DNA - TRANSCRIPTION - IN EUKARYOTES

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TRANSCRIPTION IN EUKARYOTIC CELLS

Transcription in eukaryotic cells involves the same four stages –Binding, Initiation, Elongation & Termination.

The process in eukaryotes is more complicated than that in bacteria. The newly formed mRNA in case of prokaryote is in active form *i.e.* no further processing is required but in case of eukaryotes the newly form RNA is in inactive form which is termed as preRNA or hnRNA. They must be processed before they can take part in translation.

The main differences are as follows:

1. **Three different RNA polymerases –(RNA-*pol I, pol-II & pol-III*)** transcribe the nuclear DNA of eukaryotes. Each synthesizes one or more classes of RNA.

2. **Eukaryotic promoters** are more varied than bacterial promoters. Not only are different types of promoters employed for the three polymerases. Some eukaryotic promoters are actually located **downstream** from the transcription startpoint.

3. Binding of eukaryotic RNA polymerases to DNA requires the participation of additional proteins,called **transcription factors**. Unlike the bacterial sigma factor, eukaryotic transcription factors are not part of the RNA polymerase molecule. Rather, some of them must bind to DNA before RNA polymerase can bind to the promoter and initiate transcription.

4. **Protein–protein interactions** play a prominent role in the first stage of eukaryotic transcription. Although some transcription factors bind directly to DNA, many attach to other proteins—either to other transcription factors or to RNA polymerase itself.

5. **RNA cleavage** is more important than the site where transcription is terminated in determining the location of the end of the RNA product.

6. Newly forming eukaryotic RNA molecules typically undergo extensive **RNA processing** (chemical modification) both during and, to a larger extent, after transcription.

Properties of 3 different Eukaryotic RNA Polymerases:

RNA Polymerase	**Location**	**Main Products**
I	Nucleolus	Precursor for 28S **rRNA,** 18S rRNA, and 5.8S rRNA
II	Nucleoplasm	Pre-**mRNA**,most snRNA & microRNA
III	Nucleoplasm	Pre-**tRNA**, 5S rRNA,& other small RNAs
Mitochondrial	Mitochondrion	Mitochondrial RNA
Chloroplast	Chloroplast	Chloroplast RNA

PROMOTER:

The promoters that eukaryotic RNA polymerases bind to are more varied than bacterial promoters. They can be grouped into three main categories, one for each type of polymerase.

1). The promoter used by RNA polymerase I

The promoter used by RNA polymerase I—has two parts

a). **Core promoter**— It is the smallest set of DNA sequences that able to direct the accurate initiation of transcription by RNA polymerase. The core promoter is sufficient for proper initiation of transcription.

b). **Upstream control element(UCE)**, To made transcription more efficient their present **upstream control element(UCE)** which is a fairly long sequence similar (though not identical) to the core promoter. Attachment of transcription factors to both parts of the promoter facilitates the binding of RNA polymerase I to the core promoter and enables it to initiate transcription at the start point.

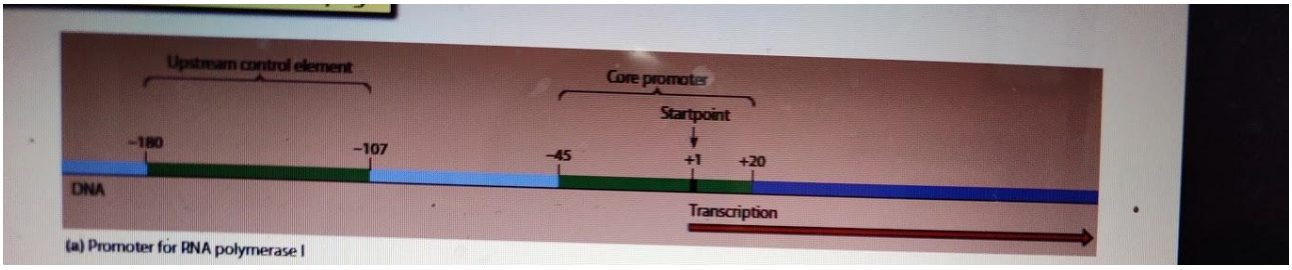


Fig: promoter for RNA polymerase I

2). The promoter used by RNA polymerase II

In the case of RNA polymerase II, at least four types of DNA sequences are involved in core promoter function. These four elements are

(1) a short **initiator (Inr)** sequence surrounding the transcription startpoint (which is often an A, as in bacteria);

(2) the **TATA box(Hogness Box),** which consists of a consensus sequence of TATA followed by two or three more A's, usually located about 25 nucleotides upstream from the startpoint;

(3) the **TFIIB recognition element (BRE)** located slightly upstream of the TATA box;

and

(4) the **downstream promoter element (DPE)** located about 30 nucleotidesdownstream from the startpoint.

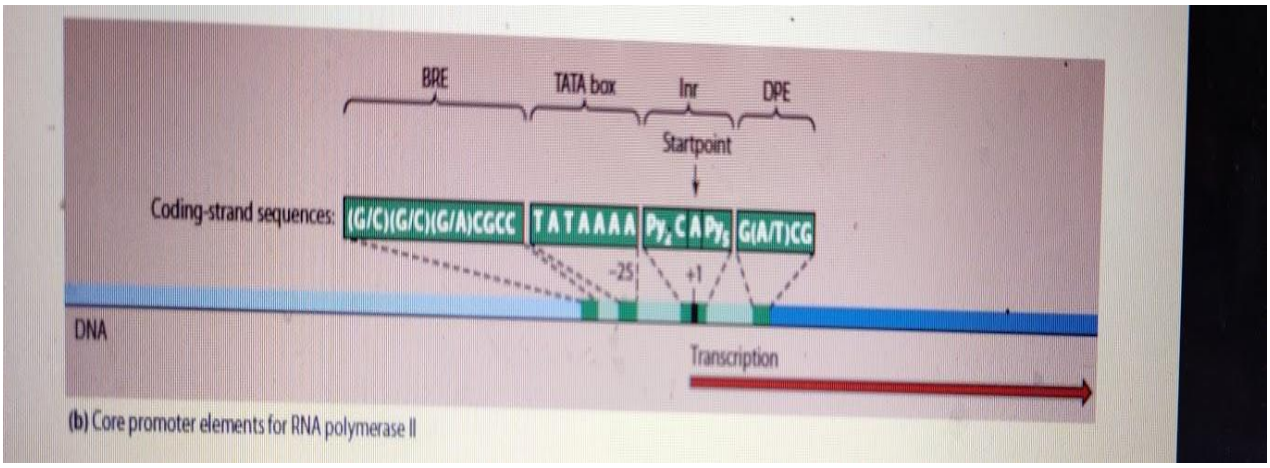


Fig 2: core promoter of RNA polymerase -II

These four elements are organized into two general types of core promoters:

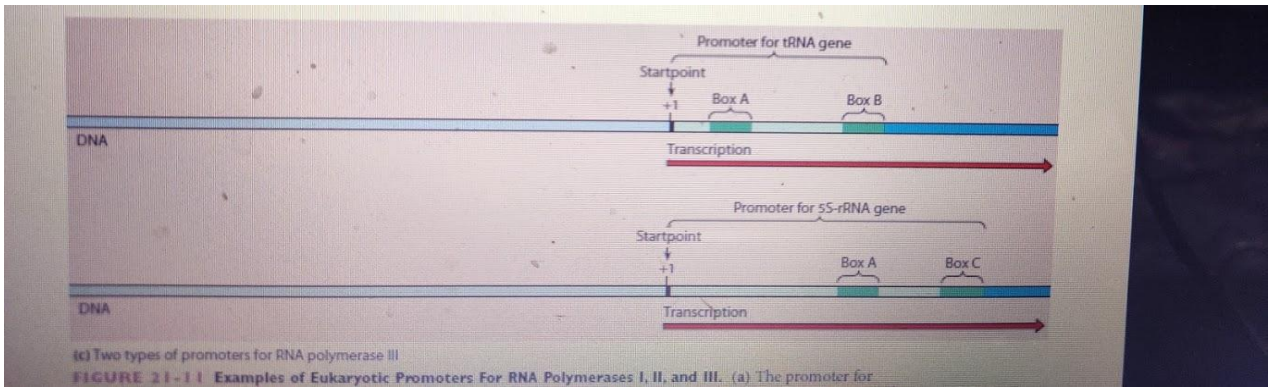
i) TATA-driven promoters, which contain an **Inr** sequence and a **TATA box with or without** an **associated BRE**, and

ii) DPE-driven promoters, which contain **DPE** and **Inr** sequences but no **TATA box** or **BRE.**

A core promoter alone (TATA-driven or DPEdriven) is capable of supporting only a basal (low) level of transcription. However, most protein-coding genes have additional short sequences further upstream—*UPSTREAM CONTROL ELEMENTS*—that improve the promoter's efficiency. Some of these upstream elements are common to many different genes; examples include the CAAT box (consensus sequence GCC**CAAT**CT in animals and yeasts) and the GC box (consensus sequence GG**GC**GG).

3. <u>The promoter used by RNA polymerase III</u>

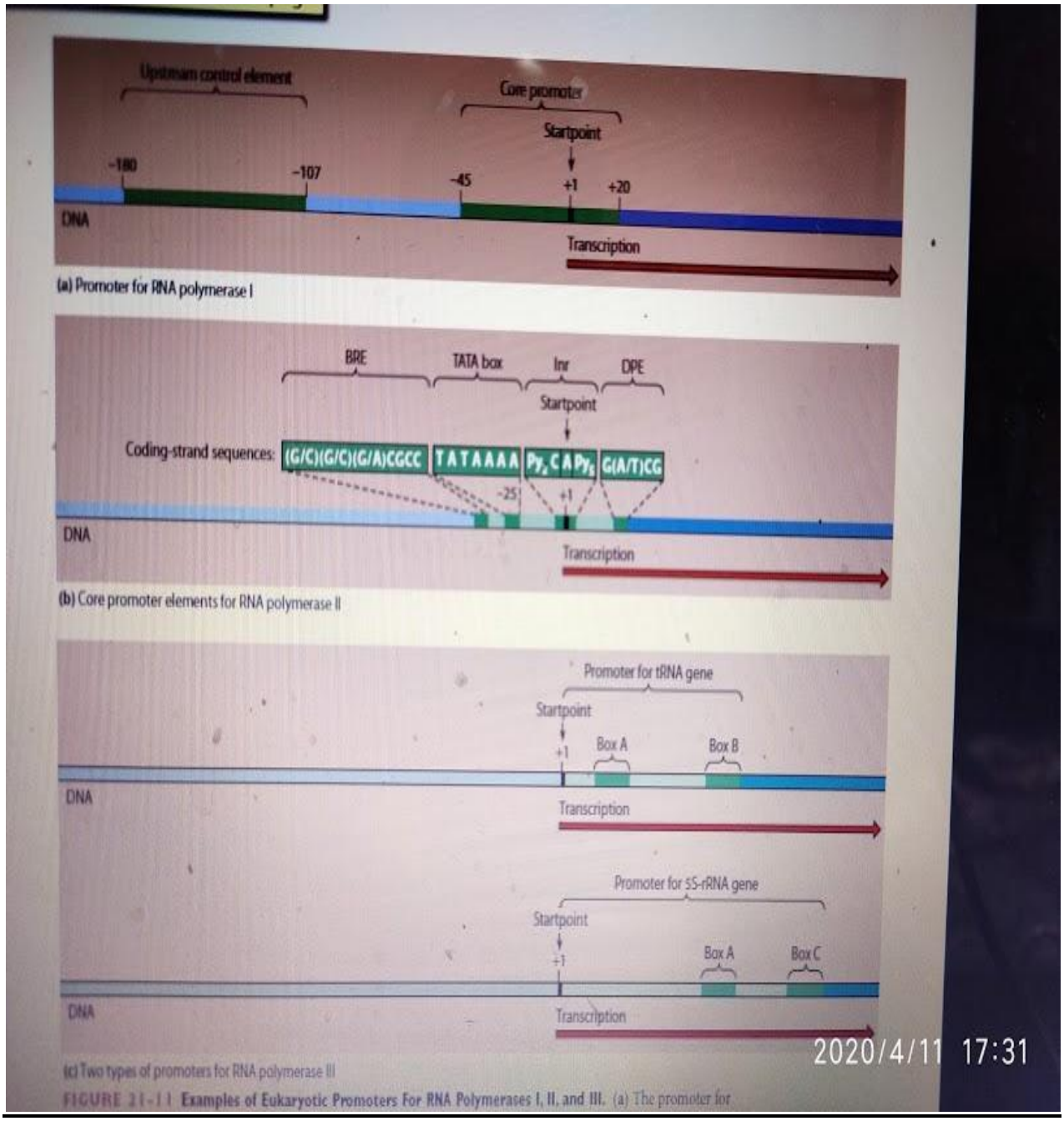
In contrast to RNA polymerases I and II, the RNA polymerase III molecule uses promoters that are entirely downstream of the transcription unit's startpoint when transcribing genes for tRNAs and 5S rRNA. The promoters used by tRNA and 5S-rRNA genes are different, but in both cases the consensus sequences fall into two blocks of about 10 bp each .The tRNA promoter has consensus sequences called box A and box B. The promoters for 5S-rRNA genes have box A (positioned farther from the startpoint than in tRNA-gene promoters) and another critical sequence, called box C.



(c) Two types of promoters for RNA polymerase III

FIGURE 21-11 Examples of Eukaryotic Promoters For RNA Polymerases I, II, and III. (a) The promoter for

COMPARISION OF PROMOTERS FOR EACH RNA POLYMERASE:



(b) Core promoter elements for RNA polymerase II

(c) Two types of promoters for RNA polymerase III

FIGURE 21-11 Examples of Eukaryotic Promoters For RNA Polymerases I, II, and III. (a) The promoter for

GENERAL TRANSCRIPTION FACTORS:

A **general transcription factor** is a protein that is always required for an RNA polymerase molecule to bind to its promoter and initiate RNA synthesis, regardless of the identity of the gene involved. Eukaryotes have many such transcription factors; their names usually include “TF” (for transcription factor), a roman numeral identifying the polymerase they aid, and a capital letter that identifies each individual factor (for example, TFIIA, TFIIB, and so forth). In humans, about six transcription factors have been identified (TFllD, TFllA, TFllB, TFllF, TFllE, TFIIH).It is postulated that the TFs bind to each other, and in turn to the enzyme RNA polymerase.

Initiation of transcription

The molecular events required for the initiation of transcription in eukaryotes are complex, and broadly involve three stages.

1. Chromatin containing the promoter sequence made accessible to the transcription machinery.
2. Binding of transcription factors (TFs) to DNA sequencesin the promoter region.
3. Stimulation of transcription by enhancers.

Enhancer can increase gene expression by about 100 fold. This is made possible by binding of enhancers to transcription factors to form activators. It is believed that the chromatin forms a loop that allows the promoter and enhance' to be close together in space to facilitate transcription

Involvement of general transcription factors

Here we are RNA polymerase II as an example to illustrates the involvement of general transcription factors in the binding of RNA polymerase to a TATA containing promoter site in DNA.

General transcription factors bind to promoters in a defined order, starting with TFIID. While TFIID binds directly to a DNA sequence (the TATA box in this example or the DPE sequence in the case of DPE-driven promoters), the other transcription factors interact primarily with each other. Hence, protein-protein interactions play a crucial role in the binding stage of eukaryotic transcription. RNA polymerase II does not bind to the DNA until several steps into the process. Eventually, a large complex of proteins, including RNA polymerase, becomes bound to the promoter region to form a preinitiation complex.

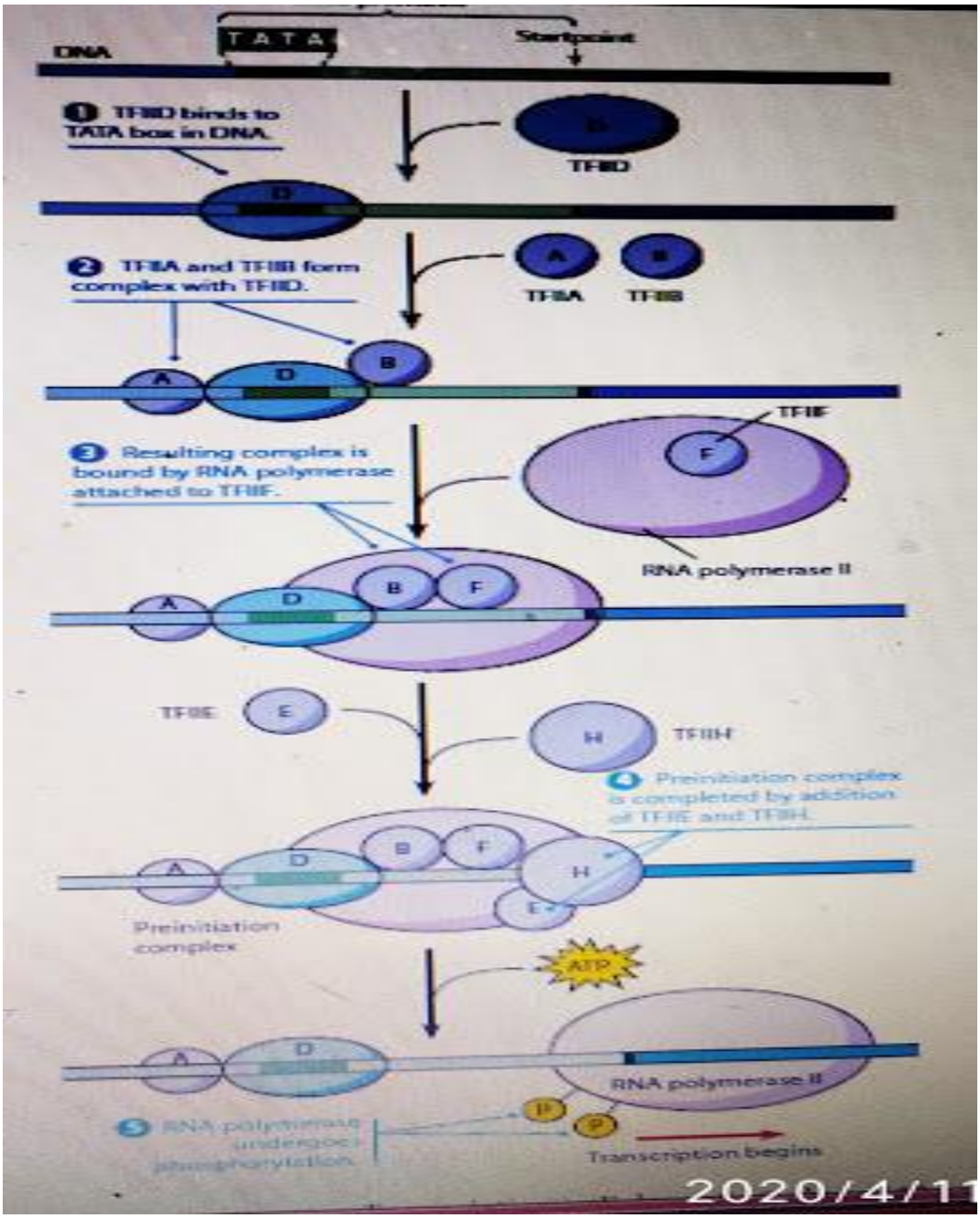


Fig: Role of General Transcription Factors in Binding RNA Polymerase II to DNA

Before RNA polymerase II can actually initiate RNA synthesis, it must be released from the preinitiation complex. A key role in this process is played by the general transcription factor TFIIH, which possesses both a helicase activity that unwinds DNA and a protein kinase activity that catalyzes the phosphorylation of RNA polymerase II. Phosphorylation changes the shape of RNA polymerase, thereby releasing it from the transcription factors so that it can initiate RNA synthesis at the startpoint. At the same time, the helicase activity of TFIIH is thought to unwind the DNA so that the RNA polymerase molecule can begin to move.

TFIID, the initial transcription factor to bind to the promoter has ability to recognize and bind to DNA promoter sequences is conferred by one of its subunits, the **TATA-binding protein**

(TBP), which combines with a variable number of additional protein subunits to form TFIID. TBP can also bind to promoters lacking a TATA box, including promoters used by RNA polymerases I and III. Depending on the type of promoter, TBP associates with different proteins, and for promoters lacking a TATA box, much of TBP's specificity is probably derived from its interaction with these associated proteins.

In addition to general transcription factors and RNA polymerase II, several other kinds of proteins are required for the efficient transcription and regulated activation of specific genes. Some of these proteins are involved in opening up chromatin structure to facilitate the binding of RNA polymerase to DNA. Others are regulatory transcription factors, which activate specific genes by binding to upstream control elements and recruiting co activator proteins that in turn facilitate assembly of the RNA polymerase pre initiation complex.

Elongation

After initiating transcription, RNA polymerases move along the DNA and synthesize a complementary RNA copy of the DNA template strand. Special proteins facilitate the disassembly of nucleosomes in front of the moving polymerase and their immediate reassembly after the enzyme passes. If an area of DNA damage is encountered, RNA polymerase may become stalled temporarily while the damage is corrected by special proteins.

TERMINATION

Termination of transcription is governed by an assortment of signals that differ for each type of RNA polymerase. For example, transcription by RNA polymerase I is terminated by a protein factor that recognizes an 18-nucleotide termination signal in the growing RNA chain. Termination signals for RNA polymerase III are also known; they always include a short run of U's (as in bacterial termination signals), and no ancillary protein factors are needed for their recognition. Hairpin structures do not appear to be involved in termination by either polymerase I or polymerase III. For RNA polymerase II, transcripts destined to become mRNA are often cleaved at a specific site before transcription is actually terminated. The cleavage site is 10–35 nucleotides downstream from a special AAUAAA sequence in the growing RNA chain. The polymerase may continue transcription for hundreds or even thousands of nucleotides beyond the cleavage site, but this additional RNA is quickly degraded. The cleavage site is also the site for the addition of a poly(A) tail, a string of adenine nucleotides found at the end of almost all eukaryotic mRNAs.

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RNA PROCESSING:

An RNA molecule newly produced by transcription, called a **primary transcript,** frequently must undergo chemical changes before it can function in the cell. We use the term **RNA processing** to mean all the chemical modifications necessary to generate a final RNA product from the primary transcript that serves as its precursor. Processing typically involves removal of portions of the primary transcript, and it may also include the addition or chemical modification of specific nucleotides.

In case of eukaryotes Transcription takes place in the nucleus, whereas translation occurs mainly in the cytoplasm. So, processing is required in the nucleus to convert primary transcripts into mature mRNA molecules that are ready to be transported to the cytoplasm and translated. The primary transcript is also known as **heterogeneous nuclear RNA (hnRNA).** HnRNA consists of a mixture of mRNA molecules and their precursors, **pre-mRNA and introns.** Conversion of pre mRNA molecules into functional mRNAs usually requires the removal of nucleotide sequences and the addition of caps and tails. RNA polymerase II plays a key role in coupling these RNA processing events to transcription

1. 5′ Capping: Most eukaryotic mRNA molecules bear distinctive modifications at both ends. At the 5′ - end, they all possess a modified nucleotide called a 5′-**cap,** and at 3′- the end they usually have a long stretch of adenine ribonucleotides known as a **poly(A) tail.**

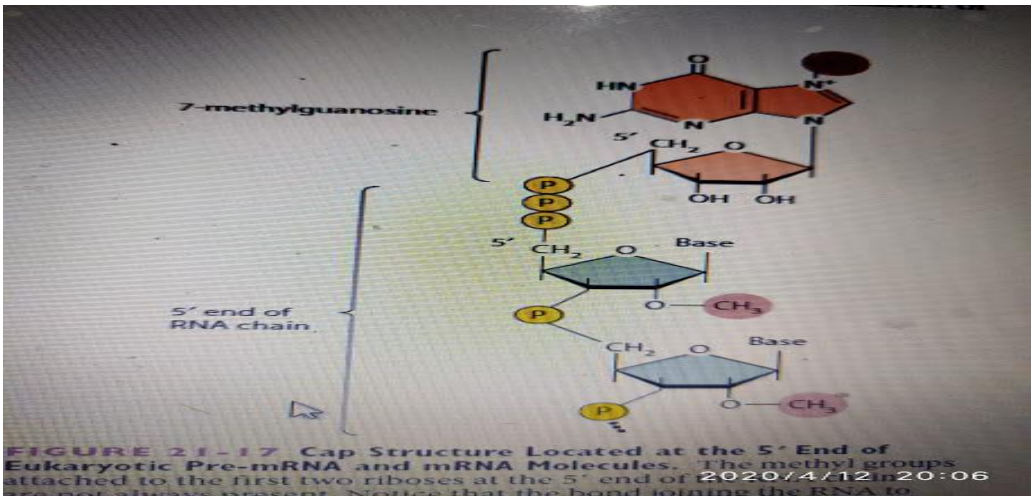

Fig: 5′-**cap structure**

A 5′- cap is simply a guanosine nucleotide that has been methylated at position 7′ of the purine ring and is "backward"—that is, the bond joining it to the 5′- end of the RNA molecule is a 5′→5′ linkage rather than the usual 3′→5′ bond . This distinctive feature of eukaryotic mRNA is added to the primary transcript shortly after initiation of RNA synthesis. As part of the capping process, the ribose rings of the first, and often the second, nucleotides of the RNA chain can also

become methylated. The 5′ cap contributes to mRNA stability by protecting the molecule from degradation by nucleases that attack RNA at the 5′ end. The 5′ cap also plays an important role in positioning mRNA on the ribosome for the initiation of translation.

2. Poly-A tail : A large number of eukaryotic mRNAs possess an adenine nucleotide chain at the 3'-end. This poly-A tail, as such, is not produced during transcription. lt is later added to stabilize mRNA. However, poly-A chain gets reduced as the mRNA enters cytosol. enzyme poly(A) polymerase, which catalyzes the addition of poly(A) sequences to RNA without requiring a DNA template.

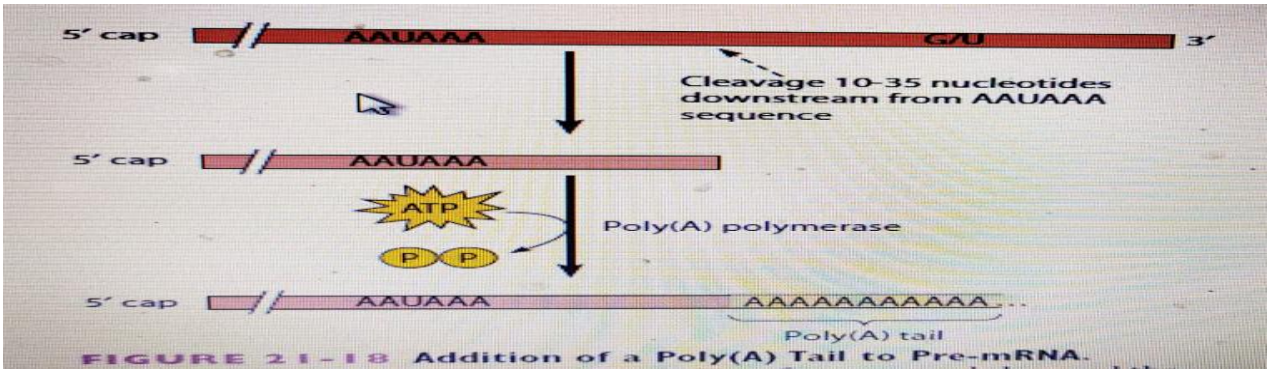

Fig: addition of poly(A) tail

3. Splicing: To produce a functional mRNA from a pre-mRNA that contains introns, eukaryotes must somehow remove the introns and splice together the remaining RNA segments (exons). The entire process of removing introns and rejoining the exons is termed **RNA splicing.**

Analysis of the base sequences of hundreds of different introns has revealed that the end of an intron typically starts with the sequence GU and the end terminates with AG. Intron removal is catalyzed by **spliceosomes,** which are large, molecular complexes consisting of five kinds of RNA combined with more than 200 proteins. Spliceosomes are assembled on pre-mRNAs from a group of smaller RNA-protein complexes called **snRNPs** (small nuclear ribonucleoproteins) and additional proteins. Each snRNP (pronounced “snurp”) contains one or two small molecules of a special type of RNA known as **snRNA** (small nuclear RNA).

Spliceosomes are assembled by the sequential binding of snRNPs to premRNA. This is happen in following ways:

1. **The first step** is the binding of a snRNP called **U1,** whose RNA contains a nucleotide sequence that allows it to base-pair with the splice site.
2. A second snRNP, called **U2,** then binds to the branch-point sequence.
3. Finally, another group of snRNPs (U4/U6 and U5) brings the two ends of the intron together to form a mature spliceosome, a massive complex comparable in size to a ribosome.

4. At this stage the pre-mRNA is cleaved at the splice site, and the newly released end of the intron is covalently joined to an adenine residue located at the branch-point sequence, creating a looped structure called a lariat.
5. The splice site is then cleaved, and the two ends of the exon are joined together, releasing the intron for subsequent degradation.
6. A multiprotein complex called an **exon junction complex (EJC)** is deposited near the boundary of each newly formed exon-exon junction. EJCs are required for the efficient export of mRNA from the nucleus. They also influence various regulatory events, including mRNA localization and translation.

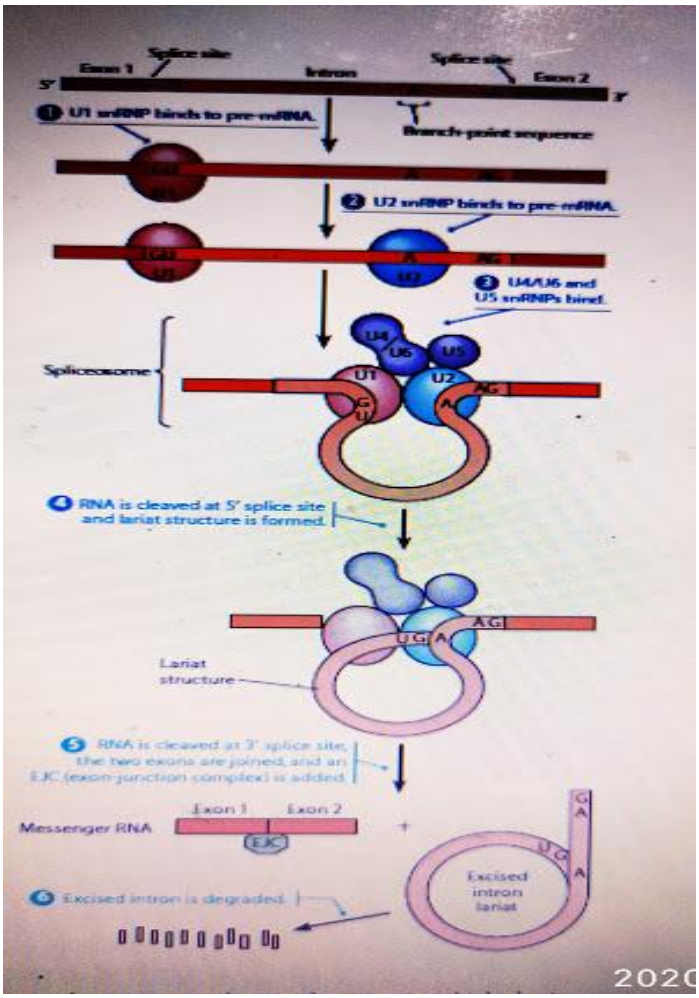

Fig: Splicing Process

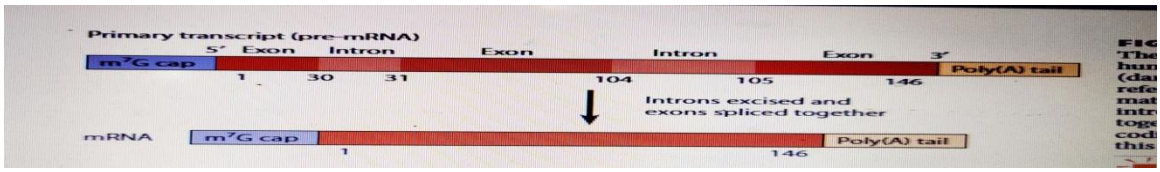

Fig: showing primary trancript before and after RNA processing

Books used as References to prepare the notes:

1. Cell and molecular Biology- concepts and experiments; 8th Ed. ---Geralad Karp
2. Becker's World of the Cell- 8th Ed.
3. Biochemistry –U satynarayan-4 th Ed.

Mitochondrial DNA

Mitochondria have their own genome which is separate from that contained within the cell nucleus. It contain the genes necessary for mitochondrial formation - the mitochondria. It is thought to have evolved separately from nuclear DNA, when bacteria containing circular DNA became part of the precursors to cells that exist today - this is shown by the observation of similarities between mitochondrial and bacterial genomes. Mitochondrial DNA is only a small portion of the DNA in a eukaryotic cell.

Mitochondrial DNA is a double stranded circular molecule, which is inherited from the mother in all multi-cellular organisms, though some recent evidence suggests that in rare instances mitochondria may also be inherited via a paternal route. Typically, a sperm carries mitochondria in its tail as an energy source for its long journey to the egg. When the sperm attaches to the egg during fertilization, the tail falls off. Consequently, the only mitochondria the new organism usually gets are from the egg its mother provided. There are about 2 to 10 transcripts of the mt-DNA in each mitochondrion.

Compared to chromosomes, it is relatively smaller, and contains the genes in a limited number. It is free of the chromosomal proteins. An exception is found in some ciliated protozoans, in which the DNA is linear. In size, mtDNA is much smaller than cpDNA and varies greatly among organisms,. In a variety of animals, including humans, mtDNA consists of about 16,000 to 18,000 bp (16 to 18 kb). However, yeast (*Saccharomyces*) mtDNA consists of 75 kb. Vertebrates have 5 to 10 such DNA molecules per organelle, while plants have 20 to 40 copies per organelle. With only rare exceptions, introns are absent from mitochondrial genes, and gene repetitions are seldom present.

Mitochondrial DNA consists of 5-10 rings of DNA and appears to carry 16,569 base pairs with 37 genes (13 proteins, 22 t-RNAs and two r-RNA) which are concerned with the production of proteins involved in respiration. Out of the 37 genes, 13 are responsible for making enzymes, involved in oxidative phosphorylation, a process that uses oxygen and sugar to produce adenosine tri-phosphate .The other 14 genes are responsible for making molecules, called transfer RNA (t-RNA) and ribosomal RNA (r-RNA). In some metazoans, there are about 100 – 10,000 separate copies of mt-DNA present in each cell.

As mitochondria have their own DNA, transcription and translation takes place for the synthesis of relatively small set of polypeptides mainly focused in ATP production.

Mitochondria use slightly a different genetic code. Most of the genes for mitochondrial proteins are present in nuclear DNA, translated in cytosol and consequently to mitochondria.

The limited genetic function of mtDNA is well-conserved across eukaryotes: mtDNA encodes a small number of proteins whose mRNAs are translated by a distinctive mitochondrial protein-synthesizing system, some of whose components are always (rRNAs), usually (tRNAs) or occasionally (ribosomal proteins) specified by the mitochondrial genome. Unlike nuclear DNA, mitochondrial DNA doesn't get shuffled every generation, so it is presumed to change at a slower rate, which is useful for the study of human evolution. Mitochondrial DNA is also used in forensic science as a tool for identifying corpses or body parts and has been implicated in a number of genetic diseases, such as Alzheimer's disease and diabetes. Changes in mt-DNA can cause maternally inherited diseases, which leads to faster aging process and genetic disorders.

The expression of mitochondrial genes uses several modifications of the standard genetic code. The replication in mitochondria is dependent on enzymes encoded by nuclear DNA. Mitochondrial-encoded gene products are present in all of the protein complexes of the electron transport chain found in the inner membrane of mitochondria. In most cases, these polypeptides are part of multichain proteins, many of which also contain subunits that were encoded in the nucleus, synthesized in the cytoplasm, and then transported into the organelle. Thus, the protein-synthesizing apparatus and the molecular components for cellular respiration are jointly derived from nuclear and mitochondrial genes. Another interesting observation is that in vertebrate mtDNA, the two strands vary in density, as revealed by centrifugation. This provides researchers with a way to isolate the strands for study, designating one heavy (H) and the other light (L).While most of the mitochondrial genes are encoded by the H strand, several are encoded by the complementary L strand.

The majority of proteins that function in mitochondria are encoded by nuclear genes. In fact, over 1000 nuclear-coded gene products are essential to biological activity in the organelle. They include, for example, DNA and RNA polymerases, initiation and elongation factors essential for translation, ribosomal proteins, aminoacyl tRNA synthetases, and several tRNA species. These imported components are distinct from their cytoplasmic counterparts, even though both sets are coded by nuclear genes.

RECOMBINATION

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Recombination

Recombination is the process in which one or more nucleic acids molecules are rearranged or combined to produce a new nucleotide sequence. Usually genetic material from two parents is combined to produce a recombinant chromosome
with a new, different genotype. Recombination results in a new arrangement of genes or parts of genes and normally is accompanied by a phenotypic change.

Micro-organisms carry out several types of recombination. The common are - **General recombination**,**site-specific recombination and Replicative recombination**

General recombination:- It is the most common form. It usually involves a reciprocal exchange between a pair of homologous DNA sequences. It can occur any place on the chromosome, and it results from DNA strand breakage and reunion leading to crossing-over. General recombination is carried out by the products of *rec* genes (Recombinant gene) such as the recA protein so important for DNA repair (*see pp. 254–55*). In bacterial transformation a nonreciprocal form of general recombination takes place
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1). General recombination (cont'd):
E.g., in bacterial transformation: **Nonreciprocal General Recombination**

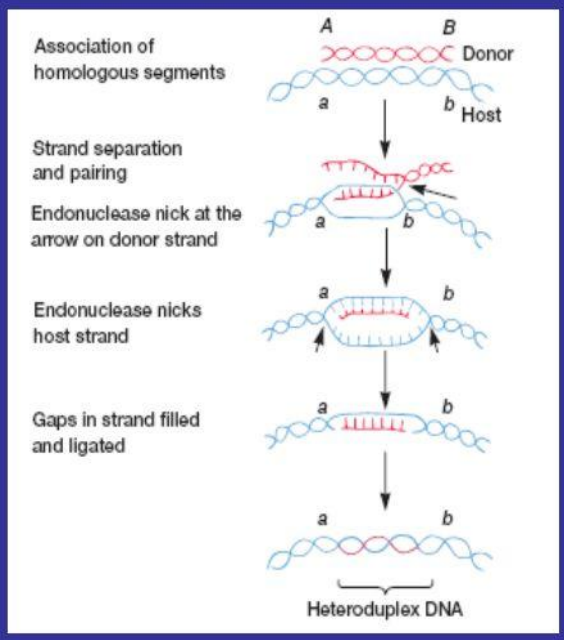

Figure 13.3 Nonreciprocal General Recombination. The Fox model for nonreciprocal general recombination. This mechanism has been proposed for the recombination occurring during transformation in some bacteria.

A piece of genetic material is inserted into the chromosome through the incorporation of a single strand to form a stretch of **heteroduplex DNA.**

Site-specific recombination:- This type of recombination is particularly important in the integration of virus genomes into bacterial chromosomes. The genetic material is not homologous with the chromosome it joins, and generally
the enzymes responsible for this event are specific for the particular virus and its host.

Replicative recombination :- It accompanies the replication of genetic material and does not depend on sequence homology. It is used by some genetic elements that move about the chromosome.

Although sexual reproduction with the formation of a zygote and subsequent meiosis is not present in bacteria, recombination can take place in several ways following **horizontal gene transfer.** It is a process in which genes are transferred from one independent, mature organism to another. In this process, a piece of donor DNA (**exogenote)** must enter the recipient cell and become a stable part of the recipient cell's genome (**endogenote)**. Sometimes the DNA exists in a form that cannot be degraded by the recipient cell's endonucleases. In this case the DNA does not need to be integrated into the host genome but must only enter the recipient to confer its genetic information on the cell. Most linear DNA fragments are not stably maintained unless they have been integrated into the bacterial
genome.

Movement of DNA from a donor bacterium to the recipient can take place in three ways:

i). Direct transfer between two bacteria temporarily in physical contact (conjugation),

ii). Transfer of a naked DNA fragment (transformation), and

iii). Transport of bacterial DNA by bacteriophages (transduction).

Whatever the mode of transfer, the exogenote has only four possible fates in the recipient :-

First, when the exogenote has a sequence homologous to that of the endogenote, integration may occur; that is, it may pair with the recipient DNA and be incorporated to yield a recombinant genome.

Second, the foreign DNA sometimes sometimes persists outside the endogenote and replicates to produce a clone of partially diploid cells.

Third, the exogenote may survive, but not replicate, so that only one cell is a partial diploid.

Finally, host cell nucleases may degrade the exogenote, a process called **host restriction.**

TRANSDUCTION

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Transduction

Transduction is a type bacterial gene transfer. Bacterial viruses or bacteriophages participate in this process. These viruses have relatively simple structures in which virus genetic material is enclosed within an outer coat, composed mainly of protein. The coat protects the genome and transmits it between host cells.

For reproductive purposes bacteriophages infect bacteria. After infecting the host cell, a bacteriophage often takes control and forces the host to make many copies of the virus. Eventually the host bacterium bursts and releases new phages. This reproductive cycle is called a lytic cycle because it ends in lysis of the host. The cycle has four phases :-

First, the virus particle attaches to a specific receptor site on the bacterial surface. The genetic material, which is often double-stranded DNA, then enters the cell.

Secondly, after adsorption and penetration, the virus chromosome forces the bacterium to make virus nucleic acids and proteins.

The third stage begins after the synthesis of virus components. Phages are assembled from these components. The assembly process may be complex, but in all cases phage nucleic acid is packed within the virus's protein coat.

Finally, the mature viruses are released by cell lysis. Bacterial viruses that reproduce using a lytic cycle often are called virulent bacteriophages because they destroy the host cell.

Many DNA phages, such as the lambda phage (*see p. 391*), are also capable of a different relationship with their host. After adsorption and penetration, the viral genome does not take control of its host and destroy it while producing new phages. Instead the genome remains within the host cell and is reproduced along with the bacterial chromosome. A clone of infected cells arises and may grow for long periods while appearing perfectly normal. Each of these infected bacteria can produce phages and lyse under appropriate environmental conditions. This relationship between the phage and its host is called **lysogeny.**

Bacteria that can produce phage particles under some conditions are said to be **lysogens** or **lysogenic,** and phages able to establish this relationship are **temperate phages.** The latent form of the virus genome that remains within the host without destroying it is called the **prophage.** The prophage usually is integrated into the bacterial genome. Sometimes phage reproduction is triggered in a lysogenized culture by exposure to UV radiation or other factors. The lysogens are then destroyed and new phages released. This phenomenon is called induction.

Transduction is the transfer of bacterial genes by viruses. Bacterial genes are incorporated into a phage capsid because of errors made during the virus life cycle. The virus containing these genes then injects them into another bacterium, completing the transfer. Transduction may be the most common mechanism for

gene exchange and recombination in bacteria. There are two very different kinds of transduction: **Generalized and Specialized.**

<u>Generalized Transduction</u>:- Generalized transduction occurs during the lytic cycle of virulent and temperate phages and can transfer any part of the bacterial genome. During the assembly stage, when the viral chromosomes are packaged into protein capsids, random fragments of the partially degraded bacterial chromosome also may be packaged by mistake. Because the capsid can contain only a limited quantity of DNA, the viral DNA is left behind. The quantity of bacterial DNA carried depends primarily on the size of the capsid The resulting virus particle often injects the DNA into another bacterial cell but does not initiate a lytic cycle. This phage is known as a **generalized transducing particle** or phage and is simply a carrier of genetic information from the original bacterium to another cell.

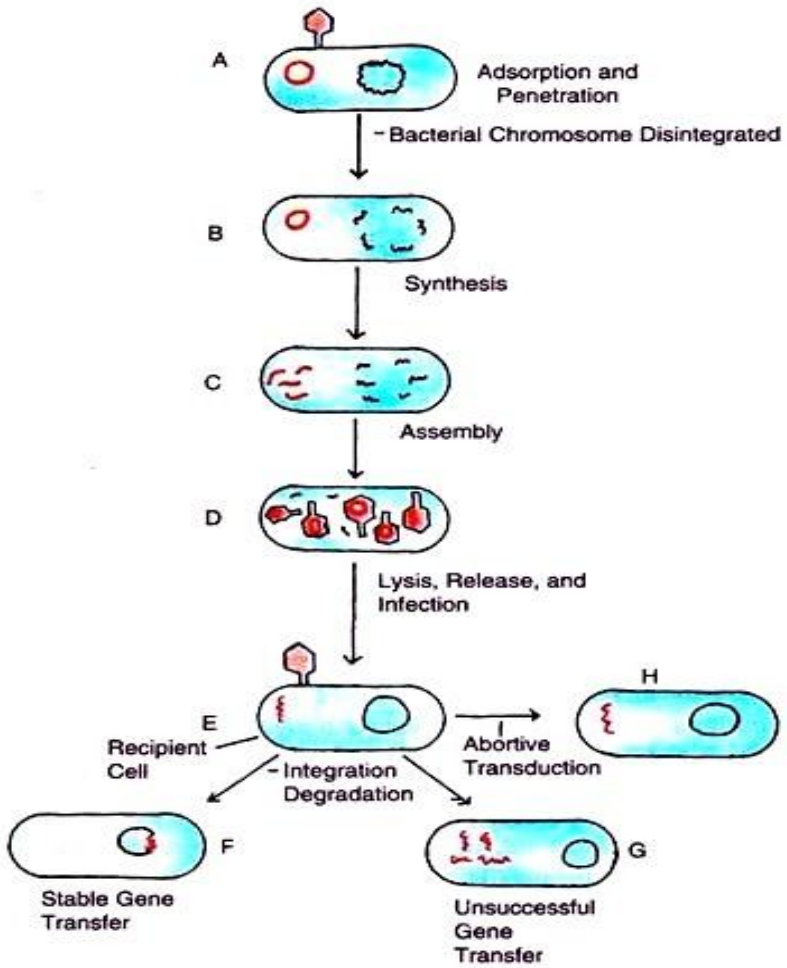
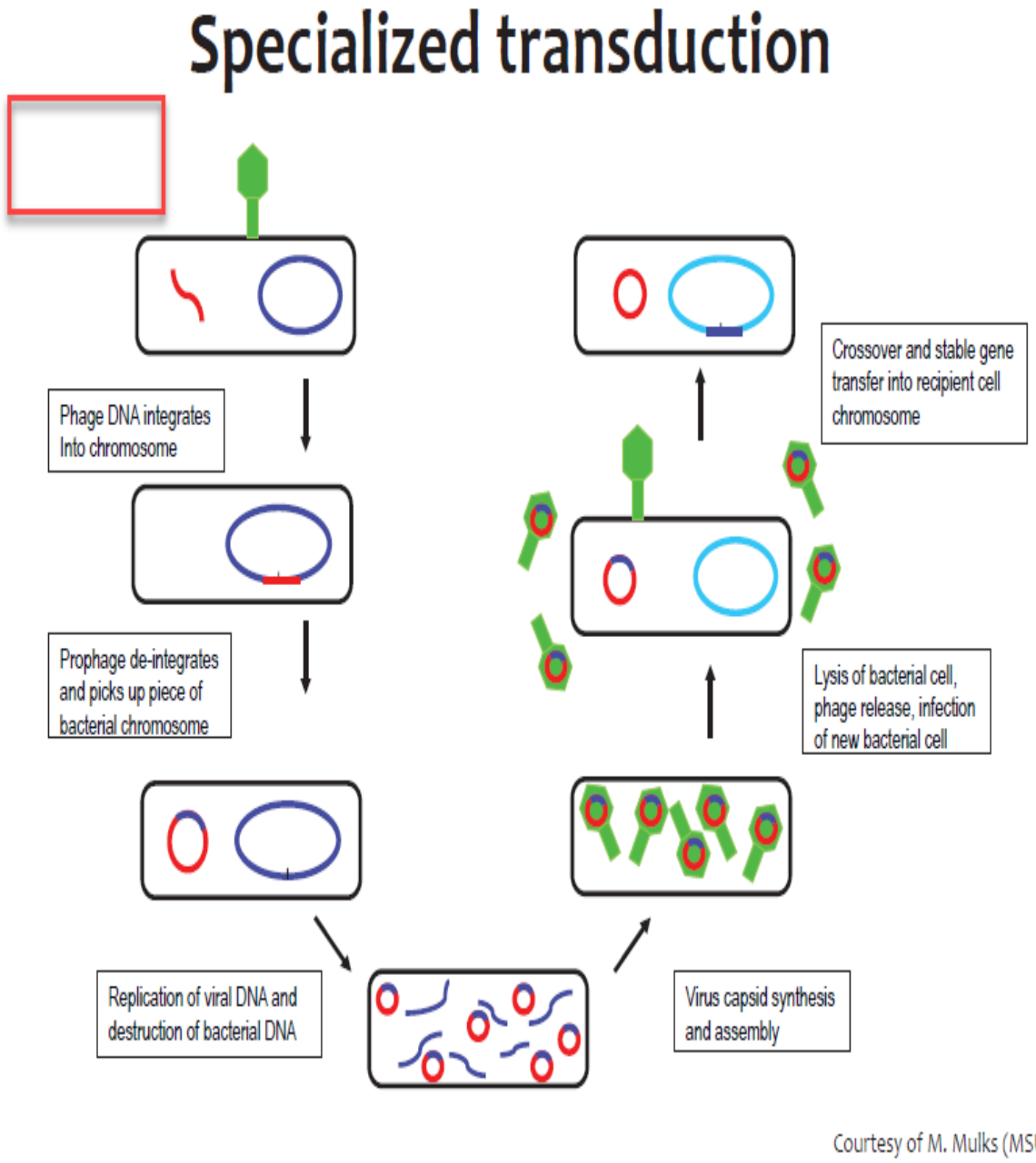


Fig. 8.18 : Outline of generalised transduction by bacteriophages.

<u>Specialized Transduction :-</u> In **specialized** or **restricted transduction,** the transducing particle carries only specific portions of the bacterial genome. Specialized transduction is made possible by an error in the lysogenic life cycle. When a prophage is induced to leave the host chromosome, excision is sometimes carried out improperly. The resulting phage genome contains portions of the bacterial chromosome (about 5 to 10% of the bacterial DNA) next to the integration site. A transducing phage genome usually is defective and lacks some part of its attachment site. The transducing particle will inject bacterial genes into another bacterium, even though the defective phage cannot reproduce without assistance.



DNA - TRANSCRIPTION - IN PROKARYOTES

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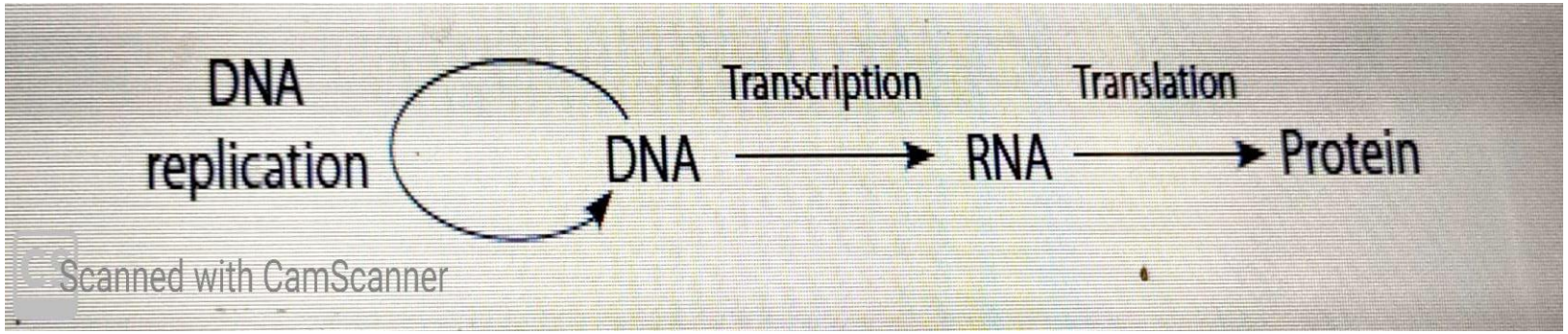
THE DIRECTION OF FLOW OF GENETIC INFORMATION

The flow of genetic information involves replication of DNA, transcription of information carried by DNA into the form of RNA, and translation of this information from RNA into protein.



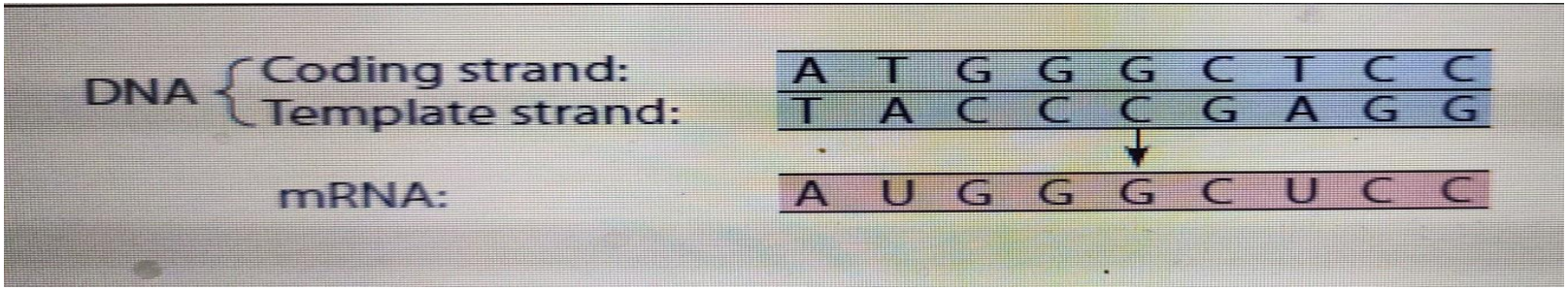
CENTRAL DOGMA OF MOLECULAR BIOLOGY

The principle of directional information flow from DNA to RNA to protein is known as the central dogma of molecular biology, a term coined by Francis Crick soon after the double-helical model of DNA was first proposed.

WHAT IS TRANSCRIPTION ?

Transcription is a process in which a DNA strand provides the information for the synthesis of an RNA strand.

DNA (more precisely, a segment of one DNA strand) first serves as a template for the synthesis of an RNA molecule, which in most cases then directs the synthesis of a particular protein.



3′ — G G C C — 3′
3′ — C C G G — 5′
↓
5′ — G G C C — 3′

5′ — T G G C A — 3′
3 — A C C G T — 5′
↓
5′ — U C C C A → 3′

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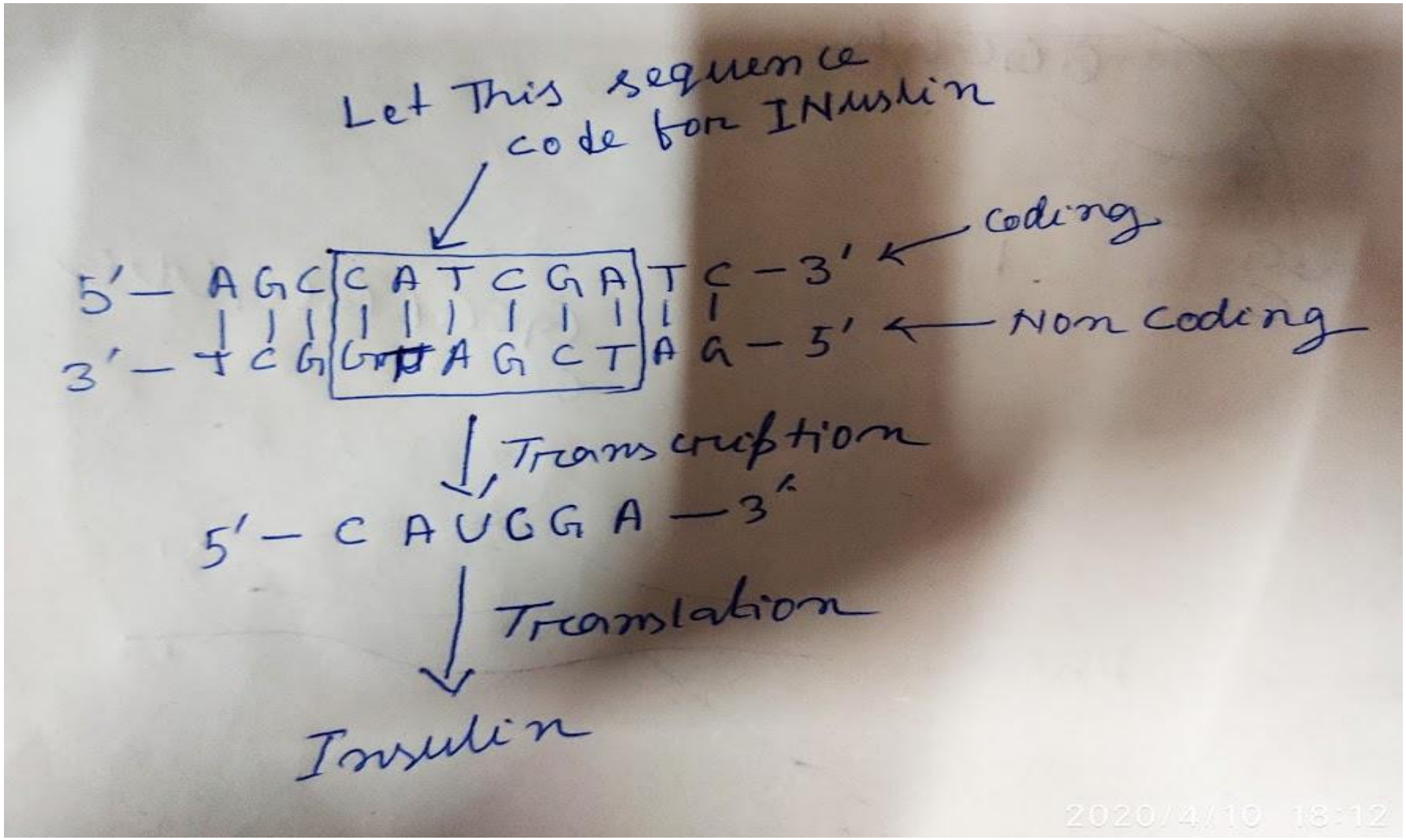
WHAT HAPPEN IN TRANSCRIPTION ?

WHAT TYPE OF INFORMATION PROVIDED BY DNA? ????



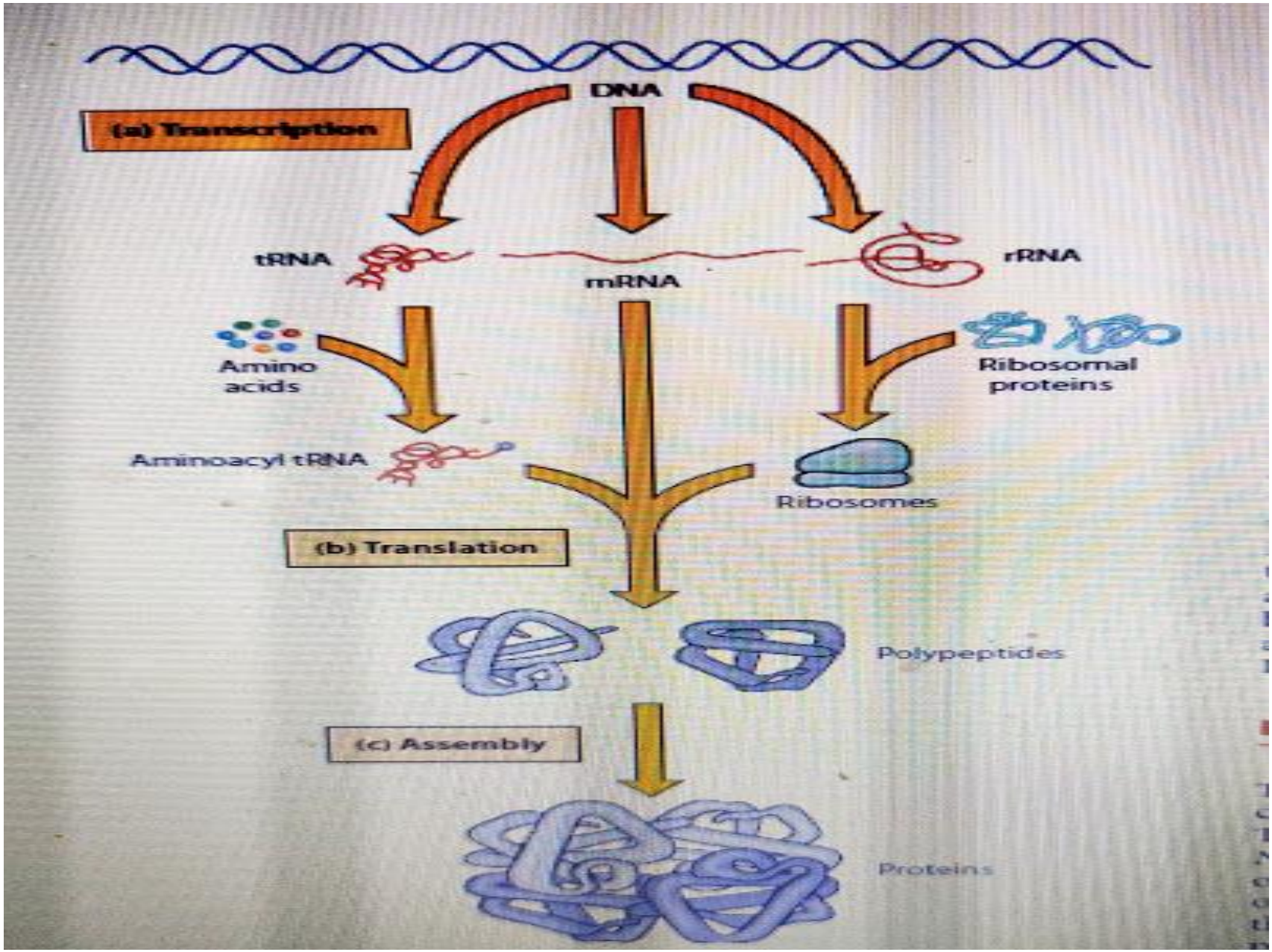
IN THE WHOLE PROCESS OF PROTEIN SYNTHESIS 3 TYPES OF RNAs ARE INVOLVED-

1. <u>mRNAs</u>: RNA that is translated into protein is called messenger RNA (mRNA) because it carries a genetic message from DNA to the ribosomes, where protein synthesis actually takes place. It is 5% of total RNAs.

2. <u>rRNAs</u> : The RNAs of a ribosome are called ribosomal RNAs (or rRNAs). rRNAs provide structural support and catalyze the chemical reaction in which amino acids are covalently linked to one another. 80% of total RNAs.

3.<u>tRNAs:</u> tRNAs are required to translate the information in the mRNA nucleotide code into the amino acid "alphabet" of a polypeptide.

Note that ribosomal and transfer RNAs do not themselves code for proteins; genes coding for these two types of RNA are examples of genes whose final products are RNA molecules rather than protein chains.


DNA
(a) Transcription
tRNA
mRNA
rRNA
Amino acids
Ribosomal proteins
Aminoacyl tRNA
Ribosomes
(b) Translation
Polypeptides
(c) Assembly
Proteins

WHO CARRIED OUT THE TRANSCRIPTION ???

Transcription of DNA is carried out by the enzyme RNA polymerase, which catalyzes the synthesis of RNA using DNA as a template. Bacterial cells have a single kind of RNA polymerase that synthesizes all three major classes ofRNA—mRNA, tRNA, and rRNA.

RNA polymerase is a large protein consisting of two α subunits, two β subunits that differ enough to be identified as β and β' , and a dissociable subunit called the **sigma σ factor.** **The sigma subunit plays a critical role in this process by promoting the binding of RNA polymerase to specific DNA sequences, called promoters, found at the beginnings of genes.**

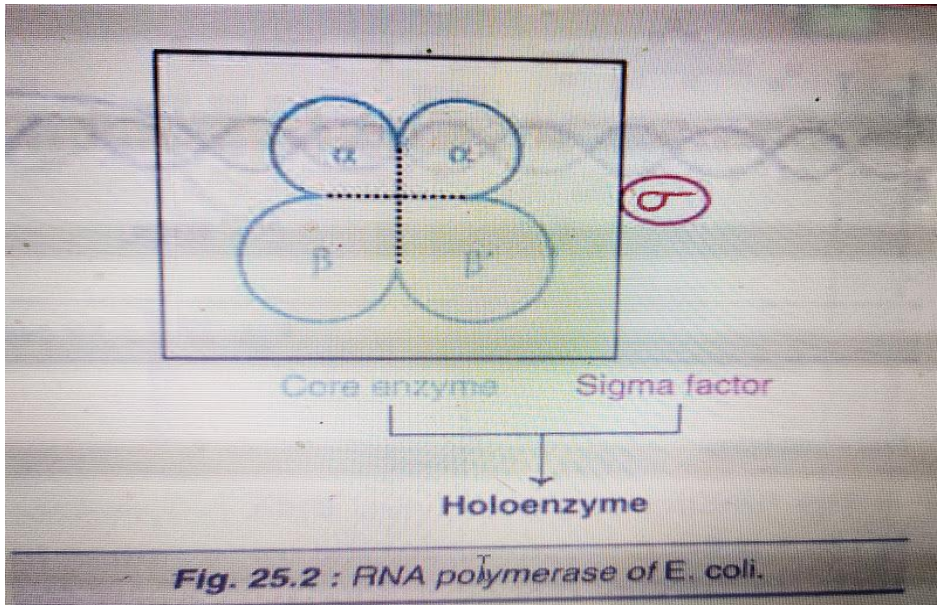


Fig. 25.2 : RNA polymerase of E. coli.

TRANSCRIPTION TAKES PLACE IN FOUR STEPS –

1. BINDING
2. INITIATION
3. ELONGATION
4. TERMINATION

HOW RNA-*pol* CARRIED OUT TRANSCRIPTION?

FIRST STEP IS THE - Binding of RNA Polymerase to a Promoter Sequence. Promoter site is a specific sequence of several dozen base pairs that determines where RNA synthesis starts and which DNA strand is to serve as the template strand.

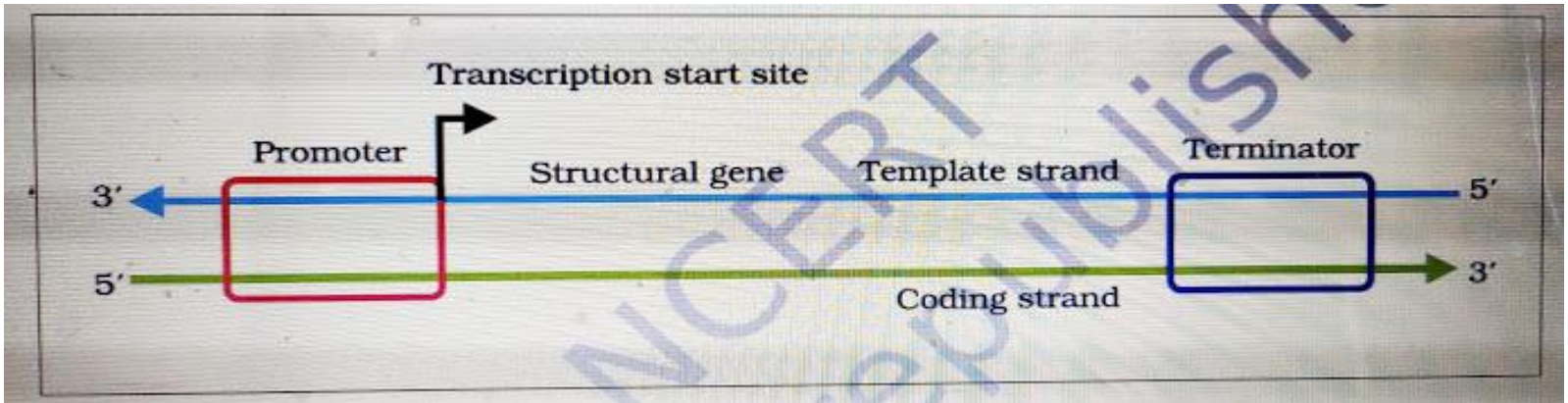


Figure 6.9 Schematic structure of a transcription unit

The terms **upstream and downstream** are used to refer to DNA sequences located toward the 5’ 3’ end of the coding strand, respectively

The point where transcription will begin, called the startpoint, is almost always a purine and often an adenine. Approximately 10 bases upstream of the startpoint is the six-nucleotide sequence TATAAT, called the -10 sequence or the Pribnow box, after its discoverer. By convention, the nucleotides are numbered from the startpoint (+1), with positive numbers to the right (downstream) and negative ones to the left (upstream). The -1 nucleotide is immediately upstream of the startpoint (there is no "0"). At or near the -35 position is the 6 nucleotide sequence TTGACA, called the –35 sequence.

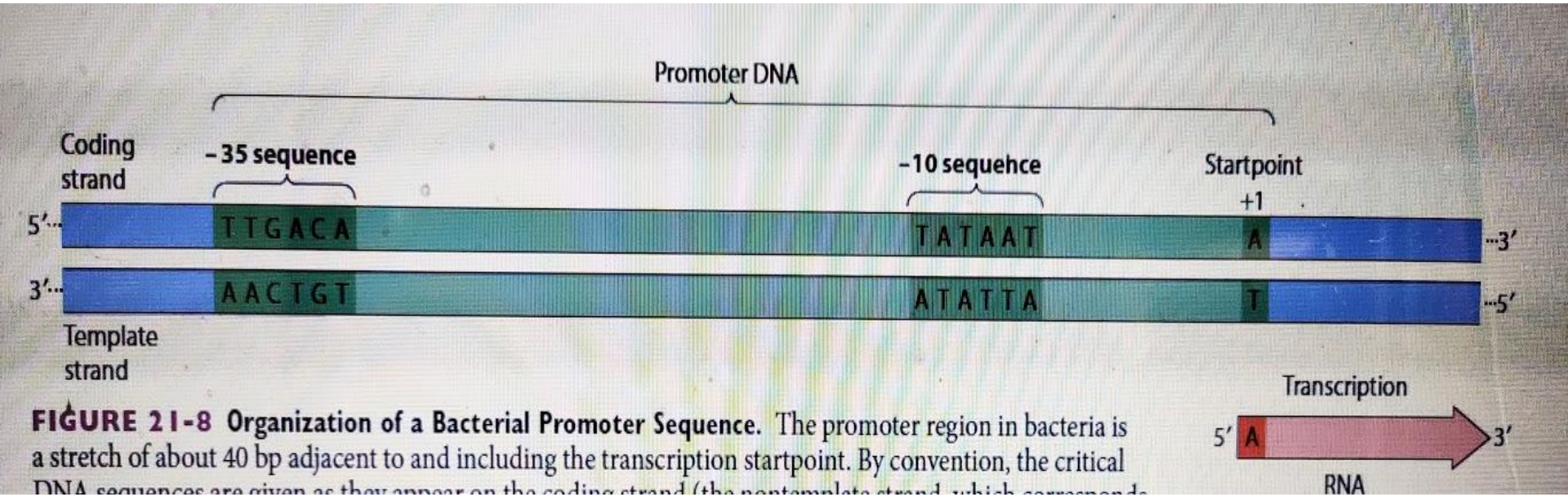
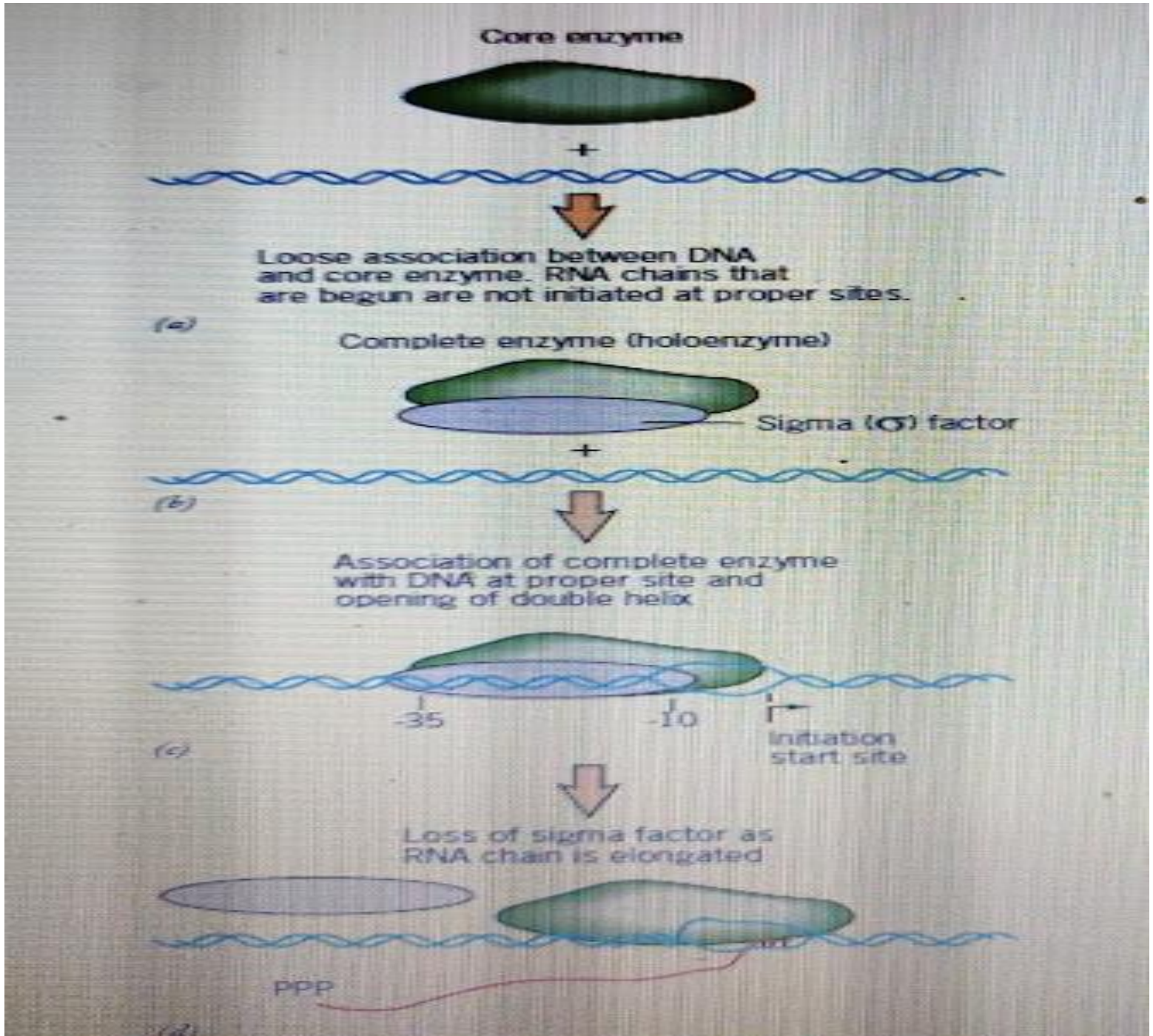
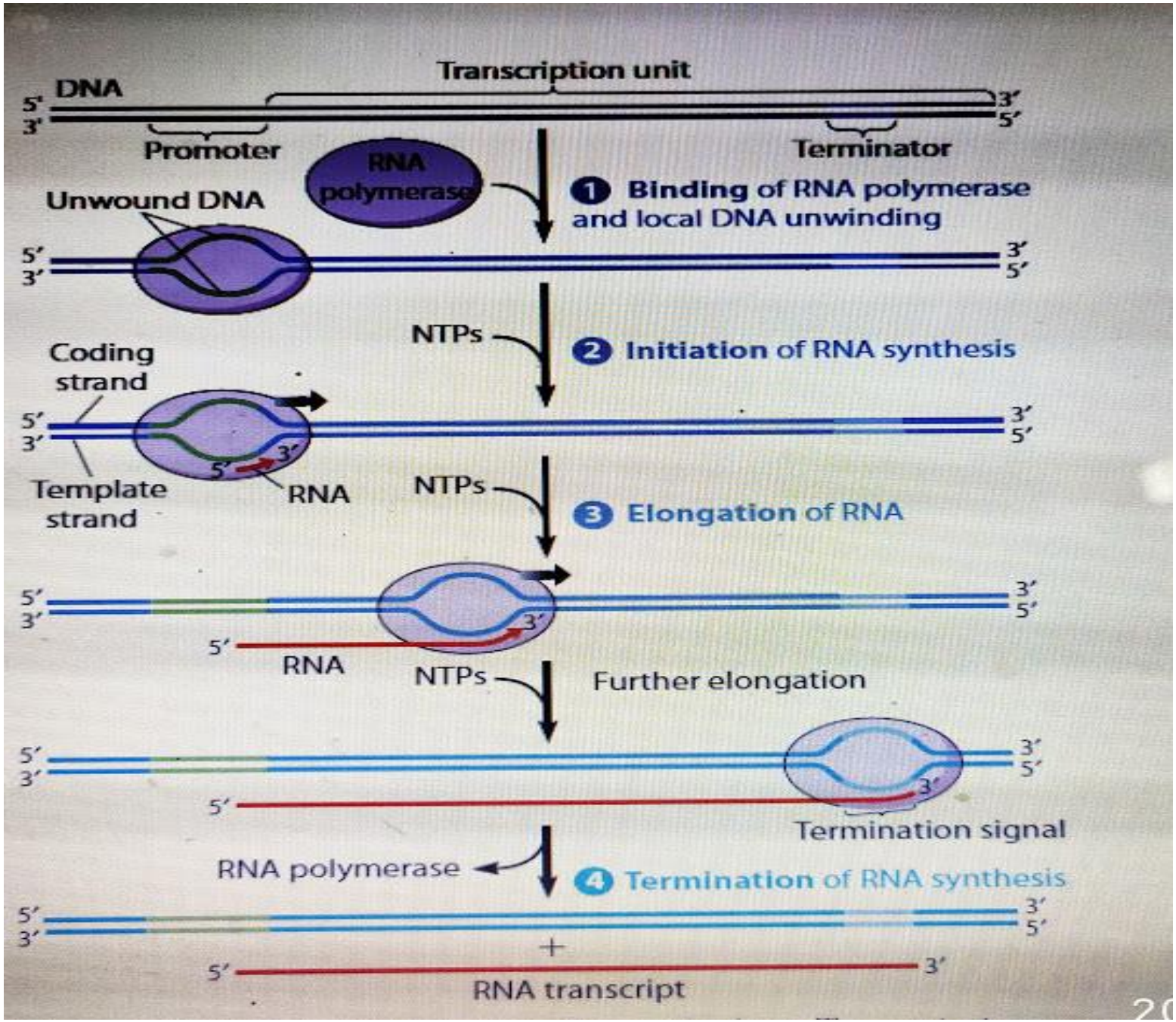
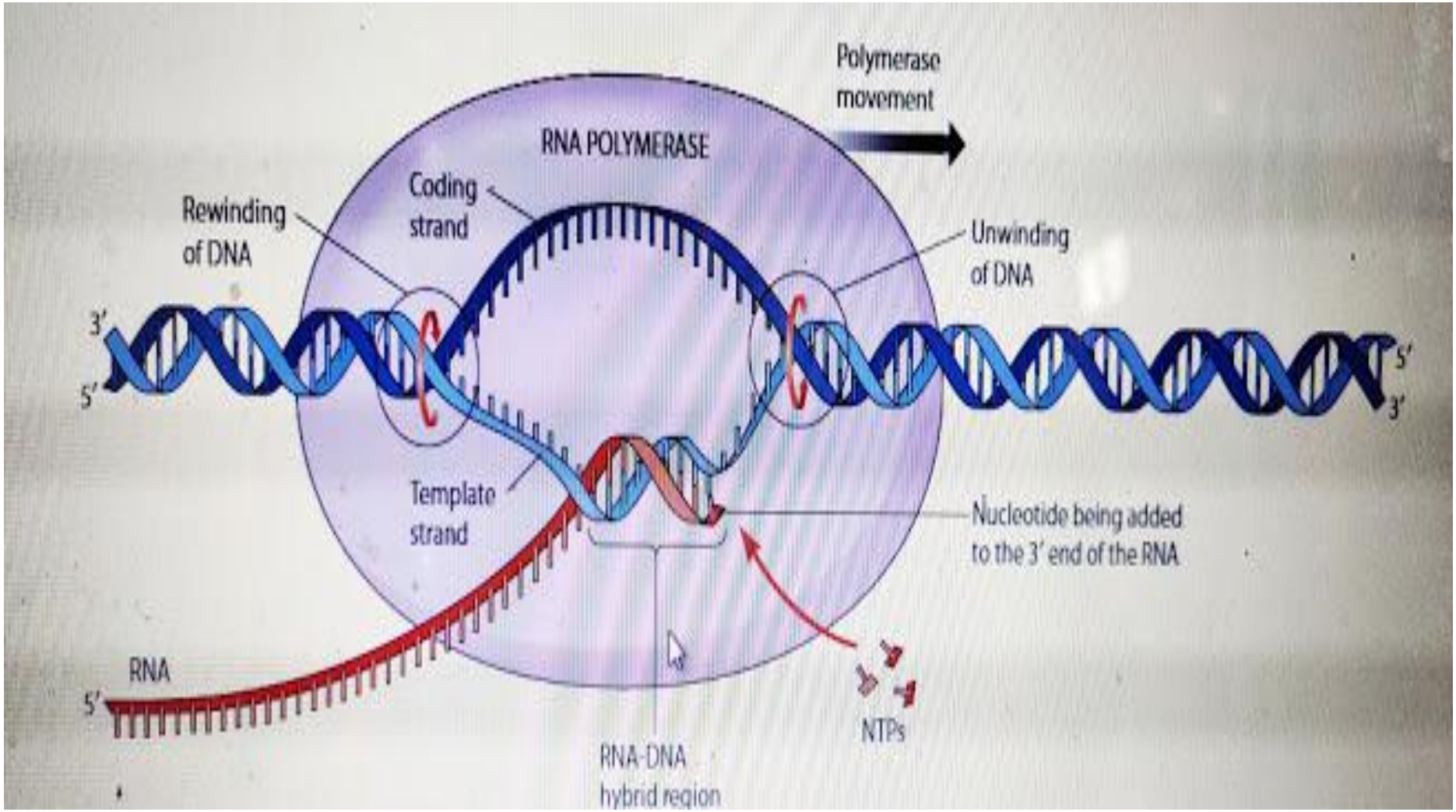


FIGURE 21-8 Organization of a Bacterial Promoter Sequence. The promoter region in bacteria is a stretch of about 40 bp adjacent to and including the transcription startpoint. By convention, the critical DNA sequences are given as they appear on the coding strand (the nontemplate strand, which corresponds


Core enzyme
+
Loose association between DNA and core enzyme. RNA chains that are begun are not initiated at proper sites.
(a)
Complete enzyme (holoenzyme)
Sigma (σ) factor
+
(b)
Association of complete enzyme with DNA at proper site and opening of double helix
-35
-10
Initiation start site
(c)
Loss of sigma factor as RNA chain is elongated
PPP
(d)

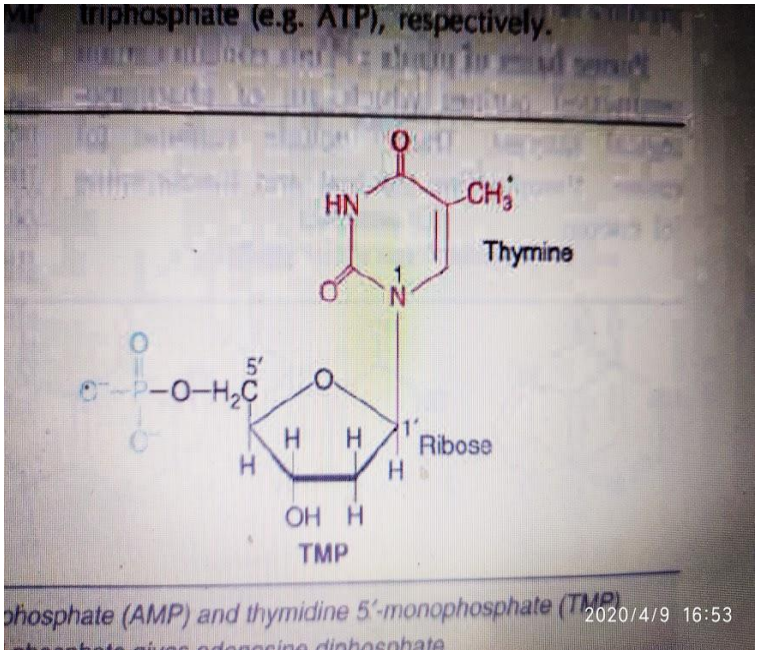

Transcription unit
DNA
5′
3′
3′
5′
Promoter
Terminator
RNA polymerase
Unwound DNA
1 Binding of RNA polymerase and local DNA unwinding
NTPs
2 Initiation of RNA synthesis
Coding strand
Template strand
RNA
NTPs
3 Elongation of RNA
RNA
NTPs
Further elongation
Termination signal
RNA polymerase
4 Termination of RNA synthesis
+
RNA transcript


Polymerase movement
RNA POLYMERASE
Coding strand
Rewinding of DNA
Unwinding of DNA
3'
5'
5'
3'
Template strand
Nucleotide being added to the 3' end of the RNA
RNA
5'
NTPs
RNA-DNA hybrid region

2. INITIATION:

1. **Once an RNA polymerase molecule has bound to a promoter site and locally unwound the DNA double helix, initiation of RNA synthesis can take place.**
2. **Template DNA strand uses NTPs (incoming Ribonucleotide triphosphates)**
3. **The first two incoming NTPs are hydrogen-bonded to the complementary bases of the DNA template strand at the start point**
4. **Then RNA polymerase catalyzes the formation of a phosphodiester bond between the 3'-hydroxyl group of the first NTP and the 5' - phosphate 5 of the second nucleotide accompanied by the release of pyrophosphate (PPi).**

$$\text{RNA}n + NTP \longrightarrow RNA_{n+1} + PPi$$


triphosphate (e.g. ATP), respectively.
Thymine
Ribose
TMP
phosphate (AMP) and thymidine 5′-monophosphate (TMP)
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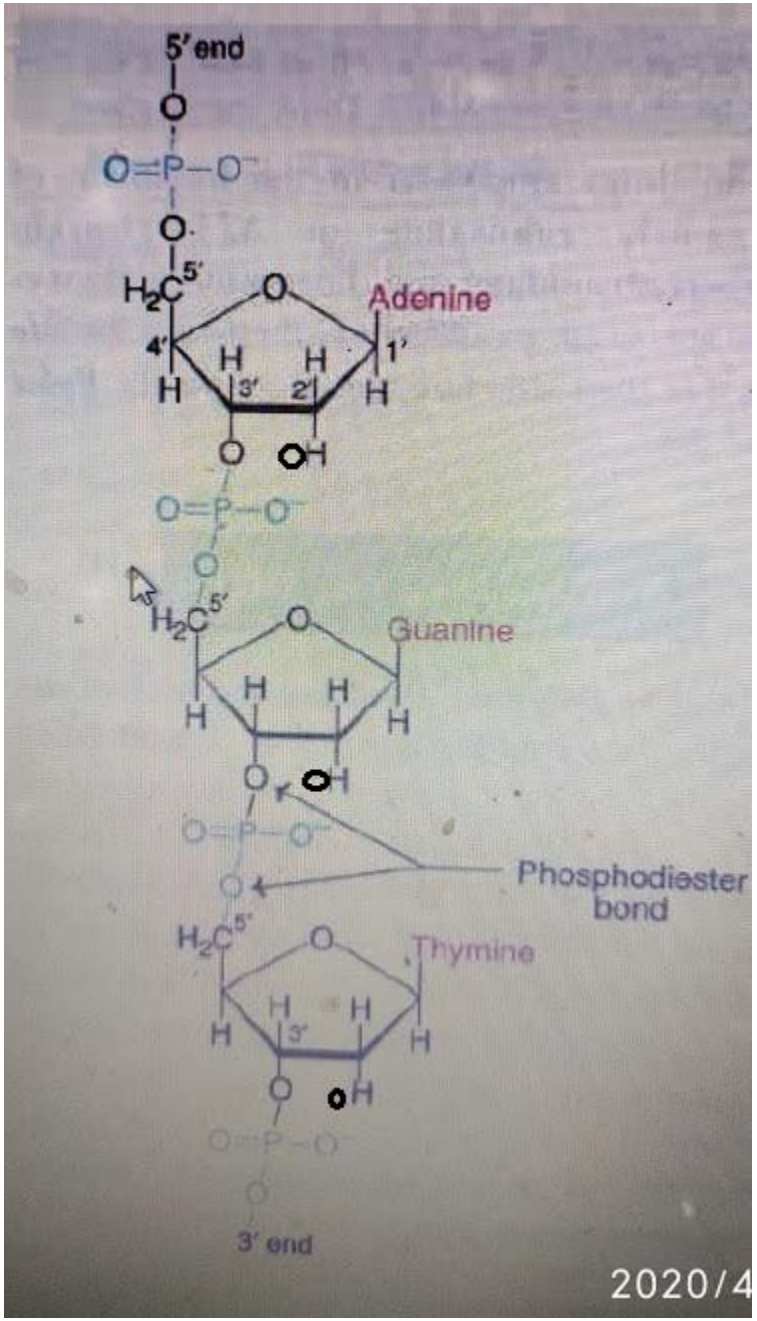
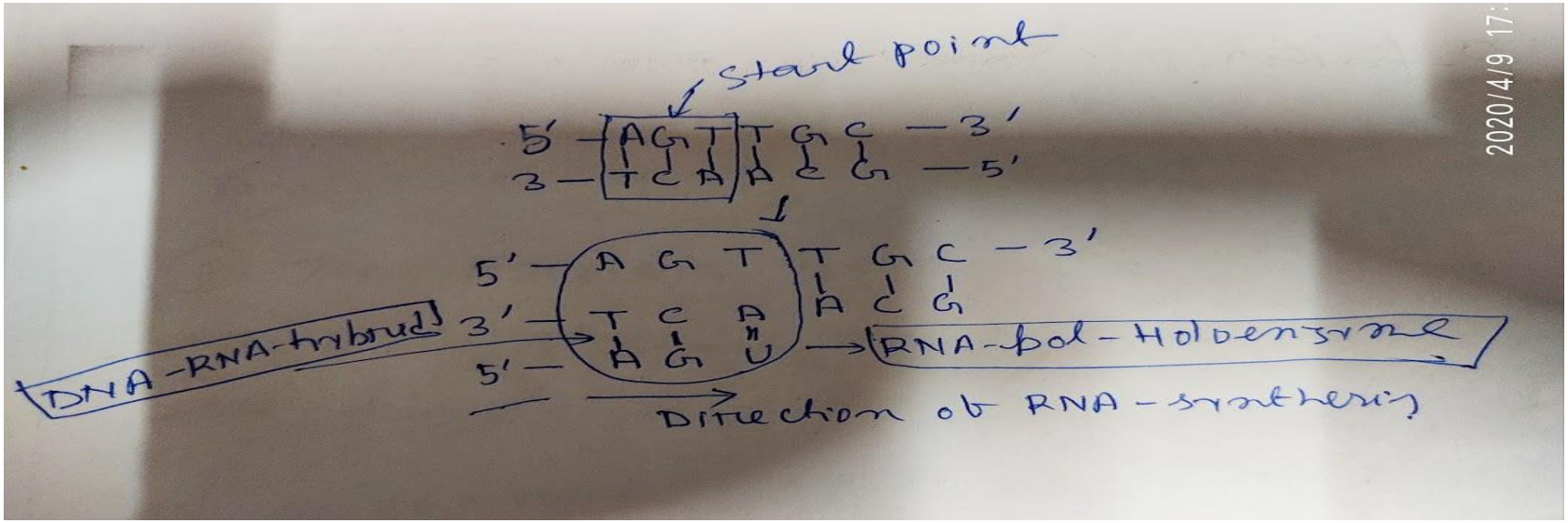

5′ end
Adenine
Guanine
Phosphodiester bond
Thymine
3′ end
2020/4

5. The polymerase then advances along the template strand as additional nucleotides are added one by one, the 5’-phosphate of each new nucleotide joining to the 3’ -hydroxyl group of the growing RNA chain, until the chain is about nine nucleotides long. This form a DNA-RNA hybrid.



6**. At this point the sigma factor generally detaches from the RNA polymerase molecule, and the initiation stage is complete.**

ELONGATION:

1. Chain elongation continues as RNA polymerase moves along the DNA molecule, untwisting the helix bit by bit and adding one complementary nucleotide at a time to the growing RNA chain.
2. The enzyme moves along the template DNA strand from the 3' toward the 5' end. Because complementary base pairing between the DNA template strand and the newly forming RNA chain is antiparallel, the RNA strand is elongated in the 5' → 3'. direction as each successive nucleotide is added to the end of the growing chain.
3. As the RNA chain grows, the most recently added nucleotides remain base-paired with the DNA template strand, forming a short RNA–DNA hybrid about 8–9 bp long.

5. As the polymerase moves forward, the DNA ahead of the enzyme is unwound to permit the RNA–DNA hybrid to form. At the same time, the DNA behind the moving enzyme is rewound into a double helix. The supercoiling that would otherwise be generated by this unwinding and rewinding is released through the action of topoisomerases, just as in DNA replication

PROOF READING

RNA polymerases possess a exonuclease activity that allow improperly base-paired nucleotides to be removed from the3’ end of a growing RNA. However, this intrinsic exonuclease activity is relatively weak, and an alternative mechanism for correcting errors is used instead. When a noncomplementary nucleotide is incorporated into a growing RNA chain by mistake, the RNA polymerase backs up slightly, and the noncomplementary nucleotide participates in catalyzing its own removal along with removal of the previously incorporated nucleotide. Such RNA proofreading appears to be sufficient for correcting mistakes that arise during transcription,

Termination of RNA Synthesis.

The process of transcription stops by terminations ignalsT. wo typeso f terminationa re identified.

1. **Rho(p) dependentent termination**: A specific protein, named p factor, binds to the growing RNA(andn ot to RNAp olymeraseo)r weakly to DNA, and in the bound state it acts as ATPase and terminatetsr anscriptioann d releaseRs NA. The p (rho) factor is also responsible for the dissociatioonf RNA polymerasfero m DNA.

2. **Rho(p) independentent termination: Since GC base pairs are held together by three** hydrogen bonds, whereas AU base pairs are joined by only two hydrogen bonds, this configuration promotes termination in the following way: First, the GC region contains sequences that are complementary to each other, causing the RNA to spontaneously fold into a **hairpin loop that** tends to pull the RNA molecule away from the DNA. Then the weaker bonds between the sequence of U residues and the DNA template are broken, releasing the newly formed RNA molecule.

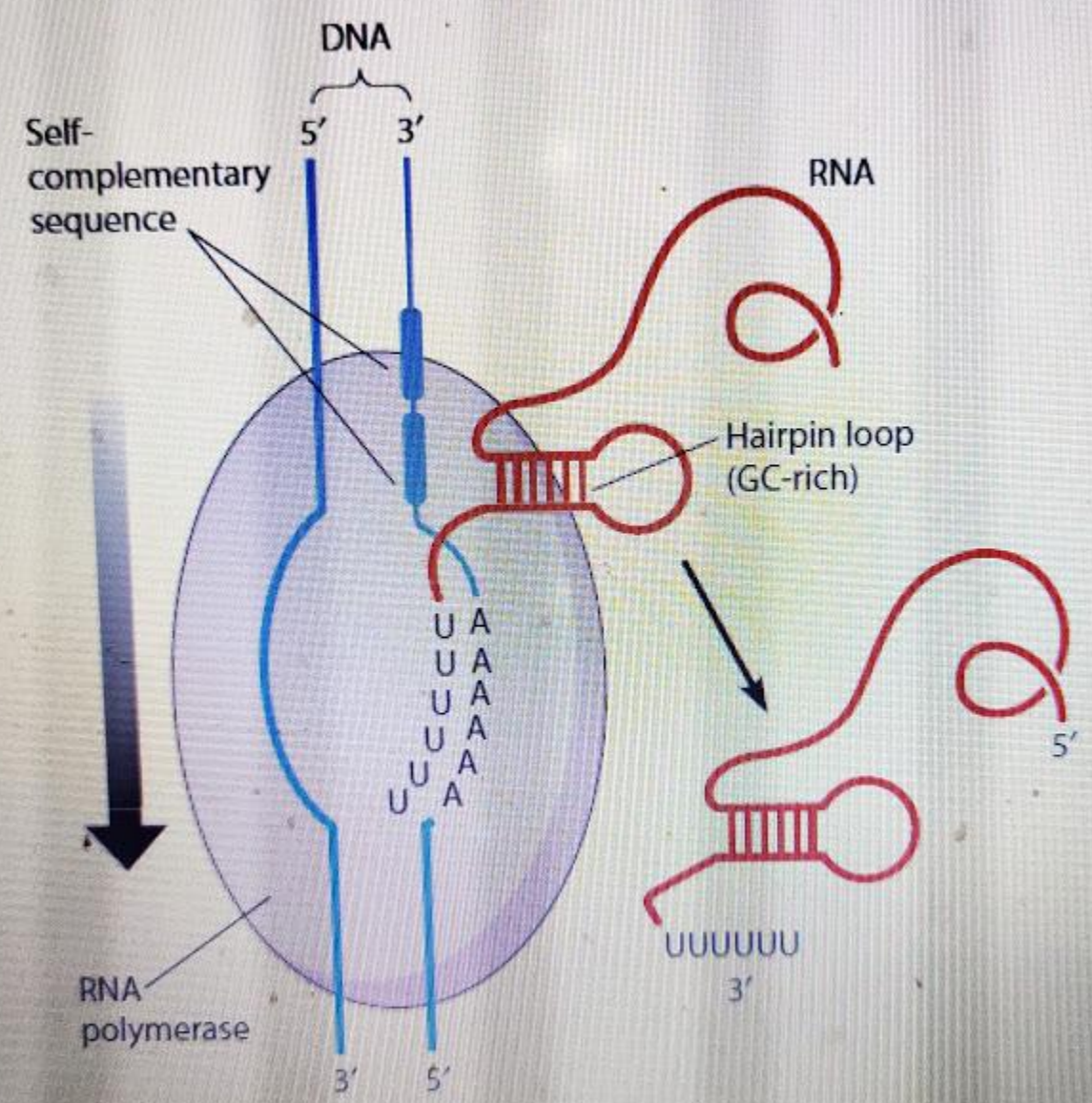


FIGURE 21-10 Termination of Transcription in Bacterial Genes That Do Not Require the Rho Termination Factor. A short

Books used as References to prepare the presentation:

1. Cell and molecular Biology- concepts and experiments; 8th Ed. ---Geralad Karp
2. Becker's World of the Cell- 8th Ed.
3. Biochemistry –U satynarayan-4 th Ed.