SY B.Sc. Life Sciences (CBSGS) **Paper II - Semester IV**
Paper Code: 70566 **Dated: 26th April 2019**

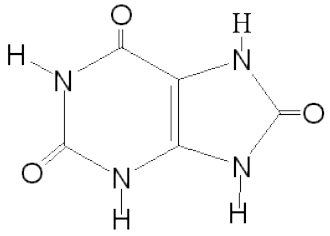
Answer key

Q. 1 A Do as directed:
Q. 1. A) Fill in the blanks: (07)
1. Pyruvate is transported from the cytosol to mitochondria by _____ of alanine. Ans.Amino group
2. Glucose-6-Phosphate is catalysed to glucose-1-phosphate by _____. Ans. Hexokinase
3. Xylulose 5 phosphate can be formed from ribulose 5 phosphate by the action of _____ enzyme. Ans. Traansketolae
4. During photosynthesis the oxygen in glucose comes from ----------Ans. CO2
5. Kranz anatomy is an adaptation to avoid _____. Ans. Photosynthesis.

6. Prostaglandins _____ gastric secretion. Ans. Inhibits
7. The storage form of fatty acids in animals is ----------Ans. Triacylglycerides.

B) Define/Explain the terms: (07)
1. Nucleic acid
Nucleic Acid: a complex organic substance present in living cells, especially DNA or RNA, whose molecules consist of many nucleotides linked in a long chain.

2. Sigma factor in transcription.
A **sigma factor** (**σ factor**) (specificity **factor**) is a protein needed for initiation of **transcription** in bacteria. It is a bacterial **transcription** initiation**factor** that enables specific binding of RNA polymerase (RNAP) to gene promoters.
3. Role of Enhancer in Transcription
an **enhancer** is a short (50–1500 bp) region of DNA that can be bound by proteins (activators) to increase the likelihood that **transcription** of a particular gene will occur. These proteins are usually referred to as **transcription** factors.
4. Structure of Uric acid



5. Lysch Nyhan syndrome is a rare inherited **disorder** caused by a deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT), produced by mutations in the HPRT gene located on the X chromosome. ... The HGPRT deficiency causes a build-up of uric acid in all body fluids.

6. Role of Rho in Transcription.
A ρ **factor** (**Rho factor**) is a prokaryotic protein involved in the termination of **transcription**. **Rho**functions as an ancillary **factor** for RNA polymerase. There are two types of **transcriptional** termination in prokaryotes, **rho**-dependent termination and intrinsic termination (also called **Rho**-independent termination).

7. Name one Post- Transcriptional modification in Eukaryotes.
Post-**transcriptional modifications** of pre-**mRNA**, such as capping, splicing, and polyadenylation, take place in the nucleus. **After** these **modifications** have been completed, the mature**mRNA** molecules have to be translocated into the cytoplasm, where protein synthesis occurs.

C) Match the columns: **(07)**

- **a. ---iii**
- **b. ---vii**
- **c. ---iv**
- **d. ---i**
- **e. ---ii**
- **f. ---v**
- **g. Vi**

Q.2. A) Answer <u>any one</u> of the following: **(10)**

1. What is Photophosphorylation? Describe Non Cyclic photophosphorylation.

1.**Photophosphorylation**

In the process of photosynthesis, the phosphorylation of ADP to form ATP using the energy of sunlight is called photophosphorylation. In photophosphorylation, light energy is used to create a high-energy electron donor and a lower-energy electron acceptor.

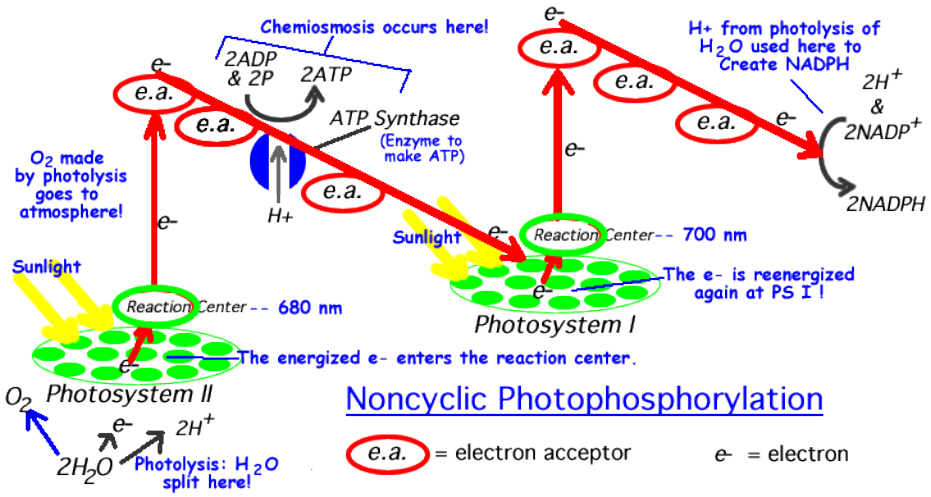
Non Cyclic photophosphorylation: Non-cyclic photophosphorylation produces ATP using the energy from excited electrons provided by photosystem II.

As electrons pass through the non-cyclic pathway, they do not return to the original photosystem. This does not create a cycle, hence the name non-cyclic.

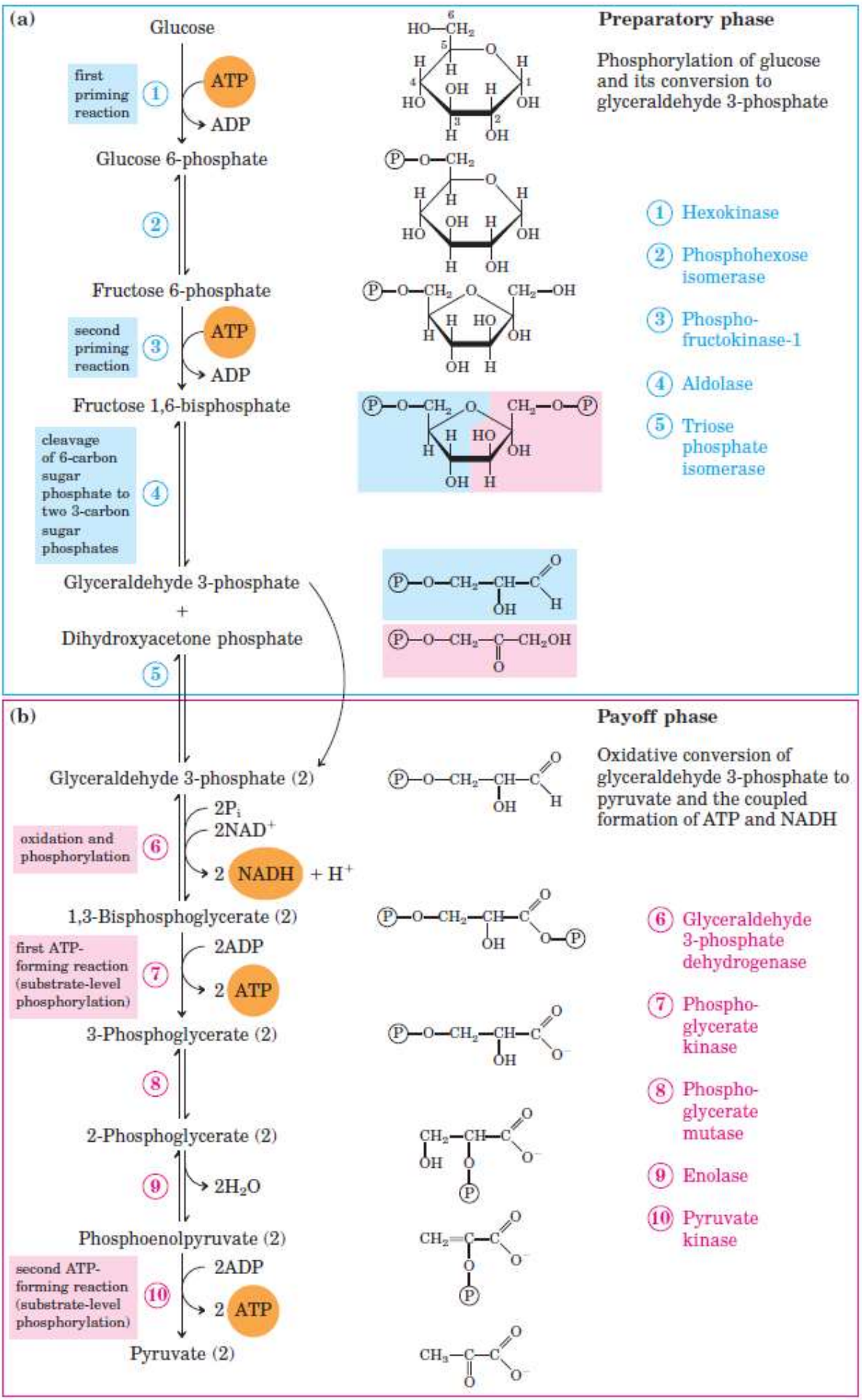
1. Photolysis provides H+ ions to replace those lost in the photosystems. The excited electrons provide energy for a proton pump to actively transport additional H+ into the thylakoid. The high concentration of H+ diffuse past ATP synthase as they pass out of the membrane to the lower H+ concentration. The energy created as H+ passes the ATP synthase forms ATP.Diffusion of H+ ions from high to low concentration through ATP synthase to form ATP is called chemiosmosis.

2. The electrons provided by non-cyclic photophosphorylation are boosted a 2nd time to a higher energy level by photosystem I to then create 2 NADPH.

Non-cyclic Photophosphorylation



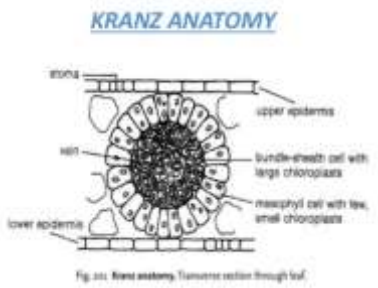
2. Explain with the help of formulae the various steps involved in the process of gluconeogenesis


(a)
Glucose
first priming reaction
1
ATP
ADP
Glucose 6-phosphate
2
Fructose 6-phosphate
second priming reaction
3
ATP
ADP
Fructose 1,6-bisphosphate
cleavage of 6-carbon sugar phosphate to two 3-carbon sugar phosphates
4
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate
1 Hexokinase
2 Phosphohexose isomerase
3 Phospho-fructokinase-1
4 Aldolase
5 Triose phosphate isomerase
(b)
Glyceraldehyde 3-phosphate (2)
oxidation and phosphorylation
6
2Pi
2NAD+
2 NADH + H+
1,3-Bisphosphoglycerate (2)
first ATP-forming reaction (substrate-level phosphorylation)
7
2ADP
2 ATP
3-Phosphoglycerate (2)
8
2-Phosphoglycerate (2)
9
2H2O
Phosphoenolpyruvate (2)
second ATP-forming reaction (substrate-level phosphorylation)
10
2ADP
2 ATP
Pyruvate (2)
Payoff phase
Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH
6 Glyceraldehyde 3-phosphate dehydrogenase
7 Phospho-glycerate kinase
8 Phospho-glycerate mutase
9 Enolase
10 Pyruvate kinase

Q. 2. B) Answer <u>any two</u> of the following: (10)

1. What is Kranz anatomy? Explain how C4 plants have increased their photosynthetic efficiency.

The anatomy of leaf of C4 plants is also called 'Kranz Anatomy', because the leaf surrounding each vascular bundle is a sheath of tightly packed,thick walled chloroplast containing cells(bundle sheath) which looks like a ring or wreath. 'Kranz' in German means wreath. The cells of bundle sheath in such plants are therefore also called as Kranz cells.



The discovery of C_4 cycle in monocots such as sugarcane, maize and sorghum has indicated that these plants have solved the problem of photorespiration. The carbon dioxide is fixed in the mesophyll cells. The initial product being a-4 carbon compound, the process is called C_4 pathway of carbon dioxide fixation.

Photorespiration occurs in C_3 plants (Calvin cycle), which leads to a 25 percent loss of the fixed CO_2. Photorespiration occurs in C_3 plants only, as the enzyme Rubisco catalysis both carboxylation and oxygenation reactions of the initial acceptor molecule that is RuBP.

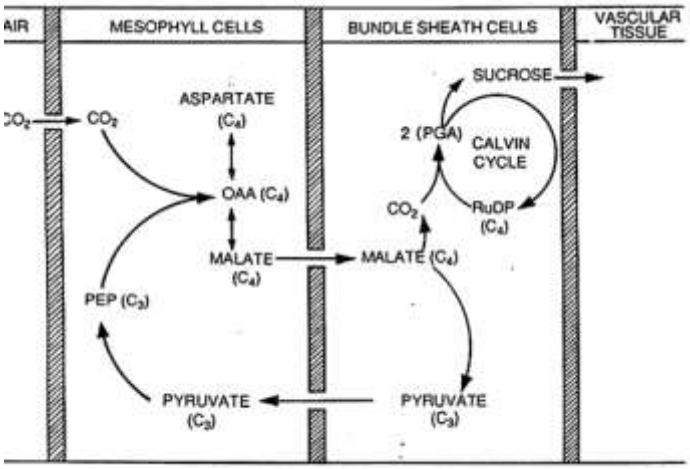


Fig. 5.11. Hatch Slack Pathway of CO_2 fixation.

In C_3 plants, photosynthesis occurs only in mesophyll cells. Photosynthesis has two types of reactions, i.e., light reactions and carbon or dark reactions.

In light reactions, ATP and $NADPH_2$ are produced, and as a result of photolysis of water O_2 is released.

During carbon or dark reactions, CO_2 is assimilated and carbohydrates are produced.

As both light reactions and carbon (dark) reactions occur in mesophyll cells in C_3 plants, it becomes essential for enzyme Rubisco to catalyse both oxygenation and carboxylation reactions of RuBP, simultaneously.

However, in category of C_4 plants, nature has evolved a mechanism to avoid occurrence of photorespiration, which is thought to be a harmful process.

C_4 pathway requires the presence of two types of photosynthetic cells, i.e., mesophyll cells and bundle sheath cells. The bundle sheath cells are arranged in a wreath like manner. This kind of arrangement of cells is called Kranz anatomy (Kranz: wreath). In Kranz anatomy, the mesophyll and bundle sheath cells are connected by plasmodesmata or cytoplasmic bridges.

The C_4 plants contain dimorphic chloroplasts. The chloroplasts in mesophyll cells are granal, whereas in bundle sheath cells they are agranal.

The granal chloroplasts contain thylakoids which are stacked to form grana, as formed in C_3 plants. However, in agranal chloroplasts of bundle sheath cells grana are absent and thylakoids are present only as stroma lamellae.

The presence of two types of cells (granal and agranal) allows occurrence of light and carbon (dark) reactions separately in each type.

Here, release of O_2 takes place in one type, while fixation of CO_2catalysed by Rubisco enzyme occurs in another type of cells.

In C_4 plants (maize, sugarcane, etc.), light reactions occur in mesophyll cells, whereas CO_2 assimilation takes place in bundle sheath cells. Such arrangement of cells does not allow O_2 released in mesophyll cells to enter in bundle-sheath cells.

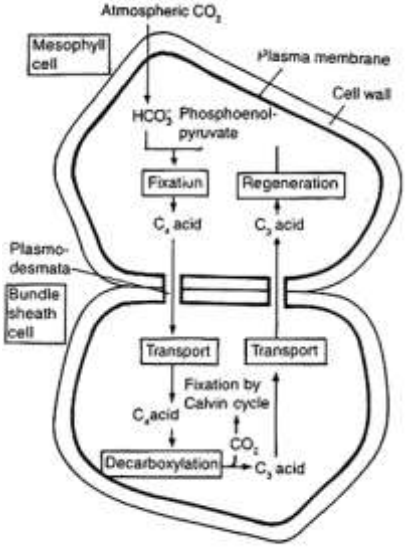


Fig. 5.12. C_4 photosynthetic carbon cycle

Hence, Rubisco enzyme, which is present only in bundle-sheath cells, does not come into contact with O_2, and thus, oxygenation of RuBP is completely avoided.

In C_4 plants, a CO_2 concentrating mechanism is present which helps in reducing the occurrence of photorespiration (i.e., oxygenation of initial acceptor RuBP). This type of CO_2 concentrating mechanism is called C_4 pathway.

For operation of C_4 pathway, both mesophyll and bundle-sheath cells are required. The main objective of C_4 pathway is to build up high concentration of CO_2 near Rubisco enzyme in bundle- sheath cells. High concentration of CO_2 near Rubisco enhances carboxylation and reduces photorespiration.

C_4 photosynthetic Carbon Cycle:

In C_4 pathway, CO_2 from the atmosphere enters through stomata into the mesophyll cells and combines with phosphoenol pyruvate (3-carbon compound). This reaction is catalysed by an enzyme known as phosphoenol pyruvate carboxylase, i.e., PEPCase. With the result, a C_4acid, oxaloacetic acid (OAA) is formed.

$$CO_2 + PEP + H_2O \xrightarrow[\text{enzyme}]{\text{PEPCase}} \text{Oxaloacetic acid (OAA)} + H_3PO_4$$

The above-mentioned reaction occurs in cytosol of the mesophyll cells and is called fixation of CO_2 or carboxylation.

Since this gives rise to the first stable product C_4 acid, and therefore, known as C_4 pathway.

The next step of reaction is transport of oxalo acetic acid (OAA – 4 C compounds) from cytosol of mesophyll cells to chloroplasts of bundle-sheath cells, where it is decarboxylated to release fixed CO_2 and high concentration of CO_2 is generated near Rubisco.

The other product of decarboxylation reaction is a 3-carbon compound called pyruvic acid.

Now, this is transported back to mesophyll cells, where if regenerates phosphoenol pyruvate to its own for continuation of C_4 pathway

2. Write a note on synthesis of Sucrose via Calvin Cycle.

2. Synthesis of Sucrose via Calvin Cycle

The metabolic pathway by which carbon dioxide (CO2) is incorporated into carbohydrate. Nobel Laureate Melvin Calvin had a major role in elucidating this cyclic series of enzyme-catalyzed reactions. The enzyme RuBisCO (ribulose bisphosphate carboxylase/oxygenase) catalyzes the initial reaction of CO2 with a five-carbon compound ribulose-1, 5-bisphosphate (RuBP). The resulting 6-carbon intermediate splits into two copies of a 3-carbon compound that is converted in subsequent steps to the carbohydrate glyceraldehyde-3-phosphate. Some of this product exits the pathway to be used for synthesis of more complex carbohydrates or other carbon compounds. The rest is converted back to RuBP (the substrate for the initial CO2 fixation reaction), completing the cycle. Most carbon compounds in the biosphere are derived from the carbohydrate product of the Calvin Cycle. The abbreviated structure of a typical carbohydrate is (H-C-OH)n. Due to unequal sharing of electrons in a C-O bond, the carbon atom in CO2 is electron deficient relative to a carbon atom in a carbohydrate, that bonds with only one oxygen atom. Carbon in CO2 is thus said to be more oxidized, while carbon in a carbohydrate is more reduced. The Calvin Cycle does not directly utilize light energy, but is part of the process of photosynthesis. Some Calvin cycle reactions require ATP (adenosine triphosphate), a compound that functions in energy transfer, and NADPH

(reduced nicotinamide adenine dinucleotide phosphate), a source of hydrogen atoms for reduction reactions. ATP and NADPH are formed during light-energized reactions of photosynthesis.

3. Schematically explain cholesterol synthesis.

Cholesterol synthesis is an expensive process for cells in terms of energy. This pathway takes place in cytoplasm. The liver and intestines are major contributors to endogenous production. Acetyl-CoAunits are joined to form a 30-carbon compound and then three carbons are removed to produce cholesterol which has 27 carbon atoms.

The cholesterol synthesis steps can be divided into:

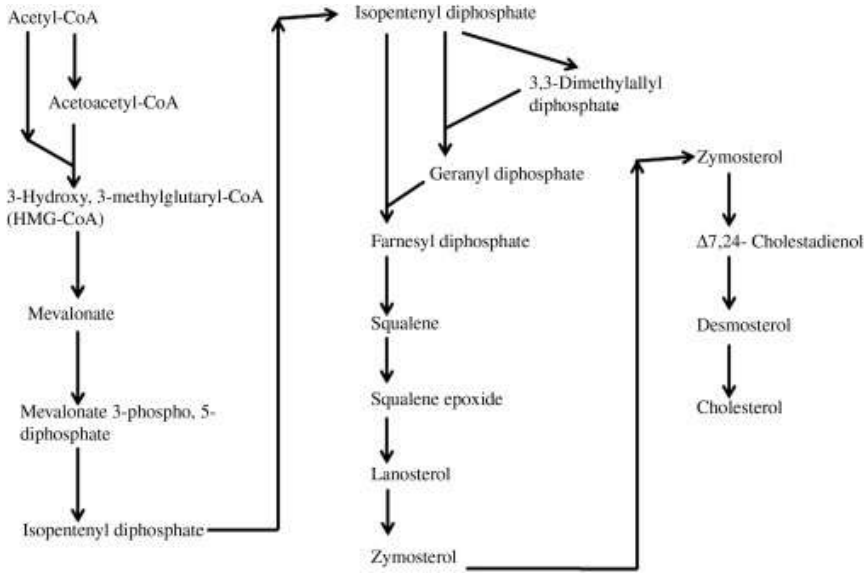
1. Mevalonate synthesis
2. Isopentenyl phosphate synthesis
3. Squalene formation
4. Lanosterol synthesis
5. Cholesterol formation.

Two acetyl-CoAs combine to form acetoacetyl-CoA, releasing CoA-SH in the presence of thiolase. Acetyl-CoA also condenses to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) catalyzed by HMG-CoA synthase. These enzymes are different from the enzymes used for ketone body synthesis in mitochondria. HMG-CoA is reduced by HMG-CoA reductase using NADPH to mevalonate. This enzyme is the regulatory enzyme of the pathway, and is inhibited by statins—the best lipid-lowering drugs.

Mevalonate is phosphorylated by three kinases sequentially utilizing three ATPs and is then decarboxylated to form isopentenyl diphosphate.

Isopentenyl diphosphate (5C) isomerizes to 3,3-dimethylallyl diphosphate (5C) by shifting a double bond and then condensation with isopentenyl diphosphate forms geranyl diphosphate (10C). Another isopentenyl diphosphate molecule joins to form the 15C compound, farnesyl diphosphate. Two such 15C molecules fuse to form 30C squalene.

Squalene is oxidized to squalene 2,3-epoxide by squalene epoxidase. During cyclization to lanosterol, a methyl group shifts from C14 to C13 and from C8 to C14. The methyl groups on C14 and C4 are removed to form 14-desmethyl lanosterol and then zymosterol. The double bond at C8–C9 is subsequently shifted to C5–C6 in two steps, forming desmosterol. The final step is the reduction of the double bond of the side chain yielding a cholesterol molecule



4. Describe the process of prostaglandin synthesis

Prostaglandins are found in most tissues and organs. They are produced by almost all nucleated cells. They are autocrine and paracrine lipid mediators that act upon platelets, endothelium, uterine and mast cells. They are **synthesized** in the cell from the fatty acid arachidonic acid.

Q.3. A) Answer <u>any one</u> of the following: (10)

1. Describe in detail the transcriptional process in Eukaryotes.

Prokaryotes and eukaryotes perform fundamentally the same process of transcription, with a few key differences. The most important difference between prokaryotes and eukaryotes is the latter's membrane-bound nucleus and organelles. With the genes enclosed in a nucleus, the eukaryotic cell must be able to transport its mRNA to the cytoplasm and must protect its mRNA from degrading before it is translated. Eukaryotes also employ three different polymerases that each transcribe a different subset of genes.

INITIATION

The eukaryotic promoters that we are most interested in are similar to prokaryotic promoters in that they contain a TATA box (Figure 1). However, initiation of transcription is much more complex in eukaryotes compared to prokaryotes. Unlike the prokaryotic RNA polymerase that can bind to a DNA template on its own, eukaryotes require several other proteins, called **transcription factors**, to first bind to the promoter region and then help recruit the appropriate polymerase.

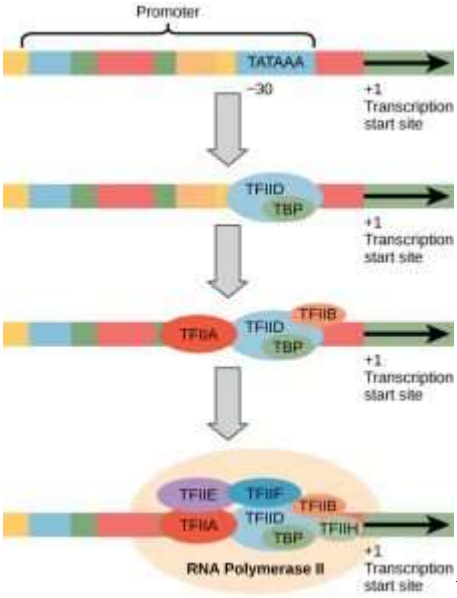


Figure 1 The generalized structure of a eukaryotic promoter and transcription factors.

In addition, there are three different RNA polymerases in eukaryotes, each of which is made up of 10 subunits or more. Each eukaryotic RNA polymerase also requires a distinct set of transcription factors to bring it to the DNA template.

RNA polymerase I is located in the nucleolus, a specialized nuclear substructure in which ribosomal RNA (rRNA) is transcribed, processed, and assembled into ribosomes. The rRNA molecules are considered structural RNAs because they have a cellular role but are not translated into protein. The rRNAs are components of the ribosome and are essential to the process of translation. RNA polymerase I synthesizes most of the rRNAs.

RNA polymerase II is located in the nucleus and synthesizes all protein-coding nuclear pre-mRNAs. Eukaryotic pre-mRNAs undergo extensive processing after transcription but before translation. For clarity, the term “mRNA” will only be used to describe the mature, processed molecules that are ready to be translated. RNA polymerase II is responsible for transcribing the overwhelming majority of eukaryotic genes.

RNA polymerase III is also located in the nucleus. This polymerase transcribes a variety of structural RNAs including transfer pre-RNAs (pre-tRNAs), and small nuclear pre-RNAs. The tRNAs have a critical role in translation; they serve as the adaptor molecules between the mRNA template and the growing polypeptide chain. Small nuclear RNAs have a variety of functions, including “splicing” pre-mRNAs and regulating transcription factors.

Each of the types of RNA polymerase recognizes a different promoter sequence and requires different transcription factors.

ELONGATION

Following the formation of the preinitiation complex, the polymerase is released from the other transcription factors, and elongation is allowed to proceed as it does in prokaryotes with the RNA polymerase synthesizing pre-mRNA in the 5′ to 3′ direction. As discussed previously, RNA polymerase II transcribes the major share of eukaryotic genes, so this section will focus on how this polymerase accomplishes elongation and termination.

Although the enzymatic process of elongation is essentially the same in eukaryotes and prokaryotes, the DNA template is more complex. When eukaryotic cells are not dividing,

their genes exist as a diffuse mass of DNA and proteins called chromatin. The DNA is tightly packaged around charged histone proteins at repeated intervals. These DNA–histone complexes, collectively called nucleosomes, are regularly spaced and include 146 nucleotides of DNA wound around eight histones like thread around a spool.

For RNA synthesis to occur, the transcription machinery needs to move histones out of the way every time it encounters a nucleosome. This is accomplished by a special protein complex called FACT, which stands for "facilitates chromatin transcription." This complex pulls histones away from the DNA template as the polymerase moves along it. Once the pre-mRNA is synthesized, the FACT complex replaces the histones to recreate the nucleosomes.

TERMINATION

The termination of transcription is different for the different polymerases. Unlike in prokaryotes, elongation by RNA polymerase II in eukaryotes takes place 1,000–2,000 nucleotides beyond the end of the gene being transcribed. This pre-mRNA tail is removed during mRNA processing. RNA polymerases I and III require termination signals. Genes transcribed by RNA polymerase I contain a specific 18-nucleotide sequence that is recognized by a termination protein. The process of termination in RNA polymerase III involves an mRNA hairpin that causes the mRNA to be released

Q. 3 A

2. Give an overview of de novo synthesis of Purines explaining the aqusition of various atoms of IMP.

Ans: Nucleotide consists of a purine or pyrimidine base plus a pentose sugar (ribose or deoxyribose) and a phosphoryl group (H_3PO_4). The purine ring consists of a 5-membered imidazol ring fused to a six-membered ring structure with two common or bridge carbon atoms (C-4 and C-5) and contains 4-N atoms. The pyrimidine ring has a simpler structure with only a six-membered ring with two N-atoms. Purine and pyrimidine nucleotides can be synthesized in living organisms either by (i) de novo pathways, or (ii) salvage pathways.

In de novo pathways, the synthesis of nucleotides begins with their metabolic precursors: amino acids, ribose-5-phosphate, CO_2, and NH_3. In salvage pathways, the breakdown products of nucleotides i.e. free bases and nucleosides are salvaged and recycled back to synthesize nucleotides again.

Salvage pathways may involve reconstruction of nucleotides from free bases by addition of ribose-phosphate moiety, or by phosphorylation of nucleosides. De novo pathways are more important quantitatively than salvage pathways. However, by using salvage pathways for nucleotides synthesis, the cells do conserve energy.

De Novo Synthesis of Purine Nucleotides (IMP, AMP & GMP):

i. Free bases are not intermediates in de novo pathways of nucleotides synthesis i.e., they are not synthesized and then attached to ribose phosphate.

ii. The purine ring structure is built up one or a few atoms at a time, and is gradually attached to ribose phosphate throughout the process.

iii. Phosphoribosyl pyrophosphate (PRPP), is an important intermediate and the starting point in purine nucleotide synthesis. It is formed from α-D-Ribose-5-phosphate (Fig. 9.34)

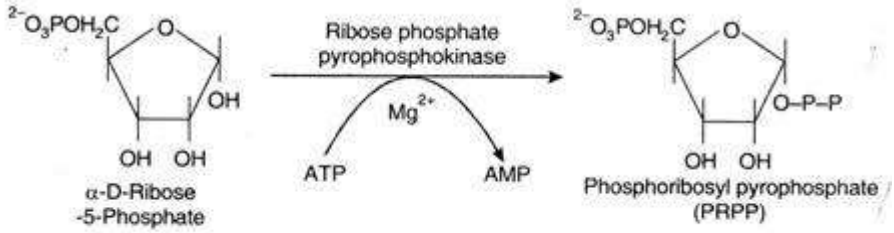


Fig. 9.34. Synthesis of PRPP from α-D-Ribose-5-phosphate

iv. (The ultimate precursors of the purine ring are shown in Fig. 9.35. These precursors were established from information obtained from isotopic experiments with ^{14}C or ^{15}N- labeled precursors that were administered into pigeons and tracing the incorporation of labeled atoms into the purine ring of their excreted uric acid).

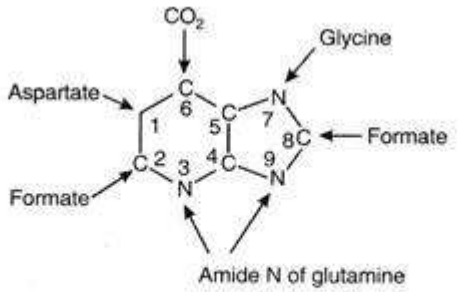


Fig. 9.35. Ultimate precursors of the atoms of purine ring as determined by isotope labeling experiments in pigeons. Formate was supplied in the form of N^{10}–formyltetrahydro folate. Glycine contributed the two bridge carbon atoms (C-4 & C-5) and also N-7.

iv. IMP (Inosine monophosphate or Inosinate) is the first purine nucleotide to be synthesized.

v. IMP is then converted into AMP and GMP.

Formation of IMP from PRPP:

a. Synthesis of IMP from PRPP (phosphoribosyl pyrophosphate) takes place in ten different steps as shown in Fig. 9.36.

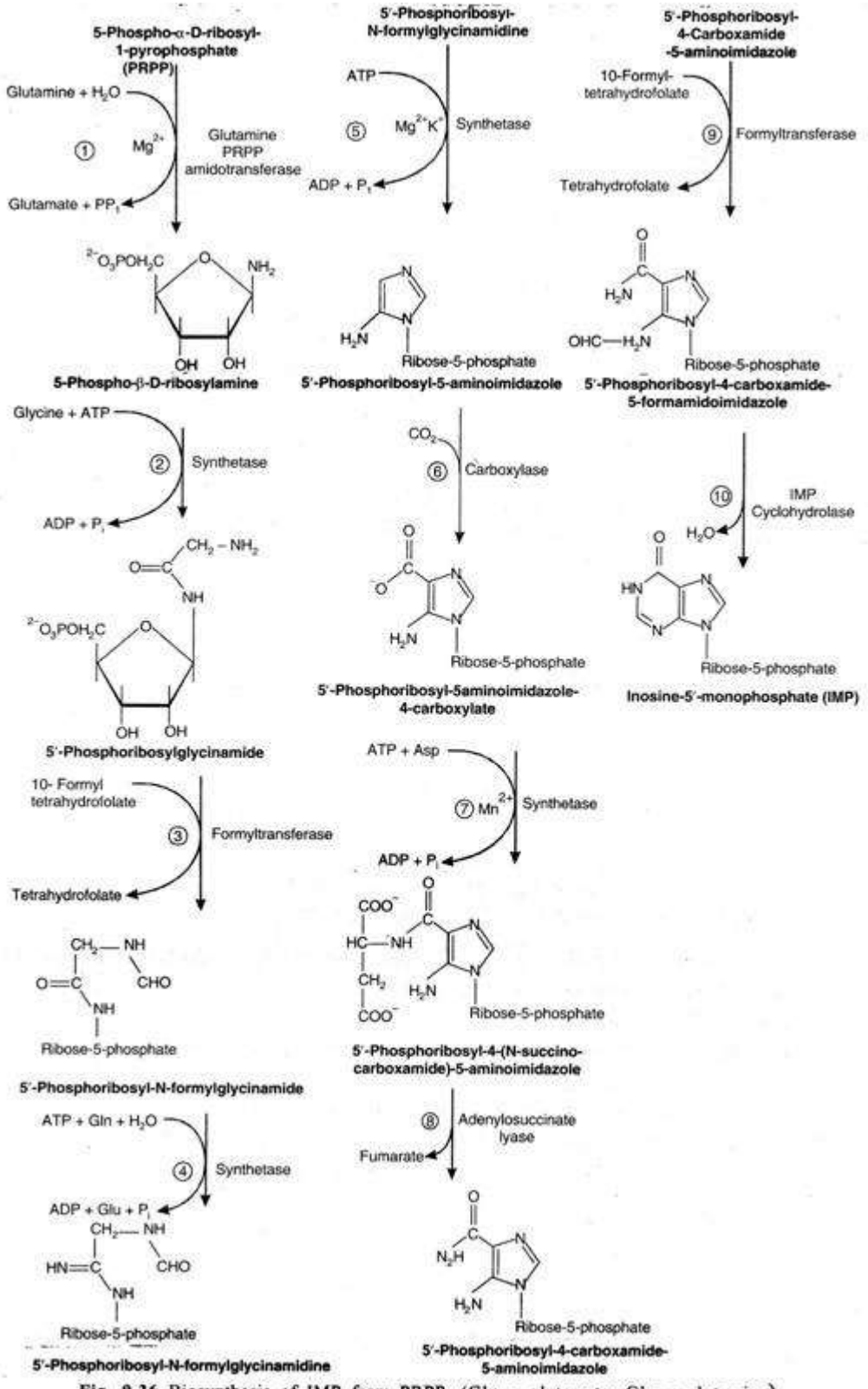


Fig. 9.36 Biosynthesis of IMP from PRPP. (Glu = glutamate; Gln = glutamine)

b. The 5-membered imidazol ring is added first to PRPP; the remaining six-membered ring of purine is built up afterwards.

(i) In the first committed step of this pathway, phosphoribosylamine is formed by the action of the enzyme glutamine phosphoribosyl pyrophosphate amidotransferase. An amino group

supplied by glutamine is attached to C-1 of PRPP and there is inversion of configuration at C-1, from a top position. The purine ring is subsequently built on this structure. Atom no. 9 (N-9) of the purine ring is introduced in this first step.

(ii) In the second step, the enzyme synthetase forms an amide bond between carboxyl group of glycine and amino group of phosphoribosylamine forming 5′-phosphoribosyl glycinamide. ATP is hydrolyzed to provide energy. The atoms 4, 5 and 7 of the purine ring are introduced in this step.

(iii) In the third step, C-8 of the purine ring is introduced as a formyl group donated by 10-formyl tetrahydrofolate, in presence of the enzyme formyl transferase, so that 5′-phosphoribosyl-N-formyl glycinamide is formed.

(iv) In the fourth step, N-3 of purine ring is introduced by transfer of another amino group from glutamine to phosphoribosyl formyl glycinamide by a synthetase enzyme, forming 5′-phosphoribosyl-N-formyl glycinamidine. ATP is hydrolyzed and provides energy.

(v) In the fifth step, cyclization reaction occurs in presence of synthetase, Mg^{2+}, and K^{+} ions, so that imidazol ring is closed. The product is 5′-phosphoribosyl-5- aminoimidazole.
(vi) In the sixth step, C-6 of the purine ring is introduced by addition of bicarbonate ($CO_2 + H_2O \rightarrow HCO_3^-$) in presence of a specific carboxylase enzyme. The product of this reaction is 5′-phosphoribosyl-5-aminoimidazole-4-carboxylate.
(vii) In the seventh step, N-8 of the purine ring is contributed by aspartate. The latter forms an amide with 4-carboxyl group in presence of synthetase, and a succinocarboxamide is formed. ATP is hydrolyzed and provides energy.

(viii) 5′-phosphoribosyl – 4 – (N-succino carboxamide) – 5-aminoimidazole is now cleaved in presence of adenylosuccinate lyase to release formate and forming 5′-phosphoribosyl- 4-carboxamide – 5 – aminoimidazole.

(ix) In the ninth step, the final atom of purine ring (i.e., C-2) is introduced which is supplied by a formyl group from 10-formyl tetrahydrofolate to the 5-amino group of the almost completed ribonucleotide.

(x) In the last step, a second ring closure takes place by elimination of water to form IMP. The enzyme involved is IMP-cyclohydrolase (IMP-synthase)

Synthesis of IMP from ribose-5-phosphate requires a total of six high energy phosphate groups from ATPs (assuming hydrolysis of pyrophosphate (P-P)) released in step (i).

Q. 3. B) Answer <u>any two</u> of the following: (10)

1. Explain HGPRT reaction and its significance.

Hypoxanthine-guanine phosphoribosyltransferase (HPRT) is the enzyme which catalyzes salvage of the purine bases guanine and hypoxanthine into their respective monophosphate nucleoside i.e., guanylic monophosphate (GMP) and inosine monophosphate (IMP). This is a PRPP-dependent reaction and HPRT is encoded by a single structural gene located on the X chromosome (Xq26-27). The entire HPRT gene has been sequenced and several different alterations in the coding region have been identified as contributing to HPRT deficiency (Camici et al., 2010). There is a spectrum of disorders related to HPRT deficiency and the three major clinical features are: (1) hyperuricemia, (2) neurologic manifestations, and (3) behavioral disturbance. The disorder that has achieved the most attention is Lesch–Nyhan disease, originally described in 1964 (Lesch and Nyhan, 1964). The expression of this disorder is almost exclusively recessive, but the small number of females reported is presumably related to nonrandom (skewed) inactivation of the normal X chromosome .

The myriad manifestations of Lesch–Nyhan disease include motor development retardation, extrapyramidal manifestations with dystonia, chorea, and athetosis, long tract signs with hyperreflexia, and positive Babinski response. The neurologic picture can mimic athetoid type of cerebral palsy. However, the hallmarks of self-injurious behavior and opisthotonic arching of the back usually allow clinical diagnosis of the disorder in older children. In addition to the self-injurious behavior, there is often cognitive impairment as well as aggressive and impulsive behavior. Physiologically, there is typically hyperuricemia and uricosuria, which may lead to gouty arthritis, tophus formation, hematuria, kidney stones, urinary tract infection, and renal failure. There is a spectrum of disease, reflective of the activity of HPRT, and, in this disorder, the erythrocyte activity is essentially undetectable

2. Name the key enzyme in DNA replication. How does it bring about polymerization of Nucleotides?

DNA REPLICATION DNA replication is the process of producing two identical replicas from one original DNA molecule. This biological process occurs in all living organisms and is the basis for biological inheritance. DNA is made up of two strands and each strand of the original DNA molecule serves as template for the production of the complementary strand, a process referred to as semiconservative replication.

REPLICATION FORK The replication fork is a structure that forms within the nucleus during DNA replication. It is created by helicases, which break the hydrogen bonds holding the two DNA strands together. The resulting structure has two branching "prongs", each one made up of a single strand of DNA. These two strands serve as the template for the leading and lagging strands, which will be created as DNA polymerase matches complementary nucleotides to the templates; the templates may be properly referred to as the leading strand template and the lagging strand template.

REPLICATION BUBBLE It is formed during replication in both eukaryotic and prokaryotic DNA. It is the place where replication occurs actively. It is otherwise known as replication bubble. Formation of the replication eye provides the theta like structure to the circular DNA during replication in prokaryotes. Each replication bubble found to have two replication forks, each at the corner of an eye.

BIDIRECTIONAL REPLICATION DNA replication typically proceeds in both directions simultaneously at any particular replicon. Note that the leading and lagging strands at the two replication forks are on opposite physical strands of the dsDNA starting at the Origin of Replication. This creates two replication forks, moving in opposite directions. Bi-directional nature of replication was identified by studying the appearance of nucleotides at the replication forks in connection with the change in the radioactive material intensity.

REPLICON Initiation of DNA replication at origins leads to the concept of a replicon. A replicon is a DNA molecule or RNA molecule, or a region of DNA or RNA, that replicates from a single origin of replication. For eukaryotic chromosomes, there are multiple replicons per chromosome. The definition of replicons is somewhat confused with mitochondria, as they use unidirectional replication with two separate origins.

SINGLE-STRANDED DNA-BINDING PROTEIN (SSB) It was called a DNA-unwinding protein (M.W. 22,000). Single-stranded DNA-binding protein, or SSB, binds to single-stranded regions of DNA to prevent premature annealing, to protect the single-stranded DNA from being digested by nucleases, and to remove secondary structure from the DNA to allow other enzymes to function effectively upon it. Single-stranded DNA is produced during all aspects of DNA metabolism: replication, recombination and repair. As well as stabilizing this single-stranded DNA, SSB proteins bind to and modulate the function of numerous proteins involved in all of these processes. SSB proteins have been identified in both viruses and organisms from bacteria to humans.

It contains four subunits. The major function of SSB is to prevent recoiling of DNA strands after it’s unwinding by helicases. Thus, SSB plays vital role in replication.

PRIMER A primer is a strand of nucleic acid that serves as a starting point for DNA synthesis. It is required for DNA replication because the enzymes that catalyze this process, DNA polymerases, can only add new nucleotides to an existing strand of DNA. The polymerase starts replication at the 3'-end of the primer, and copies the opposite strand. In most cases of natural DNA replication, the primer for DNA synthesis and replication is a short strand of RNA.

DNA PRIMASE DNA primase is an enzyme involved in the replication of DNA. DNA primase is, in fact, a type of RNA polymerase which creates an RNA primer (later this RNA piece is removed by a 5' to 3' exonuclease); next, DNA polymerase uses the RNA primer to replicate ssDNA. Primase catalyzes the synthesis of a short RNA (or DNA in some organisms) segment called a primer complementary to a ssDNA template. Primase is of key importance in DNA replication because no known DNA polymerases can initiate the synthesis of a DNA strand without an initial RNA or DNA primer (for temporary DNA elongation).

PRIMOSOME It is a complex containing Pri A, Pri B, Pri C, Dna T, Dna B, Dna C and Primase (Dna G) proteins. Primososme complex without primase referred as preprimosome complex. Primosome complex plays a vital role in lagging strand synthesis by synthesizing the primer at frequent intervals. Of all the proteins actual primer synthesizing ability relies with primase molecule. But each protein of the complex has their own role i.e. each of them necessary for the function of primosome complex in replication for example. Pri A, Pri B, Pri C necessary for the binding of primosome complex to DNA. Dna B responsible for helicase activity. Dna C aids the binding of Dna

B. Dna T helps the binding of DnaB and Dna C complex to Pri A, Pri B and Pri C complex.

Enzyme Function in DNA replication DNA Helicase Also known as helix destabilizing enzyme. Unwinds the DNA double helix at the Replication Fork. DNA Polymerase Builds a new duplex DNA strand by adding nucleotides in the 5' to 3' direction. Also performs proof-reading and error correction. DNA clamp A protein which prevents DNA polymerase III from dissociating from the DNA parent strand. Single-Strand Binding (SSB) Proteins Bind to ssDNA and prevent the DNA double helix from re-annealing after DNA helicase unwinds it, thus maintaining the strand separation. Topoisomerase Relaxes the DNA from its super-coiled nature. DNA Gyrase Relieves strain of unwinding by DNA helicase; this is a specific type of topoisomerase DNA Ligase Re-anneals the semi-conservative strands and joins Okazaki Fragments of the lagging strand. Primase Provides a starting point of RNA (or DNA) for DNA polymerase to begin synthesis of the new DNA strand. Telomerase Lengthens telomeric DNA by adding repetitive nucleotide sequences to the ends of eukaryotic chromosomes. DNA REPLICATION PROTEINS At the replication fork, many replication enzymes assemble on the DNA into a complex molecular machine called the replisome. The following is a list of major DNA replication enzymes that participate in the replisome:

DNA GYRASE DNA gyrase, often referred to simply as gyrase, is an enzyme that relieves strain while double-strand DNA is being unwound by helicase. This causes negative supercoiling of the DNA. The ability of gyrase to relax positive supercoils comes into play during DNA replication. The right-handed nature of the DNA double helix causes positive supercoils to accumulate ahead of a translocating enzyme, in the case of DNA replication, a DNA polymerase. The ability of gyrase (and topoisomerase IV) to relax positive supercoils allows superhelical tension ahead of the polymerase to be released so that replication can continue.

Negative supercoiling of bacterial DNA by DNA gyrase influences all metabolic processes involving DNA and is essential for replication. Gyrase supercoils DNA by a mechanism called sign inversion, whereby a positive supercoil is directly inverted to a negative one by passing a DNA segment through a transient double-strand break. Reversal of this scheme relaxes DNA, and this mechanism also accounts for the ability of gyrase to catenate and uncatenate DNA rings. Each round of supercoiling is driven by a conformational change induced by adenosine triphosphate (ATP) binding: ATP hydrolysis permits fresh cycles. The A subunit is particularly associated with the concerted breakage- and-rejoining of DNA and the B subunit mediates energy transduction. Gyrase is a prototype for a growing class of prokaryotic and eukaryotic topoisomerases that interconvert complex forms by way of transient double-strand breaks.

DNA HELICASES Helicases are often used to separate strands of a DNA double helix or a self-annealed RNA molecule using the energy from ATP hydrolysis, a process characterized by the breaking of hydrogen bonds between annealed nucleotide bases. They also function to remove nucleic acid-associated proteins and catalyze homologous DNA recombination.

They are proteins, which are involved in the unwinding of DNA molecule. There are four kinds of helicases namely Dna A, Dna B, Rep Proteins, and Helicases - II. Dna-A protein (Mw 48,000) - It binds to 4 of 9 mer sequence and unwinds a 3 of 13 mer sequence at Ori C site and forms an open complex during initiation of replication. It is the first protein which binds to DNA to initiate DNA replication. Dna-B proteins (Mw- 3,00,000)- It is a primosome constituent and consists of six subunits. It unwinds DNA during replication. It is responsible for the extension of open complex during replication. Rep Proteins (Mw

65,000) - It is a helicase consisting of one subunit. It binds to the 5'-- 3' template and moves in 3'-- 5' direction. It actively participates in leading strand synthesis in replication.
DNA Helicase - II (Mw 75,000) - It is helicase consisting of only one subunit. It binds with 3'--5' template stand and moves along in 5'-- 3' direction. It is involved in lagging strand synthesis in replication. The role of helicases is to unwind the duplex DNA in order to provide a single-stranded DNA for replication, transcription, and recombination for instance.
DNA LIGASE The DNA ligases are responsible for connecting DNA segments during replication, repair and recombination. They are class of enzymes that catalyze the formation of alpha-phosphodiester bond between two DNA chains. This enzyme requires the free OH group at the 3' end of other DNA strand and phosphate group at 5' end of the other. The formation of a phosphodiester bond between these groups is an endergonic (energy absorption) reaction. Hence energy source required for ligation. In E.Coli and other bacteria NAD+ supplies the energy whereas in animals i.e. eukaryotes ATP play the role. It plays a role in repairing single-strand breaks in duplex DNA in living organisms, but some forms (such as DNA ligase IV) may specifically repair double-strand breaks (i.e. a break in both complementary strands of DNA).
The DNA ligase reaction, which proceeds in three steps: adenylation (addition of AMP) of a lysine residue in the active center of the enzyme, pyrophosphate is released; transfer of the AMP to the 5' phosphate of the so-called donor, formation of a pyrophosphate bond; formation of a phosphodiester bond between the 5' phosphate of the donor and the 3' hydroxyl of the acceptor. In mammals, there are four specific types of ligase. DNA ligase I: ligates the nascent DNA of the lagging strand after the DNA polymerase I has removed the RNA primer from the Okazaki fragments.
DNA ligase II: alternatively spliced form of DNA ligase III found in non-dividing cells. DNA ligase III: complexes with DNA repair protein XRCC1 to aid in sealing DNA during the process of nucleotide excision repair and recombinant fragments. DNA ligase IV: complexes with XRCC4. It catalyzes the final step in the non-homologous end joining DNA double-strand break repair pathway. It is also required for V(D)J recombination, the process that generates diversity in immunoglobulin and T-cell receptor loci during immune system development.
TOPOISOMERASES Topoismerases are a group of enzymes which controls supercoiling of DNA thereby maintaining it in the proper topological state or superhelical tension. There are two classes of topoisomerases. They are type – I topoisomerase and type – II topoisomerase. TYPE - I TOPOISOMERASE Type-I topoisomerase (Nicking-Closing enzymes) are monomeric 100kd proteins that are widespread in both prokaryotes and eukaryotes. They can remove negative supercoils without leaving nicks in the DNA molecule.
MECHANISM After the enzyme binds to a DNA molecule and cuts on strand, the free 5' phosphate on the DNA is covalently attached to a tyrosine residue in the enzyme in the case of prokaryotes (the free 3'- phosphate on the DNA is covalently attached in the case of eukaryotes). The DNA strand that has not been cleaved is then passed through the single stranded break. The cleaved strand is then resealed. The type -I topoisomerase from E.Coli acts on negatively supercoiled molecules but not on positively supercoiled molecules. In contrast, type- I topoisomerase from eukaryotic cells can remove both positive and negative supercoils. Type-I topoisomerase reversibly catenates (interlinks) single stranded circles.
TYPE-II TOPOISOMERASES The first type II topoisomerase (Topo II) to be described was isolated from E. coli. and named DNA gyrase are 375 kd proteins that consists of two pairs of subunits designated A and B. Topo II enzymes have the ability to cut both strands

of a double- stranded DNA molecule, pass another portion of the duplex through the cut, and reseal the cut in a process that utilizes ATP. Depending on the DNA substrate, these movements will have the effect of changing a positive supercoil into a negative supercoil or of increasing the number of negative supercoils by 2.

The Topo II enzymes from mammalian cells cannot, like E. coli DNA gyrase, increase the superhelical density at the expense of ATP; presumably no such activity is required in eukaryotes, since binding of histones increases the potential superhelicity. All type II topoisomerases catalyze catenation and decatenation, that is, the linking and unlinking, of two different DNA duplexes. DNA gyrase has the ability to cut a double stranded DNA molecule, pass another portion of the duplex DNA through the cut, and reseal the cut. It changes the linking number of the DNA by 2

DNA POLYMERASES These enzymes copy DNA sequences by using one strand as a template. The reaction catalyzed by DNA polymerases is the addition of deoxyribonucleotides to a DNA chain by using dNTPs as substrates. All DNA polymerases require a template strand, which is copied. DNA polymerases also require a primer, which is complementary to the template. The reaction of DNA polymerases is thus better understood as the addition of nucleotides to a primer to make a sequence complementary to a template.

The best-studied bacterium, E. coli, has three DNA polymerase types. DNA polymerase I (Pol I) is primarily a repair enzyme, although it also has a function in replication. About 400 Pol I molecules exist in a single bacterium. DNA polymerase I only makes an average of 20 phosphodiester bonds before dissociating from the template. These properties make good sense for an enzyme that is going to replace damaged DNA. Damage occurs at separate locations so the large number of Pol I molecules means that a repair enzyme is always close at hand. DNA polymerase I has nucleolytic (depolymerizing) activities, which are an intimate part of their function. The 5′ to 3′ exonuclease activity removes base-paired sequences ahead of the polymerizing activity. During replication, this can remove primers ahead of the polymerizing function of the polymerase.

Another intimate function of DNA polymerase I (and of the other forms of DNA polymerase found in E. coli) is the 3′ to 5′ exonuclease activity. These activities can de-polymerize DNA starting from the newly synthesized end. The 3′ to 5′ exonuclease activity serves an editing function to ensure the fidelity of replication. Suppose DNA polymerase were to make a mistake and add a T opposite a G in the template strand. When the enzyme begins the next step of polymerization, the T is not properly paired with the template. The 3′ to 5′ exonucleolytic activity of DNA polymerase then removes the unpaired nucleotide, releasing TMP, until a properly paired stretch is detected. Then polymerization can resume. This cycle costs two high-energy phosphate bonds because TTP is converted to TMP. While this may seem wasteful of energy, the editing process does keep the information store of the cell intact.

DNA polymerase II is a specialized repair enzyme. Like Pol I, a large number of Pol II molecules reside in the cell (about 100). The enzyme is more processive than Pol I. Pol II has the same editing (3′ to 5′) activity as Pol I, but not the 5′ to 3′ exonuclease activity. The actual replication enzyme in E. coli is DNA polymerase III. Its properties contrast with Pol I and Pol II in several respects. Pol III is much more processive than the other enzymes, making about 500,000 phosphodiester bonds on the average. In other words, it is about 5,000 times more processive than Pol I and 50 times more processive than Pol II. Pol III is a multisubunit enzyme. It lacks a 5′ to 3′ exonucleolytic activity, although a subunit of the enzyme carries out the editing (3′ to 5′) function during replication. Finally, only about 10 molecules of Pol III reside in each cell. This remains consistent with the

function of Pol III in replication, because the chromosome only needs to be copied once per generation.

Therefore, the cell only requires a few molecules of the enzyme. Pol III synthesizes DNA at least a hundred times more rapidly than the other polymerases. It can synthesize half of the bacterial chromosome in a little more than 20 minutes, which is the fastest that the bacterium can replicate.

DNA POLYMERASE – EUKARYOTIC Following are the six important eukaryotic DNA polymerases. They are as follows: i) DNA polymerase α ii) DNA polymerase β iii) DNA polymerase γ iv) DNA polymerase δ v) DNA polymerase Ɛ vi) DNA polymerase σ

i) DNA polymerase α It is composed of four subunits of which one has the primase activity. The largest subunit of it has polymerase activity. It is supposed to exist in several forms like α 1, α 2, and α 3. DNA polymerase a in association with DNA polymerase δ, is involved in the replication of nuclear chromosomal DNA. The replication is also aided by another protein factors called Accessory Proteins and AP4A. These protein factors appear to have regulatory function. DNA pol α is supposed to carryout lagging strand synthesis. ii) DNA polymerase -β It is involved in the repair of DNA. iii) DNA polymerase - γ This polymerase is involved in the replication of mitochondria DNA.

iv) DNA polymerase -δ This polymerase has polymerase function and 3'-- >5' exonuclease activity. It is involved in leading strand synthesis. v) DNA polymerase -Ɛ It is supposed to replace DNA pol delta in some situations such as DNA repair. vi) DNA polymerase - σ This polymerase seems to be expressed only in the bone marrow cells. It is the only eukaryotic polymerase that has a deoxy ribonuclease activity

RNA PRIMERS RNA primers are single strand oligoribonucleotides with 40 to 60 base pairs. For replication RNA primers are required. This is because DNA polymerases require RNA primer for their action to start in invivo condition. In replication RNA primer is synthesized by two different enzymes namely RNA polymerase and Primosome complex. RNA polymerase synthesize RNA primer for synthesize of leading strand whereas Primosome synthesize RNA primer for lagging strand synthesis. In addition to this, RNA polymerase synthesizes only one primer whereas Primosome synthesizes many primers.

OKAZAKI FRAGMENTS Fragments synthesized during lagging strand formation of replication was identified and proved by Rejis Okazaki. Hence, by his name these fragments are called as Okazaki's fragments. They are short polynucleotides with 1000-2000 base pairs in length. These fragments are synthesized by DNA polymerases. Even though they are formed during replication, they are joined to form larger DNA at the completion of replication by the action of DNA ligase. The invention of Okazaki fragments lead to the proposal of semidiscontinuous replication concept.

RESTRICTION ENDONUCLEASE A restriction enzyme (or restriction endonuclease) is an enzyme that cuts DNA at or near specific recognition nucleotide sequences known as restriction sites. Restriction enzymes are commonly classified into three types, which differ in their structure and whether they cut their DNA substrate at their recognition site, or if the recognition and cleavage sites are separate from one another. To cut DNA, all restriction enzymes make two incisions, once through each sugar-phosphate backbone (i.e. each strand) of the DNA double helix.

These enzymes are found in bacteria and archaea and provide a defense mechanism against invading viruses. Inside a prokaryote, the restriction enzymes selectively cut up foreign DNA in a process called restriction; while host DNA is protected by a modification enzyme (a methylase) that modifies the prokaryotic DNA and blocks cleavage. Together, these two processes form the restriction modification system.

EXONUCLEASES Exonucleases are enzymes that work by cleaving nucleotides one at a time from the end (exo) of a polynucleotide chain. A hydrolyzing reaction that breaks phosphodiester bonds at either the 3’ or the 5’ end occurs. Its close relative is the endonuclease, which cleaves phosphodiester bonds in the middle (endo) of a polynucleotide chain. Eukaryotes and prokaryotes have three types of exonucleases involved in the normal turnover of mRNA: 5’ to 3’ exonuclease, which is a dependent decapping protein, 3’ to 5’ exonuclease, an independent protein, and poly (A)-specific 3’ to 5’ exonuclease.

TELOMERASE Telomerase also called telomere terminal transferase is a ribonucleoprotein that is an enzyme that adds DNA sequence repeats ("TTAGGG" in all vertebrates) to the 3' end of DNA strands in the telomere regions, which are found at the ends of eukaryotic chromosomes. This region of repeated nucleotide called telomeres contains noncoding DNA and hinders the loss of important DNA from chromosome ends. As a result, every time the chromosome is copied, only 100–200 nucleotides are lost, which causes no damage to the organism's DNA. Telomerase is a reverse transcriptase that carries its own RNA molecule, which is used as a template when it elongates telomeres, which are shortened after each replication cycle.

REVERSE TRANSCRIPTASE Reverse transcriptase is an enzyme used to generate complementary DNA (cDNA) from an RNA template, a process termed reverse transcription. It is mainly associated with retroviruses. 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.

Q. 3 B)

3. Describe the role of sigma factor in Transcription.

A **sigma factor** (**σ factor**) (specificity **factor**) is a protein needed for initiation of **transcription** in bacteria. It is a bacterial **transcription** initiation **factor** that enables specific binding of RNA polymerase (RNAP) to gene promoters.

In prokaryotic cells, a single subunit of RNA polymerase, called sigma, orchestrates the process of transcription initiation. Sigma binds to the multisubunit coreRNA polymerase ($\alpha_2\beta\beta'$), creating RNA polymeraseholoenzyme ($\alpha_2\beta\beta'\sigma$), which performs transcription initiation. Holoenzyme recognizes the two conserved hexamer sequences that constitute a prokaryotic promoter,exposes the single-stranded DNA template necessary fortranscription initiation, and begins synthesizing thenascent RNA chain. When the nascent RNA is five to tennucleotides long, sigma is released, terminating the initiation phase of transcription. Core RNA polymerase thencarries out the elongation and termination phases of transcription.

4. Write a note on termination of Prokaryotic Transcription.

Termination

RNA synthesis will continue along the DNA template strand until the polymerase encounters a signal that tells it to stop, or terminate, transcription. In prokaryotes, this signal can take two forms, rho-independent and rho-dependent.

Rho-independent Terminator

The rho-independent terminator is the more simple of the two systems and as a result is also called simple termination. The rho-independent signal is found on the DNA template strand and consists of a region that contains a section that is then repeated a few base pairs away in the inverted sequence.

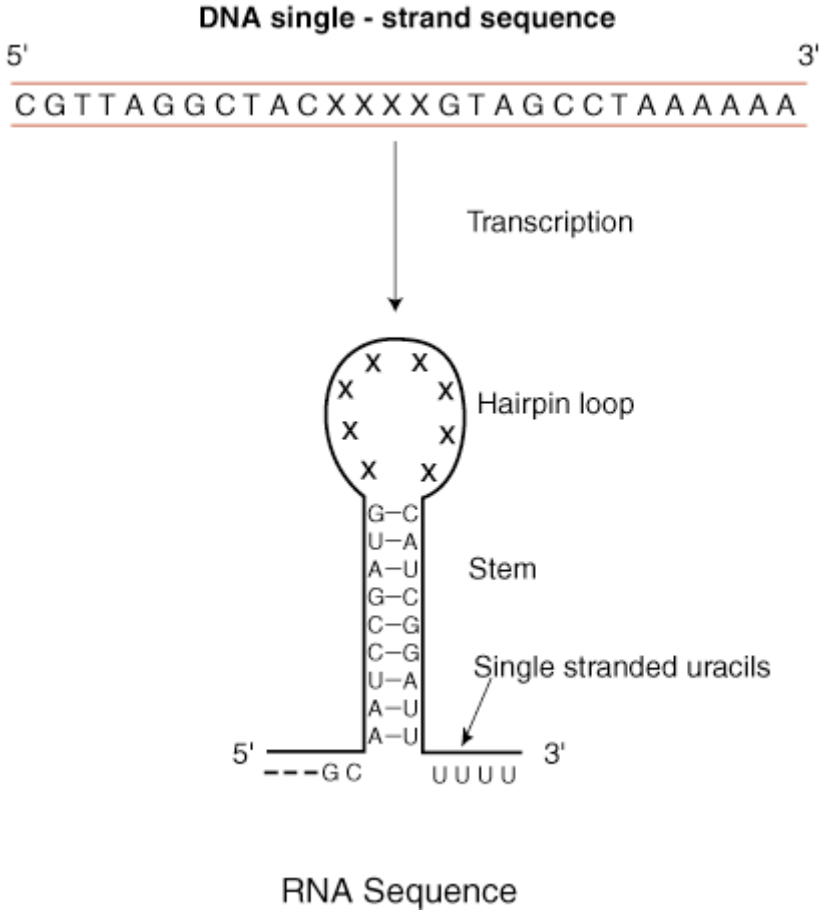


Figure %: Rho-Independent Terminator

As is shown in the figure, the patch is followed by a short string of adenines. When this stretch is transcribed into an RNA sequence, the RNA can fold back and base pair with itself forming a hairpin loop.

As you can see, the string of adenines in the DNA sequence are transcribed into uracils in the RNA sequence. Because the uracil bases will only pair weakly with the adenines, the RNA chain can easily be released from the DNA template, terminating transcription.

Rho-Dependent Terminator

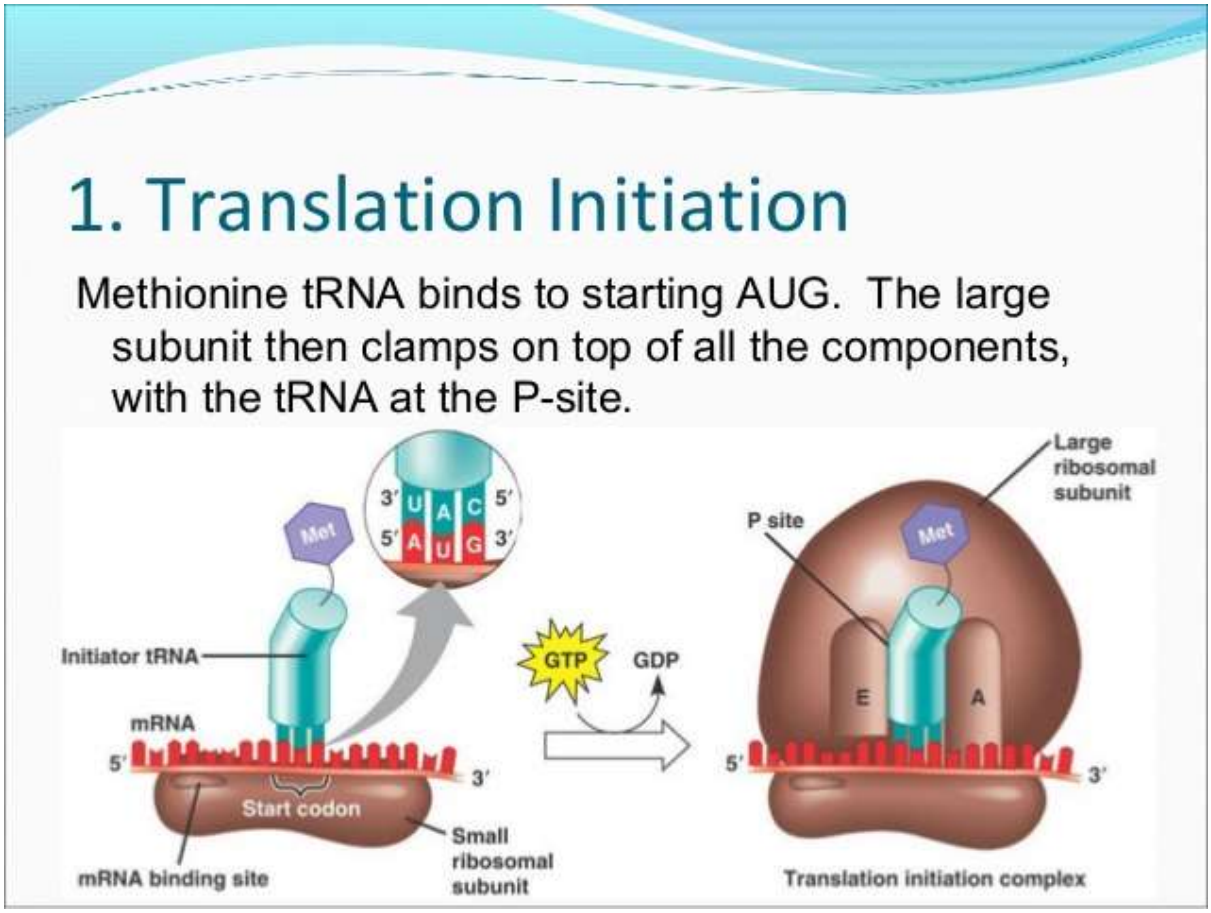
The rho-dependent terminator received its name because it is dependent on a specific protein called a rho factor. The rho factor is thought to bind to the end of the RNA chain and slide along the strand towards the open complex bubble. When the factor catches the polymerase, it causes the termination of transcription. The mechanism of this termination is unclear, but the rho factor could in some way pull the polymerase complex off of the DNA strand.

Q.4. A) Answer <u>any one</u> of the following: (10)

1. With the help of a suitable diagram, explain the process of Chain Initiation in Translation.

Protein synthesis begins with the formation of an initiation complex. In *E. coli*, this complex involves the small 30S ribosome, the mRNA template, three initiation factors (IFs; IF-1, IF-2, and IF-3), and a special initiator tRNA, called tRNA*fMet*. The initiator tRNA interacts with the start codon AUG (or rarely, GUG), links to a formylated methionine called fMet, and can also bind IF-2. Formylated methionine is inserted by fMet−tRNAf^{met} at the beginning of every polypeptide chain synthesized by *E. coli*, but it is usually clipped off after translation is complete. When an in-frame AUG is encountered during translation elongation, a non-formylated methionine is inserted by a regular Met-tRNA^{Met}. In *E. coli* mRNA, a sequence upstream of the first AUG codon, called the **Shine-Dalgarno sequence** (AGGAGG), interacts with the rRNA molecules that compose the ribosome. This interaction anchors the 30S ribosomal subunit at the correct location on the mRNA template. Guanosine triphosphate (GTP), which is a purine nucleotide triphosphate, acts as an energy source during translation—both at the start of elongation and during the ribosome's translocation.

In eukaryotes, a similar initiation complex forms, comprising mRNA, the 40S small ribosomal subunit, IFs, and nucleoside triphosphates (GTP and ATP). The charged initiator tRNA, called Met-tRNAi, does not bind fMet in eukaryotes, but is distinct from other Met-tRNAs in that it can bind IFs. Instead of depositing at the Shine-Dalgarno sequence, the eukaryotic initiation complex recognizes the 7-methylguanosine cap at the 5′ end of the mRNA. A cap-binding protein (CBP) and several other IFs assist the movement of the ribosome to the 5′ cap. Once at the cap, the initiation complex tracks along the mRNA in the 5′ to 3′ direction, searching for the AUG start codon. Many eukaryotic mRNAs are translated from the first AUG, but this is not always the case. According to **Kozak's rules**, the nucleotides around the AUG indicate whether it is the correct start codon. Kozak's rules state that the following consensus sequence must appear around the AUG of vertebrate genes: 5′-gccRccAUGG-3′. The R (for purine) indicates a site that can be either A or G, but cannot be C or U. Essentially, the closer the sequence is to this consensus, the higher the efficiency of translation. Once the appropriate AUG is identified, the other proteins and CBP dissociate, and the 60S subunit binds to the complex of Met-tRNAi, mRNA, and the 40S subunit. This step completes the initiation of translation in eukaryotes.



2. What are Post Translational Modification? Explain with any two suitable examples.
Post-translational modifications are key mechanisms to increase proteomic diversity. While the genome comprises 20,000 to 25,000 genes, the proteome is estimated to encompass over 1 million proteins. Changes at the transcriptional and mRNA levels increase the size of the transcriptome relative to the genome, and the myriad of different post-translational modifications exponentially increases the complexity of the proteome relative to both the transcriptome and genome.

a. Phosphorylation, the addition of a phosphate group, usually to serine, threonine, and tyrosine (*O*-linked), or histidine (*N*-linked)

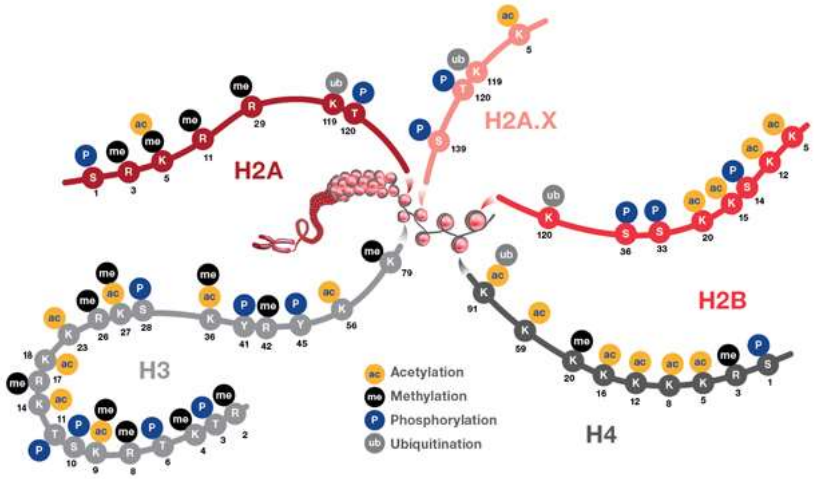
Reversible protein phosphorylation, principally on serine, threonine or tyrosine residues, is one of the most important and well-studied post-translational modifications. Phosphorylation plays critical roles in the regulation of many cellular processes, including cell cycle, growth, apoptosis and signal transduction pathways. In the following example, western blot analysis was used to evaluate phosphoprotein specificity in lysates obtained from serum-starved HeLa and NIH 3T3 cancer cell lines stimulated with epidermal growth factor (EGF) and platelet derived growth factor (PDGF), respectively.

b. Methylation is the addition of a methyl group, usually at lysine or arginine residues.

the transfer of one-carbon methyl groups to nitrogen or oxygen (N- and O-methylation, respectively) to amino acid side chains increases the hydrophobicity of the protein and can neutralize a negative amino acid charge when bound to carboxylic

acids. Methylation is mediated by methyltransferases, and S-adenosyl methionine (SAM) is the primary methyl group donor.

Methylation occurs so often that SAM has been suggested to be the most used substrate in enzymatic reactions after ATP. Additionally, while N-methylation is irreversible, O-methylation is potentially reversible. Methylation is a well-known mechanism of epigenetic regulation, as histone methylation and demethylation influences the availability of DNA for transcription. Amino acid residues can be conjugated to a single methyl group or multiple methyl groups to increase the effects of modification.



Representation showing post-translational modifications associated with histone particles. Nucleosomes are represented by red spheres wrapped by DNA (shown in gray). Also depicted are the positions of PTMs located on the histone proteins H2A (and H2A.X), H2B, H3, and H4. These PTMs impact gene expression by altering chromatin structure and recruiting histone modifiers. PTM events mediate diverse biological functions such as transcriptional activation and inactivation, chromosome packaging, and DNA damage and repair processes.

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c. N-acetylation

N-acetylation, or the transfer of an acetyl group to nitrogen, occurs in almost all eukaryotic proteins through both irreversible and reversible mechanisms. N-terminal acetylation requires the cleavage of the N-terminal methionine by methionine aminopeptidase (MAP) before replacing the amino acid with an acetyl group from acetyl-CoA by N-acetyltransferase (NAT) enzymes. This type of acetylation is co-translational, in that N-terminus is acetylated on growing polypeptide chains that are still attached to the ribosome. While 80 to 90% of eukaryotic proteins are acetylated in this manner, the exact biological significance is still unclear.

Acetylation at the ε-NH2 of lysine (termed lysine acetylation) on histone N-termini is a common method of regulating gene transcription. Histone acetylation is a reversible event

that reduces chromosomal condensation to promote transcription, and the acetylation of these lysine residues is regulated by transcription factors that contain histone acetyletransferase (HAT) activity. While transcription factors with HAT activity act as transcription co-activators, histone deacetylase (HDAC) enzymes are co-repressors that reverse the effects of acetylation by reducing the level of lysine acetylation and increasing chromosomal condensation. Sirtuins (silent information regulator) are a group of NAD-dependent deacetylases that target histones. As their name implies, they maintain gene silencing by hypoacetylating histones and have been reported to aid in maintaining genomic stability.

While acetylation was first detected in histones, cytoplasmic proteins have been reported to also be acetylated, and therefore acetylation seems to play a greater role in cell biology than simply transcriptional regulation. Furthermore, crosstalk between acetylation and other post-translational modifications, including phosphorylation, ubiquitination and methylation, can modify the biological function of the acetylated protein. Protein acetylation can be detected by chromatin immunoprecipitation (ChIP) using acetyllysine-specific antibodies or by mass spectrometry, where an increase in histone by 42 mass units represents a single acetylation.

d. Glycosylation

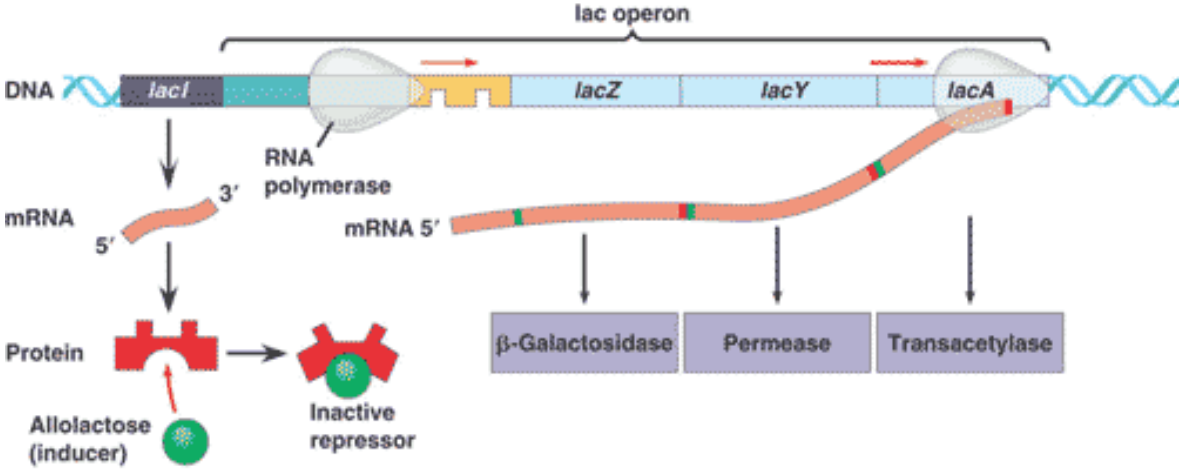
Protein glycosylation is acknowledged as one of the major post-translational modifications, with significant effects on protein folding, conformation, distribution, stability and activity.

e. Phosphorylation

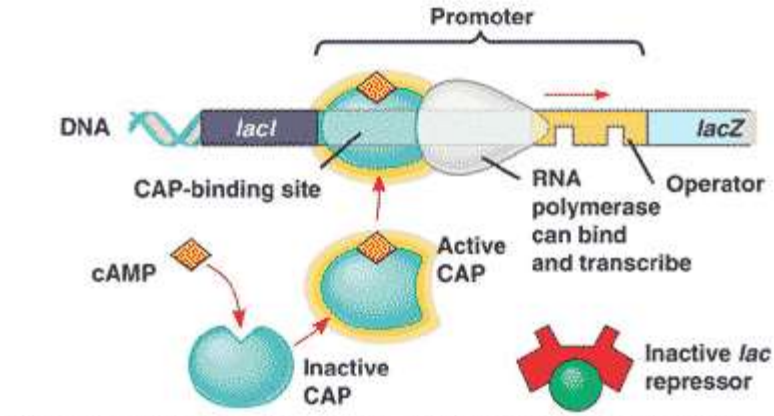
Reversible protein phosphorylation, principally on serine, threonine or tyrosine residues, is one of the most important and well-studied post-translational modifications. Phosphorylation plays critical roles in the regulation of many cellular processes, including cell cycle, growth, apoptosis and signal transduction pathways.

Q. 4. B) Answer <u>any two</u> of the following: **(10)**

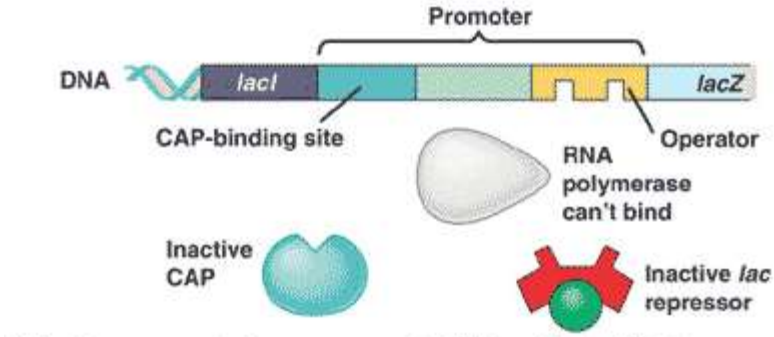
1. Diagrammatically represent working of lac operon and its regulation



(b) Lactose present, repressor inactive, operon on



(a) Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized



(b) Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized

2. **What is Alternative Splicing? Give mechanism with suitable example.**

Alternative splicing, or differential splicing, is a regulated process during gene expression that results in a single gene coding for multiple proteins. In this process, particular exons of a gene may be included within or excluded from the final, processed messenger RNA (mRNA) produced from that gene. Consequently, the proteins translated from alternatively spliced mRNAs will contain differences in their amino acid sequence and, often, in their biological functions Notably, alternative splicing allows the human genome to direct the synthesis of many more proteins than would be expected from its 20,000 protein-coding genes.

Alternative splicing occurs as a normal phenomenon in eukaryotes, where it greatly increases the biodiversity of proteins that can be encoded by the genome;[1] in humans, ~95% of multi-exonic genes are alternatively spliced. There are numerous modes of alternative splicing observed, of which the most common is exon skipping. In this mode, a particular exon may be included in mRNAs under some conditions or in particular tissues, and omitted from the mRNA in others.

The production of alternatively spliced mRNAs is regulated by a system of trans-acting proteins that bind to cis-acting sites on the primary transcript itself. Such proteins include splicing activators that promote the usage of a particular splice site, and splicing repressors that reduce the usage of a particular site. Mechanisms of alternative splicing are highly variable, and new examples are constantly being found, particularly through the use of

high-throughput techniques. Researchers hope to fully elucidate the regulatory systems involved in splicing, so that alternative splicing products from a given gene under particular conditions ("splicing variants") could be predicted by a "splicing code".

Abnormal variations in splicing are also implicated in disease; a large proportion of human genetic disorders result from splicing variants

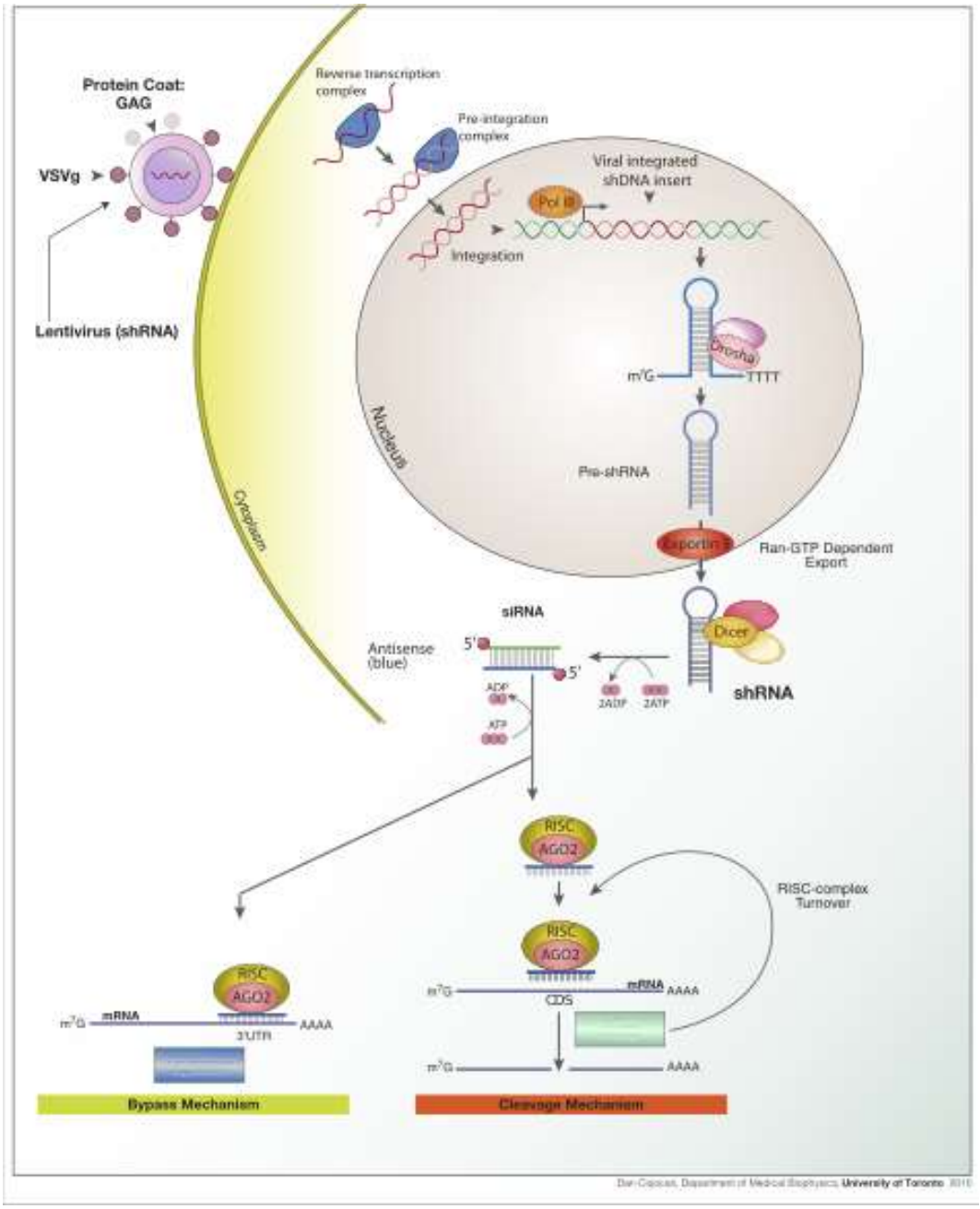
Examples of types *Exon skipping: Drosophila dsx and Alternative acceptor sites: Drosophila Transformer*

Significance: For eukaryotes alternative splicing was a very important step towards higher efficiency, because information can be stored much more economically. Several proteins can be encoded by a single gene, rather than requiring a separate gene for each, and thus allowing a more varied proteome from a genome of limited size. It also provides evolutionary flexibility. A single point mutation may cause a given exon to be occasionally excluded or included from a transcript during splicing, allowing production of a new protein isoform without loss of the original protein. Studies have identified intrinsically disordered regions (see Intrinsically unstructured proteins) as enriched in the non-constitutive exons suggesting that protein isoforms may display functional diversity due to the alteration of functional modules within these regions.

3.**Explain the role of iRNA in gene regulation**.

RNA interference (**RNAi**) is a biological process in which RNA molecules inhibit gene expression or translation, by neutralizing targeted mRNA molecules. RNAi is now known as precise, efficient, stable and better than antisense technology for gene suppression. However, antisense RNA produced intracellularly by an expression vector may be developed and find utility as novel therapeutic agents.

Two types of small ribonucleic acid (RNA) molecules – microRNA (miRNA) and small interfering RNA (siRNA) – are central to RNA interference. RNAs are the direct products of genes, and these small RNAs can bind to other specific messenger RNA (mRNA) molecules and either increase or decrease their activity, for example by preventing an mRNA from being translated into a protein. RNA interference has an important role in defending cells against parasitic nucleotide sequences – viruses and transposons. It also influences development.



3. What is Genetic code? Enlist and explain its salient features.

Genetic code Salient features

The genetic code is pretty similar in most of the organisms. It means that codon, which codes methionine in human, does the same in prokaryotes. This point is not exactly true as recently, scientists have discovered many exceptions from this rule. The genetic code is not **universal**, but it is still **predominant**.

During protein synthesis the genetic code is read **sequentially**, i.e. one codon at a time. There is no possibility to skip some of the nucleotides. The process starts from an initiation codon and then continues until it reaches termination codon in a **single translational reading frame**. This does not preclude the existence of overlapping genes, though, each with its own reading frame.

The ***genetic code*** is ***continuous*** and does not possess pauses after the triplets.

The code is universal:

Same genetic code is found valid for all organisms ranging from bacteria to man. E. coli (Bacterium), Xenopus laevis (Amphibian) and guinea pig (mammal) amino acyl-tRNA use almost the same code. and it has changed very little throughout the evolution of living organisms.

The code is degenerate:

More than one codon may specify the same amino acid; this is called degeneracy of the code. For example, except for tryptophan and methionine, which have a single codon each, all other 18 amino acids have more than one codon. Thus, nine amino acids, namely phenylalanine, tyrosine, histidine, glutamine, asparagine, lysine, aspartic acid, glutamic acid and cysteine, have two codons each. Isoleucine has three codons. Five amino acids, namely valine, proline, threonine, alanine and glycine, have four codons each. Three amino acids, namely leucine, arginine and serine, have six codons each

Complete degeneracy occurs when any of the four bases can take third position and still code for the same amino acid (e.g., UCU, UCC, UCA and UCG code for serine).

Degeneracy of genetic code has certain biological advantages. For example, it permits essentially the same complement of enzymes and other proteins to be specified by microorganisms varying widely in their DNA base composition. Degeneracy also provides a mechanism of minimising mutational lethality.

The code is non-overlapping:

In translating mRNA molecules the codons do not overlap but are "read" sequentially Thus, a non-overlapping code means that a base in a mRNA is not used for different codons.

The code is a triplet codon:

The nucleotides of mRNA are arranged as a linear sequence of codons, each codon consisting of three successive nitrogenous bases, i.e., the code is a triplet codon.

Q.5. Answer <u>any four</u> of the following: (20)

1. Give an account on biochemical actions of prostaglandin

Prostaglandins are found in most tissues and organs. They are produced by almost all nucleated cells. They are autocrine and paracrine lipid mediators that act upon platelets, endothelium, uterine and mast cells. They are synthesized in the cell from the fatty acid arachidonic acid.

Prostaglandins are one of the more potent mediators that cause increased blood flow, chemotaxis (chemical signals that summon white blood cells), and subsequent dysfunction of tissues and organs.

Prostaglandin: Suppositories are inserted into the vagina during the evening causing the uterus to go into **labor** by morning. One advantage to this method is that the mother is free to move around the **labor** room. Oxytocin: The body naturally produces the hormone oxytocin to stimulate contractions.

2. How is Wernicke-Korsakoff syndrome caused?

Wernicke-Korsakoff syndrome is a brain disorder due to vitamin B1 (thiamine) deficiency.

Causes

Wernicke encephalopathy and Korsakoff syndrome are different conditions that often occur together. Both are due to brain damage caused by a lack of vitamin B1.

Lack of vitamin B1 is common in people who have alcohol use disorder. It is also common in people whose bodies do not absorb food properly (malabsorption). This can sometimes occur with a chronic illness or after weight-loss (bariatric) surgery.

Korsakoff syndrome, or Korsakoff psychosis, tends to develop as Wernicke symptoms go away. Wernicke encephalopathy causes brain damage in lower parts of the brain called the thalamus and hypothalamus. Korsakoff psychosis results from permanent damage to areas of the brain involved with memory.

Symptoms of **Wernicke** encephalopathy include: Confusion and loss of mental activity that can progress to coma and death. Loss of muscle coordination (ataxia) that can cause leg tremor. Vision changes such as abnormal eye movements (back and forth movements called nystagmus), double vision, eyelid drooping

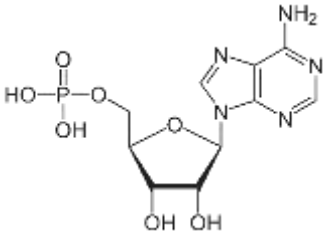
3. Describe oxidative phosphorylation

The NADH and $FADH_2$ formed in glycolysis, fatty acid oxidation, and the citric acid cycle are energy-rich molecules because each contains a pair of electrons having a high transfer potential. When these electrons are used to reduce molecular oxygen to water, a large amount of free energy is liberated, which can be used to generate ATP. *Oxidative phosphorylation is the process in which ATP is formed as a result of the transfer of electrons from NADH or* $FADH_2$ *to* O_2 *by a series of electron carriers.* This process, which takes place in mitochondria, is the major source of ATP in aerobic organisms (Figure 18.1). For example, oxidative phosphorylation generates 26 of the 30 molecules of ATP that are formed when glucose is completely oxidized to CO_2 and H_2O.

4. Give structure for AMP

Adenosine monophosphate (AMP), also known as 5'-**adenylic** acid, is a nucleotide. AMP consists of a phosphate group, the sugar ribose, and the nucleobase adenine; it is an ester of phosphoric acid and the nucleoside adenosine. As a substituent it takes the form of the prefix adenylyl-.

Chemical formula: $C_{10}H_{14}N_5O_7P$



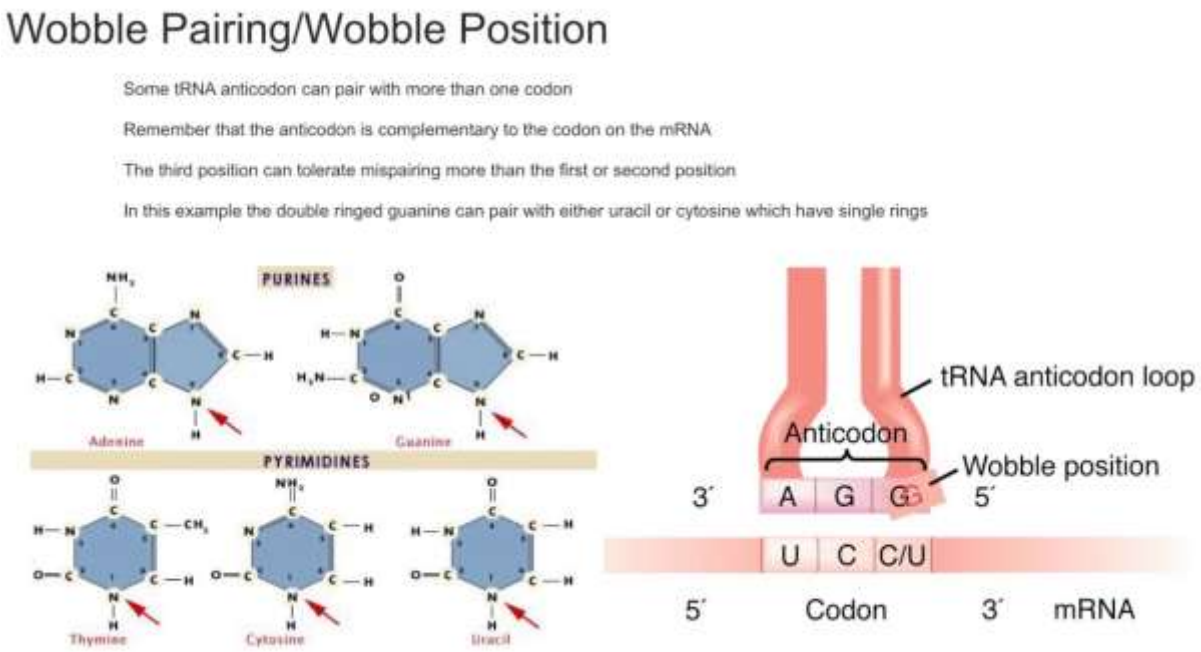
5. Wobble Hypothesis

The Wobble Hypothesis explains why multiple codons can code for a single amino acid. One tRNA molecule (with one amino acid attached) can recognise and bind to more than one codon, due to the less-precise base pairs that can arise between the 3rd base of the codon and the base at the 1st position on the anticodon.

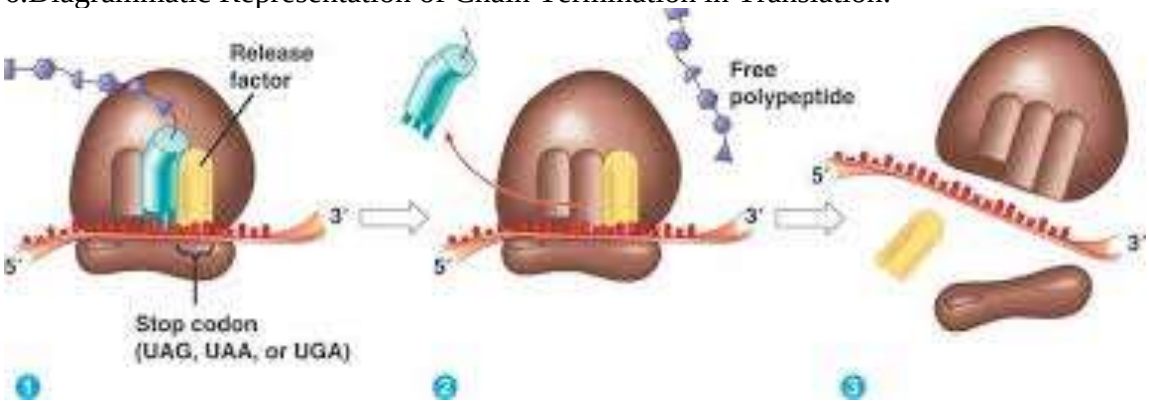
The Wobble Hypothesis. In 1966, Francis Crick proposed the Wobble Hypothesiswhich explained that the degeneracy of the genetic code is caused by the structure of the anticodon of tRNA. The anticodon is a three base sequence on the tRNA complementary to the codon on the mRNA. A wobble base pair is a pairing between two nucleotides in RNA molecules that does not follow Watson-Crick base

pair rules. The four main wobble base pairs are guanine-uracil, hypoxanthine-uracil, hypoxanthine-adenine, and hypoxanthine-cytosine.

This helps in degeneracy of genetic code.



6.Diagrammatic Representation of Chain Termination in Translation.



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