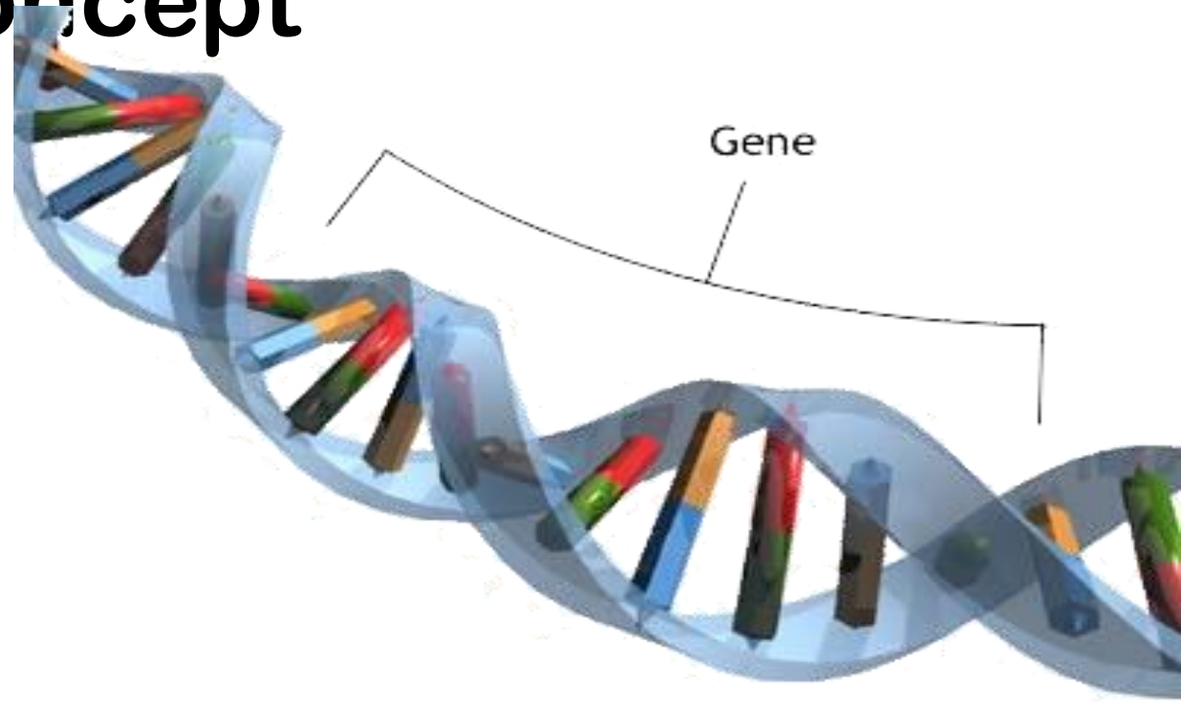
Molecular Genetics, DNA And Gene Concept



MOLECULAR GENETICS

- **Molecular genetics is the study of genetic makeup of individuals at the DNA level.**
- It is the identification and mapping of genes and genetic polymorphism.
- It employs the methods of genetics and molecular biology to elucidate molecular function and interactions among genes.

Advantages of Molecular Approach over Traditional Approach

- Assuming no genotyping errors, molecular genetic information is not affected by environmental effects and therefore has heritability equal to 1.
- Molecular genetic information can be available at an early age, in principle at the embryo stage, thereby allowing early selection and reduction of generation intervals.
- Molecular genetic information can be obtained on all selection candidates, which is especially beneficial for sex-limited traits, traits that are expensive or difficult to record, or traits that require slaughter of the animal (carcass traits).
- Molecular approach helps us to select for a wide range of traits which in turn saves time and efforts.
- Molecular genetic information enhances reliability in predicting the mature phenotype of the individual.

Need for Molecular Genetics:

- a way to select breeding animal at an early age (even embryos);
- to select for a wide range of traits and to enhance reliability in predicting the mature phenotype of the individual.
- The broad categories of existing gene based options include; molecular analysis of genetic diversity,
- animal identification and traceability,
- reproductive enhancement;
- transgenic Livestock; germ line Manipulation;
- gene based trait selection;
- animal health: diagnosis, protection and treatment
- ruminant and non-ruminant nutrition and metabolism

Nucleic Acid, DNA, Gene

Mendel Based on his experiments-

- “The particles/ factors that determine the traits are transmitted in an unaltered way from generation to generation”
- Trait/ character is not transmitted but the factors/ particles controlling them are transmitted.
- The Combination of two factors coming from both the parents determine the characteristic of the progeny.

Particulate theory of Inheritance

- This is the beginning for the concept of a gene

  [Gene = Factor/ Particle/ hereditary unit]

William Bateson (1898)

- Studied principles of inheritance in animals.
- Translated Mendel's papers in English.
- Coined the terms- Genetics, Homozygote, Heterozygote, Allelomorphs (Alleles).

Chromosomal Theory of Inheritance

- 1881- Chromosomes were observed
- 1902- Sutton and Bovery postulated that hereditary factors were physically located on chromosomes.
- Gene is part of chromosome.
- Bridges provided the experimental proof for chromosomal theory of Inheritance.
- Sturtvant produced worlds first chromosome map.

Molecular concept

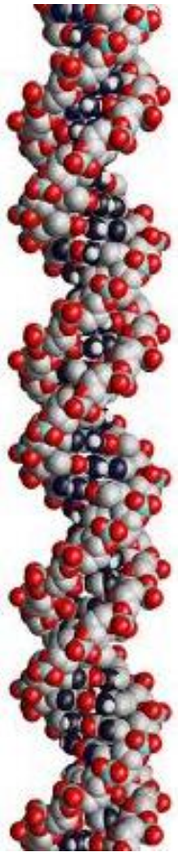
- DNA structure was discovered in 1950’s
  - But,
- 1860’s Microscopy was refined, cell and its components were under intense study
- 1866- Earnest Hackel proposed that nucleus contains factors necessary for heredity.

- Late 1860’s- Friedrich Miescher, studied heman pus cells
  - 1869-Mr.Fridrech studied WBC,discovered new term, Nuclein
  - He characterized proteins and isolated high amount of a typical substance from nucleus

Frederick Giffith, 1928

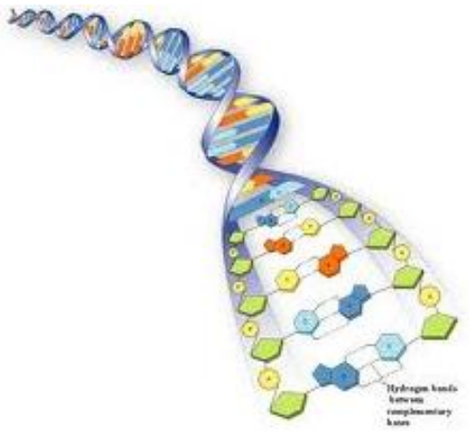
- Studied Streptococcus pneumoniae in mice.
  - Two types of bacteria smooth (S) and rough (R)
    - S injected in mice – the mice dies
    - R injected in mice – the mice survives
    - Heat killed S injected in mice – the mice survives
    - Heat killed S + R injected in mice – the mice dies

Avery, Macleod and McCarty 1944

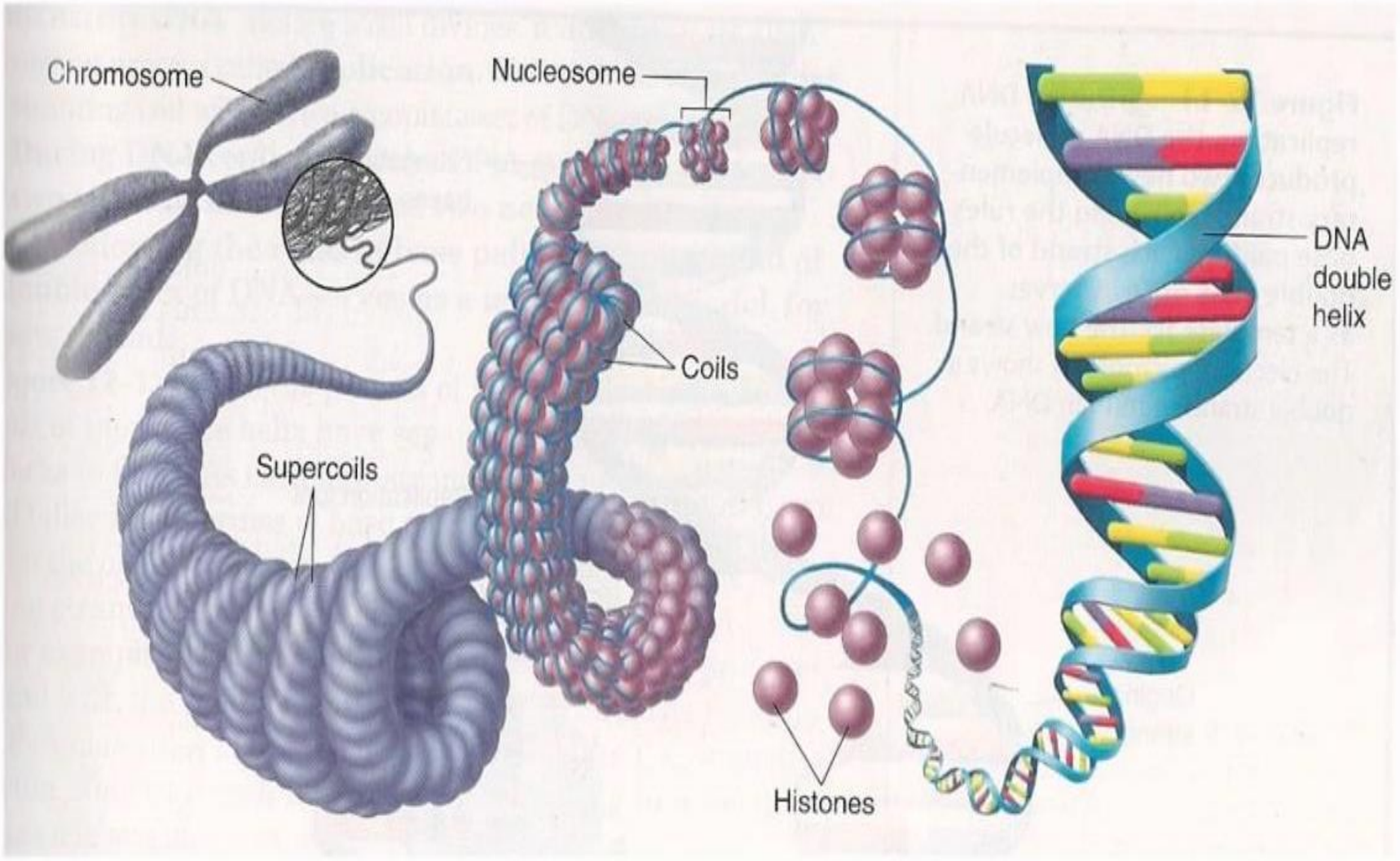


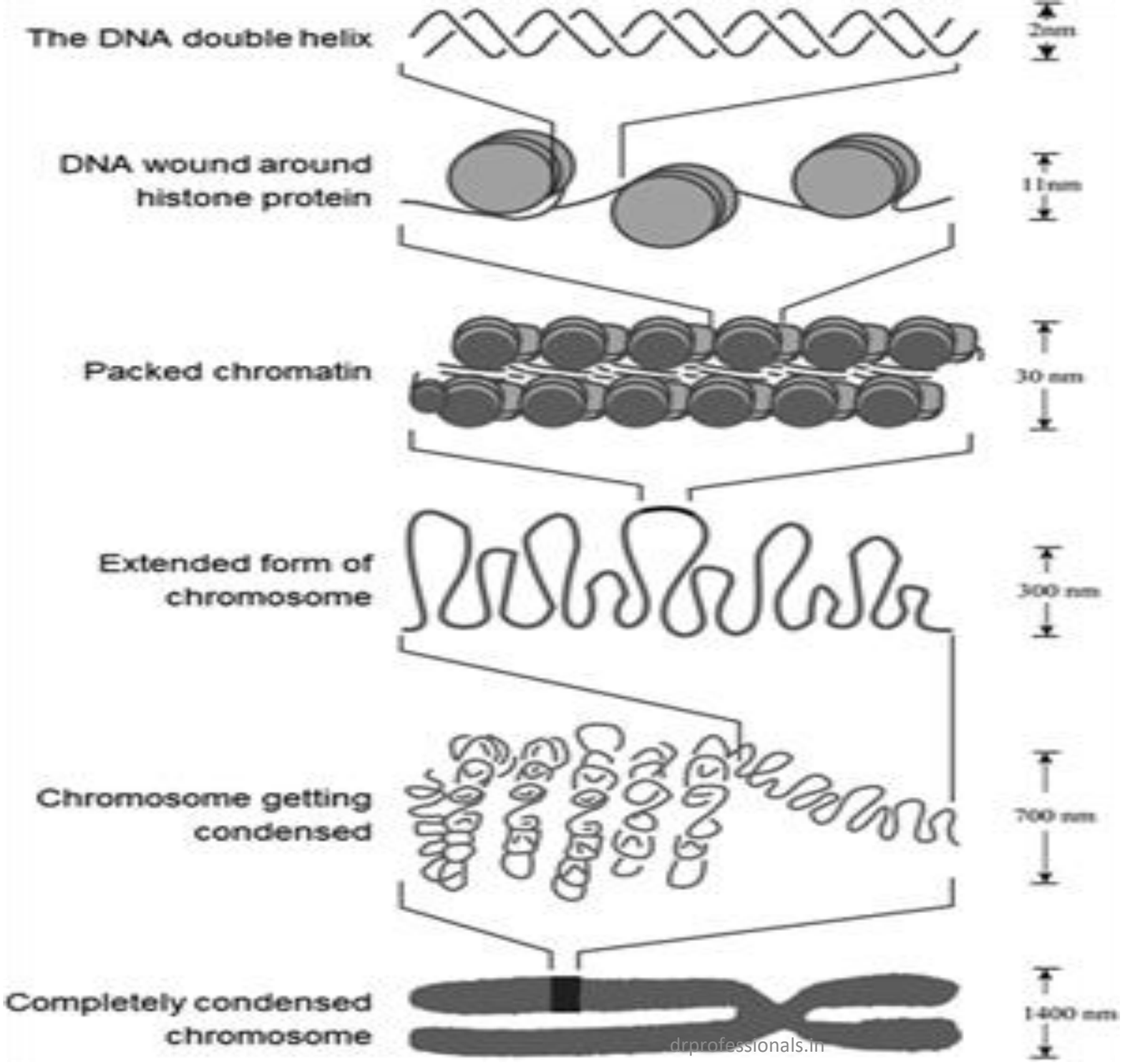
- Purified the substance observed in the heat killed S bacteria and observed that is DNA.
- Rosalind Franklin used X-ray technique found helical structure of DNA with sugar & phosphate group forming double helix

3)1953-James Watson & Francis Crick described double helix model for structure of DNA



Eukaryotic Chromosome Structure





Nucleic Acid,

- - Nucleic Acids: A group of macromolecules that:
  - a. have acidic properties
  - b. located in the nucleus
  - c. are polymers of nucleotides

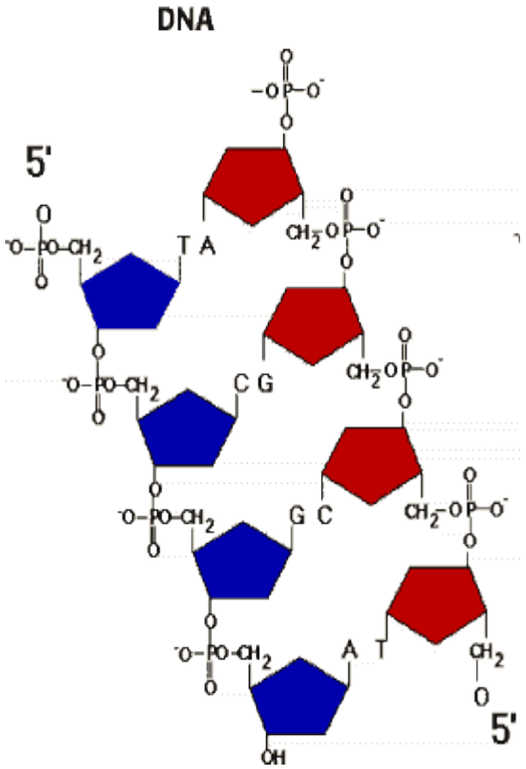
- - Required for the storage and expression of genetic information\

- - There are two classes of nucleic acid:
  - a. Deoxyribonucleic acid (DNA)
  - b. Ribonucleic acid (RNA)

- - Nucleotide: A nucleotide consists of three parts:
  - a. A nitrogenous base
  - b. A sugar
  - c. One or more phosphate

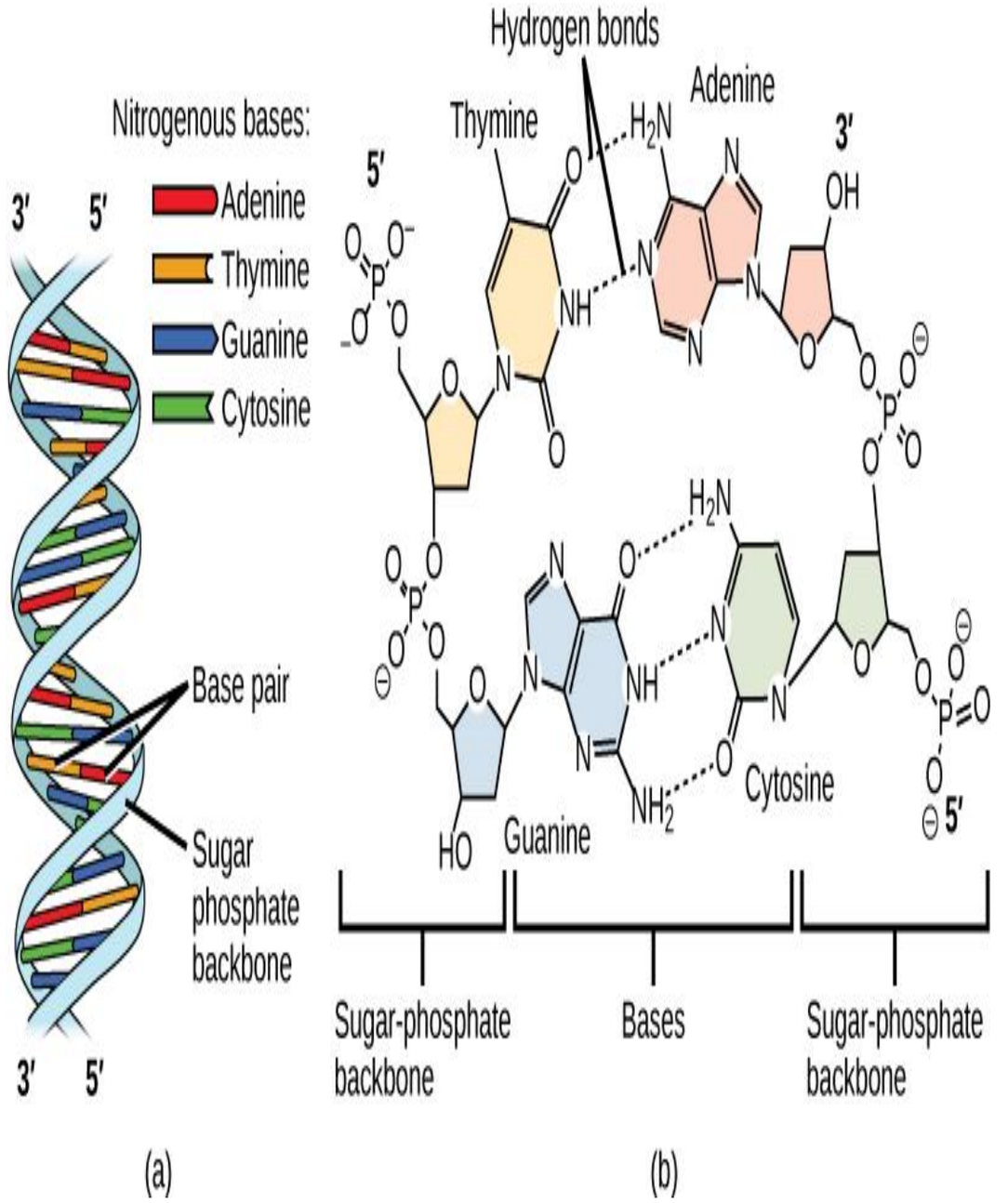
Nucleic Acids

- Sequence of Nucleotides
- Nucleotide composed of:
  - Nitrogenous Base
    - Purine
    - Pyrimidine
  - Sugar
    - Ribose
    - Deoxyribose
  - Phosphate



DNA

- Deoxyribonucleic Acid
- 4 Bases
  - Purines
    - Adenine
    - Guanine
  - Pyrimidines
    - Cytosine
    - Thymine*
- Sugar is Deoxyribose



DNA

- **Types of DNA**:-

1. Nuclear DNA-Located in cell nucleus, genetic material from both parents
2. Mitochondrial DNA-found in mitochondria, genetic material only from mother

- Base + sugar → Nucleoside
- Base + sugar + phosphates → Nucleotide

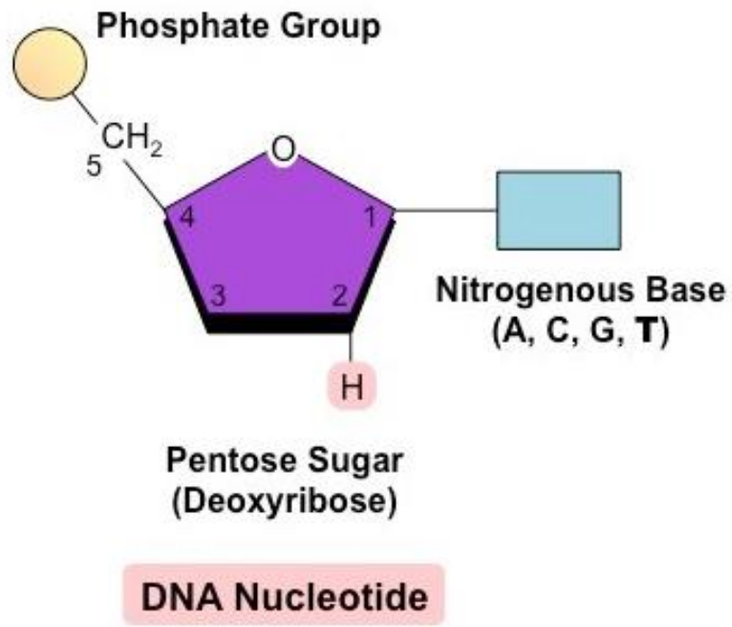
NUCLEOTIDE STRUCTURE

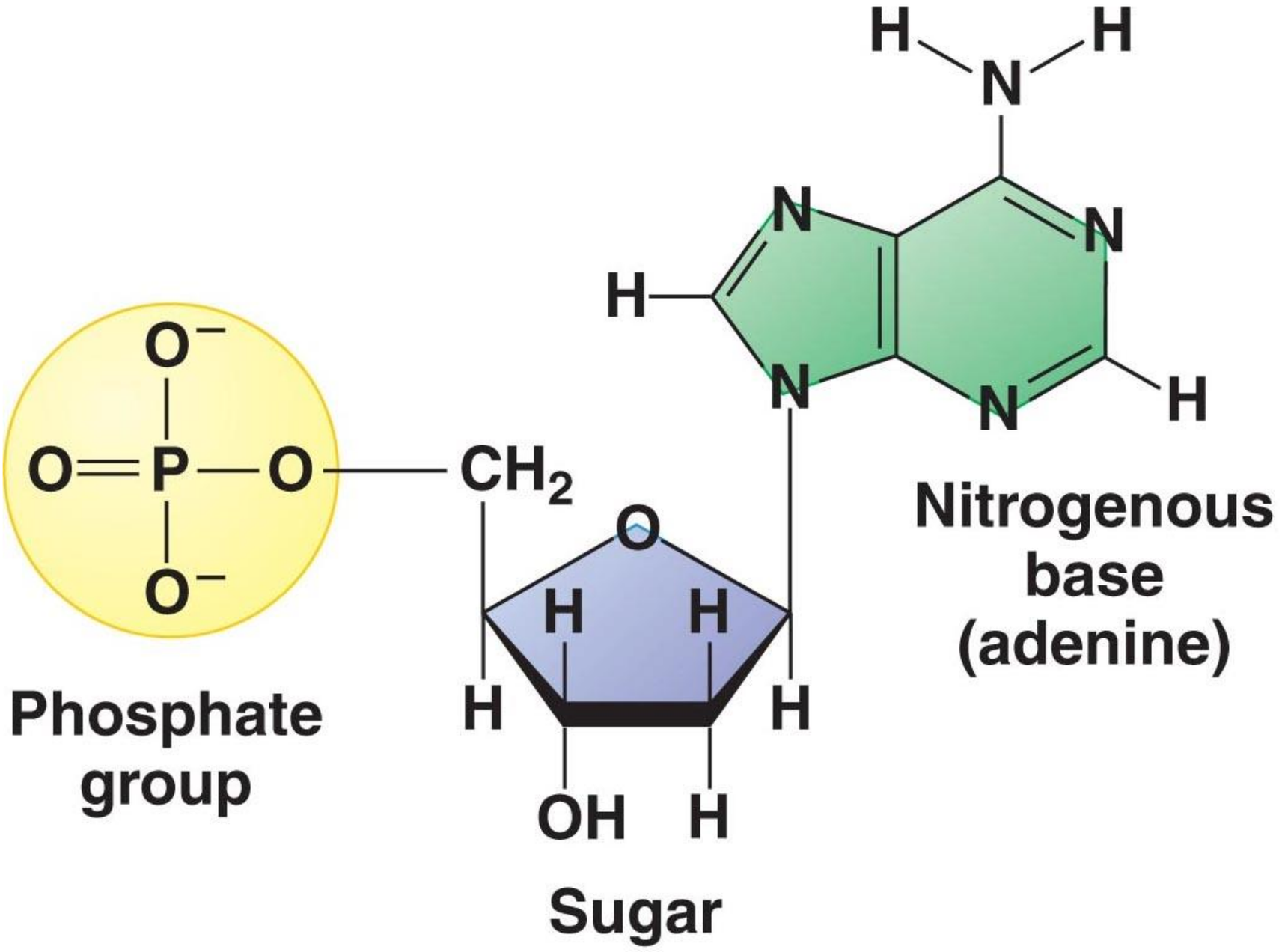
- Three main components:-

1) Condensation of Deoxyribose-pentose sugar

2) Nitrogenous bases

3) Phosphate group

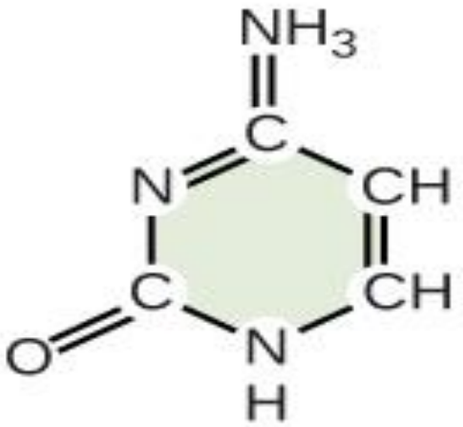




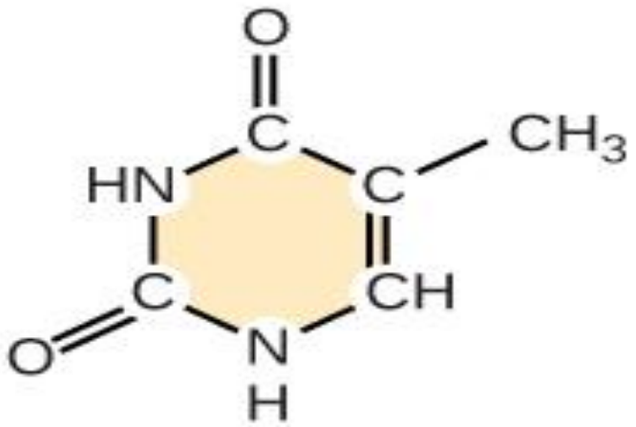
DNA STRUCTURE

- Right handed double helix,10 nucleotide pairs per helical turn
- Each spiral strand composed of sugar phosphate backbone & attached bases, is connected to a complementary strand by hydrogen bonding between paired bases,
- Adenine(A) with thymine(T)
- Guanine(G) with cytosine(C)

Pyrimidines

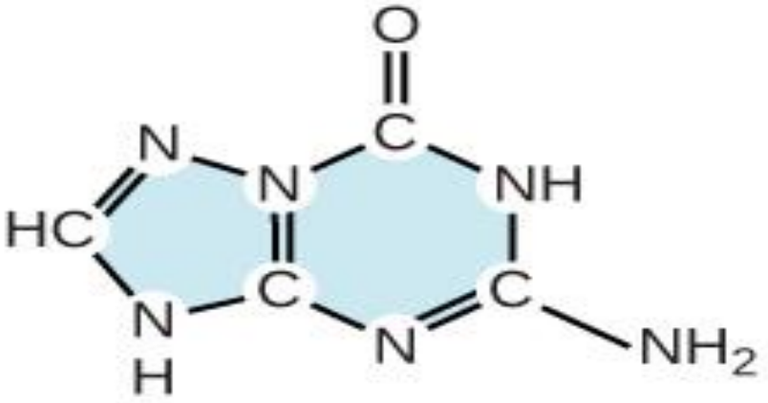


Cytosine
C

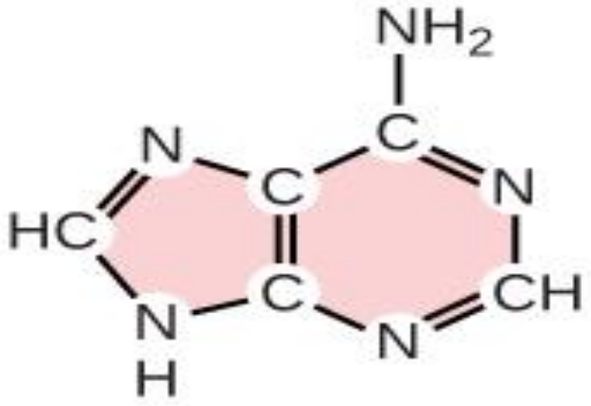


Thymine
T

Purines

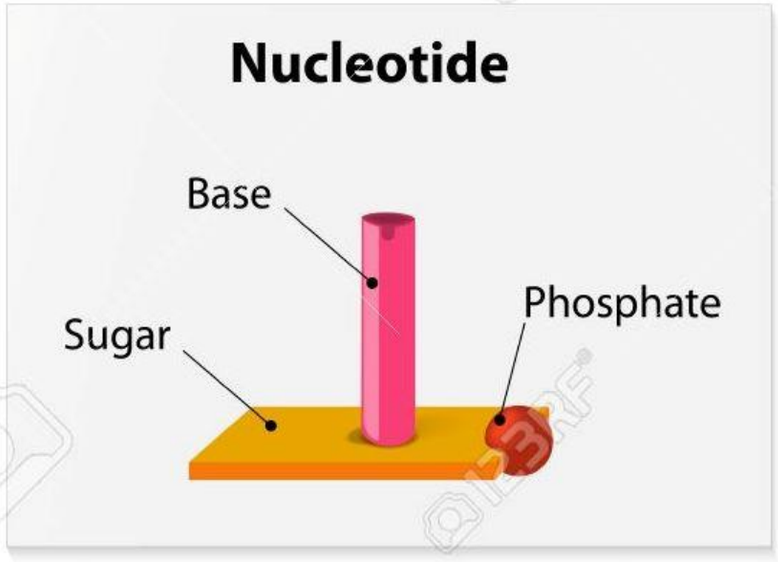


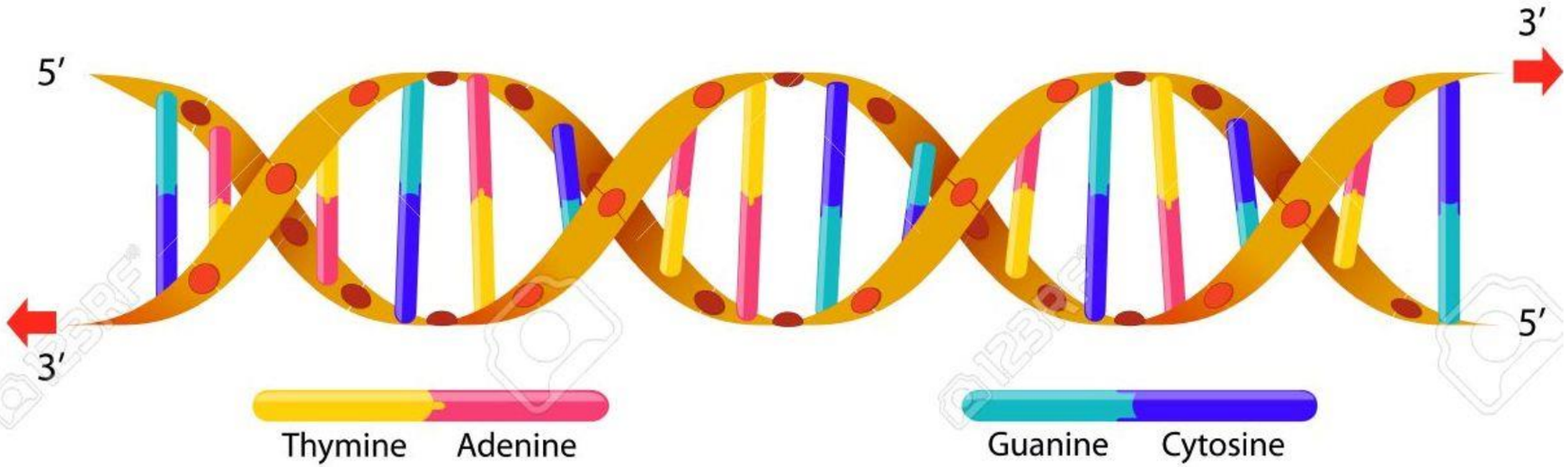
Guanine
G



Adenine
A

DNA structure





DOUBLE HELIX STRUCTURE OF DNA

Determination of double helix structure of DNA was based on X-ray diffraction pattern

Consists of two polynucleotides chain i.e.,DNA strand that wrapped to each other to form a helix chain runs in antiparallel direction

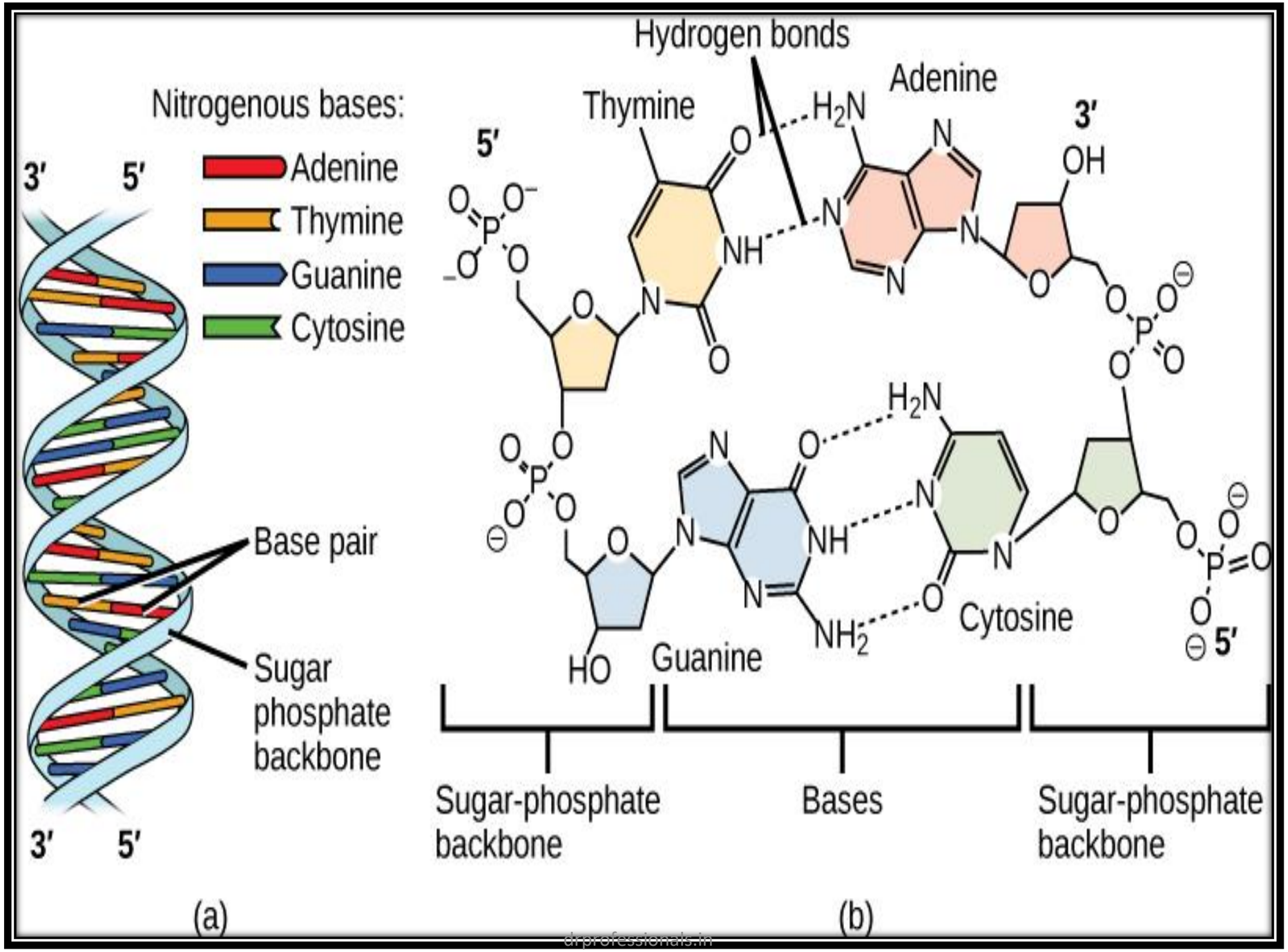
- 5’ to 3’ – sense strand
- 3’ to 5’ – antisense strand

Base pair is complementary

- A-T -2H bond
- G-C -3H bond

DNA double helix & hydrogen bonding

- There are two asymmetrical grooves on the outside of the helix- a)major groove b)minor groove
- **Groove –**any furrow(slight depression in the smoothness of a surface)or channel on a bodily structure or a part
- Certain proteins can bind within these groove
- They can interact with a particular sequence of bases
- The width of the major groove means that the edges of bases are more accessible in the major groove than in the minor groove

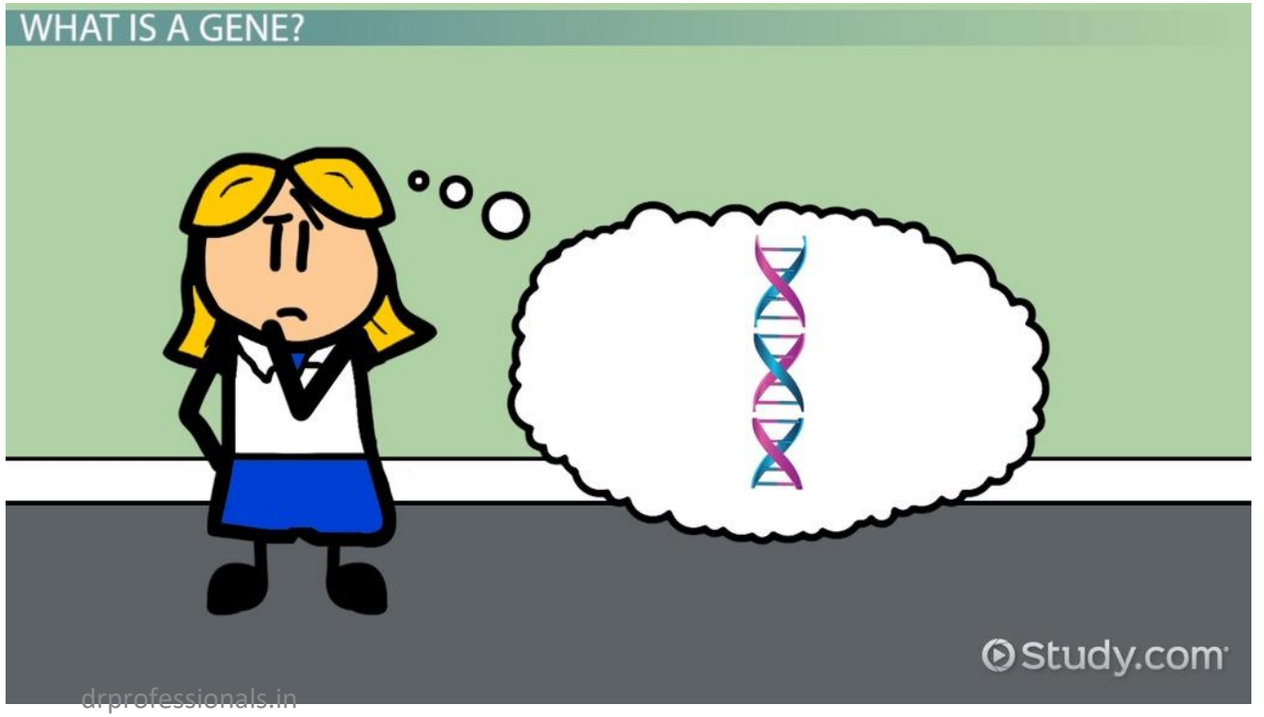


RNA

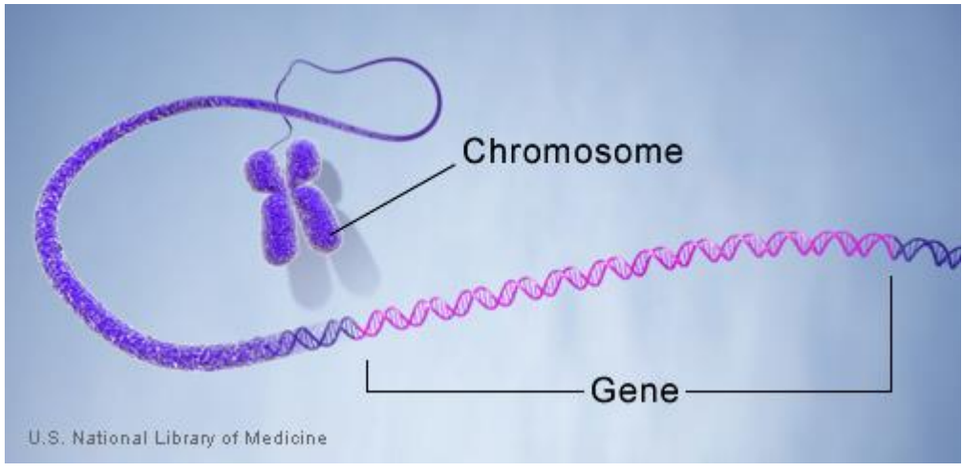
- Ribonucleic Acid
- 4 Nucleotides
  - Purine
    - Adenine
    - Guanine
  - Pyrimidines
    - Cytocine
    - Uracil*
- Sugar is Ribose

Genes

- Genes are the basic physical and functional units of heredity. Each gene is located on a particular region of a chromosome and has a specific ordered sequence of nucleotides (the building blocks of DNA).



GENES



- **A gene**: DNA sequence that is needed to encode amino acid sequence of a protein
- Composed of exons, introns and different control elements
- Exon – protein coding sequence
- Intron – intervening sequence

- Genes vary a lot in size:

  Humans: average 3000bp

  largest 2.4 million bp

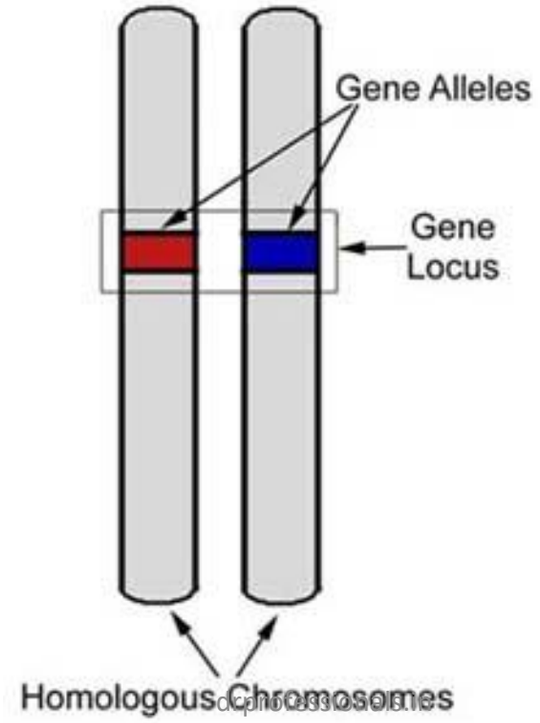
- Genes are separated by sequences with unknown function

- Only one strand of the DNA carries biological information → **template strand/ Sense / codding strand**

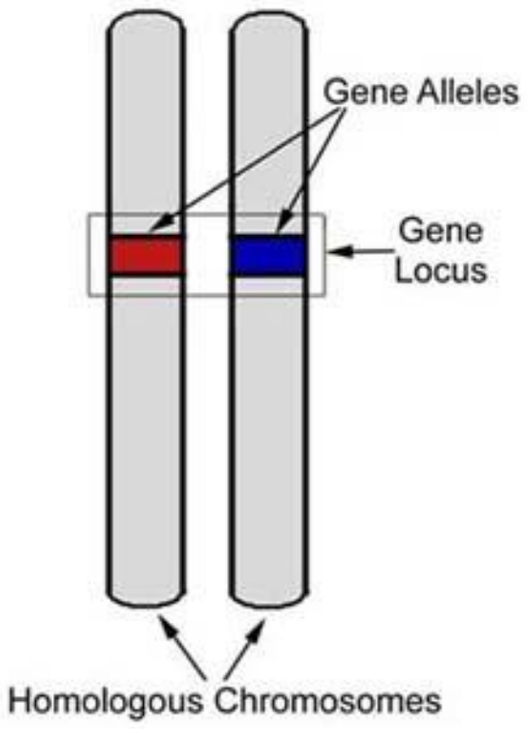
- Potential to store biological information is enormous

ALLELES

- An allele is variant form of a gene.
- The different forms of a segment of DNA that exist at an particular site in a chromosome.



What is a locus?



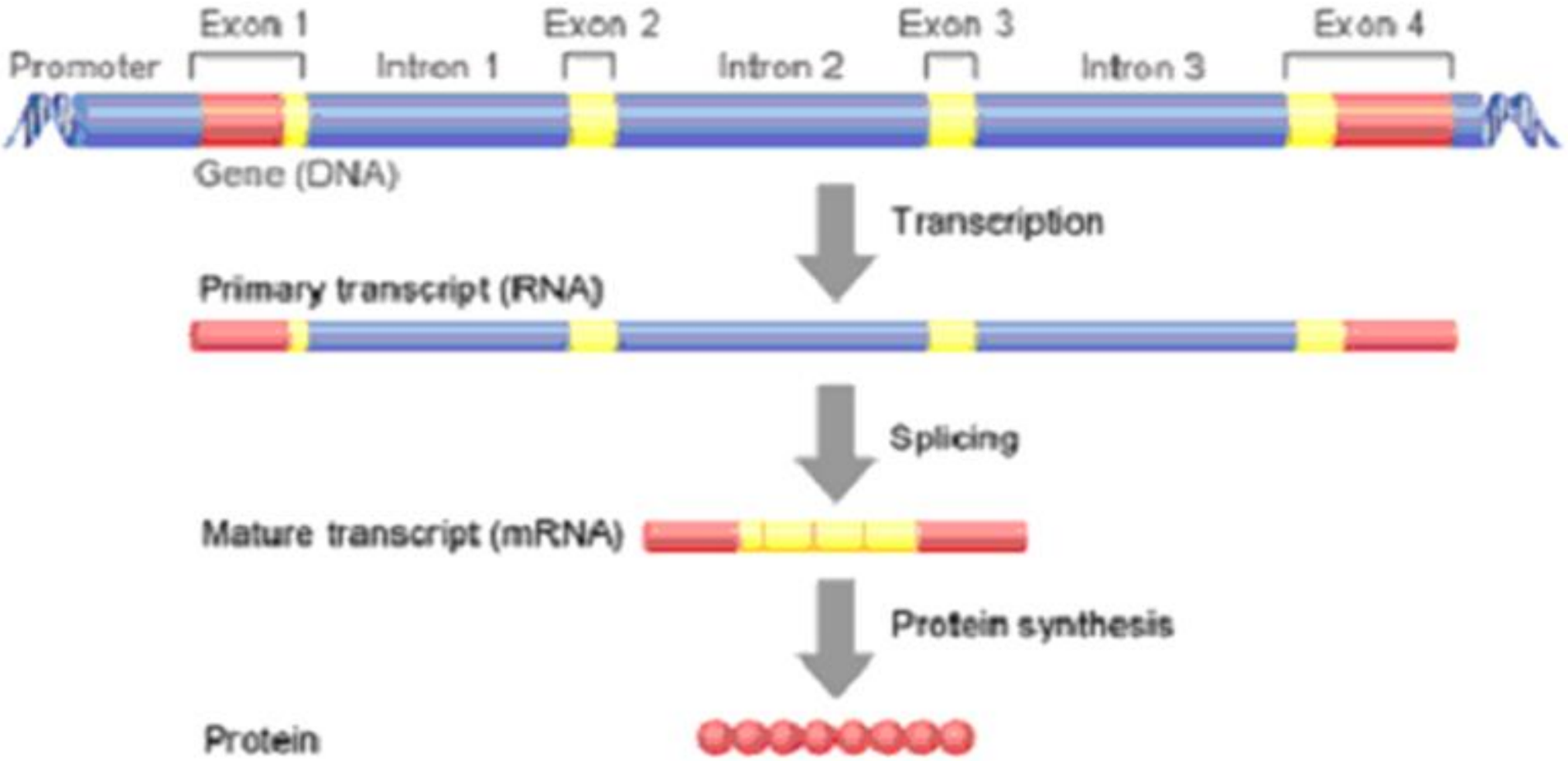
- A locus describes the region of a chromosome where a gene is located. **11p15.5** is the locus for the **human insulin gene**. **11** is the chromosome number, **p** indicates the short arm of the chromosome, and **15.5** is the number assigned to a particular region on a chromosome. When chromosomes are stained in the lab, light and dark bands appear, and each band is numbered. The higher the number, the farther away the band is from the centromere.

Gene Structure

- Eukaryotic gene structure:

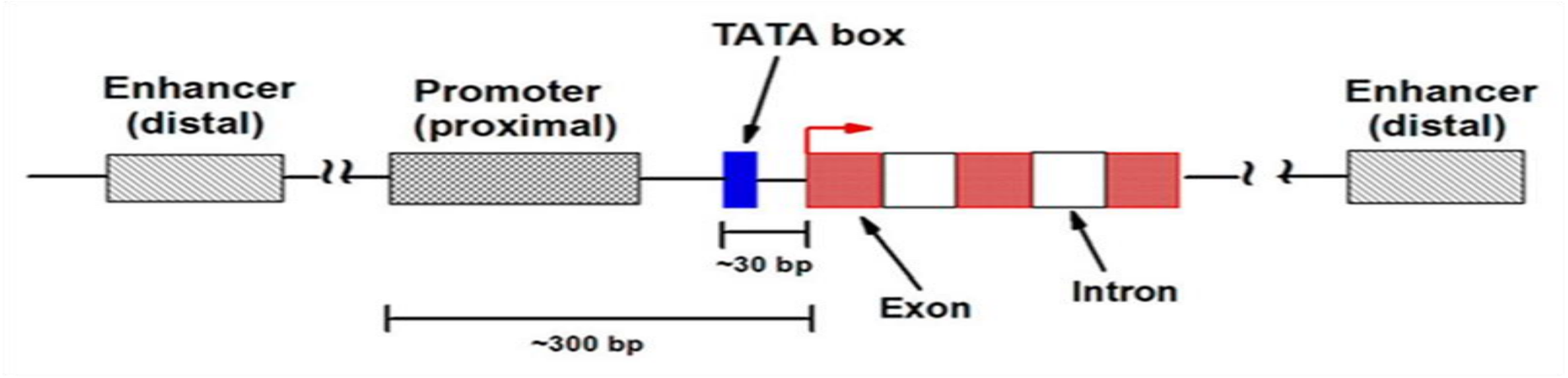
- Most eukaryotic genes in contrast to typical bacterial genes, the coding sequences (exons) are interrupted by noncoding DNA (introns).
- The gene must have (Exon; start signals; stop signals; regulatory control elements).
- The average gene 7-10 exons spread over 10-16kb of DNA.

Structure of a Gene



Enhancers

Enhancers are stretches of bases within DNA, about 50 to 150 base pairs in length; the activities of many promoters are greatly increased by **enhancers** which can exert their stimulatory actions over distances of several thousands base pairs.



Exons and Coding

- **What's the difference between exons and coding sequence?**
- Exons often are described as short segments of protein coding sequence. This is a bit of an oversimplification.
- Exons are those segments of sequence that are spliced together after the introns have been removed from the pre-mRNA. Yes, the coding sequence is contained in exons, but it is possible for some exons to contain no coding sequence.
- Portions of exons or even entire exons may contain sequence that is not translated into amino acids. These are the untranslated regions or UTRs. UTRs are found upstream and downstream of the protein-coding sequence.

- In G_1 phase of the cell cycle, many of the DNA replication regulatory processes are initiated.
- In eukaryotes, the vast majority of DNA synthesis occurs during S phase of the cell cycle, and the entire genome must be unwound and duplicated to form two daughter copies.
- During G_2, any damaged DNA or replication errors are corrected.
- Finally, one copy of the genomes is segregated to each daughter cell at mitosis or M phase.
- These daughter copies each contain one strand from the parental duplex DNA and one nascent antiparallel strand.

DNA replication

- **Eukaryotic DNA replication** is a conserved mechanism that restricts DNA replication to once per cell cycle.
- Eukaryotic DNA replication of chromosomal DNA is central for the duplication of a cell and is necessary for the maintenance of the eukaryotic genome.
- DNA replication is the action of DNA polymerases synthesizing a DNA strand complementary to the original template strand.
- To synthesize DNA, the double-stranded DNA is unwound by DNA helicases ahead of polymerases, forming a replication fork containing two single-stranded templates.
- Replication processes permit the copying of a single DNA double helix into two DNA helices, which are divided into the daughter cells at mitosis.

Steps of DNA replication

- Initiation
- Elongation
- Termination

I. Initiation

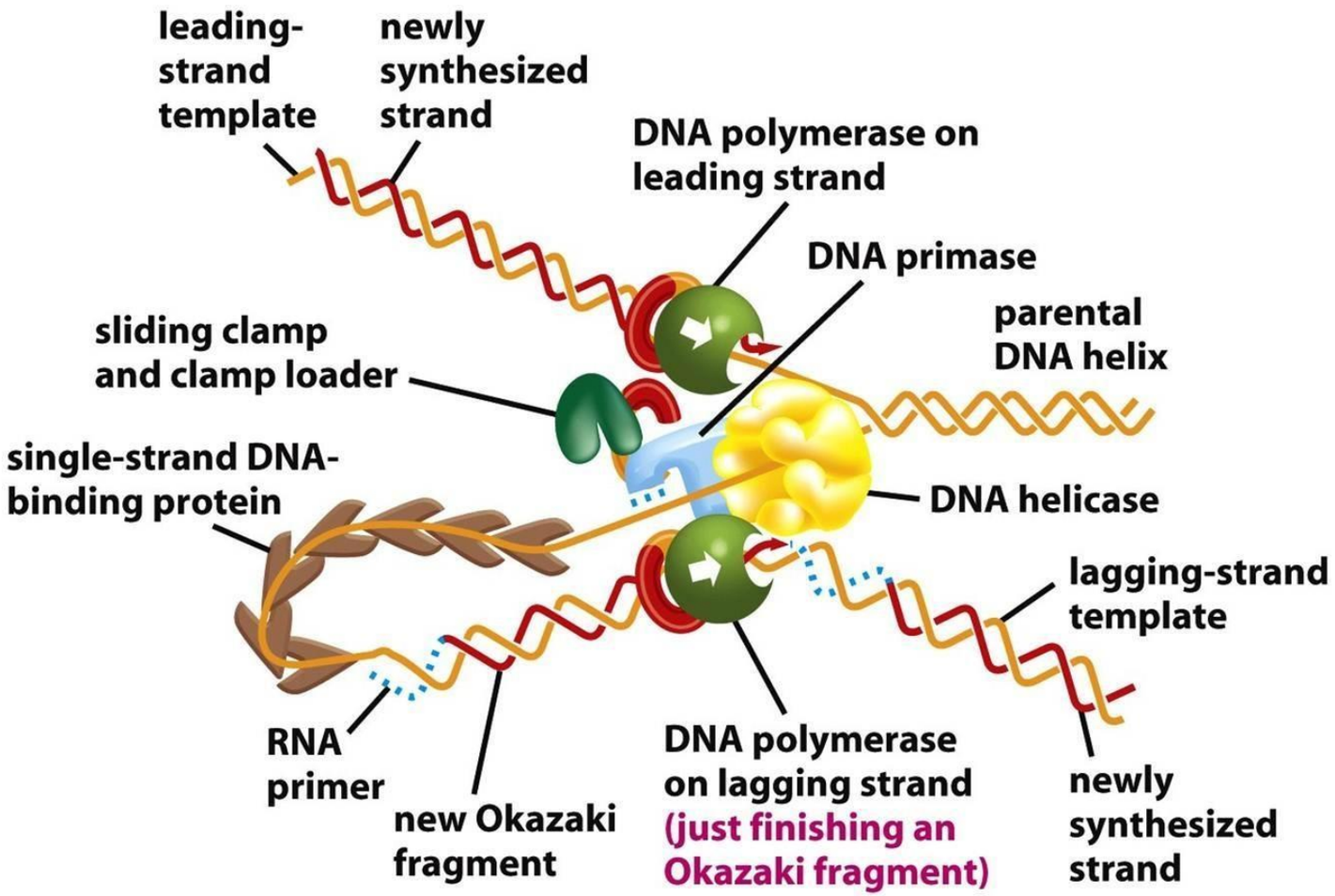
- First stage of DNA synthesis where the DNA double helix is unwound and an initial priming event by DNA polymerase α occurs on the leading strand.
- The priming event on the lagging strand establishes a replication fork.
- Priming of the DNA helix consists of synthesis of an RNA primer to allow DNA synthesis by DNA polymerase α.
- Priming occurs once at the origin on the leading strand and at the start of each Okazaki fragment on the lagging strand.
- DNA replication is initiated from specific sequences called origins of replication ("oriC"), and eukaryotic cells have multiple replication origins.

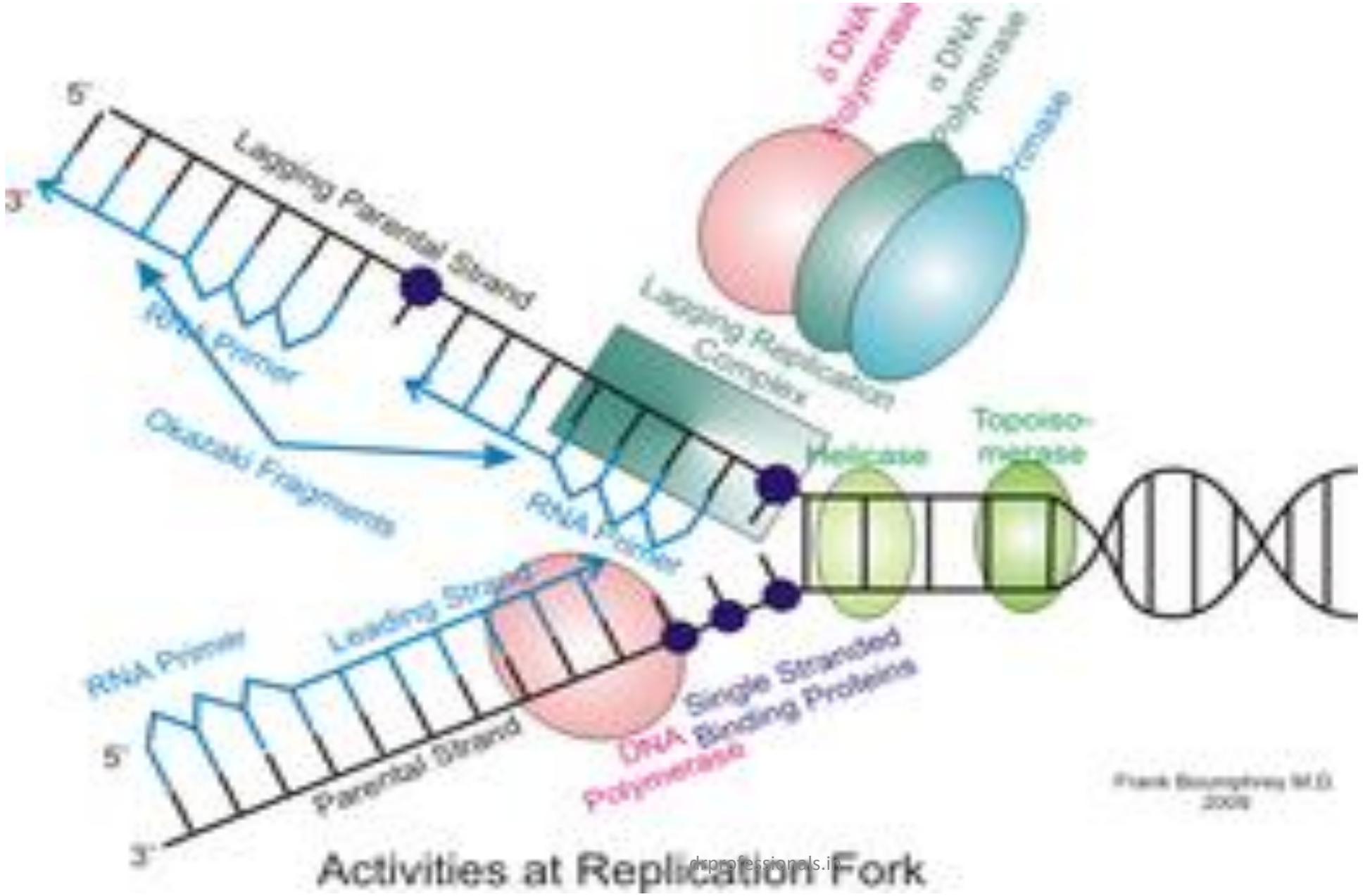
Location – “oriC” at which initiator proteins bind & trigger unwinding.

- Helicase enzyme : Separates the double helix and Single Strand Binding Protein (SSB) keeps strands separate.
- Entire DNA unwound producing segments of Single Stranded DNA

which can be copied by enzyme DNA polymerase.

- Unwinding of begins at a distinct position called as replication origin
- It is gradually progress along with molecule in both directions.
- replication origin at weak A-T rich regions.
- Replication Fork : Region where helix unwinds and new DNA synthesized.
- The cell prepares the next step, elongation, by creating short sequences of RNA called primers that provides starting point of elongation





Activities at Replication Fork

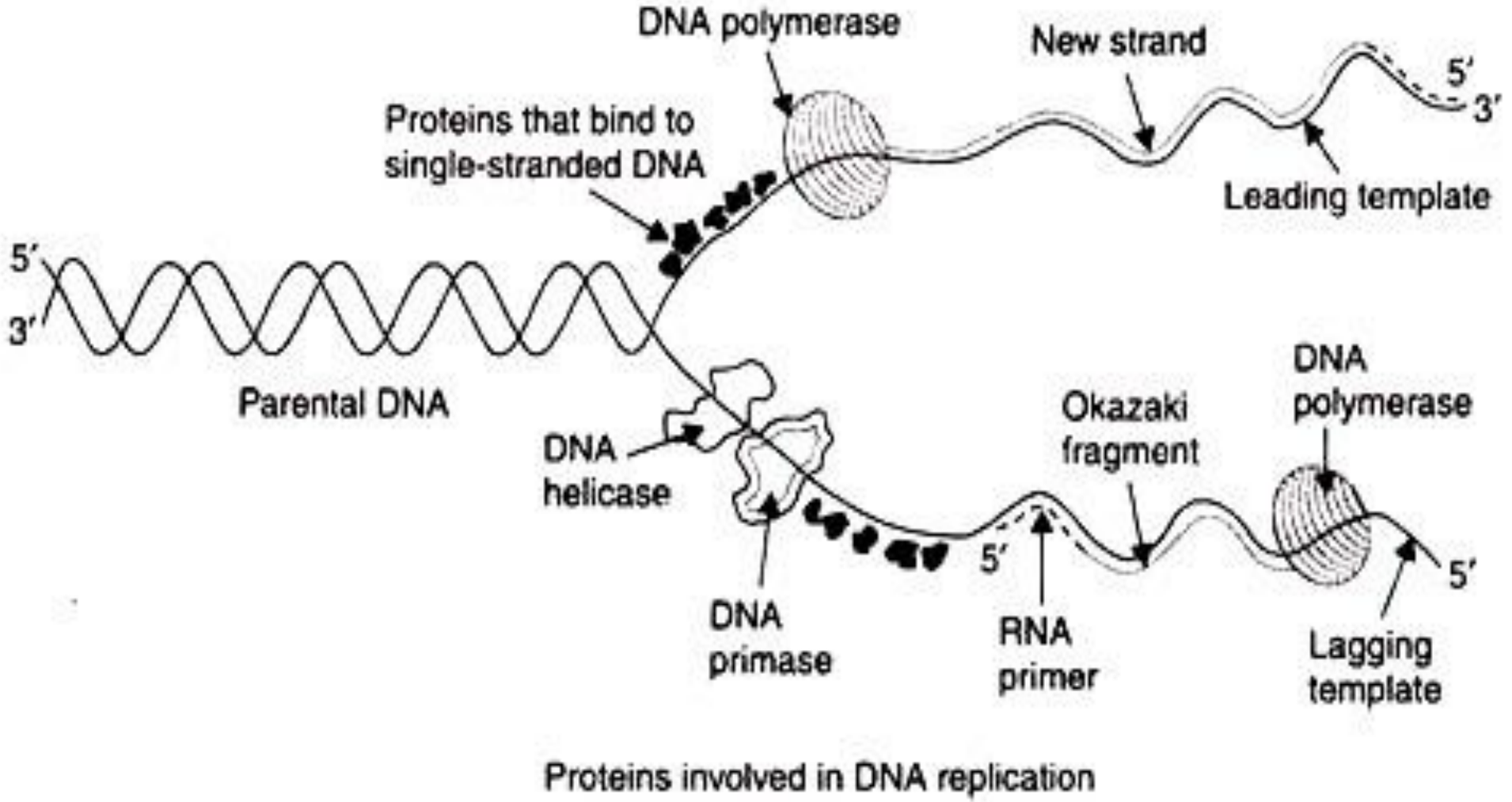


Fig. 14.11 Diagram giving an overview of DNA replication, binding of RNA polymerase, formation of Okazaki fragment, and involvement of a variety of proteins.

2.Elongation –

Synthesis of leading strand and lagging strand

- Synthesis of DNA by DNA polymerase
- Direction : 5’-3’ direction always,
- Leading Strand : Synthesized in same direction of unwinding helix

  Continuously Synthesized.
- Lagging Strand : Synthesized in opposite direction of unwinding helix

  Dis-continuously Synthesized.
- Fragments known as OKAZAKI fragments

Priming :

- DNA polymerases require a short double stranded region to imitate or prime DNA synthesis.
- This is produced by DNA dependent RNA polymerase called primase, which is able to initiate synthesis on ssDNA.
- The primase synthesizes a short RNA primer sequences on the DNA template creating a short double stranded region.
- With the primer as the starting point for the leading strand, a new DNA strand grows one base at a time.

- DNA polymerase α enzyme responsible for initiating DNA sizing the lagging strand and DNA polymerase δ synthesizing the leading strand.
- On the lagging strand synthesis ends with next RNA primer is encountered.
- DNA polymerase ε involved in DNA repair and DNA polymerase γ replicates mitochondrial DNA.

Ligation :

- Final stage of DNA replication
- OKAZAKI fragments of lagging strand joined by phosphodiester bonds with help of DNA ligase enzyme.

Termination –

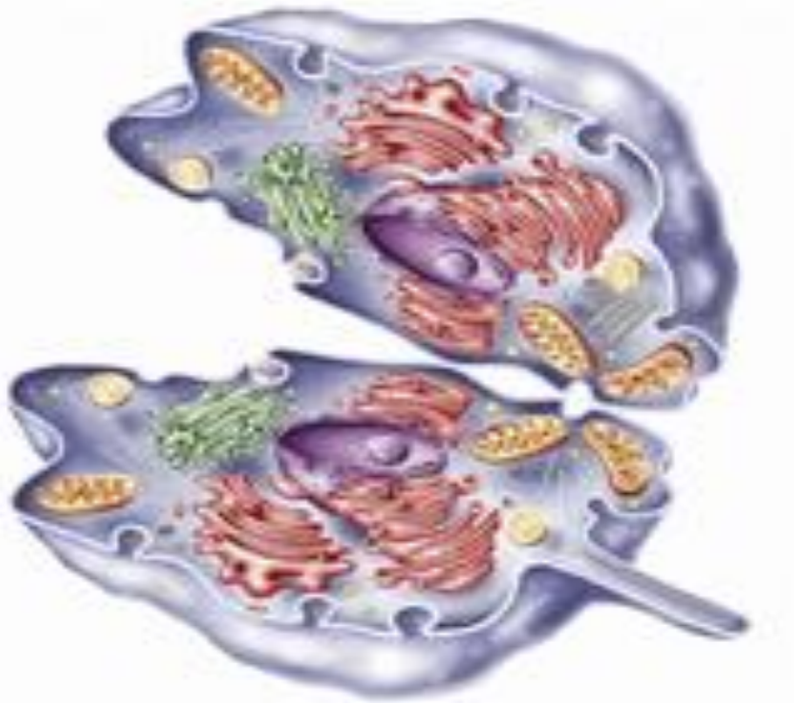
- After elongation is complete, two new double helicase have replaced the original helix, the last primer sequence must be removed from the end of the lagging strand
- Replaced with new DNA nucleotides and the backbone is sealed by DNA ligase

Gene Expression

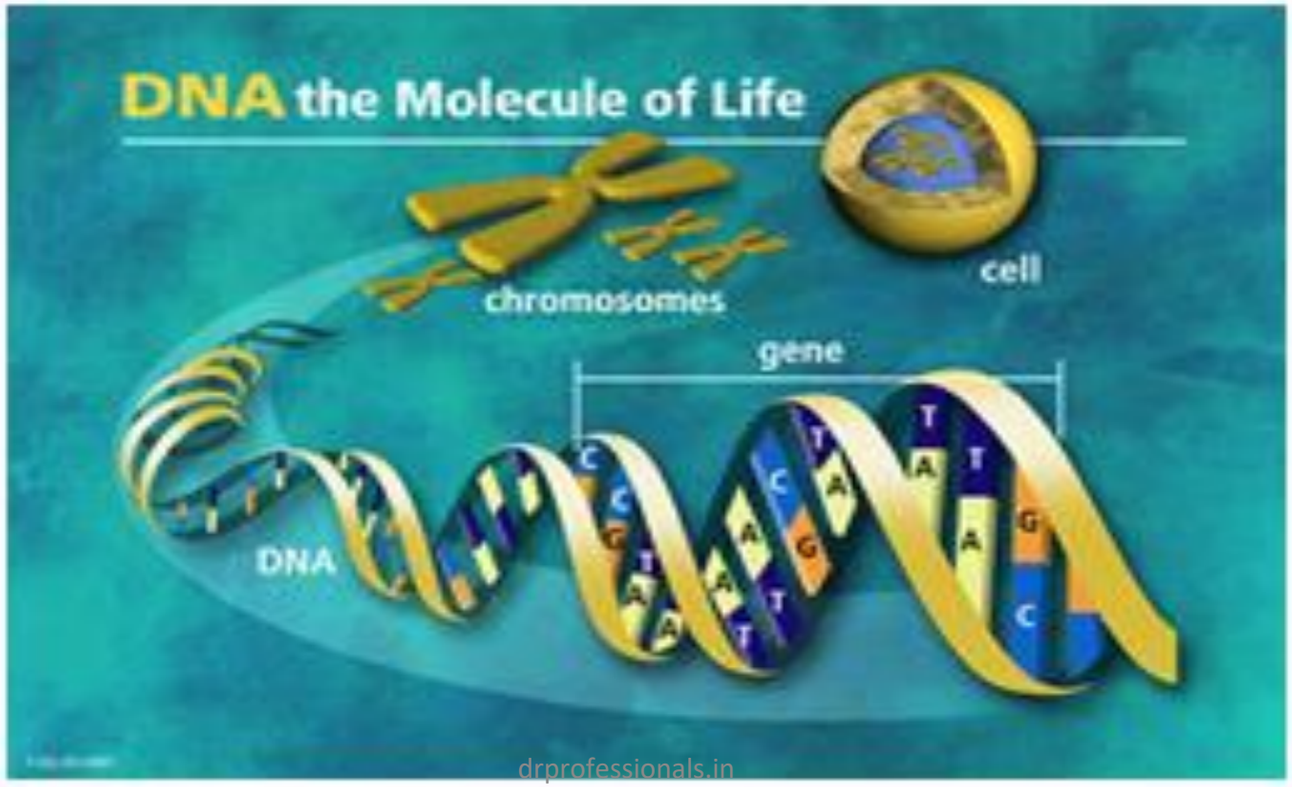
- The process by which a gene's information is converted into the structures and functions of a cell by a process of producing a biologically functional molecule of either protein or RNA (gene product) is made.
  - Gene expression is assumed to be controlled at various points in the sequence leading to protein synthesis.

The Cell

- Basic component of life
- Two main categories, prokarytic and eukaryotic cells
- Differences in the nucleus



- Genetic material is located in nucleus
- The genetic information is stored in Deoxyribonucleic acid, DNA
- DNA contains the information needed to build an individual

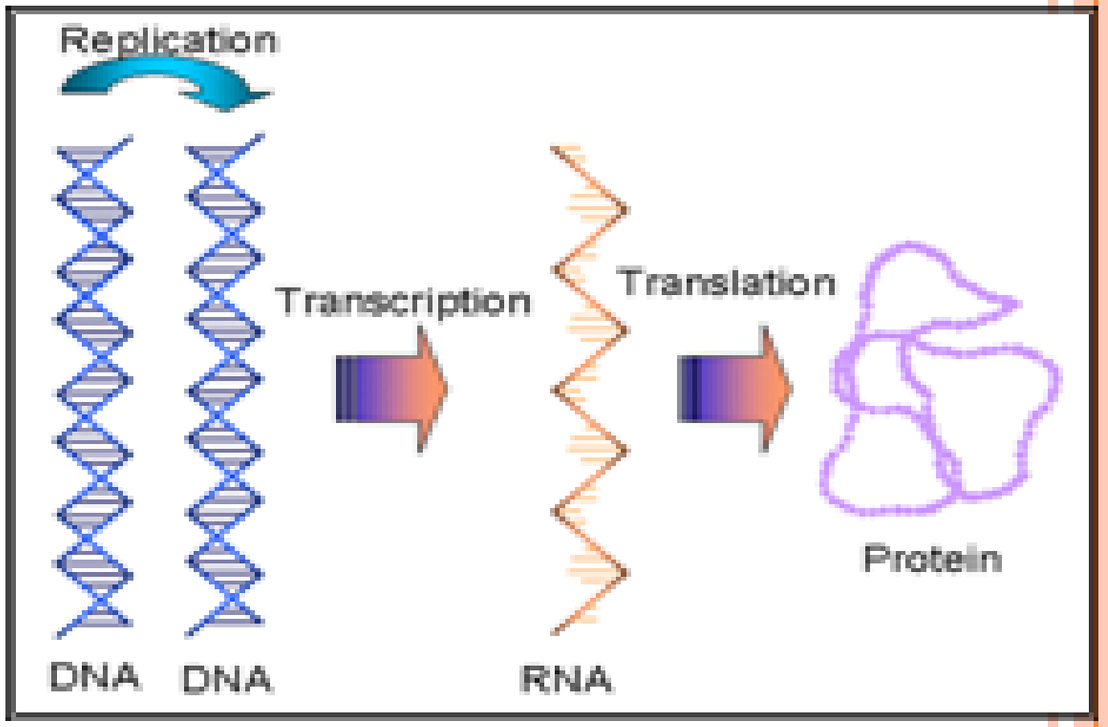


- Information of the DNA is copied to a RNA molecule in **transcription**

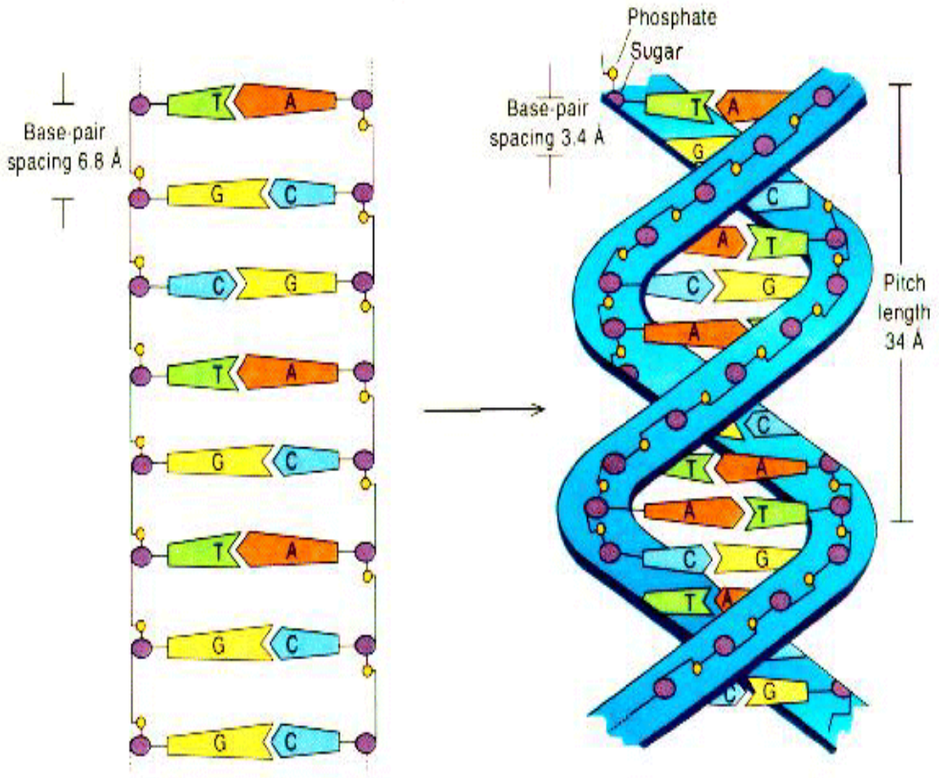
- RNA directs the protein synthesis in a **translation**

- Protein's 3D structure determines it's function

- Information transfer only in one direction



- ”ladder-structure”
  - –Bases = steps
  - –Sugars and phosphates = supporting pilars



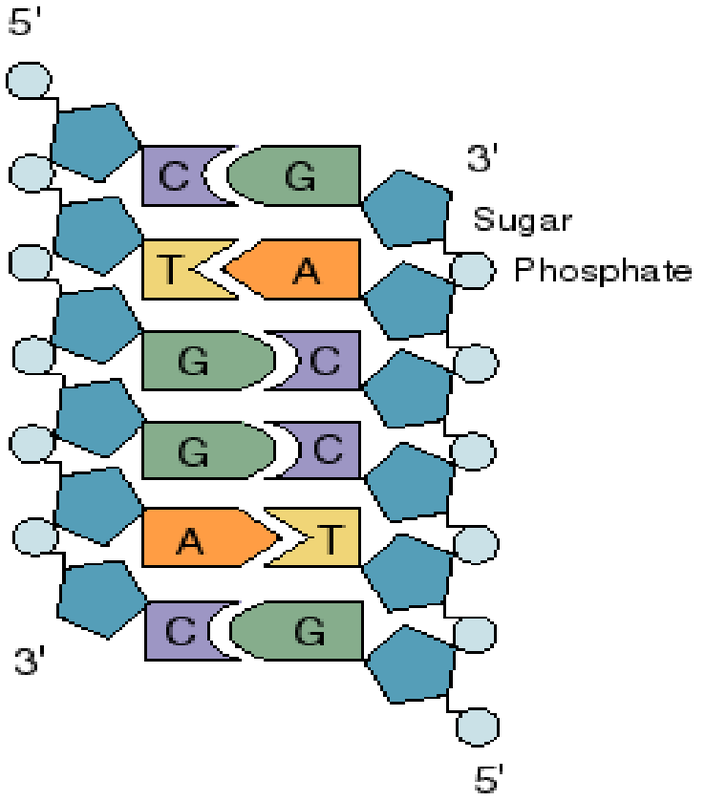
- Two nucleotide chains run in opposite directions

→chemical direction (5´-3´)

Complementary Pairing

- Bases pair with other bases
  - Space between the chains is limited → Purines with two carbon rings pair only with single ring pyrimidines
    A + T
    G + C
- Complementary pairing is vital for the use and storage of the genetic information!

- Interaction is stabilized by hydrogen bonds



The Genetic Code

- Describes how nucleotide sequence is converted to protein sequence
- Unit of three nucleotides = a codon
- A codon codes for a specific amino acid (structural component of protein)

1st	2nd position T	C	A	G	3rd
T	Phe	Ser	Tyr	Cys	T
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	T
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	T
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	T
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

The Genetic Code

Amino acids

ala - alanine
arg - arginine
asn - asparagine
asp - aspartic acid
cys - cysteine

gln - glutamine
glu - glutamic acid
gly - glycine
his - histidine
ile - isoleucine

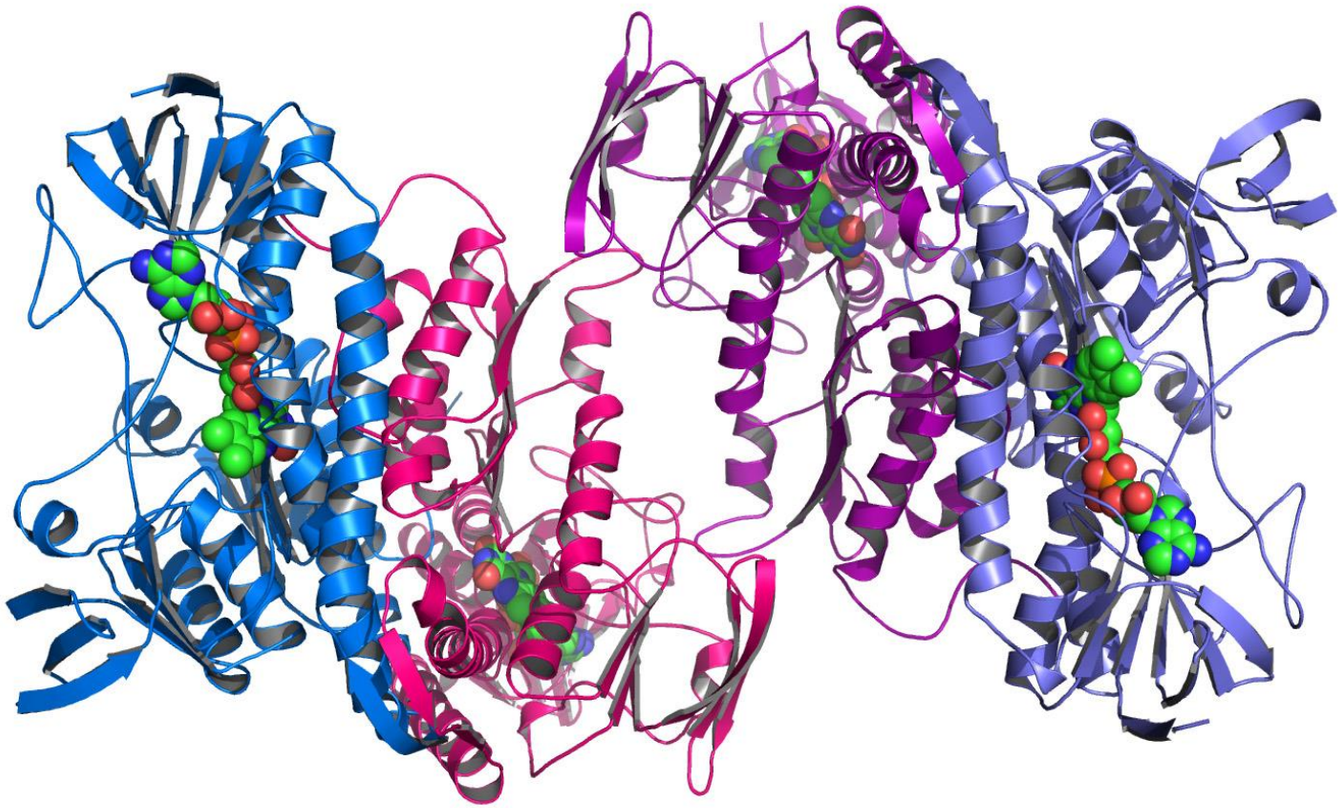
leu - leucine
lys - lysine
met - methionine
phe - phenylalanine
pro - proline

ser - serine
tthr - threonine
trp - tryptophan
tyr - tyrosine
val - valine

- The four bases can form 64 different codons
- 20 amino acids are found from the nature
- Regulatory codons

Proteins

- Polymer made of monomers – Amino Acids
- 20 Naturally occurring Amino Acids



Proteins

- Special cellular components called ribosomes use the triplet genetic code to translate the nucleotides of a mRNA sequence into the amino acid sequence of a protein.

- There are 20 different amino acids. Proteins are created by linking amino acids together in a linear fashion to form polypeptide chains.

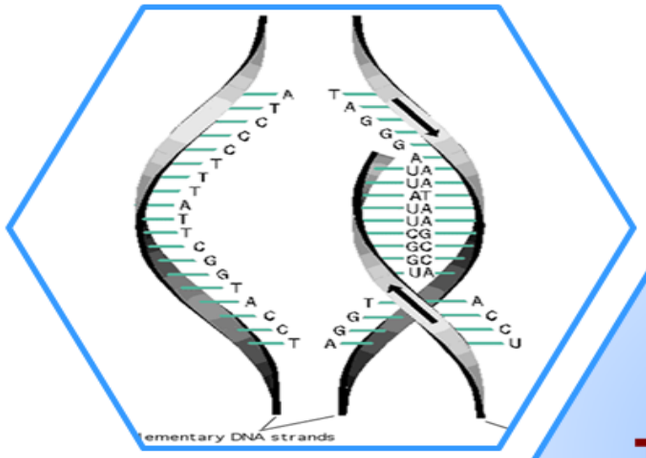
- Protein polypeptide chains fold into three-dimensional structures that can associate with other protein structures to perform specific functions.

Central dogma of molecular biology: DNA ➔ RNA ➔ Protein

Genetic information is stored in DNA.

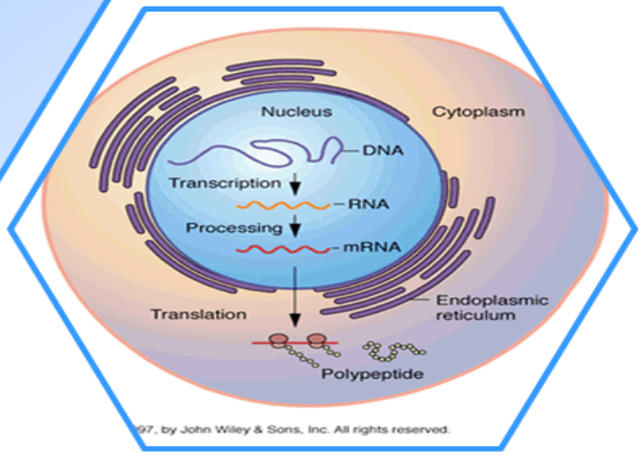
1. Segments of DNA that encode proteins or other functional products are called genes.
2. Gene sequences are transcribed into messenger RNA intermediates (mRNA).
3. mRNA intermediates are translated into proteins that perform most life functions.

Gene Expression



Transcription

Translation



Gene Expression

Transcription

- Synthesis of an RNA that is complementary to one of the strands of DNA.

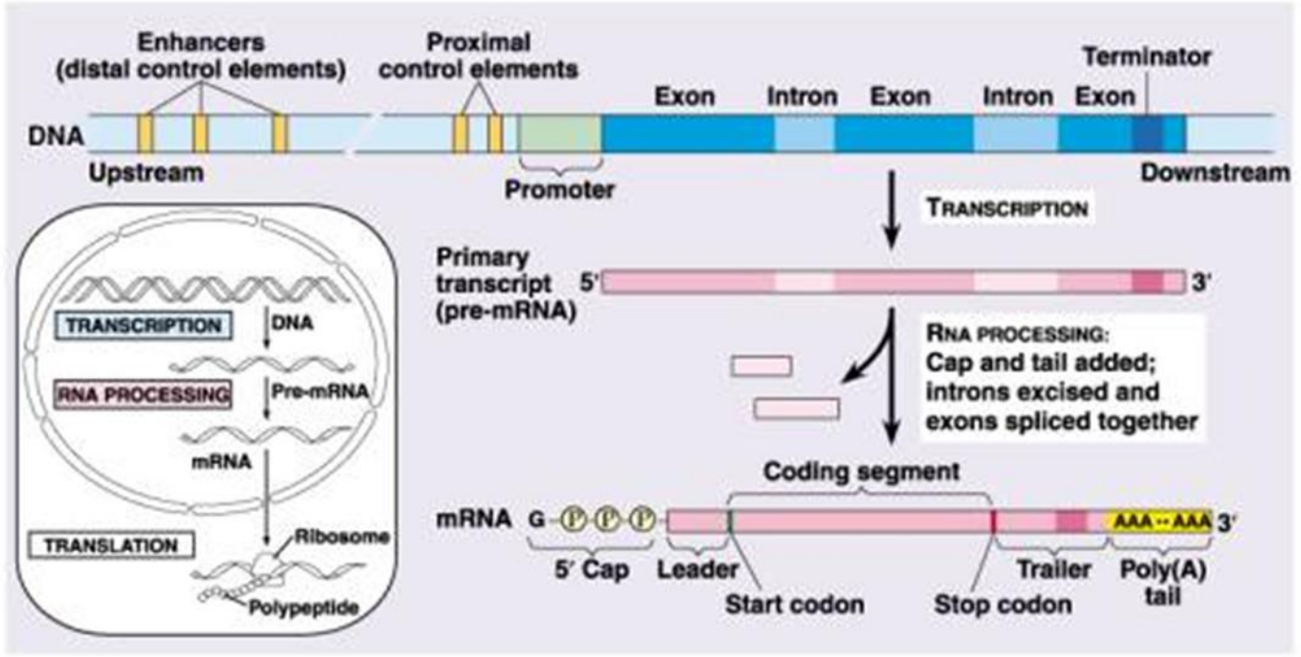
Translation

- Ribosomes read a messenger RNA and make protein according to its instruction.

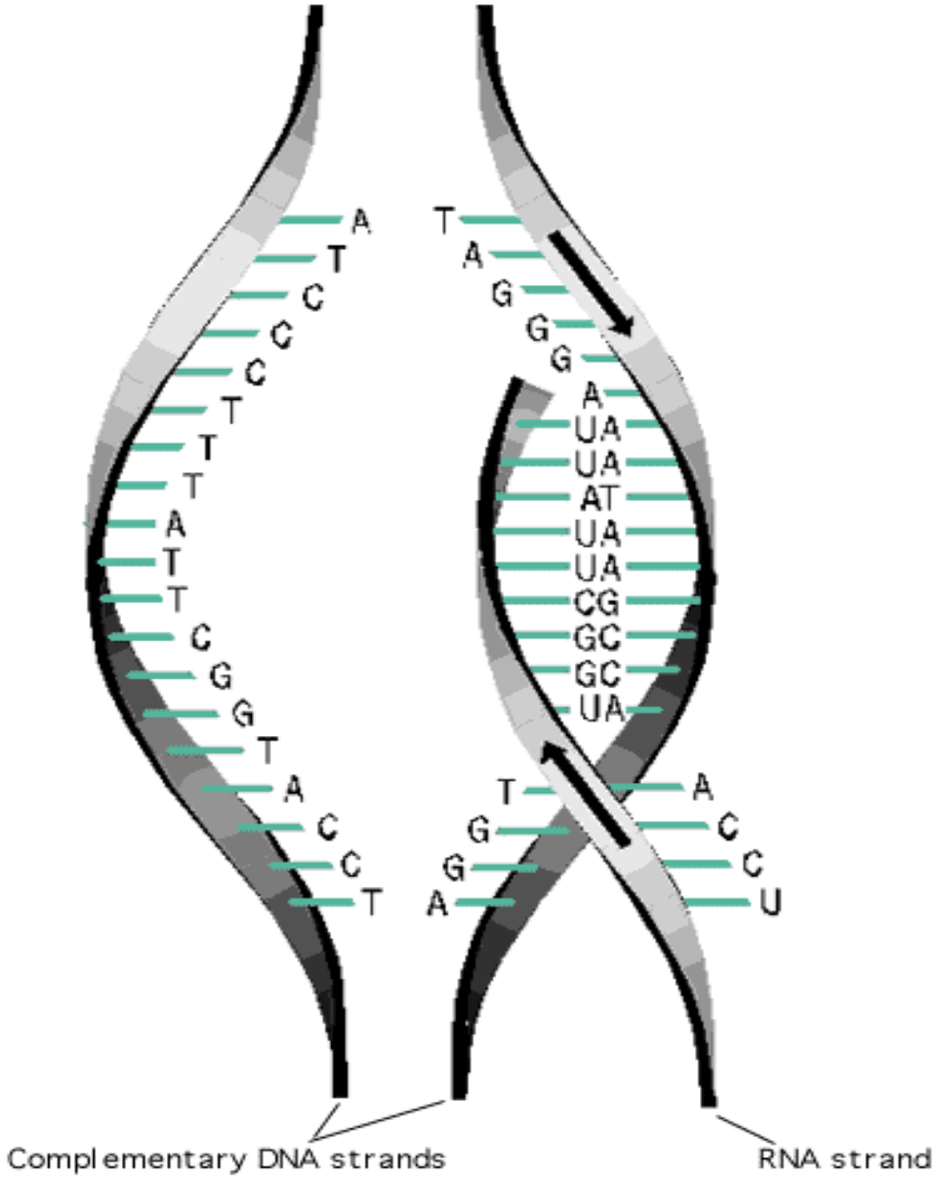
Protein Synthesis: Four stages

- Transcription
- RNA processing
- Translation
- Post-translation processing

Protein Synthesis



Transcription



Transcription Enzymes

RNA polymerase: The enzyme that controls transcription and is characterized by:

- Search DNA for initiation site,
- It unwinds a short stretch of double helical DNA to produce a single-stranded DNA template,
- It selects the correct ribonucleotide and catalyzes the formation of a phosphodiester bond,
- It detects termination signals where transcript ends.

Eukaryotic RNA polymerases have different roles in transcription

Polymerase I	nucleolus	Makes a large precursor to the major rRNA (5.8S,18S and 28S rRNA in vertebrates
Polymerase II	nucleoplasm	Synthesizes hnRNAs, which are precursors to mRNAs. It also make most small nuclear RNAs (snRNAs
Polymerase III	Nucleoplasm	Makes the precursor to 5SrRNA, the tRNAs and several other small cellular and viral RNAs.

Eukaryotic Promoter

Eukaryotic Promoter lies upstream of the gene. There are several different types of promoter found in human genome, with different structure and different regulatory properties class/I/II/III.

Conserved eukaryotic promoter elements	Consensus sequence
CAAT box	GGCCAATCT
TATA box	TATAA
GC box	GGGCGG
CAP site	TAC

Transcription Factors

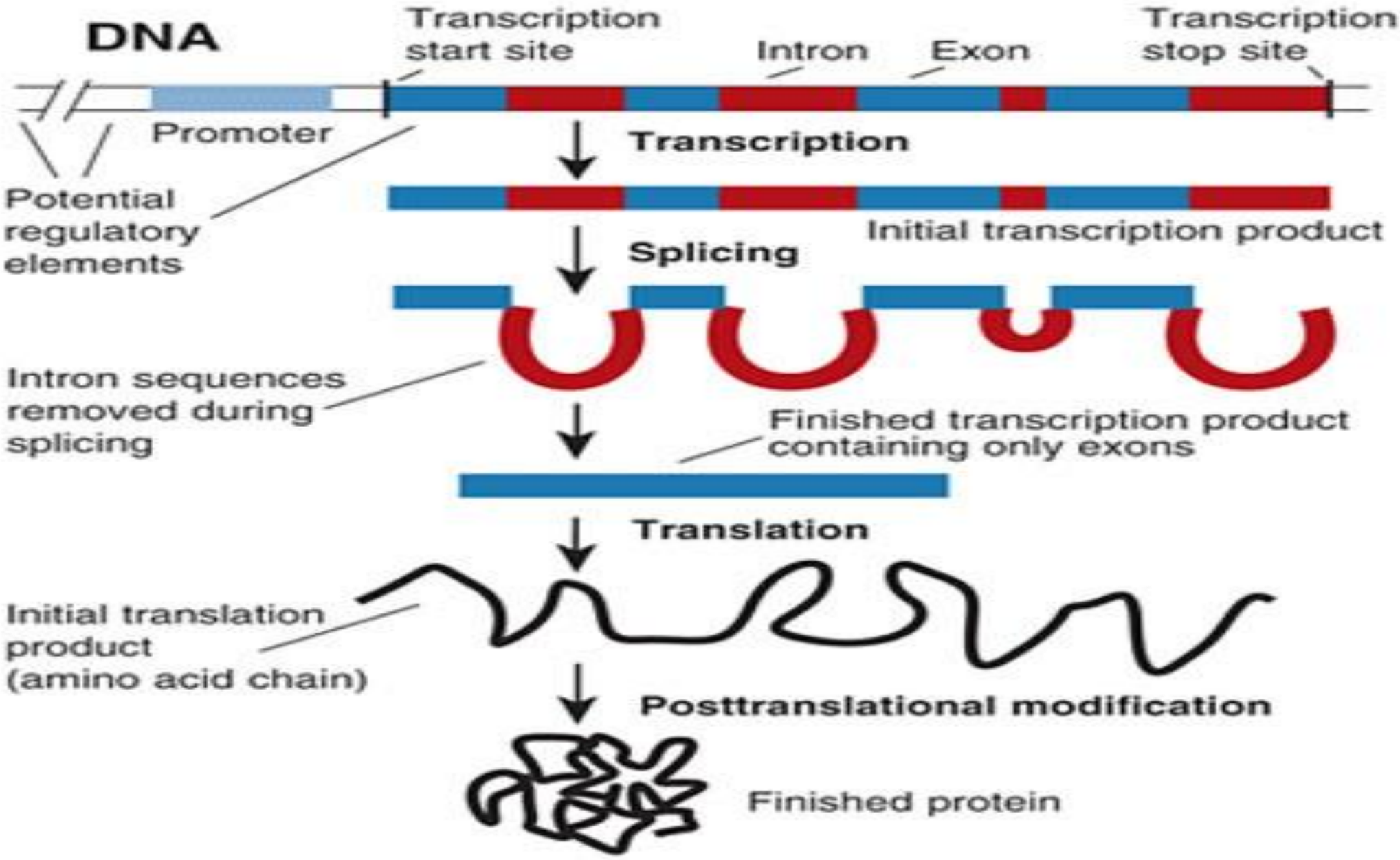
- Transcription factors are proteins that bind to DNA near the start of transcription of a gene.

- Transcription factors either inhibit or assist RNA polymerase in initiation and maintenance of transcription.

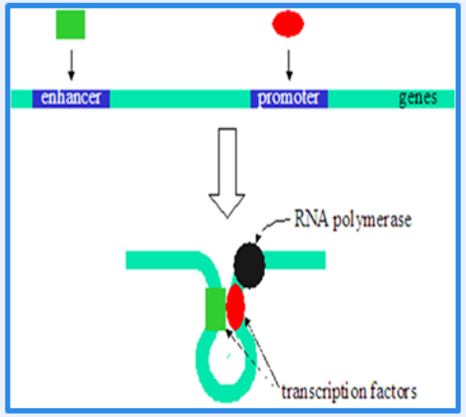
Preinitiation Complex

- The general transcription factors combine with RNA polymerase to form a preinitiation complex that is competent to initiate transcription as soon as nucleotides are available.

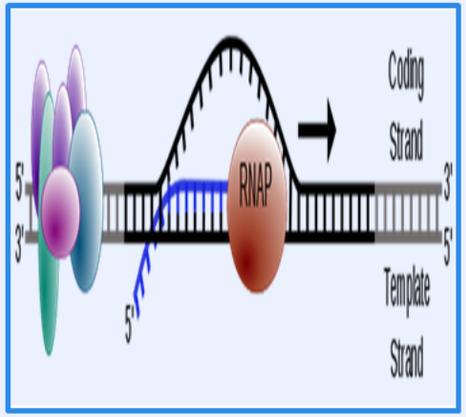
- The assembly of the preinitiation complex on each kind of eukaryotic promoter (class II promoters recognized by RNA polymerase II) begins with the binding of an assembly factor to the promoter.



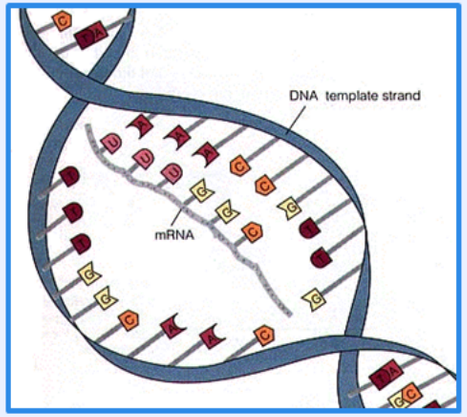
Transcription is divided into 3 phases:



Initiation



Elongation



Termination

Initiation

- The polymerase binding causes the unwinding of the DNA double helix which expose at least 12 bases on the template.

- This is followed by initiation of RNA synthesis at this starting point.

Initiation

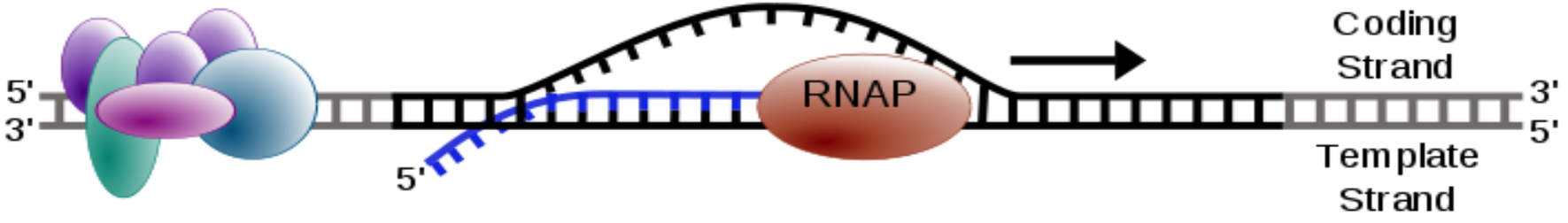
- The RNA polymerase starts building the RNA chain; it assembles ribonucleotides triphosphates: ATP; GTP; CTP and UTP into a strand of RNA.

- After the first nucleotide is in place, the polymerase joins a second nucleotide to the first, forming the initial phosphodiester bond in the RNA chain.

Elongation

- **RNA polymerase** directs the sequential binding of ribonucleotides to the growing RNA chain in the 5' - 3' direction.

- Each ribonucleotide is inserted into the growing RNA strand following the rules of base pairing. This process is repeated utill the desired RNA length is synthesized……..



Termination

- Terminators at the end of genes; signal termination. These work in conjunction with RNA polymerase to loosen the association between RNA product and DNA template. The result is that the RNA dissociate from RNA polymerase and DNA and so stop transcription.

- The product is immature RNA or pre mRNA (Primary transcript

- The primary product of RNA transcription; the hnRNAs contain both intronic and exonic sequences.

- These hnRNAs are processed in the nucleus to give mature mRNAs that are transported to the cytoplasm where to participate in protein synthesis.

RNA Processing (Pre-mRNA → mRNA

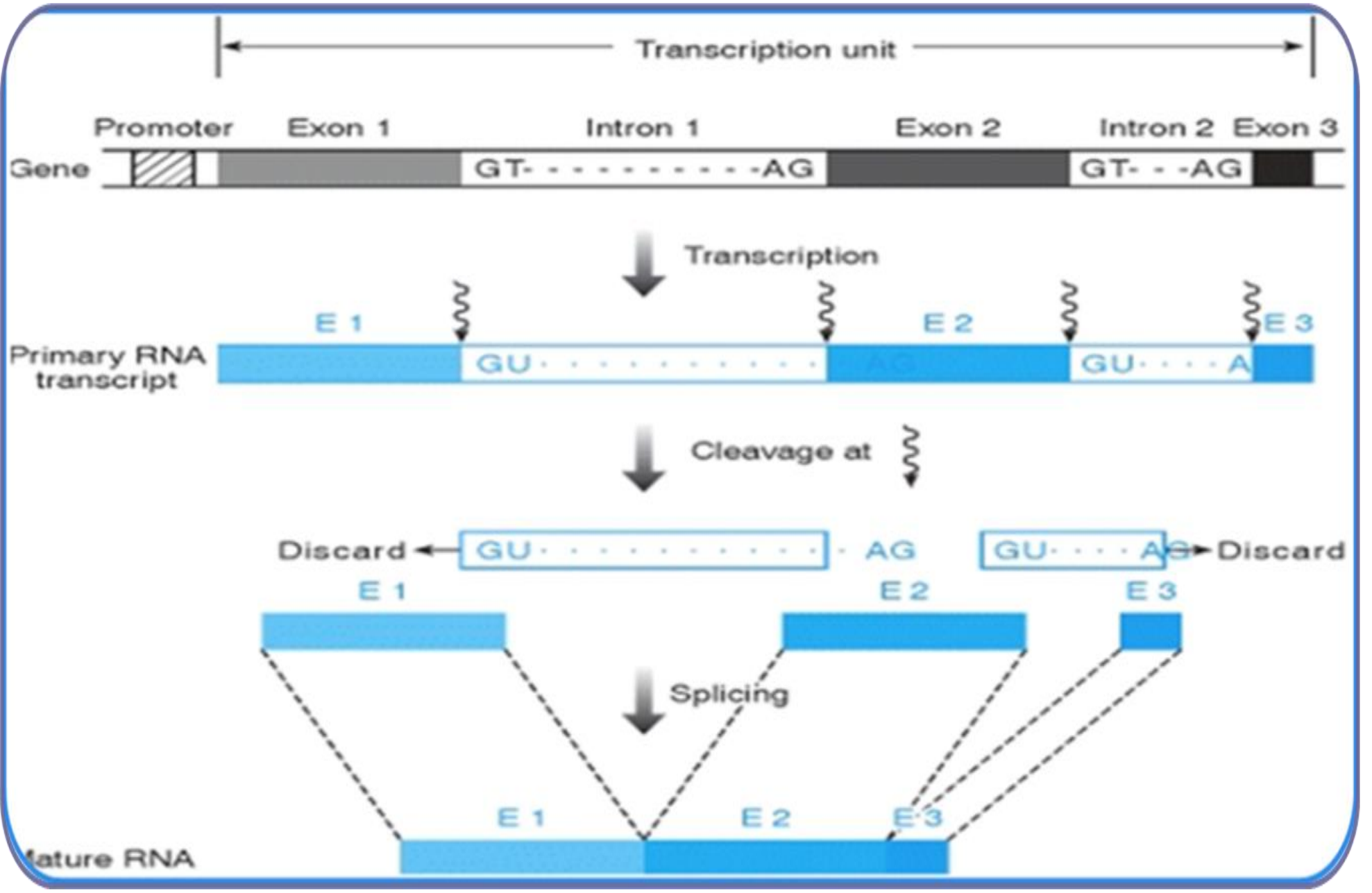
- Capping
- Splicing
- Addition of poly A tail

RNA Processing

- Capping
  - The cap structure is added to the 5' of the newly transcribed mRNA precursor in the nucleus prior to processing and subsequent transport of the mRNA molecule to the cytoplasm.
- Splicing:
  - Step by step removal of pre mRNA and joining of remaining exons; it takes place on a special structure called spliceosomes.

RNA Processing

- Addition of poly A tail:
  - Synthesis of the poly (A) tail involves cleavage of its 3' end and then the addition of about 40- 200 adenine residues to form a poly (A) tail.

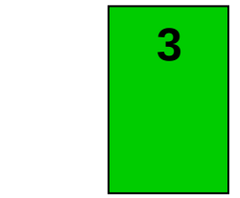

Transcription unit
Promoter
Exon 1
Intron 1
Exon 2
Intron 2
Exon 3
Gene
GT- - - - - - - - - -AG
GT- - -AG
Transcription
E 1
E 2
E 3
Primary RNA transcript
GU- - - - - - - - - - -
GU- - - -A
Cleavage at
Discard
GU- - - - - - - - - - -
AG
GU- - - -A
Discard
E 1
E 2
E 3
Splicing
E 1
E 2
E 3
fature RNA

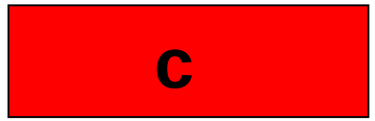
Exons vs Introns

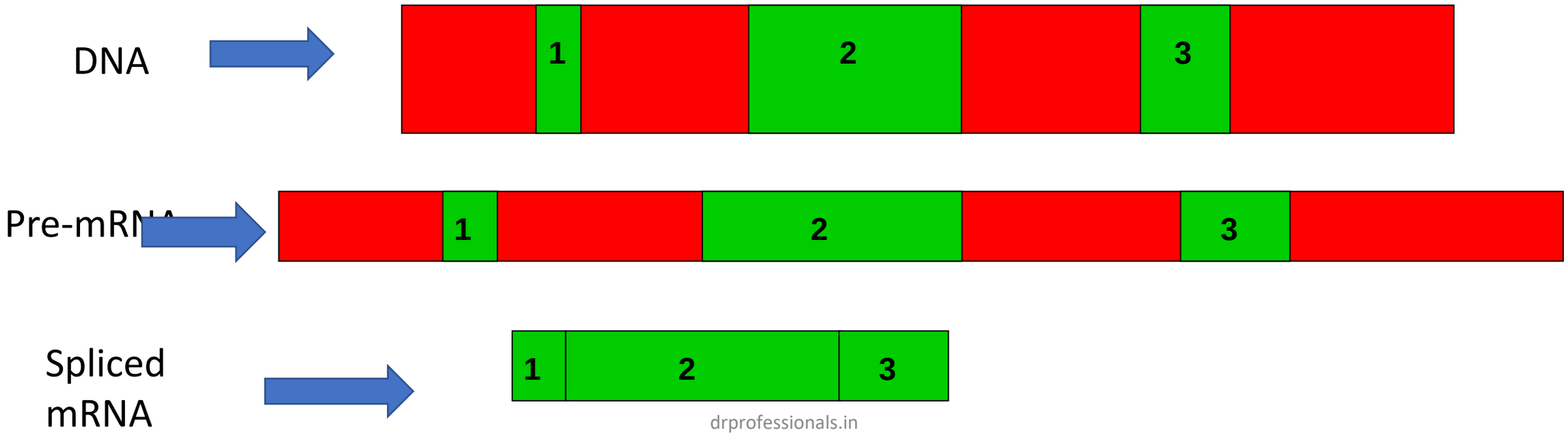
- Eukaryotic genes have introns and exons. Exons contain nucleotides that are translated into amino acids of proteins. Exons are separated from one another by intervening segments of junk DNA called introns. Introns do not code for protein. They are removed when eukaryotic mRNA is processed. Exons make up those segments of mRNA that are spliced back together after the introns are removed; the intron-free mRNA is used as a template to make proteins.

Splicing

- **Ex**ons are sequences of DNA that are **ex**pressed into protein.
- **In**trons are **in**tervening sequences that are not translated into protein

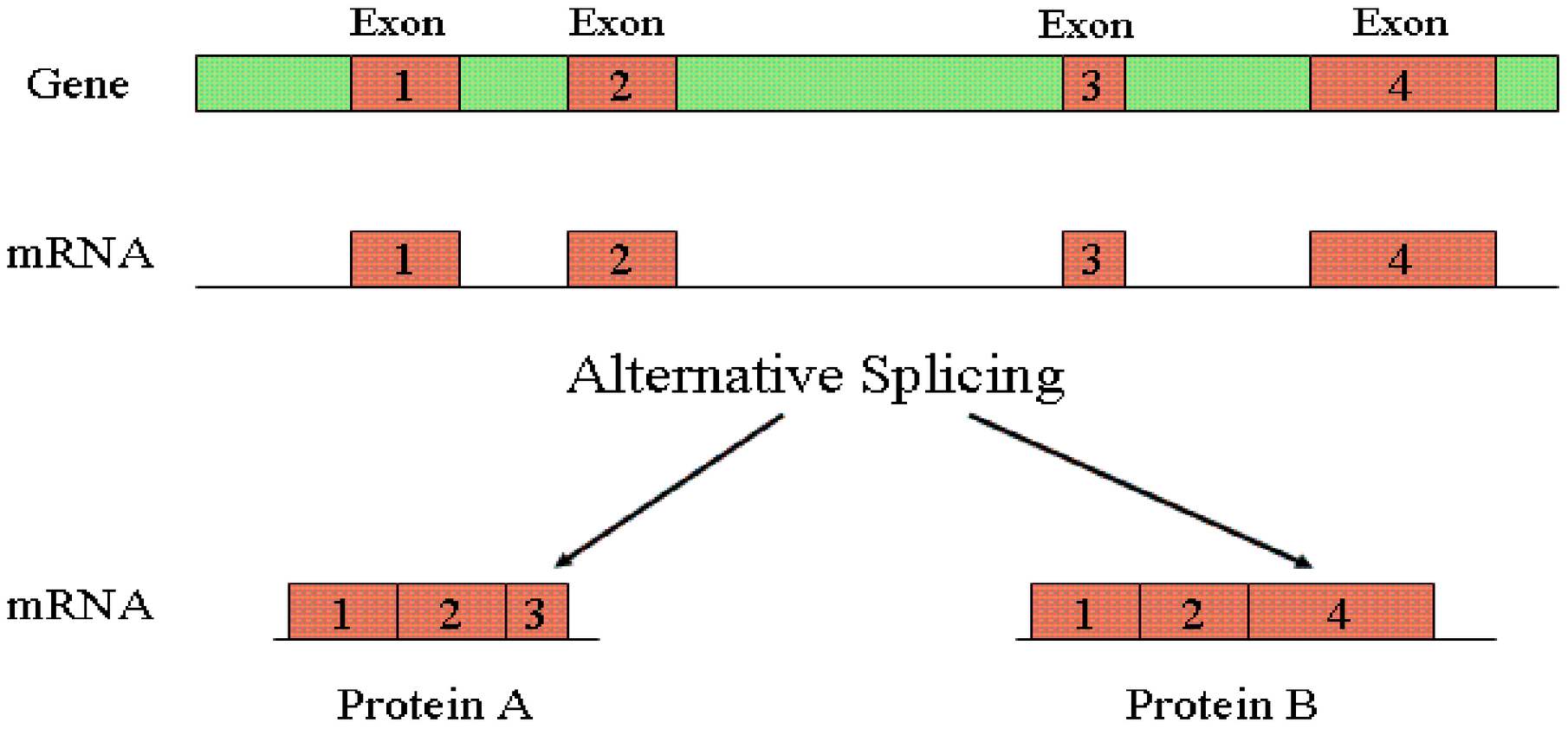






Alternative Splicing

- Alternative splicing: is a very common phenomenon in higher eukaryotes. It is a way to get more than one protein product out of the same gene and a way to control gene expression in cells.

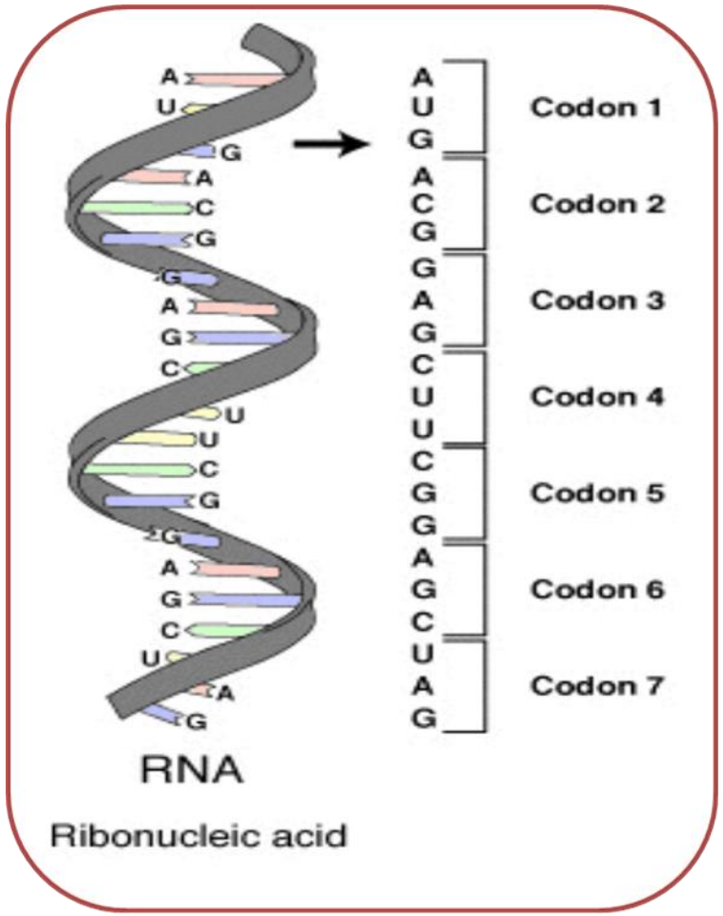


The Genetic Code

- The sequence of codons in the **mRNA** defines the primary structure of the final protein.

- Three nucleotides in mRNA (a codon) specify one amino acid in a protein.

The Genetic Code



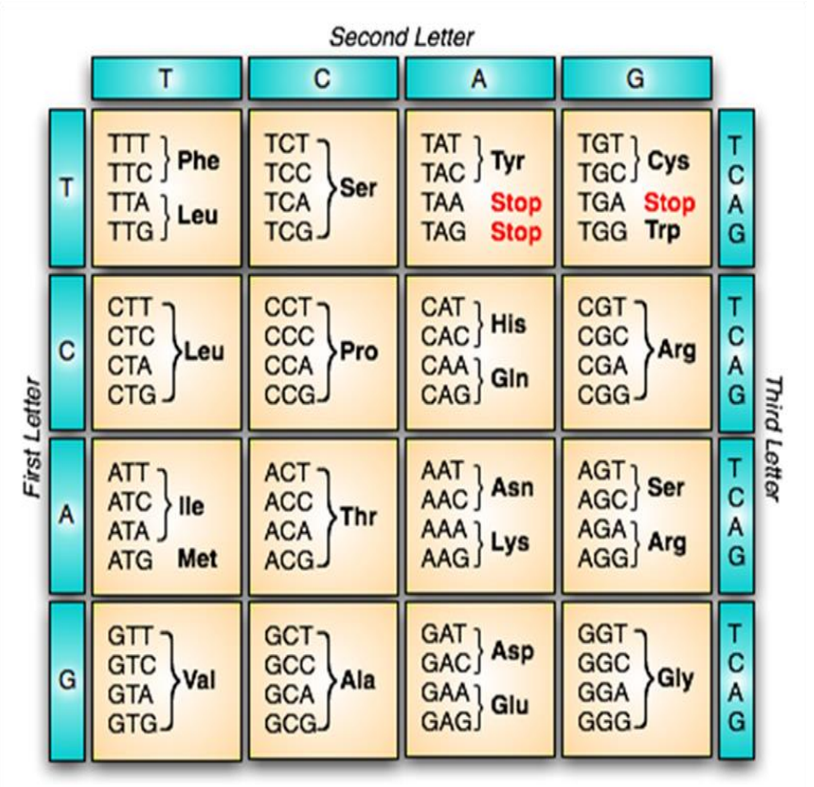
The Genetic Code

- **The triplet sequence of mRNA that specify certain amino acid.**
  - 64 different combination of bases; 61 of them code for 20 amino acids (AA); the last three codon (UAG,UGA,UAA) don not code for amino acids; they are termination codons.

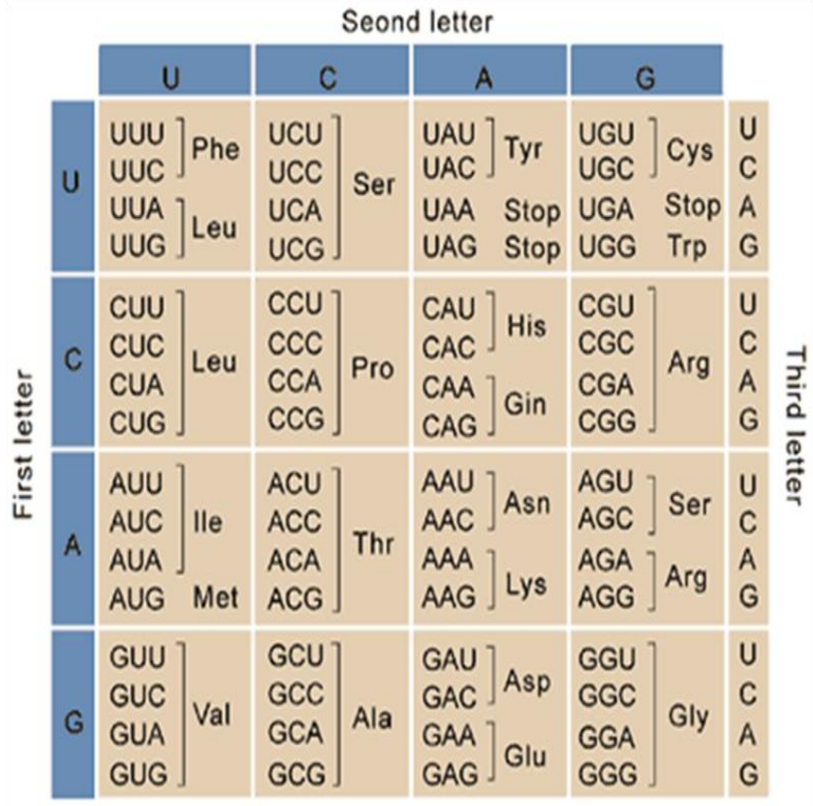
- **Degenerate**
  - More than on triplet codon specify the same amino acid.

The Genetic Code

- **Unambiguous**
  - Each codon specifies a particular amino acid, the codon ACG codes for the amino acid threonine, and only threonine.
- **Non overlapping**
  - This means that successive triplets are read in order. Each nucleotide is part of only one triplet codon.

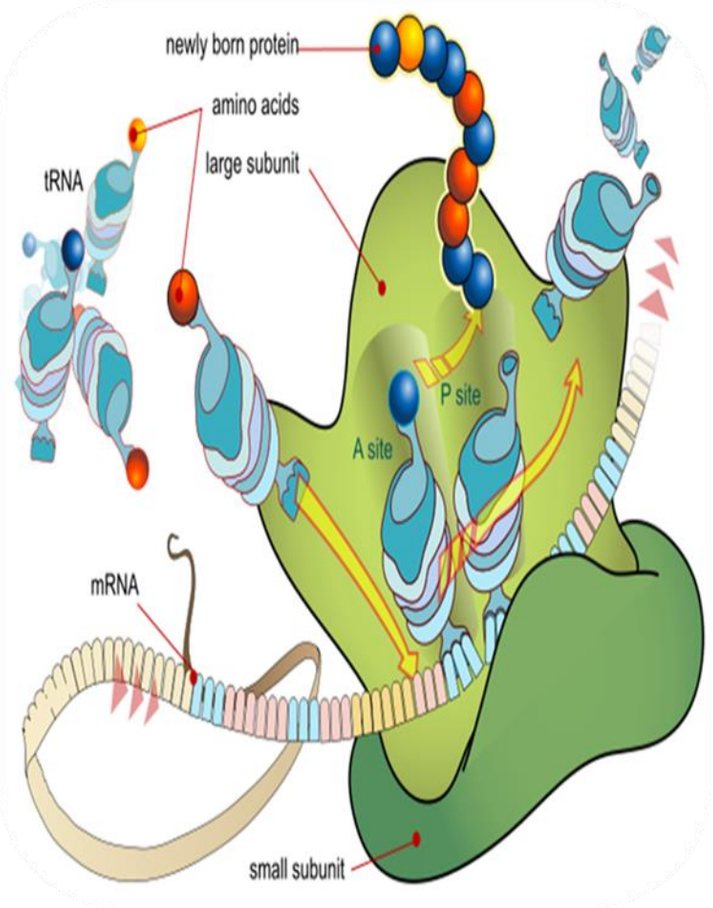


DNA Codon



RNA Codon

Translation



Translation

- Translation is the process by which ribosomes read the genetic message in the mRNA and produce a protein product according to the message's instruction.

Requirement for Translation

- Ribosomes
- tRNA
- mRNA
- Amino acids
- Initiation factors
- Elongation factors
- Termination factors
- Aminoacyl tRNA synthetase enzymes:
- Energy source:

The large ribosomal subunit contains three tRNA binding sites, designated A, P, and E.

- The A site binds an aminoacyl-tRNA (a tRNA bound to an amino acid);
- P site binds a peptidyl-tRNA (a tRNA bound to the peptide being synthesized).
- The E site binds a free tRNA before it exits the ribosome.

Ribosomes

- Eukaryotic ribosomes are larger. They consist of two subunits, which come together to form an 80S particle;
  - 60S subunit holds (three rRNAs 5S, 5.8S, 28S and about 40 proteins).
  - 40S subunit contains (an18S rRNA and about 30 proteins).

Preparatory Steps for Protein Synthesis

- First, aminoacyl tRNA synthetase joins amino acid to their specific tRNA.

- Second, ribosomes must dissociate into subunits at the end of each round of translation.

The protein synthesis occur in 3 phases

- Accurate and efficient initiation occurs; the ribosomes binds to the mRNA, and the first amino acid attached to its tRNA.
- Chain elongation, the ribosomes adds one amino acid at a time to the growing polypeptide chain.
- Accurate and efficient termination, the ribosomes releases the mRNA and the polypeptide.

Initiation

- The initiation phase of protein synthesis requires over 10 eukaryotic Initiation Factors (eIFs): Factors are needed to recognize the cap at the 5'end of an mRNA and binding to the 40s ribosomal subunit.

- Binding the initiator Met-tRNAiMet (methionyl- tRNA) to the 40S small subunit of the ribosome.

Initiation

- Scanning to find the start codon by binding to the 5'cap of the mRNA and scanning downstream until they find the first AUG (initiation codon).
- The start codon must be located and positioned correctly in the P site of the ribosome, and the initiator tRNA must be positioned correctly in the same site.
- Once the mRNA and initiator tRNA are correctly bound, the 60S large subunit binds to form 80s initiation complex with a release of the eIF factors.

Elongation

- **Transfer of proper aminoacyl-tRNA from cytoplasm to A-site of ribosome;**

- **Peptide bond formation**; Peptidyl transferase forms a peptide bond between the amino acid in the P site, and the newly arrived aminoacyl tRNA in the A site. This lengthens the peptide by one amino acids.

Elongation

- **Translocation;** translocation of the new peptidyl t-RNA with its mRNA codon in the A site into the free P site occurs. Now the A site is free for another cycle of aminoacyl t-RNA codon recognition and elongation. Each translocation event moves mRNA, one codon length through the ribosomes.

Termination

- Translation termination requires specific protein factors identified as releasing factors, RFs in E. coli and eRFs in eukaryotes.

- The signals for termination are the same in both prokaryotes and eukaryotes. These signals are termination codons present in the mRNA. There are 3 termination codons, UAG, UAA and UGA.

Termination

- After multiple cycles of elongation and polymerization of specific amino acids into protein molecules, a nonsense codon = termination codon of mRNA appears in the A site. The is recognized as a terminal signal by eukaryotic releasing factors (eRF) which cause the release of the newly synthesized protein from the ribosomal complex.

Polysomes

- Most mRNA are translated by more than one ribosome at a time; the result, a structure in which many ribosomes translate a mRNA in tandem, is called a **polysomes.**

Control of Gene Expression

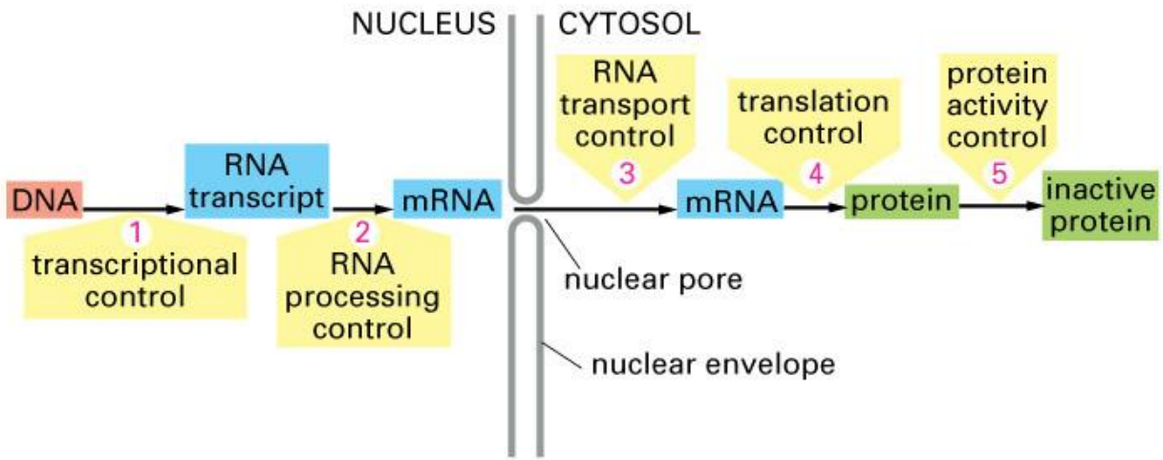


Figure 8-3 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Control of gene expression depends various factors including:

- Chromosomal activation or deactivation.
- Control of initiation of transcription.
- Processing of RNA (e.g. splicing).
- Control of RNA transport.
- Control of mRNA degradation.
- Control of initiation of translation (only in eukaryotes).
- Post-translational modifications.

Gene Expression Analysis

- Polymerase Chain Reaction
- Quantitative PCR
- Microarray

THANK YOU FOR YOUR TIME..