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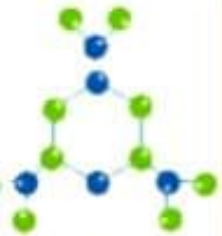

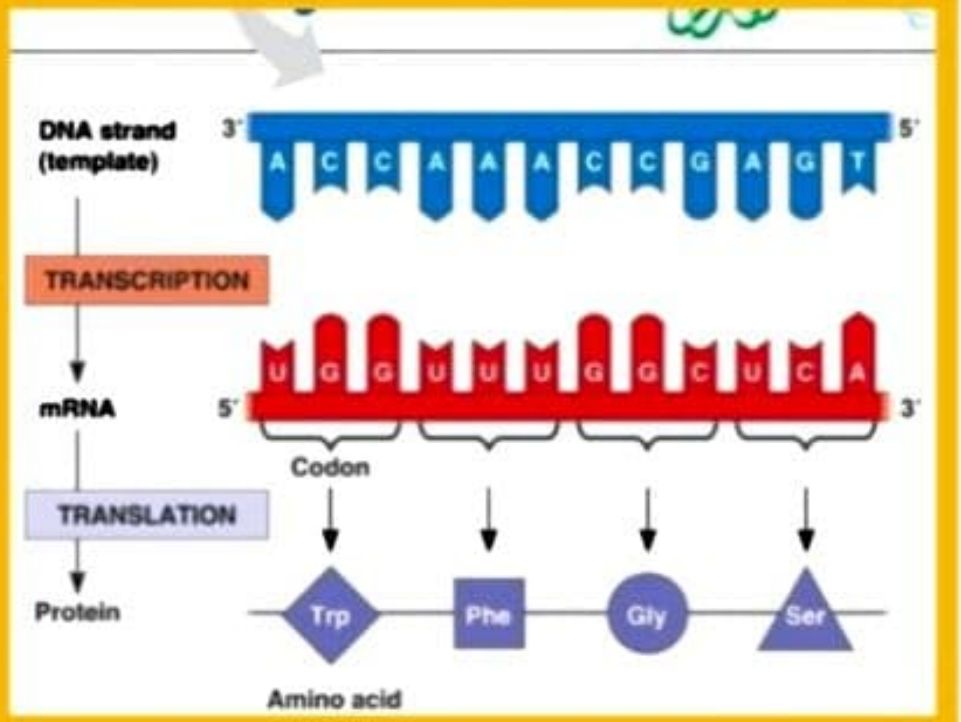

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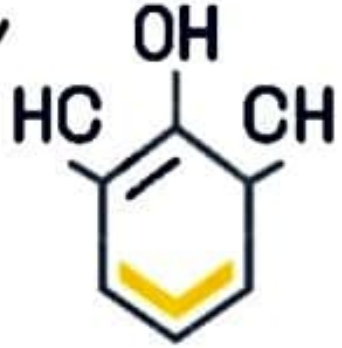


Molecular Basis Of Inheritance

Chapter - 6

CLASS - 12

DNA REPLICATION

DNA replication is the formation of two identical copies from one DNA molecule. Every time a cell divides, it needs to carry out DNA replication prior to cell division. DNA replication occurs during S-phase of the cell cycle when chromatin remains in extended form.

Semiconservative Method of Replication

According to Watson and Crick (1953), during replication, the weak hydrogen bonds between the nitrogenous bases of nucleotides separate and the two polynucleotide chains of DNA uncoil and separate. Because of the specificity of base pairing, each nucleotide of separated chains attracts its complementary nucleotide from the cell cytoplasm. Once the nucleotides are attached by their hydrogen bonds, their sugar radicals unite through their phosphate components, completing the formation of a new polynucleotide chain.

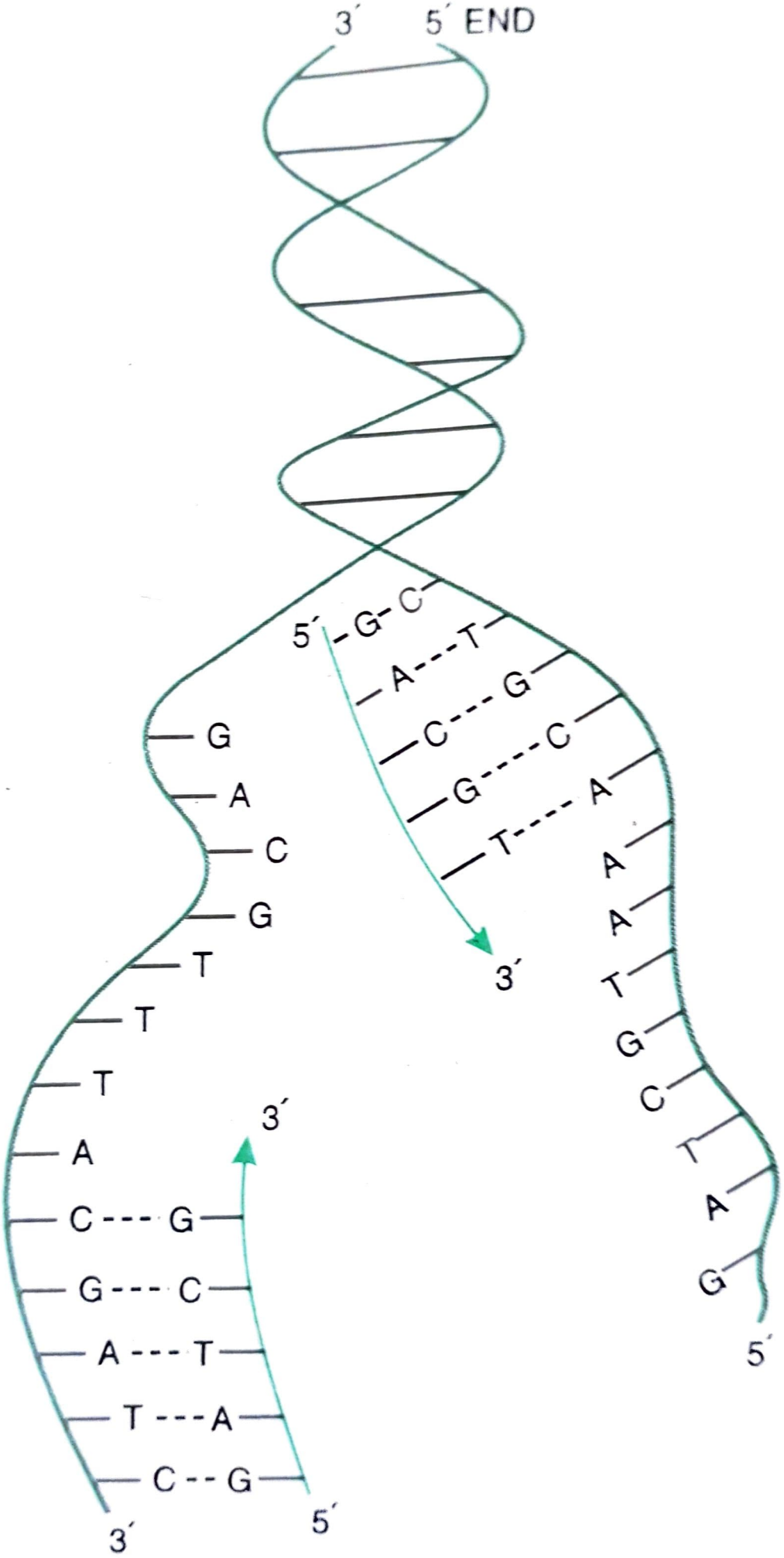


Fig. 11.12 Diagram showing DNA replication

Thus, each polynucleotide strand of the double helix of DNA serves as a template on which its complementary strand is built in 5′ → 3′ direction.

This method of DNA replication is described as **semiconservative method**. Each daughter DNA molecule is a hybrid conserving one parental polynucleotide chain and synthesising the other one.

Experimental Proof for Semiconservative Replication of DNA

1. Meselson and Stahl's Experiment on *Escherichia coli*

DNA replication is semiconservative was demonstrated by **Meselson** and **Stahl** (1958) in *Escherichia coli*.

1. *E. coli* bacteria were grown in a culture medium containing ^{15}N isotopes of nitrogen using $^{15}NH_4Cl$. This produced a population of *E. coli* with both the strands of their DNA containing ^{15}N. They represented the parental generation and their DNA with ^{15}N in both the strands was the heaviest.
2. These bacteria with ^{15}N were transferred in cultural medium containing ^{14}N and allowed to multiply only for one generation. The DNA from these first generation of bacteria which was isolated after about 20 minutes was found to be hybrid having one strand heavier (with ^{15}N) and the other lighter (with ^{14}N). The heavier strand represents the parental strand and lighter one is the new one indicating semiconservative method of DNA replication.
3. DNA extracted from this culture after 40 minutes, represented the second generation of DNA. It had equal amounts of hybrid DNA (^{15}N-^{14}N) and normal light DNA (^{14}N-^{14}N).

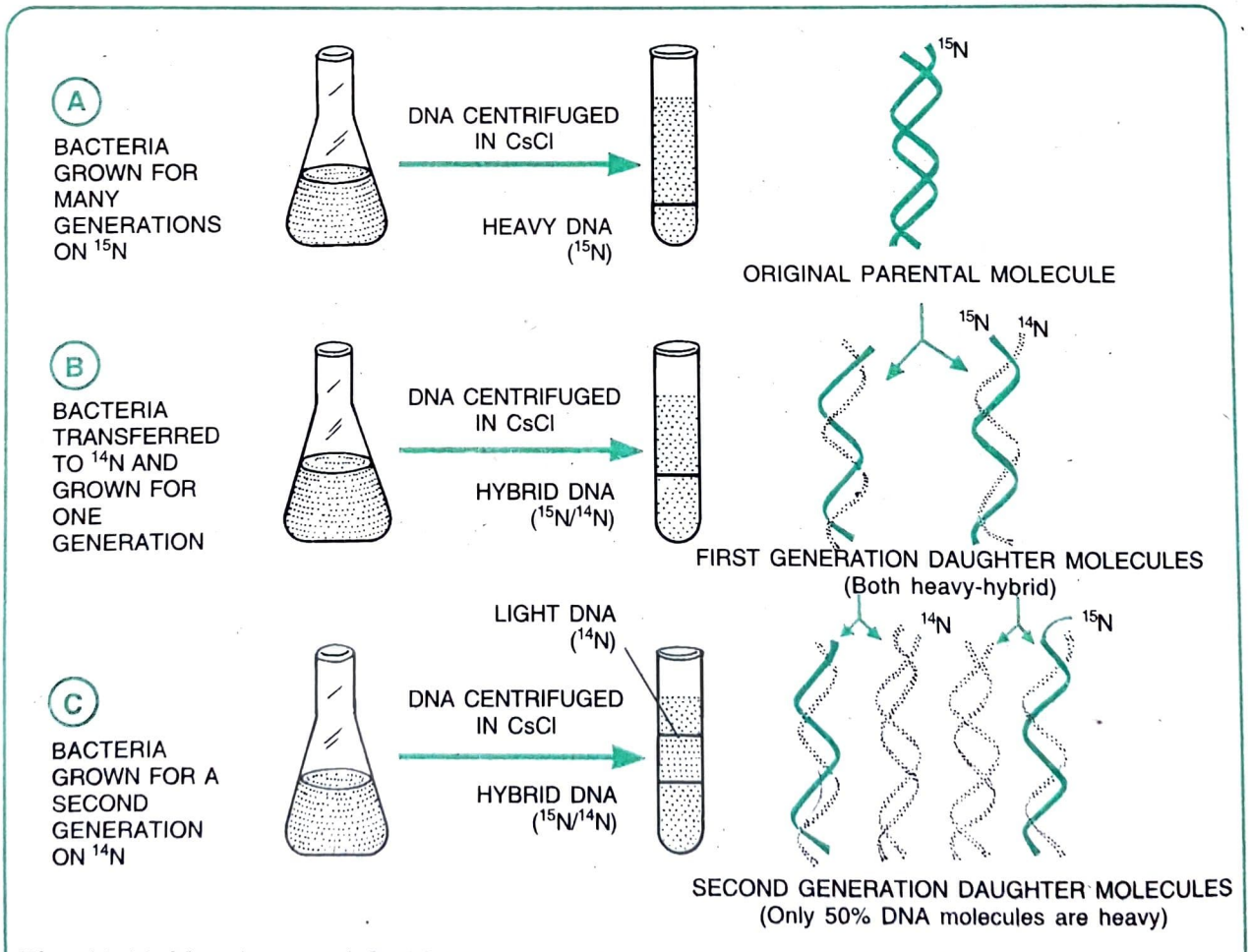


Fig. 11.13 Meselson and Stahl's experiment to demonstrate semiconservative replication of DNA. In the first replication, the daughter DNA molecules are lighter than parental strands and on second replication two bands are formed, one showing light DNA and other band showing hybrid DNA.

When DNA was extracted from *E. coli* cells after 60 minutes, it belonged to the third generation and only 25% DNA molecules was hybrid (^{15}N-^{14}N), the remaining 75% was normal DNA molecules with ^{14}N-^{14}N.

^{15}N is heavier isotope of ^{14}N. It is not a radioactive isotope. The heavy DNA molecules with ^{15}N are separated from DNA with ^{14}N only on the basis of their densities by centrifugation in a Cesium chloride (CsCl) density gradient.

Process of Replication of DNA

The process of DNA replication involves a number of steps. Each step is governed by a specific enzyme. More than a dozen enzymes and protein factors control DNA replication. These are:

1. **Activation of Deoxyribonucleotides:** The deoxyribonucleoside monophosphates (AMP, GMP, CMP, TMP) occur in the nucleoplasm. These are activated into triphosphates (ATP, GTP, CTP and TTP) by uniting with ATP. The process is known as **phosphorylation** and is catalysed by the enzyme *phosphorylase.*

2. **Recognition of Initiation Point:** DNA replication is initiated at a defined sequence of nucleotides, called **initiation point** or **origin of replication** (ori C). This consists of a specific sequence of 100 to 200 or more base pairs. Specific **initiator protein DnaA** recognises the initiation point and binds to it.

In viruses and bacteria, there is only one origin of replication, where replication begins. Since bacterial and viral chromosomes are circular and small, replication ends at the same point where it started. Thus, complete molecule of circular DNA is one unit of replication. It is called a **replicon.**

As the unwinding of DNA moves ahead of origin of replication, coiling tension develops on the separated strands. A number of **single-stranded binding proteins** (SSB) get attached and prevent the renaturation of separated strands and stabilise them.

3. **Unwinding of DNA and Exposure of Bases of Parent DNA Strands:** Initiator protein DnaA binds to origin of replication and denatures DNA. This process requires ATP and the bacterial **histone-like protein (HU).** Two hexamers of *helicase* enzyme then bind to each strand of unwounded DNA close to DnaA. Protein DnaB along with DnaC unwind DNA bidirectionally and create two potential replication forks. DNA unwinding creates tension on the two strands. This is released by enzyme *topoisomerase-I* and DNA molecule uncoils or unwinds. *Topoisomerase-II* causes recoiling of DNA. In bacteria, *topoisomerase* is called *DNA gyrase.*

The two strands of DNA start separating by the breakdown of hydrogen bonds between the paired nucleotides exposing their nitrogenous bases, so that each can serves as a template for the synthesis of a new strand.

In eukaryotes, the DNA molecules are large and cannot be separated along its entire length. They have several origins of replication and an equal number of replicons (perhaps over a thousand). During replication, each origin of replication is marked as **replication fork** due to appearance of a fork on either side of origin of replication.

4. **Formation of RNA Primer or Priming:** The *DNA-directed RNA polymerase* synthesises a short **priming strand** of RNA called **RNA primer** on the DNA template. This enzyme is also called *primase*. This RNA primer is a short strand of RNA, formed on DNA template because enzyme **DNA polymerase** cannot initiate synthesis of new DNA strand but it can polymerise the growth of DNA chain on RNA primer. It consists of about 10–60 nucleotides. The enzymes *primase* and *DNA helicase* together form **primosome.**

5. **Base Pairing or Assembly of Complementary Strands:** Deoxyribonucleoside triphosphates pair with the appropriate nitrogenous bases of template DNA strands according to base pairing rule. This is facilitated by *DNA polymerase-III.*

6. Conversion of Deoxyribonucleoside Triphosphates to Monophosphates: The deoxyribonucleoside triphosphate molecules on pairing with the nitrogenous bases of template strand, set free pyrophosphate (P~P) molecules and change into deoxyribonucleotides. The enzyme pyrophosphatase hydrolyses the pyrophosphates into inorganic phosphate groups (P_i) and releases energy.

7. Formation of New DNA Chains on RNA Primers (Polymerisation): The energy released is utilised in joining the adjacent nucleotides to form the polynucleotide chain. The process is catalysed by the enzyme *DNA polymerase-III* along with Mn^{++} and Mg^{++} ions.

The new strands of DNA are formed in the 5′→3′ direction on 3′→5′ template DNA by the addition of deoxyribonucleotides to the 3′ end of primer RNA. The addition is affected by *DNA polymerase-III* in presence of ATP. Once the synthesis of DNA strand has been initiated, it proceeds continuously, keeping pace with the unwinding of DNA at the replication fork.

Leading and Lagging Strands of DNA

DNA polymerase can polymerise the deoxyribonucleotides in the 5′ → 3′ direction, i.e., from carbon 5′ end to carbon 3′ end of the sugar molecules. Because the two DNA strands are antiparallel and the new strands must be formed on the old (parent) strands. The replication of 3′ → 5′ strand on 5′ → 3′ strand occurs differently and is called lagging strand synthesis.

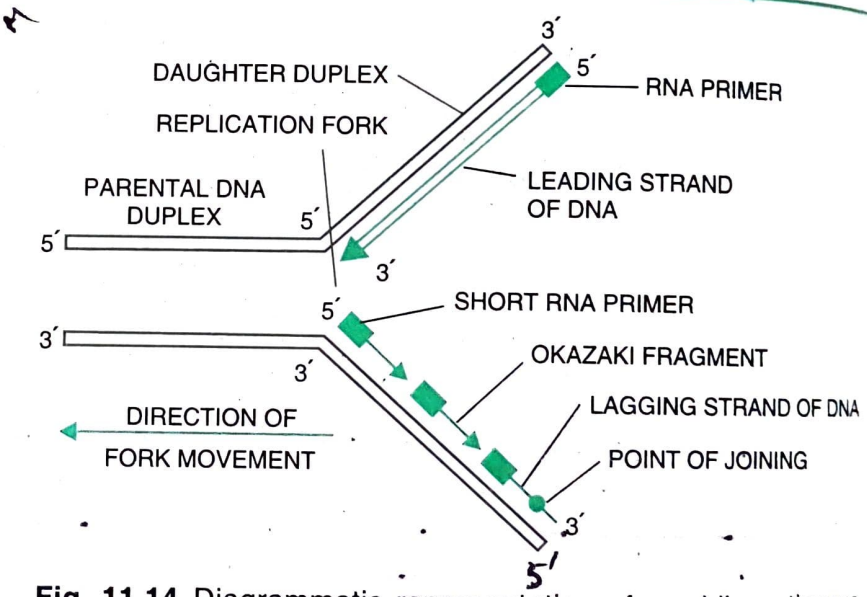


Fig. 11.14 Diagrammatic representation of semidiscontinuous replication of DNA synthesis of leading strand on 3′ → 5′ template and of lagging strand on 5′ → 3′ template strand

1. Leading Strand Synthesis: It occurs in 5′ → 3′ direction using 3′ → 5′ strand of parental DNA as template. The leading strand is synthesised as one piece. The process allows rapid synthesis with about 1,000 nucleotides added per second to each new strand.

2. Lagging Strand Synthesis: Lagging strand is also synthesised in 5′ → 3′ direction but on 5′ → 3′ strand of DNA in short segments of 1,000 to 2,000 nucleotides. These segments are called **Okazaki fragments**. This was discovered by Reiji Okazaki and colleagues. Synthesis of each Okazaki fragment begins with RNA primer synthesised by primase and is carried out by *polymerase-III* as in leading strand. The synthesis of lagging strand of DNA is discontinuous and needs one *polymerase-III* and one RNA primer for each Okazaki fragment. It involves following two additional steps:

(a) **Excision of RNA Primers:** Once a small segment of an Okazaki fragment is formed, the nucleotides of RNA primer are removed from the 5′ end by the action of 5′-3′ exonuclease activity of *DNA polymerase-I* and are replaced with DNA nucleotides.

(b) **Joining of Okazaki Fragments:** The gaps left between Okazaki fragments by the removal of RNA primer are filled with complementary deoxyribonucleotide residues by *DNA polymerase-I*. Finally, the adjacent 3′ and 5′ ends of Okazaki fragments are joined by *DNA ligase*.

TABLE 11.5 Differences between Leading and Lagging Strands of Replicating DNA

Leading Strand	Lagging Strand
1. It grows continuously as a single piece.	1. It is formed initially as short segments called Okazaki fragments and is discontinuous.
2. It needs a single RNA primer to start its growth.	2. Each segment needs a separate RNA primer to start.
3. It does not need *DNA ligase* for its growth.	3. *DNA ligase* is needed to join Okazaki fragments.
4. Direction of growth is 5′ → 3′.	4. Direction of growth of the complete strand is 3′ → 5′, but each Okazaki fragment grows in 5′ → 3′ direction.
5. It is formed on 3′ → 5′ strand.	5. The template DNA strand is formed on 5′ → 3′
6. It grows rapidly.	6. Its growth is slow.

Editing or Proofreading and DNA Repairs

The specificity of base pairing ensures accurate replication. But, sometimes, wrong bases do enter in the new chain during its synthesis. *DNA polymerase-I* enzyme identifies and replaces these forbidden base pairs with correct nitrogenous base pairs. This is called proofreading activity of RNA polymerase enzyme.

Even the forbidden base pairs introduced in DNA helix due to mutation are identified and removed by enzymes called **endonucleases** and **exonucleases**.

Inspite of proofreading and editing, some errors do creep in. But their frequency is very low—about less than one in a billion nucleotides is added in the growing chains.

Semidiscontinuous Replication of DNA

DNA replication is said to be **semidiscontinuous** because the leading strand is synthesised continuously on 3′ → 5′ strand and the lagging strand is formed discontinuously on 5′ → 3′ strand in short pieces that are called **Okazaki fragments**. These join to form a complete 3′ → 5′ strand.

Unidirectional and Bidirectional DNA Replication

John Cairns (1963) found that DNA replication in a DNA starts at one point and proceeds in one direction. This is called **unidirectional DNA replication**. However, in eukaryotes and many prokaryotes, DNA replication occurs in both directions from the origin of replication. Therefore, in them two replication forks are formed at each origin of replication. This is called **bidirectional replication**.

TRANSCRIPTION OF RNA

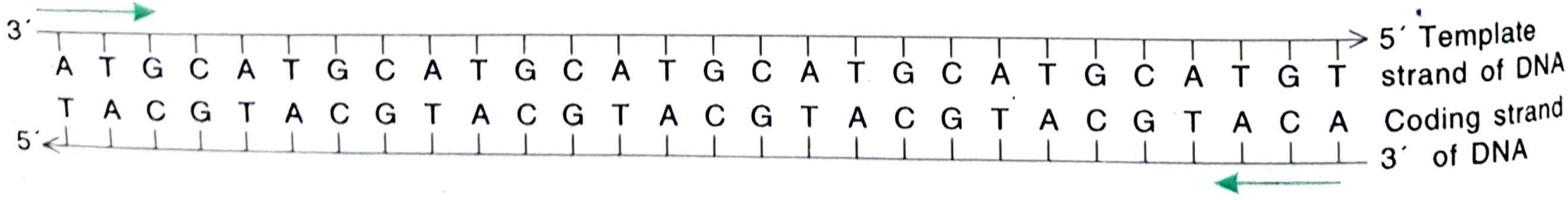
DNA transcription is defined as the process of copying the genetic message coded in DNA into mRNA molecule. The transcribed RNA moves out of the nucleus to the ribosomes in the cytoplasm to direct protein synthesis.

Necessity of Transcription

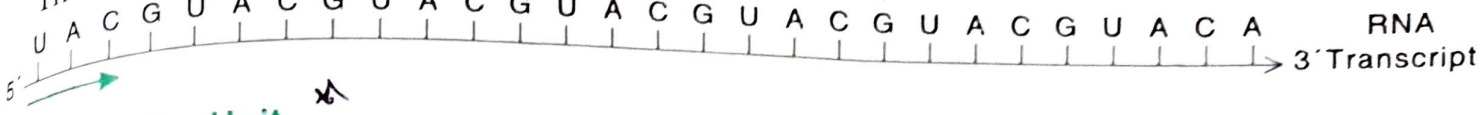
DNA contains information for the synthesis of cell's specific proteins. DNA is located in the nucleoid (in prokaryotes) or nucleus (in eukaryotes) while protein synthesis occurs in the cytoplasm. DNA does not move to the site of protein synthesis (ribosomes) to directly guide the process. Instead, it transfers its information to single-stranded mRNA molecules which move to the ribosomes to direct protein synthesis. The process of formation of RNA from the DNA template is called **transcription**.

Site and Time of Occurrence of Transcription

Transcription occurs in the nucleus during G_1 and G_2 phases of cell cycle during interphase. DNA has **promoter** and **terminater sites**. Transcription starts at the promoter site and stops at the terminater site. Only one of the two strands of DNA duplex is transcribed. Since RNA is synthesised in 5′ → 3′ direction, it is transcribed only on DNA strand having 3′ → 5′ polarity. The 3′ → 5′ DNA strand (from which RNA is transcribed) is called **template strand** or **antisense strand** or **noncoding strand**. Its complementary strand having polarity 5′ → 3′ is called **antitemplate strand** or **sense strand** or **coding strand**. The coding DNA strand has the same sequence of nitrogenous bases as the RNA transcript except that in RNA T is substituted by U. It has the same polarity: 5′ → 3′ as the RNA transcript.



The sequence of bases in RNA transcribed from template strand of above DNA will be:



Transcription Unit

The segment of DNA template strand which transcribes RNA is called **transcription unit**. It comprises three regions: **promoter, structural gene** and **terminater**.

1. **Promoter Region**: Promoter region is located just upstream of the initiation codon of the structural gene towards the 5′ end of coding strand. The promoter sequence of nitrogenous bases provides site for the binding of *RNA polymerase* for the initiation of transcription. It also helps in the differentiation of template and coding strands of DNA. The functional sequences in promoter region are called **consensus sequences**. These functional sequences are variants of 5′ TATAAT3′ and are called **TATA box**. It is known as **Pribnow box** (after its discoverer **David Pribnow**) in prokaryotes and **Goldberg Hogness box** in eukaryotes.

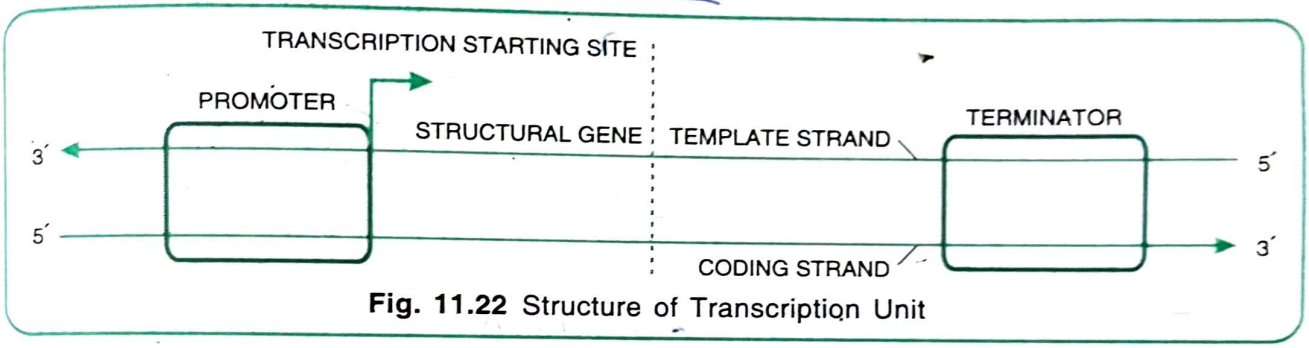


Fig. 11.22 Structure of Transcription Unit

2. **Terminator Region**: Terminator region is located downstream, i.e., towards 3′ end of the nontemplate or coding strand. It defines the end of the transcription of structure gene. The termination sites have two or more stretches of G ≡ C pairs arranged as inverted repeats followed by a string of adenine (poly A).

3. **Structure Gene (Transcription Unit and Gene)**: Structure gene is the functional unit of inheritance. It is a segment of DNA with specific sequence of nucleotides which codes for a protein or polypeptide needed for morphological or functional trait of the cell. **Benzer** (1955) used the term **cistron** for such a functional segment of DNA. Therefore, according to this modern concept, a gene is considered a unit of function (**cistron**), a unit of recombination (**recon**), a unit of mutation (**muton**) or a unit of regulation (**operon**).

The structure genes or cistrons are of two types:

(a) **Polycistronic Genes:** The genes that transcribe mRNA which codes for more than one type of polypeptide chains are called **polycistronic genes** and RNA as **polycistronic mRNA**. These are found only in prokaryotes.

(b) **Monocistronic Genes:** These genes transcribe mRNAs which code only for one polypeptide. Such monocistronic genes and monocistronic mRNA are found in eukaryotes.

Split Genes

In eukaryotes, the message coded in cistrons is discontinuous or interrupted. It is split into several coding units separated by noncoding base sequences. The coding units are called **exons**, intervening DNA segments as **introns** and such genes as **interrupted** or **split genes** by **Roberts** and **Sharp** (1993). The processed or **mature RNA** contains only the copies of exon sequences. The intron sequences are spliced out during RNA processing. Thus transcription of an interrupted gene produces the **primary transcript RNA** or **premessenger RNA** or **heterogeneous RNA** (hnRNA). It is a faithful copy of the interrupted gene. The functional RNA is formed by the removal of introns

or unwanted base sequences and the rejoining of its exons or essential sequences. This process is called RNA splicing.

Mechanism of Transcription in Prokaryotic Cells

The mechanism of transcription involves following steps:

1. **Activation of Ribonucleotides:** The ribonucleotides present in the nucleoplasm are converted to active triphosphates by phosphorylation. ATP provides energy for phosphorylation.

2. **Recognition of Promoter Region and Binding of RNA Polymerase to Promoter Region on DNA:** On a signal from the cytoplasm, the histones associated with the DNA double helix from the gene to be transcribed are removed, exposing the base sequence in this region of DNA. The sigma factor of RNA polymerase enzyme recognises the concensus sequence in the promoter region and enzyme RNA polymerase binds to the promoter in the template strand of the DNA double helix.

3. **Exposure of DNA Bases and Initiation of Transcription:** *RNA polymerase* causes local unwinding and separation of two strands of DNA. The separation begins from the middle of Pribnow box and produces a transcription bubble. The separation of strands exposes the bases of DNA for initiation of transcription. The 3′ → 5′ strand of DNA functions as template.

4. **Base Pairing:** The ribonucleotide triphosphate molecules, i.e., adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP) and uridine triphosphate (UTP) start binding to the nucleotides of template strand of DNA from the initiation point onward according to base pairing rule of Watson and Crick. This is brought about by *RNA polymerase*. Thus, enzyme *RNA polymerase* not only initiates but also causes polymerisation, i.e., elongation of polynucleotide chain in 5′ → 3′ direction.

5. **Conversion of Ribonucleotide Triphosphates to Ribonucleoside Monophosphate:** The various ribonucleoside triphosphates, on linking to the DNA template chain, break off their high-energy bonds. This changes them to ribonucleoside monophosphate and pyrophosphate groups (P~P) are set free. Pyrophosphate undergoes hydrolysis by the enzyme pyrophosphatase releasing energy. The first ribonucleotide triphosphate retains all the three phosphates.

$$P \sim P + H_2O \xrightarrow{Pyrophosphatase} 2\ Pi + Energy$$

6. **Formation and Elongation of RNA Polynucleotide Chain:** With the energy so released, each ribonucleoside monophosphate joined to DNA template chain then joins the ribonucleotide arrived earlier, making the RNA chain longer. The enzyme *RNA polymerase* catalyses the formation of phosphodiester bonds between successive nucleotides and requires divalent ions Mg^{++} and Mn^{++}.

7. **Termination of Transcription and Separation of RNA Chain:** As transcription proceeds, the hybrid DNA-RNA molecule dissociates partly, freeing the RNA molecule under synthesis. When the polymerase

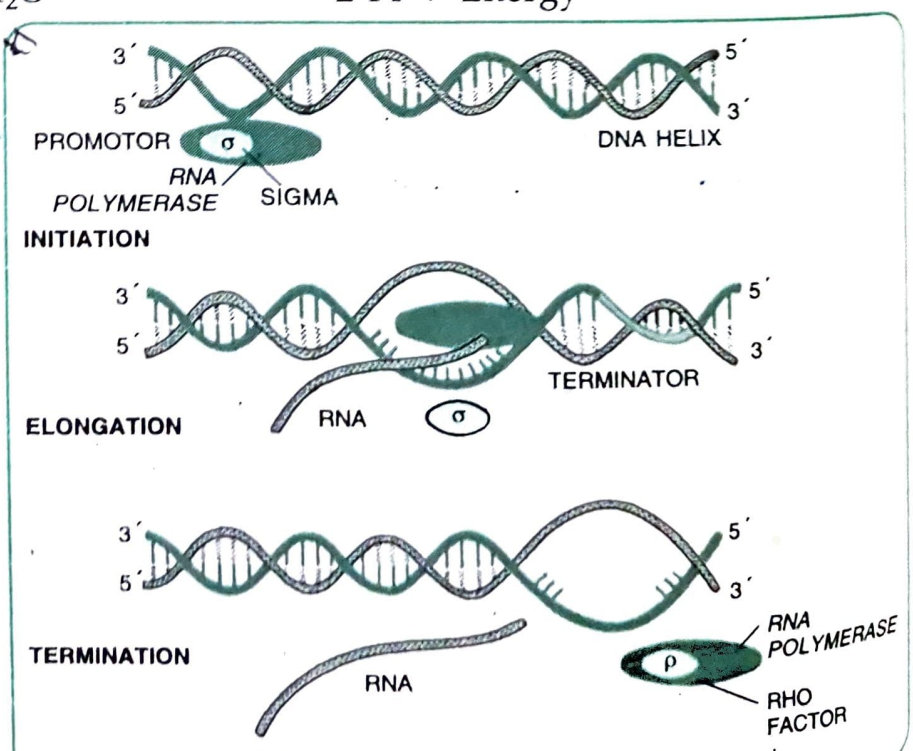


Fig. 11.23 Process of transcription in bacteria

reaches a terminater signal on the DNA, it leaves the DNA. The fully formed RNA chain is now released. One gene forms several molecules of RNA, which are released from the DNA template one after the other on completion.

Termination of Transcription

In some cases such as *E. coli*, a specific chain terminating protein, called rho factor (ρ), stops synthesis of RNA chain, but in most of the cases, *RNA polymerase* can stop transcription. Thus there are two methods of transcription:

- termination without termination factor and
- termination with a termination factor (rho r).

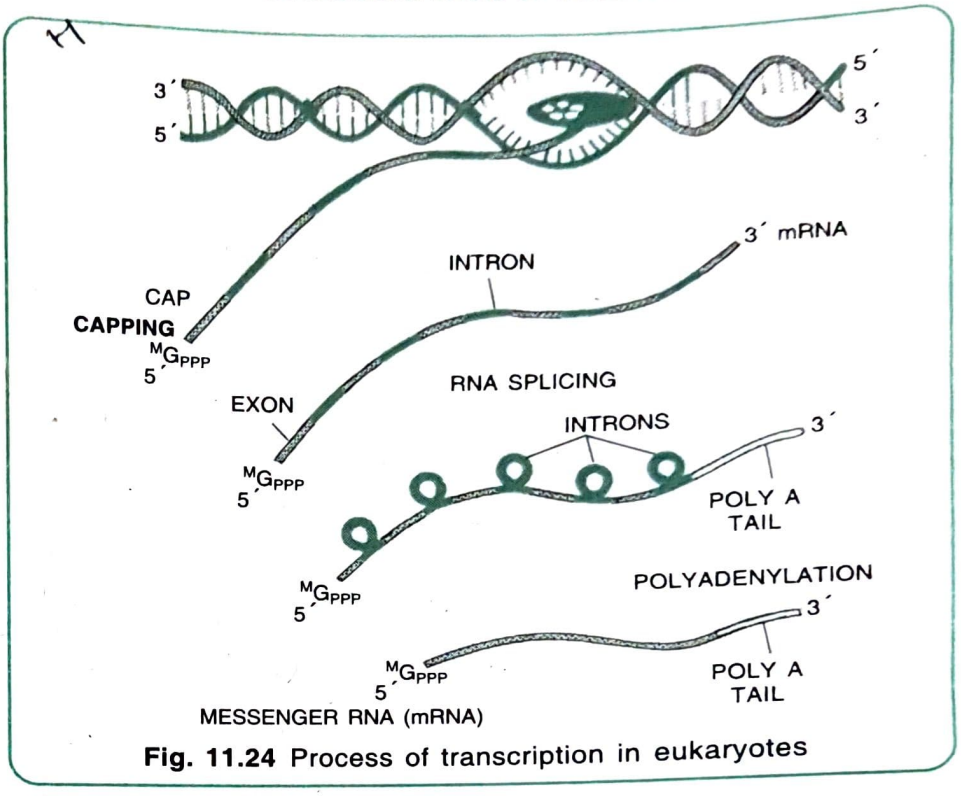


Fig. 11.24 Process of transcription in eukaryotes

Reformation of DNA Helix: As the RNA chain grows, the transcribed region of the DNA molecule restores the original double helical form.

(a) mRNA synthesis on DNA template takes place in 5′→ 3′ direction so that successive nucleotides are attached at 3′ end of the growing RNA strand. Core enzyme moves along DNA, unwinds the two strands exposing the nucleotides of template DNA. As a result complementary copy of RNA is transcribed on 3′→ 5′ strand of DNA. The transcribed RNA molecule is called RNA-transcript.

(b) There are three main steps in the process of transcription: initiation, elongation and termination.

(c) Only one enzyme *RNA polymerase* catalyses all the three functions. However, *RNA polymerase* alone can catalyse the process of chain elongation. For initiation of transcription it associates with initiation factor-sigma (σ) and for termination of transcription with termination factor-rho (ρ).

Mechanism of Transcription in Eukaryotic Cells

Transcription in eukaryotic cells is more complex and it differs from that in prokaryotes in following features:

1. Three different RNA polymerases are required in the transcription of three different types of RNA:
   (a) *RNA polymerase I* transcribes rRNAs (28S, 18S and 5.8S).
   (b) *RNA polymerase II* transcribes precursor of mRNA (the heterogeneous nuclear RNA: hnRNA) and occurs in nucleoplasm.
   (c) *RNA polymerase III* catalyses transcription of tRNA, 5S rRNA and small nuclear RNAs (snRNAs). It is also found in nucleoplasm.
2. Eukaryotes have three different types of promoters for three different polymerases.
3. Eukaryotes have many additional transcription factors for binding of RNA polymerases to DNA.

TABLE 11.10 Differences between Prokaryotic and Eukaryotic Transcriptions

Prokaryotic Transcription	Eukaryotic Transcription
1. Prokaryotic transcription occurs in the cytoplasm.	1. Eukaryotic transcription occurs in the nucleus.
2. There is no definite phase for its occurrence.	2. Takes place in the G_1 and G_2 phases of cell cycle.
3. A single *RNA polymerase* synthesises all the three types of RNA (mRNA, tRNA, rRNA).	3. Three *RNA polymerases* I, II and III synthesise rRNA, mRNA and tRNA respectively.
4. Coupled transcription translation is the rule.	4. Coupled transcription translation is not possible.
5. Initiation of transcription does not need any proteins or initiation factors.	5. Initiation of transcription requires proteins called **transcription factors** to recognise TATA box. These are TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH.
6. RNAs are released and processed in the cytoplasm.	6. RNAs are released and processed in the nucleus.
7. *RNA polymerase* is a complex of 5 polypeptides.	7. RNA polymerases are complexes of 10 to 15 polypeptides.
8. Transcriptional unit has one or more genes (**Polycistronic**).	8. Transcriptional unit has only one gene. **(Monocistronic).**
9. The mRNA primary transcript has fewer surplus nucleotides.	9. The mRNA primary transcript has a large number of surplus nucleotides.
10. The 23S, 16S and 5S rRNAs are formed from a single primary transcript.	10. The 28S, 18S, 5.8S and 5S rRNAs are formed from two primary transcripts.

RNA Processing or Post-transcriptional Modification of RNA Transcript

The originally transcribed RNA molecules are called **primary transcripts** or **hn RNA.** They are biologically inactive and in eukaryotes they have exons as well as introns. Therefore, RNA transcripts undergo extensive changes to become functional. This is called **processing of RNAs** or **post-transcriptional modifications of RNAs.** During RNA processing:

1. Larger RNA precursors are cut into smaller RNAs by a *ribonuclease-P* **enzyme (Cleaving).**
2. Unwanted ribonucleotides or introns are removed by enzymes named **nucleases (Splicing).**
3. Functional regions or exons are rejoined in defined order by *Ligase* enzyme **(Union).**
4. Certain nucleotides are added at the end enzymatically **(Terminal addition).**
5. Molecule may fold on itself to assume proper shape **(Folding)** in tRNA and rRNAs
6. Some nucleotides may be modified **(Nucleotide modification)** in tRNA.

Processing of mRNA Primary Transcript: Most prokaryotic mRNA transcripts require very little or no post-transcriptional modification to become functional mRNA. Sometimes, translation of mRNA begins while it is being transcribed. But in eukaryotes, the primary-mRNA transcript molecules are called heterogeneous nuclear RNA or hn RNA. It is chemically modified in the nucleus before being transported out in the cytoplasm for translation. Following three biochemical processes occur during mRNA processing in eukaryotes:

1. **Capping at 5′ end:** addition of methyl guanosine triphosphate to 5′ end of hnRNA.
2. **Tailing at 3′ end:** addition of 200–300 adenylate residues at 3′ end of hnRNA.
3. **Splicing:** removal of introns and joining of exon segments of the transcript.

Thus a fully processed functional, mRNA has defined 5′ end with G-cap and 3′ end with poly A tail.

Processing of tRNA Transcript: Eukaryotic pre-tRNA molecules are biochemically altered to make functional tRNA molecules by:

1. Trimming of ends by the cleavage of phosphoester bonds.

2. Splicing for removing introns.
3. Addition of some terminal sequences.
4. Heterocyclic base modification, usually by **methylation.**

In some prokaryotic tRNAs some introns are removed; some terminal sequences are added and in some, a few heterocyclic and ribose rings are modified. The endonuclease enzyme **ribonuclease P** catalyses removal of a segment from 5′ end of pre-tRNA by hydrolytic cleavage.

Processing of rRNA Transcript: Involves its trimming. rRNA transcripts to smaller segments of appropriate size. It has already been discussed in biogenensis of rRNA.

TABLE 11.11 Comparison between Replication and Transcription

Replication	Transcription
1. It occurs in the S-phase of cell cycle.	1. It occurs in G_1 and G_2-phases of cell cycle.
2. It is catalysed by *DNA polymerase* enzymes.	2. It is catalysed by *RNA polymerase* enzymes.
3. Deoxyribonucleoside triphosphates (dATP, dGTP, dCTP, dTTP) serve as raw materials.	3. Ribonucleoside triphosphates (ATP, GTP, CTP, UTP) serve as raw materials.
4. Replication occurs along both the strands of DNA.	4. It takes place along one strand of DNA.
5. It involves unwinding and splitting of the entire DNA molecule (chromosome).	5. Involves unwinding and splitting of only those genes which are to be transcribed.
6. It involves copying of the entire genome.	6. It involves copying of certain individual genes only.
7. Replicated DNA strand remains hydrogen-bonded to its template DNA strand.	7. Transcribed RNA strand separates from its DNA template strand.
8. Two double-stranded DNA molecules are formed from one DNA molecule.	8. A single one-stranded RNA molecule is formed from a segment of one DNA strand.
9. Products remain within the nucleus.	9. Greater part of the products pass from the nucleus into the cytoplasm.
10. Products are not degraded.	10. Products are degraded after their function is over.
11. Serves to conserve the genome for the next generation of cells and individuals.	11. Serves to from RNA copies of individual genes for immediate use in protein synthesis.
12. It requires RNA primer to start replication.	12. No primer is required to start.
13. It produces normal DNA molecules that do not need any processing.	13. It produces primary RNA transcript molecules which need processing to acquire final form and size.

GENETIC CODE

The linear arrangement of nitrogen bases in DNA is said to determine the sequence of amino acids in a protein molecule. It means the precise sequence of only four nitrogenous bases on the DNA strand somehow directs the proper amino acids to their proper places in a polypeptide chain through the intervention of four nitrogenous bases of RNA , i.e., A, G, C and U.

Triplet Genetic Code

George Gamow, a physicist in 1954 argued that since there are only four nitrogenous bases but they have to code for 20 amino acids, each codon should have a combination of three nitrogenous bases, i.e., a combination of three bases would generate 64 codons ($4^3 = 4 \times 4 \times 4 = 64$ condons).

Codon

A codon (code word) can be defined as a triplet sequence of nitrogenous bases in mRNA copied from DNA molecule which codes for a particular amino acid, whereas the genetic code is the sequence of nitrogenous bases in mRNA molecule, which encloses the information for linking of amino acids during the synthesis of protein molecules.

TABLE 11.12 Codons of mRNA for different Amino acids

FIRST BASE	SECOND BASE: U	SECOND BASE: C	SECOND BASE: A	SECOND BASE: G	THIRD BASE
U	UUU, UUC → Phe UUA, UUG → Leu	UCU, UCC, UCA, UCG → Ser	UAU, UAC → Tyr UAA → Terminater UAG → Terminater UGA → Terminater	UGU, UGC → Cys UGA, UGG → Try	U C A G
C	CUU, CUC, CUA, CUG → Leu	CCU, CCC, CCA, CCG → Pro	CAU, CAC → His CAA, CAG → Gln	CGU, CGC, CGA, CGG → Arg	U C A G
A	AUU, AUC, AUA → IIU AUG → Met	ACU, ACC, ACA, ACG → Thr	AAU, AAC → Asn AAA, AAG → lys	AGU, AGC → Ser AGA, AGG → Arg	U C A G
G	GUU, GUC, GUA, GUG → Val	GCU, GCC, GCA, GCG → Ala	GUA, GAC → Asp GAA, GAG → Glu	GGU, GGC, GGA, GGG → Cys	U C A G

Although, informations are coded and stored in the form of nitrogenous base sequences in DNA molecule, the message from DNA is carried out in the cytoplasm by mRNA and the code on mRNA is translated into the sequence of amino acids in polypeptide chain.

(a) Protein synthesis in cell-free medium by Marshall Nirenberg helped in cracking the genetic code or deciphering codons for different amino acids.

(b) Severo Ochoa's work on polymerisation of RNA with defined base sequences in a template-free medium (i.e. enzymatic synthesis of RNA) completed the deciphering of genetic code.

The existence of a triplet code was proved by Nirenberg (Nobel Prize winner) and Mathaei in 1961. They were able to synthesise artificial mRNA which contained molecules of only one base uracil. It was named as polyuridylic (poly-U) molecule. The synthetic poly-U was placed in a cell-free system containing protein synthesising enzyme, all the twenty amino acids and necessary ATP. After sometime a small protein-like molecule was produced which was formed by the linking of phenylalanine. It means UUU is the codon for phenylalanine.

Similarly, poly-A mRNA gives polylysine peptide chain and poly-C gives polyproline. Therefore, codon-AAA was assigned for lysine and CCC to proline.

Essential Features of Genetic Code (4 - Green)

1. **Triplet:** A codon comprises three nitrogenous bases of mRNA in a specific sequence.

2. **Genetic Code is Commaless:** There is no punctuation (comma) between the adjacent codons, i.e., each codon is immediately followed by the next codon with no intervening spaces of letters for comma.

3. **Genetic Code is Non-Overlapping:** Initially it was disturbing for some geneticists to think of degeneracy in connection with genetic code. Therefore, a triplet code with overlapping sequence was suggested. Under the overlapping triplet code the number of codons could be reduced to twenty. But recent evidences support the existence of a non-overlapping code.

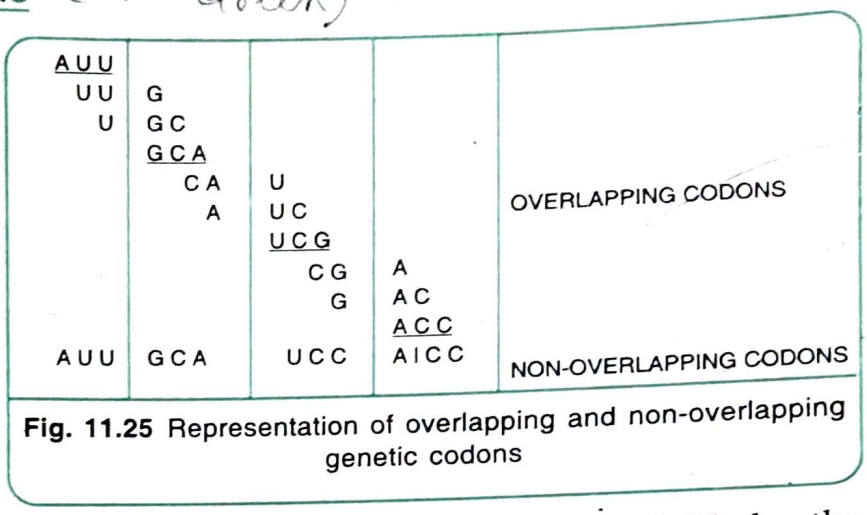


Fig. 11.25 Representation of overlapping and non-overlapping genetic codons

4. **Genetic Code is Degenerative:** More than one codons can be used for a particular amino acid. This multiple system of coding is known as **degenerate system** or **degenerate code**.

The degenerate code provides a protection to organisms against many harmful mutations, stabilises phenotypes by lessening the effect of random mutations and minimises the consequences of base pairing erros.

The major degeneracy occurs at the third position (5′ end of the triplet codon). When first two bases are specified, the same amino acid may be coded for whether the third base is U, C, A or G. This third base is described as **Wobbly base**. The hypothesis which states that tRNA anticodon can wobble at its 5′ end by pairing with noncomplementary base of mRNA codon is called **Wobble Hypothesis**. It was proposed by **Crick** in 1966. This is evident from genetic codes for the following amino acids:

(a) **Serine:** UCU, UCC, UCA, UCG and AGU, AGC.
(b) **Arginine:** CGU, CGC, CGA, CGG, and AGA, AGG.
(c) **Leucine:** CUU, CUC, CUA, CUG and UUA, UUG.
(d) **Valine:** GUU, GUC, GUA, GUG.

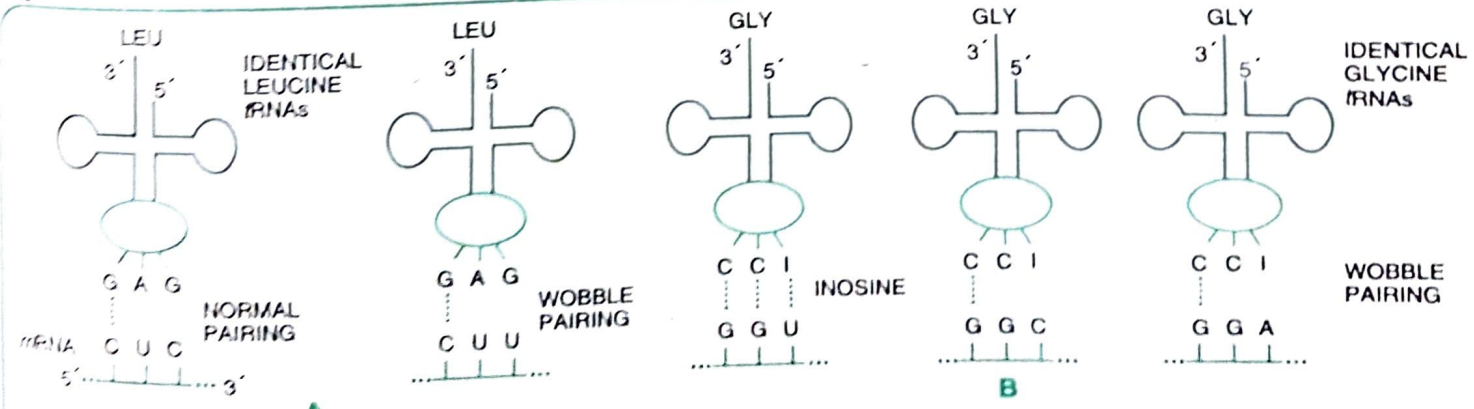


Fig. 11.26 Two examples of Wobble base pairing: A–Two different leucine codons CUC and CUU can be read by the same anticodon of leucine tRNA; B–Three different glycine codons GGU, GGC and GGA can be read by the same anticodon of glycine tRNA molecule having abnormal or wobble base (I)

5. **Genetic Code is Unambiguous and Specific:** It is because a particular codon always codes for the same amino acid throughout the living world.

6. **Collinearity:** Genetic code represents sequence of codons in mRNA and the corresponding amino acid residues of a polypeptide chain are arranged in the same linear sequence. mRNA is linear with DNA and with amino acids in polypeptide chain.

7. **Genetic Code has Chain Initiation and Chain Termination Codons:** The codon present in the beginning of the cistron is known as **initiation codon.** It marks the beginning of the message for a polypeptide chain. The initiation codon is **AUG** in majority of cases and it codes for amino acid methionine.

Similarly, the last codon of a cistron helps in reading the termination of polypeptide chain. This is known as **termination codon.** There are three termination codons: **UAA, UGA** and **UAG.** Earlier, when the function of these codons was not known, these were called **nonsense codons** because these do not code for any of the 22 essential amino acids.

The initiator and terminater codons are known as **signals** and this phenomenon is known as **punctuation.** Punctuation helps in delimiting the different cistrons on a polycistronic mRNA.

8. **Genetic Code is Universal:** It is because the same genetic code is present in all living organisms including viruses, bacteria, unicellular and multicellular organisms.

A. Given below is the sequence of nitrogenous bases in mRNA. You can predict the sequence of amino acids coded by it.

Segment of mRNA:	AUG	UUU	UUC	UUC	CGU	GCU	AAA	UUC
Polypeptide copied:	Met-	Phe-	Phe-	Phe-	Arg-	Ala-	Lys-	Phe

B. Follow the sequence of amino acids in above polypeptide chain, predict the sequence of nucleotides in the mRNA for these amino acids.

Polypeptide:	Met-	Phe-	Phe-	Phe-	Arg-	Ala-	Lys-	Phe
mRNA:	AUG	UUU	UUU	UUU	CGU	GCU	AAA	UUC

C. Compare the nucleotide sequence in mRNA in **A** and **B.** What is the difference and why? Amino acid phenylalanine (Phe) is coded by two codons UUU and UUC (The genetic code is degenerate).

1. Describe the salient features of the double helical model of DNA.
2. Explain the process of DNA replication.
3. What is reverse transcription? Explain how single stranded RNA of viruses give rise to double stranded DNA?
4. How does operon works in bacteria?
5. Explain the role of regulatory gene in the concept of operon.
6. Genetic material is DNA and not protein. How did Griffith prove this?
7. What are the advantages and probable risks of genetic engineering?
8. What are retroviruses? How do they modify the central dogma in molecular biology?
9. What does the lac operon consist of? How is the operator switch turned on and off in the expression of genes in this operon?
10. Who demonstrated the semiconservative replication of DNA? Explain the procedure in detail.
11. (a) What are the three types of RNA?
    (b) Which one of these has the shape of a clover leaf in two dimensional structure?