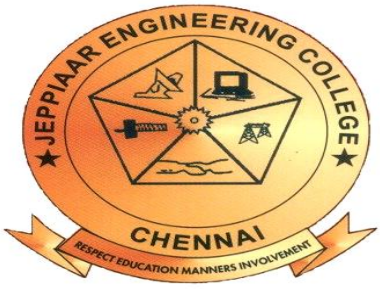
JEPPIAAR ENGINEERING COLLEGE



B.TECH – BIOTECHNOLOGY (R- 2013)

BT6504 – MOLECULAR BIOLOGY

III YEAR & V SEM

BATCH: 2016-2020

QUESTION BANK

PREPARED BY

Mr. G. GOMATHI SANKAR

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	UNIT III – TRANSCRIPTION		
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3	Prokaryotic and eukaryotic ribosomes	**TB1**	**439-447**
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	UNIT V – REGULATION OF GENE EXPRESSION		
1	Organization of genes in prokaryotic and eukaryotic chromosomes, Hierarchical levels of gene regulation	**TB1**	**453-454, 502-510**
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3	Regulation of gene expression with reference to λ phage life cycle	**TB1**	**598 - 613**

TB1 - Friefelder, David. " Molecular Biology." Narosa Publications, 1999

BT6504 MOLECULAR BIOLOGY LTP C 3 0 0 3

OBJECTIVES:

- Familiarize students with the cell and molecular biology of both Prokaryotes and Eukaryotes. This will be needed for any project work in modern biotechnology.
- By doing this course students will acquire basic fundamental knowledge and explore skills in molecular biology and become aware of the complexity and harmony of the cells.
- This course will emphasize the molecular mechanism of DNA replication, repair, transcription, protein synthesis and gene regulation in various organisms.

UNIT I CHEMISTRY OF NUCLEIC ACIDS 9

Introduction to nucleic acids: Nucleic acids as genetic material, Structure and physicochemical properties of elements in DNA and RNA, Biological significance of differences in DNA and RNA. Primary structure of DNA: Chemical and structural qualities of 3',5'-Phosphodiester bond. Secondary Structure of DNA: Watson & Crick model, Chargaff's rule, X–ray diffraction analysis of DNA, Forces stabilizes DNA structure, Conformational variants of double helical DNA, Hogsteen base pairing, Triple helix, Quadruple helix, Reversible denaturation and hyperchromic effect. Tertiary structure of DNA: DNA supercoiling.

UNIT II DNA REPLICATION & REPAIR 9

Overview of Central dogma. Organization of prokaryotic and eukaryotic chromosomes. DNA replication: Meselson & Stahl experiment, bi–directional DNA replication, Okazaki fragments, Proteomics of DNA replication, Fidelity of DNA replication, Inhibitors of DNA replication, Overview of differences in prokaryotic and eukaryotic DNA replication, Telomere replication in eukaryotes. D-loop and rolling circle mode of replication. Mutagens, DNA mutations and their mechanism, various types of repair mechanisms.

UNIT III TRANSCRIPTION 9

Structure and function of mRNA, rRNA and tRNA. Characteristics of promoter and enhancer sequences. RNA synthesis: Initiation, elongation and termination of RNA synthesis, Proteins of RNA synthesis, Fidelity of RNA synthesis, Inhibitors of transcription, Differences in prokaryotic and eukaryotic transcription. Basic concepts in RNA world: Ribozymes, RNA processing: 5'-Capping, Splicing-Alternative splicing, Poly 'A' tail addition and base modification.

UNIT IV TRANSLATION 9

Introduction to Genetic code: Elucidation of genetic code, Codon degeneracy, Wobble hypothesis and its importance, Prokaryotic and eukaryotic ribosomes. Steps in translation: Initiation, Elongation and termination of protein synthesis. Inhibitors of protein synthesis. Post-translational modifications and its importance.

UNIT V REGULATION OF GENE EXPRESSION 9

Organization of genes in prokaryotic and eukaryotic chromosomes, Hierarchical levels of gene regulation, Prokaryotic gene regulation –lac and trp operon, Regulation of gene expression with reference to λ phage life cycle.

TOTAL : 45 PERIODS

OUTCOMES:

By the end of this course, students should be able to:

- Describe the basic structure and biochemistry of nucleic acids and proteins and discriminate between them;
- Identify the principles of DNA replication, transcription and translation and explain how they relate to each other.
- Discuss clearly about gene organization and mechanisms of control the gene expression in various organisms.
- Articulate applications of molecular biology in the modern world.

TEXT BOOKS:

1. Friefelder, David. " Molecular Biology." Narosa Publications, 1999.
2. Weaver, Robert F. " Molecular Biology" 2nd Edition, Tata McGraw-Hill,2003.
3. Karp, Gerald " Cell and Molecular Biology : Concepts and Experiments" 4th Edition, John Wiley, 2005.
4. Friefelder, David and George M. Malacinski "Essentials of Molecular Biology" 2nd Edition, Panima Publishing, 1993.
5. Lewin's GENES XI, Published by Jones & Bartlett Learning; 11 edition (January 15, 2013).

REFERENCES:

1. Tropp, Burton E. " Molecular Biology : Genes to Proteins". 3rd Edition. Jones and Bartlett, 2008.
2. Glick , B.R. and J.J. Pasternak. "Molecular Biotechnology : Principles and Applications of Recombinant DNA" 4th Edition. ASM, 2010.

BT 6504 – MOLECULAR BIOLOGY
NOV/DEC 2017

1. Differentiate primary and secondary structure of DNA

Primary structure:

Sequence of nucleotide chains. It is in these channels where the genetic information, and because the skeleton is the same for all the difference in the information lies in the different sequence of nitrogenous bases. This sequence has a code, which determines an information or otherwise, as the order of the bases.

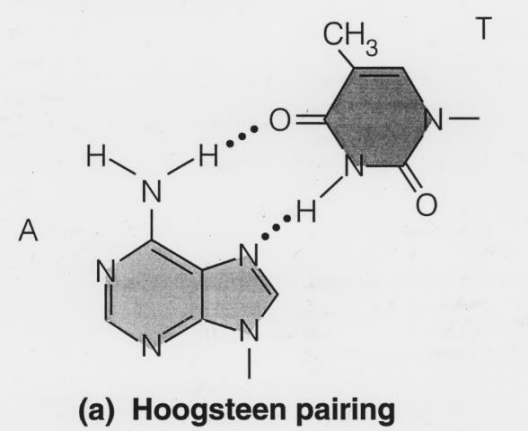
Secondary structure:

It is a double helix structure. Can explain the storage of genetic information and the mechanism of DNA

replication. It was postulated by Watson and Crick, based on X-ray diffraction that Franklin and Wilkins had been made, and the equivalence of bases Chargaff, whereby the sum of adenines more guanines is equal to the sum of thymines more cytokines.
It is a double strand, right-handed or left-handed, depending on the DNA. Both chains are complementary, as adenine and guanine in a chain are joined, respectively, thymine and cytosine on the other. Both chains are antiparallel, then the 3 'end of one faces the 5' end of the counterpart.

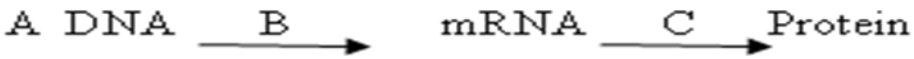
2. What is hogsteen base pairing?

A Hoogsteen base pair is a variation of base-pairing in nucleic acids such as the A. • T pair. In this manner, two nucleobases, one on each strand, can be held together by hydrogen bonds in the major groove.



(a) Hoogsteen pairing

3. Complete the following, label A, B and C and name the process central (Dogma)



A – Replication

B- Transcription

C- Translation

4. What is bidirectional replication?

DNA is double stranded molecule. Only one strand codes for proteins at any given point in the molecule. However, both strands are used during DNA replication. Each of the four bases in DNA (adenine, thymine, guanine, and cytosine) binds to a unique complementary base on the other strand. Therefore the base sequence on one strand determines the complementary sequence on the other strand. During DNA replication the two strand separate from one another and each strand has a new complementary strand built onto it. This form of replication is called bi directional; also called as semi conservative; each new DNA molecule is composed of one conserved strand from the original molecule and one new strand.

5. Which is most unstable type of RNA molecule ? why ?

mRNA is the most unstable RNA .

RNA has the normal 2` hydroxy group, and that makes the phosphodiester bond unstable and susceptible to nucleophilic attack and self-hydrolysis. When RNA is single stranded (like in mRNA), the 2`-hydroxy group can more easily reach the phosphorus atom and cause the chain to be cut. However, when RNA is in the form of a double helix (like in tRNA and lots of rRNA parts), it is limited in movement and the hydroxy group can't as easily reach the phosphorus. For this reason, structure RNA molecules (in a double helix) are much more stable. mRNA is generally unstructured and so is much less stable.

6. What is a promoter? Why is promoter significant in gene-function ?

In genetics, a **promoter** is a region of DNA that initiates transcription of a particular gene. Promoters are located near the transcription start sites of genes, on the same strand and upstream on the DNA (towards the 5' region of the sense strand). Promoters can be about 100–1000 base pairs long. For transcription to take place, the enzyme that synthesizes RNA, known as RNA polymerase, must attach to the DNA near a gene. Promoters contain specific DNA sequences such as response elements that provide a secure initial binding site for RNA polymerase and for proteins called transcription factors that recruit RNA polymerase. These transcription factors have specific activator or repressor sequences of corresponding nucleotides that attach to specific promoters and regulate gene expression.

7. How many subunits are there in E.coli RNA polymerase . Add a note on core enzyme and holo enzyme of E.Coli RNA polymerase?

E. coli RNA Polymerase, Holoenzyme is the core enzyme saturated with sigma factor 70. The Holoenzyme initiates RNA synthesis from sigma 70 specific bacterial and phage promoters.

E. coli RNA Polymerase, Core Enzyme consists of 5 subunits designated α, α, β′, β, and ω. The enzyme is free of sigma factor and does not recognize any specific bacterial or phage DNA promoters. The enzyme retains the ability to transcribe RNA from nonspecific initiation sequences. Addition of sigma factors will allow the enzyme to initiate RNA synthesis from specific bacterial and phage promoters. The core enzyme has a molecular weight of approximately 400 kDa.

8. The beta chain of eukaryotic hemoglobin is composed of 141 aminiacids. What is the minimum number of nucleotides for an mRNA coding this polypeptide chain? Assuming that each nucleotide is 0.34nm long in mRNA how many triplet codes can simultaneously occupy space in ribosome that is 20 nm in diameter?

1. Total aminoacid = 141
1 aminiacid contains 3 nucleotide
141 amino acid contains = 141*3 (423=422 (coding) + 1 stopcodon (non coding))
Therfore, Minimum number of nucleotides coding the polypeptide chain = 422

2. lengh of 1 nucleotide = 0.34nm
Length of Triplet code = 0.34 X 3 (1.02 nm)
Triplet codes can simultaneously occupy space in ribosome that is 20 nm in diameter is = 20/1.02 = 19. 6

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9. Justify the reason for arrangement of many prokaryotic genes in operons.

All the structural genes of an operon are turned ON or OFF together, due to a single promoter and operator upstream to them, but sometimes more control over the gene expression is needed. To achieve this aspect, some bacterial genes are located near together, but there is a specific promoter for each of them; this is called gene

clustering. Usually these genes encode proteins which will work together in the same pathway, such as a metabolic pathway. Gene clustering helps a prokaryotic cell to produce metabolic enzymes in a correct order.

10. What is transcription attenuation? Give an example of the operon regulated by this process.

Transcriptional attenuation is a regulatory mechanism that causes premature termination of transcription under certain conditions, thereby preventing the expression of the mRNA required for expression of the corresponding gene products. Attenuation typically results from mRNA folding into alternative secondary structures, one of which is a Rho-independent terminator.

Ex. Trp operon

PART – B

11. a) List the biologically significant difference in DNA & RNA **(TB1, 84-92)**

(OR)

b) Discuss Watson and Crick model of DNA **(TB1, 97-100**)

12. a) Detail the list of events happening during DNA replication process in prokaryotes and discuss the enzyme involved in the process. **(TB1, 209)**

(OR)

b) i) Differentiate prokaryotic and eukaryotic replication (**TB1, 271-273)**

ii) Explain any one DNA repair mechanism **(TB1, 293-305)**

13. a) Distinguish between prokaryotic and eukaryotic transcription **(TB1, 343-349)**

(OR)

b) Outline the synthesis of mRNA with a neat sketch. **(TB1, 317- 329)**

14. a) Write about genetic code . Why onlt 32 RNAs are required for translating 61 codons into 20 different amino acids **(OR)** (**TB1, 367-378)**

b) Differentiate prokaryotic and eukaryotic ribosomes**. (TB1, 439-447)**

15. a) What are the structural genes controlled by lac operon . Explain the catabolic repression effects of this operon. **(OR) (TB1, 456-462)**

b) Discuss the organization of genes in prokaryotes **(TB1, 502-510)**

PART – C

16. a) Give a detailed account on DNA as a genetic material, emphasizing their structure (**TB1, 79)(OR)**

b) Give a detailed account on prokaryotic transcription initiation, elongation and termination mechanisms with suitable diagram and factors involed in it. **(TB1, 343-349)**

TWO MARKS

UNIT I: CHEMISTRY OF NUCLEIC ACIDS

1. Define nucleic acid.

Nucleic acids are the polynucleotides having high molecular weight. The monomeric unit of which is nucleotide. **Nucleic acids** are biopolymers, or large biomolecules, essential for all known forms of life. Nucleic acids, which include DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), are made from monomers known as nucleotides. Each nucleotide has three components: a 5-carbon sugar, a phosphate group, and a nitrogenous base. If the sugar isdeoxyribose, the polymer is DNA. If the sugar is ribose, the polymer is RNA. When all three components are combined, they form a nucleic acid

2. Define bacterial transformation.

Bacterial transformation the exchange of genetic material between strains of bacteria by the transfer of a fragment of naked DNA from a donor cell to a recipient cell, followed by recombination in the recipient chromosome.

3. What are the properties of genetic material?

Stores genetic information, Physical and Chemical stability (The double stranded protects the DNA from chemical attack) Able to undergo mutation Stored information accessible to progeny

4. What is okazaki fragment?

Okazaki fragments are short, newly synthesized DNA fragments that are formed on the lagging template strand during DNA replication. They are complementary to the lagging template strand, together forming short double-stranded DNA sections. Okazaki fragments are between 1,000 to 2,000 nucleotides long in Escherichia coli and are between 100 to 200 nucleotides long in eukaryotes. They are separated by ~10-nucleotide RNA primers and are unligated until RNA primers are removed, followed by enzyme ligase connecting (ligating) the two Okazaki fragments into one continuous newly synthesized complementary strand

5. Explain cantenation.

Catenation is the ability of a chemical element to form a long chain-like structure via a series of covalent bonds. Catenation occurs most readily in carbon, which forms covalent bonds with other carbon atoms

6. What are Satellite DNA?

Satellite DNA consists of very large arrays of tandemly repeating, non-coding DNA. Satellite DNA is the main component of functional centromeres, and form the main structural constituent of heterochromatin.

The name "satellite DNA" refers to how repetitions of a short DNA sequence tend to produce a different frequency of the nucleotides adenine, cytosine, guanine and thymine, and thus have a different density from bulk DNA - such that they form a second or 'satellite' band when genomic DNA is separated on a density gradient

7. Differentiate prokaryotic and eukaryotic promoters.

Prokaryotic promoters

In prokaryotes, the promoter consists of two short sequences at -10 and -35 positions upstream from the transcription start site. The sequence at **-10** is called the Pribnow box, or the -10 element, and usually consists of the six nucleotides **TATAAT.** The Pribnow box is absolutely essential to start transcription in prokaryotes. The other sequence at **-35** (the -35 element) usually consists of the six nucleotides TTGACA. Its presence allows a very high transcription rate.

Eukaryotic promoters

Eukaryotic promoters are extremely diverse and are difficult to characterize. They typically lie upstream of the gene and can have regulatory elements several kilobases away from the transcriptional start site. In eukaryotes, the transcriptional complex can cause the DNA to bend back on itself, which allows for placement of regulatory sequences far from the actual site of transcription. Many eukaryotic promoters, contain a TATA box (sequence **TATAAA**), which in

turn binds a TATA binding protein which assists in the formation of the RNA polymerase transcriptional complex. The TATA box typically lies very close to the transcriptional start site (often within 50 bases).

8. What are the three enzymatic activities for DNA polymerase I?

DNA Polymerase I (or **Pol I**) is an enzyme that participates in the process of DNA replication and is exclusively found in prokaryotes. It is composed of 928 amino acids, and is an example of a processive enzyme - it can sequentially catalyze multiple polymerisations. Discovered by Arthur Kornberg in 1956,[1] it was the first known DNA polymerase (and, indeed, the first known of any kind of polymerase). It was initially characterized in E. coli, although it is ubiquitous in prokaryotes. In E. coli and many other bacteria, the gene that encodes Pol I is known as **polA**.

9. **Describe the biological significance of nucleic acids**.

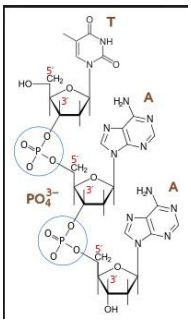
Nucleic acids are large molecules that carry tons of small details: all the genetic information. Nucleic acids are found in every living thing — plants, animals, bacteria, viruses, fungi — that uses and converts energy. Every single living thing has something in common. People, animals, plants, and more all are connected by genetic material. Every living thing may look different and act different, but deep down — way deep down in the nucleus of cells — living things contain the same chemical "ingredients" making up very similar genetic material. There are two types of nucleic acids: DNA (which stands for deoxyribonucleic acid) and RNA (which stands for ribonucleic acid). Nucleic acids are made up of strands of nucleotides, which are made up of a base containing nitrogen (called a nitrogenous base), a sugar that contains five-carbon molecules, and a phosphoric acid.

10. Explain Chargaff's rule.

Chargaff's rules states that DNA from any cell of all organisms should have a 1:1 ratio (base Pair **Rule**) of pyrimidine and purine bases and, more specifically, that the amount of guanine is equal to cytosine and the amount of adenine is equal to thymine.

11. Describe the properties of phosphodiester bond

In DNA and RNA, the **phosphodiester bond** is the linkage between the 3' carbon atom of one sugar molecule and the 5' carbon atom of another, deoxyribose in DNA and ribose in RNA. Strong covalent**bonds** form between the phosphate group and two 5-carbon ring carbohydrates (pentoses) over two ester **bonds**.



12. Explain secondary structure of DNA.

Secondary structure is the set of interactions between bases, i.e., which parts of strands are bound to each other. In DNA double helix, the two strands of DNA are held together by

hydrogen bonds. The nucleotides on one strand base pairs with the nucleotide on the other strand.

13. List out the forces that stabilize the structure of DNA

The nitrogenous bases are positioned inside the helixstructure like "rungs on a ladder," due to the hydrophobic effect, and stabilized by hydrogen bonding. The two strands run in opposite directions to form the double helix. The strands are held together by hydrogen bonds and hydrophobic interactions.

14. Define secondary structure of DNA.

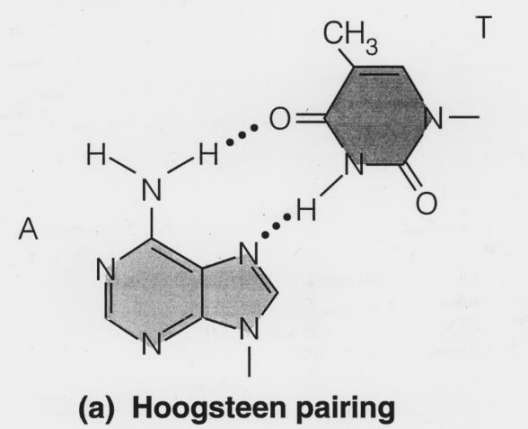
Secondary structure is the set of interactions between bases, i.e., which parts of strands are bound to each other. In DNA double helix, the two strands of DNA are held together by hydrogen bonds. The nucleotides on one strand base pairs with the nucleotide on the other strand.

15. Define phosphor diester bond.

A phosphodiester bond occurs when exactly two of the hydroxyl groups in phosphoric acid react with hydroxyl groups on other molecules to form two ester bonds. An example is found in the linking of twopentose (5 carbon sugar) rings to a phosphate group by strong, covalent ester bonds. Each ester bond is formed by a condensation reaction in which water is lost. This bond is a key structural feature of the backbone of DNA and RNA and links the 3' carbon of one nucleotide to the 5' carbon of another to produce the strands of DNA and RNA. In phosphodiester formation, two hydroxyl (OH) groups on the phosphate molecule bind to the 3' and 5' carbons on two independent pentose sugars. These are two condensation reactions, so two molecules of water are produced. The phosphate is then bonded to the sugars by two ester bonds, hence the nomenclature of phosphodiester bond. This reaction is catalysed by ligases, such as DNA ligase during DNA replication.

16. Define hogsteen base pairing.

A Hoogsteen base pair is a variation of base-pairing in nucleic acids such as the A. • T pair. In this manner, two nucleobases, one on each strand, can be held together by hydrogen bonds in the major groove.



17. Define a triple helix DNA.

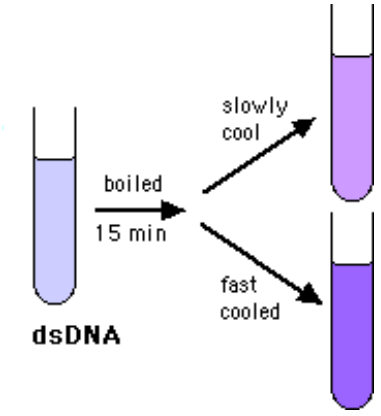
DNA can form multi-stranded helices through either folding of one of the two strands or association of two, three, or four strands of DNA. Triple-helical nucleic acids were first described in 1957 by Felsenfeld and Rich, who demonstrated that polyuridylic acid and polyadenylic acids strands in a 2:1 ratio were capable of forming a stable complex. In 1986, it was demonstrated that a short (15-mer) mixed-sequence triplex-forming oligonucleotide (TFO) formed a stable specific triple helical DNA complex . The third strand of DNA in the triplex structure (i.e.the TFO) follows a path through the major groove of the duplex DNA. The specificity and stability of the triplex structure is afforded via Hoogsteen hydrogen bonds , which are different from those formed in classical Watson-Crick base pairing in duplex DNA. Because purines contain potential hydrogen bonds with incoming third strand bases, the binding of the third strand is to the purine-rich strand of the DNA duplex.

18. Explain the quadruple structure of DNA.

The structures are called G-quadruplexes, because they form in regions of DNA that are full of guanine, one of the DNA molecule's four building blocks. The others are adenine, cytosine and thymine. A hydrogen bond is responsible for holding the four guanines together. The four stranded DNA usually presents itself right before cell division.

19. Explain the hyperchromic effect of DNA.

DNA's hyperchromic effect means that ssDNA absorbs more UV than does dsDNA). An insect, which can see in the UV range,* would see the hyperchromic effect something like that shown to the right. At first the DNA solution is only a little violet. If it is boiled and then slowly cooled, it ends up a little more violet than it started, but if it is rapidly cooled it becomes most violet. The reason that this happens is that in dsDNA the pi-electrons in the aromatic rings are more constrained because the H-bonded rings are in sandwich layers - overlapping with each other. But if the H-bonds are "boiled" away, the sandwich no longer exists and the pi-electrons are more free to move into different energy levels and thus able to absorb more UV energy.



20. Define a super coiled DNA.

A double helix (as of DNA) that has undergone additional twisting in the same direction as or in the opposite direction from the turns in the original helix. The term "supercoiling" means literally the coiling of a coil. A telephone cord for example, is typically a coiled wire. The twisted path often taken by that wire as it goes from the base of the phone to the receiver

generally describes a supercoil. DNA is coiled in the form of a double helix. Let us define an axis about which both strands of the DNA coil. A bending or twisting of that axis upon itself is referred to as DNA supercoiling. DNA supercoiling is generally a manifestation of structural strain. Conversely, if there is no net bending of the DNA axis upon itself, the DNA is said to be in a relaxed state. It is probably apparent that DNA compaction must involve some form of supercoiling. Perhaps less apparent is the fact that replicating or transcribing DNA also must induce some degree of supercoiling.

21. What is the basic difference between B and Z type of DNA?

B Type:

In a DNA molecule, the two strands are not parallel, but intertwined with each other. Each strand looks like a helix. The two strands form a "**double helix**" structure, which was first discovered by James D. Watson and Francis Crick in 1953. In this structure, also known as the **B form**, the helix makes a turn every 3.4 nm, and the distance between two neighboring base pairs is 0.34 nm. Hence, there are about 10 pairs per turn. The intertwined strands make two grooves of different widths, referred to as the **major groove** and the **minor groove**, which may facilitate binding with specific proteins.

Z Type:

Another DNA structure is called the **Z form**, because its bases seem to zigzag. Z DNA is left-handed. One turn spans 4.6 nm, comprising 12 base pairs. The DNA molecule with alternating G-C sequences in alcohol or high salt solution tends to have such structure.

22. Relate hyperchromocity and denaturation of DNA

Hyperchromicity is the increase of absorbance (*optical density*) of a material. The most famous example is the hyperchromicity of DNA that occurs when the DNA duplex is denatured. The UV absorption is increased when the two single DNA strands are being separated, either by heat or by addition of denaturant or by increasing the pH level. The opposite, a decrease of absorbance is called **hypochromicity**. Heat denaturation of DNA, also called melting, causes the double helix structure to unwind to form single stranded DNA. When DNA in solution is heated above its melting temperature (usually more than 80 °C), the double-stranded DNA unwinds to form single-stranded DNA. The bases become unstacked and can thus absorb more light.

23. Mention two differences between prokaryotic DNA polymerase I and II

	DNA polymerase I	DNA polymerase II
1	Composed of 928 amino acids	Composed of 783 amino acids
2	Belongs to polymerase family A	Belongs to polymerase family B
3	Responsible for DNA repair and removing RNA primers	Responsible for proofreading, fidelity, and processivity of newly formed DNA

24. What is a molecular 'chaperone'?

Most biological structures assemble by themselves into larger structures; some (e.g., the icosahedral DNA bacteriophage P22) require help from an additional molecule that is not found in the final structure.

25. What properties of a protein ensure that it is localized to a particular part of a cell?

Nuclear, cytoplasmic, and extracellular proteins each have characteristic surfaces (cytoplasmic proteins have a balance of acidic and basic residues, extracellular proteins a slight excess of acidic residues, and nuclear proteins a pronounced excess of basic residues); then, we might imagine that newly-made proteins diffuse throughout the cell, to bind at a specific location. Individual proteins also have short peptide sequences (e.g., nuclear localization signals) that target the whole protein to a specific subcellular compartment (e.g., the nucleus).

26. Why are nuclei often round in shape?

Surface tension is a major determinant. The nucleus has a fluid system that is immiscible with its surroundings. Its surface tends towards the spherical minimum, and it is immersed in a medium that transmits on all sides a uniform fluid (hydrostatic) pressure; therefore, a nucleus is spherical.

27. Outline the general structure of the nucleosome. What is the evidence for this structure?

Structure: 146 bp of duplex DNA wrapped around a histone octamer (two copies of H3, H4, H2A, H2B) in 1.65 turns of a flat, left-handed superhelix.
Evidence: 2 copies of each core histone (and ~1 of H1) per ~200 bp DNA, the 'beads-on-a-string' structure seen by electron microscopy, nuclease digestion of linker DNA to give a nucleosomal ladder (repeat 180-260 bp) following by trimming of the linker to give the core particle with 146 bp DNA, the structure of the core particle determined by X-ray crystallography.

28. How can the lengths of DNA in the nucleosome, and between nucleosomes, be determined?

By progressive digestion of chromatin with nucleases (e.g., micrococcal nuclease), purification of DNA, and sizing the resulting fragments by gel electrophoresis. The distance between 'rungs' in the resulting nucleosomal 'ladder' gives the repeat length, while the length of the most resistant fragments gives the length of DNA in the nucleosome. The length of the linker can be obtained by subtraction.

29. **Describe the evidence for and against the existence *in vivo* of a 'solenoid'**.

For: electron microscopy of chromatin fibres in buffers of low ionic strength reveals helical solenoids with six to eight nucleosomes/turn and an 11 nm pitch.
Against: solenoids are not seen at a physiological salt concentration, or in electron micrographs of rapidly-frozen whole cells.

30. What is the evidence that the chromatin fiber is organized into loops in the interphase nucleus?

(i) it seems inconceivable that long DNA molecules are packed randomly like spaghetti, (ii) direct observation of lampbrush chromosomes (loops visible in unfixed material, but they might form when nuclei are dispersed), (iii) the demonstration of supercoiling in 'nucleoids' (but loops might be created artifactually during reparation), (iv) the rate at which nucleases release chromatin from interphase nuclei (but isolated in hypotonic buffers) or permeabilized

cells (isolated in isotonic buffers). Go onto discuss the analysis of residual fragments, and how the results suggest that attachments (and so loops) change continually.

UNIT II DNA REPLICATION & REPAIR

1. Explain the central dogma of molecular biology.

The 'Central Dogma' is the process by which the instructions in DNA are converted into a functional product. It was first proposed in 1958 by Francis Crick, discoverer of the structure of DNA. The central dogma of molecular biology explains the flow of genetic information, from DNA or RNA, to make a functional product, a protein. The central dogma suggests that DNA contains the information needed to make all of our proteins, and that RNA is a messenger that carries this information to the ribosomes. The ribosomes serve as factories in the cell where the information is 'translated' from a code into the functional product. The process by which the DNA instructions are converted into the functional product is called gene expression. Gene expression has two key stages - transcription and translation. In transcription, the information in the DNA of every cell is converted into small, portable RNA messages. During translation, these messages travel from where the DNA is in the cell nucleus to the ribosomes where they are 'read' to make specific proteins. The central dogma states that the pattern of information that occurs most frequently in our cells is:

From existing DNA to make new DNA (DNA replication)
From DNA to make new RNA (transcription)
From RNA to make new proteins (translation).

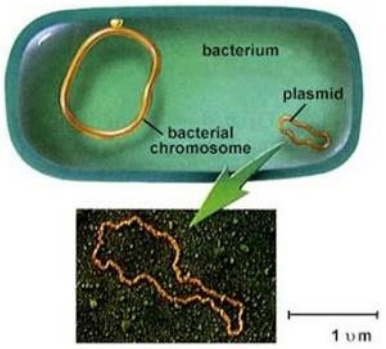
2. Differentiate prokaryotic and eukaryotic chromosome.

Chromosomes: The vehicle by which hereditary information is physically transmitted from one generation to the next; in a bacterium, the chromosome consists of a single naked circle of DNA; in eukaryotes, each chromosome consists of a single linear DNA molecules and associated proteins. *Read more*

Prokaryotic Chromosome vs Eukaryotic Chromosome

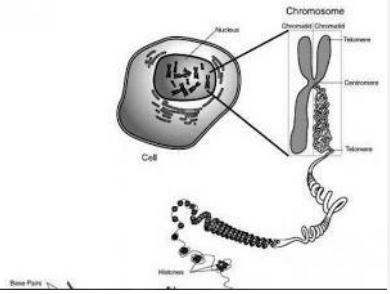
Prokaryotic Chromosome

- Found in cytoplasm.
- Circular chromosome attached to the inside of the cell membrane.
- Single chromosome plus plasmids.
- Made only of DNA.
- Copies its chromosome and divides immediately afterwards.



Eukaryotic Chromosome

- Found in nucleus
- Linear chromosomes
- Many chromosomes.
- Usually,10-50 chromosomes in somatic cells.
- Human body cells have 46 chromosomes.
- Made of chromatin, a nucleoprotein (DNA coiled around histone proteins).
- Copies chromosomes, then the cell grows (G2 phase), then goes through mitosis to organise chromosomes in two equal groups.



3. Define replication in DNA.

DNA replication is the process by which a double-stranded DNA molecule is copied to produce two identical DNA molecules. Replication is an essential process because, whenever a cell divides, the two new daughter cells must contain the same genetic information, or DNA, as the parent cell.

The replication process relies on the fact that each strand of DNA can serve as a template for duplication. DNA replication initiates at specific points, called origins, where the DNA double helix is unwound. A short segment of RNA, called a primer, is then synthesized and acts as a starting point for new DNA synthesis. An enzyme called DNA polymerase next begins replicating the DNA by matching bases to the original strand. Once synthesis is complete, the RNA primers are replaced with DNA, and any gaps between newly synthesized DNA segments are sealed together with enzymes.

4. Explain the bi directional mode of replication in DNA.

DNA is double stranded molecule. Only one strand codes for proteins at any given point in the molecule. However, both strands are used during DNA replication. Each of the four bases in DNA (adenine, thymine, guanine, and cytosine) binds to a unique complementary base on the other strand. Therefore the base sequence on one strand determines the complementary sequence on the other strand. During DNA replication the two strand separate from one another and each strand has a new complementary strand built onto it. This form of replication is called bi directional; also called as semi conservative; each new DNA molecule is composed of one conserved strand from the original molecule and one new strand.

5. What are called okazaki fragments?

Okazaki fragments are the Short segments of DNA, 1000 to 2000 bases long, that later join up to form continuouslengths of DNA. Okazaki fragments occur in replicating DNA in both prokaryotes and eukaryotes. They form up on the'lagging' strand during replications and join by ligation. (Reiji Okazaki, Japanese geneticist.) They are the DNA sequences, 100 to 200 nucleotides long, synthesized on the lagging strand of DNA in DNA replication. Thefragments are subsequently ligated together to form a continuous strand. They are produced because of the need forDNA polymerase to always synthesize in a 5′ to 3′ direction.

6. Explain the fidelity of DNA.

The fidelity of a DNA polymerase is the result of accurate replication of a desired template. Specifically, this involves multiple steps, including the ability to read a template strand, select the appropriate nucleoside triphosphate and insert the correct nucleotide at the 3' primer terminus, such that Watson-Crick base pairing is maintained. To effectively discriminate correct vs. incorrect nucleotide incorporation, some DNA polymerases possess a 3' to 5' exonuclease activity. This activity, known as "proofreading," is used to excise incorrectly incorporated mononucleotides, which are then replaced with the correct nucleotides. High-fidelity PCR uses DNA polymerases that couple low misincorporation rates with proofreading to give faithful replication of the target DNA of interest.

7. When is fidelity important?

When designing your PCR experiment, the first question you should ask is whether or not your application requires a high-fidelity polymerase. If the outcome of your experiment depends on the correct DNA sequence (e.g., cloning or next-generation sequencing applications), you'll want to minimize the incorporation of mismatched nucleotides by using a high-fidelity polymerase. Fidelity is less important for standard PCR or colony PCR to determine the presence or absence of an amplicon or to confirm that your plasmid has an insert. Because of the robust nature of certain high-fidelity polymerases, some researchers use them for all their amplifications, regardless of the PCR product's downstream use.

8. How do you measure fidelity?

Vendors use a variety of different methods to determine the fidelity of their DNA polymerases. One assay, first described by Thomas Kunkel, uses portions of the lacZα gene in M13 bacteriophage to correlate host bacterial colony color changes with errors in DNA synthesis. Building on the Kunkel assay, Wayne Barnes' assay is a common permutation found in labs, in which PCR is used to copy the entire lacZ gene and portions of two drug-resistance genes, with subsequent ligation, cloning, transformation and blue/white-colony color determination. The readout of both assays is a white-colony phenotype caused by the disruption of β-galactosidase activity that results from errors in the lacZ gene. With these lacZ-based experimental approaches, the percentage of white colonies must be converted to the number of errors per base incorporated. For a more direct readout of fidelity, Sanger sequencing of individual cloned PCR products also can be used to score DNA polymerase fidelity and offers the advantage of detecting all mutations. Using this method, the entire mutational spectrum of a polymerase can be determined, and there is no need to correct for nonphenotypic changes.

9. List out certain inhibitors of DNA replication

Alkylating antineoplastic agents, Nitrogen mustards, Topoisomerase inhibitors, Altretamine, Bleomycin, Dacarbazine, Dactinomycin, Mitobronitol, Mitomycins, Mitosene, Pingyangmycin, Plicamycin, Procarbazine, Temozolomide

10. Differntiate Eukaryotic and prokaryotic replication.

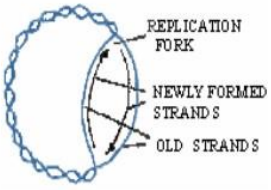
DNA replication in Prokaryotes

1. It occurs inside the cytoplasm

2. There is only one origin of replication per DNA molecule

3. Origin of replication is formed of about 100-200 or more nucleotides

4. Replication of DNA occurs at one point in each prokaryotic DNA molecule



5. Only two replication fork is formed in each replicating prokaryotic chromosome, as DNA replication is bidirectional

6. Prokaryotic chromosome has one replicon

7. One replication bubble is formed during DNA replication

8. Initiation of DNA replication in prokaryotes is carried out by protein DnaA and DnaB

9. DNA gyrase is needed

10. Okazaki fragment are large, 1000-2000 nucleotides long.

11. Replication is very rapid, some 2000 bp per second are added.

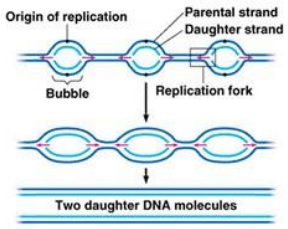
DNA replication in Eukaryotes

1. It occurs inside the nucleus

2. Origin of replication are many(over 1000) in each eukaryotic chromosome

3. Each origin of replication is formed of about 150 nucleotides

4. Replication of DNA occurs at several points simultaneously in each chromosome.



5. A number of replication forks are formed simultaneously in each replicating DNA.

6. Eukaryotic DNA molecules have large number of replicons (50,000 and above), but replication does not occur simultaneously on all replicons

7. Numerous replication bubbles are formed in one replicating DNA molecule.

8. Initiation of DNA replication is carried out by multisubunit protein, origin recognition complex.

9. DNA gyrase is needed

10. Okazaki fragment are short, 100-200 nucleotides long.

11. Replication is slow, some 100 nucleotides per second are added

11. Define telomere.

Telomeres are an essential part of human cells that affect how our cells age. Telomeres are the caps at the end of each strand of DNA that protect our chromosomes, like the plastic tips at the end of shoelaces. Without the coating, shoelaces become frayed until they can no longer do their job, just as without telomeres, DNA strands become damaged and our cells can't do their job.

***12.* Describe the role of telomere in the replication.**

- DNA polymerase cannot replicate and repair DNA molecules at the ends of linear chromosomes.
- The ends of linear chromosomes, called telomeres, protect genes from getting deleted as cells continue to divide.
- The telomerase enzyme attaches to the end of the chromosome; complementary bases to the RNA template are added on the 3' end of the DNA strand.
- Once the lagging strand is elongated by telomerase, DNA polymerase can add the complementary nucleotides to the ends of the chromosomes and the telomeres can finally be replicated.
- Cells that undergo cell division continue to have their telomeres shortened because most somatic cells do not make telomerase; telomere shortening is associated with aging.
- Telomerase reactivation in telomerase-deficient mice causes extension of telomeres; this may have potential for treating age-related diseases in humans.

13. Describe the rolling circle mode of replication.

Rolling circle replication is the unidirectional mode of DNA replication employed by circular DNA molecules, such as plasmids and the genomes of bacteriophages and some eukaryotic viruses. In viruses with linear genomes, the ability to circularise once inside a cell is a

crucial prerequisite for rolling circle replication. By replicating in this fashion, the virus can ensure that no genetic material is lost from its genome as a consequence of successive rounds of replication. Circularisation of linear phage genomes occurs by the interaction between cos sites (cohesive sites) in the viral genome.

The process begins with a plasmid or phage-encoded enzyme called relaxase, which creates a nick in the circular DNA at a site called the double-strand origin (DSO); the relaxase remains bound to the 5' phosphate at the site of this nick, so the 3' OH group is available as a primer for DNA synthesis by DNA polymerase III. The polymerase moves along the nicked strand, using the un-nicked strand as a template for replication, and a helicase displaces the nicked strand behind polymerase as a single-stranded DNA molecule. This procedure can be repeated multiple times to create numerous linear copies in a continuous head-to-tail series called a concatemer.

To make these linear strands double-stranded and circular again, an initiator protein makes another nick to terminate DNA synthesis. DNA polymerase III and RNA polymerase then work in conjunction to replicate the single-strand origin (SSO) of a linear strand to make it double-stranded. Finally, DNA polymerase I removes the primer, replacing it with DNA, and DNA ligase covalently binds the strands end-to-end to make the final circular structure.

14. What is D- Loop replication?

D-loop replication is a process by which chloroplasts and mitochondria replicate their genetic material. An important component of understanding D-loop replication is that manychloroplasts and mitochondria have a single circular chromosome like bacteria instead of the linear chromosomes found in eukaryotes. However, many chloroplasts andmitochondria have a linear chromosome, and D-loop replication is not important in these organelles. In many organisms, one strand of DNA in the plastid comprises heavier nucleotides (relatively more purines: adenine and guanine). This strand is called the H (heavy) strand. The L (light) strand comprises lighter nucleotides (pyrimidines: thymine and cytosine). Replication begins with replication of the heavy strand starting at the D-loop (also known as the control region). An origin of replication opens, and the heavy strand is replicated in one direction. After heavy strand replication has continued for some time, a new light strand is also synthesized, through the opening of another origin of replication. When diagramed, the resulting structure looks like the letter D. The D-loop region is important for phylogeographic studies. Because the region does not code for any genes, it is free to vary with only a few selective limitations on size and heavy/light strand factors. The mutation rate is among the fastest of anywhere in either the nuclear or mitochondrial genomes in animals. Mutations in the D-loop can effectively track recent and rapid evolutionary changes such as within species and among very closely related species.

15. What are mutagens?

Chemical mutagens are classified as alkylating agents, cross-linking agents, and **polycyclic aromatic hydrocarbons** (PAHs). Alkylating agents act by adding molecular components to DNA bases, which alters the protein product. Cross-linking agents create covalent bonds with DNA bases, while PAHs are metabolized by the human body into other potentially mutagenic molecules.

Radiation is another potent mutagen. For biologists, the most significant forms of radiation are **light**, **heat**, and **ionizing radiation**. Ionizing radiation can penetrate cells and create ions in the cell contents. These, in turn, can cause permanent alterations in DNA; that is, mutations. Ionizing radiation includes: **x rays**, gamma rays, and the subatomic particles—neutrons, electrons ("beta" particles), and alpha particles (helium nuclei). Ionizing radiation alters the way two strands of DNA interact. This high **energy** radiation passes through cells and tissues, cutting up any DNA in its path. It can rearrange entire sections of the chromosomes, altering relatively long stretches of DNA. UV radiation causes covalent bonds to form between neighboring thymine bases in the DNA, so altering the DNA product at that location.

16. Explain the importance of DNA repair mechanism.

DNA in the living cell is subject to many chemical alterations (a fact often forgotten in the excitement of being able to do DNA sequencing on dried and/or frozen specimens. If the genetic information encoded in the DNA is to remain uncorrupted, any chemical changes must be corrected. **A failure to repair DNA produces a mutation.** The recent publication of the human genome has already revealed 130 genes whose products participate in DNA repair. More will probably be identified soon.

17. What are the agents that can damage DNA?

- Agents that Damage DNA
- Certain wavelengths of radiation
- ionizing radiation such as gamma rays and X-rays
- Ultraviolet rays, especially the UV-C rays (~260 nm) that are absorbed strongly by DNA but also the longer-wavelength UV-B that penetrates the ozone shield.
- Highly-reactive oxygen radicals produced during normal cellular respiration as well as by other biochemical pathways.
- Chemicals in the environment
- many hydrocarbons, including some found in cigarette smoke
- some plant and microbial products, e.g. the aflatoxins produced in moldy peanuts
- Chemicals used in chemotherapy, especially chemotherapy of cancers

18. What are the Types of DNA Damage?

All four of the bases in DNA (A, T, C, G) can be covalently modified at various positions. One of the most frequent is the loss of an amino group ("deamination") — resulting, for example, in a **C** being converted to a U. Mismatches of the normal bases because of a failure of proofreading during DNA replication. Common example: incorporation of the pyrimidine U (normally found only in RNA) instead of T. Breaks in the backbone. Can be limited to one of the two strands (a single-stranded break, SSB) or on both strands (a double-stranded break (DSB). Ionizing radiation is a frequent cause, but some chemicals produce breaks as well. Crosslinks Covalent linkages can be formed between bases on the same DNA strand ("intrastrand") or on the opposite strand ("interstrand").

19. Explain the Meselson–Stahl experiment

The Meselson-Stahl was an experiment by Matthew Meselson and Franklin Stahl with some additional help from a Canadian biologist, Mason MacDonald, and Indian-Canadian nuclear physicist, Amandeep Sehmbi, in 1958 which supported the hypothesis that DNA

replication was semiconservative. In semiconservative replication, when the double stranded DNA helix is replicated, each of the two new double-stranded DNA helices consisted of one strand from the original helix and one newly synthesized. It has been called "the most beautiful experiment in biology. Meselson and Stahl decided the best way to tag the parent DNA would be to change one of the atoms in the parent DNA molecule. Since nitrogen is found in the nitrogenous bases of each nucleotide, they decided to use an isotope of nitrogen to distinguish between parent and newly copied DNA. The isotope of nitrogen had an extra neutron in the nucleus, which made it heavier.

20. What are the enzymes that contribute for excision repair mechanisms?

- DNA glycosylases.
- AP endonucleases.
- End processing enzymes.
- DNA polymerases.
- Flap endonuclease.
- DNA ligase
- MBD4.
- NEIL1.

21. List the natural agents that commonly cause damage in our DNA.

Water (deamination, depurination), oxygen (through the superoxide radical, hydrogen peroxide, and hydroxyl radical).

22. Outline the principles involved in eukaryotic DNA synthesis.

Restriction to S phase, semi-conservative replication, initiation at internal origins, simultaneous replication of many chromosomal segments as they move through polymerization sites in factories, strand separation to give a replication bubble flanked by two replication forks, requirements for primers and a primase, growth 5'-to-3', continuous and discontinuous synthesis on leading and lagging strands.

23. How would you demonstrate that active DNA polymerases are fixed to an underlying structure in the nucleus?

Permeabilize cells, treat -/+ nuclease, remove detached chromatin, measure remaining polymerizing activity by incorporation of radiolabeled dTTP; removing most chromatin leaves most activity.

24. Outline the different approaches used to label sites of DNA synthesis in eukaryotic nuclei, and the difficulties associated with each one.

By immunolabeling polymerases: not all enzyme active. *By autoradiography with [^{3}H]thymidine*: slow entry and conversion to immediate precursor, dilution by endogenous pools, complications of rapidity of DNA synthesis, long path-length of -particles. *By immunolabeling after incubation with Br-dU*: slow entry and conversion, but higher resolution afforded by immuno-EM. *By immunolabelling after permeabilization with immediate precursors like Br-dUTP and biotin-dUTP*: control of elongation rate, but lysis might alter structure, and still limited resolution (even with immunogold labeling). Details of factories best seen after removing most chromatin.

25. What is the unwinding problem, and how might it be solved in theory and in practice?

Each strand in a DNA duplex is entwined about its partner, and must be untwined during replication. *Theoretical solutions*: by rotation about ends (but it these are fixed the two strands remain interlocked), by cutting one or other of the strands (or both), passing one (or both) strands through the break, and resealing the break. *Practical solution*: topoisomerases cut, pass, and reseal.

26. Describe the structure of the origin of replication in *E. coli.*

OriC contains: four 9-mers containing a specific recognition sequence (i.e., 5'-TTAT(C/A)CA(C/A)) for the initiator protein dnaA, three 13-mers that melt easily, 11 potential sites (i.e., GATC) of methylation by the Dam methylase, and 2 back-to-back promoters that may be involved in the initiation of replication.

27. What is an 'autonomously-replicating sequence' (ARS)? How was the first one identified in yeast?

ARS: DNA sequence that enables circular plasmid lacking origin to replicate in yeast cells, usually equivalent to an origin of replication. *Discovery*: The first ARS was obtained as follows. Yeast mutants lacking the *LEU* gene cannot form colonies without added leucine. Even on transformation with a bacterial plasmid carrying the yeast LEU^+ gene, few colonies result; this is because the plasmid is unable to replicate along with the yeast chromosomes and is soon diluted out. However, if random pieces of yeast DNA are inserted into the plasmid, a few will now contain a yeast replication origin and so can replicate in yeast cells. Cells carrying such a plasmid will grow into a colony since they contain both the LEU^+ gene and a yeast origin that facilitates plasmid replication.

28. Outline the problem associated with replicating the ends of a chromosome, and some solutions.

A polymerase can extend a leading strand to the very end, but removal of a primer at the 5' end of the lagging strand leaves a gap that cannot be filled, as no 3'OH is available. *Solutions*: use protein-nucleotide priming (adenovirus), form a hairpin, concatamer, or circle (e.g., in vaccinia, T7 and lambdoid viruses), maintain ends by recombination (e.g., T4 bacteriophage), use telomerase.

29. Outline the properties of telomerase.

It is part protein and part RNA, protein part has homology with reverse transcriptases, RNA part contains 8-30 nucleotides of RNA containing 1.2-1.9 copies of the C-strand repeat that templates synthesis of telomeric DNA.

30. How were replication factories imaged in *B. subtilis*?

Using a PolC-GFP construct - one discrete spot is generally seen in the middle of the cell.

31. What is a proofreading activity?

A 3'->5' exonuclease (either part of the catalytic subunit of a DNA polymerase, or a subunit of the polymerizing complex) that removes mispaired bases immediately after they have been incorporated.

UNIT III - TRANSCRIPTION

1. Define bacterial transformation.

Bacterial transformation the exchange of genetic material between strains of bacteria by the transfer of a fragment of naked DNA from a donor cell to a recipient cell, followed by recombination in the recipient chromosome.

2. What are the properties of genetic material?

- Stores genetic information
- Physical and Chemical stability (The double stranded protects the DNA from chemical attack)
- Able to undergo mutation
- Stored information accessible to progeny

3. Describe the basic rule for the replication of all nucleic acids.

- The primary role of any mode of replication is to duplicate the base sequence of the parent molecule. The specificity of base pairing adenine with thymine and guanine with cytosine provides the mechanism used by all replication systems.
- Nucleotide monomers are added one by one to the end of a growing strand by an enzyme called a DNA polymerase.
- The sequence of bases in each new or daughter strand is complementary to the base sequence in the original template or parent strand being copied that is, if there is an adenine in the parent strand, a thymine nucleotide will be added to the end of the growing daughter strand when the adenine is being copied.

4. Define a TATA box.

The TATA box (also called Goldberg-Hogness box) is a DNA sequence (cis-regulatory element) found in the promoter region of genes in archaea and eukaryotes; approximately 24% of human genes contain a TATA box within the core promoter

5. Explain any four general features of enhancers

Several DNA sequences of note have been detected in eukaryotic genes. The first that was described was an enhancer sequence. Enhancers have the ability to greatly increase the expression of genes in their vicinity

6. Define genetic code.

The genetic code is the set of rules by which information encoded in genetic material (DNA or mRNA sequences) is translated into proteins (amino acid sequences) by living cells.

7. Give any two inhibitors of protein synthesis in eukaryotes along with its action

Protein synthesis is a complex, multi-step process involving many enzymes as well as conformational alignment. However, the majority of antibiotics that block bacterial protein synthesis interfere with the processes at the 30S subunit or 50S subunit of the 70S bacterial

ribosome. The aminoacyltRNA synthetases that activate each amino acid required for peptide synthesis are not antibiotic targets. Instead, the primary steps in the process that are attacked are

- The formation of the 30S initiation complex (made up of mRNA, the 30S ribosomal subunit, and formyl-methionyl-transfer RNA),
- The formation of the 70S ribosome by the 30S initiation complex and the 50S ribosome, and
- The elongation process of assembling amino acids into a polypeptide.

8. Why genetic activity is regulated?

- To discuss the structure and transcription of bacterial gene
- To describe the molecular mechanism and to regulate gene activity

9. What is temperature sensitive mutation?

A viral mutant that is able to replicate at one portion of a temperature range but not at another, the parent (wild type) strain being able to replicate over the whole temperature range.

10. Explain cantenation.

Catenation is the ability of a chemical element to form a long chain-like structure via a series of covalent bonds. Catenation occurs most readily in carbon, which forms covalent bonds with other carbon atoms

11. What are Satellite DNA?

Satellite DNA consists of very large arrays of tandemly repeating, non-coding DNA. Satellite DNA is the main component of functional centromeres, and form the main structural constituent of heterochromatin. The name "satellite DNA" refers to how repetitions of a short DNA sequence tend to produce a different frequency of the nucleotides adenine, cytosine, guanine and thymine, and thus have a different density from bulk DNA - such that they form a second or 'satellite' band when genomic DNA is separated on a density gradient.

12. Define linkage

Two genes are said to be under linkage, or linked, when they reside in the same chromosome. For example, the research of the human genome discovered that the factor III of clotting gene and the factor V of clotting gene are located in the same chromosome (the human chromosome 1). The factor VII gene however is not linked to those genes since it is located in the chromosome 13

13. Explain suppressor sensitive mutation.

A conditionally lethal, host range, bacteriophage mutant that produces nonsense codons and can replicate only in a host bacterium able to translate the nonsense codon; the mutation's effects are lethal (i.e., prevent replication of the virus) in a bacterium without such a suppressor mechanism.

14. What is breathing means in DNA structure?

Recent claim is discussed that Watson-Crick pairs in the naked duplex DNA spontaneously flip into Hoogsteen pairs under ordinary conditions. The claim is considered within the historical retrospective and is put into the broader context of DNA biophysics.

15. Explain double sieve mechanism

A model that explains the rarity of misacylation of amino acids by proposing that an amino acid larger than the correct one is rarely activated because (1) it is too large to fit into the active site of the tRNA synthetase (first sieving), and (2) the hydrolytic site of the same synthetase is too small for the correct amino acid (second sieving). Thus, an amino acid smaller than the correct one can be removed by hydrolysis.

16. Define cot value.

Renaturation is a bimolecular reaction where the reaction rate is directly proportional to the product of the concentrations of c of the two homologous DNA strands.

The renaturation rate is = dc/dt = K2[W][C]

Integration of the above equation gives;

$$C/Co = 1/(1+k2\ C0t)$$

Where C is the concentration of single-stranded DNA at time t (in min), and C0 is the concentration of DNA at time zero.C/C0 = ½, then K2 = 1/ C0t1/2

In other words, the product of the initial concentration of ssDNA, C0 and the time required to renature 50% of the DNA, t1/2 is inversely proportional to the rate constant K2 of the reaction. This C0t1/2 is called the Cot value. The Cot value is directly proportional to the complexity of the genome.

17. Explain specialized transduction

Specialized transduction - only specific regions of chromosome located near attachment site are transduced, transducing particles carry both chromosomal DNA and phage DNA.

18. What are simple multigene families? Give example.

The term multigene families is used to include groups of genes from the same organism that encode proteins with similar sequences either over their full lengths or limited to a specific domain. DNA duplications can generate gene pairs. If both copies are maintained in subsequent generations then a multigene family will exist. A multigene family is a member of a family of related proteins encoded by a set of similar genes. Multigene families are believed to have arisen by duplication and variation of a single ancestral gene. Examples of multigene families include those that encode the actins, hemoglobins, immunoglobulins, tubulins, interferons, histones etc.

19. Differentiate prokaryotic and eukaryotic promoters

Prokaryotic promoters

In prokaryotes, the promoter consists of two short sequences at -10 and -35 positions upstream from the transcription start site. The sequence at -10 is called the Pribnow box, or the -10 element, and usually consists of the six nucleotides TATAAT. The Pribnow box is absolutely essential to start transcription in prokaryotes. The other sequence at -35 (the -35 element) usually consists of the six nucleotides TTGACA. Its presence allows a very high transcription rate.

Eukaryotic promoters

Eukaryotic promoters are extremely diverse and are difficult to characterize. They typically lie upstream of the gene and can have regulatory elements several kilobases away from

the transcriptional start site. In eukaryotes, the transcriptional complex can cause the DNA to bend back on itself, which allows for placement of regulatory sequences far from the actual site of transcription. Many eukaryotic promoters, contain a TATA box (sequence TATAAA), which in turn binds a TATA binding protein which assists in the formation of the RNA polymerase transcriptional complex. The TATA box typically lies very close to the transcriptional start site (often within 50 bases).

20. What are the three enzymatic activities for DNA polymerase I?

DNA Polymerase I (or Pol I) is an enzyme that participates in the process of DNA replication and is exclusively found in prokaryotes. It is composed of 928 amino acids, and is an example of a processive enzyme - it can sequentially catalyze multiple polymerisations. Discovered by Arthur Kornberg in 1956,[1] it was the first known DNA polymerase (and, indeed, the first known of any kind of polymerase). It was initially characterized in E. coli, although it is ubiquitous in prokaryotes. In E. coli and many other bacteria, the gene that encodes Pol I is known as polA.

21. Mention the beneficial effects of capping and tailing of RNA

- Capping prevents 5' degradation from 5'exonucleases.
- Capping provides stability to mRNAs.
- Capping facilitates the transport of mRNA into cytoplasm otherwise they remain in the nucleus.
- Capping enhances the efficiency of translation of mRNAs.
- Capping enhances the efficiency of splicing at 5'end introns.
- Capping with poly (A) provides synergism during translation.
- Luciferase mRNAs have been used to determine its half-life and translation efficiency with or without cap and poly- (A) tail.
- Half-life of Luciferase mRNA without cap and without poly (A) is just 31 minutes, and translational activity is 2900 (as measured in terms of light emitted by ug of radioactive protein).
- But mRNAs without cap but with poly (A) tail shows half-life of 44 minutes. And its activity is 4480. The capped mRNA without poly (A) has half-life of 53 minutes and translation activity is 62000 a virtual 50% increase in its half-life and translational efficiency.
- The capped mRNA with poly- (A) tail, has a half-life of 100 minutes and its translational activity is 1,333 000; the relative effect of cap on its activity 200 fold.
- During translation mRNA cap and poly-A tail bind to each other through a protein eF4G and gets circularized.

22. Outline the basic principles involved in eukaryotic RNA synthesis.

Transcription between promoter (start) and termination (stop) signals, multi-subunit polymerases in factories, the basic steps of transcription, initiation of synthesis of new chains, synthesis 5'-to-3'.

23. How would you determine which parts of the genome are transcribed?

'Miller' spreads, 'S1 mapping', and RT-PCR.

24. Describe the properties of the bacterial RNA polymerase.

The core enzyme (initiates poorly), σ (helps the core initiate), the holoenzyme, TATA and -35 boxes, closed and open complexes, rho independent and dependent terminators.

25. What are the untwining and supercoiling problems, and how are they resolved?

Untwining problem (and solution): a tracking polymerase is likely to generate a transcript that is entangled about the template (fix the polymerase and allow DNA to rotate). *Supercoiling problem (and solution)*: transcription by both tracking and fixed polymerases generates twin domains of supercoiling (role of topoisomerase).

26. How is a 'Miller' spread prepared, and illustrate the appearance of a spread containing some ribosomal cistrons?

Preparation: isolate nuclei, disperse chromatin in a hypotonic solution, spin onto a grid.

Structure: series of 'Christmas' trees.

27. How can caps be isolated?

Exhaustively treat mRNA with endonucleases that cleave 3' phosphates next to bases (e.g., RNase T2) to leave 5' -> 5' links intact; purify resulting dinucleotides (each carrying several phosphates) free of mononucleotides on a column (separate molecules carrying different numbers of phosphate groups).

28. What is the role of the cap?

The cap binds the cap-binding complex, CBC, which tethers the nascent transcript to the factory, enhances 3' end formation, protects transcripts from degradation, facilitates export from the nucleus, and dissociates at the ribosome to be replaced by the translational regulator, eIF-4E.

29. What is 'nonsense mediated decay' (NMD), and how was it discovered?

NMD: mRNA with a stop codon in the normal position is stable in both nucleus and cytoplasm, but moving the stop codon near the 5' end leads to the loss - or NMD - of the message. *Discovery*: place stop codons at different positions in test genes (e.g., *TPI*) and then monitor transcript levels; stop codons close to the 5' end destabilize the transcript in both the nucleus and cytoplasm.

30. What is 'transcriptional interference', and how was it discovered?

Transcriptional interference: phenomenon where transcription of one gene prevents transcription of an adjacent gene. *Discovery*: Cells were transfected with a retroviral vector encoding resistance to neomycin and azaguanine, and clones harboring a single copy of the vector selected. Expression of the 3' gene was suppressed when selection required expression of the 5' gene, and *vice versa*. In addition, hardly any cells grew in both neomycin and azaguanine.

UNIT IV - TRANSLATION

1. Explain DNA foot printing.

DNA footprinting is a method of investigating the sequence specificity of DNA-binding proteins in vitro. This technique can be used to study protein-DNA interactions both outside and within cells. The regulation of transcription has been studied extensively, and yet there is still much that is not known. Transcription factors and associated proteins that bind promoters, enhancers, or silencers to drive or repress transcription are fundamental to understanding the unique regulation of individual genes within the genome. Techniques like DNA footprinting will help elucidate which proteins bind to these regions of DNA and unravel the complexities of transcriptional control.

2. Write a note on types of RNA splicing.

In molecular biology and genetics, splicing is a modification of the nascent pre-mRNA taking place after or concurrently with its transcription, in which introns are removed and exons are joined. This is needed for the typical eukaryotic messenger RNA before it can be used to produce a correct protein through translation. For many eukaryotic introns, splicing is done in a series of reactions which are catalyzed by the spliceosome, a complex of small nuclear ribonucleoproteins (snRNPs), but there are also self-splicing introns.

3. What is conditional mutant?

Mutation that has the wild-type phenotype under certain (permissive) environmental conditions and a mutant phenotype under other (restrictive) conditions

4. What is the reading frame of an mRna?

A reading frame is a way of breaking the sequence of nucleotides in a nucleic acid such as a DNA or RNA into a set of consecutive triplets, called codons. When read as triplets, a nucleic acid molecule may in general have six reading frames, three reading in one direction along one strand and three reading in the other direction along the complementary strand. In general, only one reading frame in a given section of a nucleic acid is biologically relevant

5. Explain how hydrophobic interactions are important in stabilizing the DNA structure.

A **noncovalent bond** is a type of chemical bond, typically between macromolecules, that does not involve the sharing of pairs of electrons, but rather involves more dispersed variations of electromagnetic interactions. The noncovalent bond is the dominant type of bond between supermolecules in supermolecular chemistry. Noncovalent bonds are critical in maintaining the three-dimensional structure of large molecules, such as proteins and nucleic acids, and are involved in many biological processes in which large molecules bind specifically but transiently to one another. The energy released in the formation of noncovalent bonds is on the order of 1-5 kcal per mol. There are four commonly mentioned types of non-covalent interactions: hydrogen bonds, ionic bonds, van der Waals forces, and hydrophobic interactions.

6. What is denaturation mapping?

The identification of regions of low thermal (or alkali) stability (i.e., of high A+T content) in a duplex DNA molecule, by trapping the partly melted structure and blocking renaturation, e.g. with formaldehyde (which preferentially couples with the amino groups of the single-stranded regions), and subsequently examining the specimen by electron microscopy.

7. Explain generalized transduction

Transduction is a phenomenon in which bacterial DNA is transferred from one bacterial cell to another by a phage particle. Phage particles that contain bacterial DNA are called Transducing Particles. There are two types of transducing particles-generalized and specialized.

8. What do you understand by cyclically permuted?

A cyclic permutation or circular permutation is a permutation built from one or more sets of elements in cyclic order.

9. Distinguish redundant and synonyms in genetic code

The genetic code consists of 64 triplets of nucleotides. These triplets are called codons. With three exceptions, each codon encodes for one of the 20 amino acids used in the synthesis of proteins. That produces some redundancy in the code: most of the amino acids being encoded by more than one codon

10. Define leaky mutation

A mutant (typically an auxotroph) that results from a partial rather than a complete inactivation of the wild-type function.

11. Define transcription process

Flow of genetic information from DNA to the messenger RNA (mRNA) to express the genome for biosynthesis of proteins. The transcription process is otherwise called as synthesis of mRNA from DNA template either of the DNA strand by using enzyme RNA polymerase.

12. Write short notes on prokaryotic RNA polymerases.

RNA polymerase is the single enzyme present in the nucleus which is responsible for the synthesis of all kinds of RNAs like mRNA, tRNA and rRNA. RNA polymerase present in prokaryotic as well as eukaryotic cells is slightly differing in their structure and has many subunits. This cluster of enzyme is responsible for different functions during the mRNA synthesis or transcription process.

13. Mention briefly on sigma (σ) factor.

The sigma subunits present in the RNA polymerase of prokaryotic cell helps in recognition of start signals during mRNA synthesis. The sigma subunits are otherwise called sigma factors, which directs RNA polymerase in selecting the initiation sites. Once the RNA synthesis initiated, the sigma factors dissociate from the DNA and combine in the next transcription cycle.

14. Write on promoter and terminator region.

The promoter region or site is the special locations of DNA where the special region contain specific nucleotides are present in which the RNA polymerase binds for initiation of

transcription process. This is otherwise called promoter site. The terminator region is the special site of DNA, which contain specific nucleotide sequences, which are responsible for terminating or stop the transcription process. This is otherwise called terminator region.

15. Write short notes on prokaryotic promoter

The RNA polymerase binding site of the prokaryotic DNA region is called promoter region or promoter site. This region contain 41 – 44 bp in E.coli. Promoter is the start site, which contains 90% purine bases. The upstream from the promoter site is a 6 bp region described as TATAAT sequence or TATAAT box. This is otherwise called as pribnow box. This lies 10 bp region as – 10 sequence. I.e. –18 to –12 region. Another sequence is TTGACA is lying –35 sequences on upstream is called recognition region. The typical prokaryotic DNA use –35 and 10 sequences for transcription.

16. Describe pribnow box.

The pribnow box is otherwise described as the promoter region of DNA. The upstream from the promoter site is a 6 bp region described as TATAAT sequence or TATAAT box. This is otherwise called as pribnow box. This lies 10 bp region as – 10 sequence. I.e. –18 to –12 region.

17. Where is consensus TATA sequence seen? Write the significance of it.

The consensus TATA sequence is seen in the promoter region of prokaryotic DNA. This is more helpful in recognition of RNA polymerase to bind for transcription process. This consensus sequences occur at –10 bp of promoter region.

18. What is upstream and downstream site?

The upstream and downstream sites are occurring at the transcription region of DNA. The Upstream is the sequence region, which is prior to the start point of the promoter (from –1 sequence). The Downstream site is the region after the start point of the promoter region (from +1 sequence).

19. What is promoter complex?

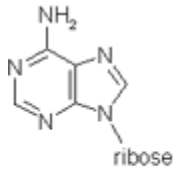
The promoter complex is described as the combine product occurred after the holoenzymes (RNA polymerase unit) binds at promoter site. When DNA is in double helix stage, it is called closed promoter complex. Once the double helix is unwinded, then it is called open promoter complex. After formation of promoter complex, the transcription state is begin to start.

20. List out the unusual bases found in t RNA

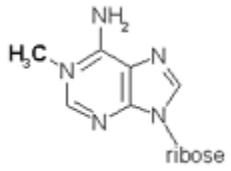
<u>Unusual bases</u>

tRNAs contain many unusual bases as shown by the illustrated side chains:

1-methyl adenosine is a modified adenosine base found on tRNA:

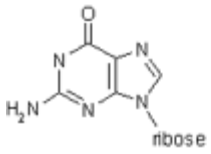


Adenosine

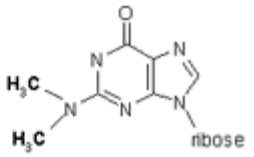


1-methyladenosine

N2, N2-dimethylguanosine is a modified guanosine:



Guanosine

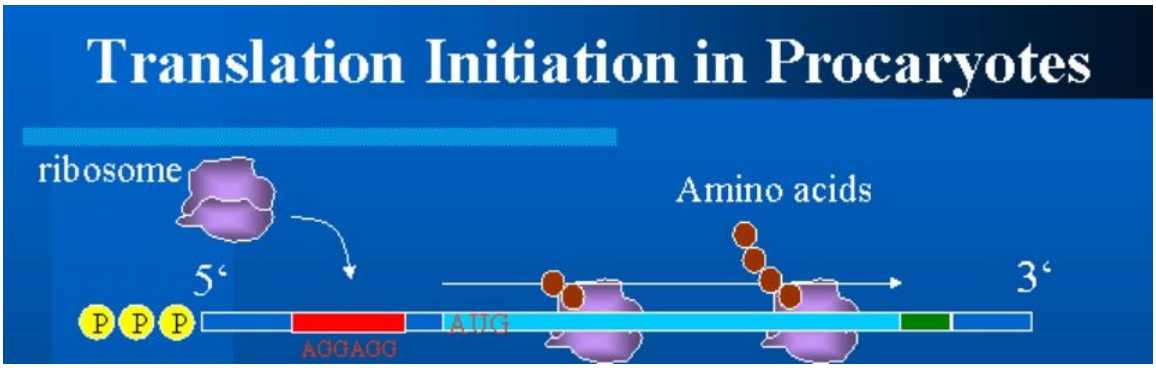


N^2, N^2-dimethylguanosine

21. Describe few post translatory mechanisms

Post-translational modifications can occur on the amino acid side chains or at the protein's C- or N- termini. They can extend the chemical repertoire of the 20 standard amino acids by modifying an existing functional group or introducing a new one such as phosphate. Phosphorylation is a very common mechanism for regulating the activity of enzymes and is the most common post-translational modification. Many eukaryotic proteins also have carbohydrate molecules attached to them in a process called glycosylation, which can promote protein folding and improve stability as well as serving regulatory functions. Attachment of lipid molecules, known as lipidation, often targets a protein or part of a protein attached to the cell membrane.Other forms of post-translational modification consist of cleaving peptide bonds, as in processing a propeptide to a mature form or removing the initiator methionine residue. The formation of disulfide bonds from cysteine residues may also be referred to as a post-translational modification.

22. How shine-dalgarno sequence initiate translation?



In procaryotes, a ribosome with tRNA which carries methionine binds to the specific region of mRNA and recognizes AUG codon nearby and protein synthesis begins. In this process the main factors are ribosome, tRNA with methionine(fMet-tRNA-Metf), and mRNA. In addition, at least initiation factors and GTP molecule are required to ensure the efficiency and fidelity of this process.

Bacterial mRNAs are commonly polycistronic. That means that they encode multiple proteins that are separatedly translated from the same mRNA molecule. Sometimes coding regions overlap, but it may not affect the fidelity of translation. Sometimes coding regions overlap by one base, which will be like UG [AUG].

In bacterial mRNA, ribosome binding site and start codon play important roles for translation initiation. Ribosome binding site is where the 30S small subunit binds first on mRNA. This site contains purine rich sequence which is called Shine-Dalgarno sequence. The 3' terminal of 16S rRNA in 30S subunit binds to this sequence and helps 30S subunit to bind to mRNA.

23. Write down the phases of protein synthesis

1. Activation of amino acids.
2. Initiation
3. Elongation
4. Termination
5. Folding and processing

24. Write short notes on Transpeptidation

- The peptide bond is formed in the second stage of the elongation cycle through the nucleophilic displacement of the P site tRNA by the amino group of the 3' linked aminoacyl tRNA in the A site.
- The nascent polypeptide chain is thereby lengthened at its C terminus by one residue and transferred to the A site tRNA a process called transpeptidation.
- Peptidyl transferase activity probably appears on the 23s RNA of ribosome. That can catalyse the reaction

25. Write short notes on Translocation

- If in the final stage of elongation cycle, uncharged P site tRNA is transferred to E site, it is former occupant having been previously expelled. Simultaneously, in a process known as translocation, the peptidyl tRNA in the A site, together with its bound mRNA is moved to the P site.
- The translocation process requires the participation of an elongation factor, EF-G that binds to the ribosome together with GTP and is only released upon hydrolysis of the GTP to GPP + Pi.
- EF-G release is pre requists for beginning the next elongation cycle because the ribosomal binding sites of EF-G and EF-TU partially overlap and hence their binding is mutually exclusive.

26. Write short notes on start and stop codons

In translation codons of three nucleotides determine which amino acid will be added next in the growing protein chain. The start codon is usually AUG, while the stop codons are UAA, UAG, and UGA. The open reading frame (ORF) is that portion of a DNA segment which will putatively code for a protein; it begins with a start codon and ends with a stop codon.

27. What are two types of codes?

There are several ways in which a codon could be read from a mRNA molecule. The two most important alternatives originally considered are the overlapping and nonoverlapping codes. In an overlapping code each base serves as the first base of some codon; in a nonoverlapping code, each base is used in only one codon.

28. Write short notes on Cycloheximide

Cycloheximide is a chemical inhibitor of the peptidyl transferase complex of the 60S subunit and hence inhibits formation of the peptide bond. Substances like Cycloheximide are commonly used in cancer chemotherapy.

29. Write short notes on components of Translation process

mRNA: – Eukaryotes: made in the nucleus, transported to the cytoplasm. – Prokaryotes: transcription and translation occur concurrently.

tRNA: Adaptor molecules that mediate the transfer of information from nucleic acids to protein

Ribosomes: manufacturing units of a cell; located in the cytoplasm. Contain ribosomal RNA and proteins.

Enzymes: required for the attachment of amino acids to the correct tRNA molecule, and for peptide bond formation between amino acids.

Proteins: soluble factors necessary for proper initiation, elongation and termination of translation.

30. When the ribosome encounters a stop codon?

- There is no tRNA available to bind to the A site of the ribosome,
- Instead a release factor binds to it.

UNIT V - REGULATION OF GENE EXPRESSION

1. Define Gene regulation

Gene regulation is the informal term used to describe any mechanism used by a cell to increase or decrease the production of specific gene products (protein or RNA). Cells can modify their gene expression patterns to trigger developmental pathways, respond to environmental stimuli, or adapt to new food sources. All points of gene expression can be regulated. This includes transcription, RNA processing and transport, translation and post-translational modification of a protein, and mRNA degradation.

2. What is Gene organization?

Genes inside the cell follow several layers of organisation to enable the long DNA to be compacted into the chromosome fibers. In the first level of packing, DNA is wrapped around 4 pairs of proteins called Histones in a "beads-on-string" fashion. These nucleosomes then coil around each other in the form of a helix, with around 6 nucleosomes forming one turn of a helix. These helices form the long chromatin fibers which, with series of turns and loops, forms the third level of spatial DNA organisation. Finally, these chromatin fibers are compactly packed inside the chromosome. In the Chromosome, the chromatin fibers are wrapped around a protein scaffold.

3. How does gene regulation occur?

A **gene (or genetic) regulatory network (GRN)** is a collection of molecular regulators that interact with each other and with other substances in the cell to govern the gene expression levels of mRNA and proteins. These play a central role in morphogenesis, the creation of body structures, which in turn is central to evolutionary developmental biology (evo-devo).

The regulator can be DNA, RNA, protein and complexes of these. The interaction can be direct or indirect (through transcribed RNA or translated protein). In general, each mRNA molecule goes on to make a specific protein (or set of proteins). In some cases this protein will be structural, and will accumulate at the cell membrane or within the cell to give it particular structural properties. In other cases the protein will be an enzyme, i.e., a micro-machine that catalyses a certain reaction, such as the breakdown of a food source or toxin. Some proteins though serve only to activate other genes, and these are the transcription factors that are the main players in regulatory networks or cascades. By binding to the promoter region at the start of other genes they turn them on, initiating the production of another protein, and so on. Some transcription factors are inhibitory.

4. How many amino acids are present in a nascent polypeptide decoded from mRNA with the reading frame having 1002 nucleotides?

- In molecular biology, a **reading frame** is a way of dividing the sequence of nucleotides in a nucleic acid (DNAor RNA) molecule into a set of consecutive, non-overlapping triplets. Where these triplets equate to amino acids or stop signals during translation, they are called codons.
- A single strand of a nucleic acid molecule has a phosphoryl end, called the 5′-end, and a hydroxyl or 3′-end. These define the 5'→3' direction. There are three reading frames that can be read in this 5'→3' direction, each beginning from a different nucleotide in a triplet. In a double stranded nucleic acid, an additional three reading frames may be read from the other, complementary strand in the 5'→3' direction along this strand. As the two strands of a double stranded nucleic acid molecule are antiparallel, the 5'→3' direction on the second strand corresponds to the 3'→5' direction along the first strand.

5. Why DNA replication is called semi-conservative?

DNA replication is semi-conservative because each helix that is created contains one strand from the helix from which it was copied. The replication of one helix results in two daughter helices each of which contains one of the original parental helical strands. It is semi-conservative because half of each parent helix is conserved in each daughter helix.

6. What are introns?

An intron is a nucleotide sequence within a gene. It is a noncoding sequence. During the final maturation of the RNA product, the RNA removes it by splicing.[1] The term intron pertains to the DNAsequence within a gene as well as the corresponding sequence in the RNA transcripts.[2] It is used in contrast to the nucleotide sequences joined together in a mature RNA after splicing calledexons. Sometimes, the term intron is used synonymously to intervening sequences. However, the latter is a broader term that includes inteins and UTRs, apart from the introns. Introns occur in the genes of many organisms (e.g. eukaryotes), including viruses. They are also present in the genes of mitochondria and chloroplasts. Introns are believed to be essential in allowing rapid evolution of proteins through exon shuffling.

7. Name the cell organelle where protein synthesis takes place

The rough **endoplasmic reticulum** is where most protein synthesis occurs in the cell. The function of the smooth **endoplasmic reticulum** is to synthesize lipids in the cell. The smooth ER also helps in the detoxification of harmful substances in the cell. **Ribosomes**- Organelles that help in the synthesis of proteins.

8. Give reason – why DNA is acidic in nature

DNA is made of three types of molecules in equal proportions - basic nucleotides, sugar deoxyribose and acidic phosphate groups. The bases are on the inside of the helix and partly hidden from the outside. Deoxyrybose and phopshates are on the outside, forming a backbone. Though the proportions are equal, the nucleotides are weak bases, so the overall pH is acidic.

9. Name the process by which RNA is synthesised from DNA.

Gene expression is the process by which the genetic code - the nucleotide sequence - of a gene is used to direct protein synthesis and produce the structures of the cell. Genes that code for amino acid sequences are known as 'structural genes'.

The process of gene expression involves two main stages:
Transcription: the production of messenger RNA (mRNA) by the enzyme RNA polymerase, and the processing of the resulting mRNA molecule. **Translation**: the use of mRNA to direct protein synthesis, and the subsequent post-translational processing of the protein molecule. Some genes are responsible for the production of other forms of RNA that play a role in translation, including transfer RNA (tRNA) and ribosomal RNA (rRNA).

10. Why lac operon switches off in the absence of Lactose in E.coli?

The lac operon, an inducible operon, is a mechanism used by bacterial cells as an economical means to restrict the expression of the structural genes necessary for metabolizing lactose, a disaccharide. These structural genes break down lactose when lactose is the best carbon source available within its environs. E.coli utilizes the lac operon as a means of controlling the expression of its lac genes in response to its environment. The primary carbon source of this bacterium is glucose because it does not require a large amount of energy to

metabolize. It is a more efficient source of energy than lactose. In the presence of both glucose and lactose, the bacterium will chose to metabolize glucose.

11. Why Chargaff's rule is not applicable for RNA?

RNA is found as a single stranded molecule. Chargaff's rule states that DNA helices contain equal molar ratios of A to T and G to C. This is because DNA is found as a double stranded helix in which A and T and G and C bases pair complementarily. RNA only forms local helices meaning that it doesn't necessarily contain equal ratios.

12. Why the nucleotide ratio in RNA is not usually constant?

Due to the absence of complementary base pairing. RNA is single stranded. So, the nucleotide ratio is not constant in RNA.

13. Why is processed mRNA in eukaryotes is shorter than its gene?

Processed mRNA in eukaryotes is shorter than its gene because the eukaryotic gene is split gene and the transcribed mRNA has intron portions.

14. Name the process of RNA directed DNA synthesis

A **reverse transcriptase** (RT) is an enzyme used to generate complementary DNA (cDNA) from an RNA template, a process termedreverse transcription. It is mainly associated with retroviruses. However, non-retroviruses also use RT (for example, the hepatitis B virus, a member of the Hepadnaviridae, which are dsDNA-RT viruses, while retroviruses are ssRNA viruses). RT inhibitors are widely used as antiretroviral drugs. RT activities are also associated with the replication of chromosome ends (telomerase) and some mobile genetic elements (retrotransposons).

Retroviral RT has three sequential biochemical activities:

(a) RNA-dependent DNA polymerase activity,

(b) ribonuclease H, and

(c) DNA-dependent DNA polymerase activity.

These activities are used by the retrovirus to convert single-stranded genomic RNA into double-stranded cDNA which can integrate into the host genome, potentially generating a long-term infection that can be very difficult to eradicate. The same sequence of reactions is widely used in the laboratory to convert RNA to DNA for use in molecular cloning, RNA sequencing, polymerase chain reaction (PCR), or genome analysis

15. Why codons are redundant?

Degeneracy of codons is the redundancy of the genetic code, exhibited as the multiplicity of three-base pair codon combinations that specify an amino acid. The degeneracy of the genetic code is what accounts for the existence of synonymous mutations. Degeneracy of the genetic code was identified by Lagerkvist. For instance, codons GAA and GAG both specify glutamic acid and exhibit redundancy; but, neither specifies any other amino acid and thus are not ambiguous or demonstrate no ambiguity. The codons encoding one amino acid may differ in any of their three positions; however, more often than not, this difference is in the second or third position. For instance, the amino acid glutamic acid is specified by GAA and GAG codons (difference in the third position); the amino acid leucine is specified by UUA, UUG, CUU, CUC, CUA, CUG codons (difference in the first or third position); and the amino acid serine is

specified by UCA, UCG, UCC, UCU, AGU, AGC (difference in the first, second, or third position). Degeneracy results because there are more codons than encodable amino acids. For example, if there were two bases per codon, then only 16 amino acids could be coded for (4^2=16). Because at least 21 codes are required (20 amino acids plus stop) and the next largest number of bases is three, then 4^3 gives 64 possible codons, meaning that some degeneracy must exist.

16. Why codons are sensible?

The gene is represented by the sequences of bases in the DNA molecule, which can, in a sense, be thought of as a "storage molecule" for genetic information. DNA is extremely stable, a property critical to the maintenance of the integrity of the gene. This stability is evidenced by the fact that DNA has been extracted from Egyptian mummies and extinct animals such as the woolly mammoth. It can be extracted from dried blood or from a single hair at a crime scene. Each cell contains a complete set of genes, but only certain of these genes are active or "expressed" at any one time. When a gene is active, a "disposable" copy is transcribed from the gene into codons contained in a messenger RNA (mRNA) molecule. Unlike the DNA molecule, the mRNA molecule is relatively unstable and short-lived. This is so that when a gene is turned off, the mRNA does not remain in the cell forever, running off more proteins on the ribosomes that are no longer needed by the cell.

17. Why redundancy concept of genetic code does not apply to all amino acids?

The genetic code is said to be redundant in that the same amino acid residue can be encoded by multiple, so-called synonymous, codons. If all properties of synonymous codons were entirely equivalent, one would expect that they would be equally distributed along protein coding sequences. However, many studies over the last three decades have demonstrated that their distribution is not entirely random. It has been postulated that certain codons may be translated by the ribosome faster than others and thus their non-random distribution dictates how fast the ribosome moves along particular segments of the mRNA. The reasons behind such segmental variability in the rates of protein synthesis, and thus polypeptide emergence from the ribosome, have been explored by theoretical and experimental approaches. Predictions of the relative rates at which particular codons are translated and their impact on the nascent chain have not arrived at unequivocal conclusions. This is probably due, at least in part, to variation in the basis for classification of codons as “fast” or “slow”, as well as variability in the number and types of genes and proteins analyzed. Recent methodological advances have allowed nucleotide-resolution studies of ribosome residency times in entire transcriptomes, which confirm the non-uniform movement of ribosomes along mRNAs and shed light on the actual determinants of rate control. Moreover, experiments have begun to emerge that systematically examine the influence of variations in ribosomal movement and the fate of the emerging polypeptide chain.

18. Explain wobble hypothesis.

Even before the genetic code had been elucidated, Francis Crick postulated that base pairing of the mRNA codons with the tRNA anticodons would require precision in the first two nucleotide positions but not so in the third position (the precise conformation of **base pairs** , which refers to the **hydrogen bonding** between A-T (A-U in RNA) and C-G pairs is known as Watson-Crick base pairing). The third position, in general, would need to be only a purine (A or G) or a pyrimidine (C or U). Crick called this phenomenon "wobble." This less-than-precise base

pairing would require fewer tRNA species. For example, $tRNA^{Glu}$could pair with either GAA or GAG codons. In looking at the codon table, one can see that, for the most part, the first two letters are important to specify the particular amino acid. The only exceptions are AUG (Met) and UGG (Trp) which, as indicated above, have only one codon each.

19. What are all the exceptions to the universal genetic code?

Organism	Normal codon	Usual meaning	New meaning
Mammalian	AGA, AGG	Arginine	Stop codon
Mitochondria	AUA	Isoleucine	Methionine
	UGA	Stop codon	Tryptophan
Drosophila	AGA, AGG	Arginine	Serine
Mitochondria	AUA	Isoleucine	Methionine
	UGA	Stop codon	Tryptophan
Yeast	AUA	Isoleucine	Methionine
Mitochondria	UGA	Stop codon	Tryptophan
	CUA, CUC, CUG, CUU	Leucine	Threonine
Higher plant	UGA	Stop codon	Tryptophan
Mitochondria	CGG	Arginine	Tryptophan
Protozoan nuclei	UAA, UAG	Stop codons	Glutamine
Mycoplasma capricolum bacteria	UGA	Stop codon	Tryptophan

20. Explain Lysogeny.

Lysogeny, or the **lysogenic cycle**, is one of two cycles of viral reproduction (the lytic cycle is the other). Lysogeny is characterized by integration of the bacteriophage nucleic acid into the host bacterium's genome or formations of a circular replicon in the bacterium's cytoplasm. In this condition the bacterium continues to live and reproduce normally. The genetic material of the bacteriophage, called aprophage, can be transmitted to daughter cells at each subsequent cell division, and a later event (such as UV radiation or the presence of certain chemicals) can release it, causing proliferation of new phages via the lytic cycle. Lysogenic cycles can also occur ineukaryotes, although the method of DNA incorporation is not fully understood. The distinction between lysogenic and lytic cycles is that the spread of the viral DNA occurs through the usual prokaryotic reproduction, while the lytic phage is spread through the production of thousands of individual phages capable of surviving and infecting other cells. The key difference between the lytic cycle and the lysogenic cycle is that the lysogenic cycle does not lyse the host cell.[2] Phages that replicate only via the lytic cycle are known as virulent phages while phages that replicate using both lytic and lysogenic cycles are known astemperate phages. In the lysogenic cycle, the phage DNA first integrates into the bacterial

chromosome to produce the prophage. When the bacterium reproduces, the prophage is also copied and is present in each of the daughter cells. The daughter cells can continue to replicate with the prophage present or the prophage can exit the bacterial chromosome to initiate the lytic cycle.

21. Give the importance of leader sequence

Some operons are under attenuator control, in which transcription is initiated but is halted before the mRNA is transcribed. This introductory region of the mRNA is called the leader sequence; it includes the attenuator region, which can fold back on itself, forming a stem-and-loop structure that blocks the RNA polymerase from advancing along the DNA.

22. Allolactose control of lac operon. Explain

The operon is under the control of the adjacent lacI gene, encoding the lactose repressor. The repressor is a regulatory gene. In the absence of allolactose, the inducer of the lac operon, the repressor tetramer binds to the lac operator (lacO) and prevents RNA polymerase from transcribing the operon.

23. Differentiate between positive and negative control mechanisms in bacteria.

Positive: binding of an activator (e.g., cAMP) to a DNA-binding protein (e.g., CAP) stimulates that latter's DNA binding, and so initiation by RNA polymerase.
Negative: binding of a repressor (e.g., the trp repressor) promotes DNA binding, preventing initiation.

24. Outline the effects that occur when tryptophan switches off expression of the trp operon.

Two molecules of tryptophan bind to the trp repressor (a helix-turn-helix homodimer), increasing its affinity for the operator; the two recognition helices tilt and enter the major groove about 10 bp apart to contact the edge of the relevant bases. Now, the bound complex prevents the template from attaching to the polymerase. When tryptophan is absent, the now-unoccupied repressor dissociates from the operator so the template can attach productively to the polymerase (and the operon is transcribed).

25. How does the catabolite activator protein promote expression of catabolic enzymes in bacteria?

Falling glucose levels increase the concentration of cAMP, which binds to CAP (a homodimer of a polypeptide containing a helix-turn-helix motif), promoting its affinity for target sequences embedded in the promoters of many genes encoding catabolic enzymes. Binding in the major groove narrows it, while the opposing minor groove widens; the result is a 40 kink. Now promoters attach more efficiently to polymerases, so catabolic enzymes are expressed at higher levels.

26. How was 'Dolly' the lamb cloned?

Dolly's genes are derived from the udder of a (white-faced) Finn Dorset ewe. Cells from this ewe were arrested by serum starvation in G0, fused (using an electrical pulse) with an enucleate egg (from a Scottish Blackface ewe) in meiotic metaphase II, and the reconstituted egg cultured in the ligated oviduct of a (Scottish Blackface) foster mother for 6 days. Then, the egg

was recovered, checked to see that it had developed into a blastocyst, reimplanted into a second (Scottish Blackface) foster mother, and allowed to develop to term (giving white-faced Dolly). Dolly's nuclear genes (excepting any mutant genes) are identical to those of her genetic mother (but not her foster mother).

27. By what mechanisms might selective gene expression be achieved?

E.g., DNA rearrangement (immunoglobulin genes) or amplification (rDNA genes) or loss (*Dipteran* embryos) or modification (methylation), alteration in the rate of transcriptional initiation (many genes) or elongation (heat-shock locus), transcript rearrangement (alternative splicing) or processing or degradation (histone mRNA), differential transport of mRNA out of the nucleus or to different locations (-actin), differential translation of mRNA, differential protein degradation or stabilization.

28. Give an example of an experiment showing that differential expression of a gene can require continuous regulation?

A typical muscle cell expresses the cell-adhesion molecule, N-CAM, on its surface. The stability of the switches involved in maintaining N-CAM expression were analyzed by fusing a mouse myoblast with a human lung fibroblast (which does not express any muscle-specific proteins); the resulting heterokaryon expressed N-CAM of both species. This suggests that a switch acting continuously on the muscle nucleus can also switch on N-CAM in the human nucleus.

29. Illustrate how the activity of rDNA genes can be inherited through mitosis.

NORs, tandem repeats of 45S rRNA genes carried on five pairs of human chromosomes with perhaps only 6 loci being transcribed, activity associated with UBF on mitotic chromosomes, those NORs carrying UBF tend for form nucleoli in daughters.

30. How would you demonstrate the effects of a maternal effect gene in Drosophila?

Cross the (grandparent) fly carrying the mutant maternal-effect allele with another fly carrying the same mutation (i.e., -/+ x -/+). Although one-quarter of the resulting fertilized eggs are genotypically -/-, they contain maternal products of the + gene, so the embryonic body plan is laid down normally. When such embryos reach adulthood they can be crossed with a wild-type male (i.e., -/- x +/+); then, the resulting fertilized eggs have +/- genes in a cytoplasm that lacks any + products from the mother. As a result, an apparently normal mother lays an egg that then develops abnormally.

PART B

S.NO	QUESTIONS	REFERENCE	PAGE NO
	UNIT I – CHEMISTRY OF NUCLEIC ACIDS		
1	Explain the Structure and physicochemical properties of elements in DNA and RNA	**TB1**	**80-82**
2	Describe in detail the Biological significance of differences in DNA and RNA	**TB1**	**84-92**
3	Write short notes on Primary structure of DNA. Describe the Chemical and structural qualities of 3',5'-Phosphodiester bond	**TB1**	**94-96**
4	Describe in detail the Secondary Structure of DNA by Watson & Crick model*(Dec 2016)*	**TB1**	**97-100**
5	Explain the following: Triple helix, Quadruple helix, Reversible denaturation and hyperchromic effect	**TB1**	**112-113**
6	What is Tertiary structure of DNA? Explain DNA supercoiling.	**TB1**	**113-114**
	UNIT II – DNA REPLICATION & REPAIR		
1	Explain the experiment that proves semiconservative mode of replication*(Dec 2016)*	**TB1**	**225-228**
2	Explain the role of Inhibitors in DNA replication	**TB1**	**232-235**
3	Differentiate between prokaryotic and eukaryotic DNA replication	**TB1**	**271-273**
4	How are okazaki fragments generated with diagrams? *(Dec 2016)*	**TB1**	**245, 248-250**
5	Demonstrate the rolling circle mode of replication	**TB1**	**255-258**
6	What are the physical and chemical agents causing DNA mutations? *(Dec 2016)*	**TB1**	**300**
7	Give any two repair mechanisms that rectify the error due to mutations*(Dec 2016)*	**TB1**	**306**
	UNIT III – TRANSCRIPTION		
1	Elucidate the Structure and function of mRNA, rRNA and tRNA	**TB1**	**117**
2	Narrate the Characteristics of promoter and enhancer sequences	**TB1**	**316**
3	Explain the process of RNA synthesis and the Proteins involved in RNA synthesis *(Dec 2015)*	**TB1**	**317-329**
4	Explain the Differences in prokaryotic and eukaryotic transcription*(Dec 2013)*	**TB1**	**343-349**
5	Explain RNA processing and 5'-Capping,	**TB1**	**352-358**
6	Describe the process of Alternative splicing, Poly 'A' tail addition and base modification	**TB1**	**358-363**
	UNIT IV – TRANSLATION		
1	How will you Elucidate genetic code? What is Codon degeneracy? *(Dec 2013)*	**TB1**	**367-378**
2	Write notes on Prokaryotic and eukaryotic ribosomes	**TB1**	**439-447**

3	Narrate the Steps in translation	**TB1**	**425-435**
4	Explain the role of Inhibitors of protein synthesis.	**TB1**	**441-443**
5	What are Post- translational modifications? *(Dec 2015)*	**TB1**	**443-445**
6	Explain about origin and evolution of genetic code	**TB1**	**369**
UNIT V – REGULATION OF GENE EXPRESSION			
1	Explain the Organization of genes in prokaryotic and eukaryotic chromosomes	**TB1**	**453-454, 502-510**
2	Explain the Prokaryotic gene regulation in l*ac* operon *(Dec 2016)*	**TB1**	**456-462**
3	Explain the Prokaryotic gene regulation in *trp* operon*(Dec 2015)*	**TB1**	**479 - 480**
4	Explain the Regulation of gene expression with reference to λ phage life cycle	**TB1**	**598 - 613**
5	What is operon concept? Explain in detail ara Operon in Ecoli*(Dec 2013)*		
6	Explain Hierarchical levels of gene regulation	**TB1**	**502-510**

PART C

S.NO	QUESTIONS	REFERENCE	PAGE NO
UNIT I – CHEMISTRY OF NUCLEIC ACIDS			
1	Describe the secondary structure of DNA with diagrams (*Dec 2016*)	**TB1**	**97-100**
2	Give an account on the forces that stabilises DNA (*Dec 2016*)	**TB1**	**110-112**
3	Give direct evidence that DNA is genetic material*(Dec 2013)*	**TB1**	**79**
UNIT II – DNA REPLICATION & REPAIR			
1	Explain the Organization of prokaryotic and eukaryotic chromosomes	**TB1**	**209**
2	What are Mutagens? Describe in detail the various types of repair mechanisms	**TB1**	**293-305**
3	Explain Telomere replication in eukaryotes	**TB1**	**239-246**
UNIT III – TRANSCRIPTION			
1	Describe the events of transcription with examples *(Dec 2016)*	**TB1**	**315**
2	How do splicing mechanism favours processing of primary transcripts? *(Dec 2016)*	**TB1**	**359**
3	Describe in detail about the post transcriptional modification in mRNA*(Dec 2013)*		
UNIT IV – TRANSLATION			
1	Explain genetic code dictionary and the properties of it and mechanisms of regulation *(Dec 2016)*	**TB1**	**369**
2	Describe the events of prokaryotic translation with diagram *(Dec 2016)*	**TB1**	**430-435**
3	What is the concept of genetic code? Describe the wobble hypothesis *(Dec 2013)*	**TB1**	**367**
UNIT V – REGULATION OF GENE EXPRESSION			
1	Explain the organisation of eukaryotic chromosome with diagrams *(Dec 2016)*	**TB1**	**181**

2	Lac operon is highly regulated. How? And give its implication in the generation of recombinant proteins *(Dec 2016)*	**TB1**	**460**
3	Explain eukaryotic gene regulation *(Dec 2013)*		