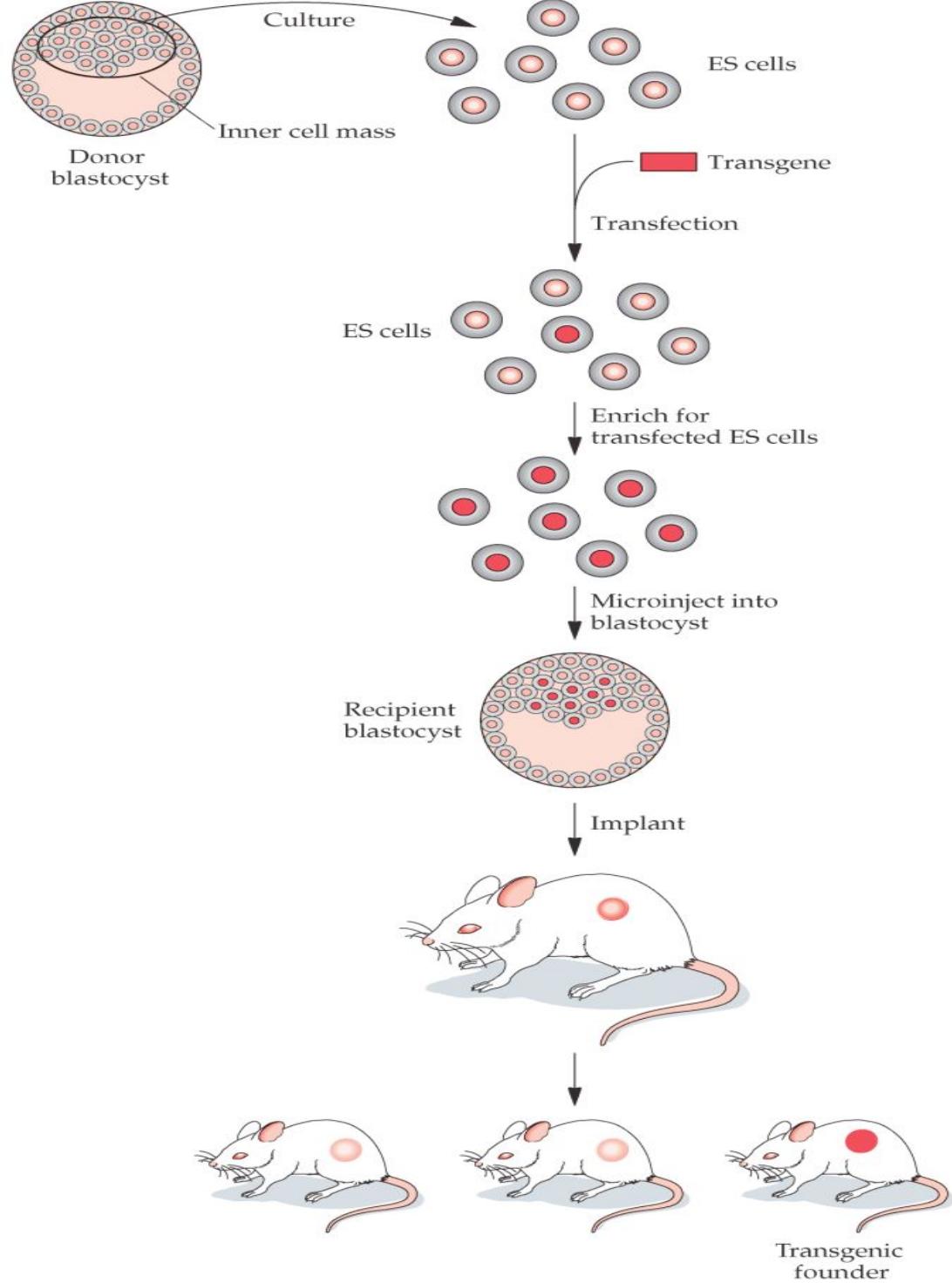
RETROVIRUS-MEDIATED GENE TRANSFER

Retroviral vectors can be used to create transgenic animals



EMBRYONIC STEM CELL-MEDIATED GENE TRANSFER

- Genetically engineered embryonic stem (ES) cells can be used to create transgenic animals
- This method allow for gene targeting via homologous recombination.



THE MOST COMMON TYPES OF GMO'S

I.

FOODS

- Crops are modified to develop resistance to herbicides and increase their nutrient content, for example corn and soybeans .
- Fruits are modified to make them ripen later. This help them available fresh in marketplace during a longer time or for fruits that ripen after being picked, make it easier to transport them.

THE MOST COMMON TYPES OF GMO'S

II.

MEDICINES

- These can be produced cheaper and easier
some are: insulin, thyroid hormones and the Hepatitis B vaccine
- GM Bacteria's have been particularly important in producing large amounts of pure human proteins for use in medicine like clotting factors for hemophilia and human growth hormones to treat dwarfism

OTHER TYPES OF GMO'S ARE

III.

MAMMALS



- Research human diseases
(To develop animal models for many diseases.)
- Produce industrial or consumer products
(pharmaceutical products or tissue implantation)
- Enhance production or food quality traits
(faster growth fish, pigs that digest food more efficiently)
- Improve animal health(disease resistance)

GENETICALLY MODIFIED PIGS

- MEDICINE
 - Production of pharmaceuticals (human hemoglobin in blood of pigs for treating Trauma patients)
 - Organs for Xenotransplantation into humans
 - development of models for human diseases

Applications

- To research human diseases (for example, to develop animal models for these diseases)
- To produce industrial or consumer products
- To produce products intended for human therapeutic use (pharmaceutical products or tissue)
- To enhance production or food quality traits (faster growing fish, pigs that digest food more efficiently)
- To improve animal health (disease resistance)



THANK YOU

THE *lac* OPERON

Rudrakshi B.Raut

The Institute Of Science,Mumbai

M.sc-2 (sem:3)

Paper-2

Roll no.17

CONTENT

- Introduction
- Concept of lac operon
- Operon model
- Functioning of lac operon
- Different Scenarios
- Lac mutations :
 - Structural Mutation
 - Operator Mutation
 - Promoter Mutation
- Positive and Negative control
- References

INTRODUCTION

- *Operon* is operating units which can be defined as the cluster of genes located together on the chromosomes & transcribed together.
- It is group of closely linked *structure genes* & associated *control gene* which regulate the metabolic activity.
- All the genes of an operon are coordinately controlled by a mechanism 1st described in 1961 by *Francois Jacob & Jaques Monod* of the Pasteur institute of Paris.

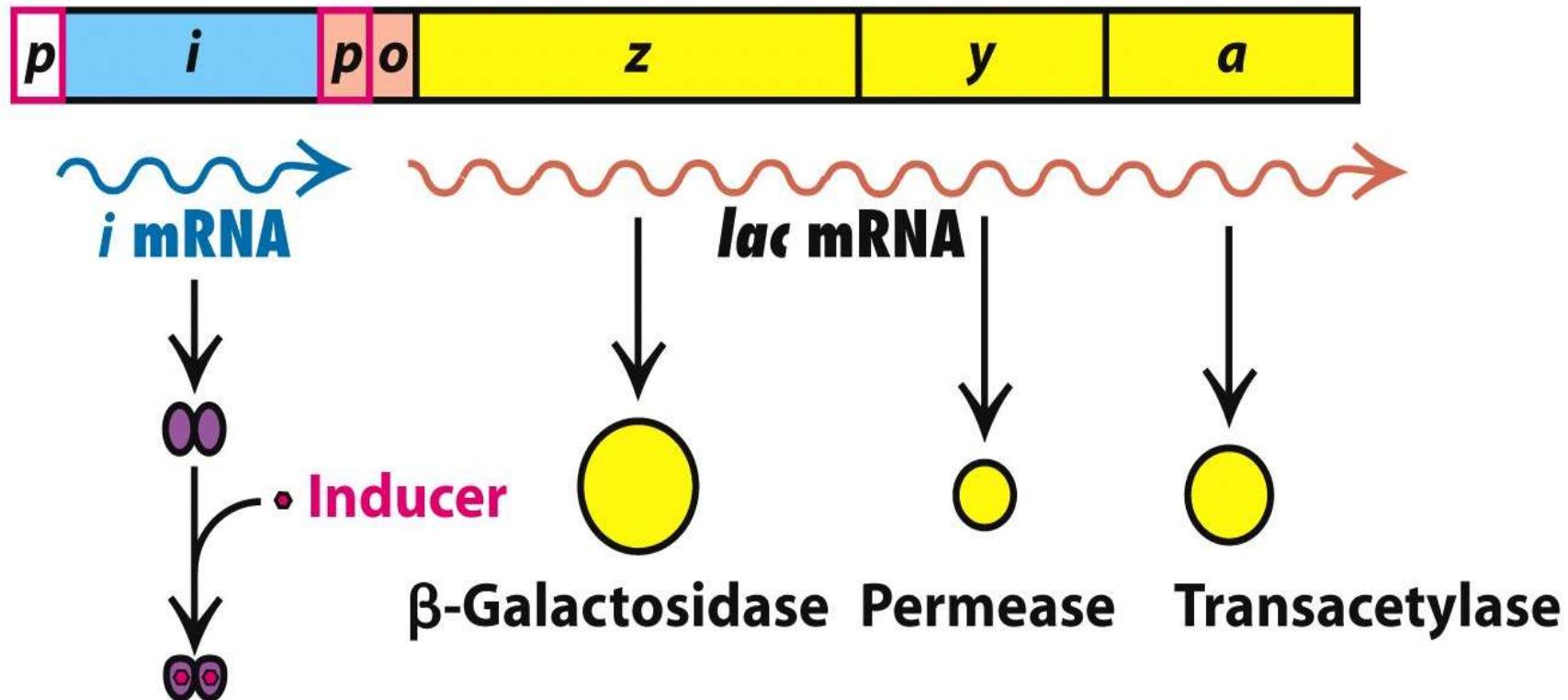


Jacob, Monod & Lwoff

The *lac* operon

- The lactose operon designated as *lac operon*.
- The lac operon codes for enzymes involved in the catabolism (degradation) of lactose.
- lactose is the disaccharide which is made up of glucose & galactose.
- It is the inducible operon since the presence of lactose induce the operon to switched on.

Operon model



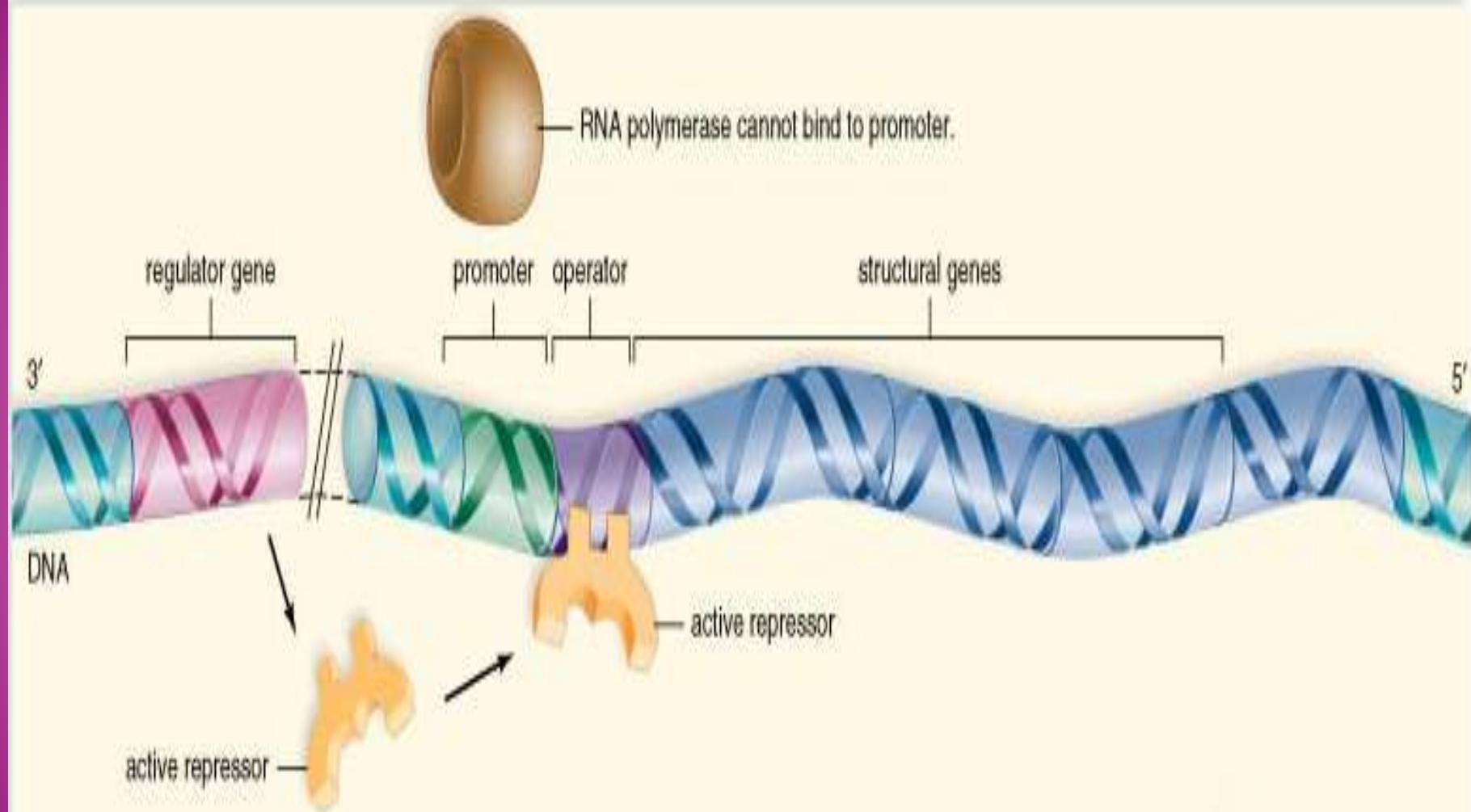
Repressor-inducer
complex does not
bind DNA

Designation of gene	Codes for enzyme	Function of the enzyme
lac Z	β -galactosidase	Breaks down lactose into glucose & galactose.
lac y	galactose permease	This protein, found in the E.coli cytoplasmic membrane, actively transports lactose into the cells
lac a	Thio-galactoside trans acetylase	The function of this enzyme is not known. It is coded for by the gene lacA.

<u>Element</u>	<u>Purpose</u>
Operator (lacO)	Binding site for repressor
Promoter (lacP)	Binding site for RNA Polymerase
Repressor	Gene encoding the lac repressor protein. Binds to DNA at the operator & blocks binding of RNA Polymerase at the promoter.
lacI	Controls production of the repressor protein

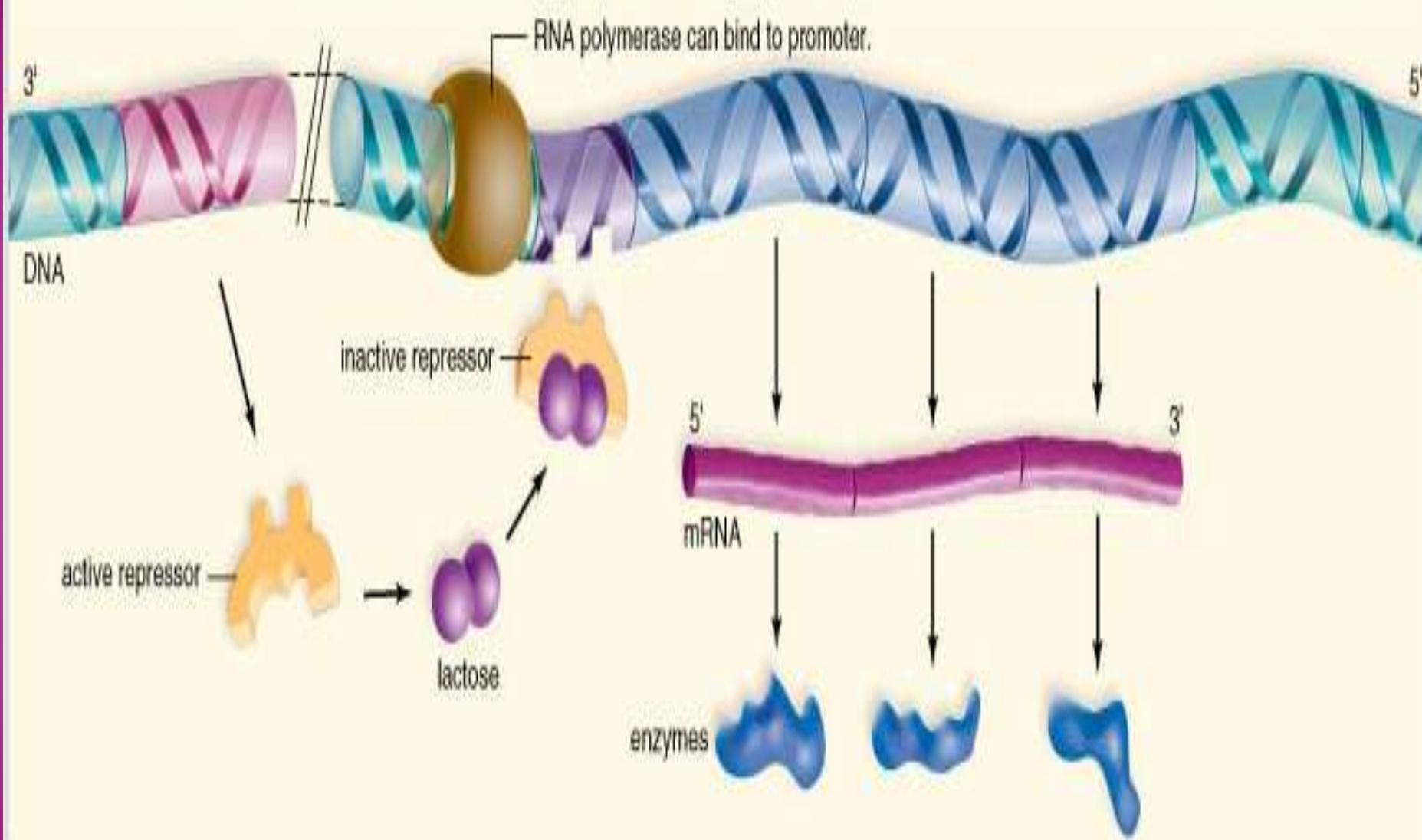
FUNCTIONING OF LAC OPERON

- In the absence of lactose(inducer), the regulator gene produce a repressor protein which bind to the operator site & prevent the transcription as a result, the structural gene do not produce mRNA & the proteins are not formed.



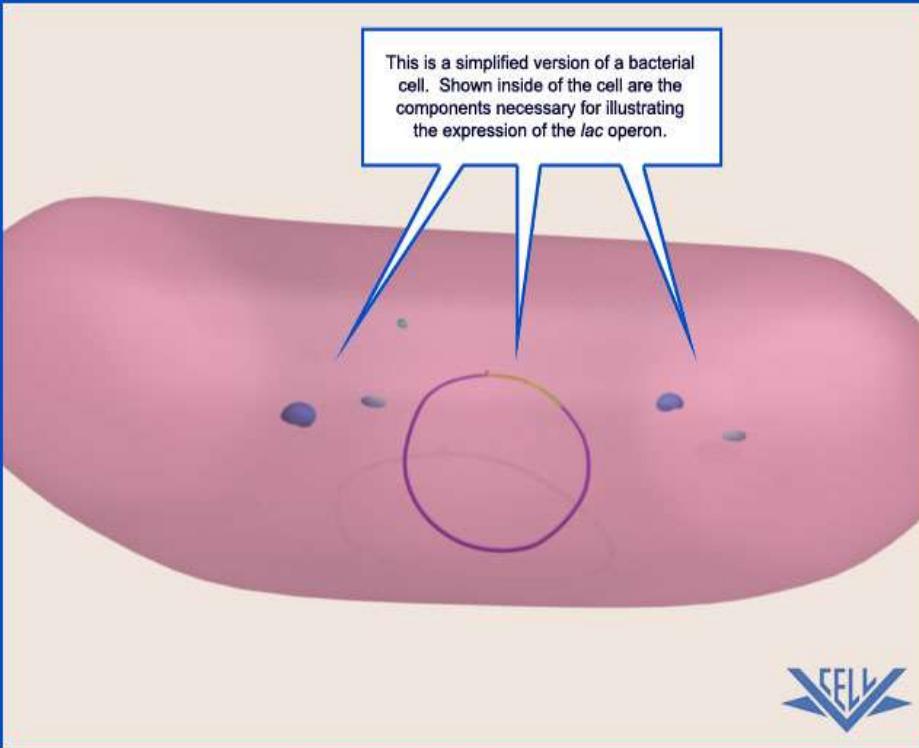
- a. Lactose absent. Enzymes needed to take up and use lactose are not produced.

- When lactose(inducer), introduce in the medium, binds to the repressor the repressor now fails to binds to the operator.
- Therefore the operoter is made free & induces the RNA polymerase to bind to the initiation site on promoter which results in the synthesis of *lac* mRNA.
- This mRNA codes for three enzyme necessary for lactose catabolism.

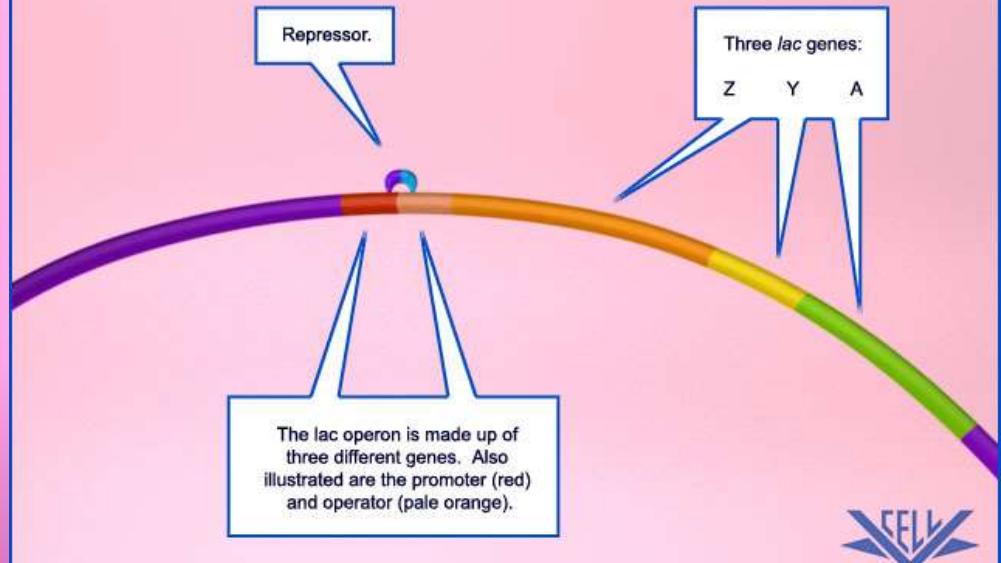


b. Lactose present. Enzymes needed to take up and use lactose are produced only when lactose is present.

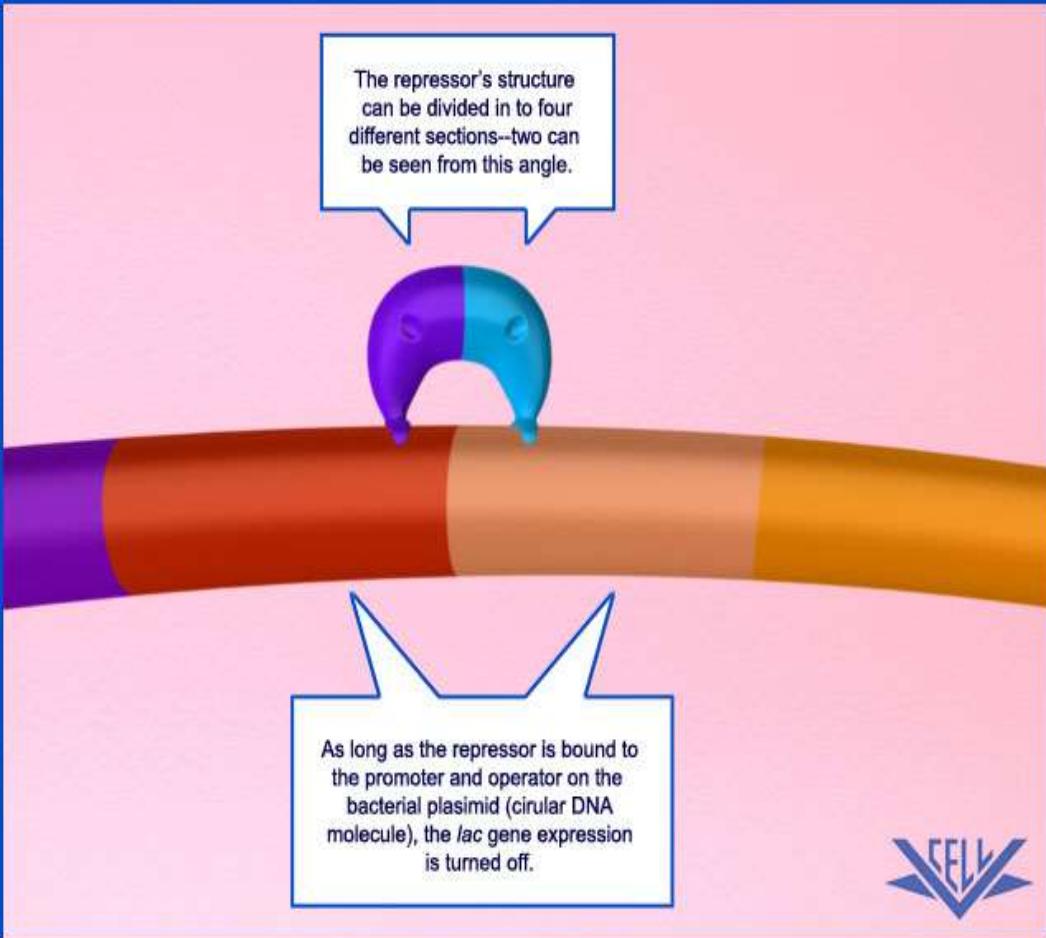
The *lac operon* gene sequence.



A simplified *E. coli* bacterial cell.



Lactose molecules added to the environment outside of the cell.



The **repressor** molecule, bound to the **controlling region**.

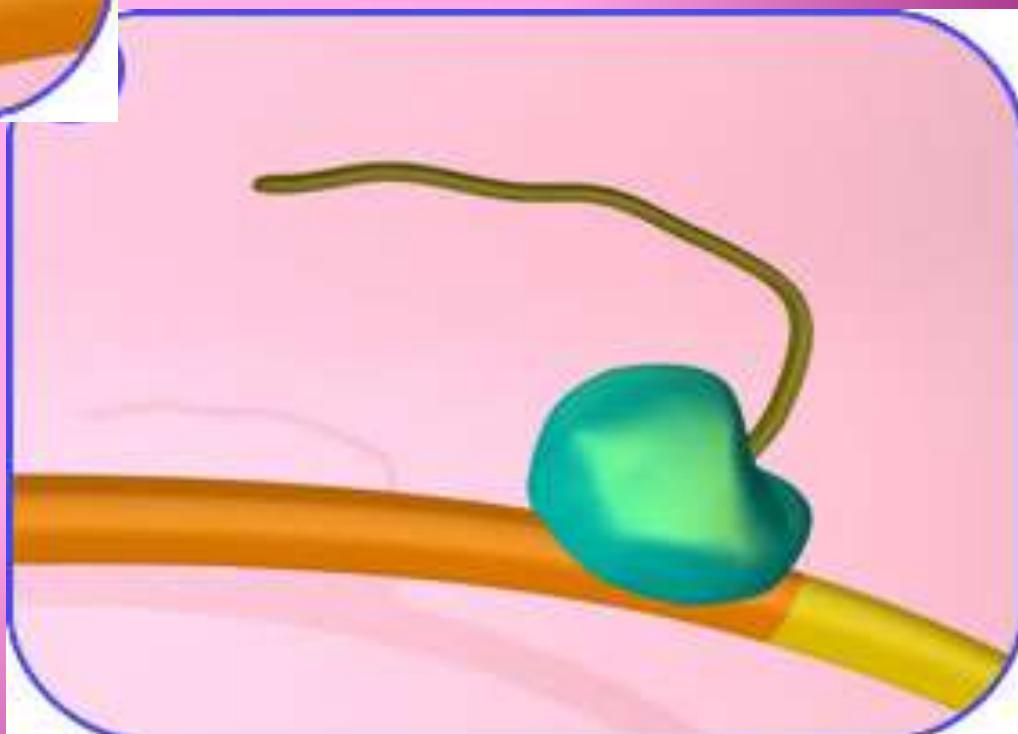


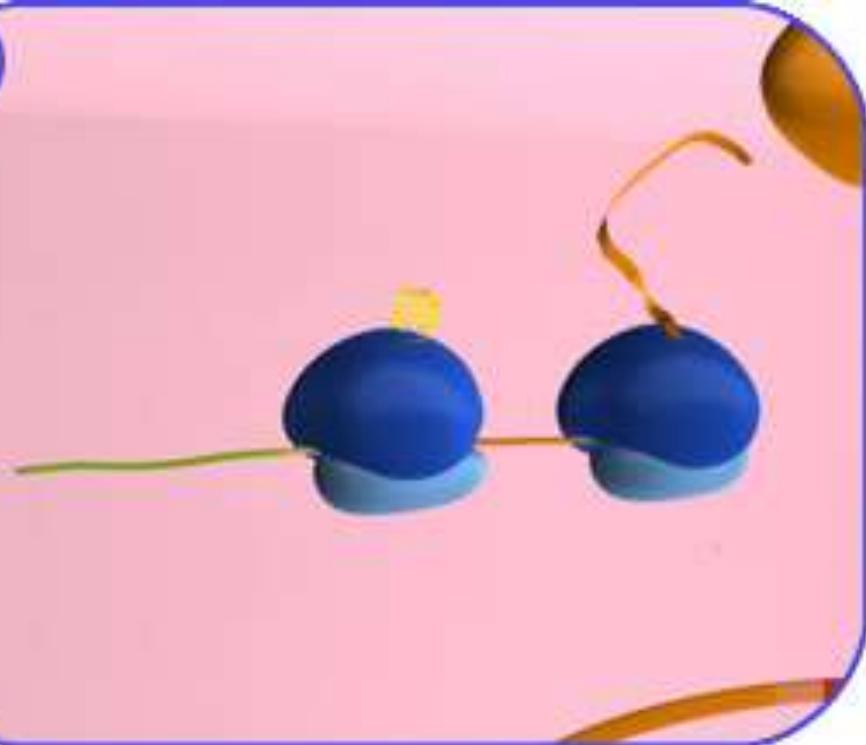
5



RNA polymerase
transcribing the genes in
the *lac operon* into *mRNA*.

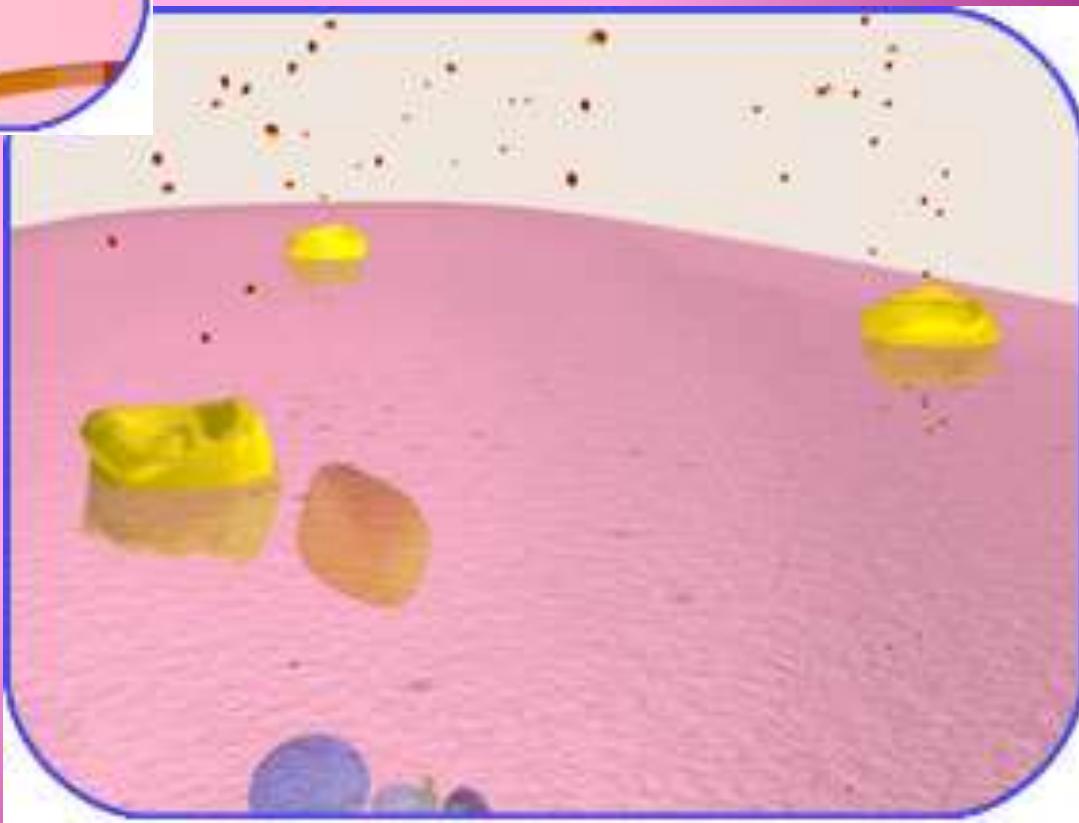
Lactose molecules bound
to the *repressor*.
This releases the
repressor from the DNA.



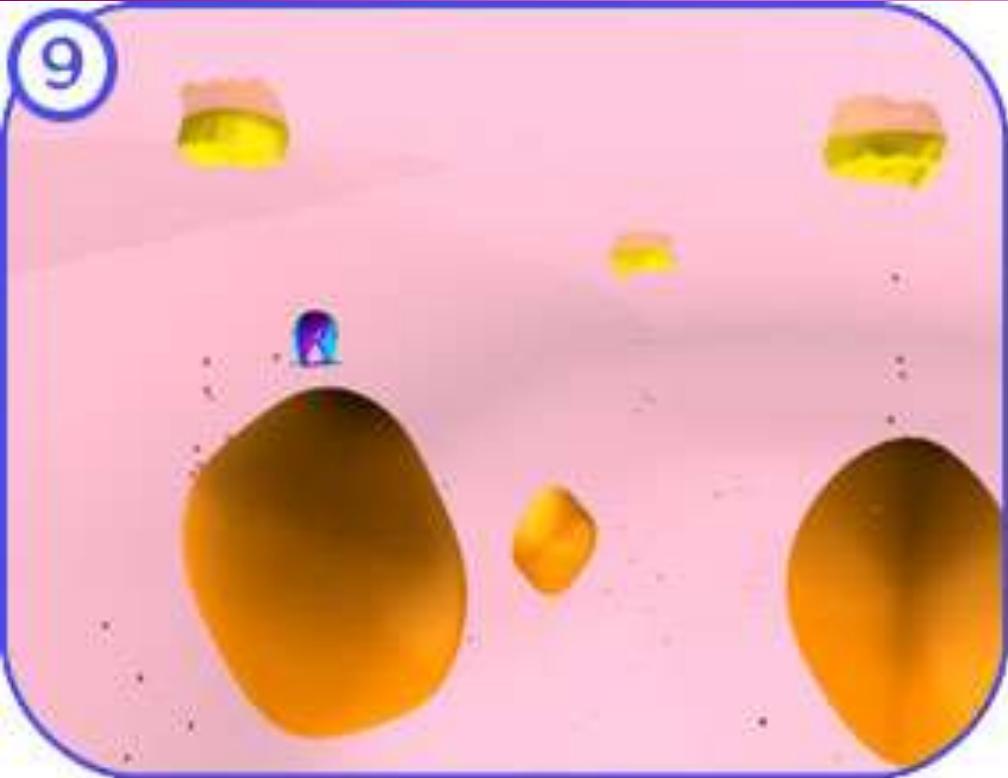


Ribosomes translating the **mRNA** into proteins.

One of the proteins (yellow) encoded by the ***lac operon*** allows ***lactose*** to enter the cell at a high rate.



9

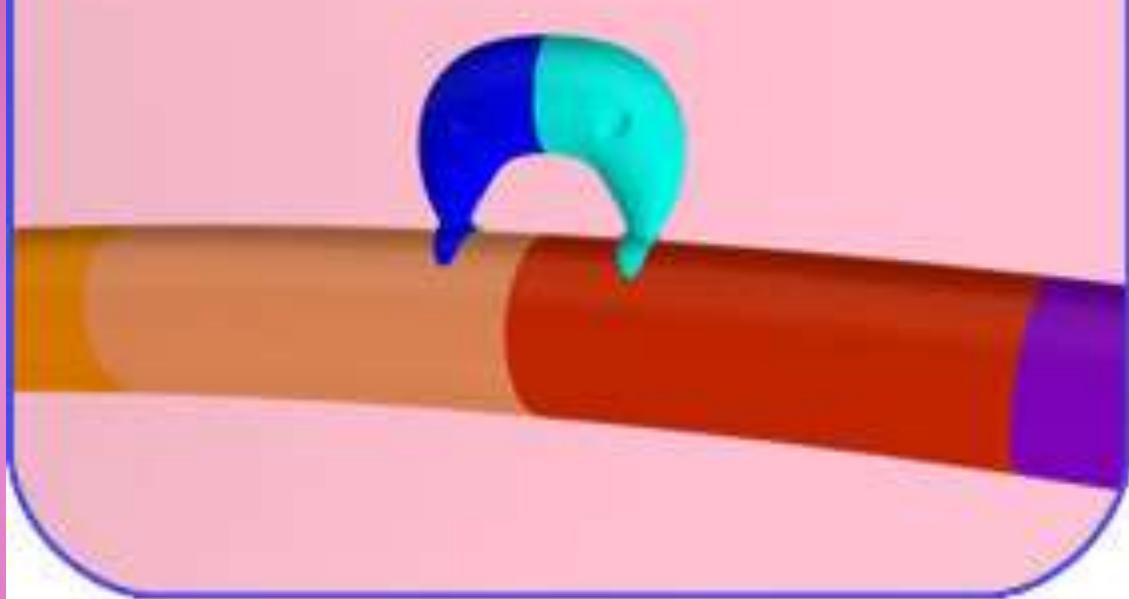


A second protein (orange) digests the *lactose* as it enters the cell.

The *lactose* molecules bound to the *repressor* are released.



11



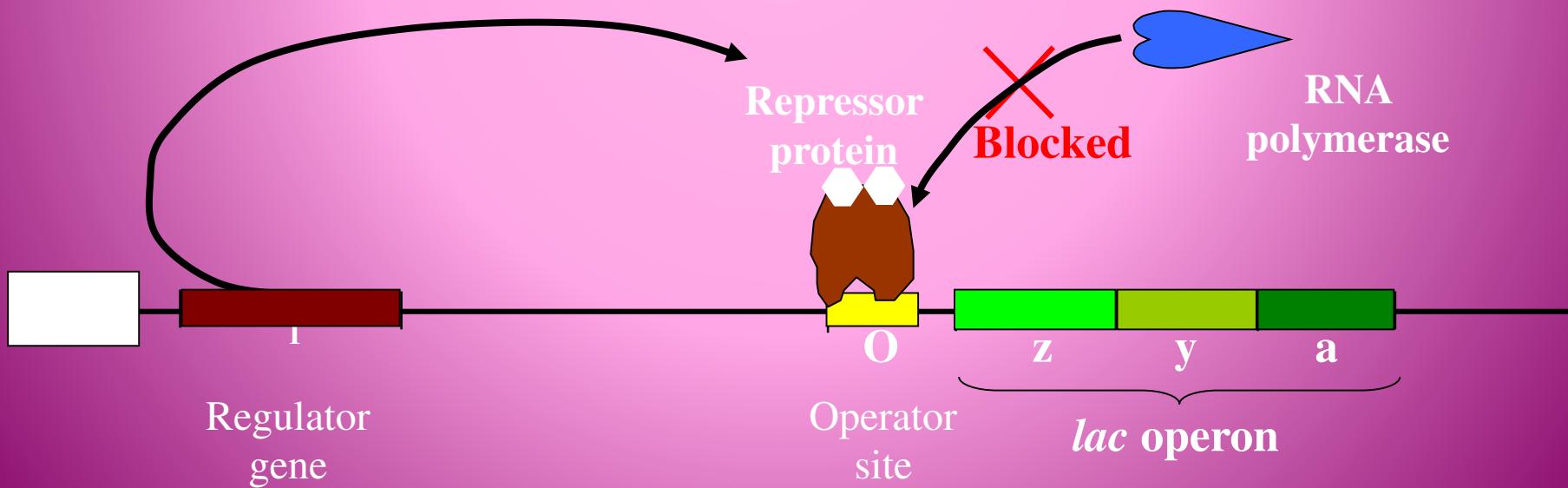
Repressor again binds to the *controlling region* of the DNA.

Different Scenarios

1. Lactose (-)
2. Lactose (+)
3. Lactose (+) and glucose (+)
4. Lactose (+) and glucose (-)

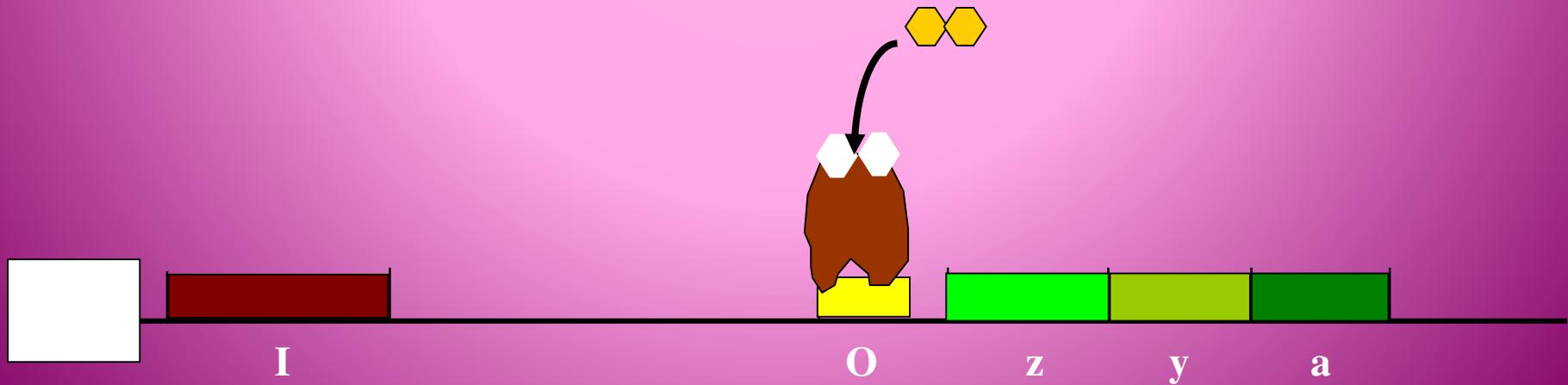
1. When lactose is absent

- A repressor protein is continuously synthesised. It sits on a sequence of DNA just in front of the *lac* operon, the **Operator site**
- The **repressor protein** blocks the **Promoter site** where the RNA polymerase settles before it starts transcribing



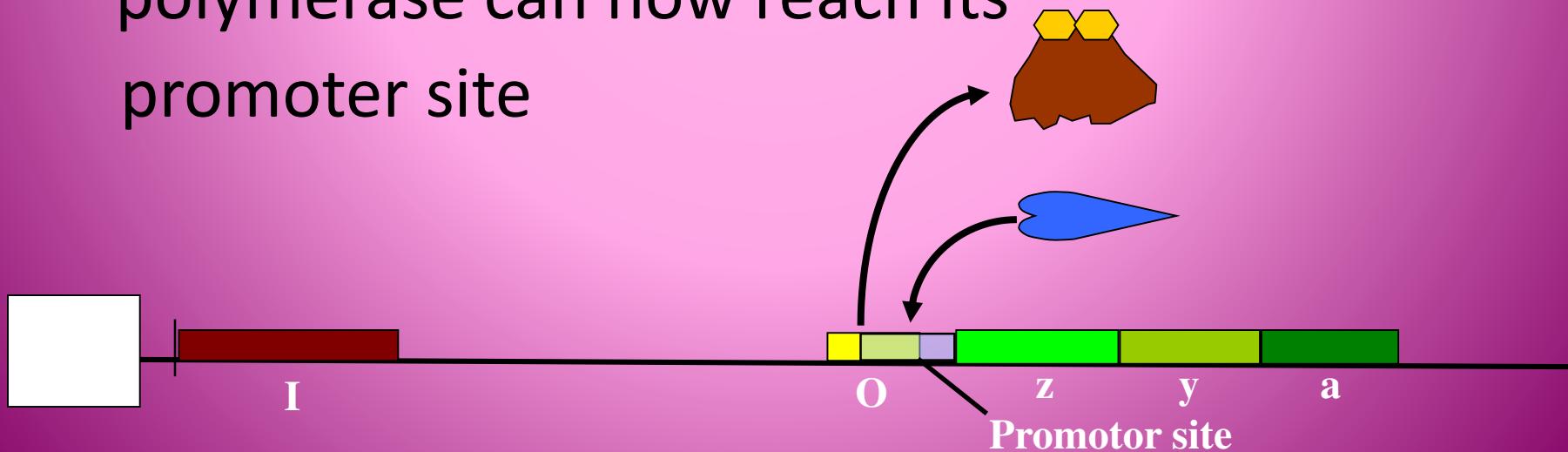
2. When lactose is present

- A small amount of a sugar allolactose is formed within the bacterial cell. This fits onto the repressor protein at another active site (**allosteric site**)
- This causes the repressor protein to change its shape (a **conformational change**). It can no longer sit on the operator site. RNA polymerase can now reach its promoter site



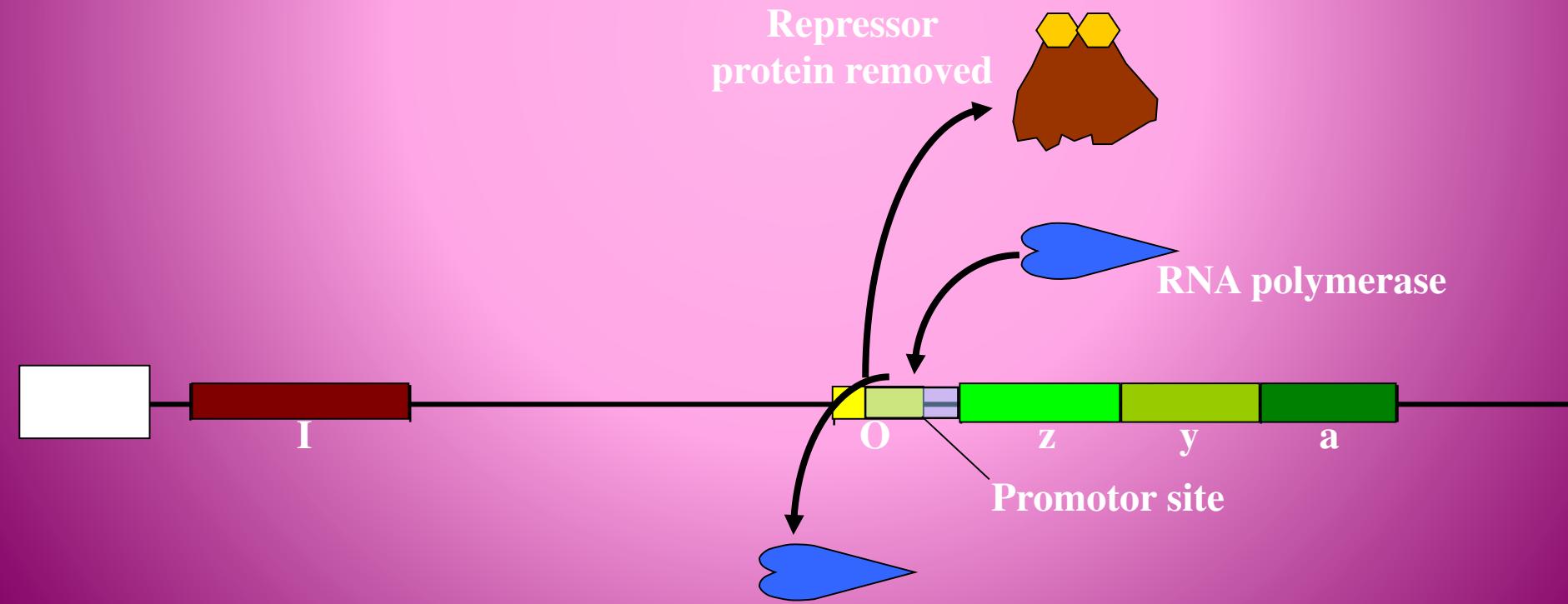
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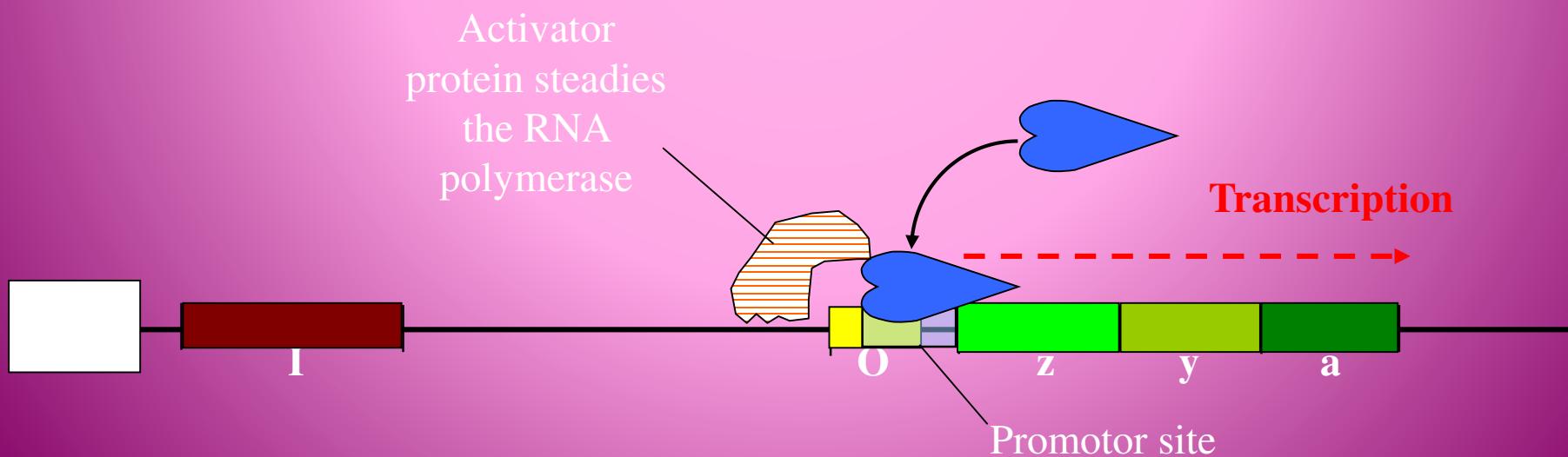
3. When both glucose and lactose are present

- When glucose and lactose are present RNA polymerase can sit on the promoter site but it is unstable and it keeps falling off.



4. When glucose is absent and lactose is present

- Another protein is needed, an **activator protein**. This stabilises RNA polymerase.
- The activator protein only works when glucose is absent
- In this way *E. coli* only makes enzymes to metabolise other sugars in the absence of glucose



Summary

Carbohydrates	Activator protein	Repressor protein	RNA polymerase	lac Operon
+ GLUCOSE + LACTOSE	Not bound to DNA	Lifted off operator site	Keeps falling off promoter site	No transcription
+ GLUCOSE - LACTOSE	Not bound to DNA	Bound to operator site	Blocked by the repressor	No transcription
- GLUCOSE - LACTOSE	Bound to DNA	Bound to operator site	Blocked by the repressor	No transcription
- GLUCOSE + LACTOSE	Bound to DNA	Lifted off operator site	Sits on the promoter site	Transcription

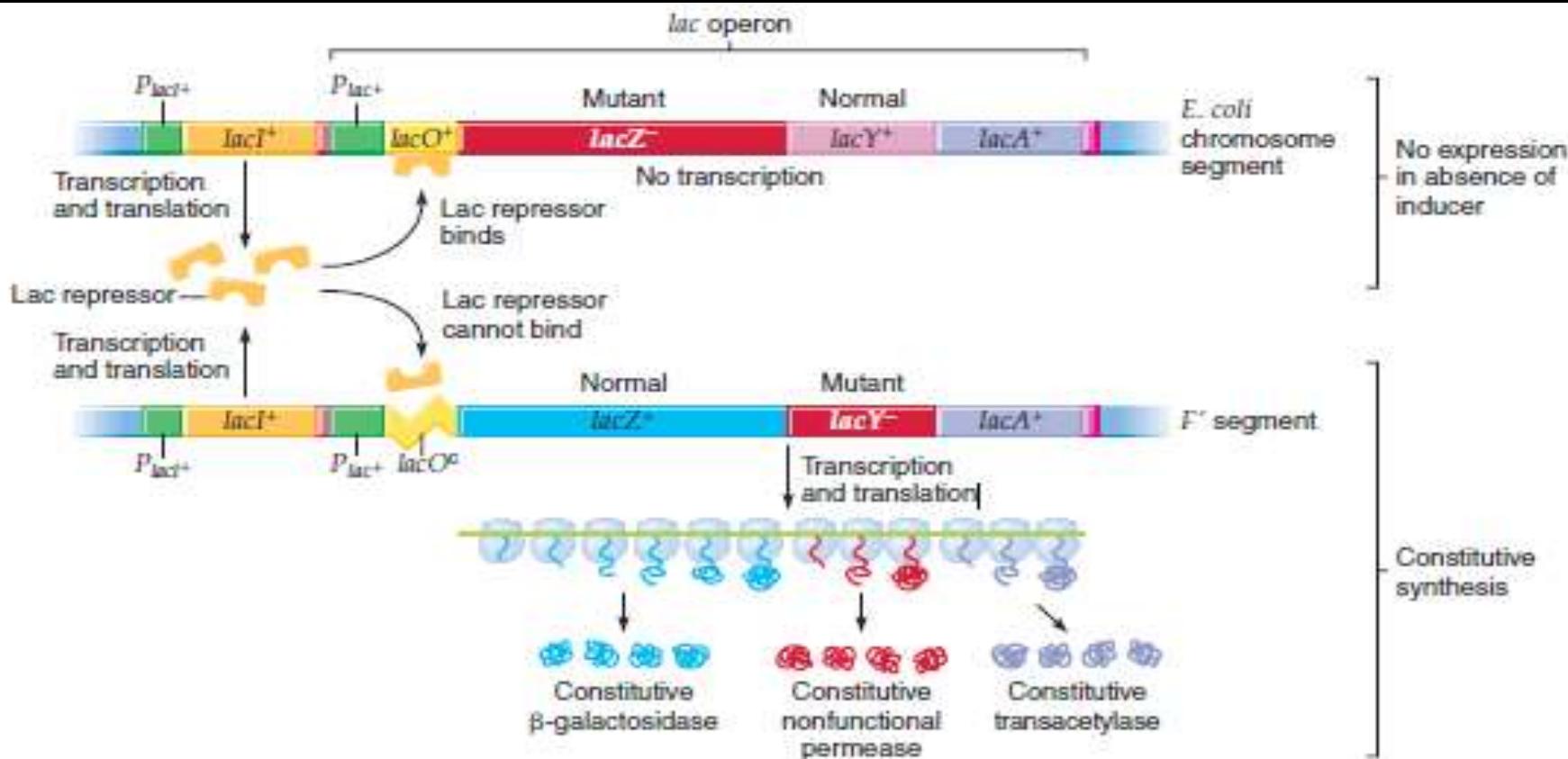
LAC MUTATIONS

- Jacob & Monod workout the structure & function of *lac* operon by analyzing mutations that affects lactose metabolism.
- To help define the role of the different components of the operon, they use partial diploid stain of *E.coli*.
- They determine that some part of the *lac* operon are *cis* acting where other are *trans* acting.

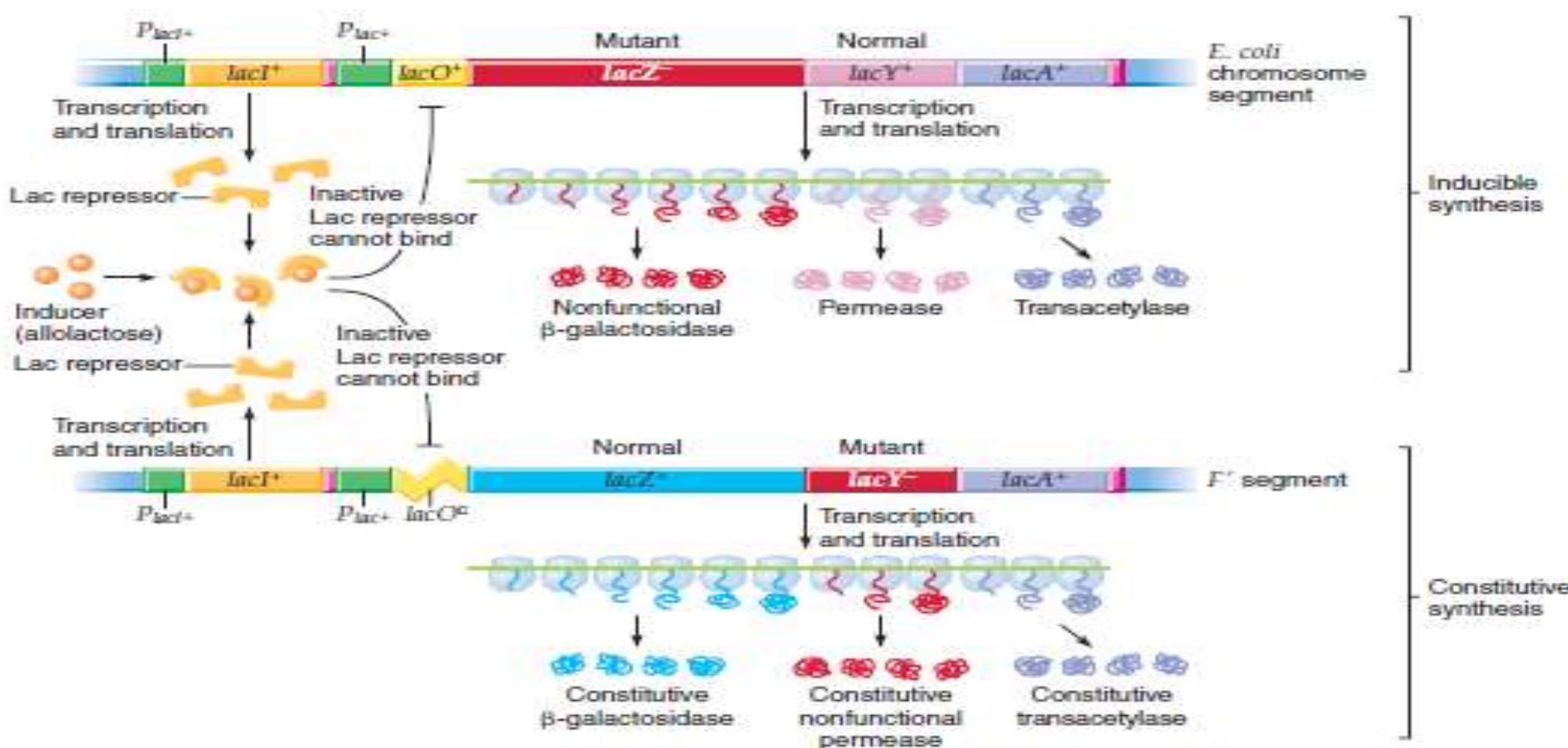
STRUCTURAL-GENE MUTATION

- Jacob and Monod first discovered some mutant strains that had lost the ability to synthesize either β -galactosidase or permease.
- The mutation which occurred on lacZ and LacY structural genes altered the amino acid sequences of the proteins encoded by the genes.

a) In the **absence of inducer**, the *lacO⁺* operon is turned off, whereas the *lacOc* operon produces functional β -galactosidase from the *lacZ⁺* gene and nonfunctional permease molecules from the *lacY⁻* gene with missense mutation.



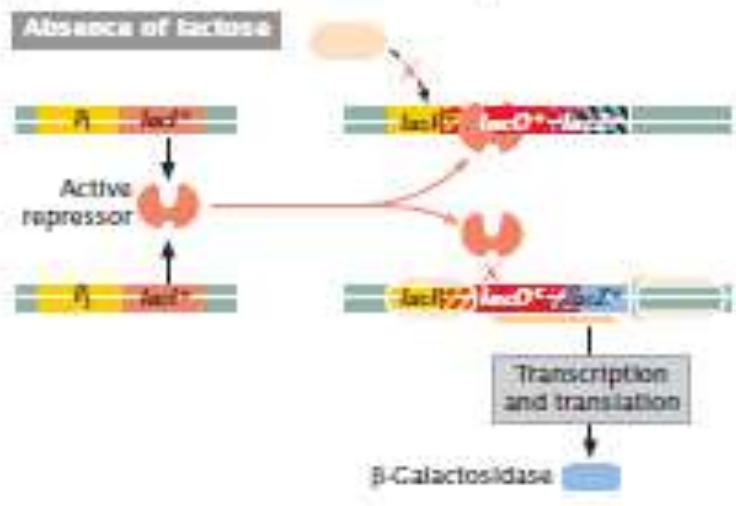
b) In the **presence of inducer** the functional β -galactosidase and defective permease are produced from the $lacO^c$ operon, whereas the $lacO^+$ operon produces nonfunctional β -galactosidase from the $lacZ^-$ gene & functional permease from $lacY^+$ gene.



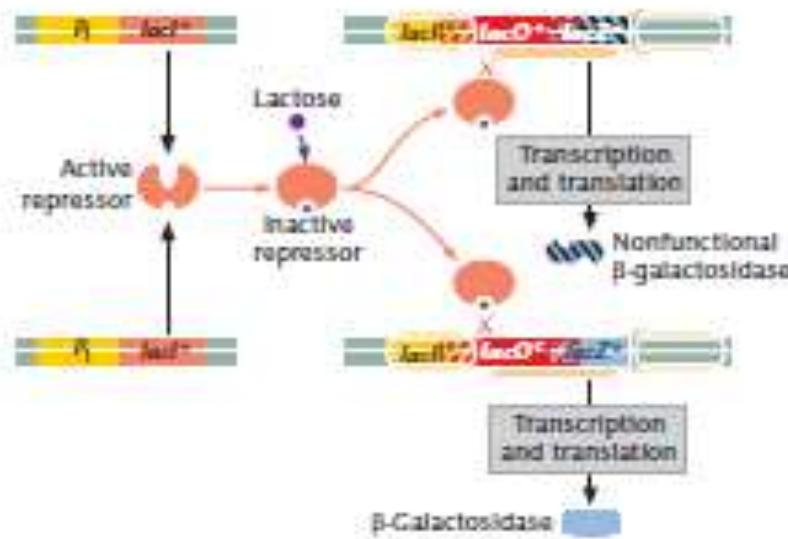
OPERATOR MUTATIONS

- Jacob & Monod find another constitutive mutants to a site adjacent to *lacZ*.
- These mutations occurred at the operator site & were referred to as *lacO*^c.
- The *lacO*^c mutations altered the sequence of DNA at the operator so that the repressor protein was no longer able to bind.
- A partial diploid with genotype *lacI*⁺ *lacO*^c *lacZ*⁺ / *lacI*⁺ *lacO*⁺ *lacZ*⁺ exhibited constitutive synthesis of β -galactosidase, indicating that *lacO*^c is dominant over *lacO*⁺.

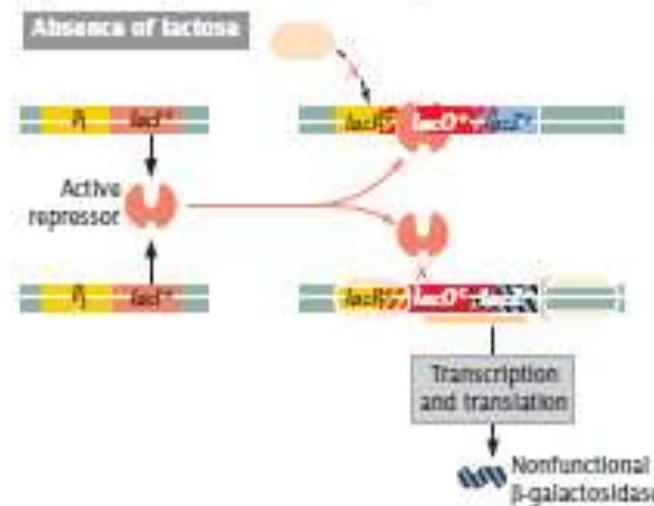
(a) Partial diploid $lacI^+$ $lacO^+$ $lacZ^+$ / $lacI^+$ $lacO^C$ $lacZ^+$



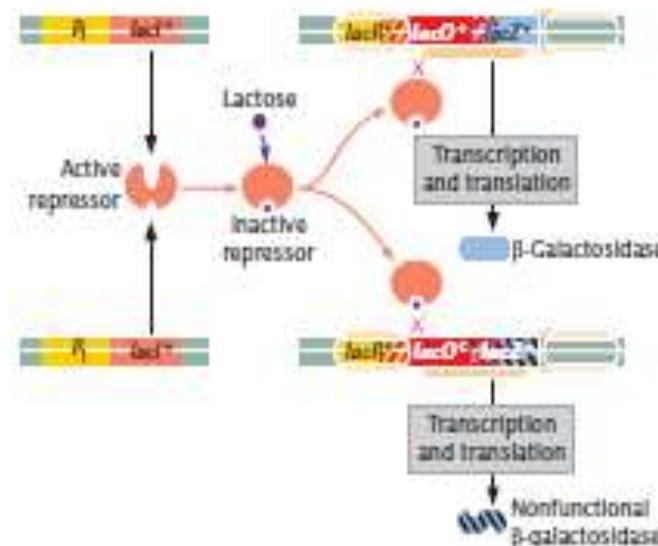
Presence of lactose



(b) Partial diploid $lacI^+$ $lacO^+$ $lacZ^+$ / $lacI^+$ $lacO^C$ $lacZ^-$



Presence of lactose



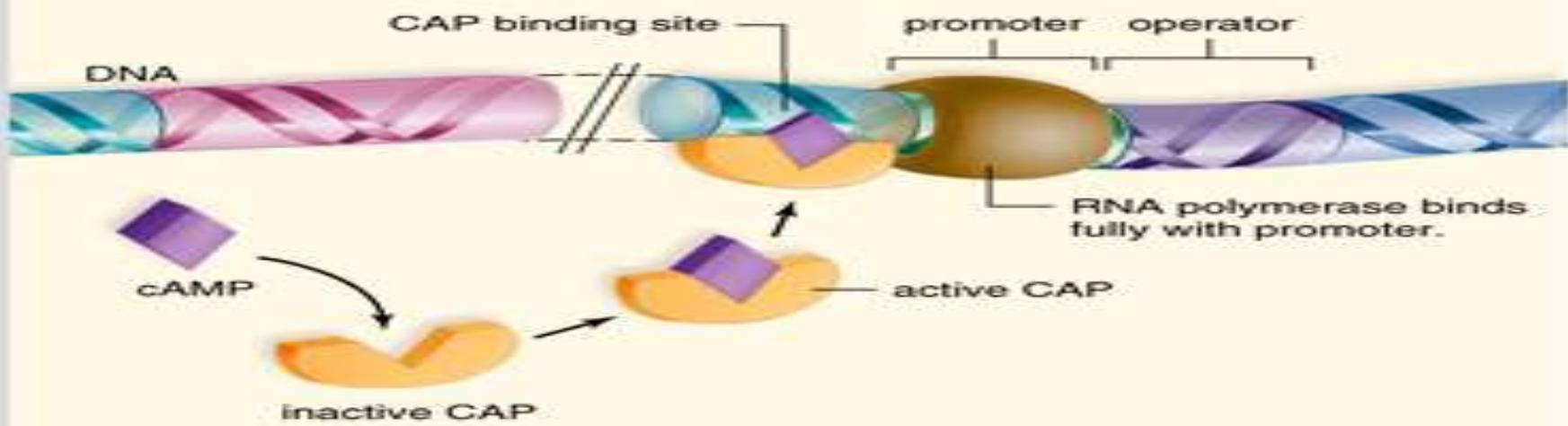
PROMOTER MUTATION:

- Mutations affecting lactose metabolism have also been isolated at the promoter site; these mutations are designated *lacP*⁻, and they interfere with the binding of RNA polymerase to the promoter.
- This binding is essential for the transcription of the structural gene.
- E.coli strain with *lacP*⁻ mutation does not produce *lac* proteins either in a presence or absence of lactose.
- *lacP*⁻ mutations are *cis* acting.

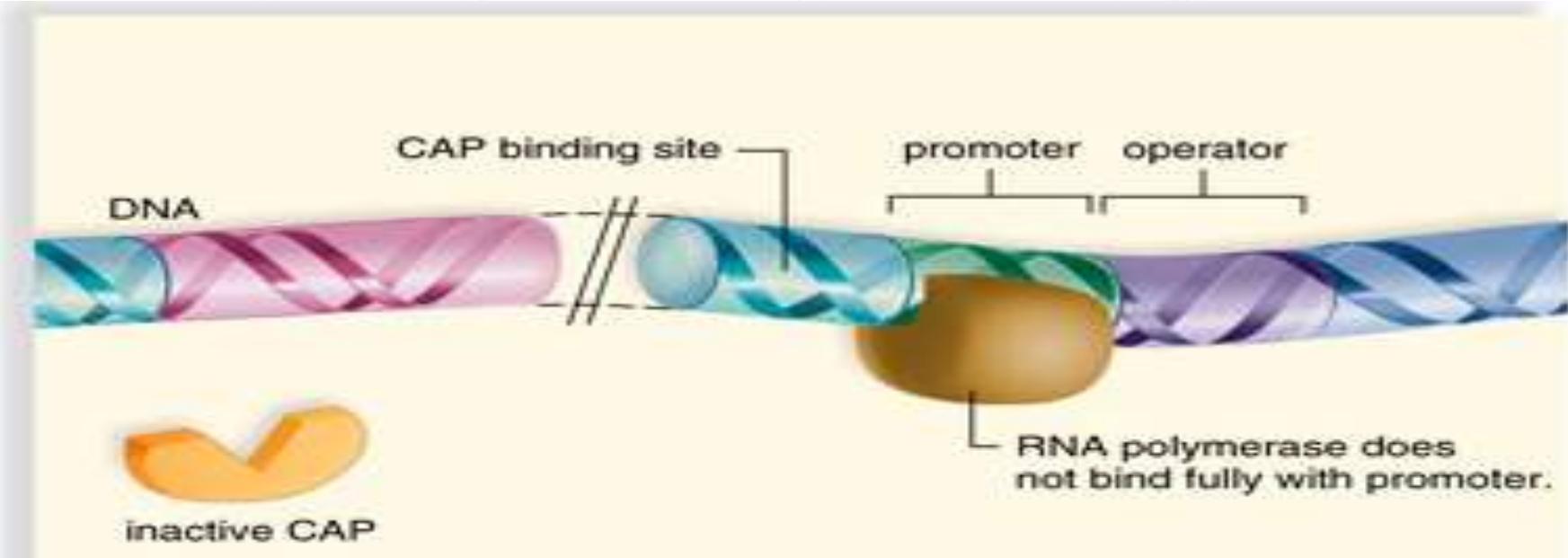
- The lac operon is under two forms of control; **positive** and **negative** control.
- Negative control occurs when the binding of a protein prevents an event.
- Positive control is when the binding causes the event.

POSITIVE CONTROL

- When glucose is available, gene that participate in the metabolism other sugars are repressed, in a phenomenon known as catabolite repression.
- Catabolite repression is a type of +ve control in the lac operon.
- The catabolite activator protein(CAP), complex cAMP, binds to a site near the promoter & stimulates the binding of RNA polymerase.
- A cellular level of cAMP are controlled by glucose; allolactose level increases the abundance of cAMP & enhance the transcription of the lac structural genes.



a. Lactose present, glucose absent (cAMP level high)



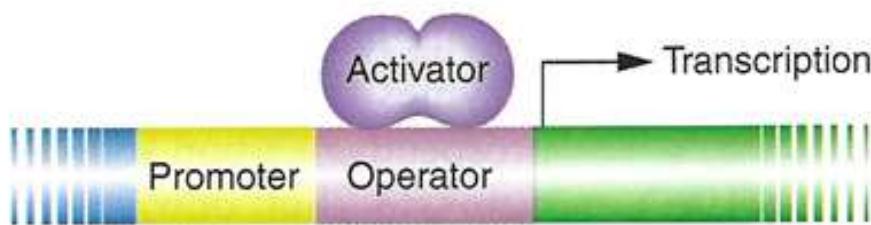
b. Lactose present, glucose present (cAMP level low)

NEGATIVE CONTROL

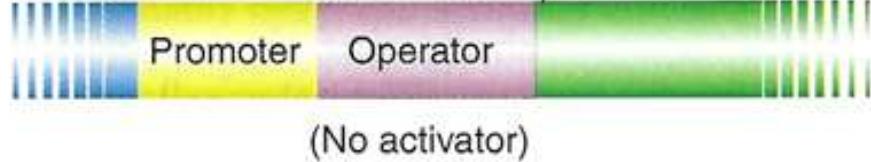
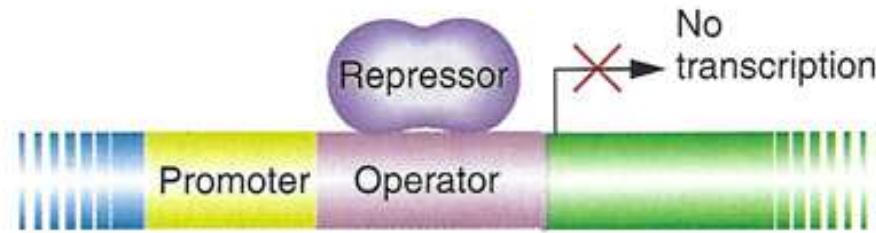
- The lac repressor bind to the operator.
- The DNA sequence cover by the repressor overlaps the DNA sequence recognized by the RNA polymerase.
- Therefore, when the repressor is bound to the operator, RNA polymerase cannot bind to the promoter & transcription can not occur, the *lac* operon is said to be under –ve control.

Positive and negative regulation

Positive regulation



Negative regulation



(No activator)



(No repressor)

POSITIVE VS NEGATIVE CONTROL

Regulatory
protein is
present

Example of
regulatory
protein

**Mutate
regulatory
gene to lose
function**

Positive control

Operon ON

Activator

Operon OFF

Negative control

Operon OFF

Repressor

Operon ON

REFERENCE

Books :

- *Genetics by Benjamin Pierce*
- *iGenetics by Peter J.Russell*

Internet :

- [Www.google.com](http://www.google.com)
- https://www.google.co.in/search?q=The+lac+operon+in+e.coli.ppt&client=opera&hs=OtG&biw=1366&bih=586&source=lnms&tbo=isch&sa=X&ei=OzQ0VJu1N42xuATqjIH4BQ&ved=0CAYQ_AUoAQ

Thanks!

MAJOR TECHNIQUES IN GENE MANIPULATION OF PLANTS



INTRODUCTION

- Plant genetic engineering has become one of the most important molecular tools in the modern molecular breeding of crops.
- Over the last decade, significant progress has been made in the development of new and efficient transformation methods in plants.
- Despite a variety of available DNA delivery methods, *Agrobacterium*- and ballistic-mediated transformation remain the two predominantly employed approaches.

- Production of transgenic plants

Isolate and clone gene of interest



Add DNA segments to initiate or enhance gene expression



Add selectable markers



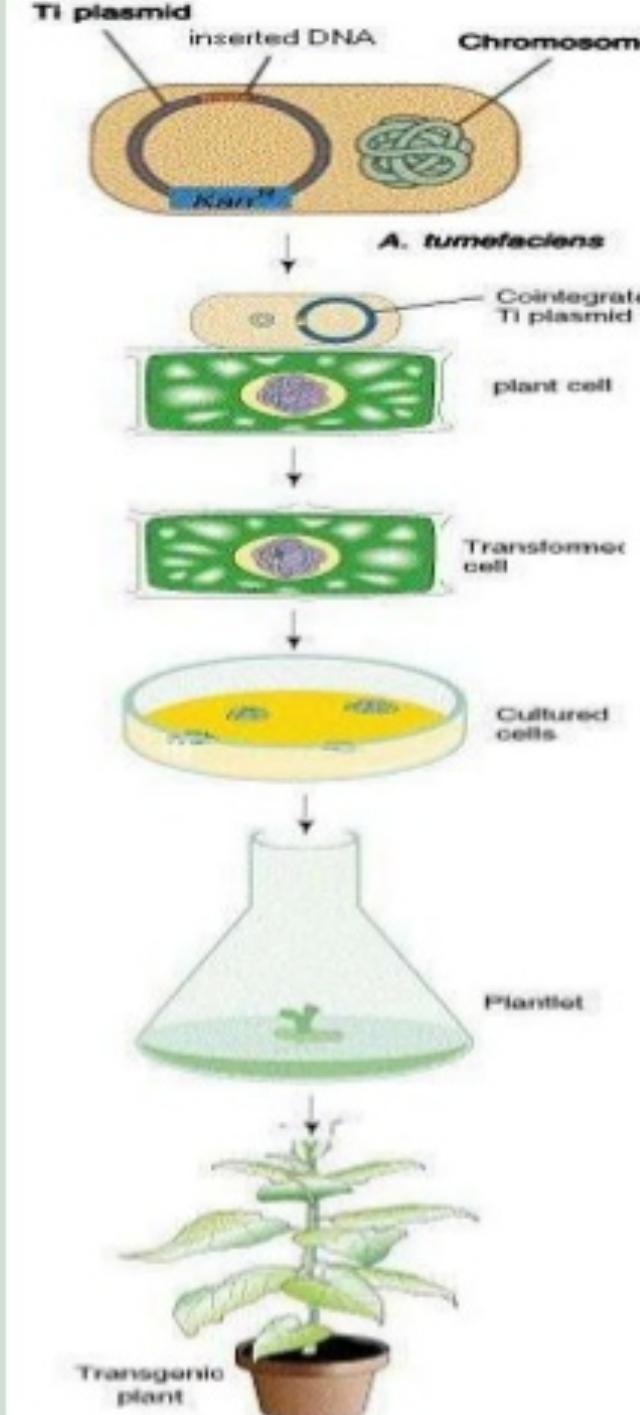
Introduce gene construct into plant cells (transformation)



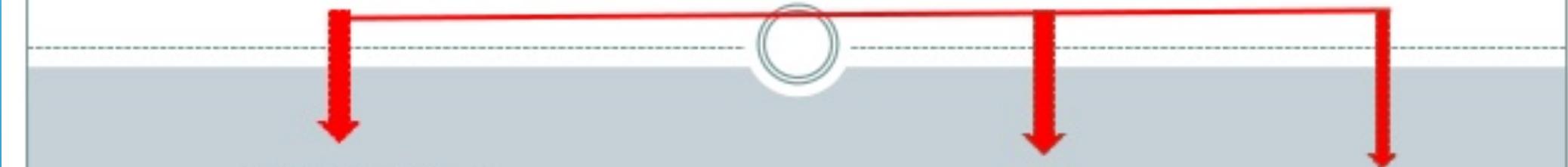
Select transformed cells or tissues



Regenerate whole plants



Plant Transformation Methods

			
(DIRECT)		(INDIRECT)	
Physical	Chemical	Biological	In Planta
<ul style="list-style-type: none">• Microinjection• Pressure• Biolistics – gene gun/particle bombardment• Electroporation• Microinjection• Silica/carbon fibers• Laser mediated	<ul style="list-style-type: none">• PEG• DEAE- dextran• Calcium phosphate• Artificial lipids• Proteins• Dendrimers	<ul style="list-style-type: none"><i>A. Tumefaciens</i><i>A. Rhizogenes</i>• Virus-mediated	<ul style="list-style-type: none">• Meristem transformation• Floral dip method• Pollen transformation

Techniques for plant genetic transformation

- Indirect method- *Agrobacterium* mediated gene transfer
- Direct methods-
 - Particle bombardment (biolistics)
 - Microprojectile gun method
 - Electroporation
 - Silicon carbide fibres
 - Polyethylene glycol (PEG)/protoplast fusion
 - Liposome mediated gene transfer

Transformation vector requirements

- Origin of replication
- Bacterial selectable marker
- Gene constructs of interest
- T-DNA borders and other *Agrobacterium* genes if using *Agrobacterium*
- Compatible with helper plasmid if using *Agrobacterium*

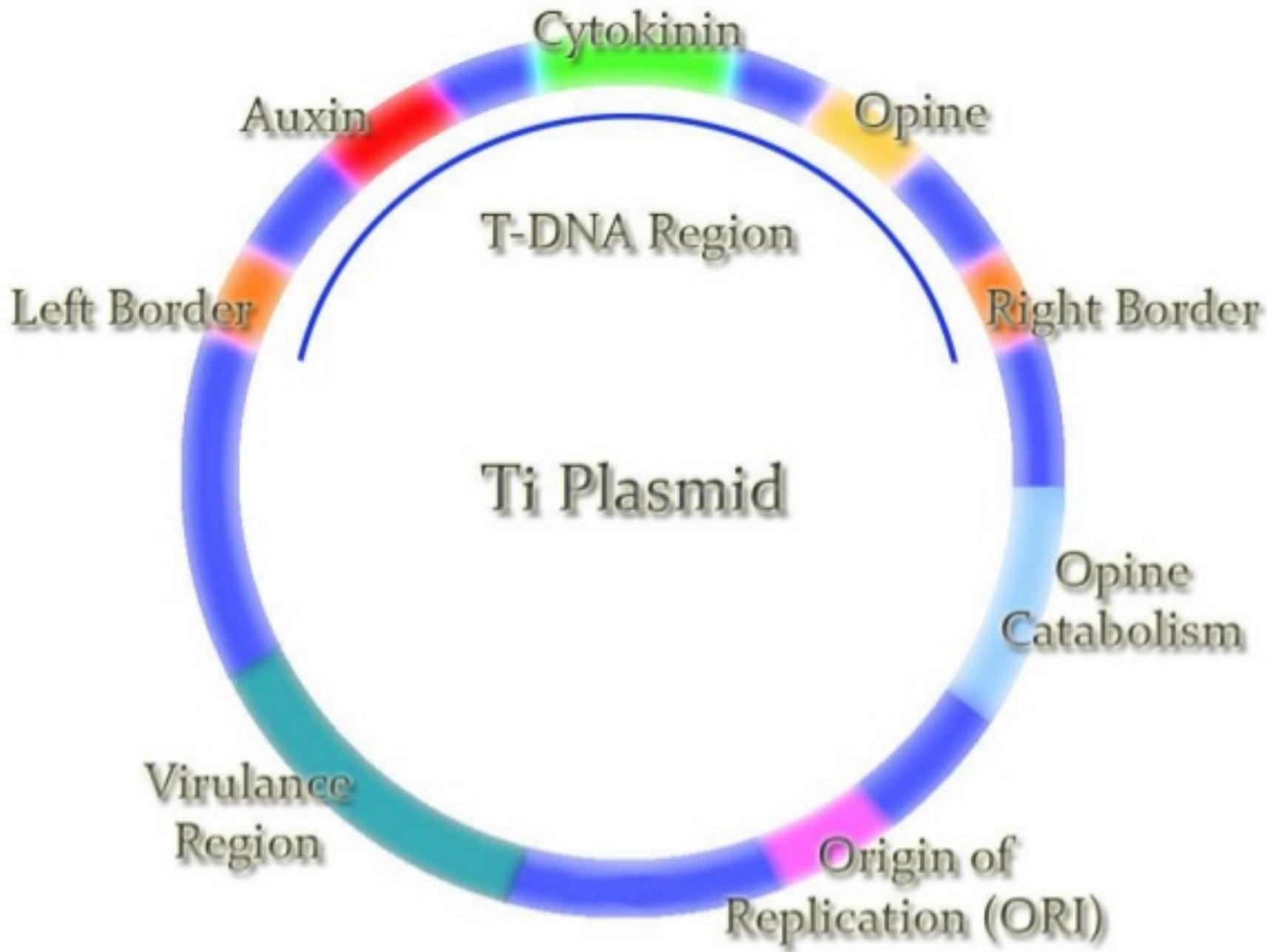
***Agrobacterium* mediated gene transfer**

***Agrobacterium*-**

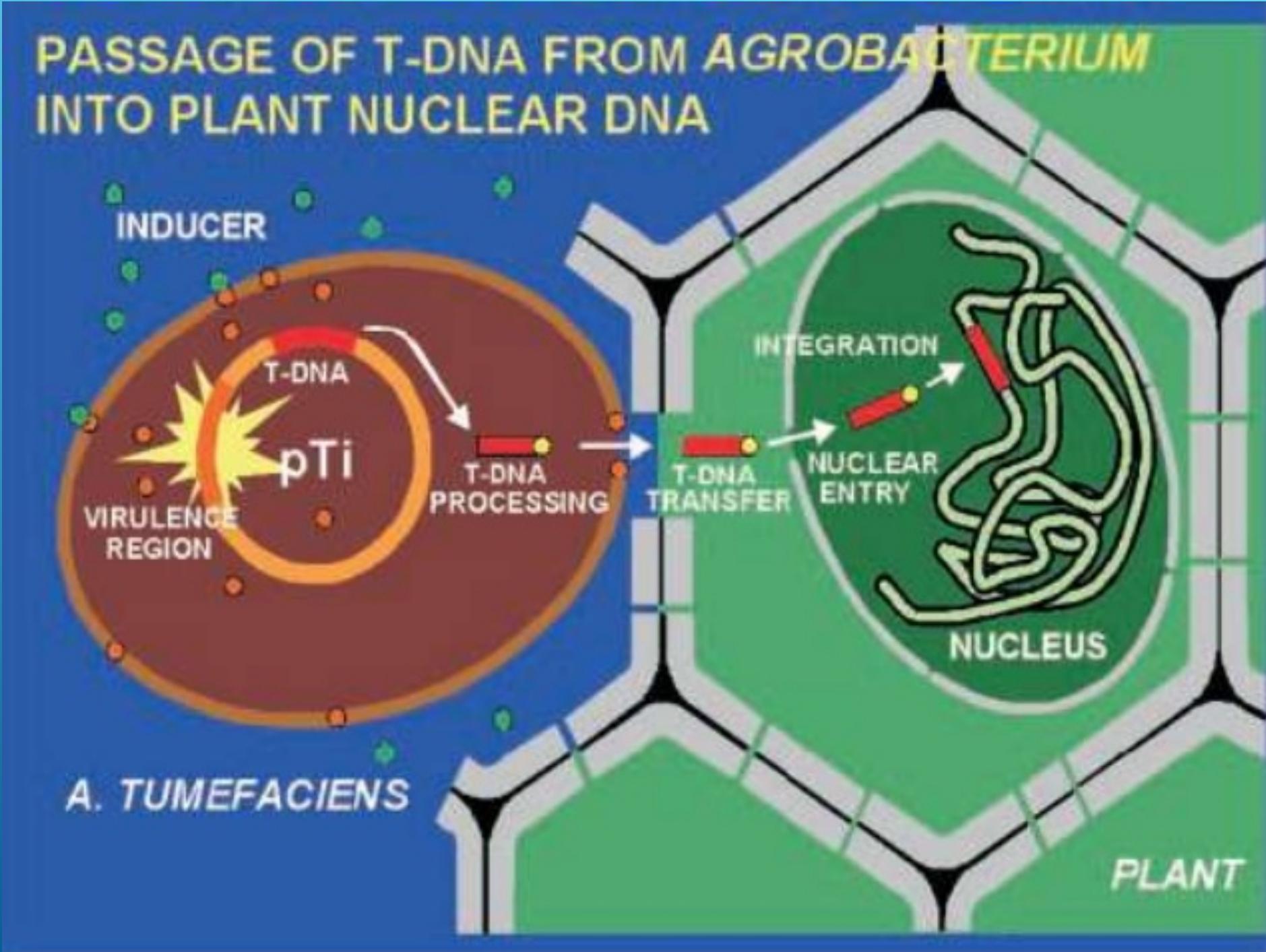
- Soil borne, gram negative, rod shaped, motile found in rhizosphere
- Causative agents of “Crown gall” disease of dicotyledones
- Have ability transfer bacterial genes to plant genome
- Attracted to wound site via chemotaxis in response to chemicals (sugar and Phenolic molecules: acetosyringone) released from damaged plant cells
- Contains Ti plasmid which can transfer its T-DNA region into genome of host plants

Ti-plasmid features

- Two strains of Ti-plasmid:
 - Octopine strains- contains two T-DNA region: T_L (14 kb) and T_R (7 kb)
 - Nopaline strains- contain one T-DNA region(20 kb)
- Size is about 200 kb
- Has a central role in Crown-gall formation
- Contains one or more T-DNA region that is integrated into the genome of host plants
- Contain a *vir* region ~ 40 kb at least 8~11 *vir* genes
- Has origin of replication
- Contains a region enabling conjugative transfer
- Has genes for the catabolism of opines



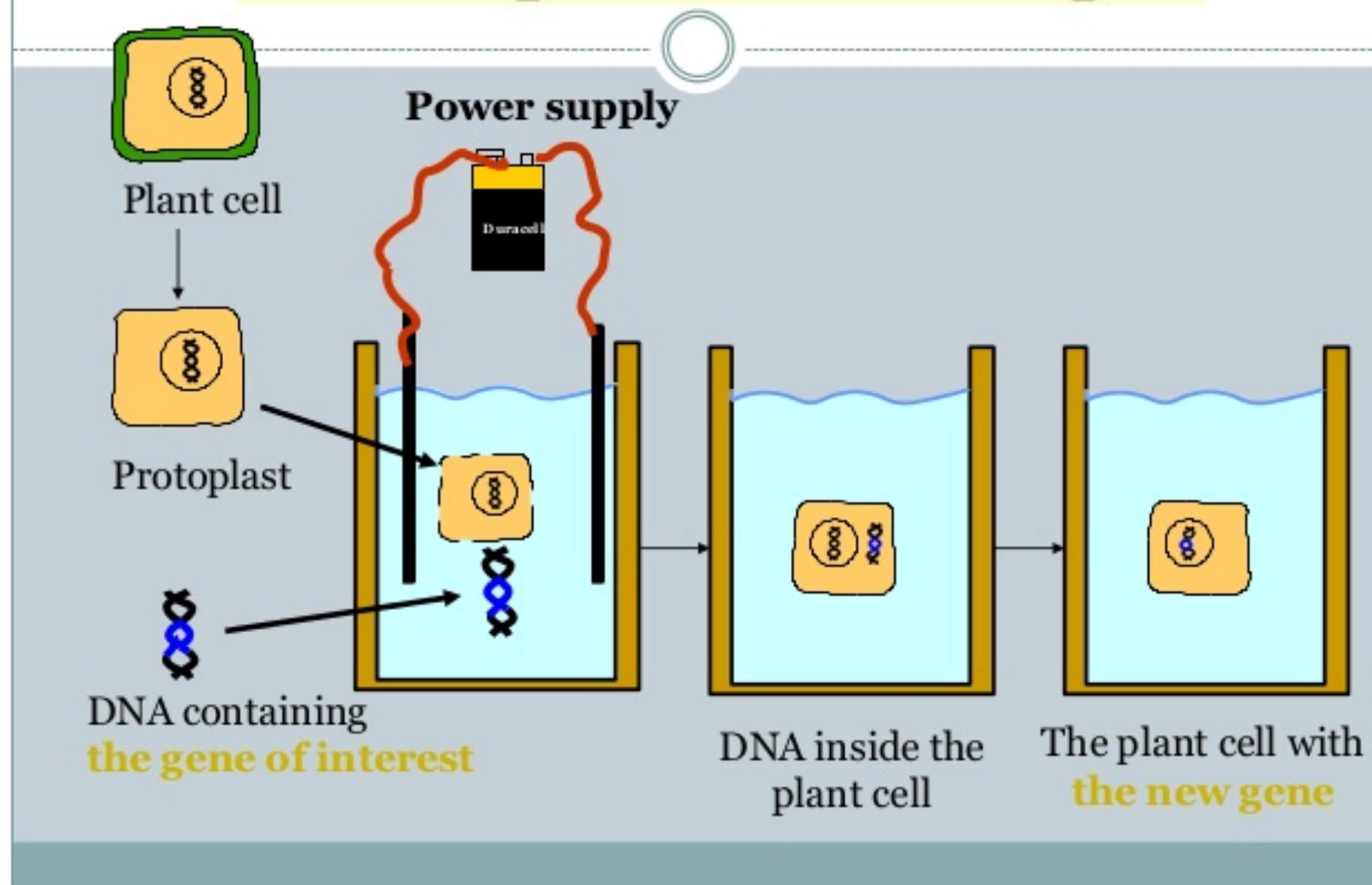
PASSAGE OF T-DNA FROM AGROBACTERIUM INTO PLANT NUCLEAR DNA



Electroporation technique.

- Is the process whereby electrical impulses of high field strength are used to reversibly permeabilize cell membrane to facilitate uptake of large molecules, including DNA.
- It has been used for long time for transient and integrative transformation of protoplasts.
- 1 to 1.5 kV. so uses low capacitance hence short decay time .

Electroporation Technique

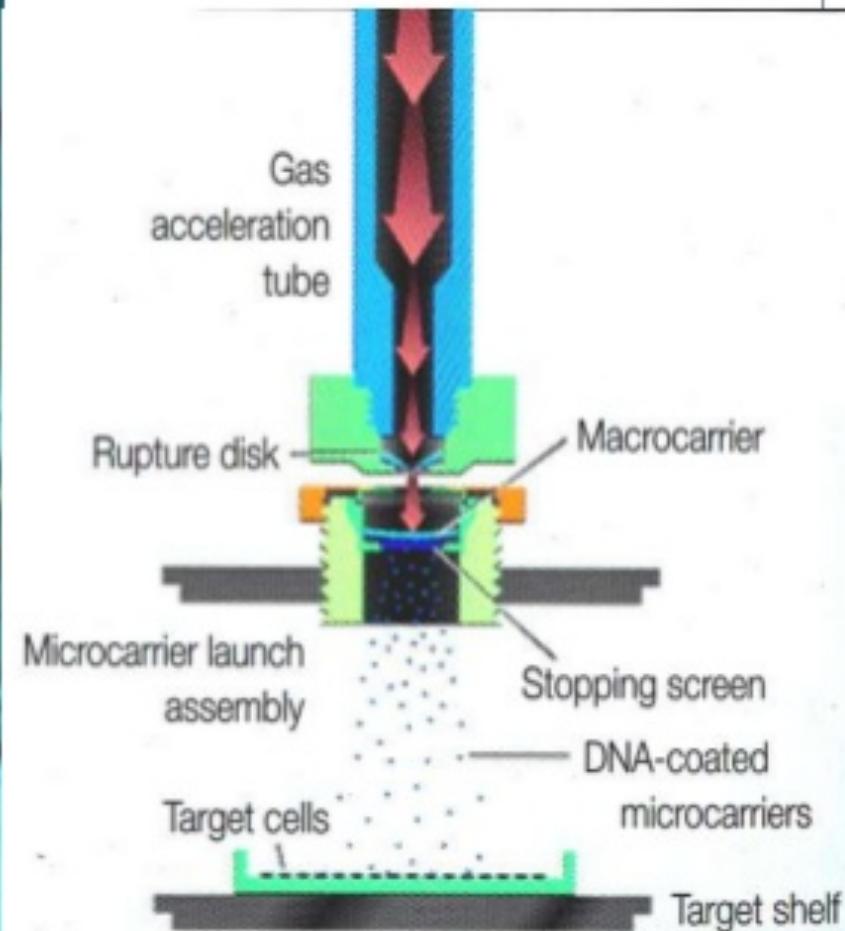


Biolistic/Particle bombardment

- High velocity micro projectile were utilize to deliver nucleic acids into living cells.
- **Advantages :**
 - Transformation of organized tissue
 - Universal delivery system
 - Transformation of recalcitrant spp
 - Study of basic plant development processes.

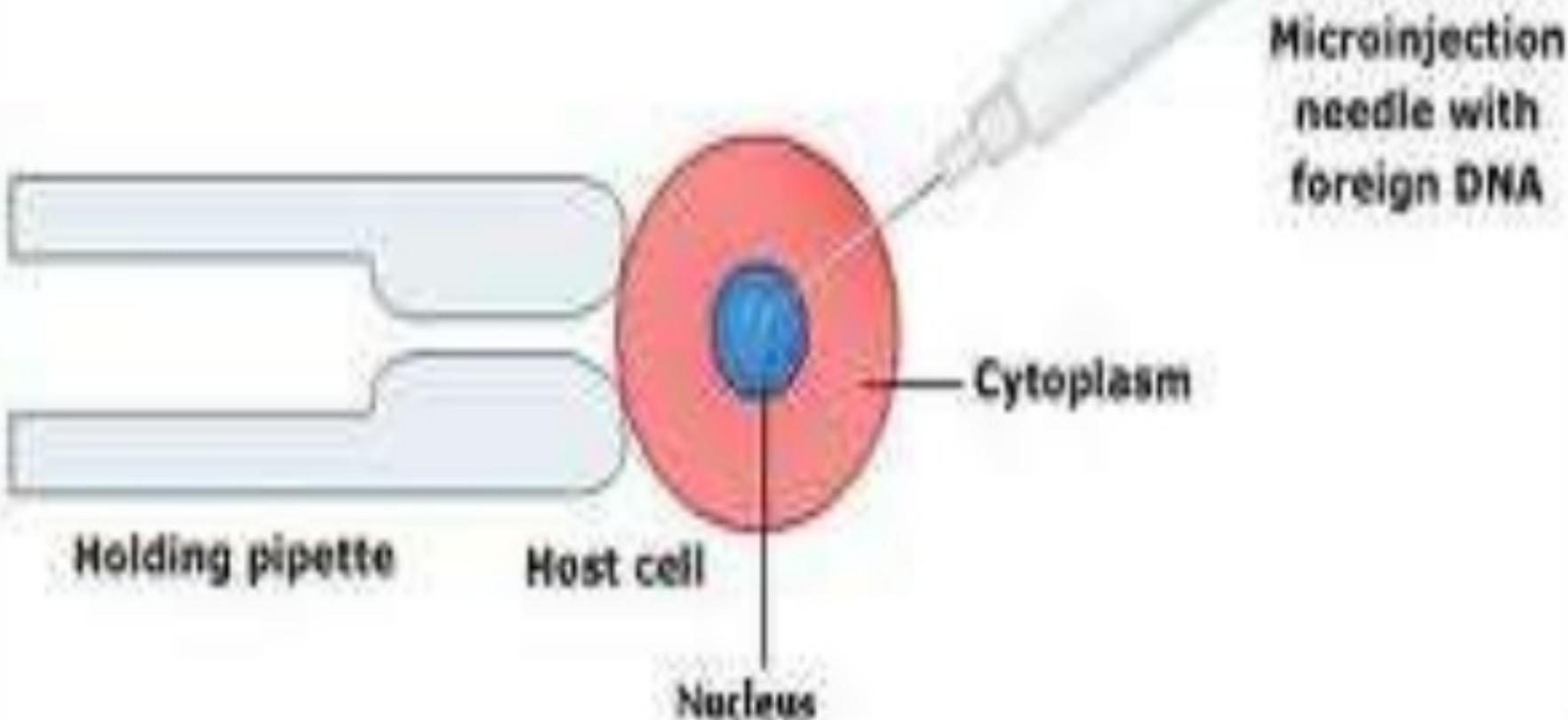
Particle Gun

The Helium Gas Gun – Circa 2000



Microinjection

- Under a microscope, a cell is manipulated to a blunt capillary. Gentle suction holds the cell in place.
- With a micromanipulator, a very fine tipped pipet is inserted into the cytoplasm or nucleus.
- DNA or RNA is injected directly into the nucleus or cytoplasm.
- Microinjection has been successfully used with large frog eggs, cultured mammalian cells, mammalian embryos, and plant protoplasts and tissues

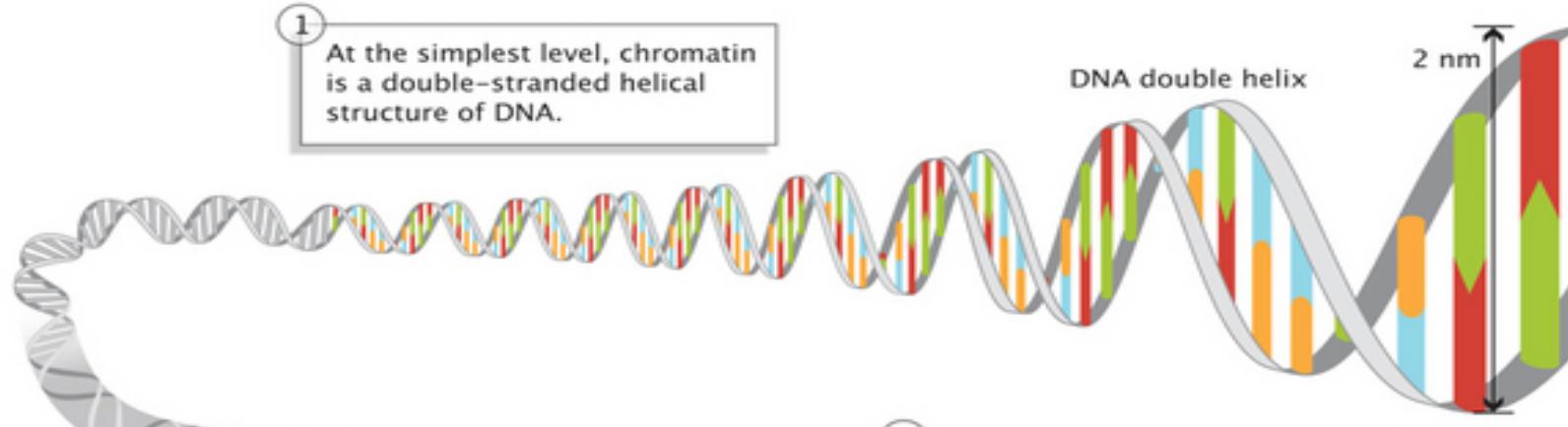


Thank you



THE ORGANIZATION AND CONTROL OF EUKARYOTIC GENOMES

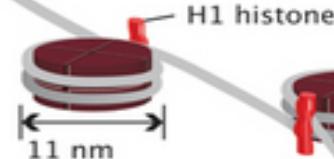
- BY ANANDI R



2 DNA is complexed with histones to form nucleosomes.

3 Each nucleosome consists of eight histone proteins around which the DNA wraps 1.65 times.

Nucleosome core of eight histone molecules



4 A chromatosome consists of a nucleosome plus the H1 histone.

Chromatosome

6 ... that forms loops averaging 300 nm in length.



250-nm-wide fiber

700 nm

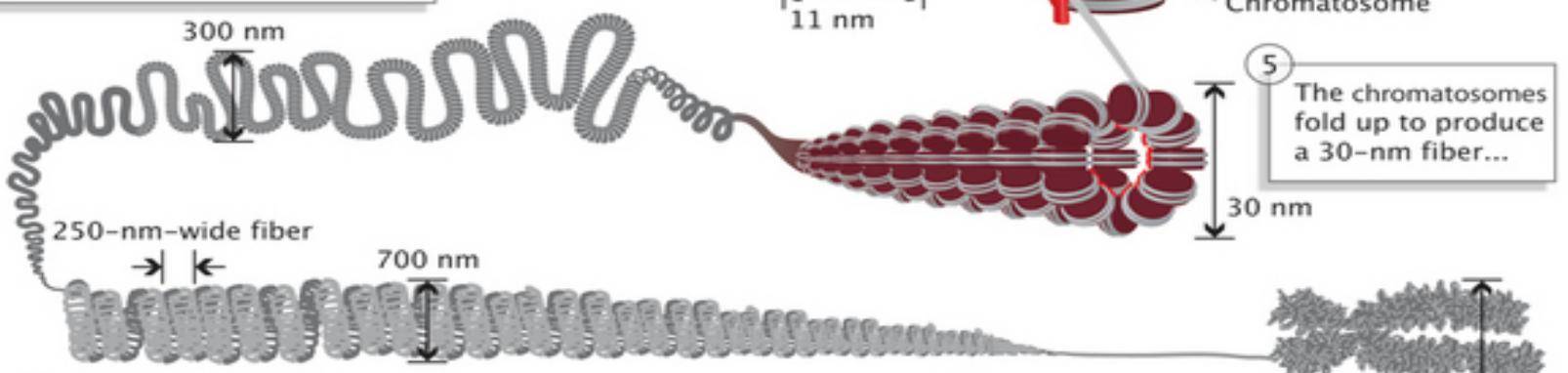
7 The 300-nm fibers are compressed and folded to produce a 250-nm-wide fiber.

5 The chromatosomes fold up to produce a 30-nm fiber...

30 nm

8 Tight coiling of the 250-nm fiber produces the chromatid of a chromosome.

1400 nm



Genome includes

- ▶ Double stranded DNA
- ▶ Histone proteins
- ▶ Nucleosomes
- ▶ Chromatosomes
- ▶ Chromatids
- ▶ Chromosomes

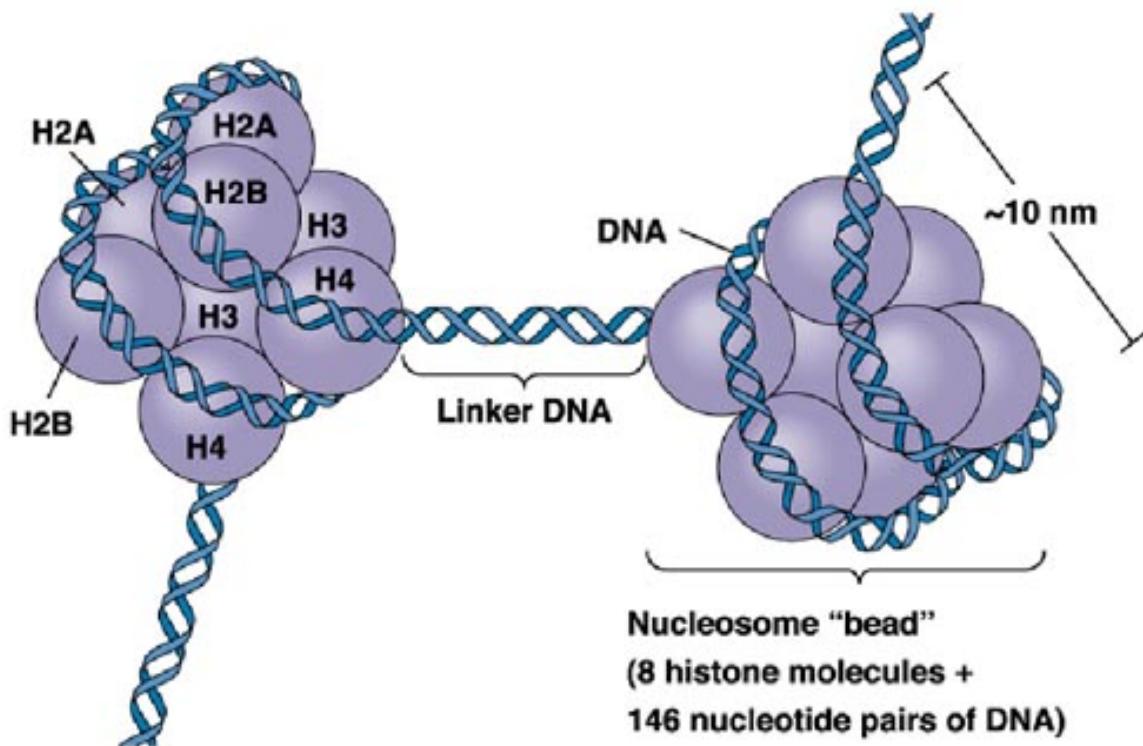
Chromatin structure

- ▶ Eukaryotic DNA is precisely combined with large amounts of protein.
- ▶ During interphase, chromatin fibers are highly extended.
- ▶ If extended, each DNA molecule would be about 6 cm long

DNA packing

- ▶ **First level - Histone** proteins
- ▶ Their positively charged amino acids bind tightly to negatively charged DNA.
- ▶ The five types of histones are very similar from one eukaryote to another .
- ▶ Unfolded chromatin has the appearance of beads on a string, a **nucleosome**, in which DNA winds around a core of histone proteins.

Nucleosomes



DNA packing

- ▶ The beaded string seems to remain essentially intact throughout the cell cycle.
- ▶ Histones leave the DNA only transiently during DNA replication.
- ▶ They stay with the DNA during transcription
- ▶ By changing shape and position, nucleosomes allow RNA-synthesizing polymerases to move along the DNA.

DNA packing

- ▶ **Level two** - As chromosomes enter mitosis the beaded string coils to form the 30-nm *chromatin fiber*.
- ▶ **Level three** - This fiber forms *looped domains* attached to a scaffold of non histone proteins.
- ▶ **Level four** - the looped domains coil and fold to produce the characteristic metaphase chromosome.

DNA packing

- ▶ Interphase chromatin is generally much less condensed than the chromatin of mitosis with the 30-nm fibers and looped domains remaining intact.
- ▶ The chromatin of each chromosome occupies a restricted area within the interphase nucleus.
- ▶ Interphase chromosomes have areas that remain highly condensed, **heterochromatin**, and less compacted areas, **euchromatin**.

Assignment

- ▶ Differentiate between heterochromatin and euchromatin.

Genome Organization at the DNA Level

- ▶ In eukaryotes, most of the DNA (about 97% in humans) does *not* code for protein or RNA.
 1. noncoding regions are regulatory sequences.
 2. introns.
 3. **Repetitive DNA, present in many copies in the genome. (Three Types)**

Types of Repeated DNA Sequences

1. Long terminal repeats (LTRs)

- ▶ Are identical sequences of DNA that repeat hundreds or thousands of times found at either end of retrotransposons or proviral DNA formed by reverse transcription of retroviral RNA.
- ▶ They are used by viruses to insert their genetic material into the host genomes.

Types of Repeated DNA Sequences

2. Tandem repeats

occur in DNA when a pattern of one or more nucleotides is repeated and the repetitions are directly adjacent to each other.

- ▶ Satellite DNA - typically found in centromeres and heterochromatin
- ▶ Minisatellite - repeat units from about 10 to 60 base pairs, found in many places in the genome, including the centromeres
- ▶ Microsatellite - repeat units of less than 10 base pairs; this includes telomeres, which typically have 6 to 8 base pair repeat units

Types of Repeated DNA Sequences

3. Interspersed repeat

- ▶ These sequences propagate themselves by RNA mediated transposition, they have been called retrotransposons.
- ▶ Some types of interspersed repetitive DNA elements allow new genes to evolve by uncoupling similar DNA sequences from gene conversion during meiosis.
- ▶ Types : Transposable elements, DNA transposons, retrotransposons

Regulation of Gene Expression in Prokaryotes

- BY ANANDI R

Introduction

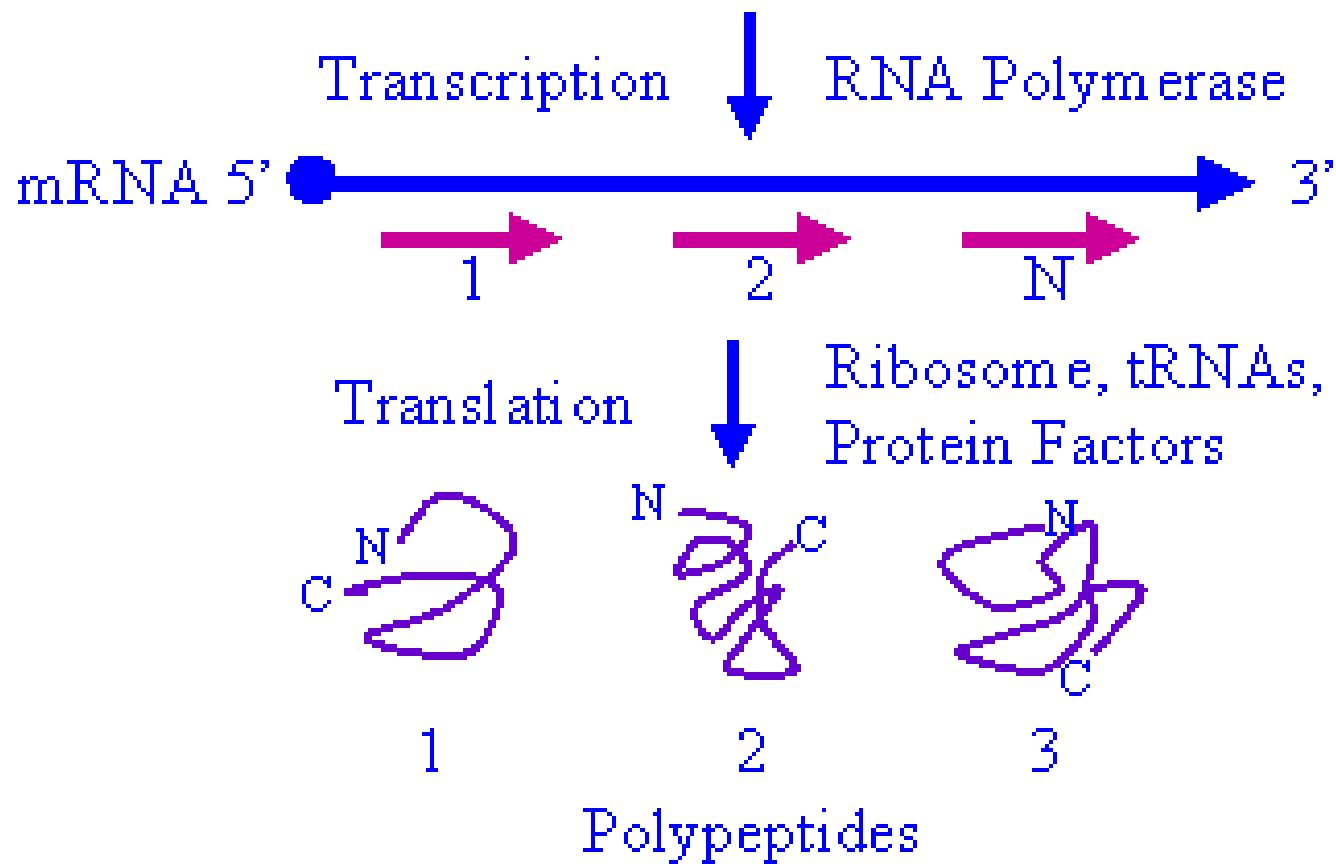
- ▶ Control of gene expression is at the level of **transcription**.
- ▶ If a gene is not transcribed then the gene product and ultimately the phenotype will not be expressed.
- ▶ We are now going to consider two systems of control of gene expression in the *E. coli* cell.
- ▶ Both of these systems are concerned with the production of enzymes involved in cell metabolism, but each exhibits a different type of control.
- ▶ **Induction** - the production of a specific enzyme (or set of enzymes) in response to the presence of a substrate.
- ▶ **Repression** - the cessation of production of a specific enzyme (or set of enzymes) in response to an increased level of a substrate.

Introduction

- ▶ All of the genes which encode the enzymes necessary for the pathway are found next to each other on the *E. coli* chromosome.
- ▶ One key feature of both systems to be discussed is that a single mRNA is transcribed with multiple translation stop codons.
- ▶ The proteins that can be translated from the mRNA are the enzymes required for a specific pathway.
- ▶ This type of mRNA is called a **Polycistronic mRNA (code for the enzymes and are translated from a single mRNA)** and is totally unique to prokaryotes.

Prokaryotic Gene Expression

Promoter | Cistron1 | Cistron2 | CistronN | Terminator



Gene Expression

- ▶ Constitutive
- ▶ Non - Constitutive
- ▶ Inducible
- ▶ Repressible
- ▶ Positive and Negative Control

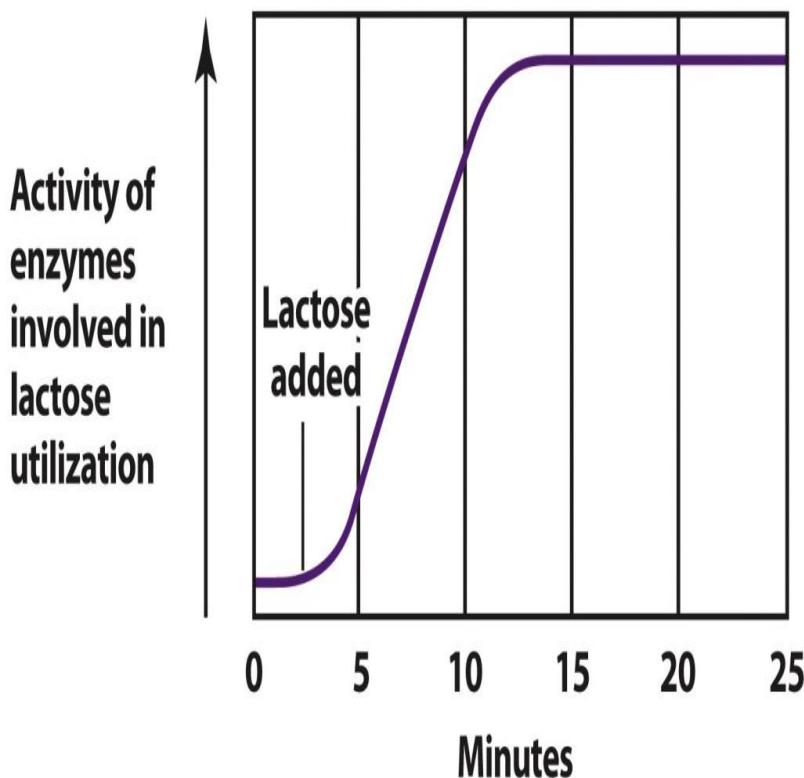
Constitutive and Non-Constitutive gene expression

- ▶ **Constitutive:** Genes that specify cellular components that perform housekeeping functions— for example, the ribosomal RNAs and proteins involved in protein synthesis are expressed **constitutively**.
- ▶ **Non-Constitutive:** Other genes often are expressed only when their products are required for growth.

Inducible (Induction of Genes)

Example : Lactose Utilization

Induction of enzyme synthesis

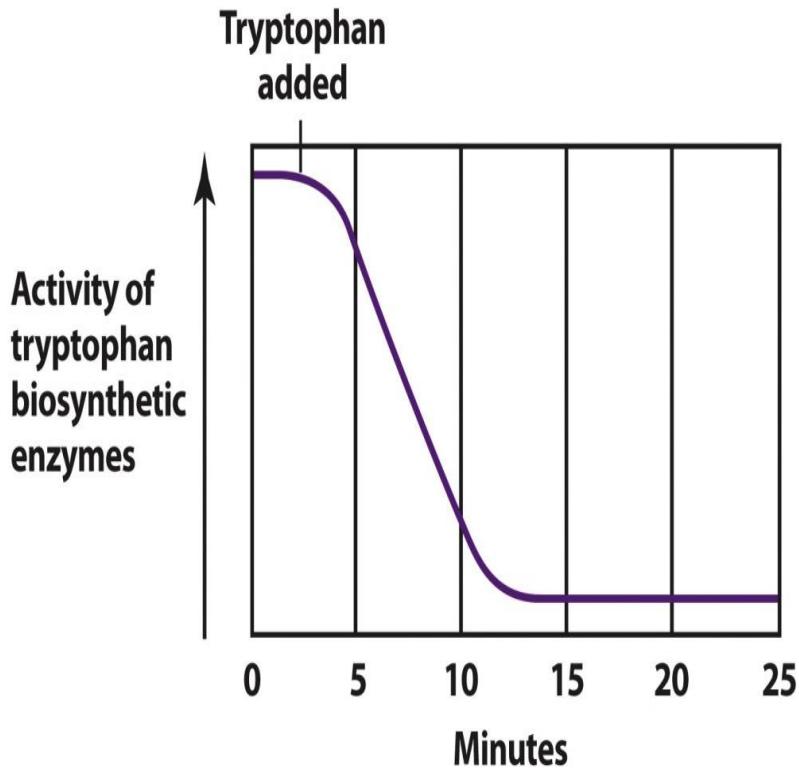


- ▶ Gene expression is induced when glucose is absent and lactose is present.
- ▶ Induction occurs at the level of transcription and alters the rate of enzyme synthesis.
- ▶ Enzymes involved in catabolic pathways are often inducible.

Repressible (Repression of Genes)

Example :Tryptophan Biosynthesis

Repression of enzyme synthesis

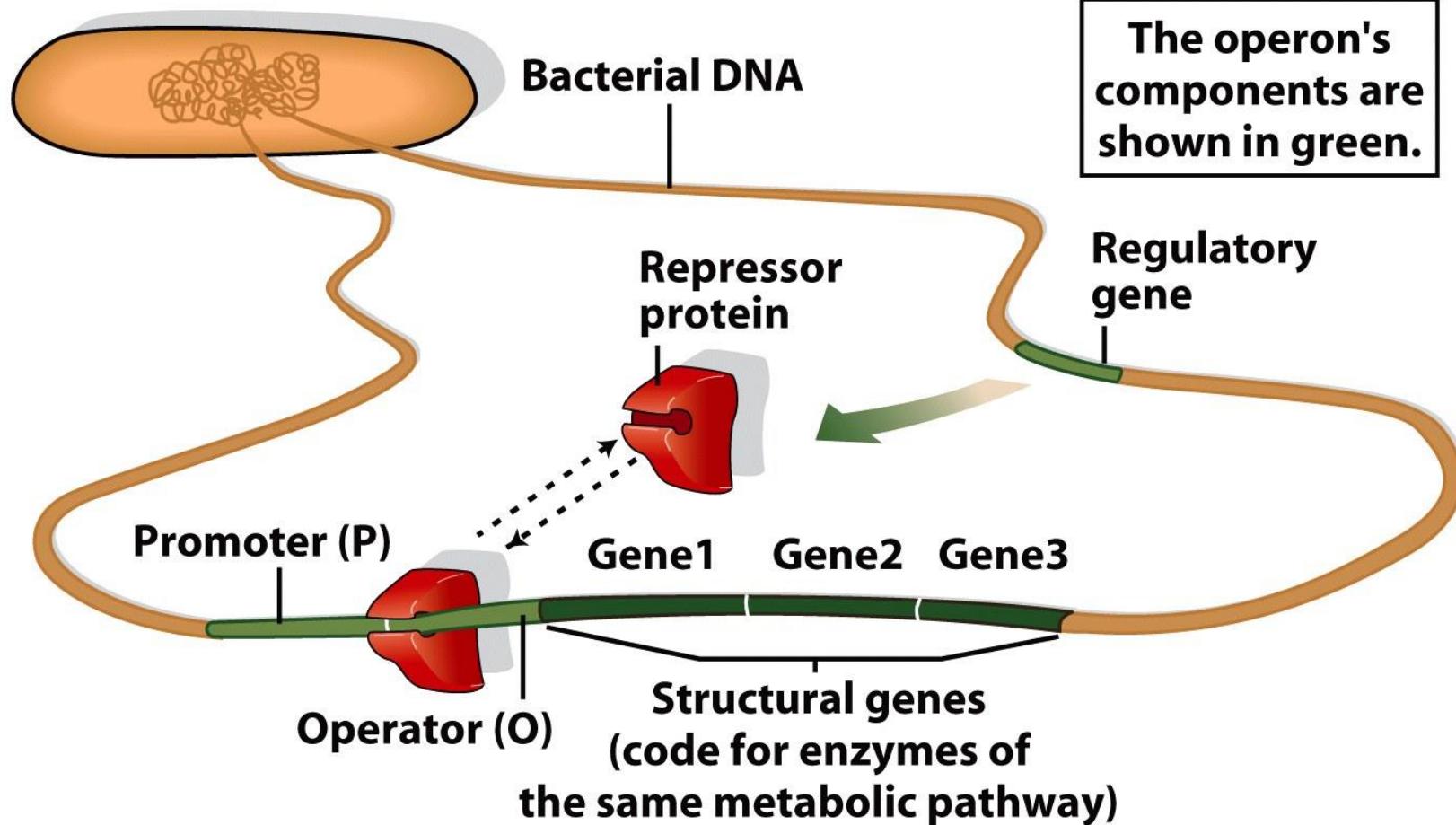


- ▶ Genes are turned on (depressed) in the absence of tryptophan and turned off (repressed) when tryptophan is available.
- ▶ Repression occurs at the level of transcription.
- ▶ Enzymes involved in anabolic pathways are often repressible.

Regulatory gene

- ▶ The product of a **regulatory gene** is required to **initiate (turn on)** the expression of one or more genes.
- ▶ The product of a **regulatory gene** is required to **turn off** the expression of one or more genes.

Regulatory gene: Organization of a bacterial operon



Positive and Negative Control Mechanisms

- ▶ **Regulator genes** encode products that regulate the expression of other genes.
- ▶ In **positive control mechanisms**, the product of the regulator gene is required to **turn on** the expression of structural genes.
- ▶ In **negative control mechanisms**, the product of the regulator gene is necessary to **shut off** the expression of structural genes.

Regulatory Mechanisms

- ▶ In a **positive control mechanism**, the activator is involved in **turning on** gene expression.
- ▶ In a **negative control mechanism**, the co-repressor is involved in **turning off** gene expression.

Operons

- ▶ Units required for Gene Expression
 - Sequence of DNA
- ▶ In prokaryotes, the **operon** includes structural genes, the operator and the promoter.

Components of the Operon Model

- ▶ The repressor gene encodes a **repressor**.
- ▶ The repressor binds (under appropriate conditions) to the **operator**. Binding is regulated by the presence or absence of the **effector molecule** (inducer or co-repressor).
- ▶ The **promoter** is the site of transcription initiation for the structural gene(s).
- ▶ Transcription of the **structural gene(s)** is regulated by binding of the repressor to the operator.

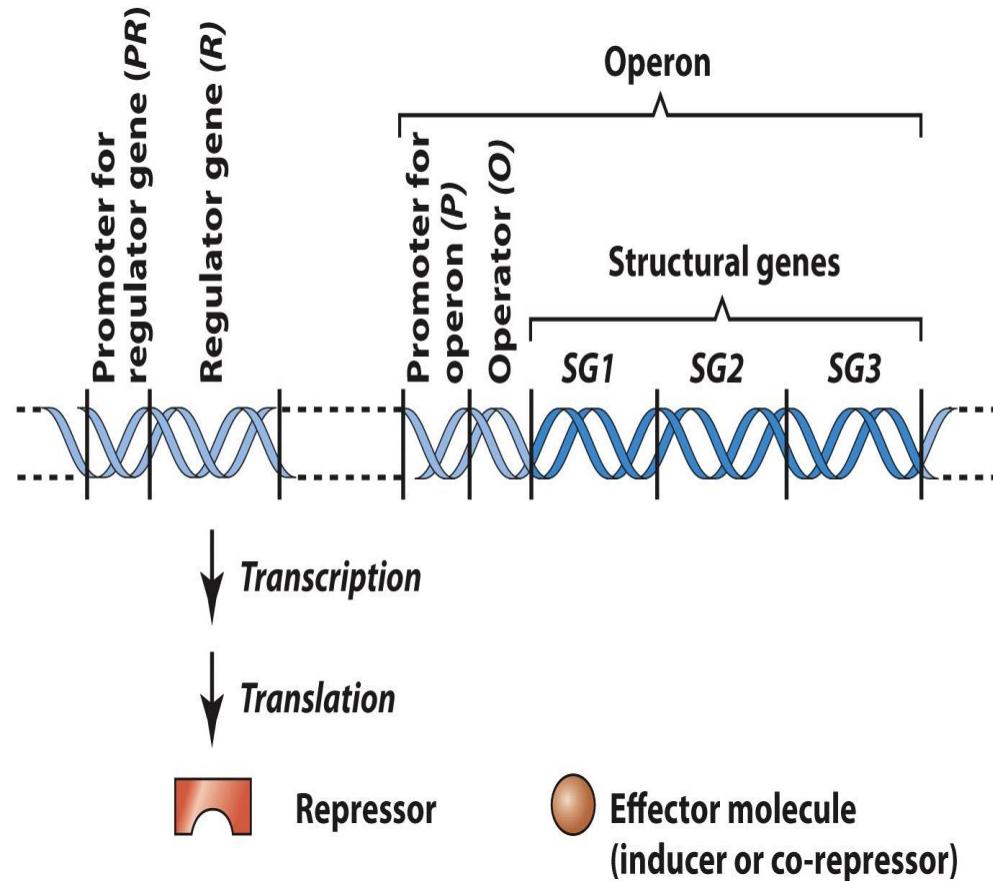
Control of Gene Expression in Bacteria (The Bacterial Operon)

An **operon** is a functional complex of genes containing the information for enzymes of a metabolic pathway. It includes:

- ▶ **Structural genes** – code for the enzymes and are translated from a single mRNA (**Polycistronic**).
- ▶ **Promoter** – where the RNA polymerase binds.
- ▶ **Operator** – site next to the promoter , where the regulatory protein can bind.
- ▶ A **repressor** (proteins) which binds to a specific DNA sequence to determine whether or not a particular gene is transcribed.
- ▶ The **regulatory gene** encodes the repressor protein.

The Operon Model

The operon: components



Each operon contains

- *several contiguous structural genes*
- *a promoter*
- *an operator*

The Structural Genes of an Operon

- ▶ **A single mRNA transcript** carries the coding information of an entire operon.
- ▶ **Operons** containing more than one structural gene are **multigenic**.
- ▶ **All structural genes** in an operon are **co-transcribed** and therefore are coordinately expressed.

The Lactose (lac) Operon in *E. coli*: Induction and Catabolite Repression

The structural genes in the *lac* operon are transcribed only when lactose is present and glucose is absent.

The *lac* Operon - an inducible system

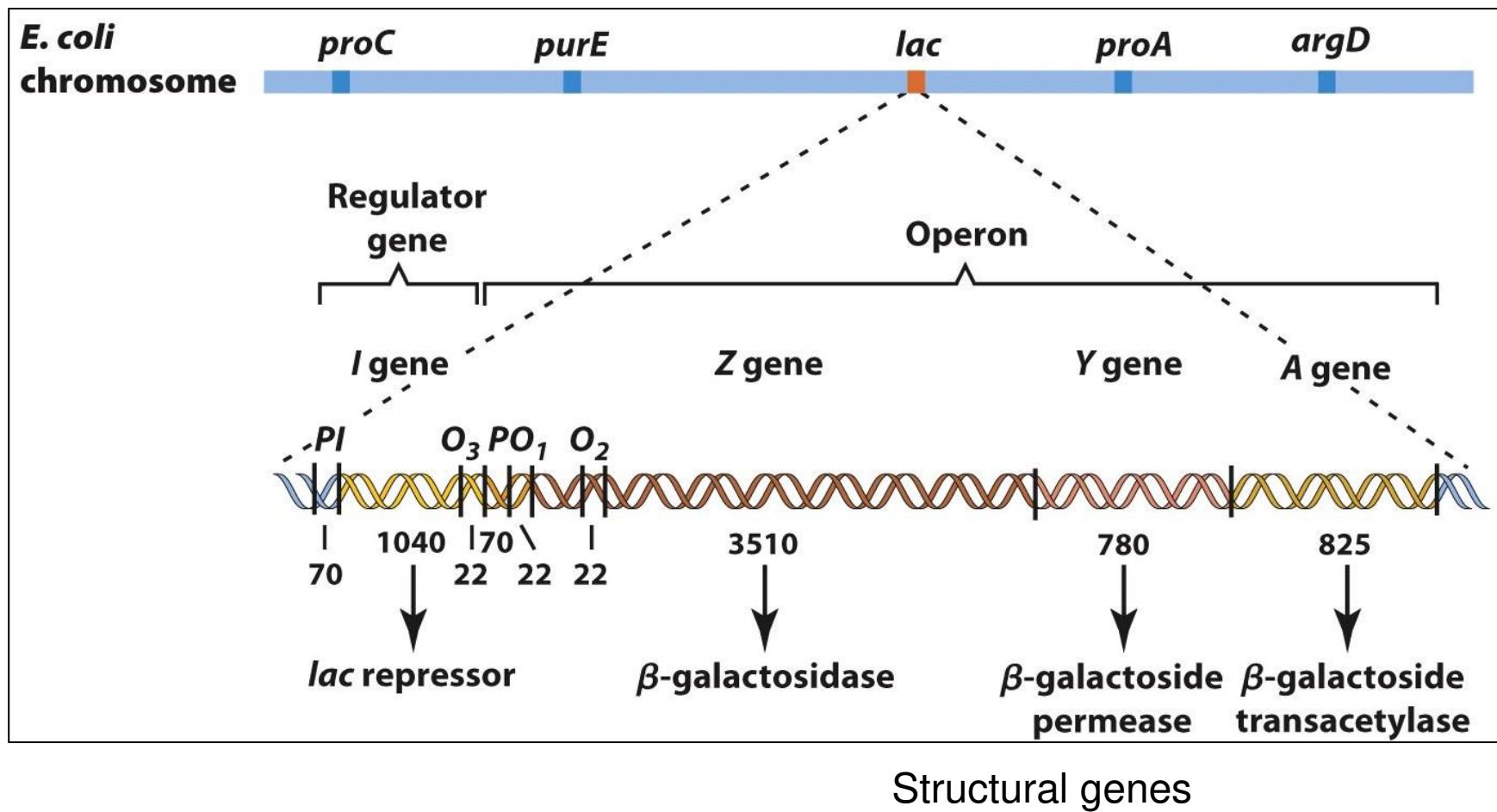
- ▶ The first control system for enzyme production worked out at the molecular level described the control of enzymes that are produced in response to the presence of the sugar lactose in *E. coli* cell.
- ▶ The work was performed by Jacob and Monod for which they were awarded the Nobel Prize.
- ▶ Pathway for production of glucose from lactose.

The *lac* Operon - an inducible system

Several proteins involved in lactose metabolism in the *E. coli* cell. They are:

- ▶ β -galactosidase - converts lactose into glucose and galactose.
- ▶ β -galactoside permease - transports lactose into the cell.
- ▶ β -galactoside transacetylase – an enzyme that transfers an acetyl group from acetyl-CoA to β -galactosides.

The *lac* Operon of *E. coli*



The *lac* Operon of *E. coli*

lac Operon Gene	Gene Function
I	Gene for repressor protein
P	Promoter
O	Operator
lac Z	Gene for β -galactosidase
lac Y	Gene for β -galactoside permease
lac A	Gene for β -galactoside transacetylase

Induction of the *lac* Operon

- ▶ In the absence of inducer, the repressor binds to the *lac* operator and represses transcription of the structural genes.
- ▶ When the repressor binds to inducer, it is released from the operator, and transcription of the structural genes is turned on.
- ▶ The inducer, allolactose, is derived from lactose in a reaction catalyzed by β -galactosidase.
- ▶ The *lac I* gene encodes a repressor.

Catabolite Repression (Glucose effect)

High glucose... low induction of lac operator

- ▶ The *lac* promoter has two components
 - ▶ The RNA polymerase binding site
 - ▶ A binding site for catabolite activator protein (CAP)
- ▶ Binding of CAP to the promoter activates transcription of the *lac* operon from being induced when glucose is absent.
- ▶ CAP binds to the promoter only when cyclic AMP (cAMP) is present at sufficient concentrations.

CAP Exerts Positive Control of the *lac* Operon; cAMP is the Effector

- ▶ When glucose is present
 - ▶ Adenylcyclase is inactive.
 - ▶ cAMP levels are low.
 - ▶ CAP cannot bind to the *lac* operon.
 - ▶ The *lac* structural genes cannot be induced at high levels.
- ▶ When glucose is absent
 - ▶ Adenylcyclase is active.
 - ▶ cAMP levels are high.
 - ▶ CAP/cAMP binds to the *lac* operon.
 - ▶ The *lac* structural genes can be induced.

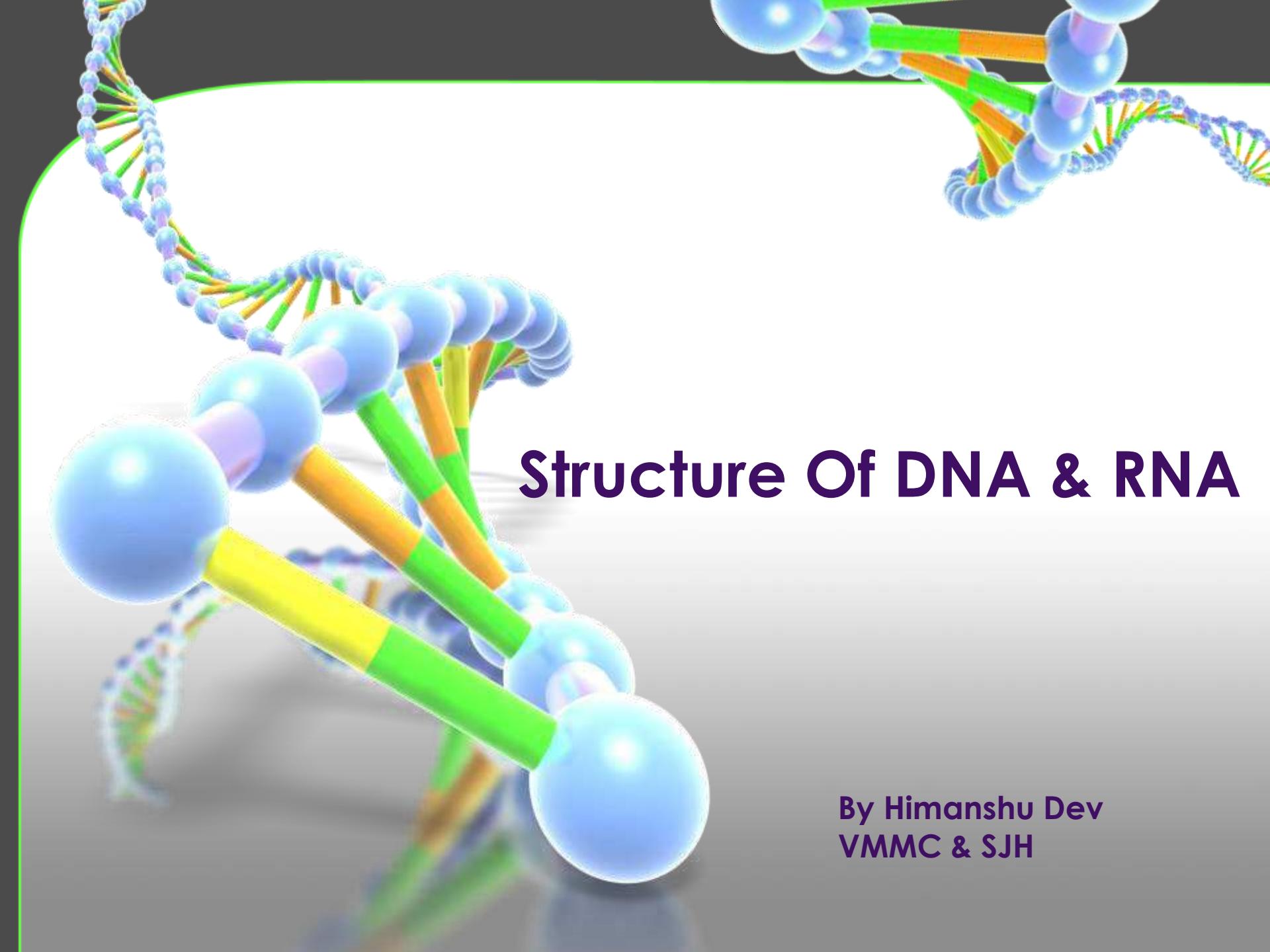
Video link

The Lac operon | Regulation of gene expression

- ▶ <https://www.youtube.com/watch?v=sc9pAk0blgo>
- ▶ trp operon
- ▶ <https://www.youtube.com/watch?v=Ay7OhRqYNvM>

Trp operon

► Assignment



Structure Of DNA & RNA

By Himanshu Dev
VMMC & SJH

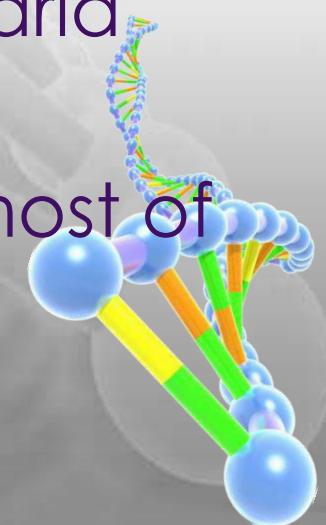
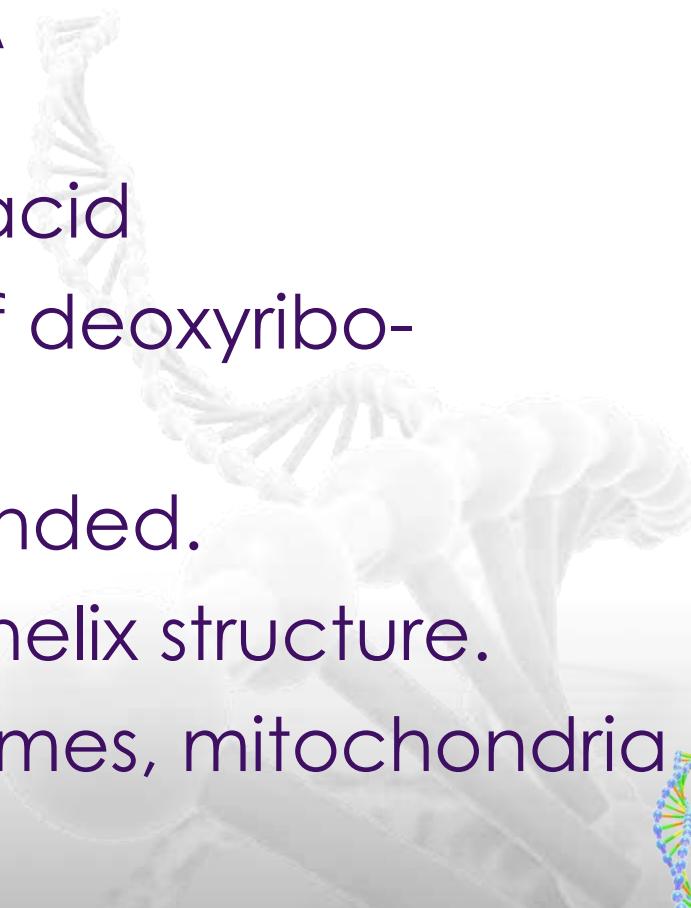
DNA





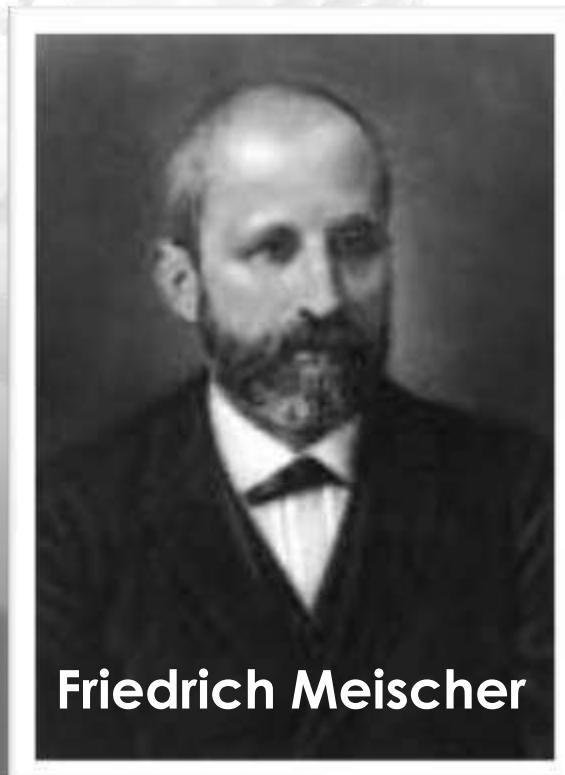
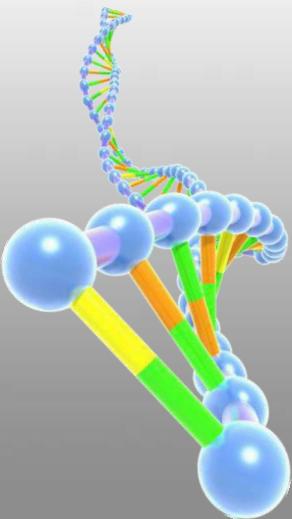
DNA

- Deoxyribonucleic acid
- DNA - a polymer of deoxyribonucleotides.
- Usually double stranded.
- And have double-helix structure.
- found in chromosomes, mitochondria and chloroplasts.
- It acts as the genetic material in most of the organisms.
- Carries the genetic information

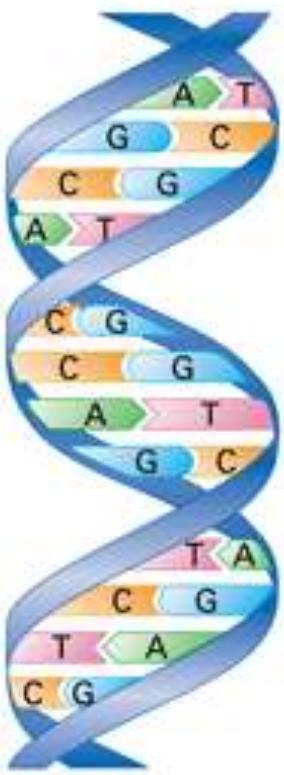
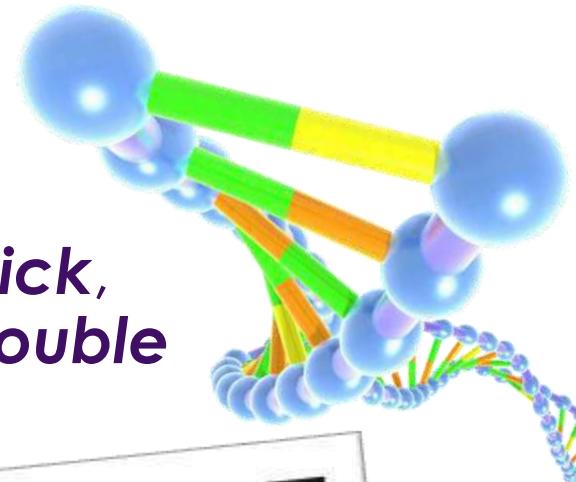


A Few Key Events Led to the Discovery of the Structure of DNA

- DNA as an acidic substance present in nucleus was first identified by **Friedrich Meischer** in 1868.
- He named it as 'Nuclein'.



➤ In 1953, **James Watson and Francis Crick**, described a very simple but famous **Double Helix** model for the structure of DNA.



FRANCIS CRICK AND JAMES WATSON

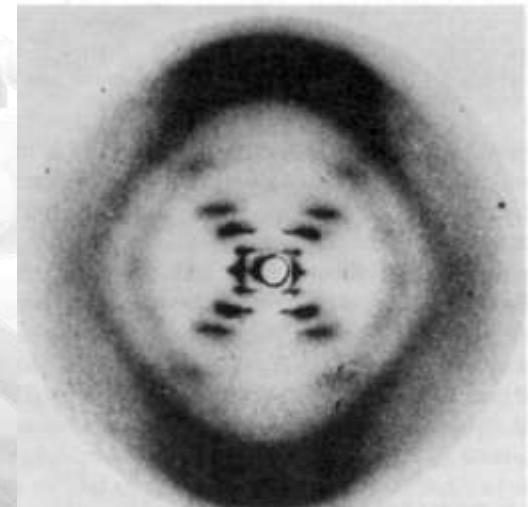
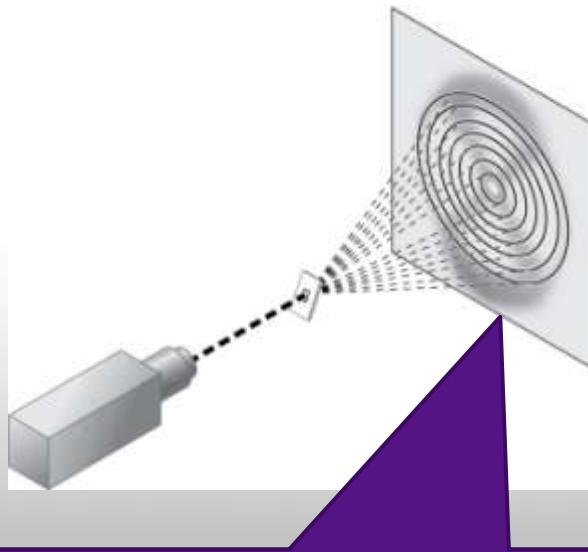


- The scientific framework for their breakthrough was provided by other scientists including
 - Linus Pauling
 - Rosalind Franklin and Maurice Wilkins
 - Erwin Chargaff

❖ Rosalind Franklin

- She worked in same laboratory as Maurice Wilkins.
- She study X-ray diffraction to study wet fibers of DNA.

X-ray diffraction
of wet DNA fibers



The diffraction pattern is interpreted
(using mathematical theory)

This can ultimately provide
information concerning the structure
of the molecule

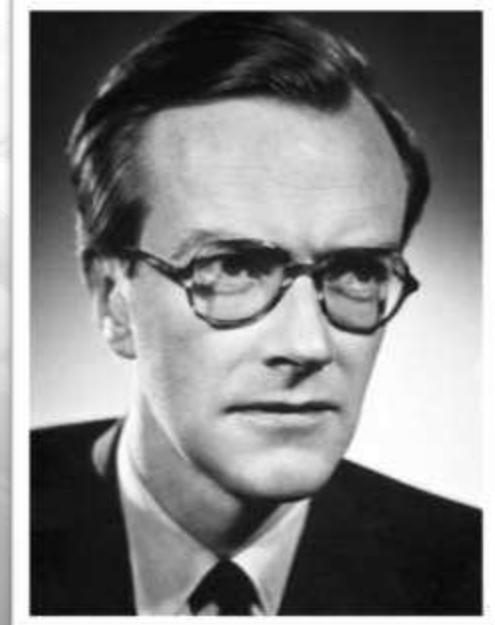
X Ray
Crystallography
Rosalind
Franklin's photo

- She made marked advances in X-ray diffraction techniques with DNA
- The diffraction pattern she obtained suggested several structural features of DNA
 - Helical
 - More than one strand
 - 10 base pairs per complete turn

Rosalind Franklin



Maurice Wilkins

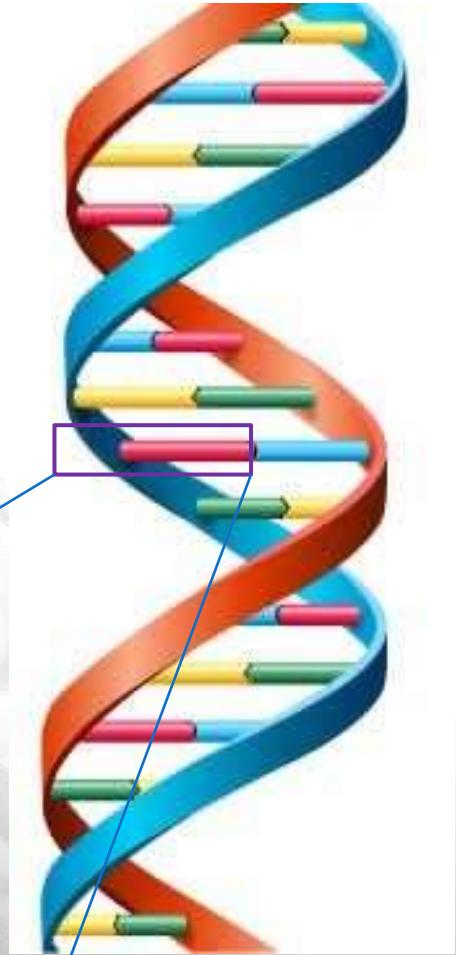
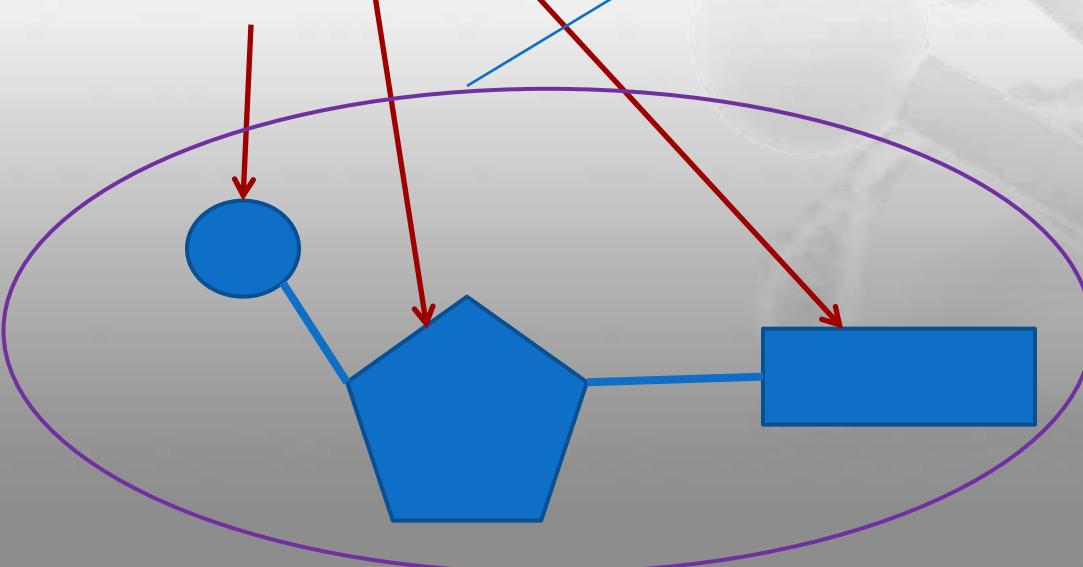


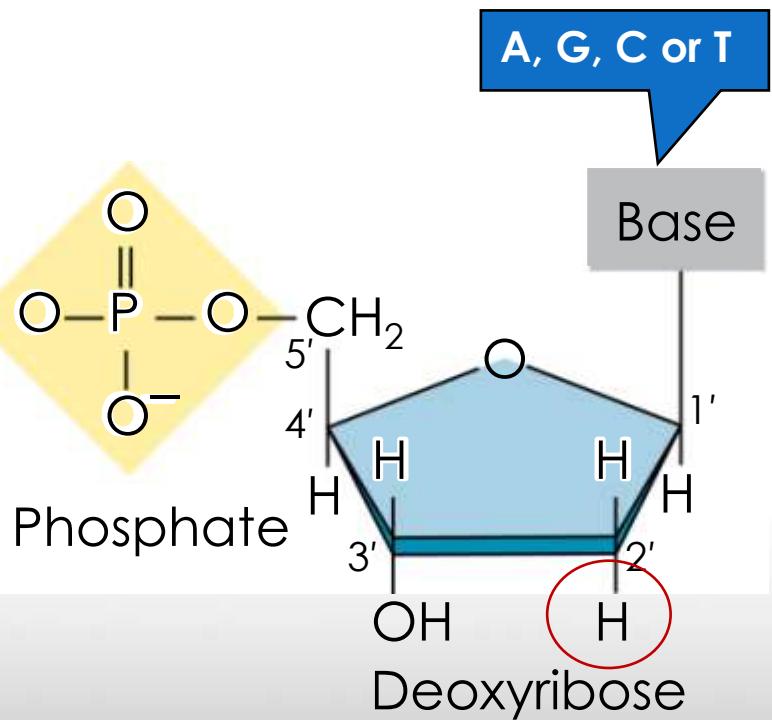
❖ DNA Structure

- DNA structure is often divided into four different levels primary, secondary, tertiary and quaternary.

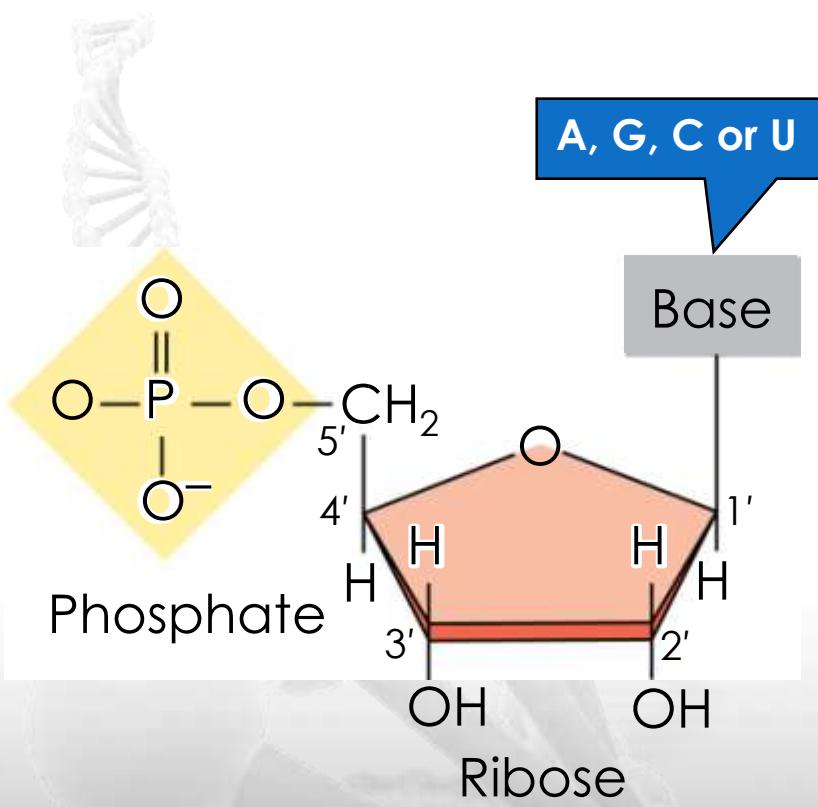
- DNA has three main components

- 1. Deoxyribose (a pentose sugar)
- 2. Base (there are four different ones)
- 3. Phosphate



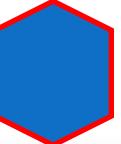
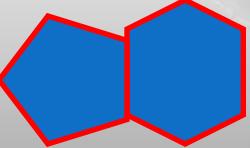


DNA Nucleotide

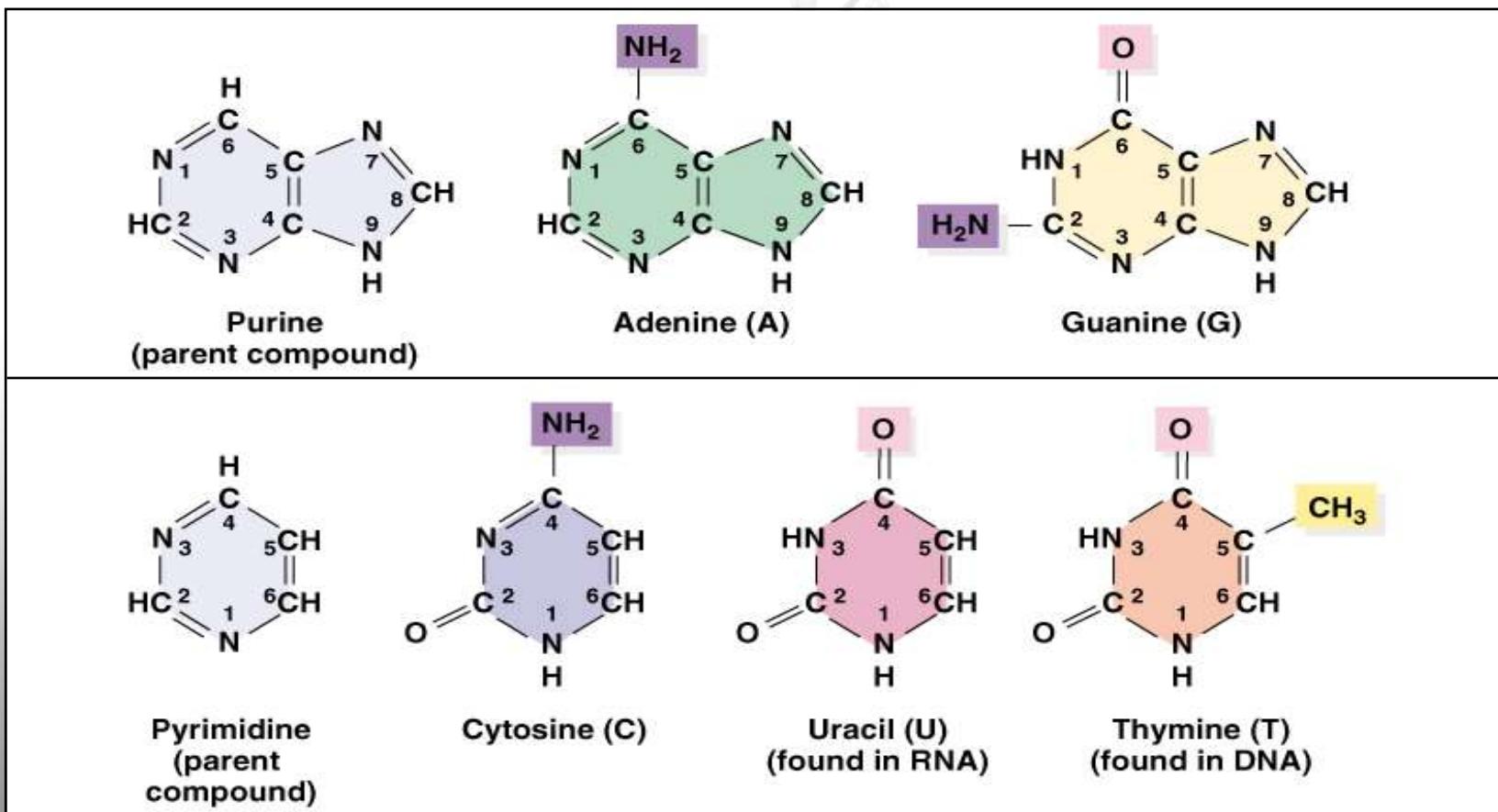


RNA Nucleotide

❖ The Nitrogenous Bases

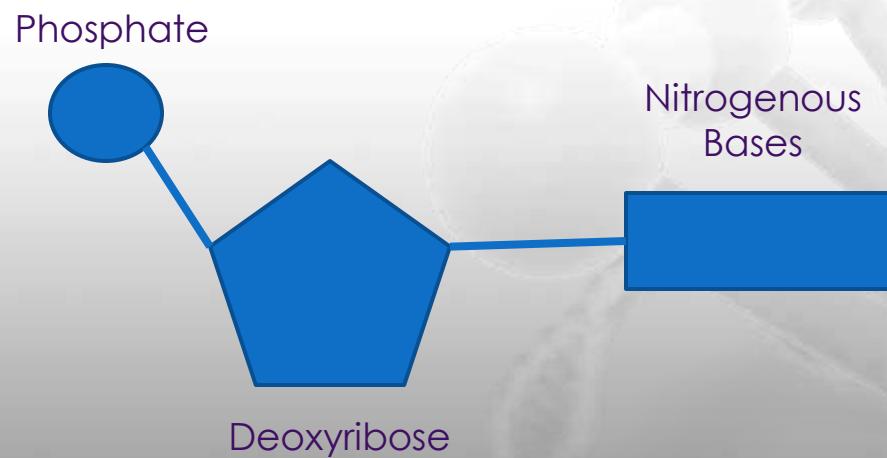
- THEY ARE DIVIDED INTO TWO GROUPS
 - Pyrimidines and purines
- PYRIMIDINES (MADE OF ONE 6 MEMBER RING)
 - Thymine 
 - Cytosine
- PURINES (MADE OF A 6 MEMBER RING, FUSED TO A 5 MEMBER RING)
 - Adenine
 - Guanine 
- THE RINGS ARE NOT ONLY MADE OF CARBON

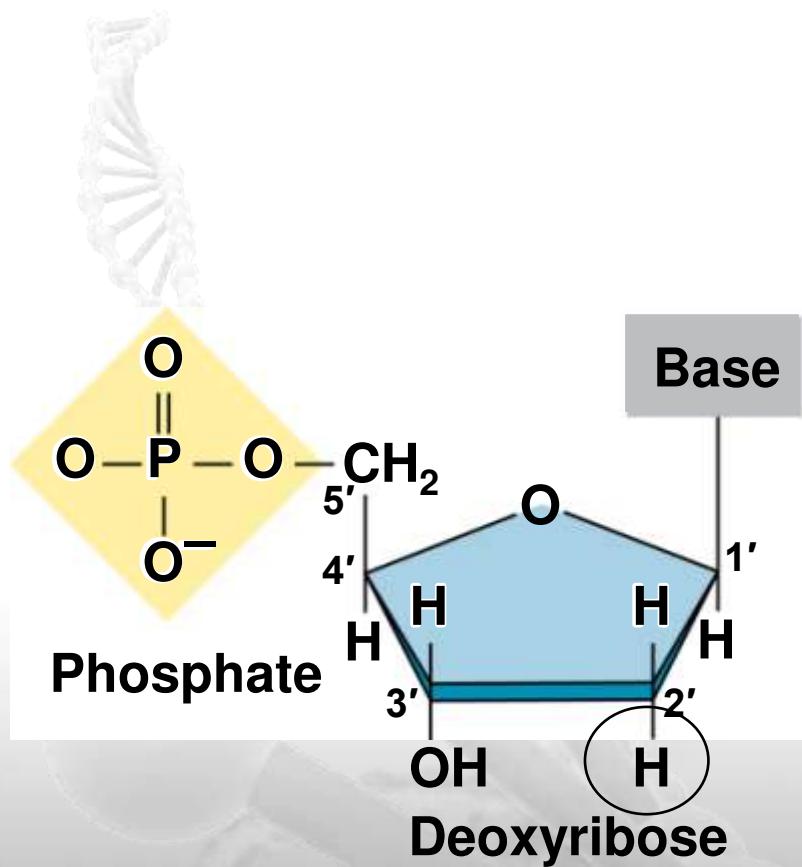
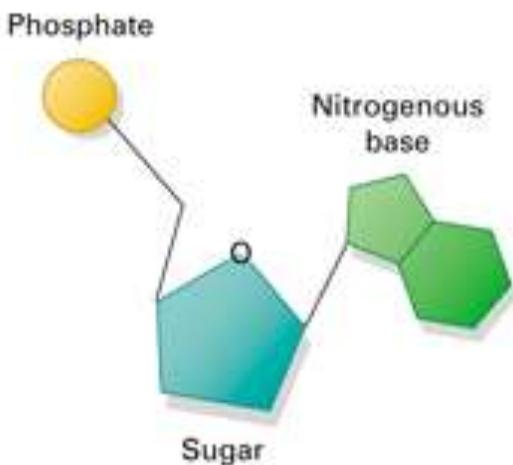
❖ Nitrogenous bases of DNA & RNA



❖ Nucleotide Structure

- Nucleotides are formed by the condensation of a sugar, phosphate and one of the 4 bases
- The following illustration represents one nucleotide





DNA nucleotide

- Base + sugar → nucleoside
 - Example
 - Adenine + ribose = Adenosine
 - Adenine + deoxyribose = Deoxyadenosine
- Base + sugar + phosphate(s) → nucleotide
 - Example
 - Deoxyadenosine monophosphate (dAMP)
 - Deoxyadenosine diphosphate (dADP)
 - Deoxyadenosine triphosphate (dATP)

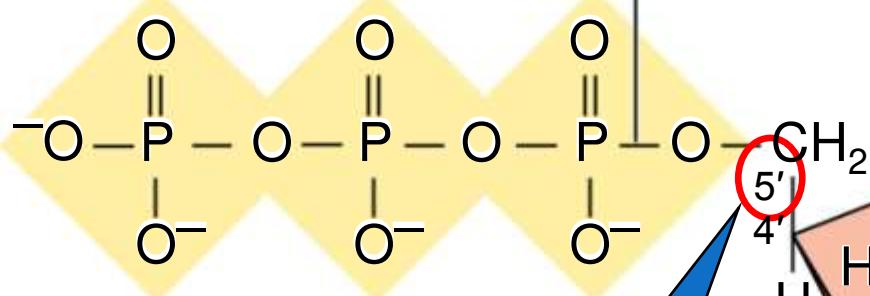
Deoxyadenosine triphosphate

Doxadenosine diphosphate

Deoxyadenosine monophosphate

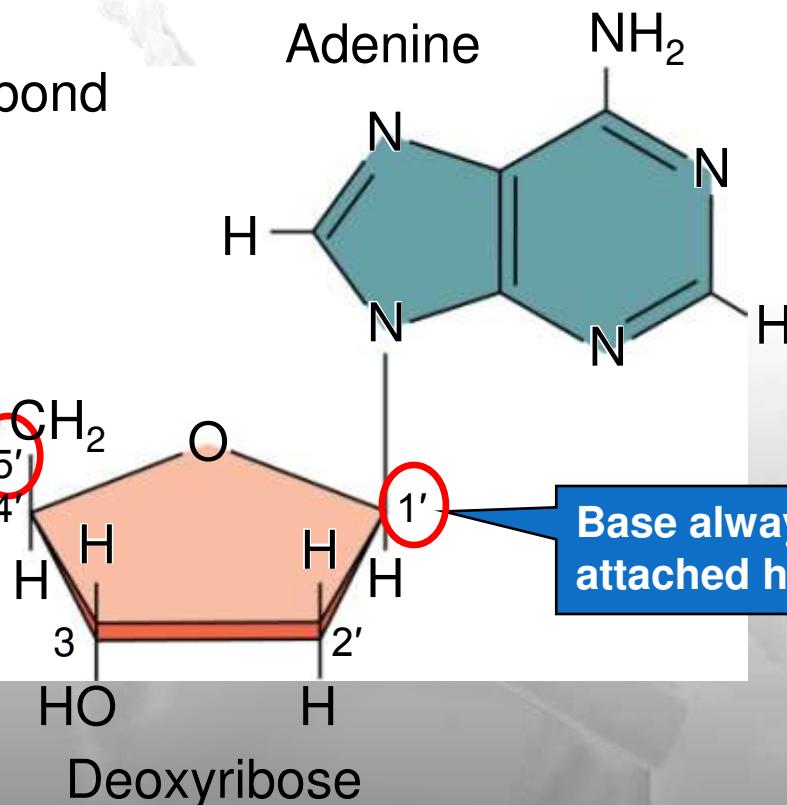
Deoxyadenosine

Phosphoester bond



Phosphate groups

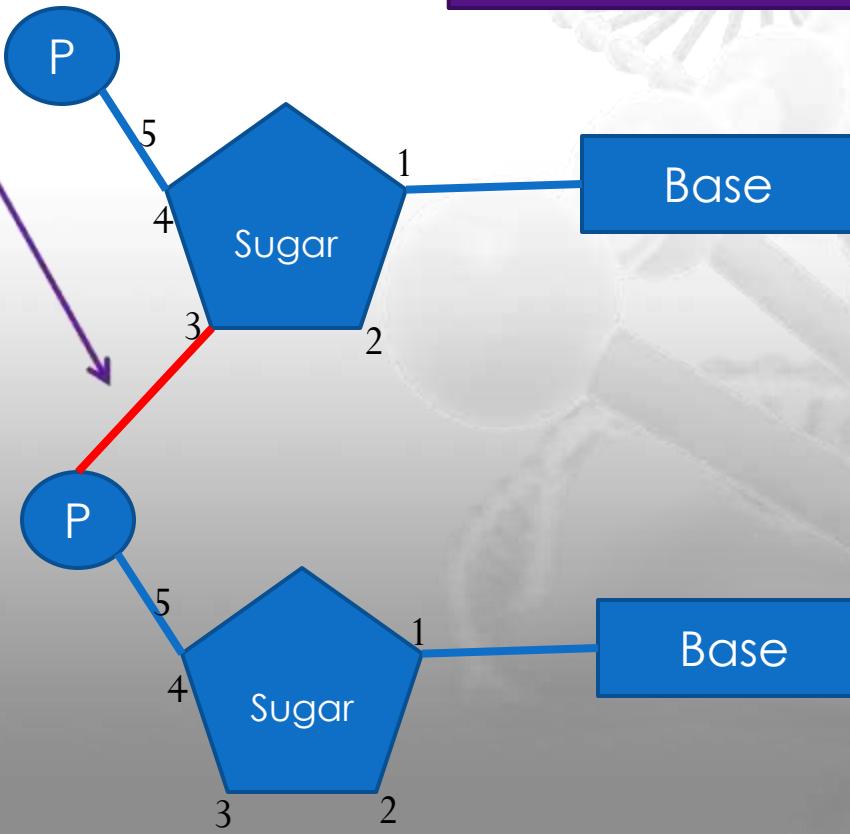
Phosphates are attached here

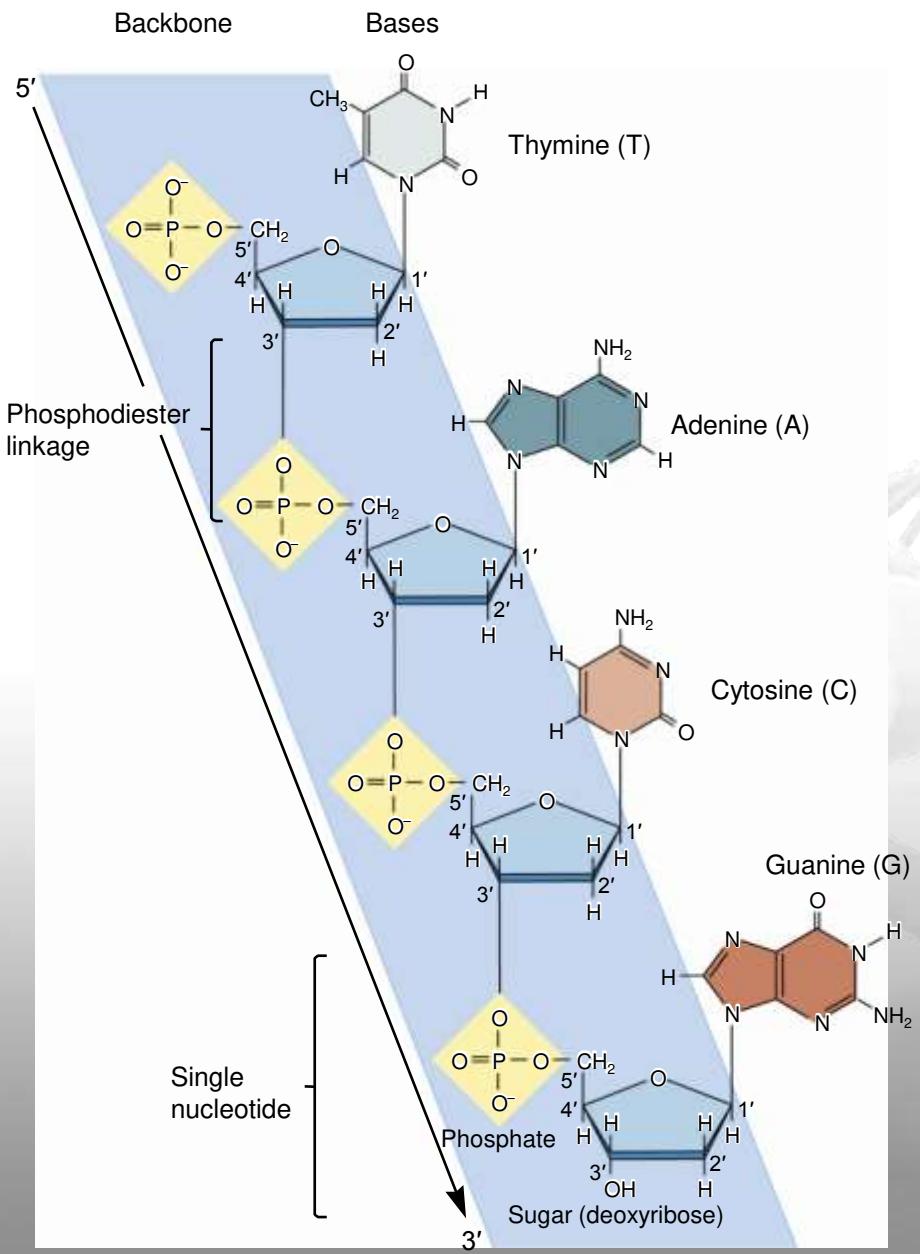


Base always attached here

- Nucleotides are linked together by covalent bonds called phosphodiester linkage.

A chemical bond that involves sharing a pair of electrons between atoms in a molecule.

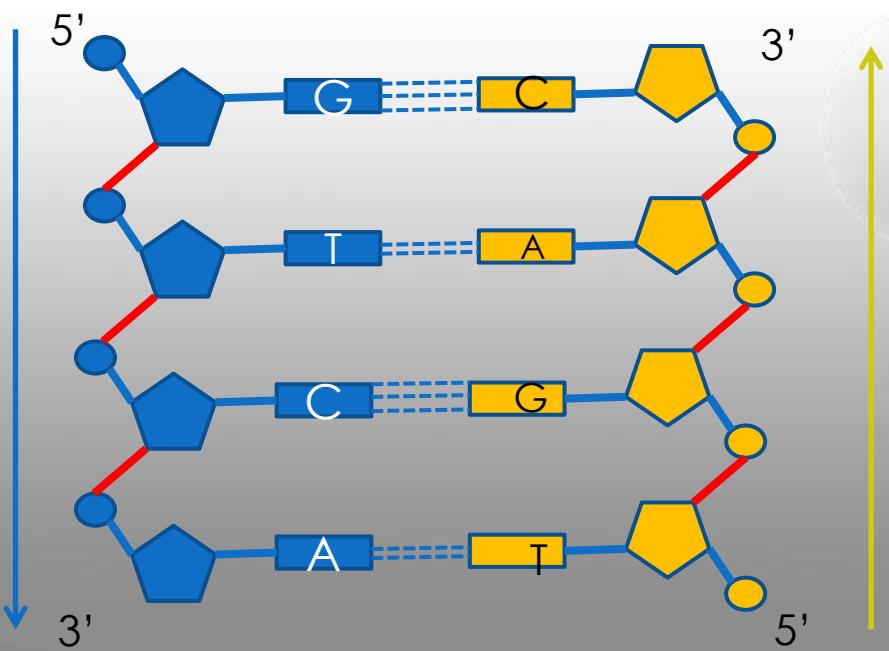


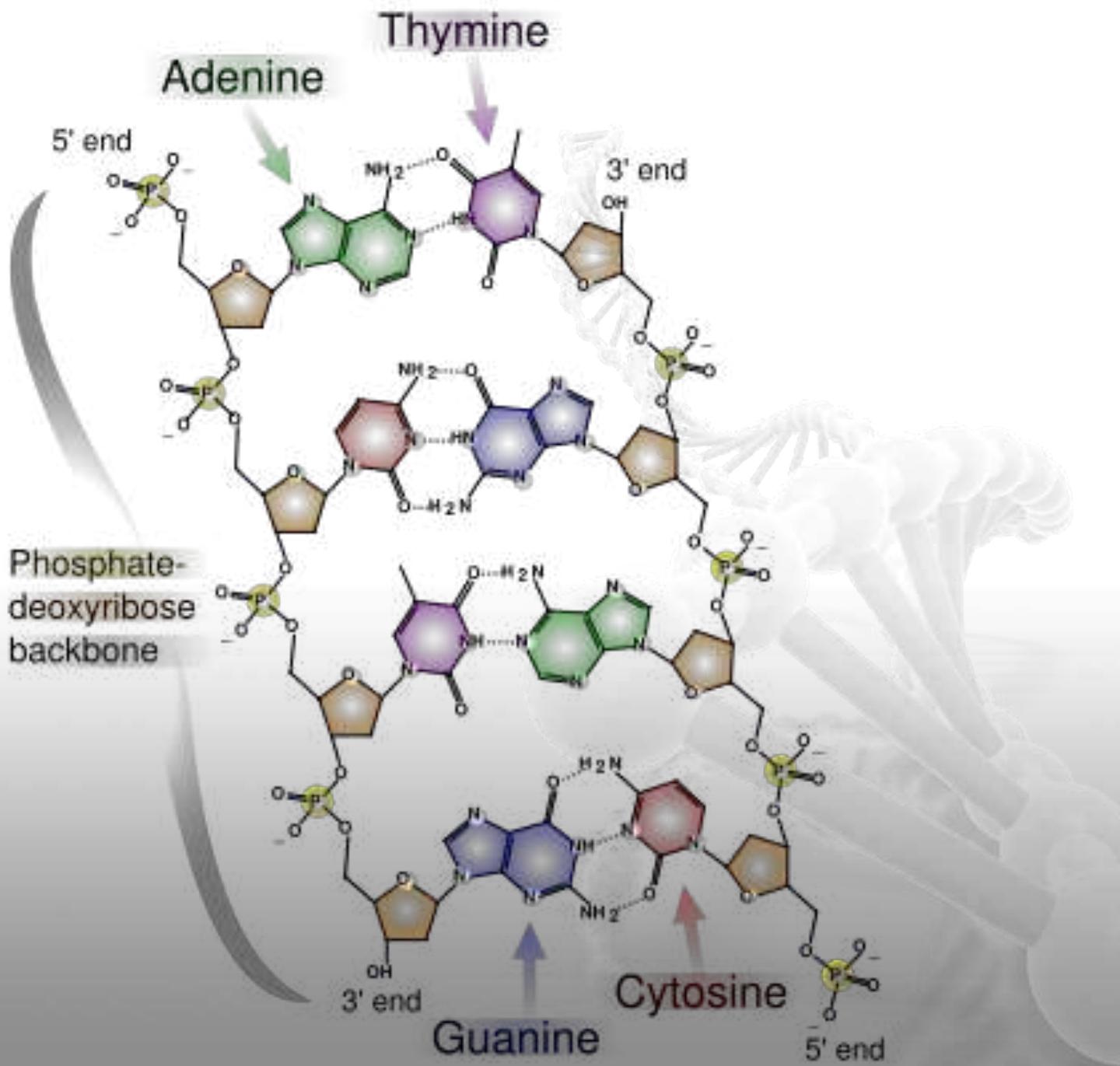


❖ DNA Double Helix & Hydrogen bonding

Salient features of the Double-helix structure of DNA:

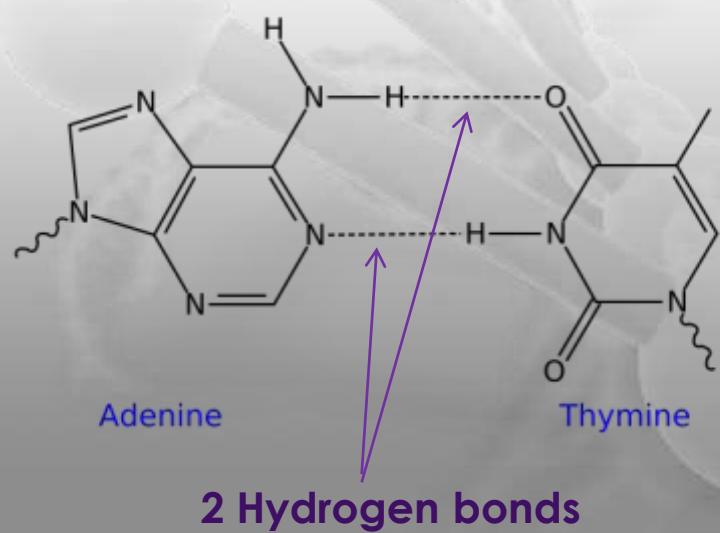
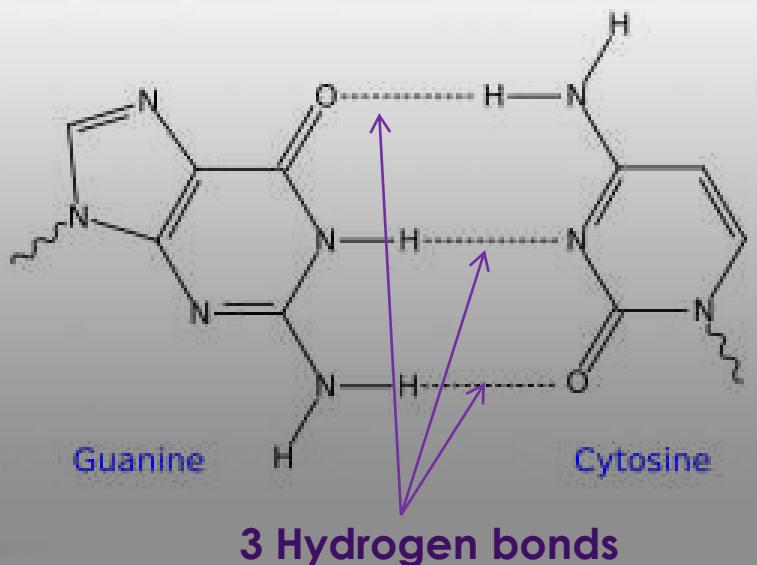
- It is made of two polynucleotide chains, where the backbone is constituted by sugar-phosphate, and the bases project inside.
- The two chains have anti- parallel polarity. It means, if one chain has the polarity $5' \rightarrow 3'$, the other has $3' \rightarrow 5'$.





❖ DNA Double Helix & Hydrogen bonding

- The bases in two strands are paired through hydrogen bond (H-bonds) forming base pairs (bp). **Adenine** forms **two** hydrogen bonds with **Thymine** from opposite strand and vice-versa. Similarly, **Guanine** is bonded with **Cytosine** with **three** H-bonds.
 - Based on the observation of **Erwin Chargaff** that for a double stranded DNA, the ratios between **Adenine** and **Thymine**; and **Guanine** and **Cytosine** are constant and equals one.
 - **Hydrogen bond**:-A chemical bond consisting of a hydrogen atom between two electronegative atoms (e.g., oxygen or nitrogen) with one side be a covalent bond and the other being an ionic bond.



❖ Erwin Chargaff's Experiment

- Chargaff pioneered many of biochemical technique for the isolation, purification and measurement of nucleic acids from living cells.
- It was known that DNA contained the four bases: A, G, C & T.
- Chargaff analyzed the base composition DNA isolated from many different species.

❖ THE HYPOTHESIS

- An analysis of the base composition of DNA in different species may reveal important features about structure of DNA.

1. For each type of cell, extract the chromosomal material. This can be done in a variety of ways, including the use of high salt, detergent, or mild alkali treatment. Note: The chromosomes contain both DNA and protein.

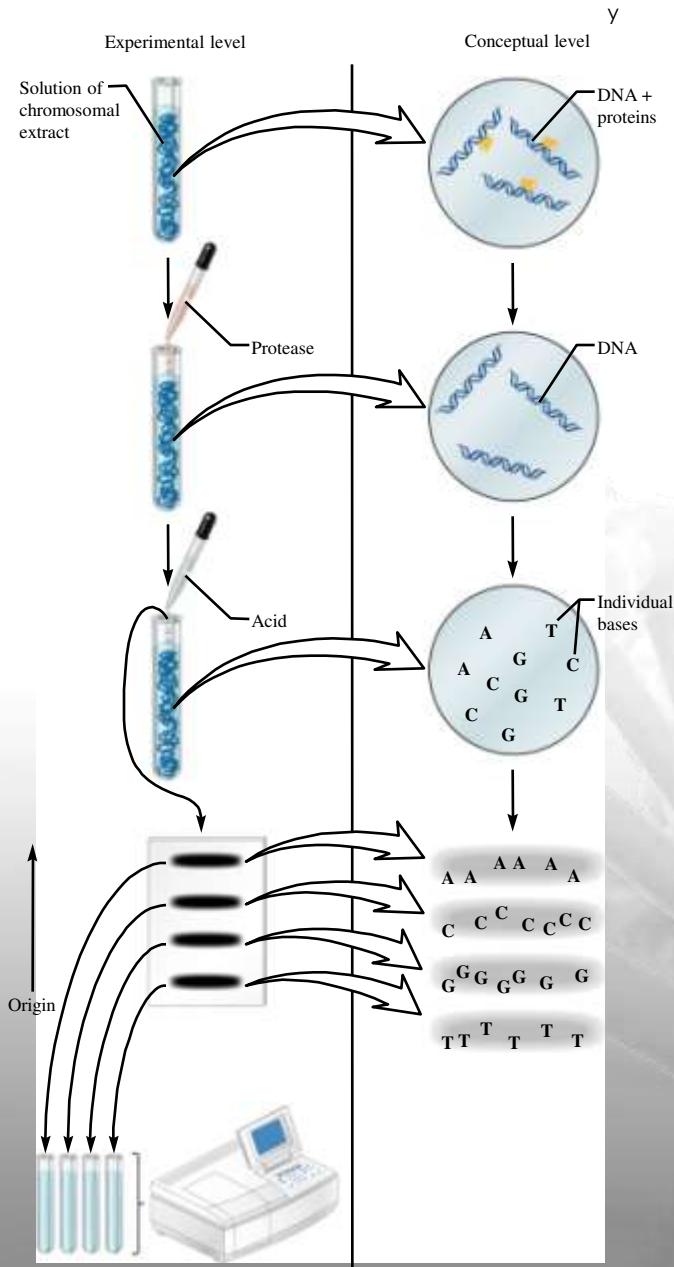
2. Remove the protein. This can be done in several ways, including treatment with protease.

3. Hydrolyze the DNA to release the bases from the DNA strands. A common way to do this is by strong acid treatment.

4. Separate the bases by chromatography. Paper chromatography provides an easy way to separate the four types of bases. (The technique of chromatography is described in the Appendix.)

5. Extract bands from paper into solutions and determine the amounts of each base by spectroscopy. Each base will absorb light at a particular wavelength. By examining the absorption profile of a sample of base, it is then possible to calculate the amount of the base. (Spectroscopy is described in the Appendix.)

6. Compare the base content in the DNA from different organisms.



The Data



THE DATA

Base Content in the DNA from a Variety of Organisms*

Percentage of Bases (based on molarity)

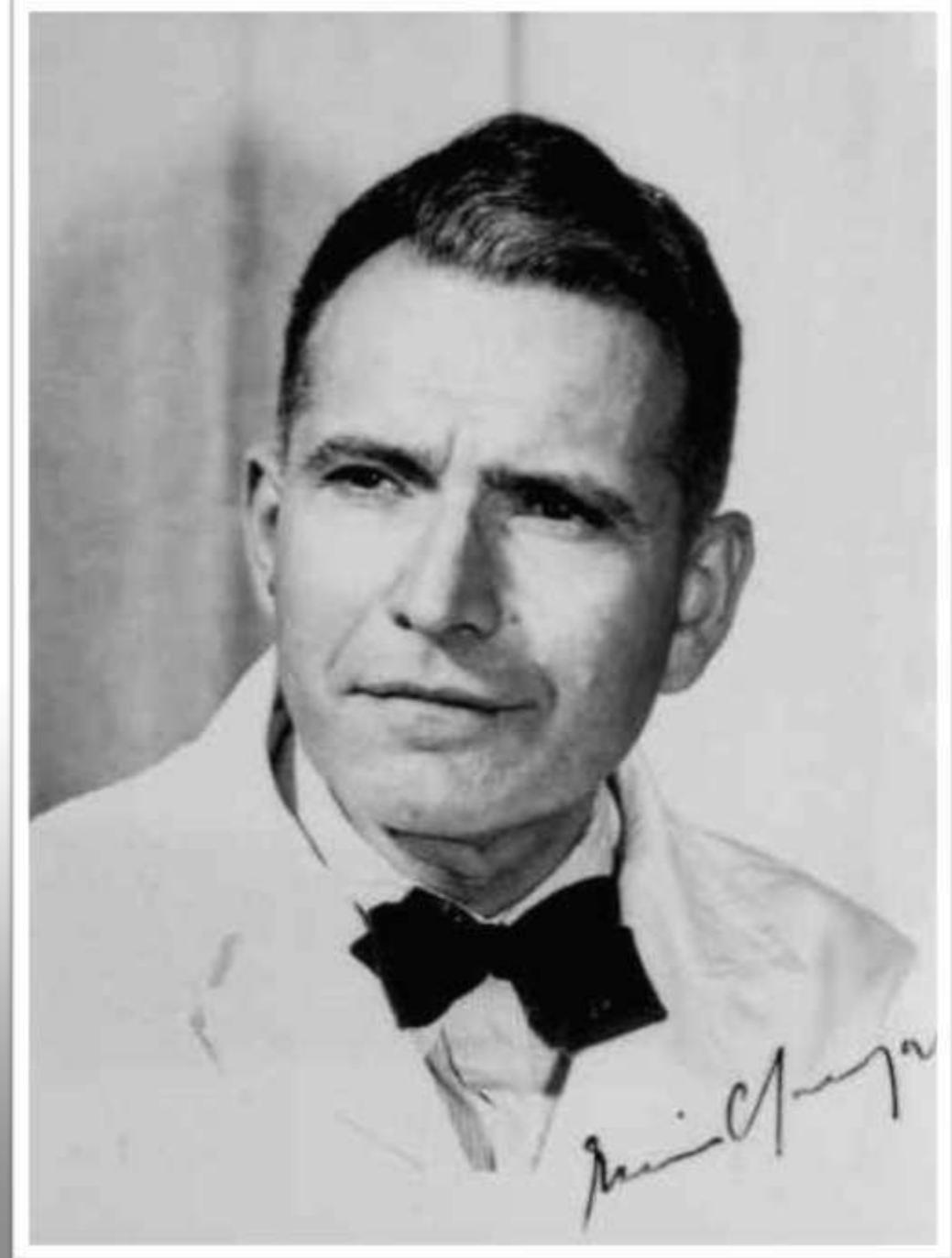
<i>Organism</i>	<i>Adenine</i>	<i>Thymine</i>	<i>Guanine</i>	<i>Cytosine</i>
<i>Escherichia coli</i>	26.0	23.9	24.9	25.2
<i>Streptococcus pneumoniae</i>	29.8	31.6	20.5	18.0
Yeast	31.7	32.6	18.3	17.4
Turtle red blood cells	28.7	27.9	22.0	21.3
Salmon sperm	29.7	29.1	20.8	20.4
Chicken red blood cells	28.0	28.4	22.0	21.6
Human liver cells	30.3	30.3	19.5	19.9

*When the base compositions from different tissues within the same species were measured, similar results were obtained. These data were compiled from several sources. See E. Chargaff and J. Davidson, Eds. (1995) *The Nucleic Acids*. Academic Press, New York.

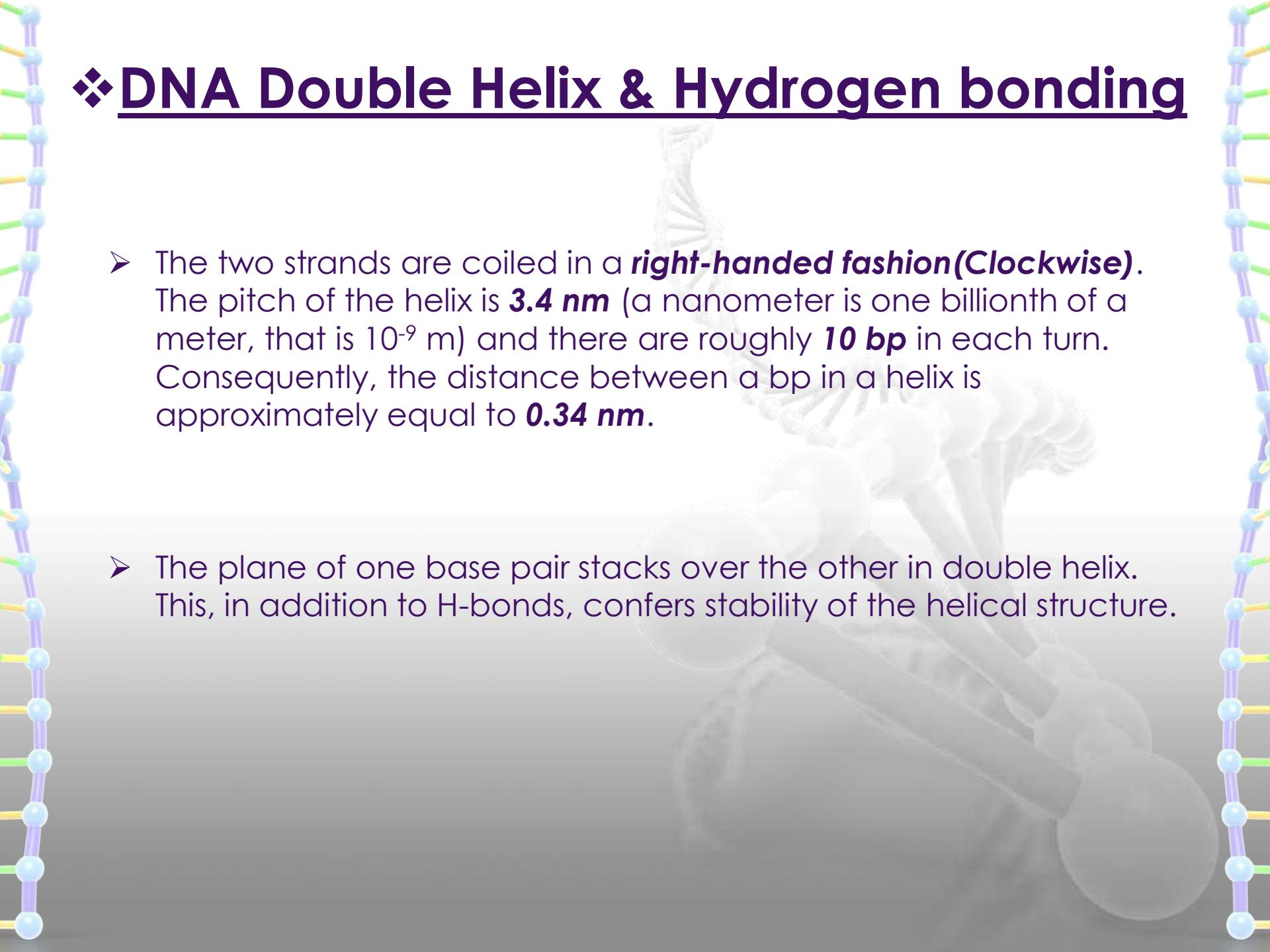
Interpretation of Data

- The compelling observation was that:
 - ✓ Percentage of adenine=percentage of thymine
 - ✓ Percentage of Cytosine=percentage of Guanine
- This observation became known as a **Chargaff's Rule.**

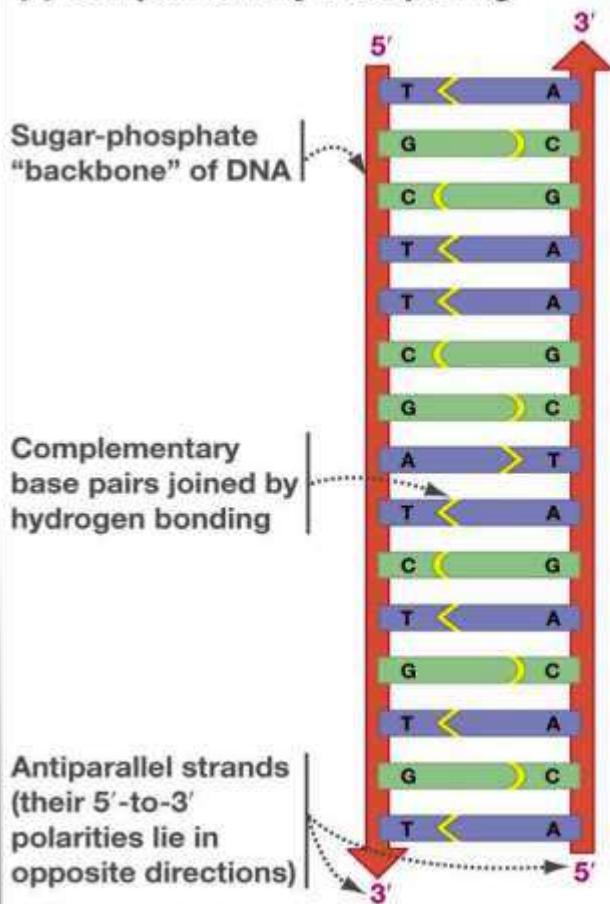
Erwin Chargaff



❖ DNA Double Helix & Hydrogen bonding

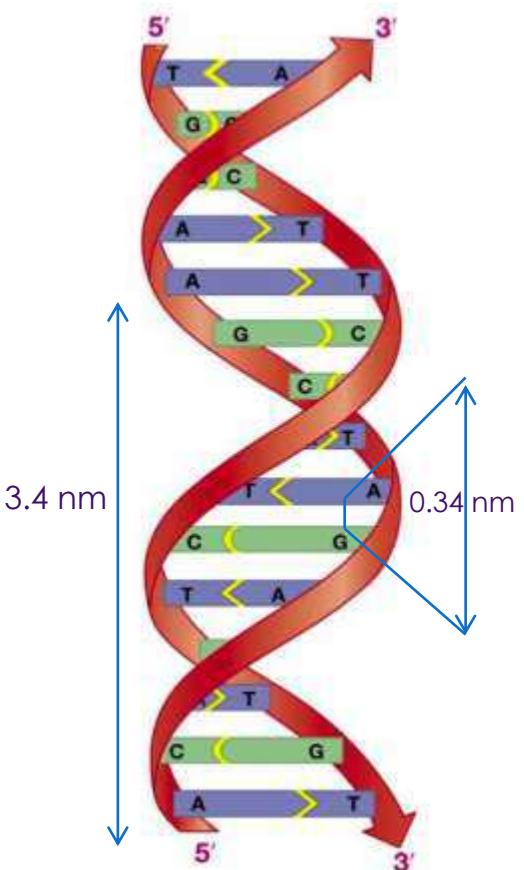
- 
- The two strands are coiled in a **right-handed fashion (Clockwise)**. The pitch of the helix is **3.4 nm** (a nanometer is one billionth of a meter, that is 10^{-9} m) and there are roughly **10 bp** in each turn. Consequently, the distance between a bp in a helix is approximately equal to **0.34 nm**.
 - The plane of one base pair stacks over the other in double helix. This, in addition to H-bonds, confers stability of the helical structure.

(a) Complementary base pairing

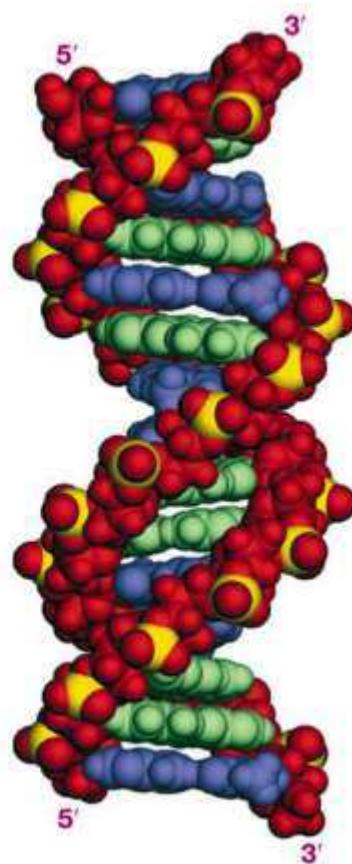


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(b) The double helix



(c) A space-filling model

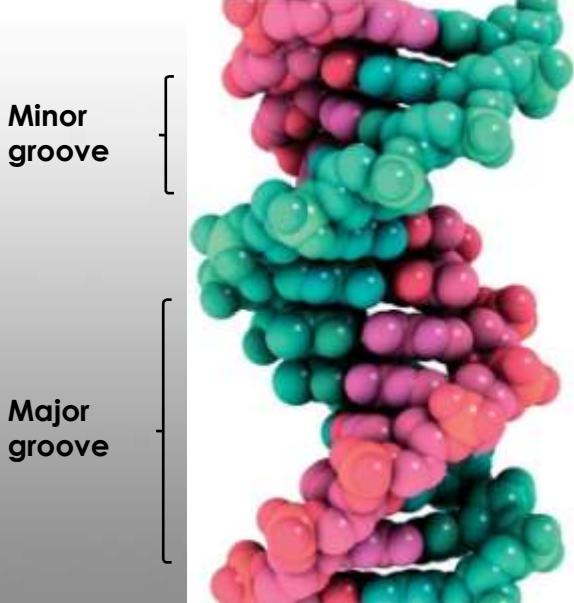
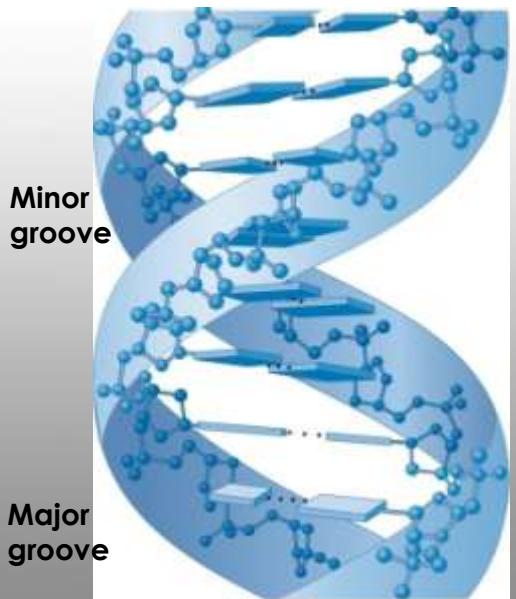


❖ DNA Double Helix & Hydrogen bonding

- There are two asymmetrical grooves on the outside of the helix:
 - Major groove
 - Minor groove

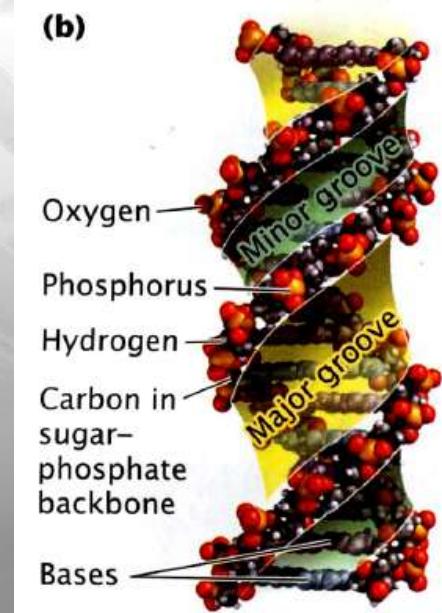
Groove:-any furrow(slight depression in the smoothness of a surface) or channel on a bodily structure or part.

- ✓ Certain proteins can bind within these groove
 - ✓ They can thus interact with a particular sequence of bases.



(a) Ball-and-stick model of DNA

(b) Space-filling model of DNA



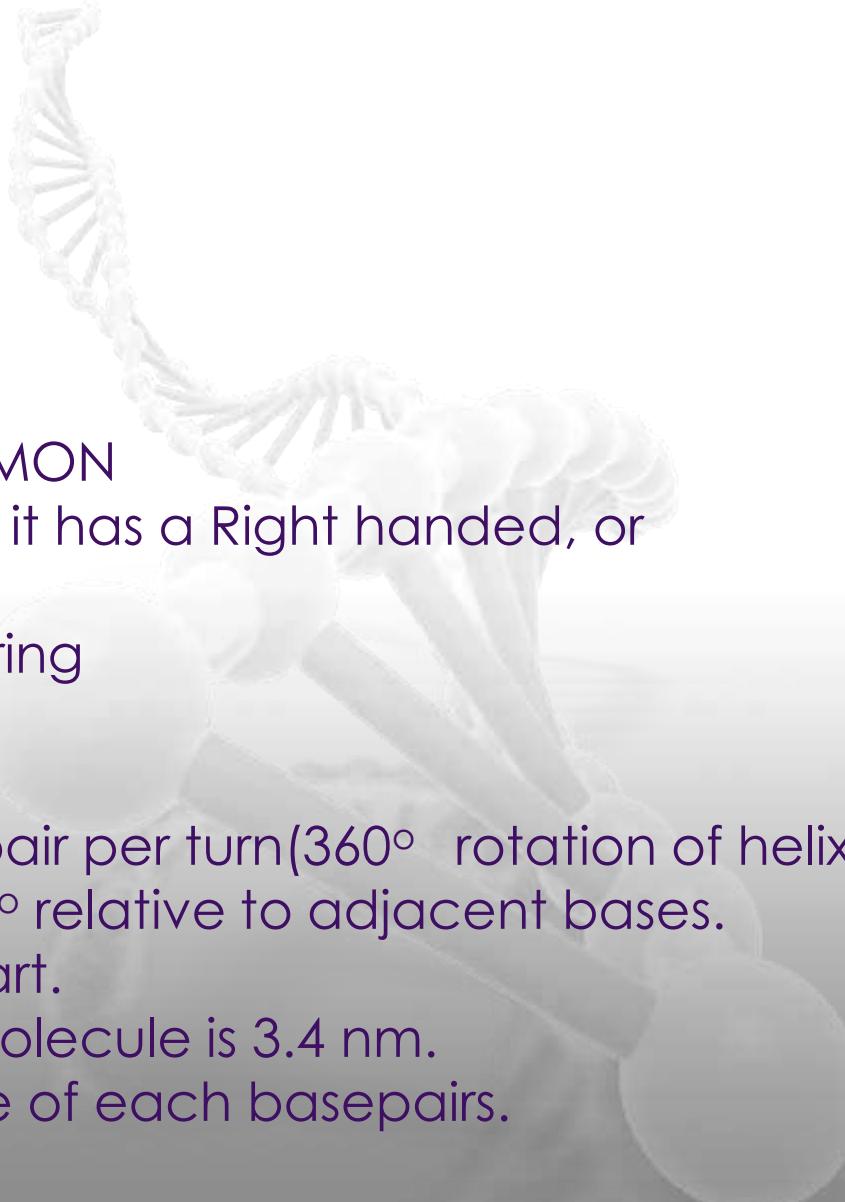
❖ Structure of Double-helix

- Three major forms:
 - ✓ B-DNA
 - ✓ A-DNA
 - ✓ Z-DNA

❖ B-DNA

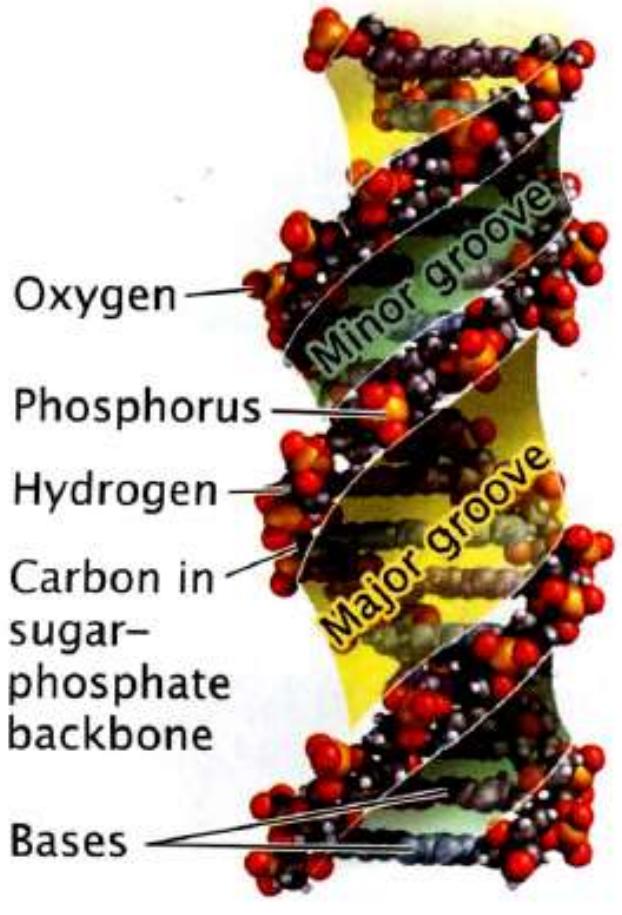
is biologically THE MOST COMMON

- ✓ It is a α -helix meaning that it has a Right handed, or clockwise, spiral.
- ✓ Complementary base pairing
 - A-T
 - G-C
- ✓ Ideal B-DNA has 10 base pair per turn (360° rotation of helix)
- ✓ So each base is twisted 36° relative to adjacent bases.
- ✓ Base pair are 0.34 nm apart.
- ✓ So complete rotation of molecule is 3.4 nm.
- ✓ Axis passes through middle of each basepairs.



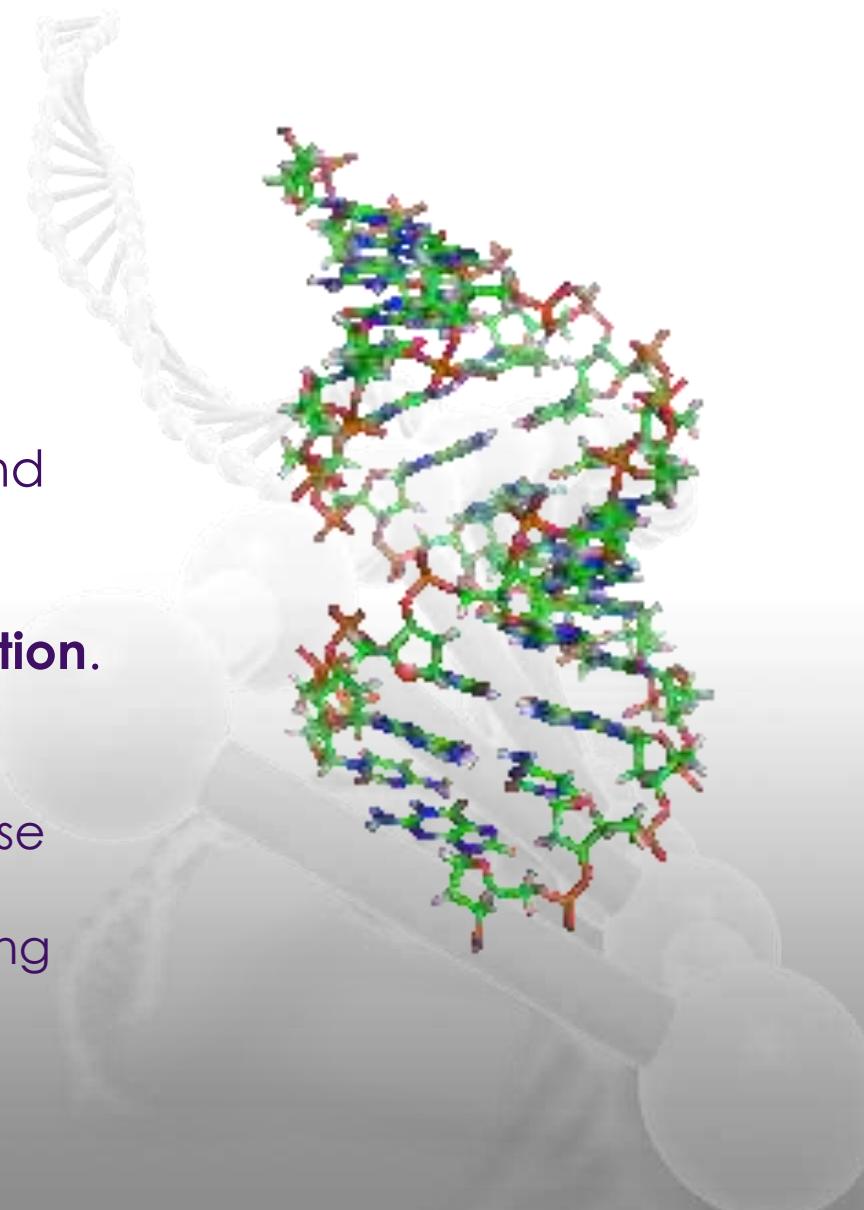
❖ B-DNA

- ✓ Minor Groove is Narrow, Shallow.
- ✓ Major Groove is Wide, Deep.
- ✓ This structure **exists** when **plenty of water** surrounds molecule and there is no unusual base sequence in DNA-Condition that are likely to be present in the cells.
- ✓ B-DNA structure is most stable configuration for a random sequence of nucleotides under physiological condition.



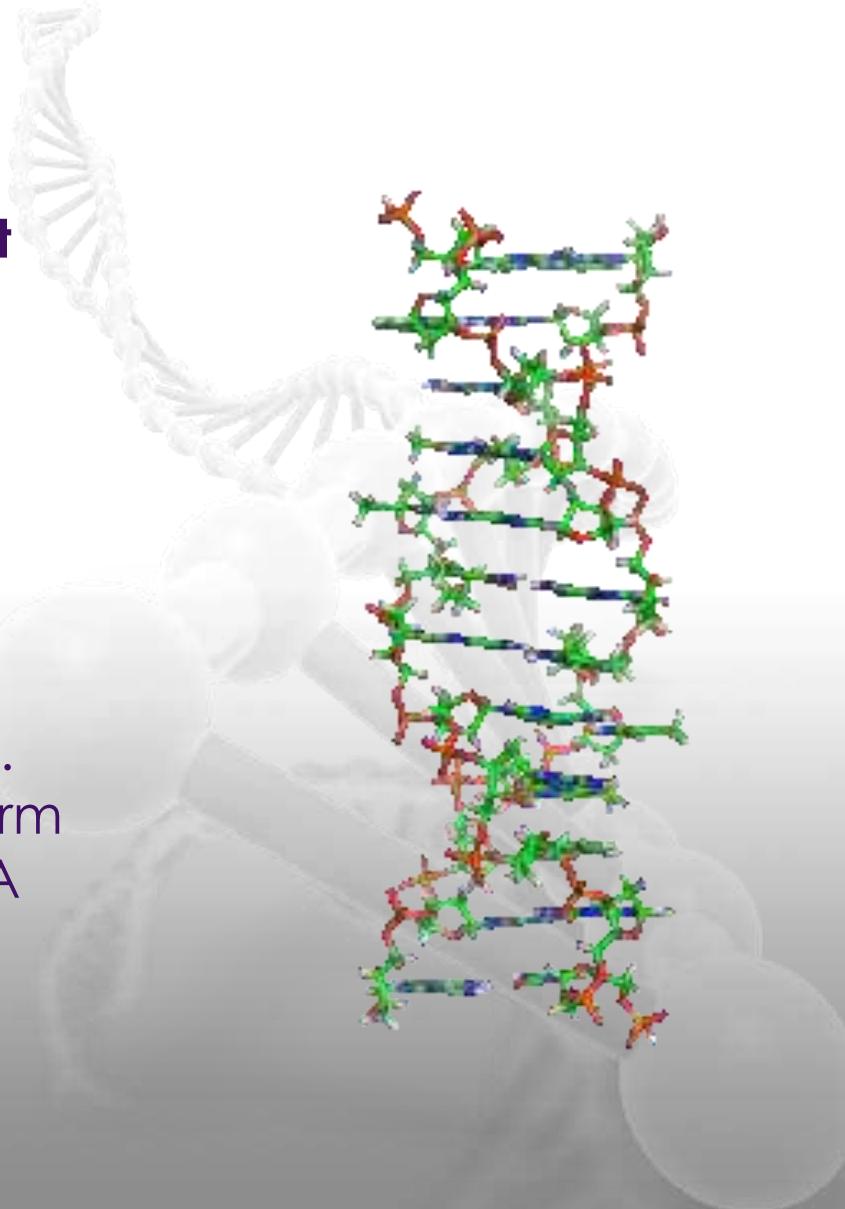
❖ A-DNA

- ✓ Right-handed helix
- ✓ Wider and flatter than B-DNA
- ✓ 11 bp per turn
- ✓ Its bases are tilted away from main axis of molecule
- ✓ Narrow Deep major Groove and Broad, Shallow minor Groove.
- ✓ Observed when less water is present. i.e. **Dehydrating condition.**
- ✓ A-DNA has been observed in two context:
 - Active site of DNA polymerase (~3bp)
 - Gram (+) bacteria undergoing sporulation



❖ Z-DNA

- ✓ A left-handed helix
- ✓ Seen in Condition of **High salt concentration.**
- ✓ In this form sugar-phosphate backbones zigzag back and forth, giving rise to the name Z-DNA(for zigzag).
- ✓ 12 base pairs per turn.
- ✓ A deep Minor Groove.
- ✓ No Discernible Major Groove.
- ✓ Part of some active genes form Z-DNA, suggesting that Z-DNA may play a role in regulating gene transcription.



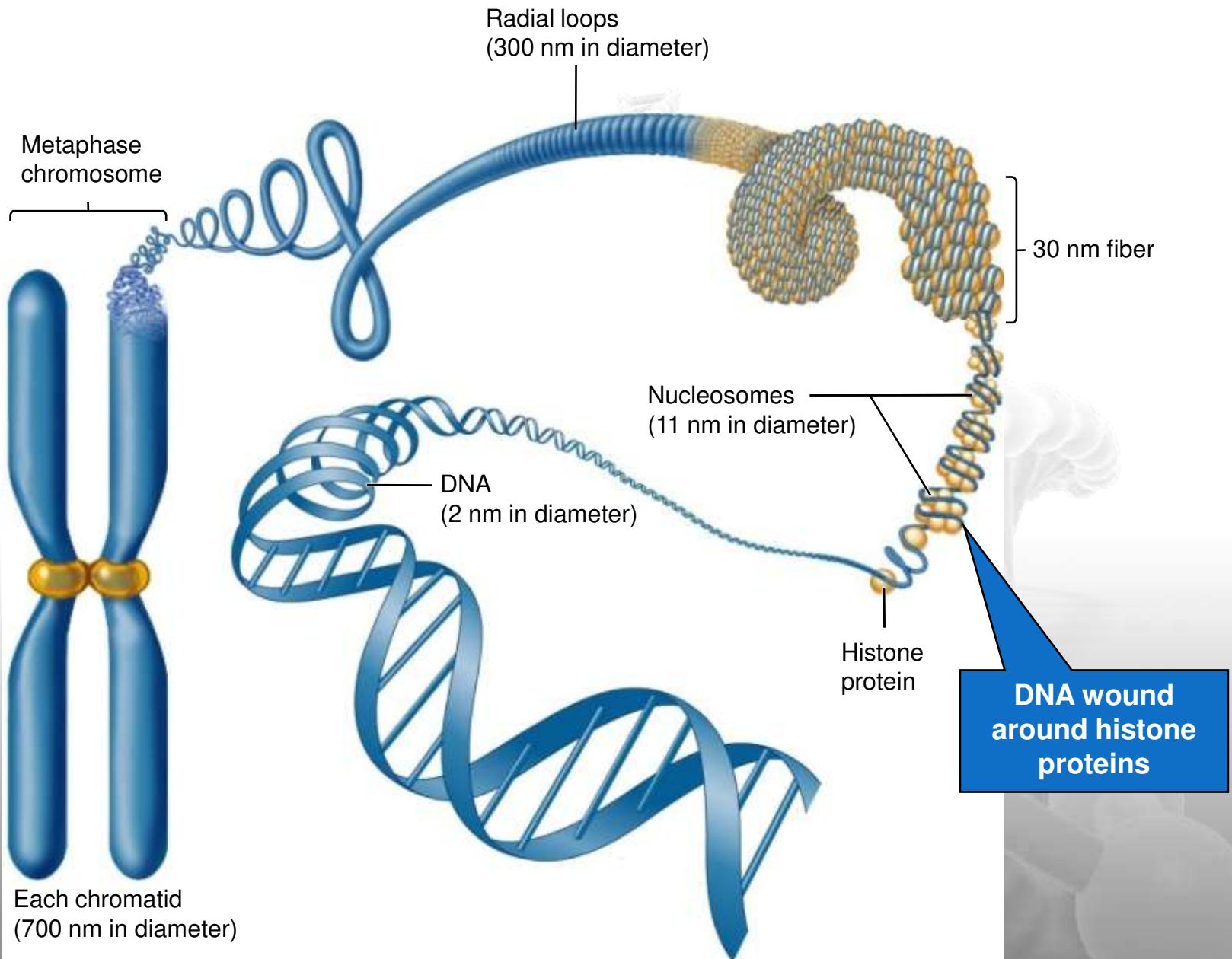
Property	B-DNA	A-DNA	Z-DNA
Strand	Antiparallel	Antiparallel	Antiparallel
Type of Helix	Right-handed	Right-handed	Left-handed
Overall shape	Long and narrow	Short and wide	Elongated and narrow
Base pair per turn	10	11	12
Distance between adjacent bases	0.34 nm	0.23 nm	0.38 nm
Pitch/turn of helix	3.40 nm	2.82 nm	4.56 nm
Helical Diameter	2.0 nm	2.3 nm	1.8 nm
Tilt/inclination of bp to axis	1°	20°	90°

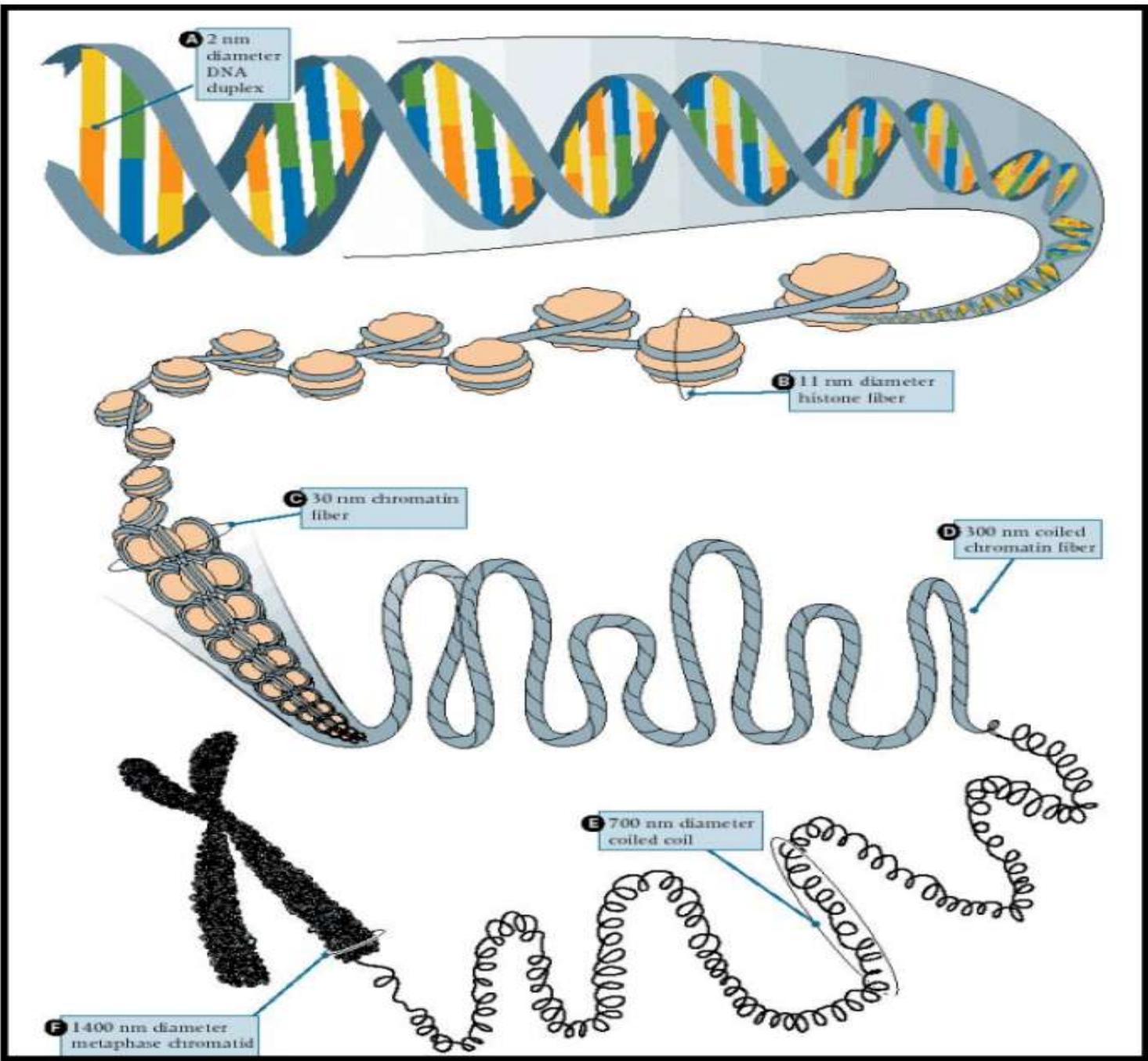
Property	B-DNA	A-DNA	Z-DNA
Major Groove	Wide & Deep	Narrow & Deep	No discernible
Minor Groove	Narrow, shallow	Broad, Shallow	Narrow, Deep



❖ DNA Supercoiling

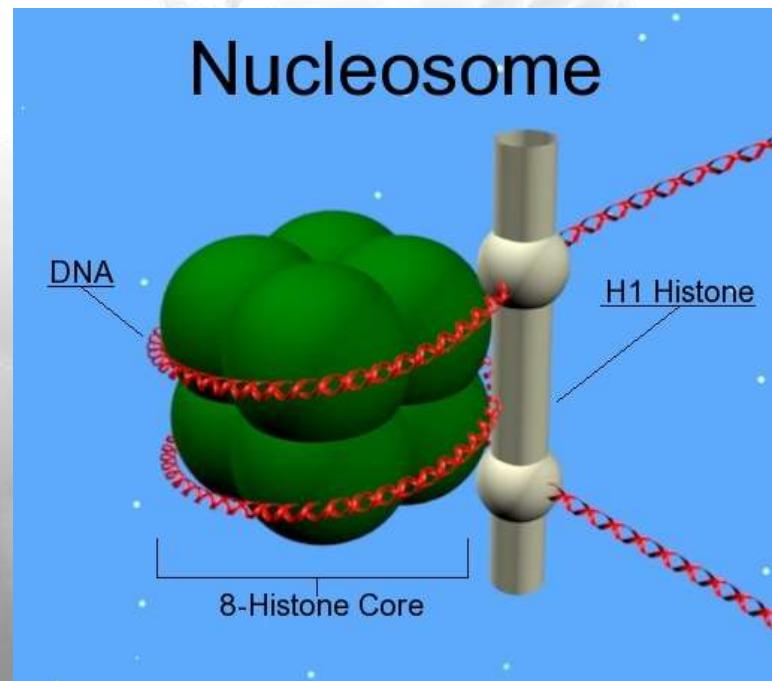
- **DNA supercoiling** refers to the over or under-winding of strands.
- DNA supercoiling is important for DNA packaging within all cells. Because the length of DNA can be of thousands of times that of a cells, packaging this material into the cell or nucleus (in Eukaryotes) is a difficult feat.
- Supercoiling of DNA reduces the space and allows for much more DNA to be packaged.



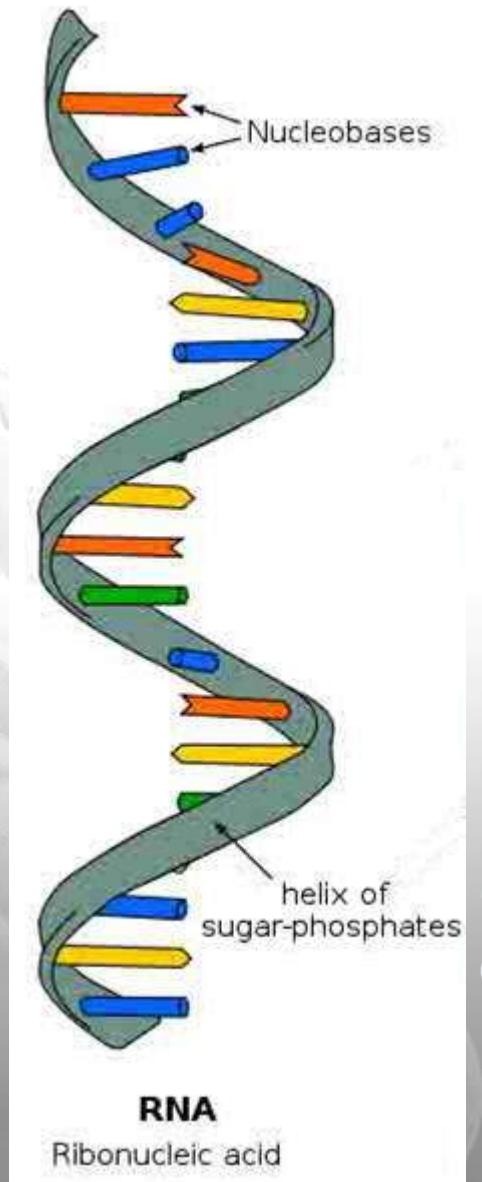
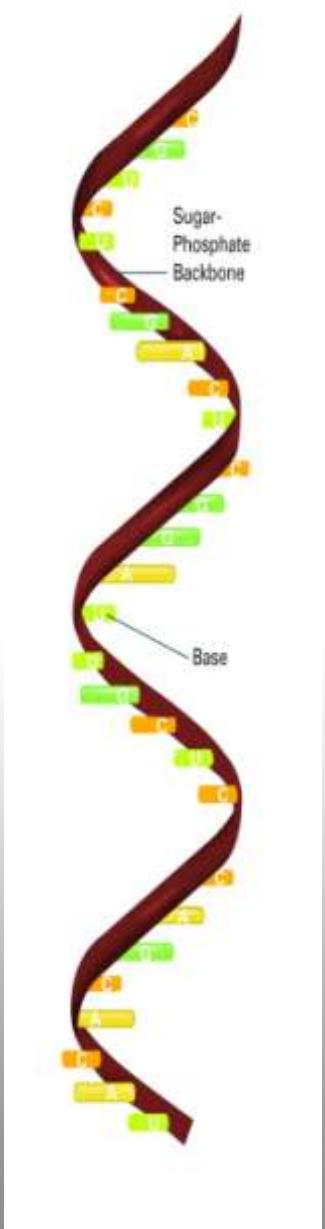


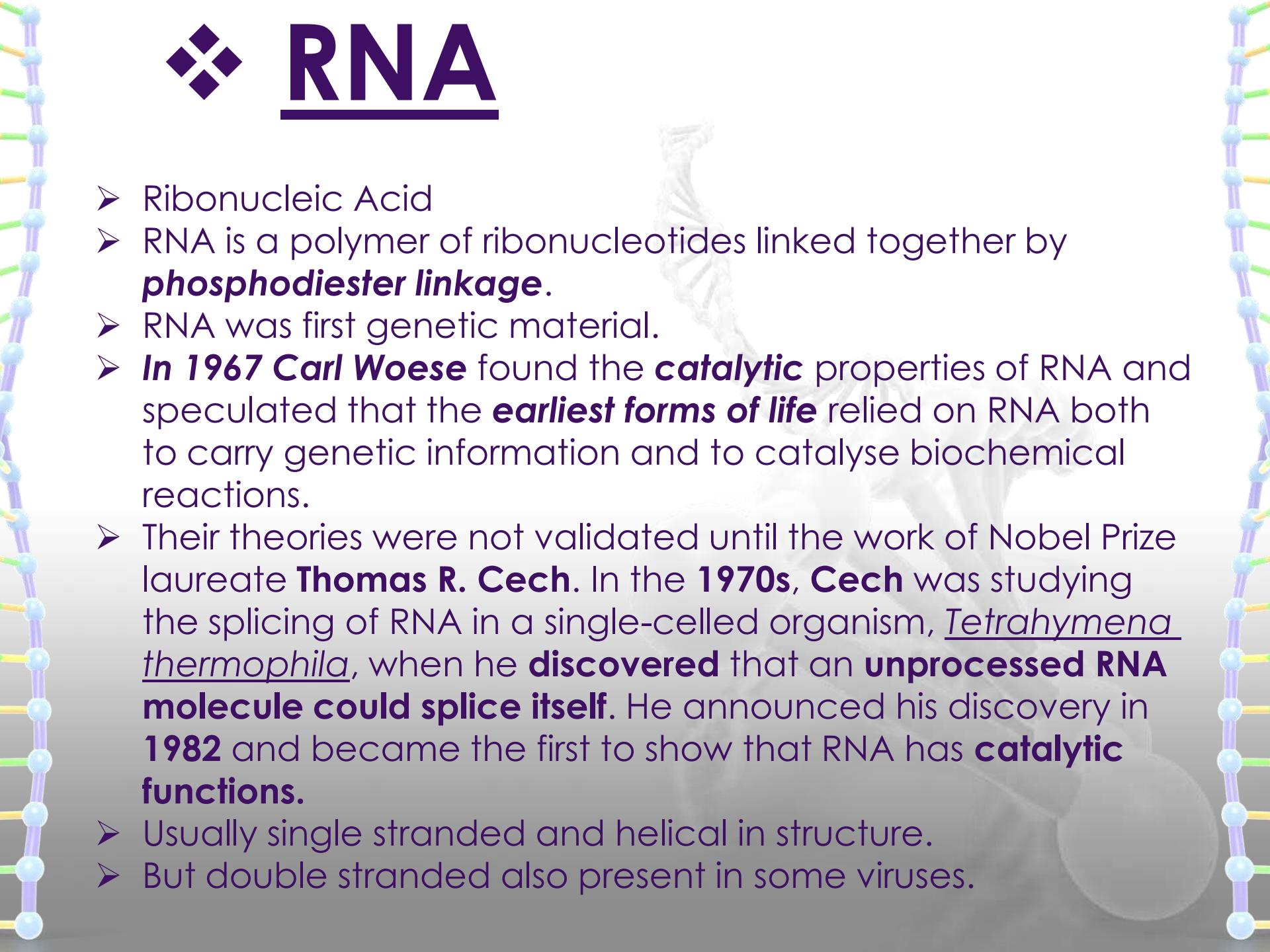
❖ Nucleosome Structure

- Nucleosome are the basic unit of the chromatin organization.
- In Eukaryotes DNA associated with Proteins.
(In prokaryotes DNA is naked)
- Nucleosomes= basic bead like unit of DNA packing
 - ✓ Made of segment of DNA wound around a protein core that is composed of 2 copies of each 4 types of Histones.
- Nucleosomes have:
 - ✓ 8 Histones in the core
 - ✓ DNA wrapped twice around the core
 - ✓ One Histone holding the Nucleosome together
 - ✓ A DNA 'linker' continues towards the next nucleosome.
- The DNA has a negatively charged backbone(because of PO_4^{3-} group)
- The Protein(Histones) are positively charged.
- The DNA and Protein are Electromagnetically attracted to each other to form chromatin.



RNA



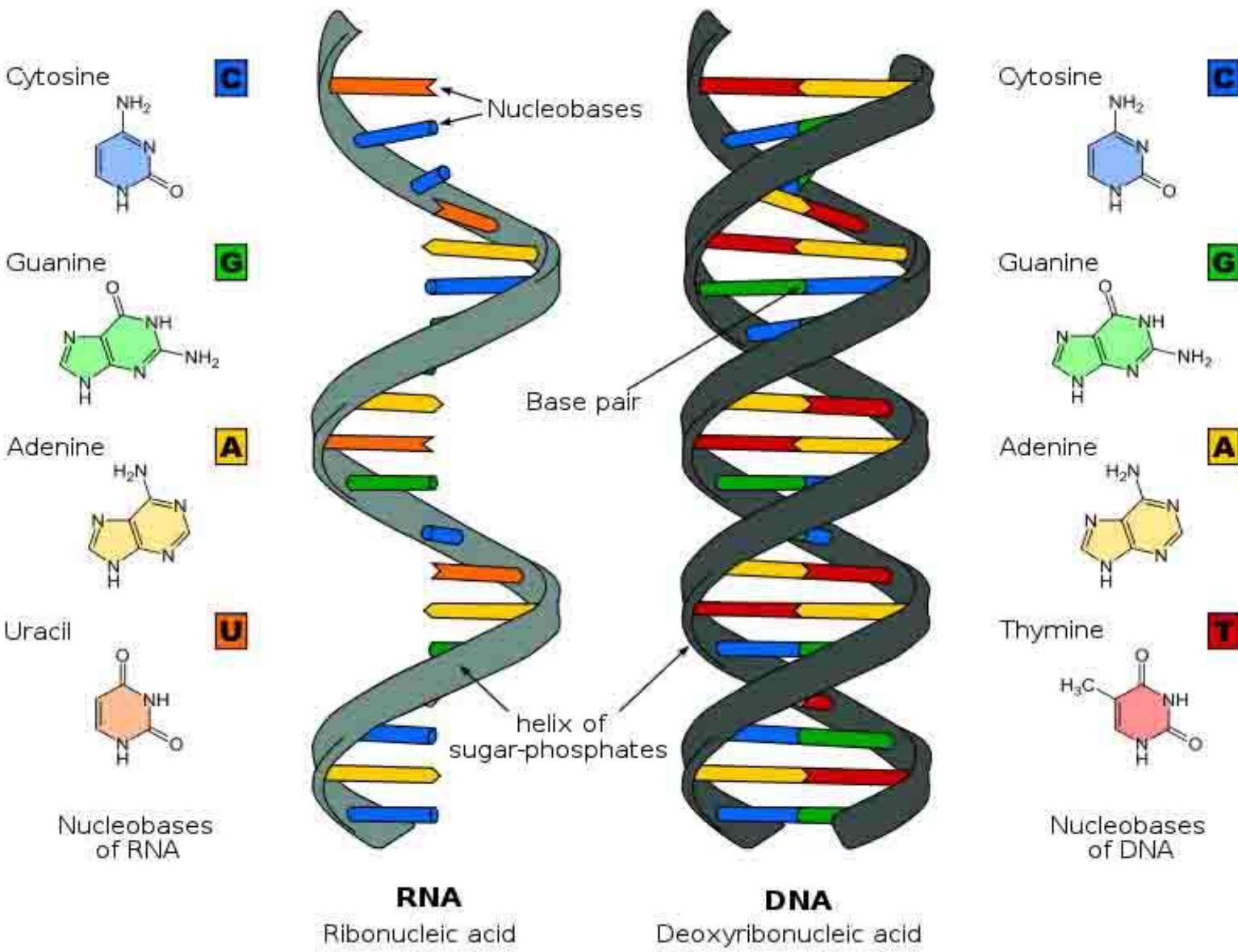


RNA

- Ribonucleic Acid
- RNA is a polymer of ribonucleotides linked together by **phosphodiester linkage**.
- RNA was first genetic material.
- **In 1967 Carl Woese** found the **catalytic** properties of RNA and speculated that the **earliest forms of life** relied on RNA both to carry genetic information and to catalyse biochemical reactions.
- Their theories were not validated until the work of Nobel Prize laureate **Thomas R. Cech**. In the **1970s**, **Cech** was studying the splicing of RNA in a single-celled organism, *Tetrahymena thermophila*, when he **discovered** that an **unprocessed RNA molecule could splice itself**. He announced his discovery in **1982** and became the first to show that RNA has **catalytic functions**.
- Usually single stranded and helical in structure.
- But double stranded also present in some viruses.

- RNA exists in several different single-stranded structures, most of which are directly or indirectly involved in protein synthesis or its regulation.
- It also acts as the genetic material in some viruses.
- It functions as messenger(mRNA), adapter(tRNA), structural(rRNA) and in some cases as a catalytic molecule(Ribozyme).
- RNA strands are typically several hundred to several thousand nucleotides in length.

❖ RNA V/S DNA

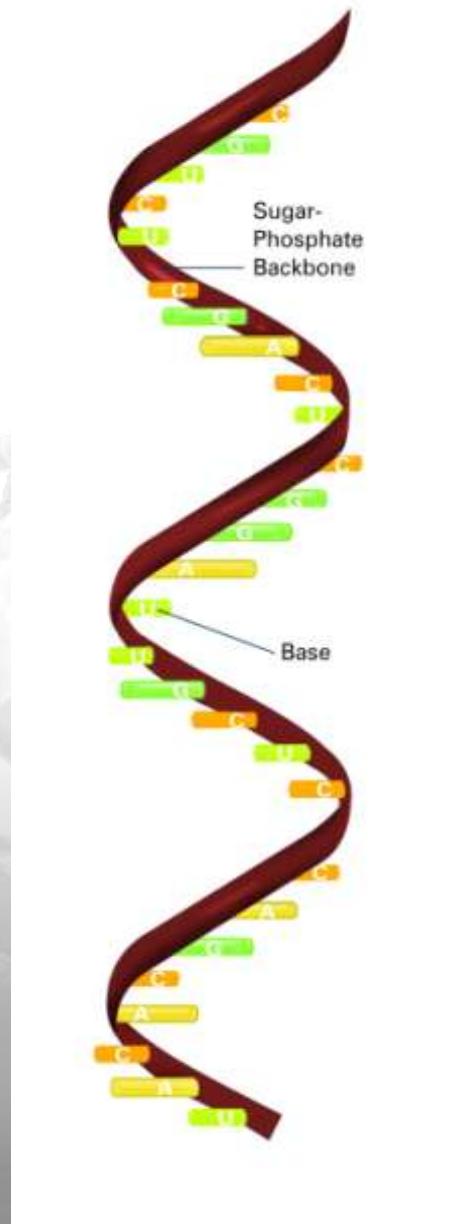


❖ RNA structure

- There are also three main component
 - a) Phosphate Group
 - b) Sugar(Ribose)
 - c) And Nitrogenous base

❖ The Nitrogenous Bases

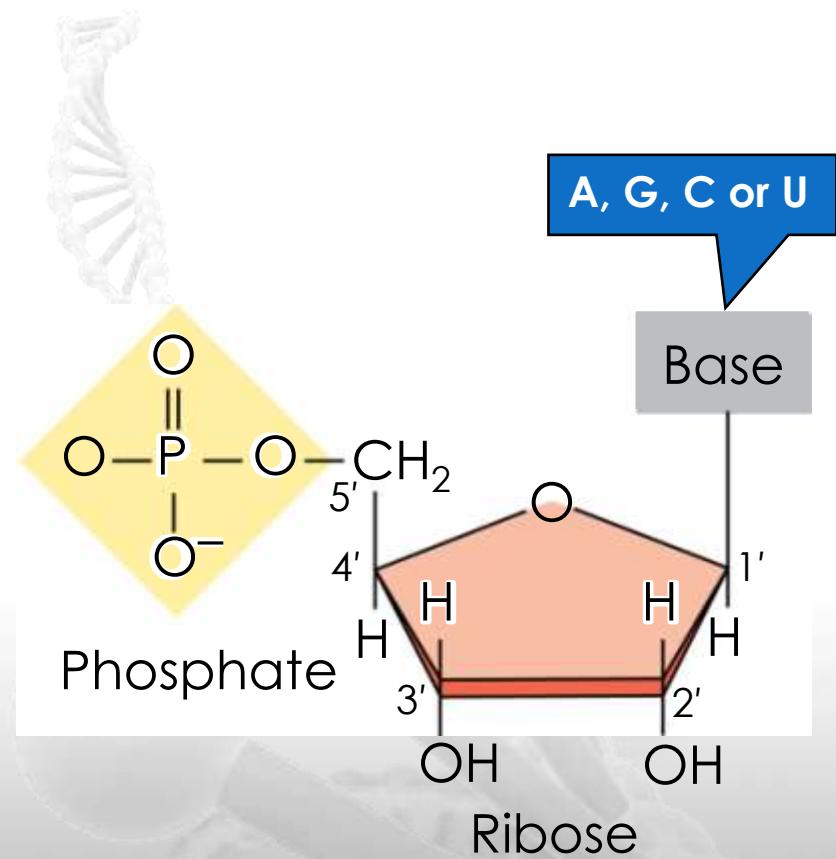
- They are divided into two groups:
 - i. Purine
 - ii. Pyrimidine
- Purines (made of a 6 member ring, fused to a 5 member ring)
 - ✓ Adenine
 - ✓ Guanine
- Pyrimidine (made of a 6 member ring)
 - ✓ Cytosine
 - ✓ Uracil



❖ RNA Structure

❖ Nucleotide

- Nucleotides are formed by the condensation of a sugar, phosphate and one of the 4 bases
- The following illustration represents one nucleotide



RNA Nucleotide

- Base + sugar → nucleoside
 - Example
 - Adenine + ribose = Adenosine
- Base + sugar + phosphate(s) → nucleotide
 - Example
 - Adenosine monophosphate (AMP)
 - Adenosine diphosphate (ADP)
 - Adenosine triphosphate (ATP)

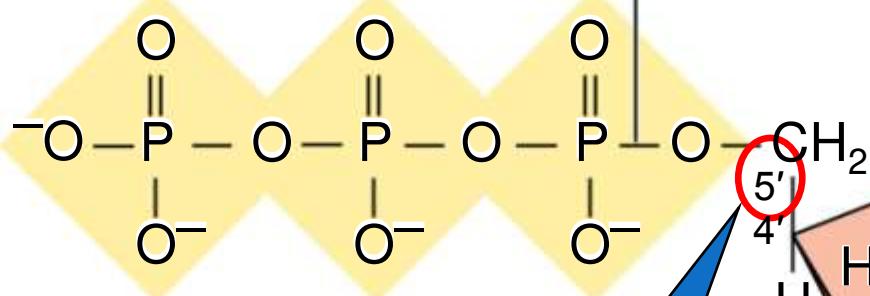
Adenosine triphosphate

Adenosine diphosphate

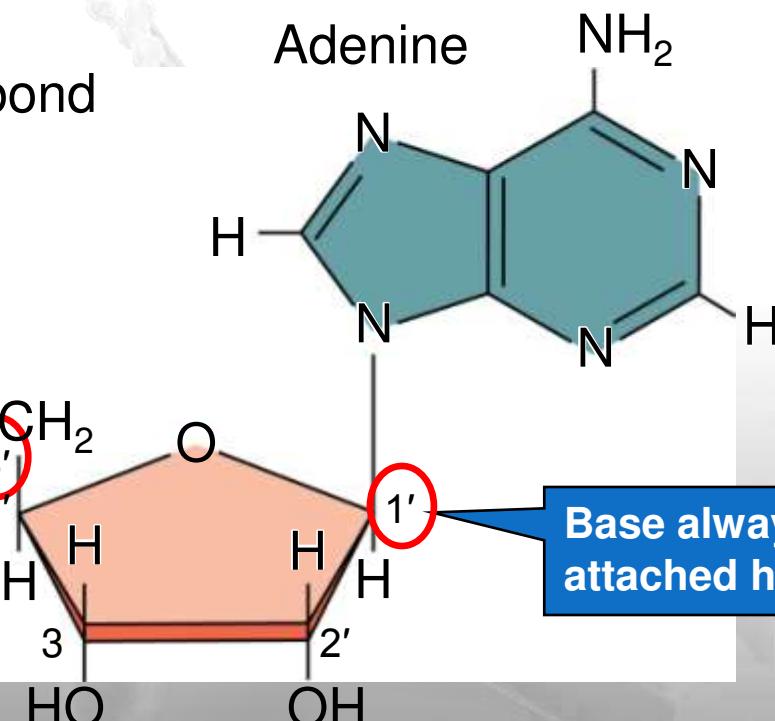
Adenosine monophosphate

Adenosine

Phosphoester bond



Phosphates are attached here

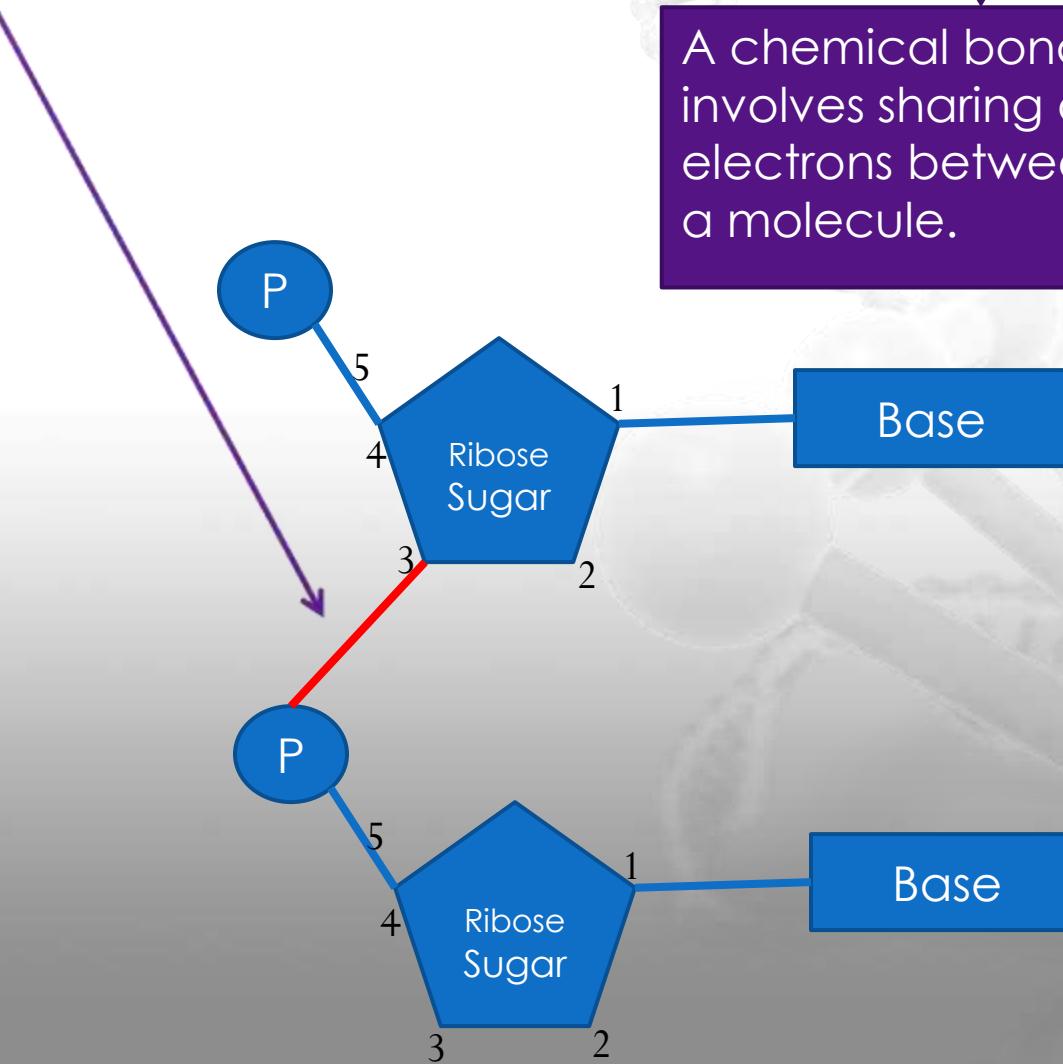


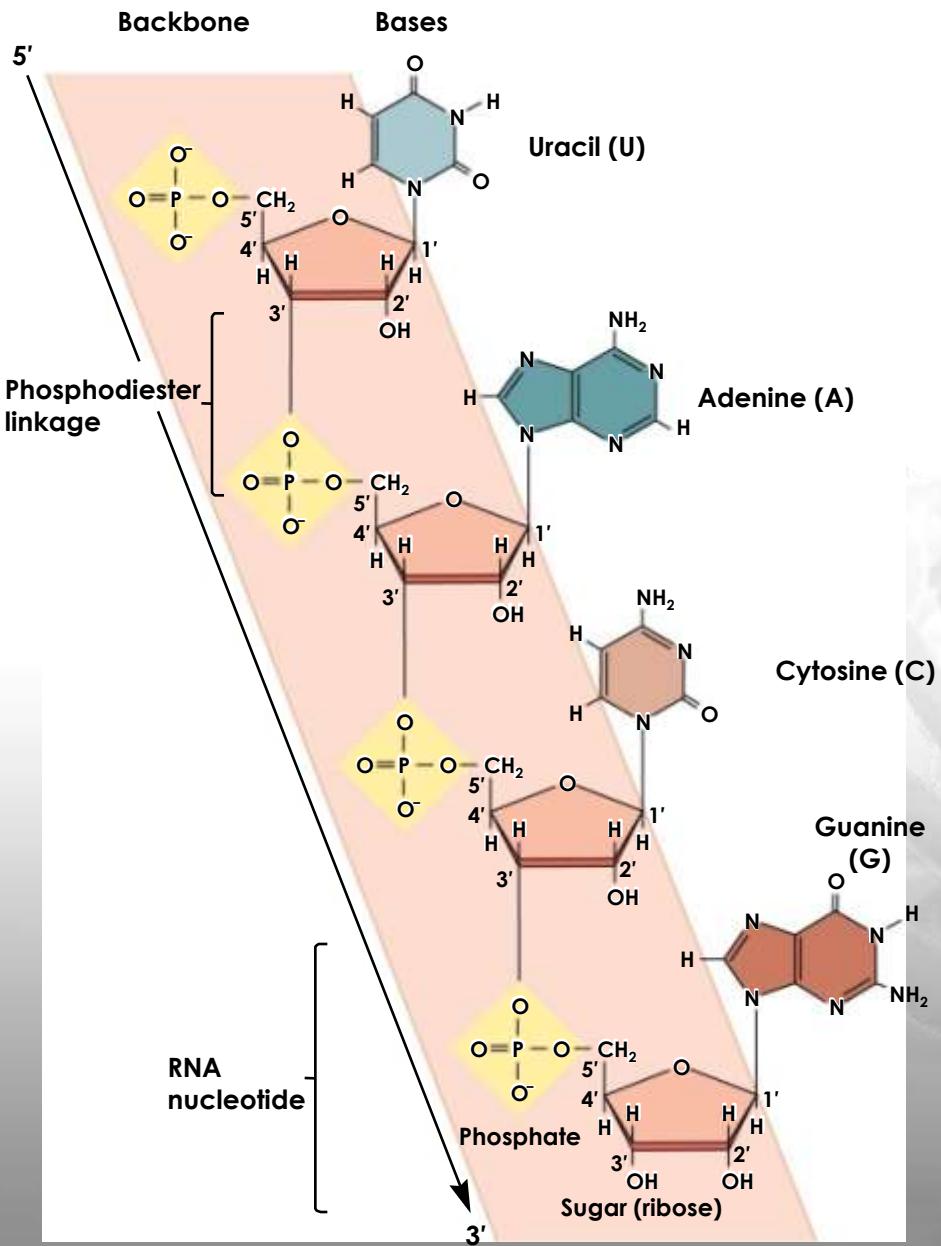
Base always attached here

❖ Covalent bonding B/W Nucleotides

- Nucleotides are linked together by covalent bonds called phosphodiester linkage.

A chemical bond that involves sharing a pair of electrons between atoms in a molecule.





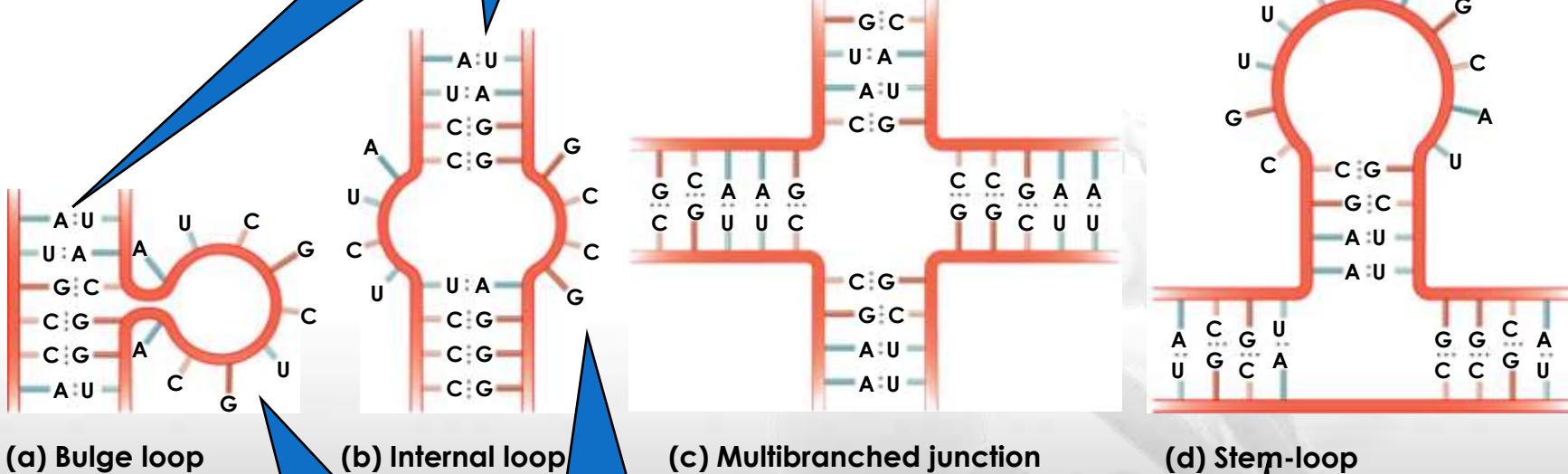
❖ Hydrogen bonding

- Usually RNA is single stranded, But in some viruses RNA present in double stranded form.
- The bases in two strands are paired through hydrogen bond (H-bonds) forming base pairs (bp). **Adenine** forms **two** hydrogen bonds with **Uracil** from opposite strand and vice-versa. Similarly, **Guanine** is bonded with **Cytosine** with **three** H-bonds.

❖ dsRNA Structure

- There are double-stranded RNA structures
 - ✓ RNA can fold back on itself
 - ✓ Depends on base sequence
 - ✓ Gives stem (double-strand) and loop (single-strand structures)
- ds RNA has an A-like conformation
 - ✓ Steric clashes between 2'-OH groups prevent the B-like conformation.

Complementary regions
Held together by
hydrogen bonds



Non-complementary regions
Have bases projecting away
from double stranded regions

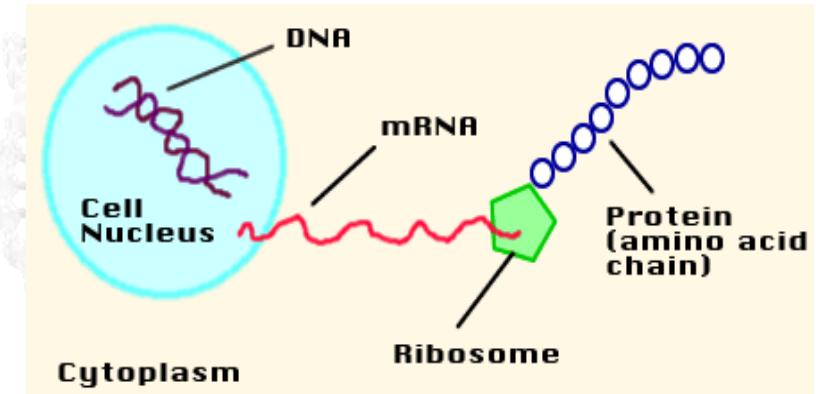
Also called
hair-pin

❖ Types of RNA

- In all prokaryotic and eukaryotic organisms, three main classes of RNA molecules exist-
 - 1) Messenger RNA(m RNA)
 - 2) Transfer RNA (t RNA)
 - 3) Ribosomal RNA (r RNA)
- The other are –
 - ✓ small nuclear RNA (SnRNA),
 - ✓ micro RNA(mi RNA) and
 - ✓ small interfering RNA(Si RNA) and
 - ✓ heterogeneous nuclear RNA (hnRNA).

❖ Messenger RNA (m-RNA)

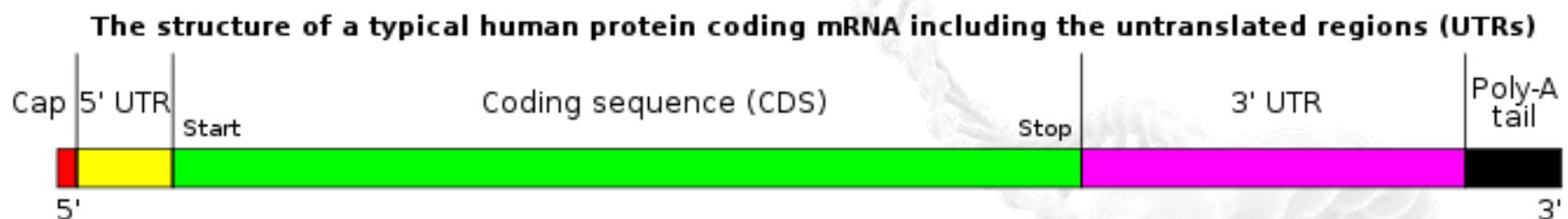
- All members of the class function as messengers carrying the information in a gene to the protein synthesizing machinery



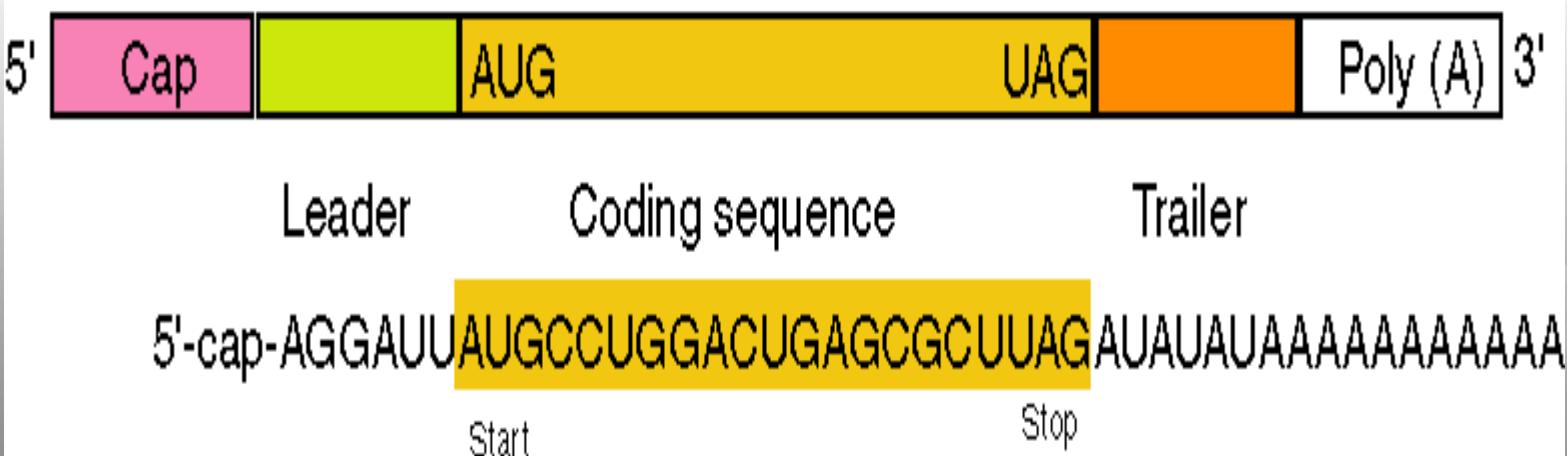
❖ Structure

- The 5' terminal end is capped by **7- methyl guanosine triphosphate cap**.
- The cap is involved in the **recognition of mRNA** by the translating machinery.
- It stabilizes m RNA by protecting it from 5' **exonuclease**.
- The 3'end of most m-RNAs have a polymer of Adenylate residues(20-250).
- The tail prevents the attack by 3' exonucleases.
- On both 5' and 3' end there are non coding sequences which are not translated (NCS)

- The intervening region between non coding sequences present between 5' and 3' end is called coding region. This region encodes for the synthesis of a protein.



Eukaryotic mRNA molecule



❖ Heterogeneous nuclear RNA (hnRNA) [Precursor mRNA]

- In mammalian nuclei , hnRNA is the immediate product of gene transcription
- The nuclear product is heterogeneous in size (Variable) and is very large.
- 75 % of hnRNA is degraded in the nucleus, only 25% is processed to mature m RNA.
- Mature m –RNA is formed from primary transcript by capping, tailing, splicing and base modification.

❖ Transfer RNA (t-RNA)

- Transfer RNA are the smallest of three major species of RNA molecules
- They have 74-95 nucleotide residues
- They transfer the amino acids from cytoplasm to the protein synthesizing machinery, hence the name t RNA.
- They are also called Adapter molecules, since they act as adapters for the translation of the sequence of nucleotides of the m RNA in to specific amino acids
- There are at least 20 species of tRNA one corresponding to each of the 20 amino acids required for protein synthesis.
- **tRNA** is the only RNA species that contains the **nucleoside thymidine**.

❖ Structure

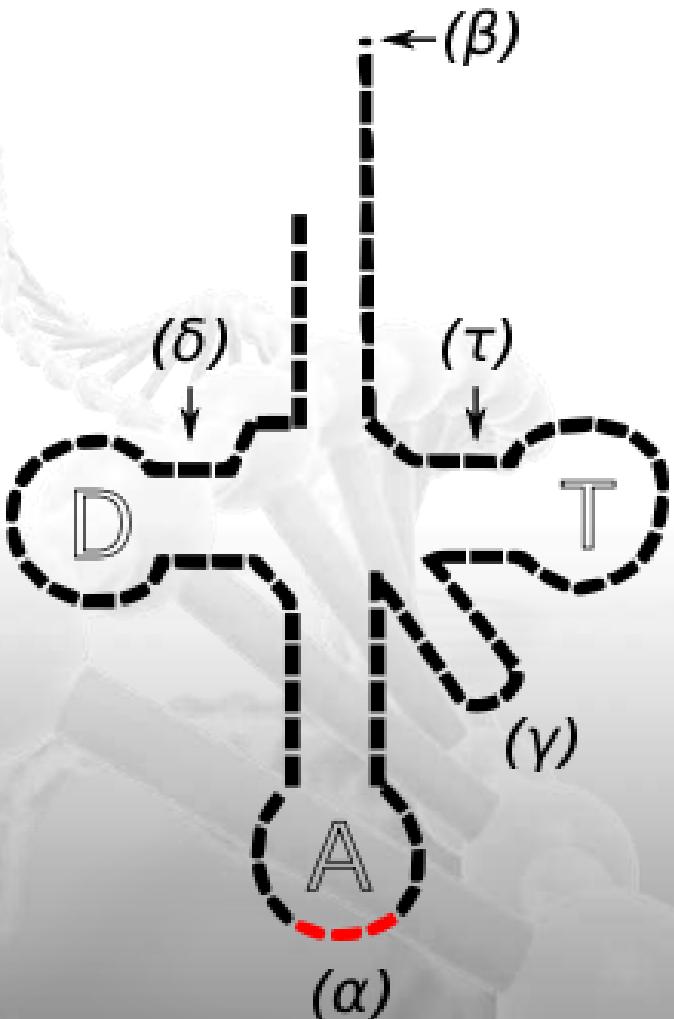
1) Primary structure- The nucleotide sequence of all the t RNA molecules allows extensive intrastand complementarity that generates a secondary structure.

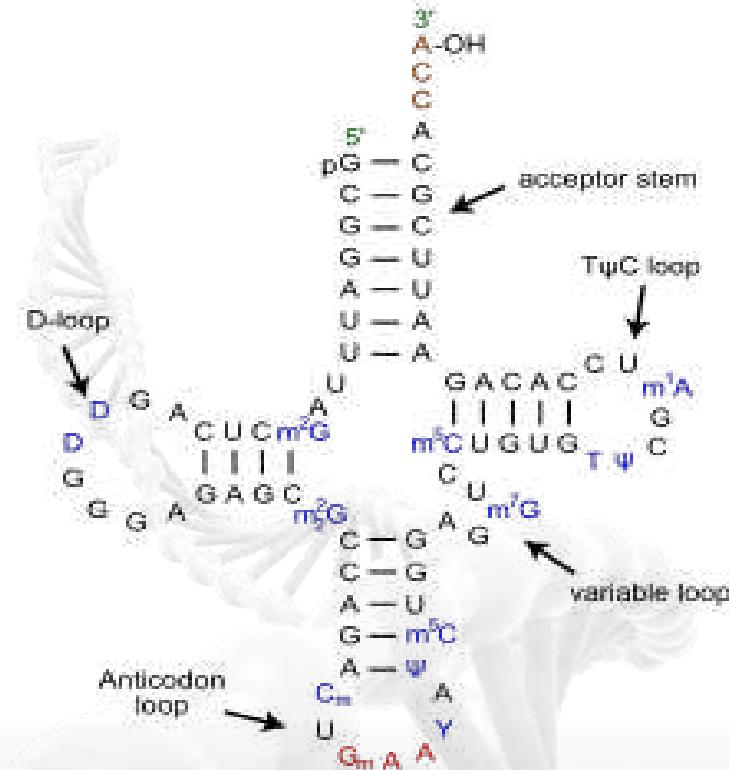
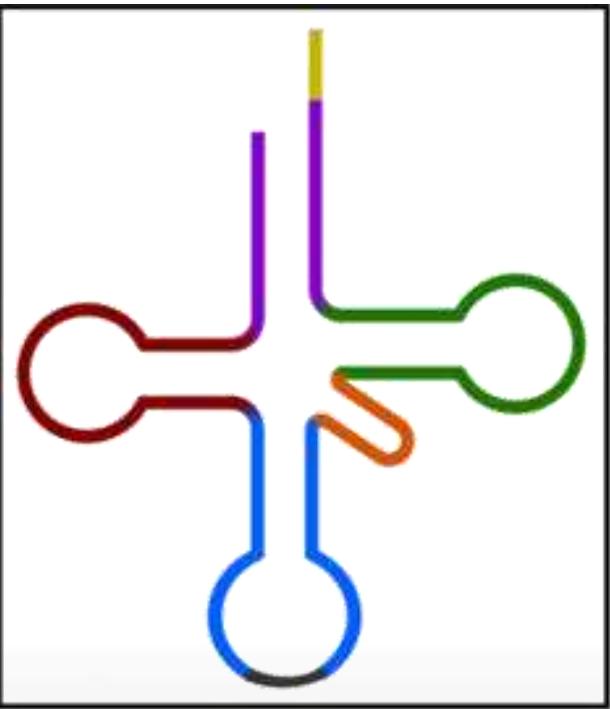
2) Secondary structure- Each single t- RNA shows extensive internal base pairing and acquires a clover leaf like structure. The structure is stabilized by hydrogen bonding between the bases and is a consistent feature.

Secondary structure (Clover leaf structure)

All t-RNA contain 5 main arms or loops which are as follows-

- a) Acceptor arm
- b) Anticodon arm
- c) D HU arm (*DihydroUracil*)
- d) TΨ C arm *Thymidine Pseudouridine Cytosine*
- e) Extra arm





Secondary structure of tRNA. **CCA tail** in **yellow**, **Acceptor stem** in **purple**, **Variable loop** in **orange**, **D arm** in **red**, **Anticodon arm** in **blue** with **Anticodon** in **black**, **T arm** in **green**.

3) Tertiary structure of t-RNA

- The L shaped tertiary structure is formed by further folding of the clover leaf due to hydrogen bonds between T and D arms.
- The base paired double helical stems get arranged in to two double helical columns, continuous and perpendicular to one another.



❖ Ribosomal RNA (rRNA)

- **Ribosomal ribonucleic acid (rRNA)** is the **RNA** component of the **ribosome**, and is essential for **protein synthesis** in all living organisms.
- The functions of the ribosomal RNA molecules in the ribosomal particle are not fully understood, but they are necessary for ribosomal assembly and seem to play key roles in the binding of mRNA to ribosomes and its translation
- Recent studies suggest that an rRNA component performs the peptidyl transferase activity and thus is an enzyme (a **ribozyme**).
- It constitutes the predominant material within the ribosome, which is approximately 60% rRNA and 40% protein by weight.
- Ribosomes contain two major rRNAs and 50 or more proteins.
- The ribosomal RNAs form two subunits, the large subunit (LSU) and small subunit (SSU). The LSU rRNA acts as a **ribozyme**, catalysing **peptide bond formation**.

❖ Small RNA molecules

□ Major types of small RNA molecules:

- Small nuclear RNA (snRNA) - involved in mRNA splicing.
- Small nucleolar RNA (snoRNA) - directs the modification of ribosomal RNAs.
- Micro RNA (miRNA) and short interfering RNA (siRNA) - regulate gene expression.

❖ Differences between RNA and DNA

S.No.	RNA	DNA
1)	Single stranded mainly except when self complementary sequences are there it forms a double stranded structure (Hair pin structure)	Double stranded (Except for certain viral DNA s which are single stranded)
2)	Ribose is the main sugar	The sugar moiety is deoxy ribose
3)	Pyrimidine components differ. Thymine is never found (Except tRNA)	Thymine is always there but uracil is never found
4)	Being single stranded structure- It does not follow Chargaff's rule	It does follow Chargaff's rule. The total purine content in a double stranded DNA is always equal to pyrimidine content.

S.No.	RNA	DNA
5)	RNA can be easily destroyed by alkalis to cyclic diesters of mono nucleotides.	DNA resists alkali action due to the absence of OH group at 2' position
6)	RNA is a relatively a labile molecule, undergoes easy and spontaneous degradation	DNA is a stable molecule. The spontaneous degradation is very too slow. The genetic information can be stored for years together without any change.
7)	Mainly cytoplasmic, but also present in nucleus (primary transcript and small nuclear RNA)	Mainly found in nucleus, extra nuclear DNA is found in mitochondria, and plasmids etc
8)	The base content varies from 100- 5000. The size is variable.	Millions of base pairs are there depending upon the organism

S.No.	RNA	DNA
9)	There are various types of RNA – mRNA, rRNA, tRNA, SnRNA, SiRNA, miRNA and hnRNA. These RNAs perform different and specific functions.	DNA is always of one type and performs the function of storage and transfer of genetic information.
10)	No variable physiological forms of RNA are found. The different types of RNA do not change their forms	There are variable forms of DNA (A, B and Z)
11)	RNA is synthesized from DNA, it can not form DNA(except by the action of reverse transcriptase). It can not duplicate (except in certain viruses where it is a genomic material)	DNA can form DNA by replication, it can also form RNA by transcription.
12)	Many copies of RNA are present per cell	Single copy of DNA is present per cell.

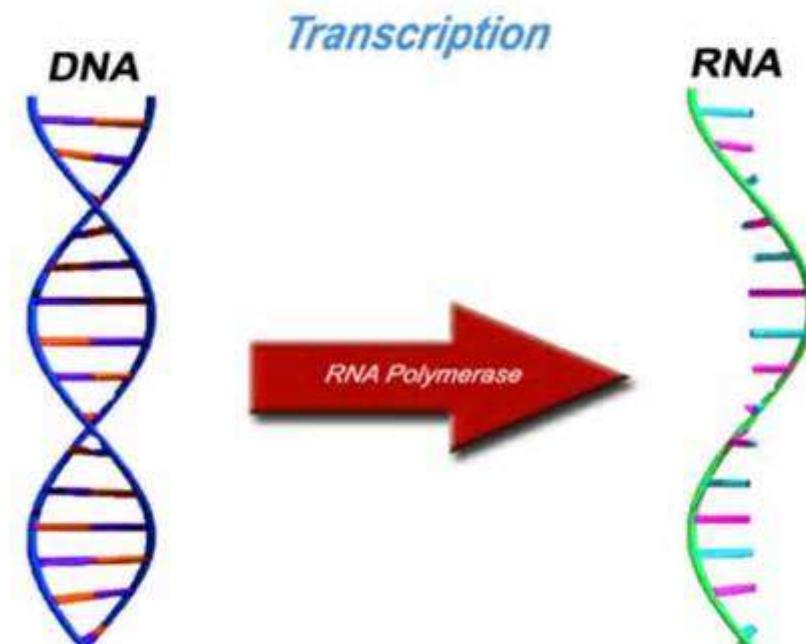


Thank

You

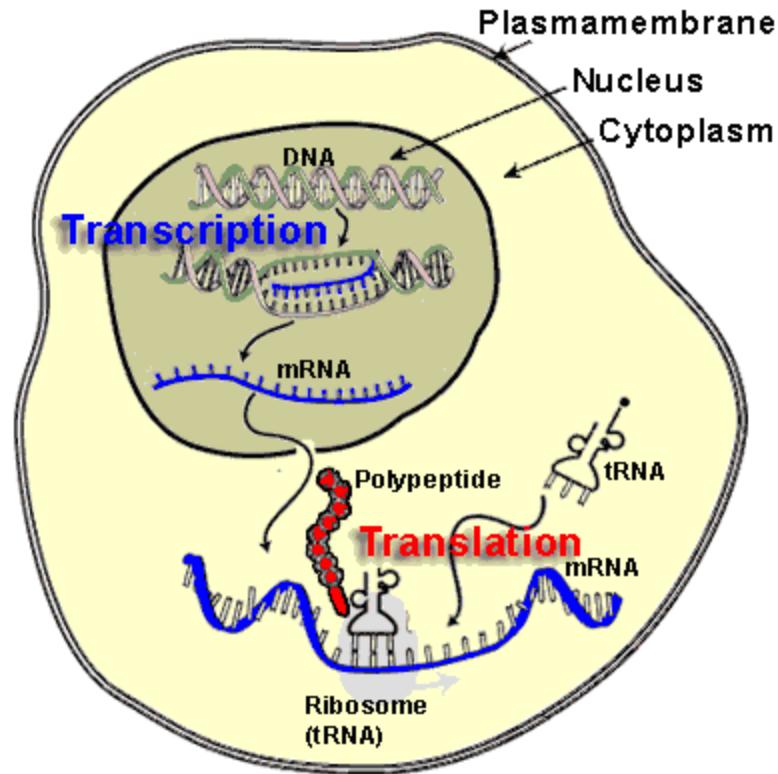


DNA Transcription (Part-1)



By- Professor (Dr.) Namrata Chhabra
Biochemistry For Medics- Lecture Notes
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Flow of genetic information



- The genetic information flows from DNA to mRNA and then to the protein synthesizing machinery.

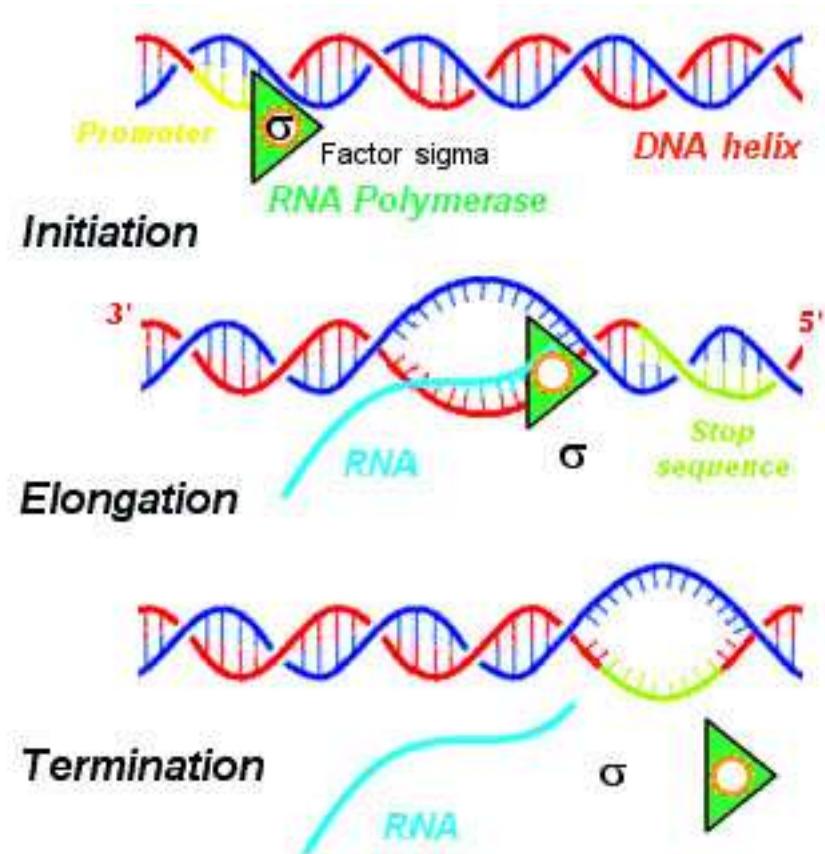
DNA Transcription- Introduction

- The synthesis of an RNA molecule from DNA is called **Transcription**.
- All eukaryotic cells have five major classes of RNA: ribosomal RNA (rRNA), messenger RNA (mRNA), transfer RNA (tRNA), small nuclear RNA and microRNA (snRNA and miRNA).
- The first three are involved in protein synthesis, while the small RNAs are involved in mRNA splicing and regulation of gene expression.

Similarities between Replication and Transcription

The processes of DNA and RNA synthesis are similar in that they involve-

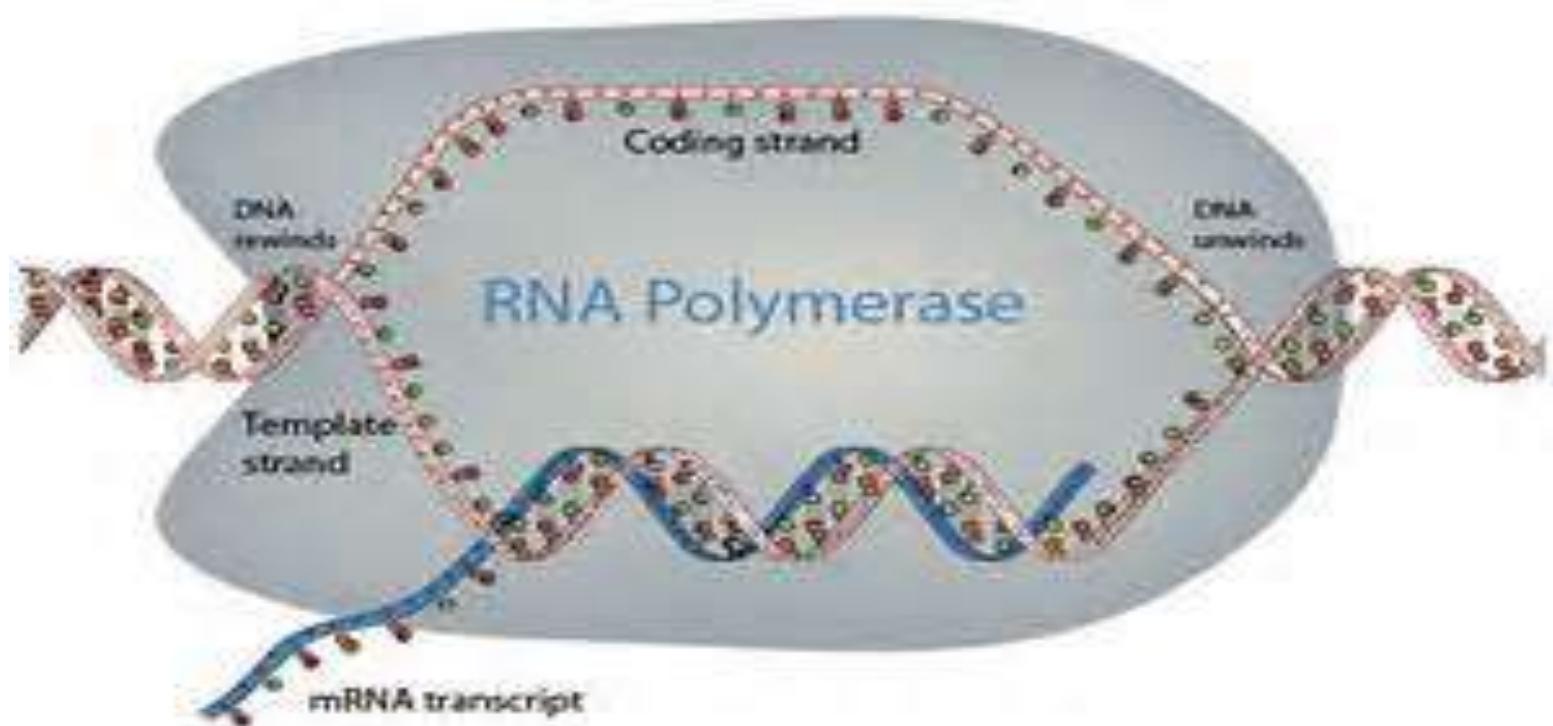
- (1) the general steps of initiation elongation, and termination with 5' to 3' polarity;
- (2) large, multicomponent initiation complexes; and
- (3) adherence to Watson-Crick base-pairing rules.



Differences between Replication and Transcription

- (1) Ribonucleotides are used in RNA synthesis rather than deoxy ribonucleotides;
- (2) U replaces T as the complementary base pair for A in RNA;
- (3) A primer is not involved in RNA synthesis;
- (4) Only a portion of the genome is transcribed or copied into RNA, whereas the entire genome must be copied during DNA replication; and
- (5) There is no proofreading function during RNA transcription.

Template strand



- The strand that is transcribed or copied into an RNA molecule is referred to as the template strand of the DNA.
- The other DNA strand, the non-template strand, is frequently referred to as the coding strand of that gene.

Template strand

- The information in the template strand is read out in the 3' to 5' direction
- The sequence of ribonucleotides in the RNA molecule is complementary to the sequence of deoxy ribonucleotides in template strand of the double-stranded DNA molecule
- In the coding strand (complementary strand) the sequence is same as that of the sequence of nucleotides in the primary transcript.

Template strand (contd.)

DNA coding strand **5' A T C C T G G T A G A T C C T T A A G**

DNA transcribed
.

m-RNA

3' T A G G A C C A T C T A G G A A T T

5' A U C C U G G U A G A U C C U U A A G

transcription 

With the exception of T for U changes, coding strand corresponds exactly to the sequence of the RNA primary transcript, which encodes the (protein) product of the gene.

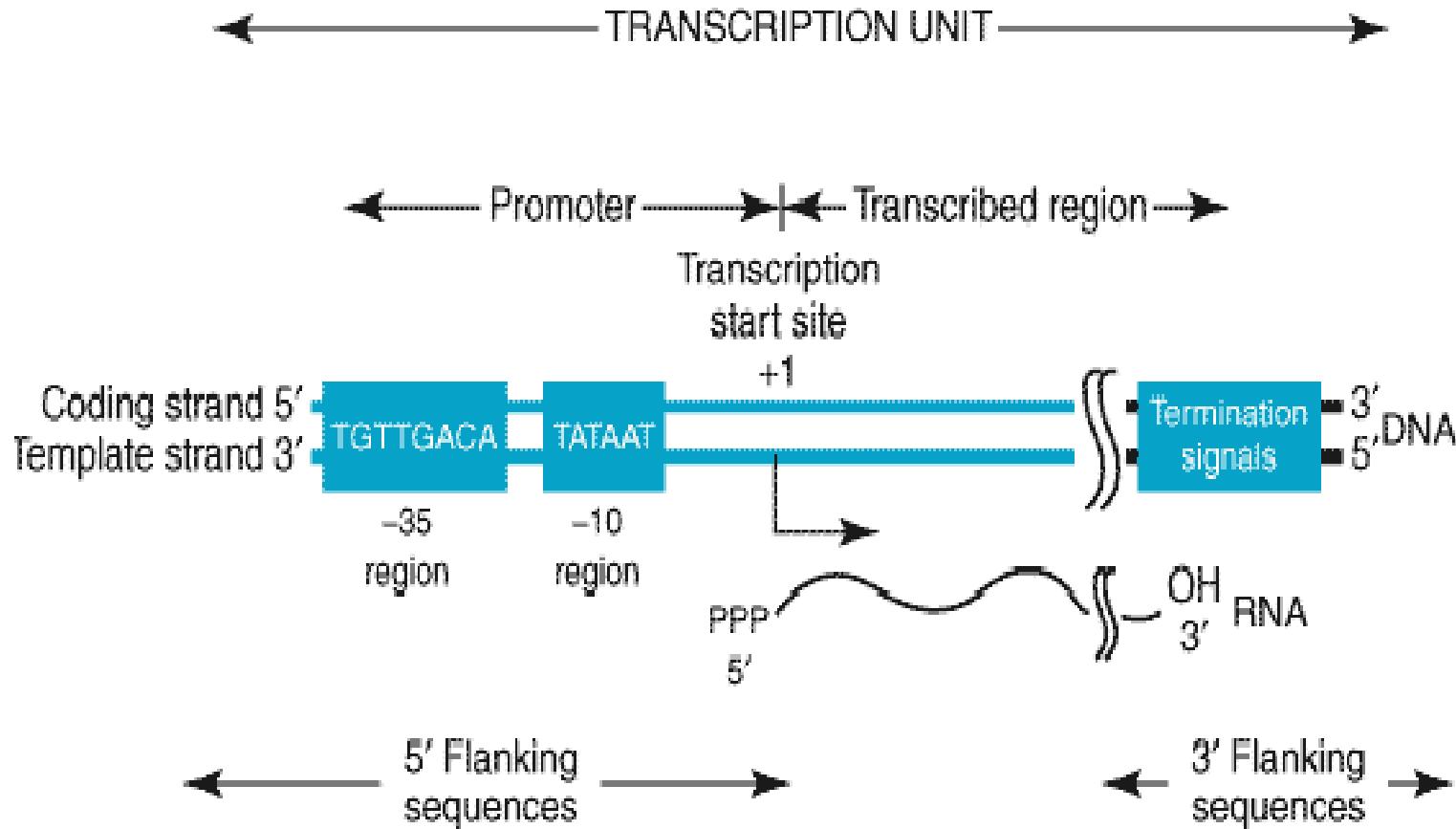
Template strand (contd.)

- In the case of a double-stranded DNA molecule containing many genes, the template strand for each gene will not necessarily be the same strand of the DNA double helix.
- Thus, a given strand of a double-stranded DNA molecule will serve as the template strand for some genes and the coding strand of other genes.

Transcription unit

- A transcription unit is defined as that region of DNA that includes the signals for transcription initiation, elongation, and termination.
- **DNA-dependent RNA polymerase** is the enzyme responsible for the polymerization of ribonucleotides into a sequence complementary to the template strand of the gene.
- The enzyme attaches at a specific site—**the promoter**—on the template strand.
- This is followed by initiation of RNA synthesis at the starting point, and the process continues until a termination sequence is reached.

Transcription unit and Primary transcript



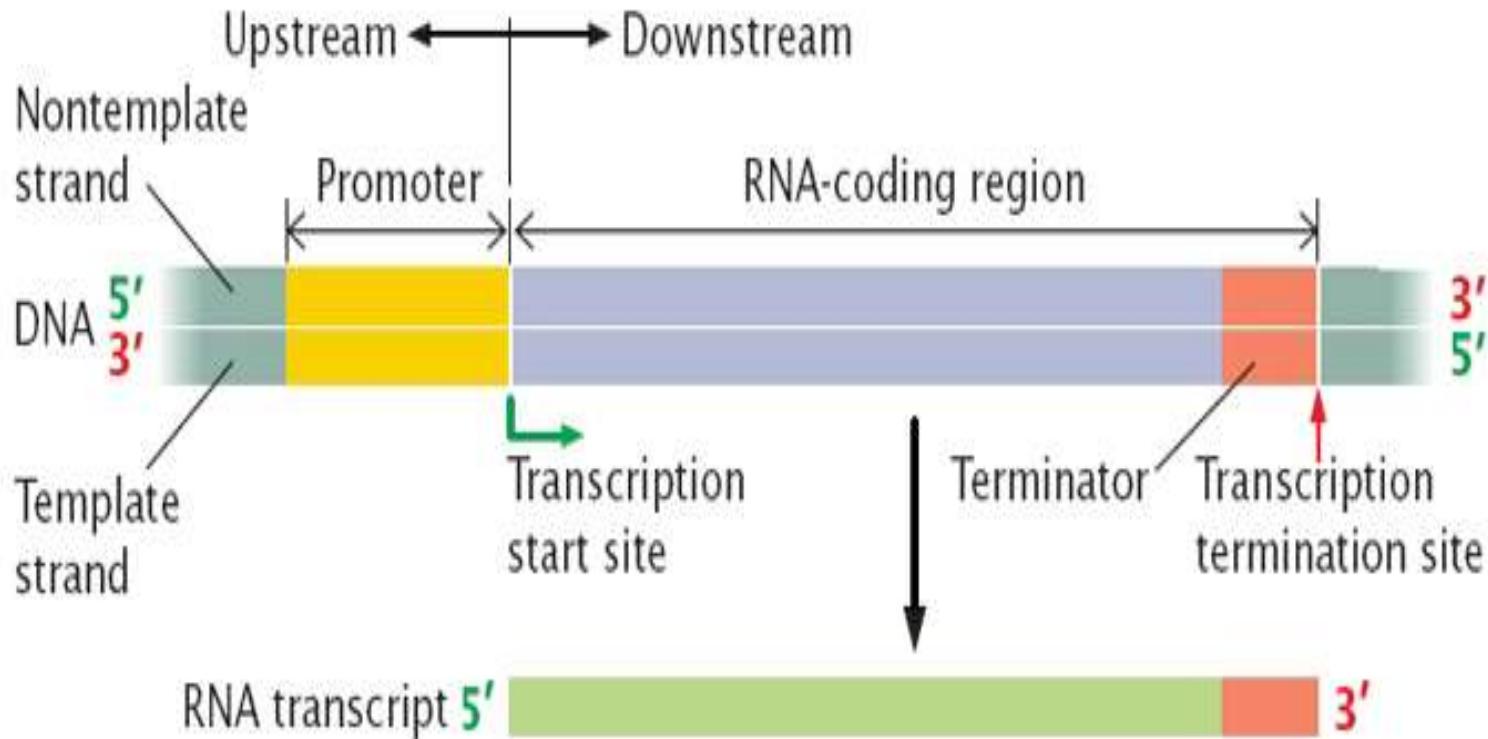
Primary transcript

- The RNA product, which is synthesized in the 5' to 3' direction, is the **primary transcript**.
- In prokaryotes, this can represent the product of several contiguous genes
- In mammalian cells, it usually represents the product of a single gene
- The 5' terminals of the primary RNA transcript and the mature cytoplasmic RNA are identical.
- **The starting point of transcription corresponds to the 5' nucleotide of the mRNA.**

Primary transcript

- This is designated position +1, as is the corresponding nucleotide in the DNA
- The numbers increase as the sequence proceeds *downstream*.
- The nucleotide in the promoter adjacent to the transcription initiation site is designated -1,
- These negative numbers increase as the sequence proceeds *upstream*, away from the initiation site.
- This provides a conventional way of defining the location of regulatory elements in the promoter.

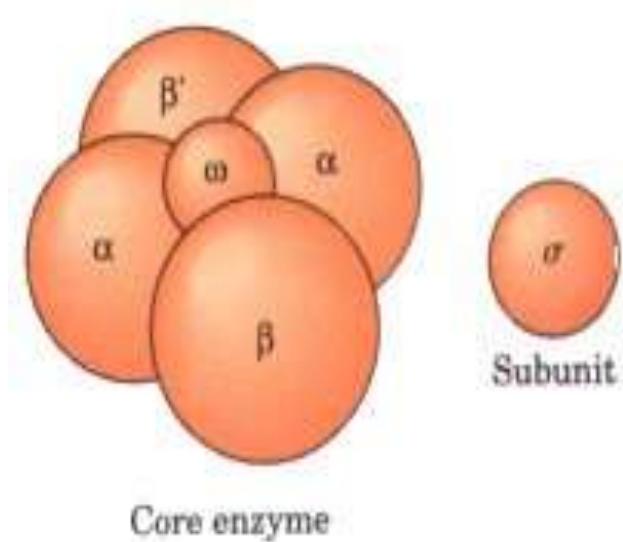
Transcription unit



Bacterial DNA-Dependent RNA Polymerase

The DNA-dependent RNA polymerase (RNAP) of the bacterium *Escherichia coli* exists as an approximately 400 kDa core complex consisting of-

- two identical α subunits,
- similar but not identical β and β' subunits, and
- an ω subunit and a
- A sigma subunit (σ)
- Beta is thought to be the catalytic subunit.



Bacterial DNA-Dependent RNA Polymerase

- RNAP, a metalloenzyme, also contains two zinc molecules.
- The core RNA polymerase associates with a specific protein factor (the sigma σ factor) that helps the core enzyme recognize and bind to the specific deoxynucleotide sequence of the promoter region to form the preinitiation complex (PIC)
- Bacteria contain multiple factors, each of which acts as a regulatory protein.

Mammalian DNA-Dependent RNA Polymerases

Mammalian cells possess three distinct nuclear DNA-Dependent RNA Polymerases

- RNA polymerase I is for the synthesis of rRNA
- RNA polymerase II is for the synthesis of mRNA and miRNA
- RNA polymerase III is for the synthesis of tRNA/5S rRNA, snRNA

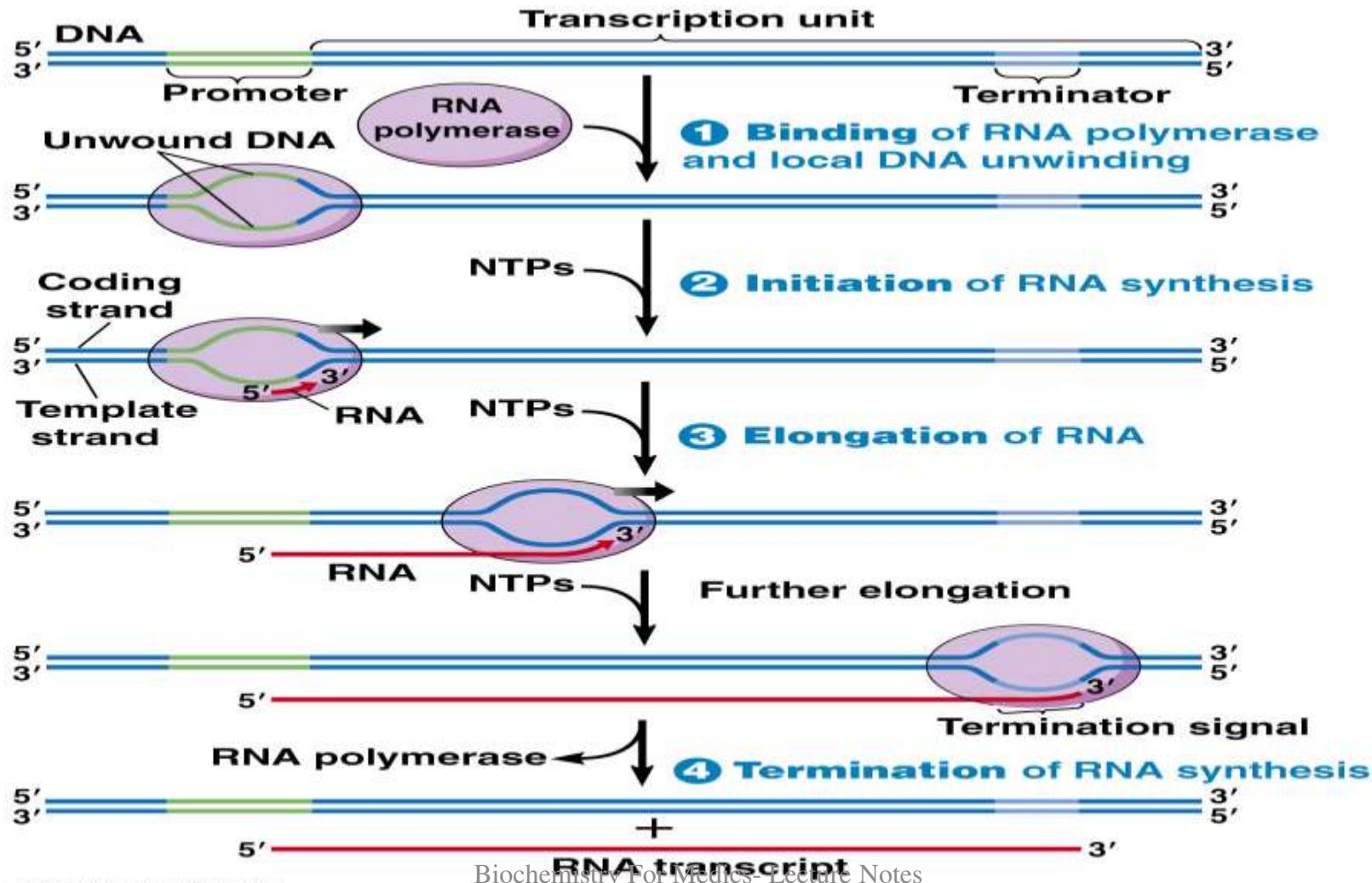
Prokaryotic transcription

Steps of RNA Synthesis-

The process of transcription of a typical gene of E. Coli can be divided in to three phases-

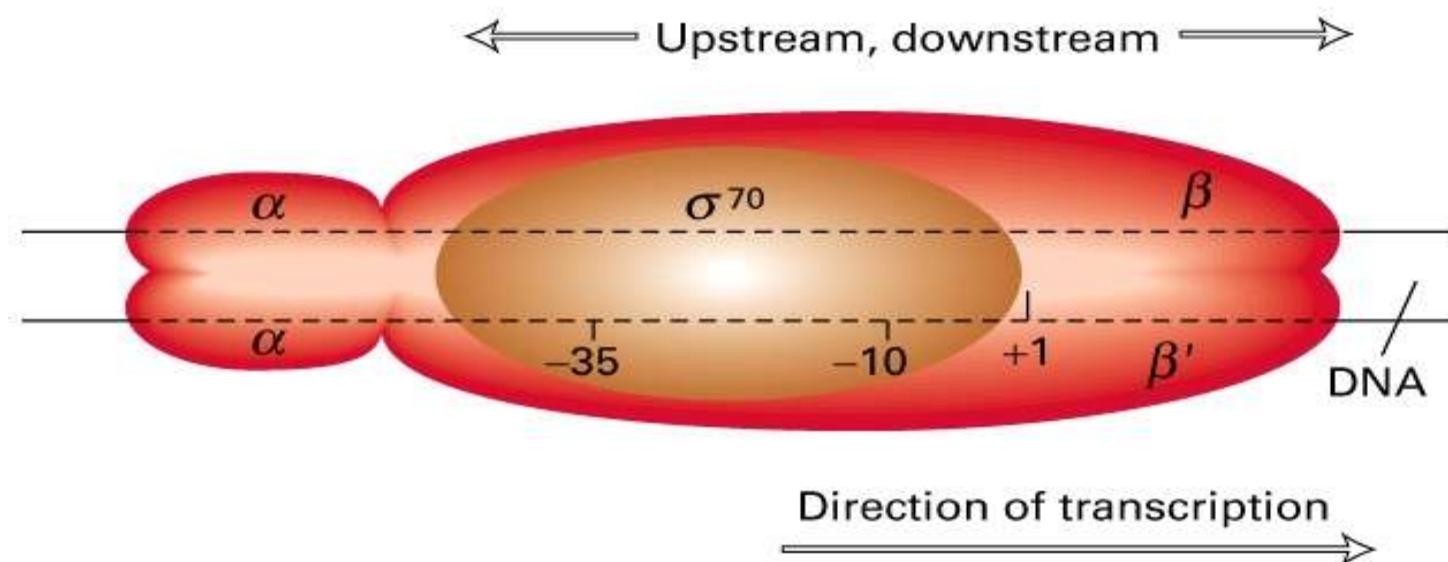
- i) Initiation
- ii) Elongation
- iii) Termination

Overview of Prokaryotic DNA Transcription



i) Initiation of Transcription

- Initiation of transcription involves the binding of the RNA polymerase holoenzyme to the promoter region on the DNA to form a **preinitiation complex, or PIC**
- Characteristic "Consensus" nucleotide sequence of the prokaryotic promoter region are highly conserved.



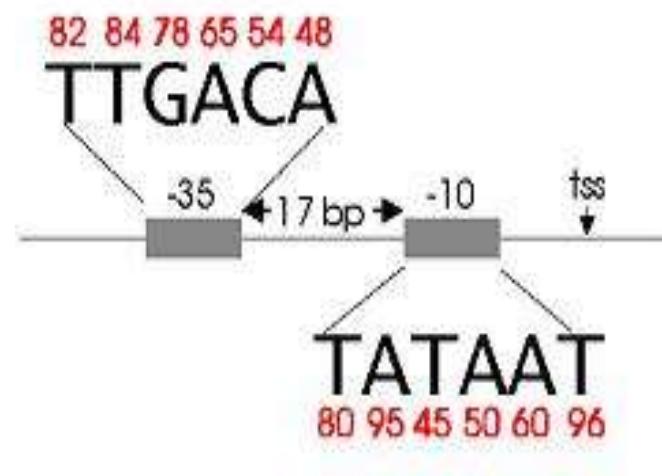
Structure of bacterial prokaryotic promoter region

Pribnow box

- This is a stretch of 6 nucleotides (5'- TATAAT-3') centered about 8-10 nucleotides to the left of the transcription start site.

-35 Sequence

- A second consensus nucleotide sequence (5'- TTGACA-3'), is centered about 35 bases to the left of the transcription start site.



i) Initiation of Transcription (contd.)

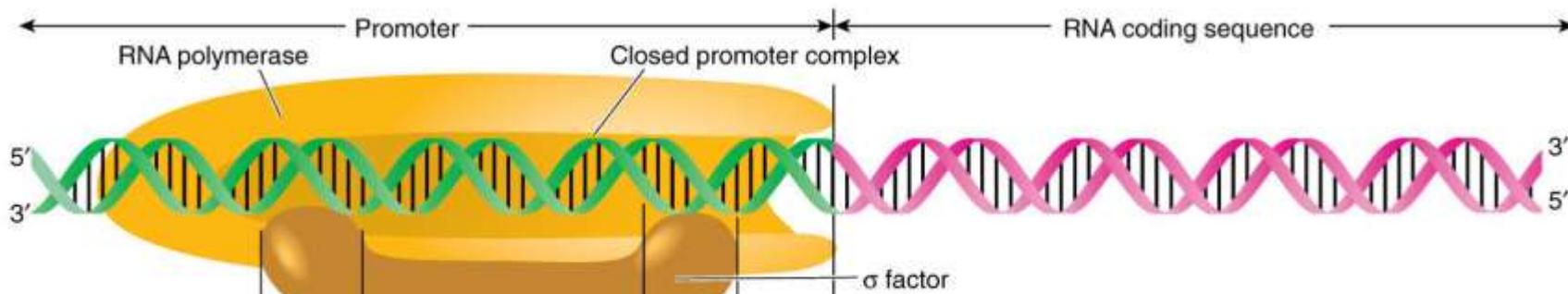
- Binding of RNA-polymerase (RNAP) to the promoter region is followed by a conformational change of the RNAP, and the first nucleotide (almost always a purine) then associates with the initiation site on the subunit of the enzyme.
- In the presence of the appropriate nucleotide, RNAP catalyzes the formation of a phosphodiester bond, and the nascent chain is now attached to the polymerization site on the subunit of RNAP.

i) Initiation of Transcription (contd.)

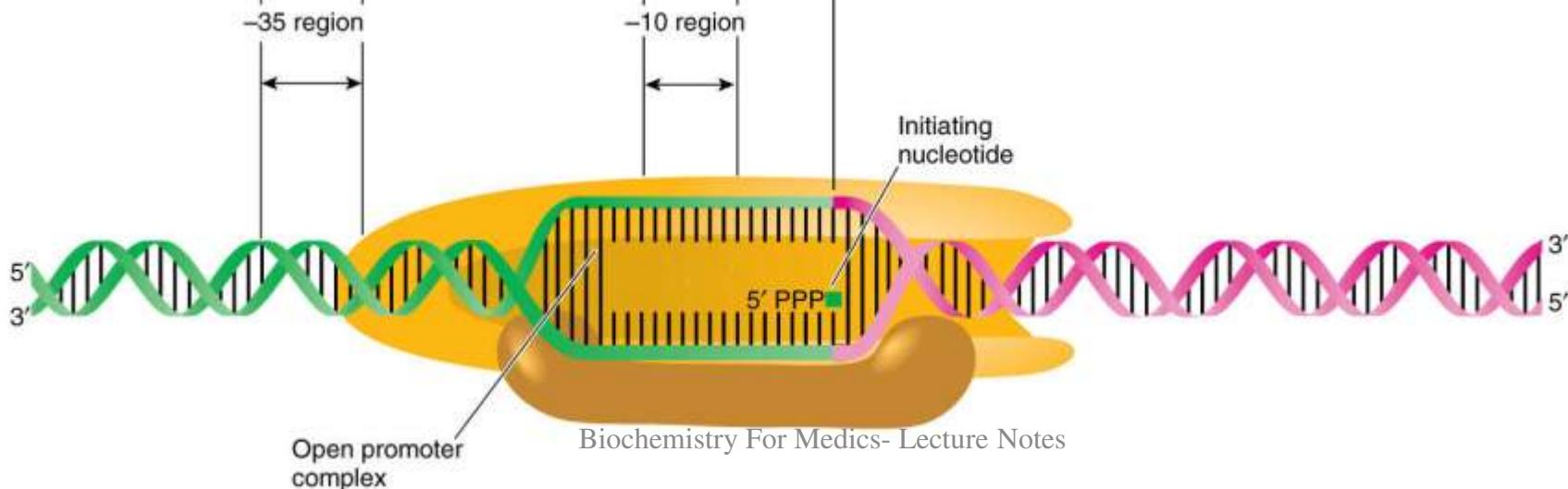
- In both prokaryotes and eukaryotes, a purine ribonucleotide is usually the first to be polymerized into the RNA molecule.
- After 10–20 nucleotides have been polymerized, RNAP undergoes a second conformational change leading to **promoter clearance**.
- Once this transition occurs, RNAP physically moves away from the promoter, transcribing down the transcription unit, leading to the next phase of the process, elongation.

i) Initiation of Transcription (contd.)

a) In initiation, the RNA polymerase holoenzyme first recognizes the promoter at the -35 region and binds to the full promoter.



b) As initiation continues, RNA polymerase binds more tightly to the promoter at the -10 region, accompanied by a local untwisting of the DNA in that region. At this point, the RNA polymerase is correctly oriented to begin transcription at $+1$.

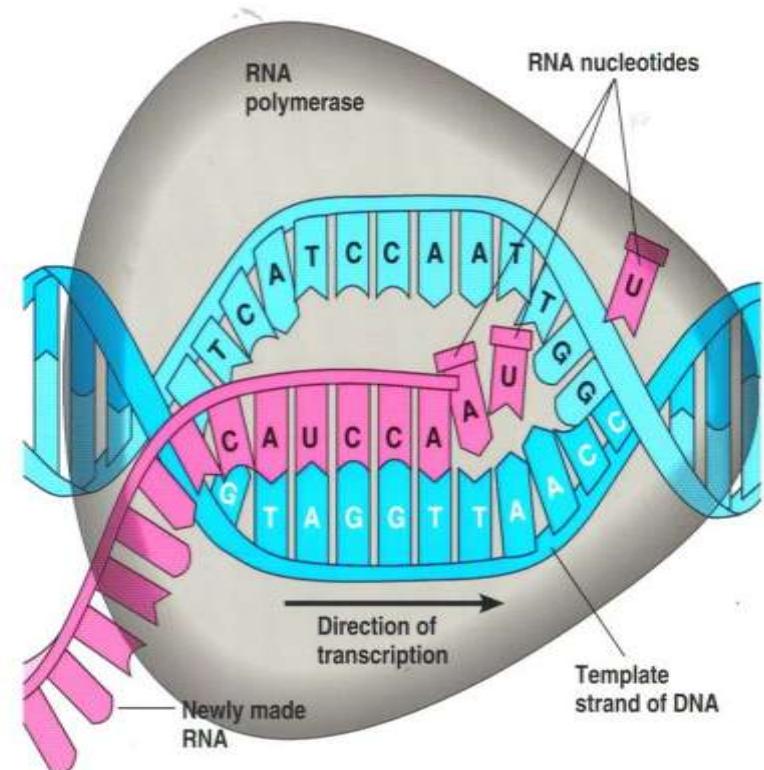


II) Elongation step of Transcription

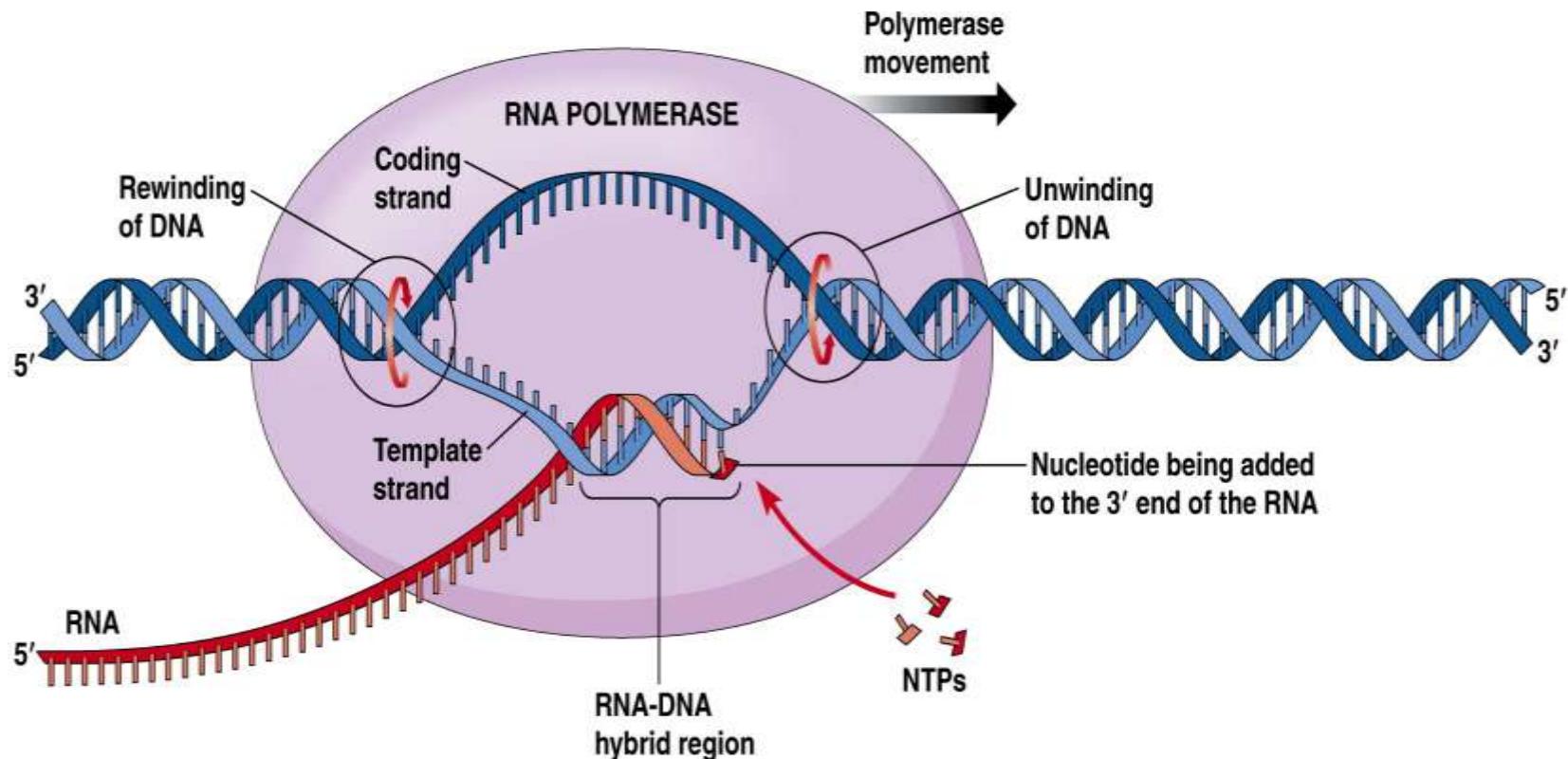
- As the elongation complex containing the core RNA polymerase progresses along the DNA molecule, DNA unwinding must occur in order to provide access for the appropriate base pairing to the nucleotides of the template strand.
- The extent of this transcription bubble (i.e., DNA unwinding) is constant throughout and is about 20 base pairs per polymerase molecule.

II) Elongation step of Transcription

- RNA polymerase has associated with it an "unwindase" activity that opens the DNA helix.
- Topo isomerase both precedes and follows the progressing RNAP to prevent the formation of super helical complexes.
- Base pairing rule is followed during the incorporation of ribonucleotides



II) Elongation step of Transcription

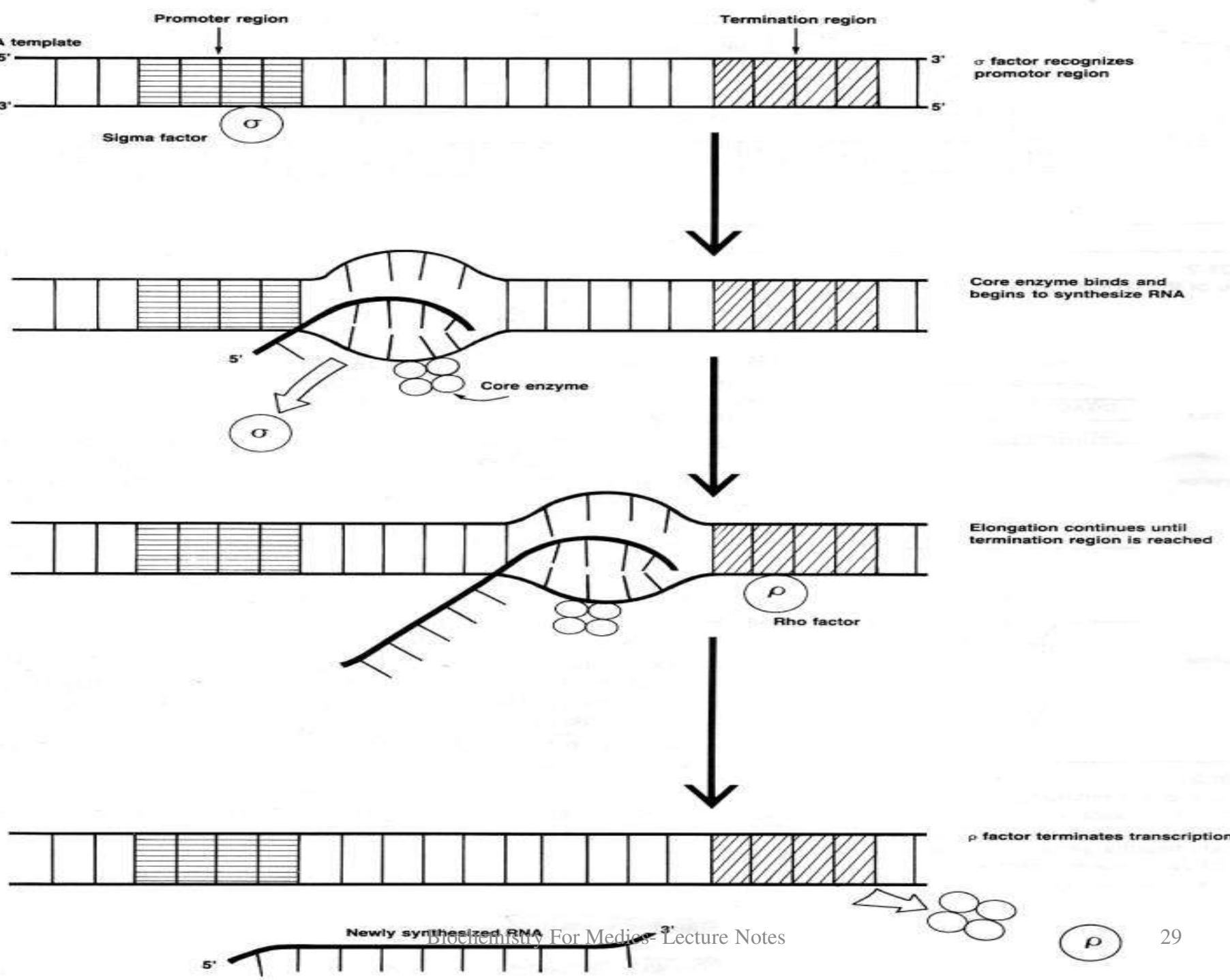


III) Termination of transcription

Termination of the synthesis of the RNA molecule in bacteria is of two types-

a) Rho (ρ) dependent termination-

- The termination process is signaled by a sequence in the template strand of the DNA molecule—a signal that is recognized by a termination protein, the rho (ρ) factor.
- Rho is an ATP-dependent RNA-stimulated helicase that disrupts the nascent RNA-DNA complex.



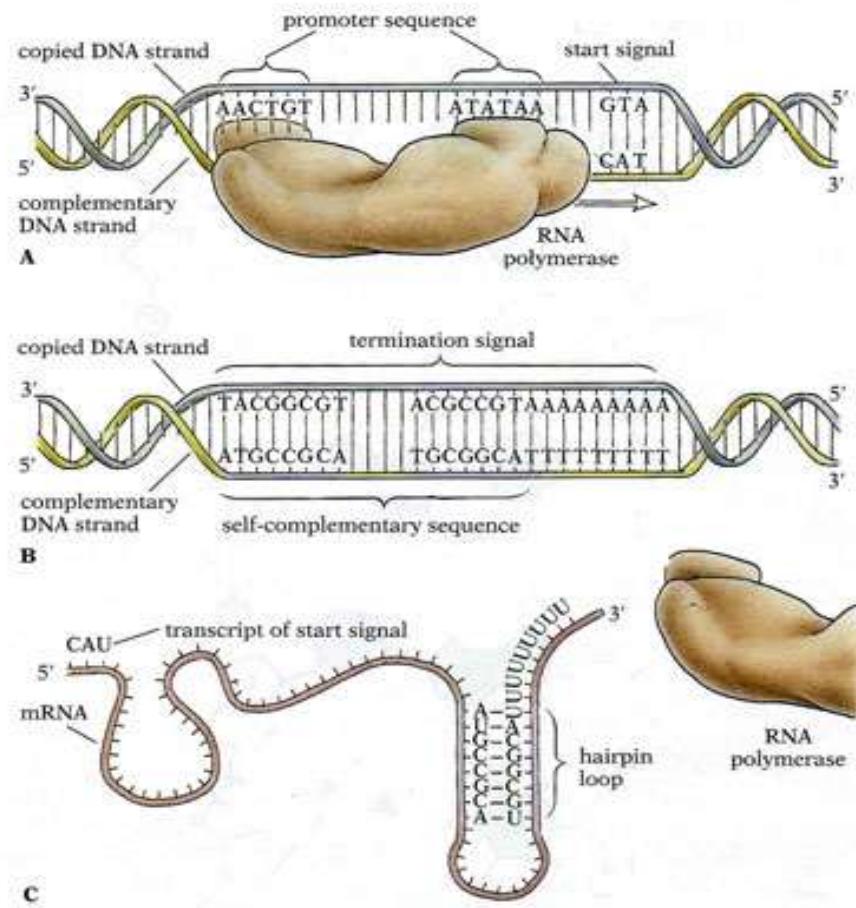
III) Termination of transcription (contd.)

b) Rho independent termination

- This process requires the presence of intrachain self complementary sequences in the newly formed primary transcript so that it can acquire a stable hair pin turn that slows down the progress of the RNA polymerase and causes it to pause temporarily.
- Near the stem of the hairpin, a sequence occurs that is rich in G and C.
- This stabilizes the secondary structure of the hair pin.

III) Termination of transcription (contd.)

- Beyond the hairpin, the RNA transcript contains a strings of Us, the bonding of Us to the corresponding As is weak.
- This facilitates the dissociation of the primary transcript from DNA.



III) Termination of transcription (contd.)

- After termination of synthesis of the RNA molecule, the enzyme separates from the DNA template.
- With the assistance of another factor, the core enzyme then recognizes a promoter at which the synthesis of a new RNA molecule commences.

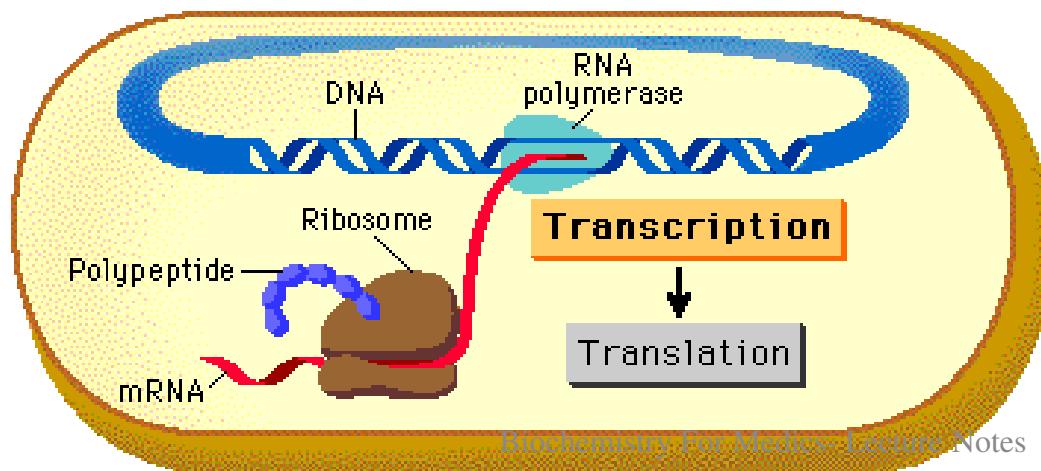
Eukaryotic transcription

- The general process of transcription can be applied to both prokaryotic cells and eukaryotic cells.
- The basic biochemistry for each is the same; however, the specific mechanisms and regulation of transcription differ between prokaryotes and eukaryotes.
- Transcription of eukaryotic genes is far more a complicated process than prokaryotes.

Prokaryotic versus Eukaryotic Transcription

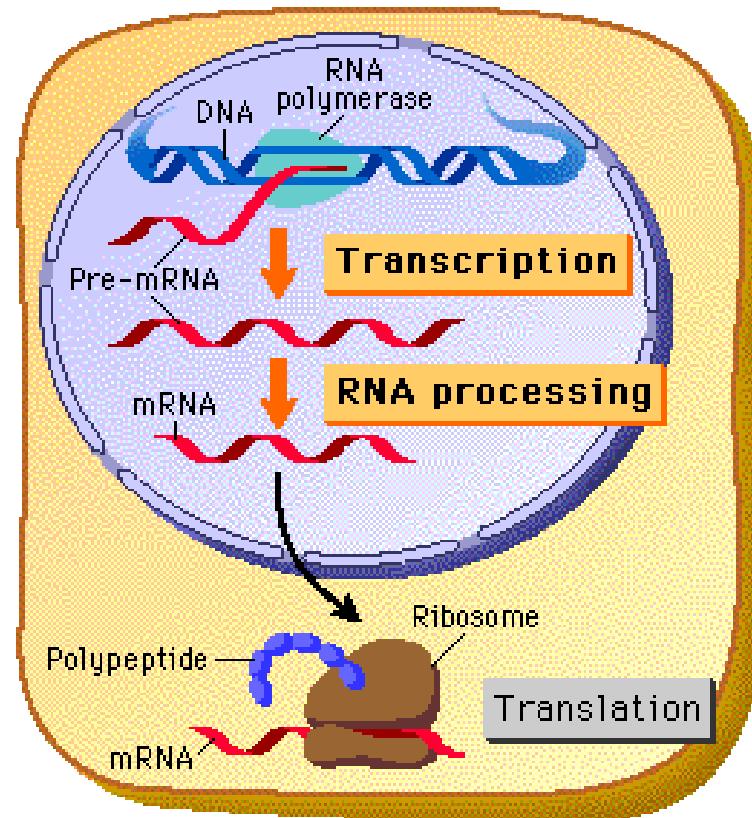
1) Location

- In prokaryotes (bacteria), transcription occurs in the cytoplasm.
- Translation of the mRNA into proteins also occurs in the cytoplasm



Prokaryotic versus Eukaryotic Transcription

- In eukaryotes, transcription occurs in the cell's nucleus, mRNA then moves to the cytoplasm for translation.



Prokaryotic versus Eukaryotic Transcription

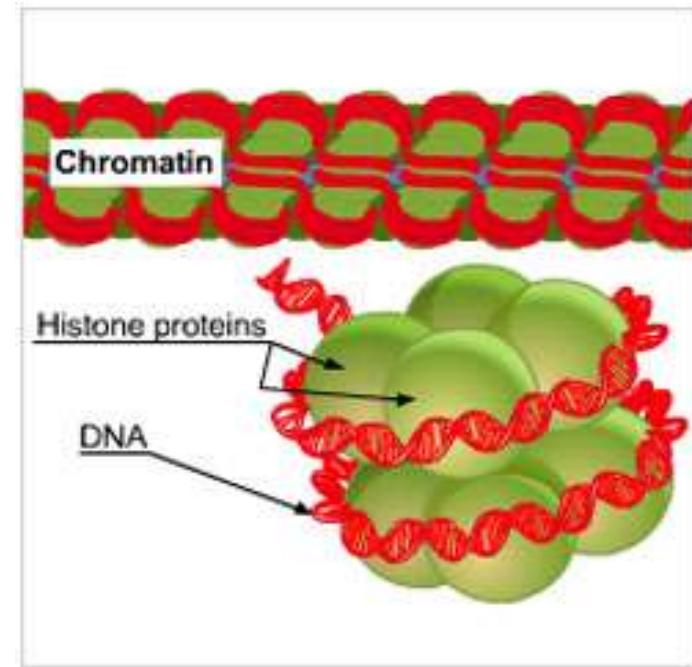
2) Genome size

- The genome size is much larger in eukaryotes,
- Greater specificity is needed for the transcription of eukaryotic genes.

Prokaryotic versus Eukaryotic Transcription

3) Chromatin Structure

- DNA in prokaryotes is much more accessible to RNA polymerase than DNA in eukaryotes.
- Eukaryotic DNA is wrapped around proteins called histones to form structures called nucleosomes
- Eukaryotic DNA is packed to form chromatin .
- While RNA polymerase interacts directly with prokaryotic DNA, other proteins mediate the interaction between RNA polymerase and DNA in eukaryotes



Prokaryotic versus Eukaryotic Transcription

4) RNA polymerases

- There are three distinct classes of RNA polymerases in eukaryotic cells. All are large enzymes with multiple subunits. Each class of RNA polymerase recognizes particular types of genes.
- RNA polymerase I- Synthesizes the precursor of the large ribosomal RNAs (28S, 18S and 5.8S).
- RNA polymerase II - Synthesizes the precursors of messenger RNA and small nuclear RNAs(snRNAs).
- RNA polymerase III- Synthesizes small RNA, including t RNAs, small 5S RNA and some snRNAs.

Prokaryotic versus Eukaryotic Transcription

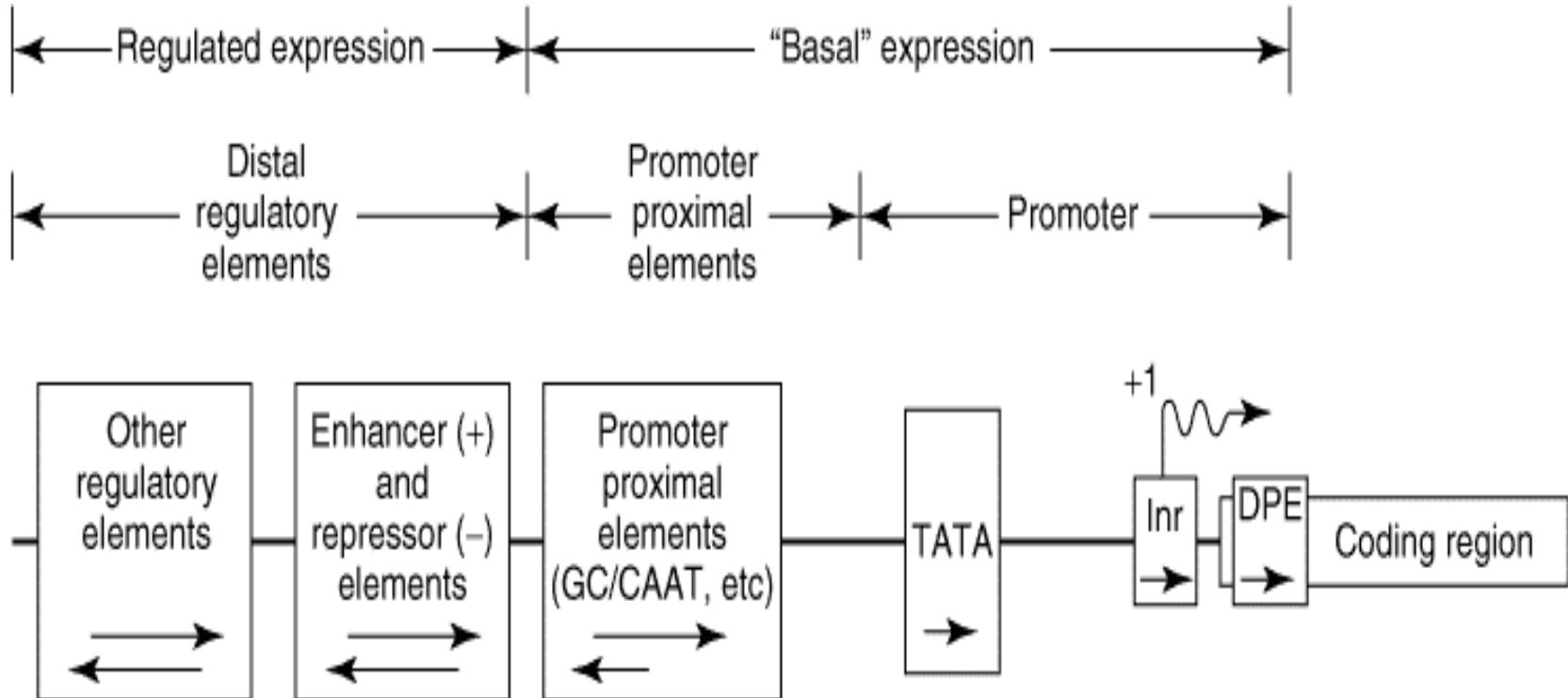
5) Promoter regions

- Eukaryotic promoters are more complex.
- Two types of sequence elements are promoter-proximal and distal regulatory elements.
- There are two elements in promoter proximal ,One of these defines **where transcription is to commence** along the DNA, and the other contributes to the mechanisms that control **how frequently** this event is to occur.
- Most mammalian genes have a TATA box that is usually located 25–30 bp upstream from the transcription start site.

Prokaryotic versus Eukaryotic Transcription

- The consensus sequence for a TATA box is TATAAA, though numerous variations have been characterized.
- Sequences farther upstream from the start site determine how frequently the transcription event occurs.
- Typical of these DNA elements are the GC and CAAT boxes, so named because of the DNA sequences involved.
- Each of these boxes binds a specific protein.
- Distal regulatory elements enhance or decrease the rate of transcription.
- They include the enhancer/ silencer regions and other regulatory elements.

Prokaryotic versus Eukaryotic Transcription

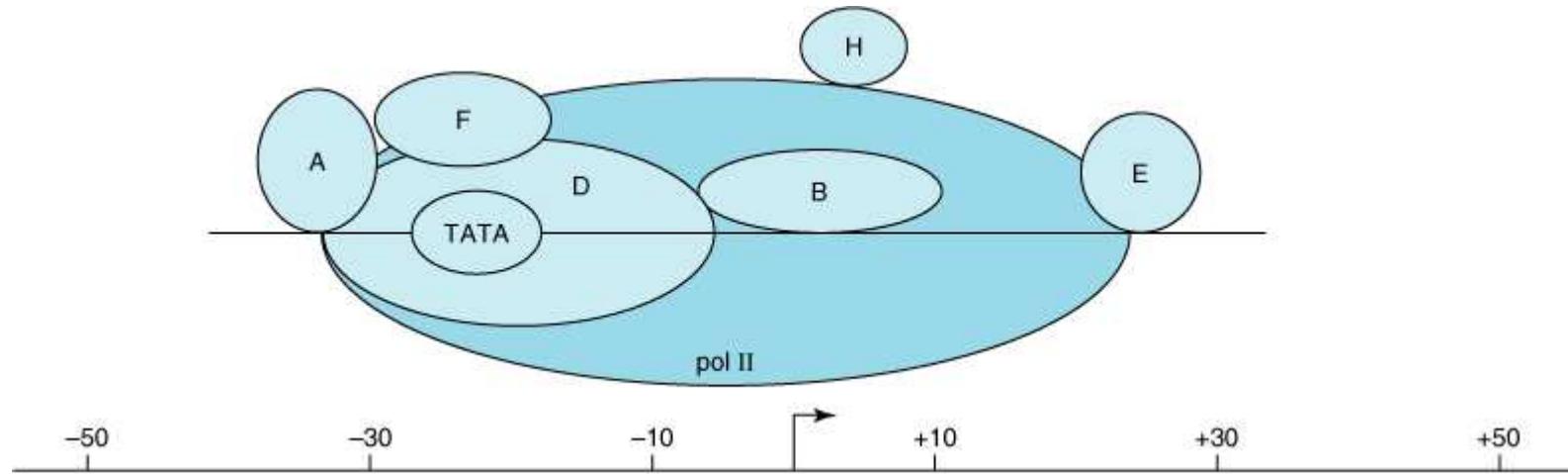


Prokaryotic versus Eukaryotic Transcription

6) Promoter identification

- In contrast to the situation in prokaryotes, eukaryotic RNA polymerases alone are not able to discriminate between promoter sequences and other regions of DNA
- The TATA box is bound by 34 kDa **TATA binding protein (TBP)**, which in turn binds several other proteins called **TBP-associated factors (TAFs)**.
- This complex of TBP and TAFs is referred to as TFIID

Prokaryotic versus Eukaryotic Transcription



- Formation of the basal transcription complex begins when TFIID binds to the TATA box.
- It directs the assembly of several other components by protein-DNA and protein-protein interactions.
- The entire complex spans DNA from position -30 to +30 relative to the initiation site.

Prokaryotic versus Eukaryotic Transcription

- Binding of TFIID to the TATA box sequence is thought to represent the first step in the formation of the transcription complex on the promoter.
- Another set of proteins—co activators—help regulate the rate of transcription initiation by interacting with transcription activators that bind to upstream DNA elements

Prokaryotic versus Eukaryotic Transcription

7) Enhancers and Repressors

- A third class of sequence elements can either increase or decrease the rate of transcription initiation of eukaryotic genes
- These elements are called either enhancers or repressors (or silencers), depending on which effect they have.

Prokaryotic versus Eukaryotic Transcription

- They have been found in a variety of locations both upstream and downstream of the transcription start site and even within the transcribed portions of some genes.
- In contrast to proximal and upstream promoter elements, enhancers and silencers can exert their effects when located hundreds or even thousands of bases away from transcription units located on the same chromosome.
- **Hormone response elements** (for steroids, T_3 , retinoic acid, peptides, etc) act as—or in conjunction with—enhancers or silencers

Prokaryotic versus Eukaryotic Transcription

7) Termination of transcription

- The signals for the termination of transcription by eukaryotic RNA polymerase II are very poorly understood.

8) Processing of primary transcript

- mRNA produced as a result of transcription is not modified in prokaryotic cells. Eukaryotic cells modify mRNA by RNA splicing, 5' end capping, and addition of a polyA tail.
- Most eukaryotic RNAs are synthesized as precursors that contain excess sequences which are removed prior to the generation of mature, functional RNA.

Transcription summary

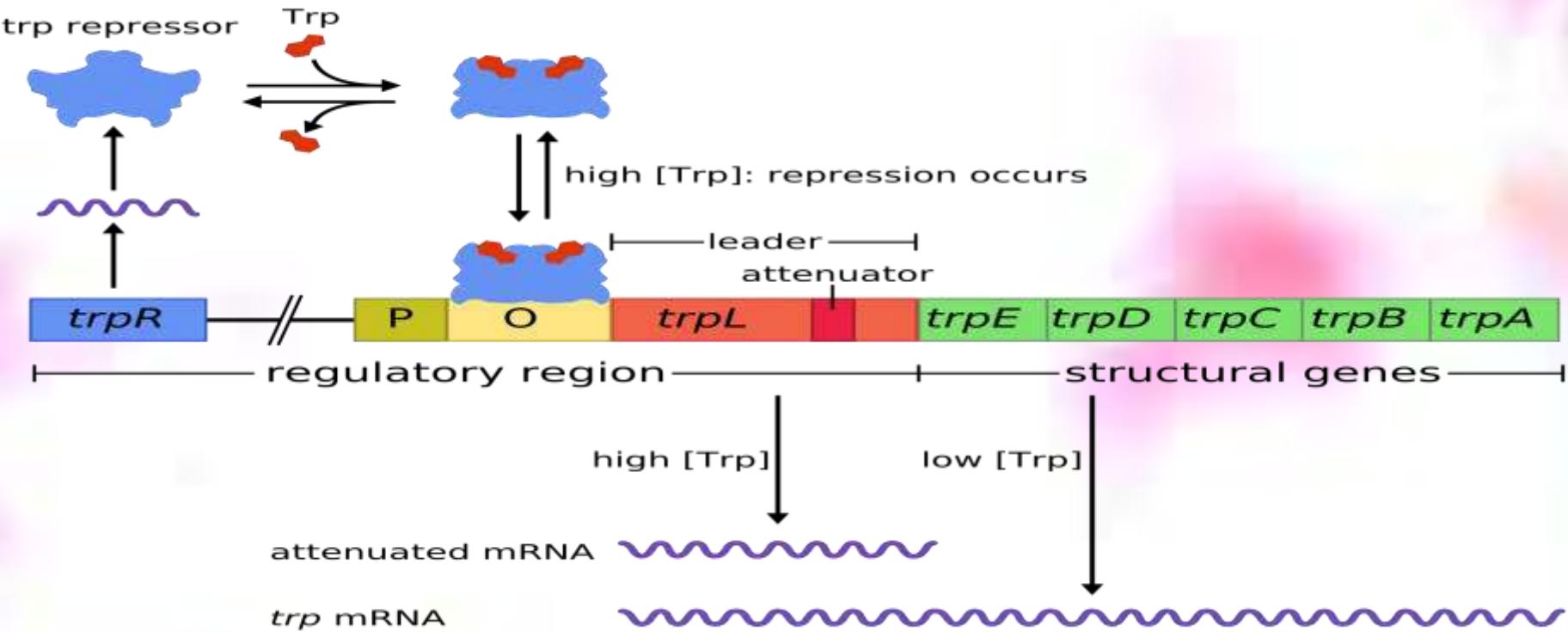
- To revise the concepts follow the links
- http://highered.mcgraw-hill.com/sites/0072507470/student_view0/chapter3/animation_mrna_synthesis_transcription_quiz_1_.html
- http://telstar.ote.cmu.edu/biology/animation/DnaTranscription/transcription_simple.html
- <http://bcs.whfreeman.com/thelifewire/content/chp12/1202001.html>

TRYPTOPHAN OPERON

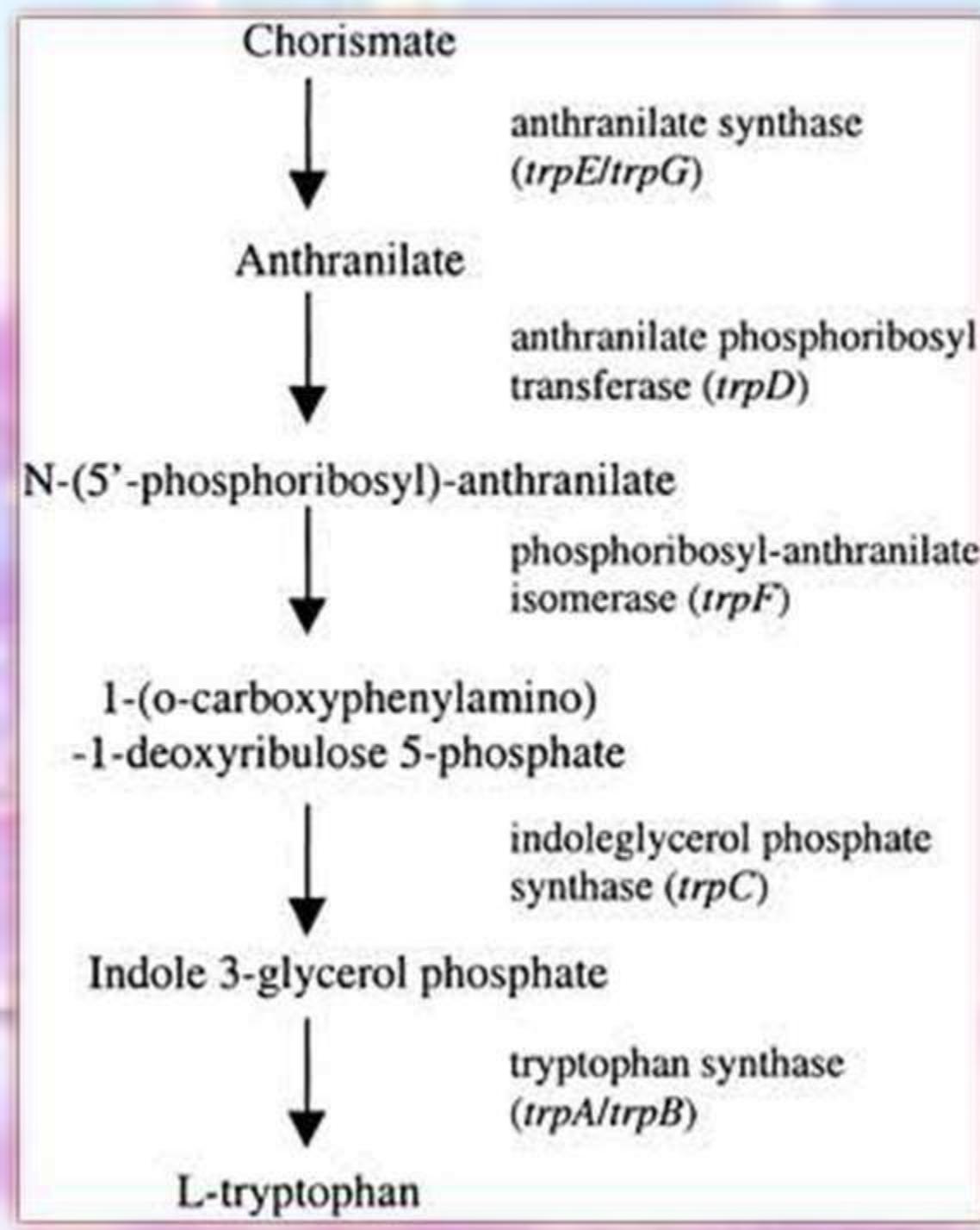
By
DEVI PRIYA SUGATHAN
MSC BIOCHEMISTRY AND
MOLECULAR BIOLOGY

The *trp* Operon

- The 20 common amino acids are required in large amount for protein synthesis, and *E.coli* can synthesize all of them.
- The genes for the enzymes needed to synthesize a given amino acid are generally clustered in an **operon** and are expressed whenever existing supplies of that amino acid are inadequate for cellular requirements.
- When the amino acid is abundant the biosynthetic enzymes are not needed and the operon is repressed.



- The *E.coli* tryptophan operon includes **five genes** for the enzymes required to convert **chorismate to tryptophan** .
- The mRNA from the tryptophan operon has a half life of only about **3 min**, allowing the cell to respond rapidly to changing needs for this amino acid.



- The biosynthesis of tryptophan by the enzymes encoded in the *trp* operon is diagrammed.

Regulatory Sequence

- This Operon is regulated by two mechanisms:
 - The repressor binds to its operator
 - The transcription of *trp* mRNA is attenuated.

The *trp* repressor

- The Trp repressor is a homodimer, each subunit containing 107 amino acid residues.
- When tryptophan is abundant it binds to the Trp repressor, causing a conformational change that permits the repressor to bind to the *trp* operator and inhibit expression of the *trp* operon.
- The *trp* operator site overlaps the promoter, so binding of the repressor blocks binding of the RNA polymerase.

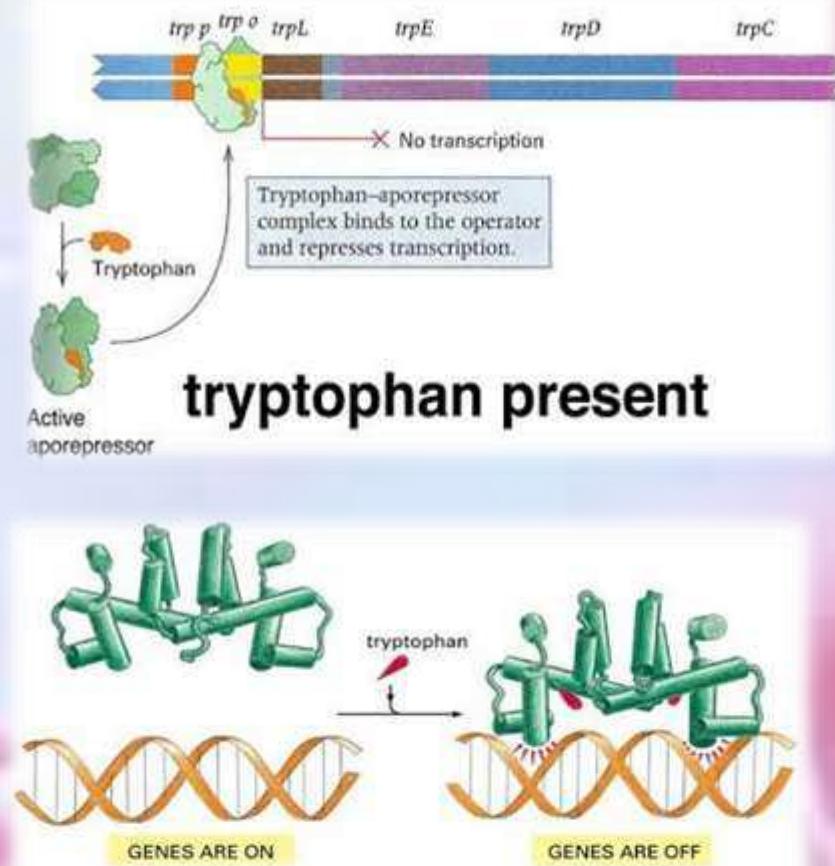


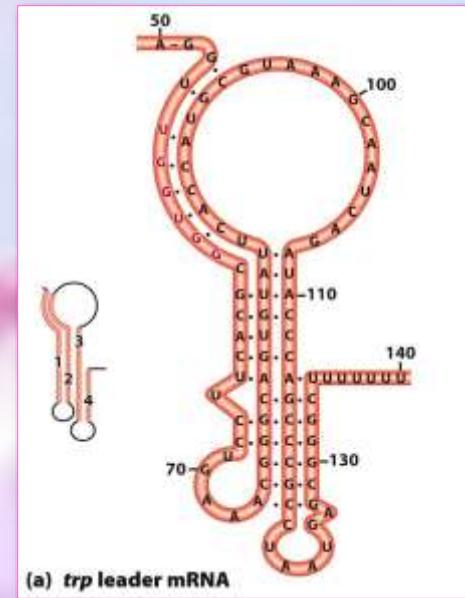
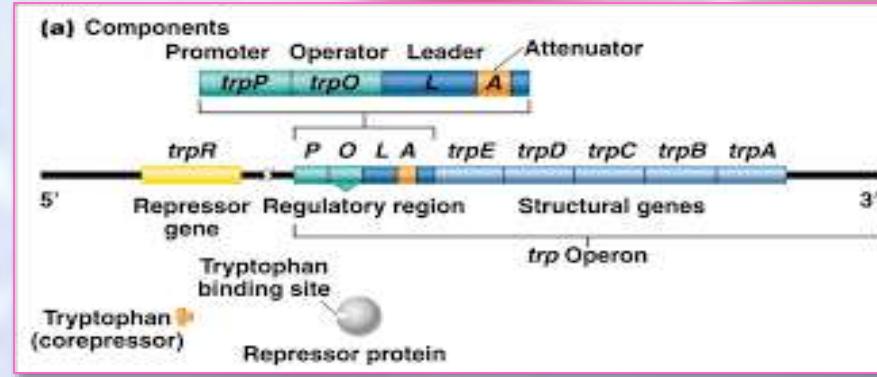
Figure 7-35. Molecular Biology of the Cell, 4th Edition.

The binding of tryptophan to the tryptophan repressor protein changes the conformation of the repressor. It is an helix-turn-helix protein.

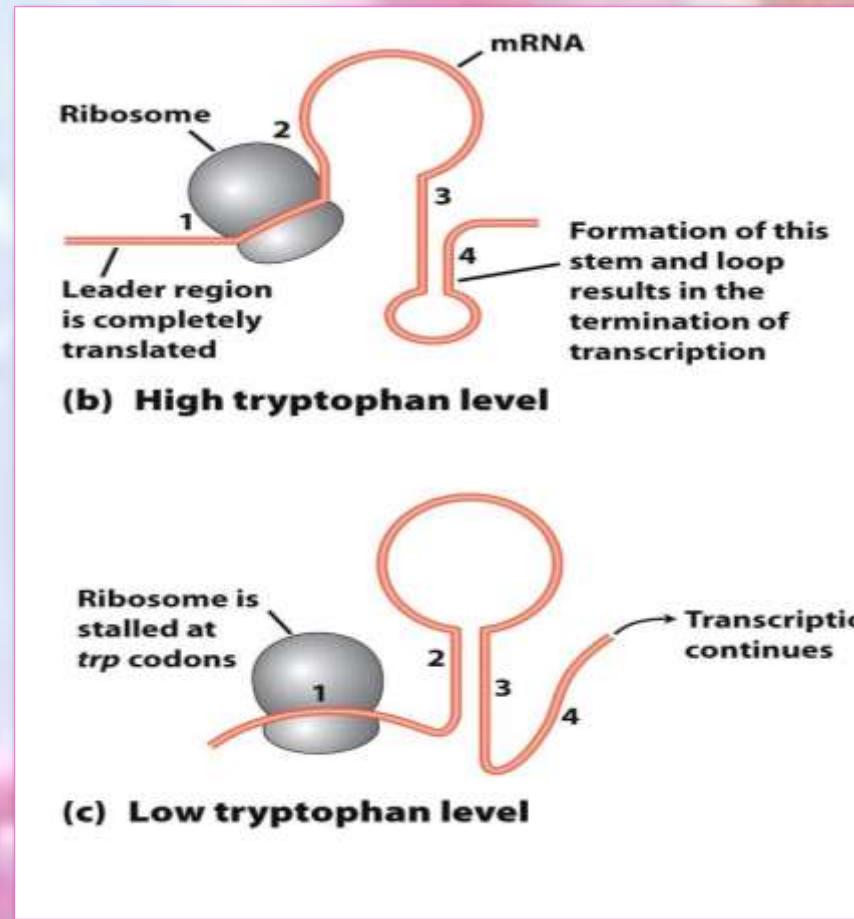
Transcriptional Attenuation

❑ It is a second regulatory process, in which transcription is initiated normally but is abruptly halted before the operon genes are transcribed.

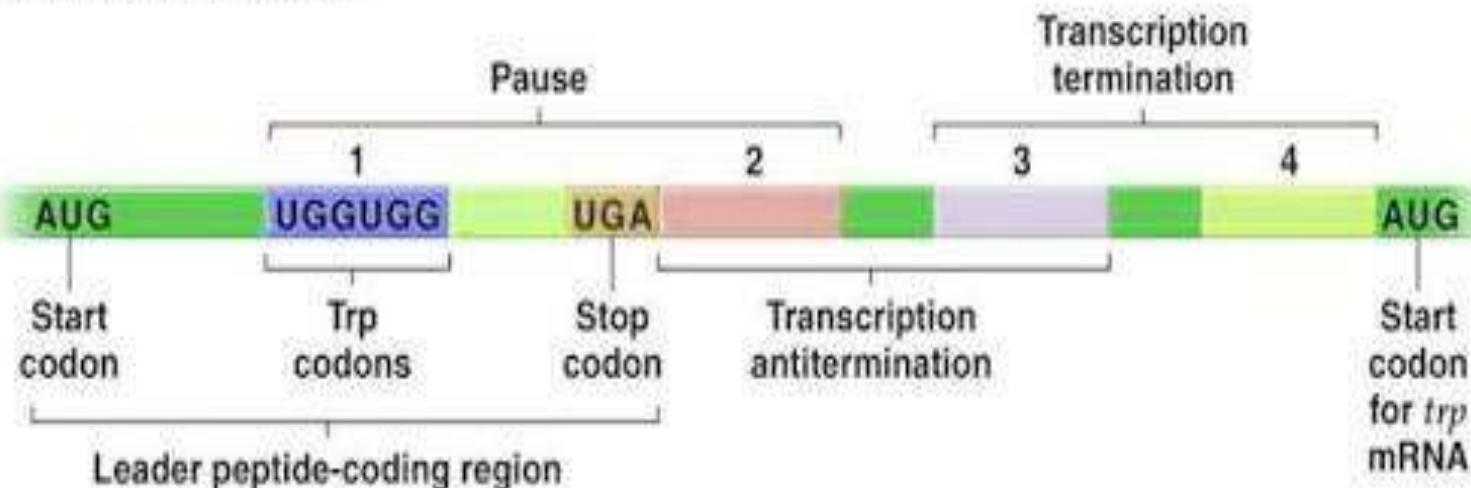
❑ The *trp* operon attenuation mechanism uses signals encoded in four sequences within a 162 nucleotide **leader region** at the 5' end of the mRNA, preceding the initiation codon of the first gene.



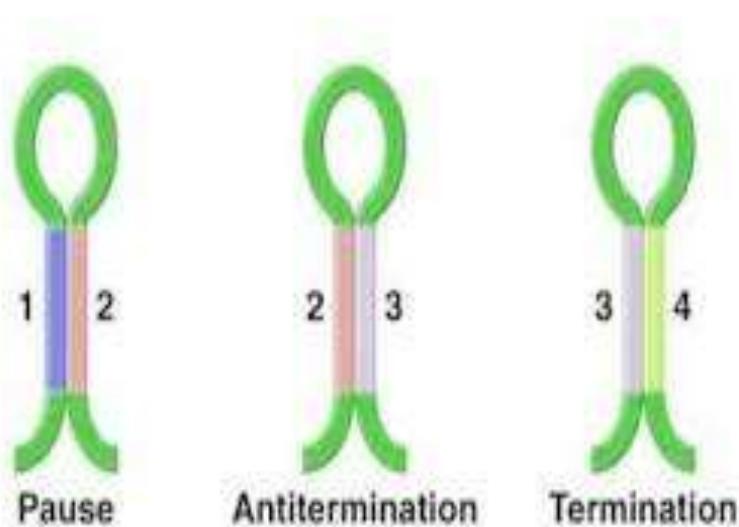
- Within the leader lies a region known as **attenuator**, made up of sequences **3** and **4**.
- These sequences base pair to form a G≡C rich stem and loop structure closely followed by a series of U residues.
- The attenuator structure act as a transcription terminator.
- If sequence 2 and 3 base pair the attenuator structure cannot form and transcription continues into the *trp* biosynthetic genes.



Organization of region:

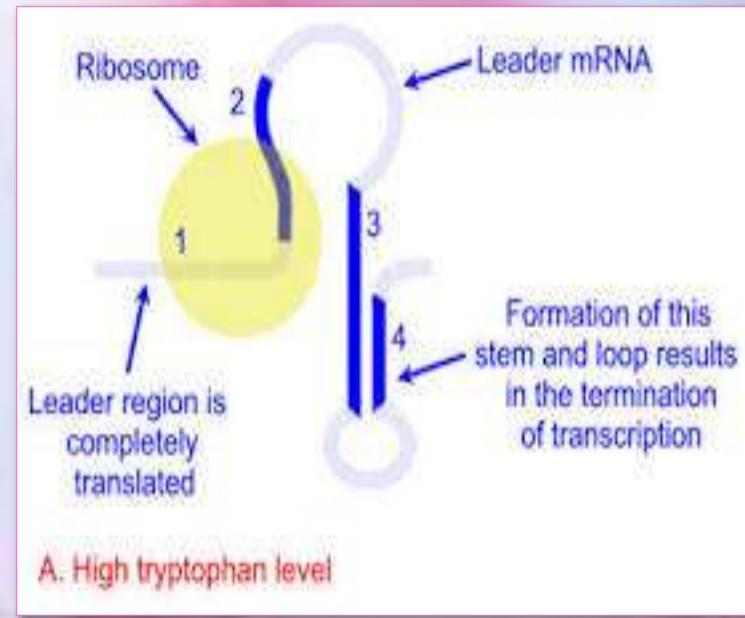


Alternative RNA structures:

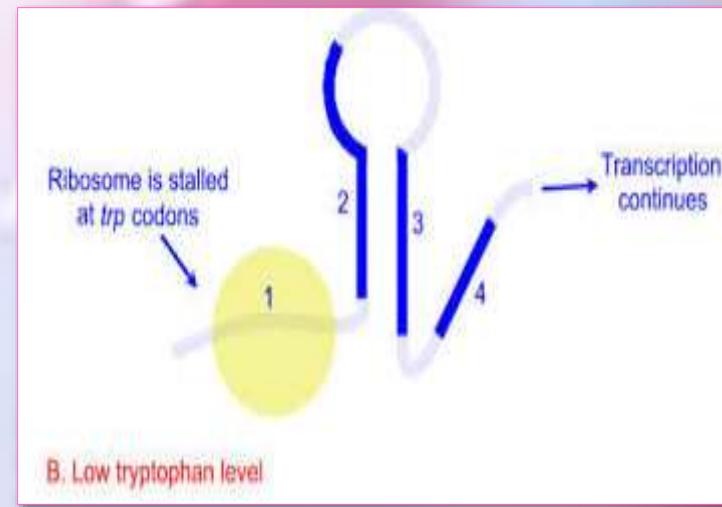


What mechanism determines whether sequence 3 pairs with sequence 2 or with sequence 4 ?

- When tryptophan concentrations are high, concentrations of charged tryptophan tRNA are also high. This allows translation to proceed rapidly past the two trp codons of sequence 1 and into sequence 2, before sequence 3 is synthesized by RNA polymerase.
- In this situation, sequence 2 is covered by the ribosome , and unavailable for paring to sequence 3 when sequence 3 is synthesized; the attenuator structure sequence 3 and 4 forms and transcription halts.



- When tryptophan concentrations are low, the ribosome stalls at the two Trp codons in sequence 1 because the charged tryptophan tRNA is unavailable.
- Sequence 2 remains free while sequence 3 is synthesized, allowing these two sequences to base pair and permitting transcription to proceed.



Summary