Fig.5.2 : The structures of adenosine 5'-monophosphate (AMP) and thymidine 5'-monophosphate (TMP)
(∗-Addition of second or third phosphate gives adenosine diphosphate (ADP)
and adenosine triphosphate (ATP) respectively).

STRUCTURE OF DNA

DNA is a *polymer of deoxyribonucleotides* (or simply deoxynucleotides). It is composed of monomeric units namely deoxyadenylate (dAMP), deoxyguanylate (dGMP), deoxycytidylate (dCMP) and deoxythymidylate (dTMP) (It may be noted here that some authors prefer to use TMP for deoxythymidylate, since it is found only in DNA).

Chargaff's rule of DNA composition

Erwin Chargaff in late 1940s quantitatively analysed the DNA hydrolysates from different species. He observed that in all the species he studied DNA had equal numbers of adenine and thymine residues (A = T) and equal numbers of guanine and cytosine residues (G = C). This is known as Chargaff's rule of *molar equivalence between the purines and pyrimidines in DNA* structure.

Single stranded DNA, and RNAs which are usually single stranded, do not obey Chargaff's rule. However, double stranded RNA which is the genetic material in certain viruses satisfies Chargaff'ss rule.

THE WATSON AND CRICK MODEL OF DNA STRUCTURE

The *double helical* structure of DNA was proposed by James Watson and Francis Crick in 1953 (Nobel Prize, 1962). The structure of DNA double helix is comparable to a twisted ladder. The

salient features of Watson-Crick model of DNA (now known as B-DNA) are given below (*Fig.5.3*).

1. The DNA is a right handed double helix. It consists of **two polydeoxyribonucleotide chains** (strands) twisted around each other on a common axis.

2. The two strands are **antiparallel**, i.e., one strand runs in the 5' to 3' direction while the other in 3'
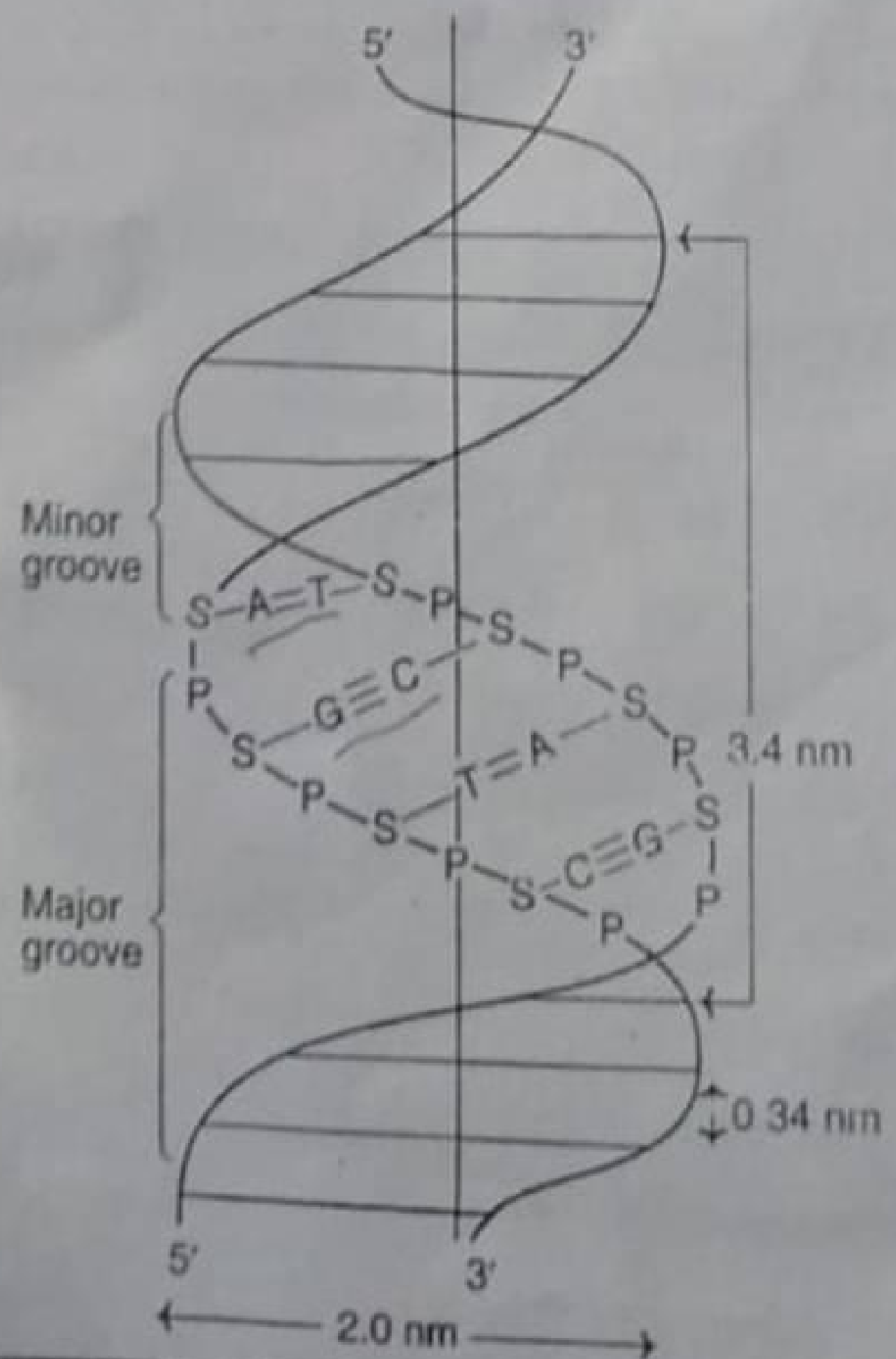

Fig.5.3 : Watson-Crick model of DNA helix.

to 5' direction. This is comparable to two parallel adjacent roads in opposite direction.

3. The width of a double helix is 20 A° (2 nm).

4. Each turn (pitch) of the helix is 34 A° (3.4 nm) with 10 pairs of nucleotides, each pair placed at a distance of about 3.4 A°.

5. The two strands are held together by *hydrogen bonds* formed by complementary base pairs. The A-T pair has 2 hydrogen bonds while G–C pair has 3 hydrogen bonds.

6. The complementary base pairing in DNA helix proves Chargaff's rule. The content of adenine equals to that of thymine $(A = T)$, and guanine equals to that of cytosine $(G = C)$.

Different forms of DNA double helix

The double helical structure of DNA exists in at least 6 different forms—A to E and Z. Among these, B, A and Z forms are important. The *B-form of DNA double helix* described by Watson and Crick is the most *predominant* form under physiological conditions. Each turn of the B-form has 10 base pairs spanning a distance of 3.4 nm. The width of the double helix is 2 nm (*Fig.5.3*).

The A-form is also a right handed helix. It contains 11 base pairs per turn. There is a tilting of the base pairs by 20° away from the central axis.

The Z-form (Z-DNA) is a left handed helix and contains 12 base pairs per turn. The polynucleotide strands of DNA move in a somewhat 'zig zag' fashion, hence the name Z-DNA.

Organization of DNA in the cell

In prokaryotic cells (e.g. bacteria) with no distinct nucleus, the DNA is rather loosely packed. In contrast, the eukaryotic DNA is found in association with basic proteins namely *histones* to form *nucleosomes*. The nucleosomes in turn coil to produce chromatin in which form DNA is present in the chromosomes.

STRUCTURE OF RNA

RNA is a polymer of ribonucleotides held together by 3',5'-phosphodiester bridges. Although RNA has certain similarities with DNA structure, they have several specific differences

1. **Pentose :** The sugar in RNA is ribose in contrast to deoxyribose in DNA.

2. **Pyrimidine :** RNA contains the pyrimidine uracil in place of thymine in DNA.

3. **Single strand :** RNA is usually a single stranded polynucleotide.

4. **Chargaff's rule—not obeyed :** Due to the single stranded nature, there is no specific relation between purine and pyrimidine contents.

5. **Orcinol colour reaction :** RNAs can be histologically identified by orcinol colour reaction due to the presence of ribose.

TYPES OF RNA

The 3 distinct types of RNAs with their respective cellular composition are given below

1. Messenger RNA (mRNA)   :   5–10%
2. Transfer RNA (tRNA)   :   10–20%
3. Ribosomal RNA (rRNA)   :   50–80%

Besides the three RNAs referred to above, human cells contain small nuclear RNA (snRNA) which are involved in RNA processing.

Messenger RNA (mRNA)

The mRNA is synthesized in the nucleus (in eukaryotes) as *heterogeneous nuclear RNA* (hnRNA). hnRNA on processing liberates the functional mRNA which enter the cytoplasm to participate in protein synthesis. mRNA has high molecular weight with short half-life.

Transfer RNA (tRNA)

Transfer RNA (soluble RNA) molecule contains 71-80 nucleotides (mostly 75) with a molecular weight of about 25,000. There are at least 20 species of tRNAs corresponding to 20 amino acids present in protein structure.

The structure of tRNA depicted in *Fig.5.4* resembles that of a clover leaf. tRNA contains mainly four arms, each arm contains a base paired stem.

1. **The acceptor arm :** This arm is capped with a sequence CCA (5' to 3'). The amino acid is attached to the acceptor arm.
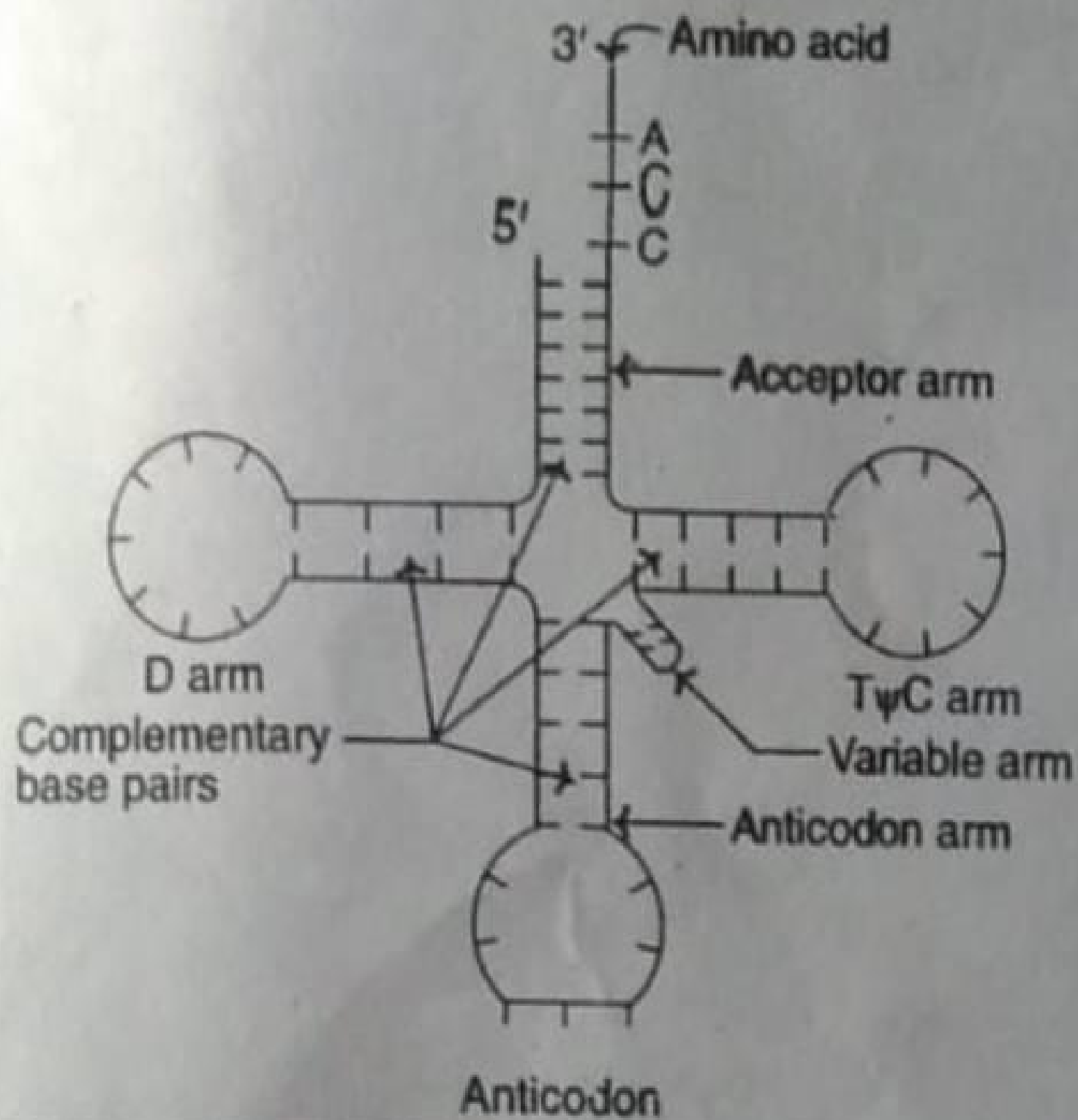
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Fig. 5.4 : Structure of transfer RNA.

2. **The anticodon arm :** This arm, with the three specific nucleotide bases (anticodon) recognizes the triplet codon of mRNA. The codon and anticodon are complementary to each other.

3. **The D arm :** It is so named due to the presence of dihydrouridine.

4. **The TψC arm :** This arm contains a sequence of T, pseudouridine (psi, Ψ) and C.

5. **The variable arm :** This arm is the most variable in tRNA.
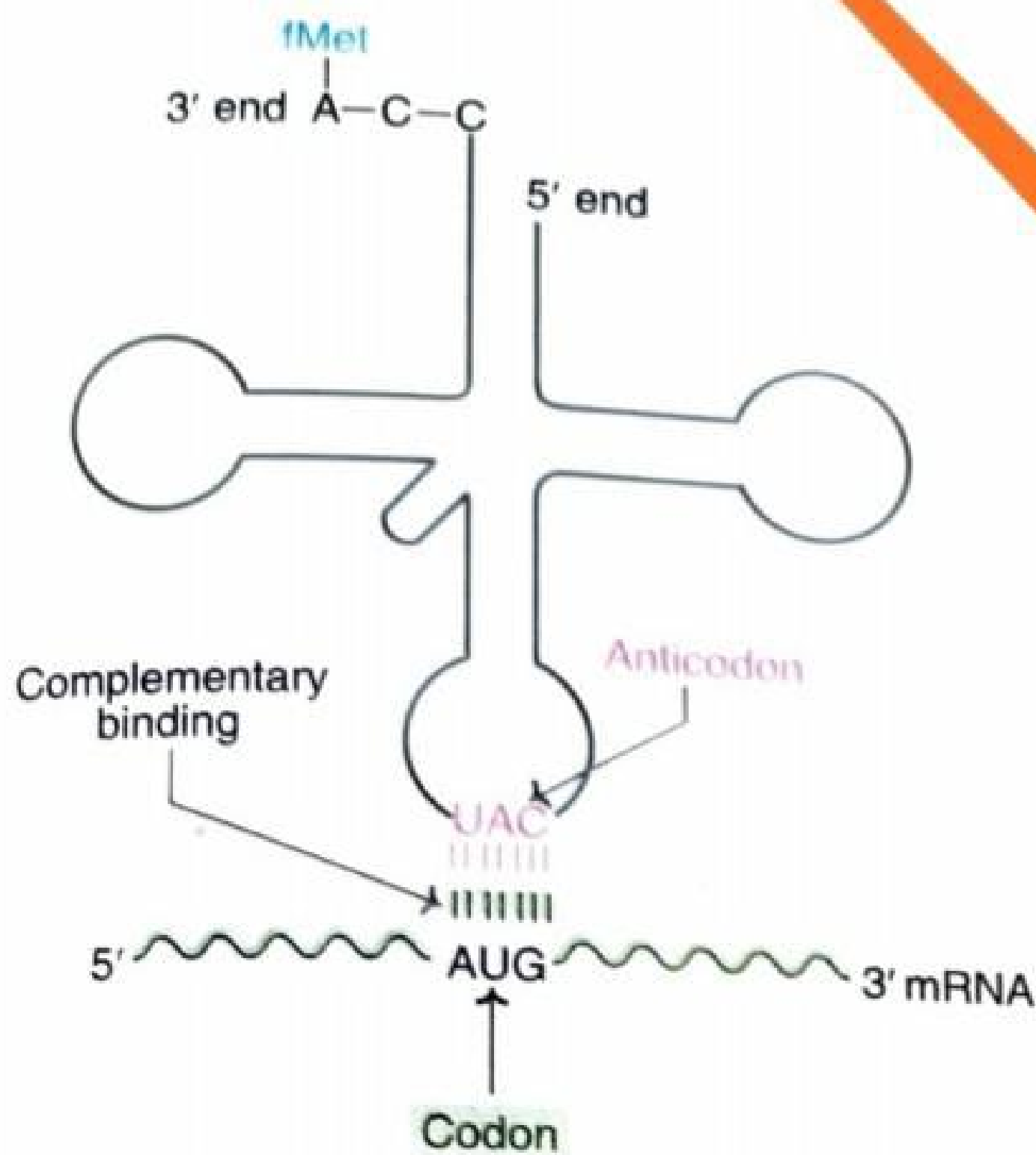
Ribosomal RNA (rRNA)

The ribosomes are the factories of protein synthesis. They are composed of two major nucleoprotein complexes–60S subunit and 40S subunit. The 60S subunit contains 28S rRNA, 5S rRNA and 5.8S rRNA while the 40S subunit contains 18S rRNA. The function of rRNAs in ribosomes is not clearly known. It is believed that they play a significant role in the binding of mRNA to ribosomes and protein synthesis.

⚕ SUMMARY AND BIOMEDICAL / CLINICAL CONCEPTS
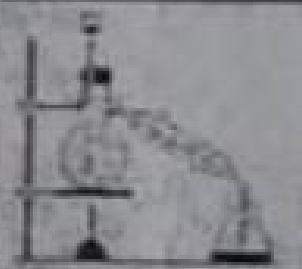
1. Nucleic acids are the polymers of nucleotides (polynucleotides) held by 3' and 5' phosphodiester bridges. A nucleotide consists of base + sugar + phosphate.

2. Besides being the constituents of nucleic acid structure, nucleotides perform a wide variety of cellular functions (e.g. energy carriers, metabolic regulators, second messengers etc.)

3. Both DNA and RNA contain the purines adenine and guanine, and the pyrimidine cytosine. The second pyrimidine is thymine in DNA while it is uracil in RNA.

4. The structure of DNA is a double helix (Watson-Crick model) composed of two antiparallel strands of polydeoxynucleotides twisted around each other. They are held together by 2 or 3 hydrogen bonds formed between bases i.e. A = T; G ≡ C.

☞ DNA, organized into genes, is the reserve bank of genetic information, ultimately responsible for the chemical basis of life and heredity.

☞ Uric acid is a purine, and the end product of purine metabolism, that has been implicated in the disorder gout.

☞ Certain purine bases from plants such as caffeine (of coffee), theophylline (of tea) and theobromine (of cocoa) are of pharmacological interest.

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Fig.25.10 : Complementary binding of codon (of mRNA) and anticodon (of tRNA).

other in antiparallel direction (5′ → 3′ of mRNA with 3′ → 5′ of tRNA). The usual conventional complementary base pairing (A=U, C≡G) occurs between the first two bases of codon and the last two bases of anticodon. The third base of the codon is rather lenient or flexible with regard to the complementary base.

Wobble hypothesis

Wobble hypothesis, put forth by Crick, is the phenomenon in which a *single tRNA can recognize more than one codon.* This is due to the fact that the third base (3′-base) in the codon often fails to recognize the specific complementary base in the anticodon (5′-base). Wobbling is attributed to the difference in the spatial arrangement of the 5′-end of the anticodon. The possible pairing of 5′-end base of anticodon (of tRNA) with the 3′-end base of codon (mRNA) is given

Anticodon		Codon	
C	—	G	} Conventional base pairing
A	—	U	
U	—	G or A	} Non-conventional base (coloured) pairing
G	—	U or C	

Wobble hypothesis explains the degeneracy of the genetic code, i.e. existence of multiple codons for a

single amino acid. Although there are 61 codons for amino acids, the number of tRNAs is far less (around 40) which is due to wobbling.

PROTEIN BIOSYNTHESIS

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

I. Requirement of the components
II. Activation of amino acids
III. Protein synthesis proper
IV. Chaperones and protein folding
V. Post-translational modifications.

I. REQUIREMENT OF THE COMPONENTS

The protein synthesis may be considered as a biochemical factory operating on the ribosomes. As a factory is dependent on the supply of raw materials to give a final product, the protein synthesis also requires many components.

1. **Amino acids** : Proteins are polymers of amino acids. Of the *20 amino acids* found *in protein structure,* half of them (10) can be synthesized by man. About *10 essential amino acids* have to be provided through the diet. Protein synthesis can occur only when all the amino acids needed for a particular protein are available.

As regards prokaryotes, there is no requirement of amino acids, since all the 20 are synthesized from the inorganic components.

2. **Ribosomes** : The functionally active ribosomes are the *centres* or *factories for protein synthesis.* Ribosomes may also be considered as workbenches of translation. Ribosomes are huge complex structures (70S for prokaryotes and 80S for eukaryotes) of proteins and ribosomal RNAs. Each ribosome consists of two subunits—one big and one small. The functional ribosome has two sites—A site and P site. Each site covers both the subunits. *A site* is for binding of aminoacyl tRNA and *P site* is for binding peptidyl tRNA, during the course of translation. Some authors consider A site as acceptor site, and P site as donor site. In case of eukaryotes, there is another site called *exit* site or *E site.* Thus,

eukaryotes contain three sites (A, P and E) on the ribosomes.

The ribosomes are located in the cytosomal fraction of the cell. They are found in association with rough endoplasmic reticulum (RER) to form clusters RER—ribosomes, where the protein synthesis occurs. The term *polyribosome* (polysome) is used when several ribosomes simultaneously translate on a single mRNA (*Fig.25.11*).

3. **Messenger RNA (mRNA) :** The specific information required for the synthesis of a given protein is present on the mRNA. The DNA has passed on the genetic information in the form of *codons* to mRNA to translate into a protein sequence.

4. **Transfer RNAs (tRNAs) :** They carry the amino acids, and hand them over to the growing peptide chain. The amino acid is covalently bound to tRNA at the 3'-end. Each tRNA has a three nucleotide base sequence—the *anticodon*, which is responsible to recognize the codon (complementary bases) of mRNA for protein synthesis.

In man, there are about 50 different tRNAs whereas in bacteria around 40 tRNAs are found. Some amino acids (particularly those with multiple codons) have more than one tRNA.

5. **Energy sources :** Both *ATP* and *GTP* are required for the supply of energy in protein synthesis.

6. **Protein factors :** The process of translation involves a number of protein factors. These are needed for initiation, elongation and termination of protein synthesis. The protein factors are more complex in eukaryotes compared to prokaryotes.

II. ACTIVATION OF AMINO ACIDS

Amino acids are activated and attached to tRNAs in a two step reaction. A group of enzymes—namely aminoacyl tRNA synthetases—are required for this process. These enzymes are highly specific for the amino acid and the corresponding tRNA.

The amino acid is first attached to the enzyme utilizing ATP to form enzyme-AMP-amino acid complex. The amino acid is then transferred to the 3' end of the tRNA to form aminoacyl tRNA (*Fig.25.12*).

III. PROTEIN SYNTHESIS PROPER

The protein or polypeptide synthesis occurs on the ribosomes (rather polyribosomes). The *mRNA is read in the 5' → 3' direction and the polypeptide synthesis proceeds from N-terminal end to C-terminal end.* Translation is directional and collinear with mRNA.

The prokaryotic mRNAs are *po........ic,* since a single mRNA has many coding regi...s that code for different polypeptides. In contras... eukaryotic mRNA is *monocistronic,* since it codes...r a single polypeptide.

In case of prokaryotes, translation co...mences before the transcription of the gene is co...pleted. Thus, simultaneous transcription and transl...on are possible. This is not so in case of eukaryotic or...nisms since transcription occurs in the nucleus w...ereas translation takes place in the cytosol. Furthe...the primary transcript (hnRNA) formed from DNA...s to undergo several modifications to generate funct...nal mRNA.

Protein synthesis is comparatively simple in ca...e of prokaryotes compared to eukaryotes. Furthe... many steps in eukaryotic translation were no... understood for quite sometime. For these reasons, majority of the textbooks earlier used to describe translation in prokaryotes in detail, and give most

INITIATION OF TRANSLATION

The initiation of translation *in eukaryotes* is complex, involving at least *ten eukaryotic initiation factors (eIFs)*. Some of the eIFs contain multiple (3-8) subunits. The process of translation initiation can be divided into four steps (*Fig.25.13*).

1. Ribosomal dissociation.

2. Formation of 43S preinitiation complex.

3. Formation of 48S initiation complex.
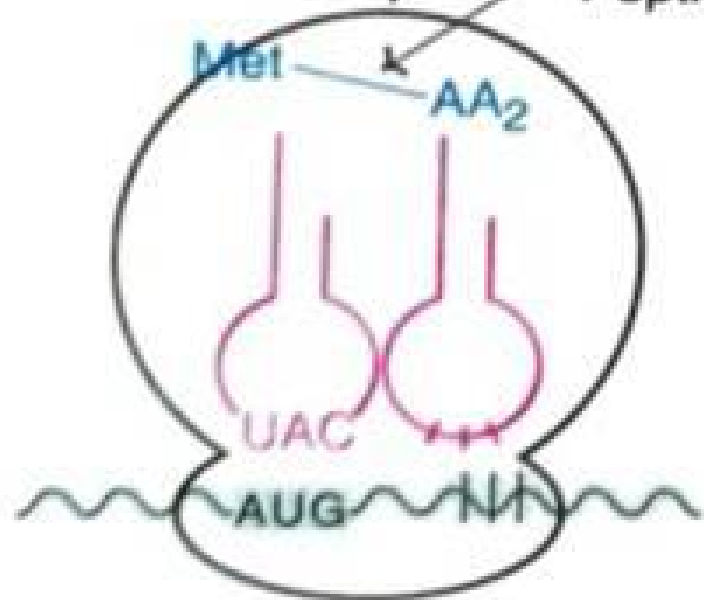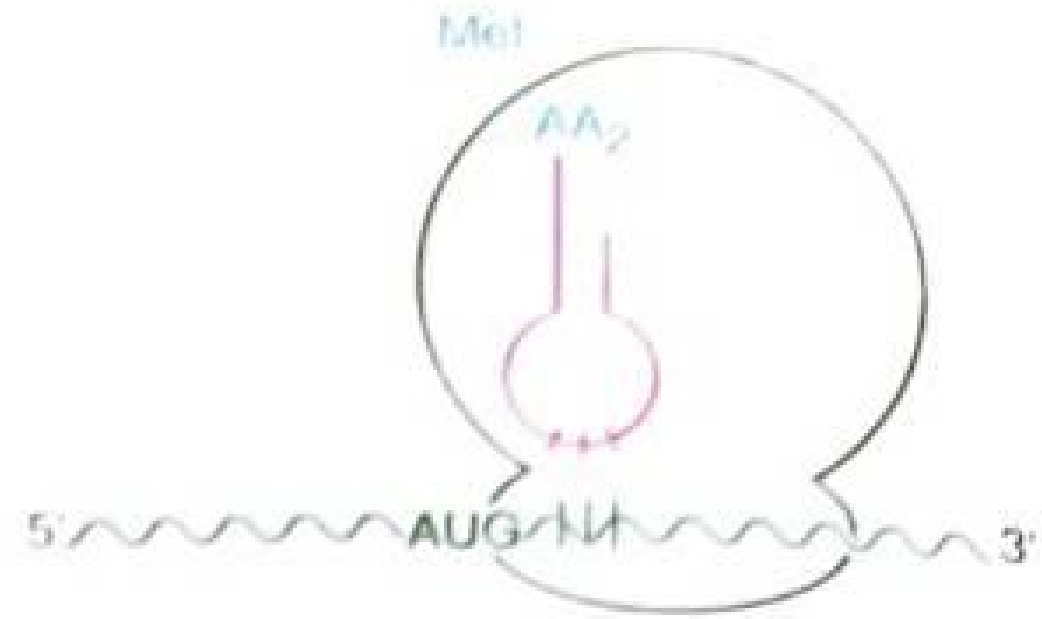
4. Formation of 80S initiation complex.
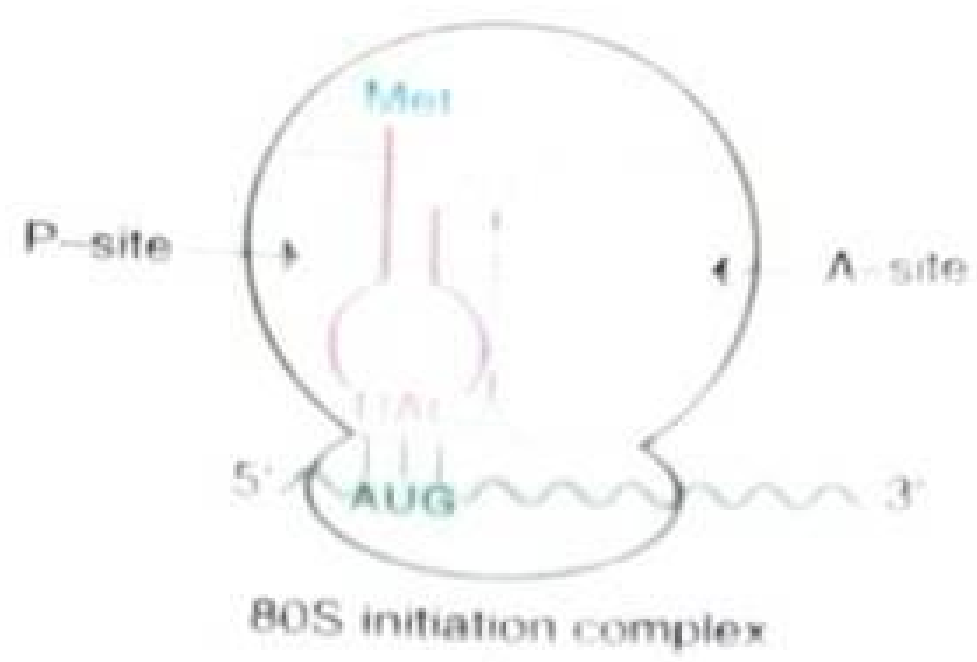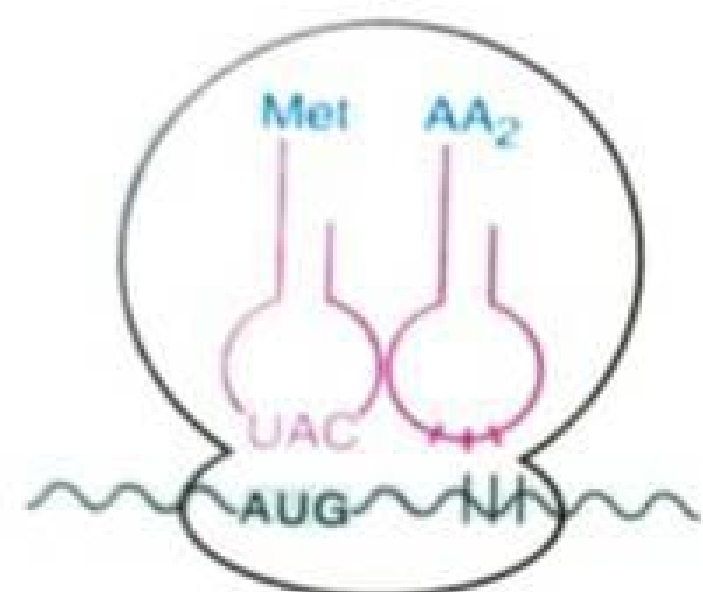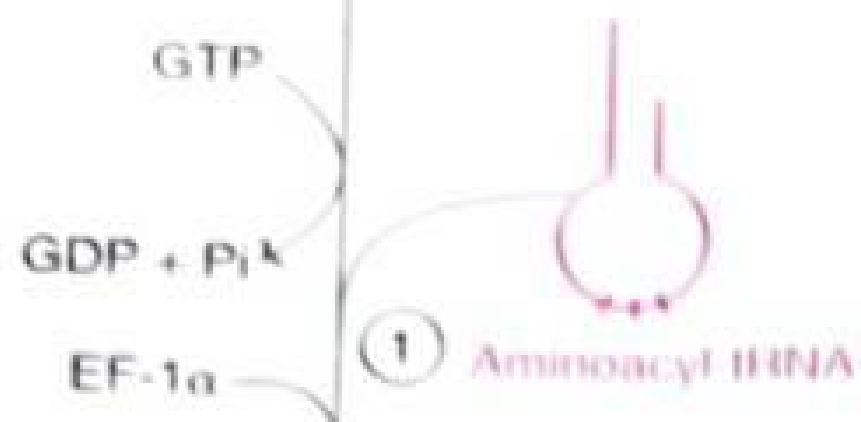
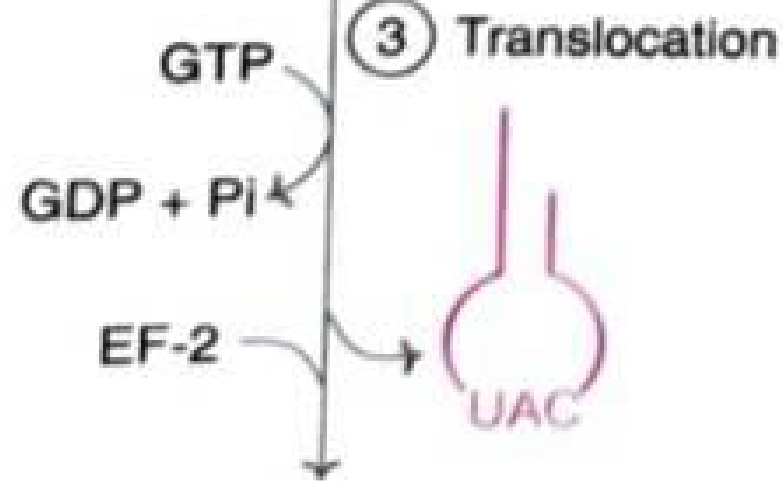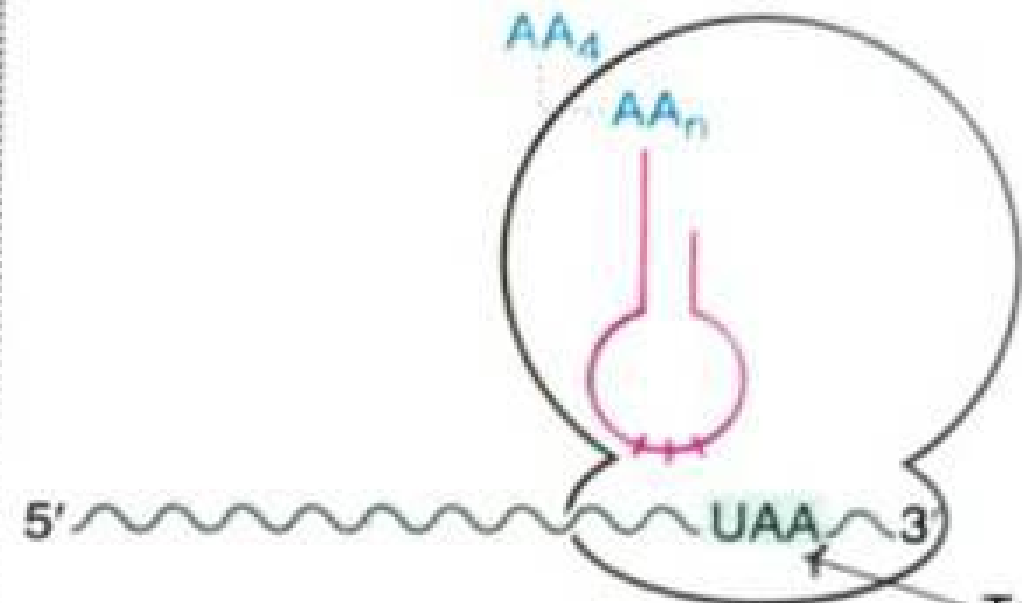**Fig.25.14 : Protein biosynthesis – Elongation and termination (for initiation See Fig. 25.13).** Met-Methionine; P-site – Peptidyl tRNA binding site; A-site – Aminoacyl tRNA binding site. AA-Amino acid; EF-Elongation factor; RF-Releasing factor.

Fig. contd. next column

DNA-REPLICATION, RECOMBINATION, AND REPAIR

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

The central dogma of life

The *biological information flows from DNA to RNA, and from there to proteins.* This is the central dogma of life (*Fig.24.1*). It is ultimately the DNA that controls every function of the cell through protein synthesis.

As the carrier of genetic information, DNA in a cell must be duplicated (replicated), maintained and passed down accurately to the daughter cells. Three distinct processes are designed for this purpose. The *'three Rs'* of DNA-replication, recombination, and repair, are dealt with in this chapter.

REPLICATION OF DNA

DNA is the genetic material. When the cell divides, the daughter cells receive an identical copy of genetic information from the parent cell.

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

The salient features of replication in prokaryotes are described first. This is followed by some recent information on the eukaryotic replication.

REPLICATION IN PROKARYOTES

Replication is semiconservative

The parent DNA has two strands complementary to each other. Both the strands undergo simultaneous replication to produce two daughter molecules. Each one of the newly synthesized DNA has one-half of the parental DNA (one strand from original) and one-half of new DNA (*Fig.24.2*). This is known as semiconservative replication since *half of the original DNA is conserved in the daughter DNA.*

Initiation of replication

The initiation of DNA synthesis occurs at a site called *origin of replication.* In case of prokaryotes, there is a single site whereas in eukaryotes, there are multiple sites of origin. These sites mostly consist of a short sequence of A-T base pairs. A specific protein called *dna A* (20-50 monomers) binds with the site of origin for replication. This causes the double-stranded DNA to separate.

Replication bubbles

The two complementary strands of DNA separate at the site of replication to form a bubble. Multiple replication bubbles are formed in eukaryotic DNA
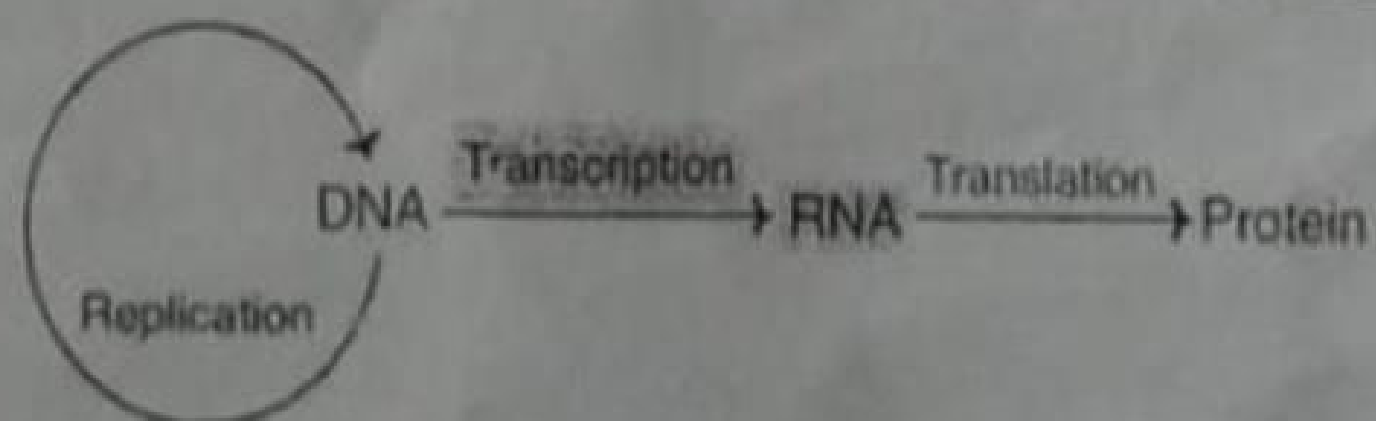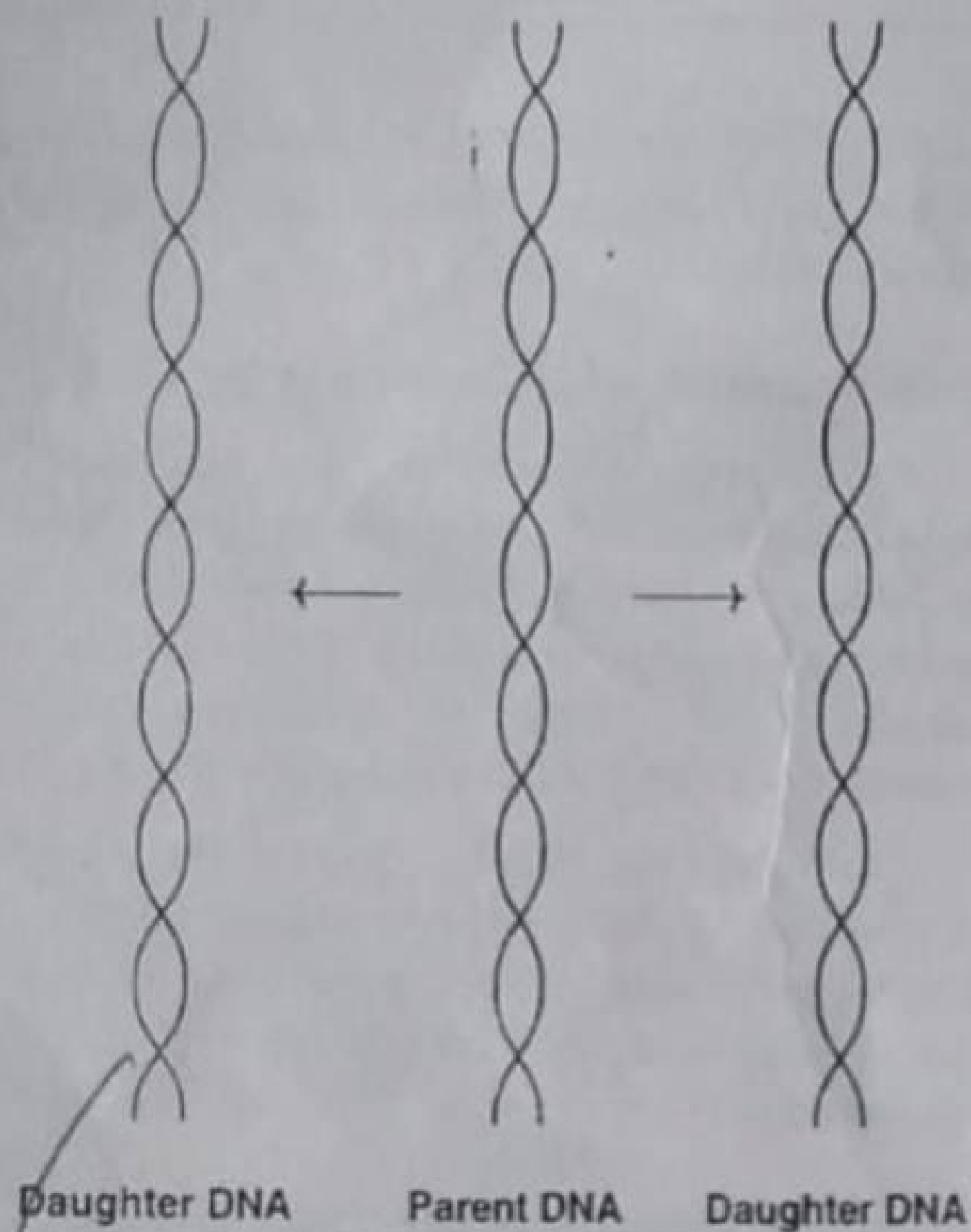

Fig.24.1 : The central dogma of life.

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Daughter DNA   Parent DNA   Daughter DNA

Fig.24.2 : DNA replication—semiconservative.

molecules, which is essential for a rapid replication process (*Fig.24.3*).

RNA primer

For the synthesis of new DNA, a short fragment of RNA (about 5-50 nucleotides, variable with species) is required as a primer.

DNA synthesis is semidiscontinuous and bidirectional

The replication of DNA occurs in 5' to 3' direction, simultaneously, on both the strands of DNA. On one strand, the *leading (continuous) strand—the DNA synthesis is continuous*. On the other strand, the *lagging (discontinuous) strand—the synthesis of DNA is discontinuous*. Short pieces of DNA (15-250 nucleotides) are produced on the lagging strand.

In the replication bubble, the DNA synthesis occurs in both the directions (bidirectional) from the point of origin.

Replication fork and DNA synthesis

The separation of the two strands of parent DNA results in the formation of a replication fork. The active synthesis of DNA occurs in this region. The replication fork moves along the parent DNA as the daughter DNA molecules are synthesized.

DNA helicases : These enzymes bind to both the DNA strands at the replication fork. Helicases move along the DNA helix and separate the strands. Their function is comparable with a *zip opener.* Helicases are dependent on ATP for energy supply.

Single-stranded DNA binding (SSB) proteins : These are also known as DNA helix-destabilizing proteins. They possess no enzyme activity. SSB proteins bind only to single-stranded DNA (separated by helicases), keep the two strands separate and provide the template for new DNA synthesis.

DNA synthesis catalysed by DNA polymerase III

The synthesis of a new DNA strand, catalysed by DNA polymerase III, occurs in 5' → 3' direction. This is antiparallel to the parent template DNA strand. The presence of all the four deoxyribonucleoside triphosphates (dATP, dGTP, dCTP and dTTP) is an essential prerequisite for replication to take place.

The synthesis of two new DNA strands, simultaneously, takes place in the opposite direction—one is in a direction (5' → 3') towards the replication fork which is continuous, the other in a direction (5' → 3') away from the replication fork which is discontinuous (*Fig.24.4*).

The incoming deoxyribonucleotides are added one after another, to 3' end of the growing DNA chain. The template DNA strand (the parent) determines the base sequence of the newly synthesized complementary DNA.

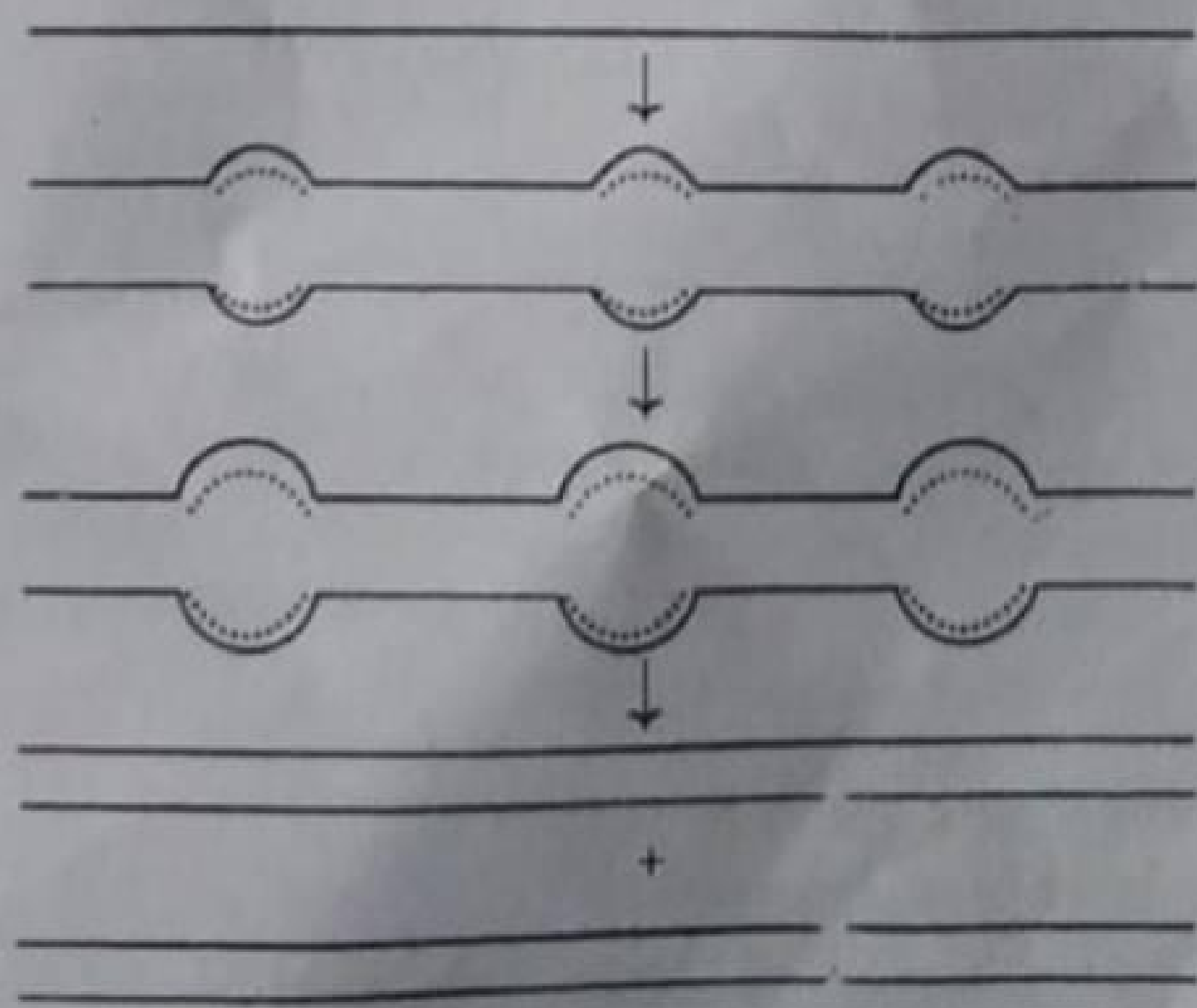**Okazaki pieces :** The small *fragments of the discontinuously synthesized DNA are called*

Fig.24.3 : Schematic representation of multiple replication bubbles in DNA replication.
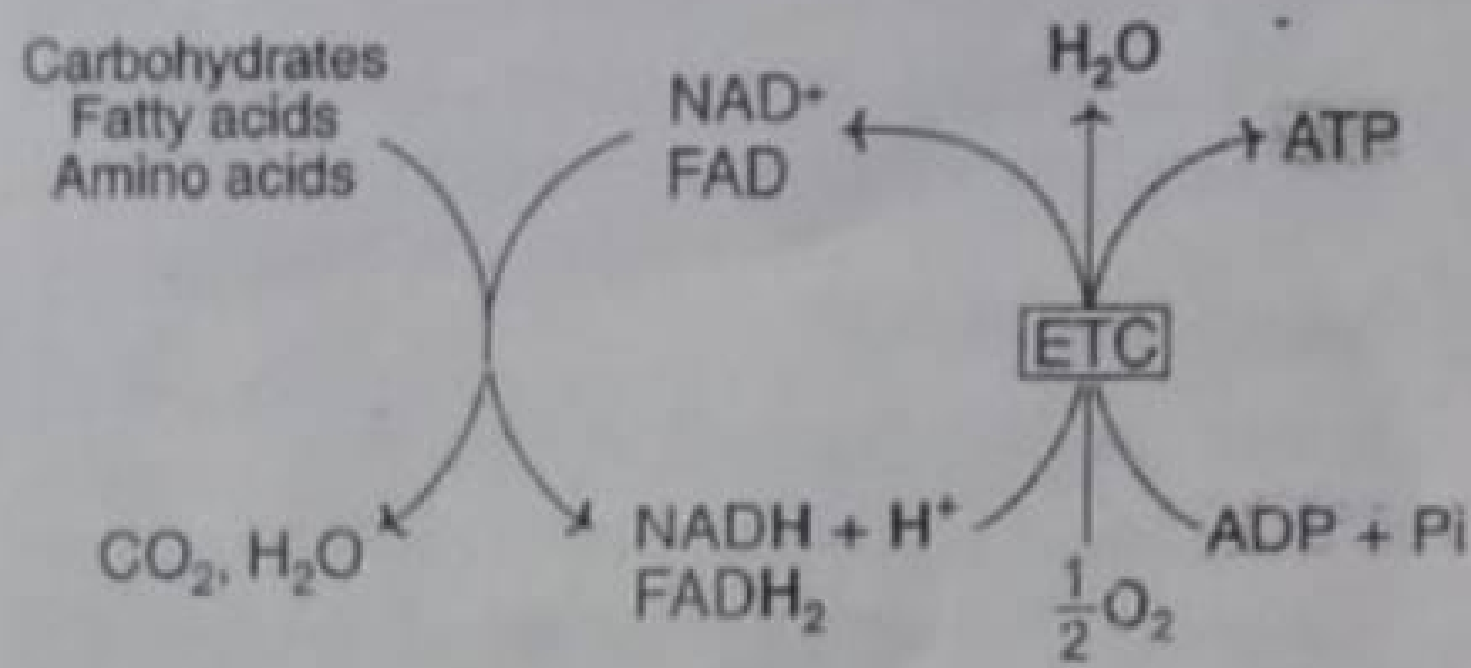
Fig.11.3 : Overview of biological oxidation (ETC-Electron transport chain).

The electron lost in the oxidation is accepted by an acceptor which is said to be reduced. Thus the oxidation-reduction is a tightly coupled process.

Redox potential (E_0)

The *oxidation-reduction* potential or, simply, redox potential, is a quantitative measure of the tendency of a redox pair to lose or gain electrons. The redox pairs are assigned specific *standard redox potential* (E_0 volts) at pH 7.0 and 25°C. e.g. NAD$^+$/NADH -0.32; FAD/FADH$_2$ -0.22; cytochrome c $+0.25$.

The electrons flow from a redox pair with more negative E_0 to another redox pair with more positive E_0.

ELECTRON TRANSPORT CHAIN

The energy-rich carbohydrates (particularly glucose), fatty acids and amino acids undergo a series of metabolic reactions and, finally, get oxidized to CO_2 and H_2O. The reducing equivalents from various metabolic intermediates are transferred to coenzymes NAD$^+$ and FAD to produce, respectively, NADH and FADH$_2$. The latter two reduced coenzymes pass through the electron transport chain (ETC) or *respiratory chain* and, finally, reduce oxygen to water. The passage of electrons through the ETC is associated with the loss of free energy. A part of this free energy is utilized to generate ATP from ADP and Pi (*Fig.11.3*).

Mitochondria
—the power houses of cell

The mitochondria are the centres for metabolic oxidative reactions to generate reduced coenzymes (NADH and FADH$_2$) which, in turn, are utilized in

ETC to liberate energy in the form of ATP. For this reason, mitochondrion is appropriately regarded as the *power house of the cell.*

Mitochondrial organization

The mitochondrion consists of five distinct parts. These are the outer membrane, the inner membrane, the intermembrane space, the cristae and the matrix (*Fig.11.4*).

Inner mitochondrial membrane : The electron transport chain and ATP synthesizing system are located on the inner mitochondrial membrane which is a specialized structure, rich in proteins. This membrane is highly folded to form cristae. The inner surface of the inner mitochondrial membrane possesses specialized particles (that look like lollipops), the *phosphorylating subunits* which are the centres for ATP production.

Mitochondrial matrix : The interior ground substance forms the matrix of mitochondria. It is rich in the *enzymes* responsible for the *citric acid cycle, β-oxidation of fatty acids* and *oxidation of amino acids*.

Components and reactions of the electron transport chain

There are five distinct carriers that participate in the electron transport chain (ETC). These carriers are sequentially arranged (*Fig.11.5*) and are responsible for the transfer of electrons from a given substrate to ultimately combine with proton and oxygen to form water.

i. Nicotinamide nucleotides

Of the two coenzymes NAD$^+$ and NADP$^+$ derived from the *vitamin niacin,* NAD$^+$ is more actively involved in the ETC. NAD$^+$ is reduced to *NADH+H$^+$* by dehydrogenases with the removal of two hydrogen atoms from the substrate (AH$_2$). The substrates include glyceraldehyde 3-phosphate, pyruvate, isocitrate, α-ketoglutarate and malate.

$$AH_2 + NAD^+ \rightleftharpoons A + NADH + H^+$$

II. Flavoproteins

The enzyme NADH dehydrogenase (NADH-coenzyme Q reductase) is a flavoprotein with FMN as the prosthetic group. The coenzyme FMN accepts two electrons and a proton to form FMNH$_2$.
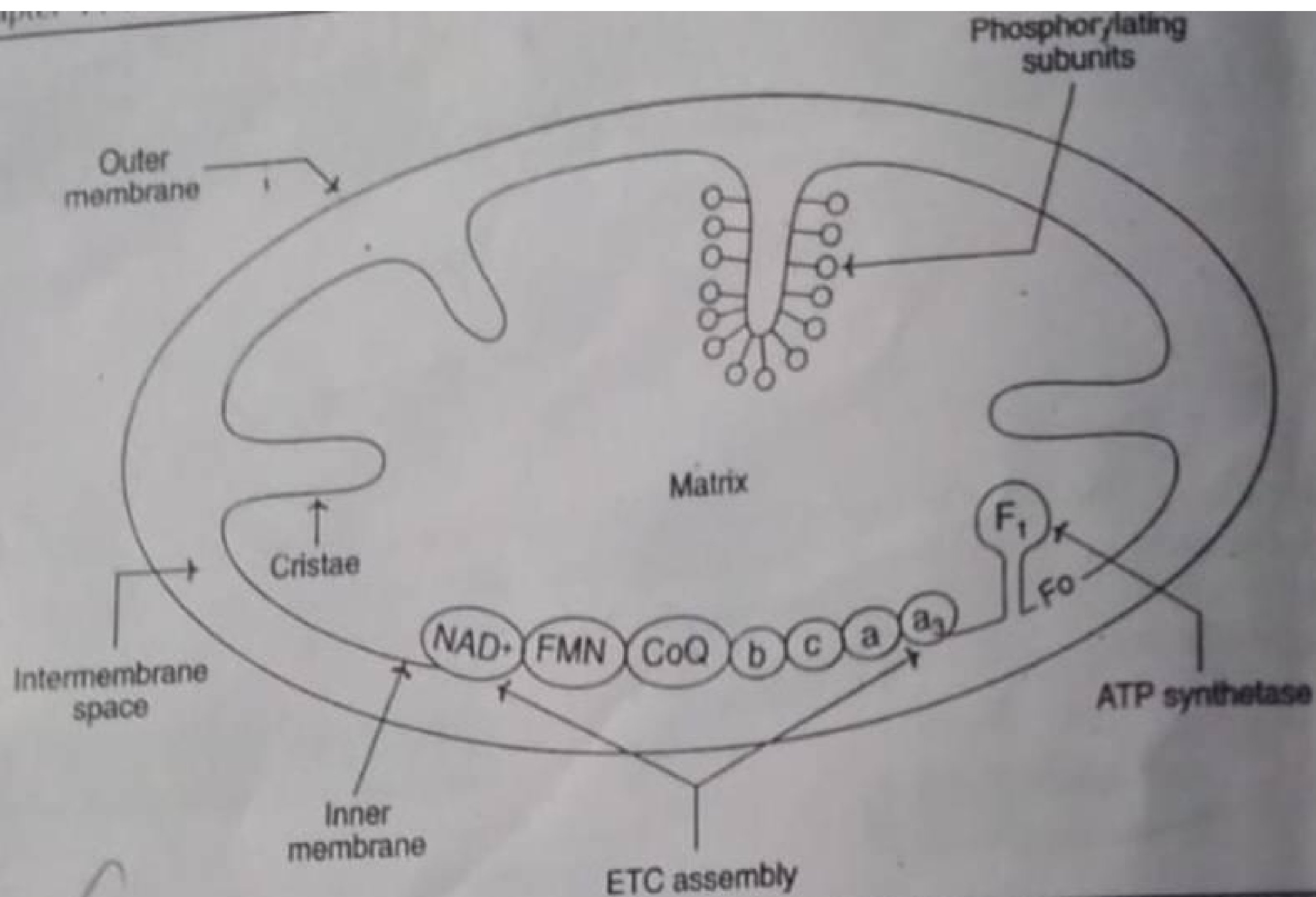
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Fig. 11.4 : Structure of mitochondrion depicting electron transport chain (ETC) (F₀, F₁ = Protein subunits).

III. Iron-sulfur proteins

The iron-sulfur (FeS) proteins exist in the oxidized (Fe^{3+}) or reduced (Fe^{2+}) state. However, the mechanism of action of iron-sulfur proteins in the ETC is not clearly understood.

IV. Coenzyme Q

Coenzyme Q is also known as *ubiquinone* since it is ubiquitous in living system. It is a quinone derivative with a variable *isoprenoid* side chain. The mammalian tissues possess a quinone with 10 isoprenoid units which is known as coenzyme Q_{10} (CoQ_{10}).

V. Cytochromes

The cytochromes are conjugated proteins containing heme group. The iron of heme in cytochromes is alternately oxidized (Fe^{3+}) and reduced (Fe^{2+}), which is essential for the transport of electrons in the ETC. This is in contrast to the heme iron of hemoglobin and myoglobin which remains in the ferrous (Fe^{2+}) state.

The *electrons* are *transported* from *coenzyme Q to cytochromes* (in the order) *b, c_1, c. a and a_3*. The property of reversible oxidation-reduction of heme iron $Fe^{2+} \rightleftharpoons Fe^{3+}$ present in cytochromes allows them to function as effective carriers of electrons in ETC.

Cytochrome a and a_3 : The term *cytochrome oxidase* is frequently used to collectively represent cytochrome a and a_3 which is the terminal component of ETC. Cytochrome oxidase is the only electron carrier, the heme iron of which can directly react with molecular oxygen.

In the final stage of ETC, the transported electrons, the free protons and the molecular oxygen combine to produce water.
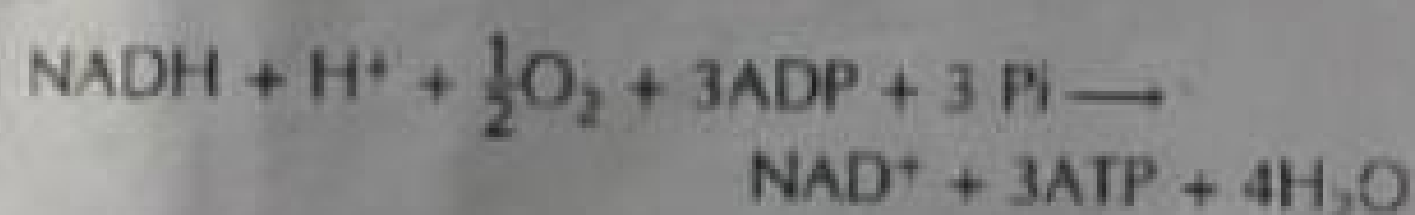
OXIDATIVE PHOSPHORYLATION·

The transport of electrons through the ETC is linked with the release of free energy. The process of *synthesizing ATP from ADP and Pi coupled with the electron transport chain* is known as oxidative phosphorylation.

P : O Ratio

The P : O ratio refers to the atoms of *phosphate* utilized to the atoms of *oxygen* consumed in oxidation. More appropriately, P : O ratio represents the number of molecules of ATP synthesized per pair of electrons carried through ETC.

The mitochondrial oxidation of NADH with a P : O ratio of 3 can be represented by the following equation :

$$NADH + H^+ + \tfrac{1}{2}O_2 + 3ADP + 3 Pi \longrightarrow$$
$$NAD^+ + 3ATP + 4H_2O$$

P : O ratio of 2 is assigned to the oxidation of $FADH_2$.

Sites of oxidative phosphorylation in ETC

The P : O ratio of 3 for NADH oxidation indicates that there are three reactions in the ETC that are exergonic to result in the synthesis of 3 ATP molecules (*See Fig.11.5*). The *three sites of ATP formation* in ETC are

1. Oxidation of $FMNH_2$ by coenzyme Q.

2. Oxidation of cytochrome b by cytochrome c_1.

3. Cytochrome oxidase reaction.

Each one of the above reactions represents a *coupling site* for ATP production. There are only two coupling sites for the oxidation of $FADH_2$ (P : O ratio 2), since the first site is bypassed.

MECHANISM OF OXIDATIVE PHOSPHORYLATION

Several hypotheses have been put forth to explain the process of oxidative phosphorylation. The most important among them—namely, chemical coupling,

conformational coupling and chemiosmotic—are discussed below.

Chemical coupling hypothesis

According to chemical coupling hypothesis, during the course of electron transfer in respiratory chain, a series of *phosphorylated high-energy intermediates* are first produced which are utilized for the synthesis of ATP. These reactions are believed to be analogous to the substrate level phosphorylation that occurs in glycolysis or citric acid cycle. However, this hypothesis lacks experimental evidence, since all attempts, so far, to isolate any one of the high-energy intermediates have not been successful.

Chemiosmotic hypothesis

This mechanism, originally proposed by Peter Mitchell (1961), is now widely accepted. It explains how the transport of electrons through the respiratory chain is effectively utilized to produce ATP from ADP + Pi.

Proton gradient : The inner mitochondrial membrane, as such, is impermeable to protons (H^+) and hydroxyl ions (OH^-). The transport of electrons through ETC is coupled with the *translocation of protons (H^+)* across the inner mitochondrial membrane (coupling membrane) from the matrix to the intermembrane space. The pumping of protons results in an *electrochemical or proton gradient*. This is due to the accumulation of more H^+ ions (low pH) on the outer side of the inner mitochondrial membrane than the inner side. The proton gradient developed due to the electron flow in the respiratory chain is sufficient to result in the synthesis of ATP from ADP and Pi (*Fig.11.6*).

Rotory motor model for ATP generation

Paul Boyer in 1964 proposed (Nobel Prize, 1997) that a conformational change in the mitochondrial membrane proteins leads to the synthesis of ATP. This hypothesis, considered as rotory motor/*engine driving model* or *binding change model*, is widely accepted for the generation of ATP.

The enzyme ATP synthase is F_0F_1 complex (of complex V) which is composed of many subunits. In response to the proton flux, conformational changes occur in the ATP synthase complex that finally lead to the generation of ATP.

For a better understanding of biological oxidation, it is worthwhile to have a basic knowledge of bioenergetics and the role of high-energy compounds in biological processes.

BIOENERGETICS

Bioenergetics or *biochemical thermodynamics* deals with the study of energy changes (transfer and utilization) in biochemical reactions. The reactions are broadly classified as *exergonic* (energy releasing) and *endergonic* (energy consuming). Bioenergetics is concerned with the initial and final states of energy component of the reactants and not the mechanism of chemical reactions.

Free energy

The energy actually *available to do work* (utilizable) is known as free energy. Changes in the free energy (ΔG) are valuable in predicting the feasibility of chemical reactions. The reactions can occur spontaneously if they are accompanied by a decrease in free energy.

Negative and positive ΔG

If free energy change (ΔG) is represented by a negative sign, there is a loss of free energy. The reaction is said to be *exergonic*, and proceeds spontaneously. On the other hand, a positive ΔG indicates that energy must be supplied to the reactants. The reaction cannot proceed spontaneously and is *endergonic* in character.

The hydrolysis of ATP is a classical example of exergonic reaction

$ATP + H_2O \longrightarrow ADP + Pi \ (\Delta G° = -7.3 \ Cal/mol)$
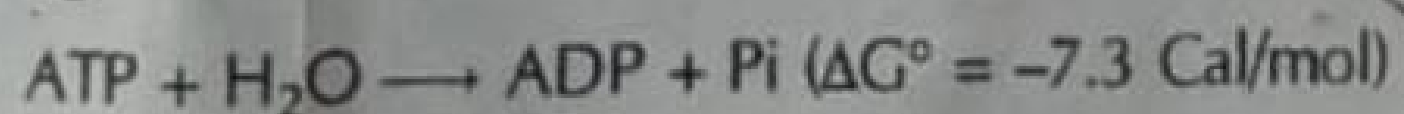
The reversal of the reaction ($ADP + Pi \longrightarrow ATP$) is endergonic and occurs only when there is a supply of energy of at least 7.3 Cal/mol ($\Delta G°$ is positive).

HIGH-ENERGY COMPOUNDS

The term high-energy compounds or *energy rich compounds* is usually applied to substances which possess sufficient free energy to liberate at least 7 Cal/mol at pH 7.0. Certain other compounds which liberate less than 7.0 Cal/mol (lower than ATP hydrolysis to ADP + Pi) are referred to as low-energy compounds. The list of important high-energy and low-energy metabolites is given in *Table 11.1*.

All the high-energy compounds—when hydrolysed—liberate more energy than that of ATP. These

TABLE 11.1 Standard free energy of hydrolysis of some important compounds

Compounds	DG (Cal/mol)
High-energy phosphates	
Phosphoenol pyruvate	−14.8
1,3-Bisphosphoglycerate	−11.8
Phosphocreatine	−10.3
S-Adenosylmethionine	−10.0
Pyrophosphate	−8.0
Acetyl CoA	−7.7
ATP → ADP + Pi	−7.3
Low-energy phosphates	
ADP → AMP + Pi	−6.6
Glucose 1-phosphate	−5.0
Glucose 6-phosphate	−3.3
Glycerol 3-phosphate	−2.2