Botany- 4th semester- paper C8T: UNIT-4- TRANSCRIPTION

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Transcription

The first step a cell takes in reading out part of its genetic instructions is to copy the required portion of the nucleotide sequence of DNA into the nucleotide of RNA. The process is called **transcription.**

- **Like DNA, RNA is a linear polymer made up of four different types of nucleotides linked together by phosphodiester bonds.**

- It differs from DNA chemically in two respects-

  -1. The nucleotides in DNA is deoxyribonucleotides that is the sugar id deoxyribose. Whereas in RNA, it is ribose sugar.

  -2. It contains the base uracil (U) instead of thymine in DNA. U like T can base pair by hydrogen bond with A.

- DNA and RNA differ significantly in structure as well. DNA is a double helical structure, whereas RNA is single-stranded. Moreover RNA can fold into a complex shape that allows it to carry out functions in cell in addition to conveying information between DNA and protein. Also, there are different types of RNA, some having structural and even catalytic functions.

Transcription produces an RNA chain identical in sequence with one strand of the DNA (known as coding strand). This new strand is made 5'→3' and is complementary (it base pairs with) the template which is 3' → 5'. The one that serves as template is called template strand. **The coding strand is also known as sense (+) strand and the template strand is known as antisense strand (-)**

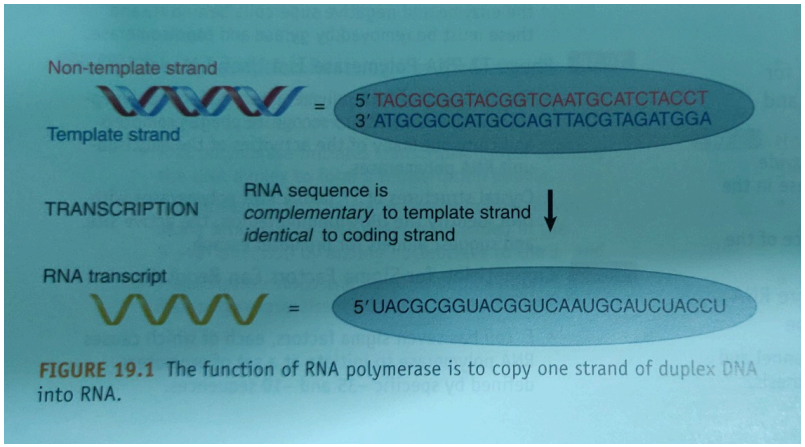
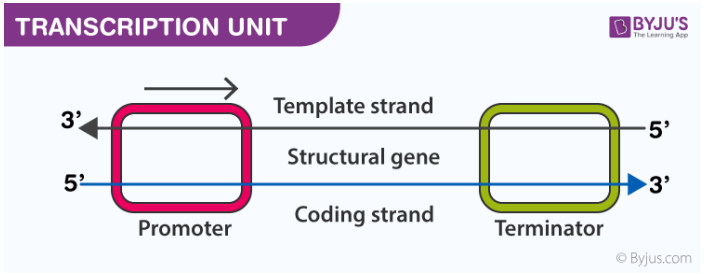


FIGURE 19.1 The function of RNA polymerase is to copy one strand of duplex DNA into RNA.

RNA synthesis is catalyzed by the enzyme **RNA polymerase.** Transcription starts when RNA polymerase binds to a special region, called the **promoter (at the start of the gene).**
The promoter includes the first base pair that is transcribed into the RNA (the startpoint) as well as the surrounding bases.



From this position, RNA polymerase moves along the template, synthesizing RNA until it reaches terminator sequence where the transcription ends.
the transcription unit extends from promoter to terminator.

In eukaryotes, a transcription unit typically carries the information of just **one gene** and it is termed as **monocistronic transcription unit**.

In prokaryotes, a set of **adjacent genes** is often transcribed as a unit termed as **polycistronic transcription unit.**

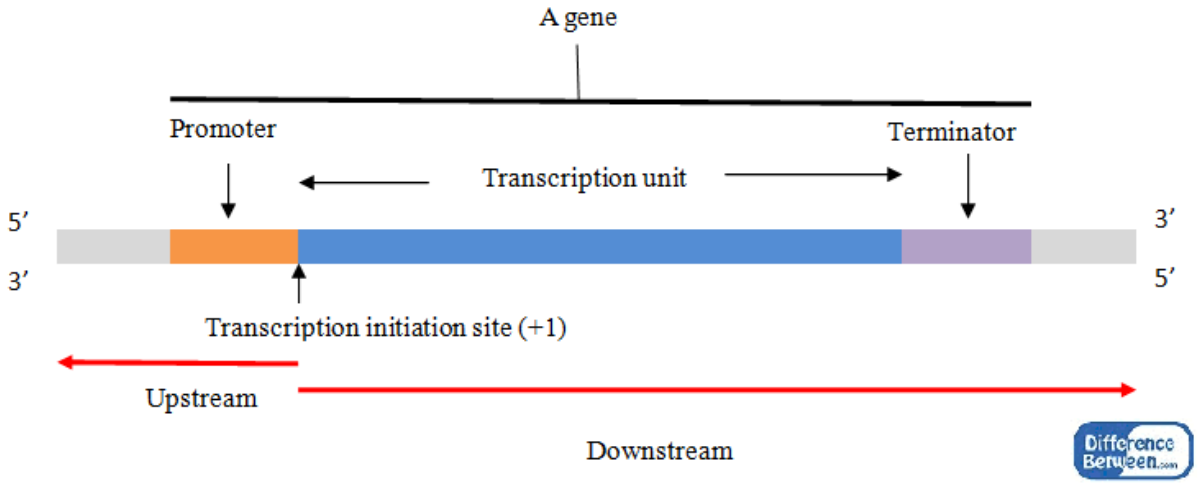
- **The immediate product of transcription is called primary transcript.**

The eukaryotic transcription unit may be **simple or complex**. The primary transcript produced from a simple transcription unit is processed to yield a single type of mRNA, encoding a single protein

- In case of **complex transcription units**, which are common in multicellular organisms, the primary RNA transcript can be processed in more than one way, leading to the formation of more than one type of mRNAs, encoding more than one type of polypeptides.

Transcription starts when RNA polymerase binds to a special region, called the **promoter**, at the start of the gene. The promoter includes the first base pair that is transcribed into the RNA, which is called the **startpoint.** From this point the RNA polymerase moves along the template, synthesizing RNA until it reaches terminator sequence where the transcription ends. The **transcription unit** extends from promoter to terminator.

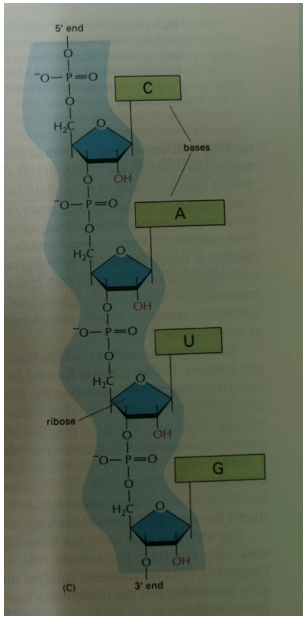
Sequence prior to the startpoint are described as upstream, and those after the startpoints are known as downstream **(and they are numbered as +1, as numbers increase as they go downstream the base before the startpoint is numbered -1, and the negative numbers increase going upstream)** of it.

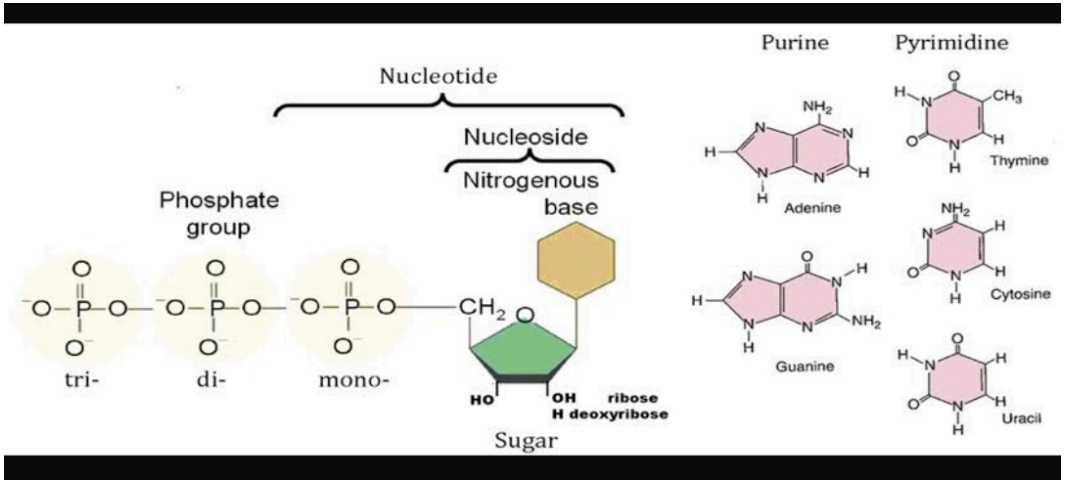


Prokaryotic transcription

In prokaryotes, RNA transcription takes place within a **transcription bubble** in which DNA is transiently separated into its single strands and the template strand is used to direct synthesis of the RNA strand.

The RNA chain is synthesized in 5’ to 3’ direction by adding new nucleotides to the 3’ end of the growing chain. the 3’-OH group of the last nucleotide added to the chain reacts with an incoming nucleoside 5’ triphosphate (ATP, GTP, UTP, CTP). The incoming nucleotide loses its terminal two phosphate groups; its α group is used in the phosphodiester bond linking it to the chain.





RNA polymerase created the transcription bubble when it binds to a promoter. And the RNA polymerase moves along the DNA, with the bubble moving with it and the RNA chain growing in length. The process of base pairing and base addition within the bubble is catalyzed and scrutinized by the RNA polymerase itself.

- The length of the transcription bubble is **12 to 14 bp** but the length of DNA-RNA duplex within the bubble is **8 to 9 bp.**
- As the enzyme moves along the template, the DNA duplex reforms, and the RNA is displaced as a free polynucleotide chain

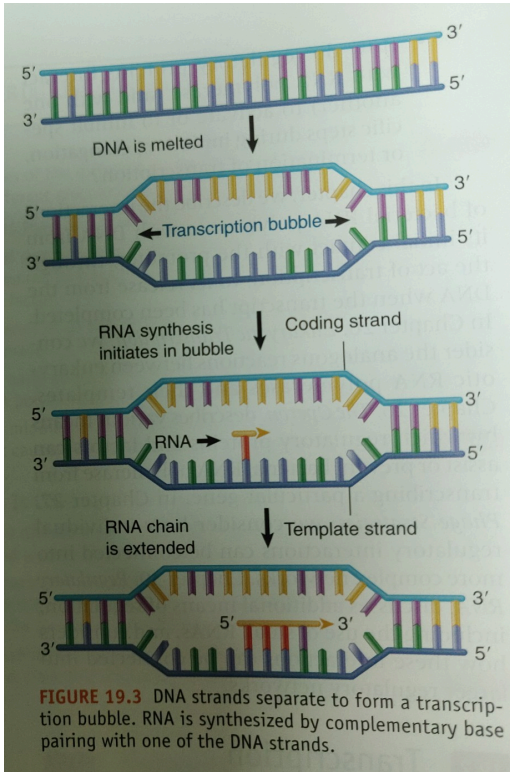


FIGURE 19.3 DNA strands separate to form a transcription bubble. RNA is synthesized by complementary base pairing with one of the DNA strands.

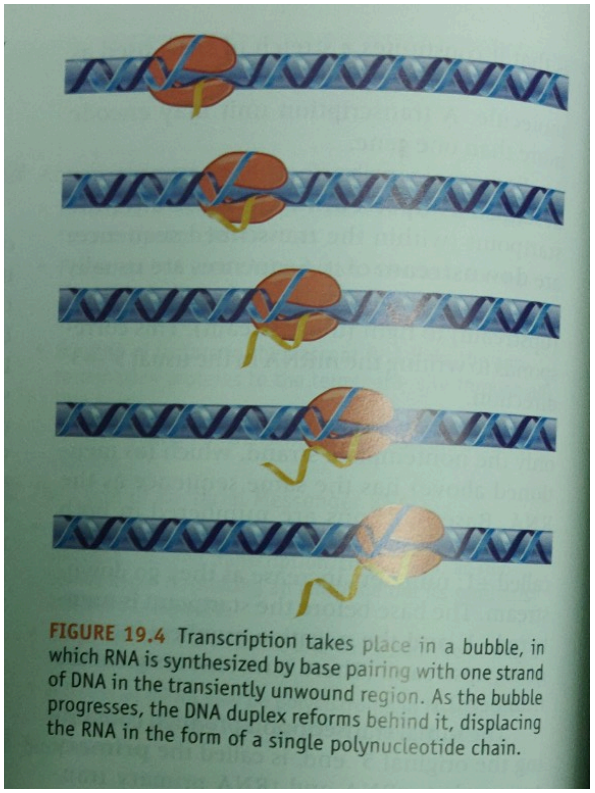


FIGURE 19.4 Transcription takes place in a bubble, in which RNA is synthesized by base pairing with one strand of DNA in the transiently unwound region. As the bubble progresses, the DNA duplex reforms behind it, displacing the RNA in the form of a single polynucleotide chain.

RNA polymerase.

DNA dependent RNA synthesis is catalyzed by the enzyme DNA dependent RNA polymerase (simply called **RNA polymerase**). In prokaryotes, single type of RNA polymerase appears to be responsible for the synthesis of all different types of RNA such as mRNA, rRNA, tRNA.

Bacterial RNA polymerase is. Multisubunit enzyme made up of five different polypeptides- α, β, β', ω, **σ** . The holoenzyme $\alpha_2 \beta\beta'\omega$ **σ** can be separated into two components-

- The core enzyme $\alpha_2 \beta\beta'\omega$
- The sigma factor- **σ** polypeptide.

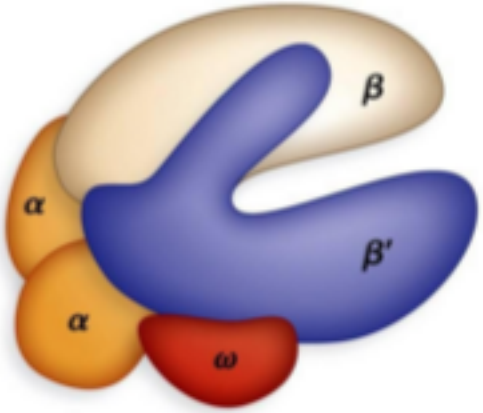
The complete holoenzyme in *E.coli* has a molecular mass of approx. 465 kDa.

Function od each subunits-

1. α subunit- this is required for assembly of the core enzyme and plays an important role in interaction of RNA polymerase with some regulatory factors.
2. β and β'- comprise the catalytic center and help in elongation.
3. **σ-** this subunit is concerned specifically with promoter recognition. Sigma factor not only ensures that bacterial RNA polymerase initiates transcription from specific sites, but it also reduces binding to non-specific sequences.
4. ω **-** this subunit facilitates assembly of RNA polymerase and stabilizes assembled RNA polymerase.

The catalytic activity of RNA pol is provided by the core complex composed of β and β' subunits, ω and two copies of α subunits. The core enzyme has the ability to synthesize RNA on a DNA template, but it cannot recognize promoters. The form of enzyme responsible for initiating transcription from promoters is called the holoenzyme $\alpha_2 \beta\beta'\omega$ **σ** .

Bacterial RNA polymerase



RNA polymerase core enzyme

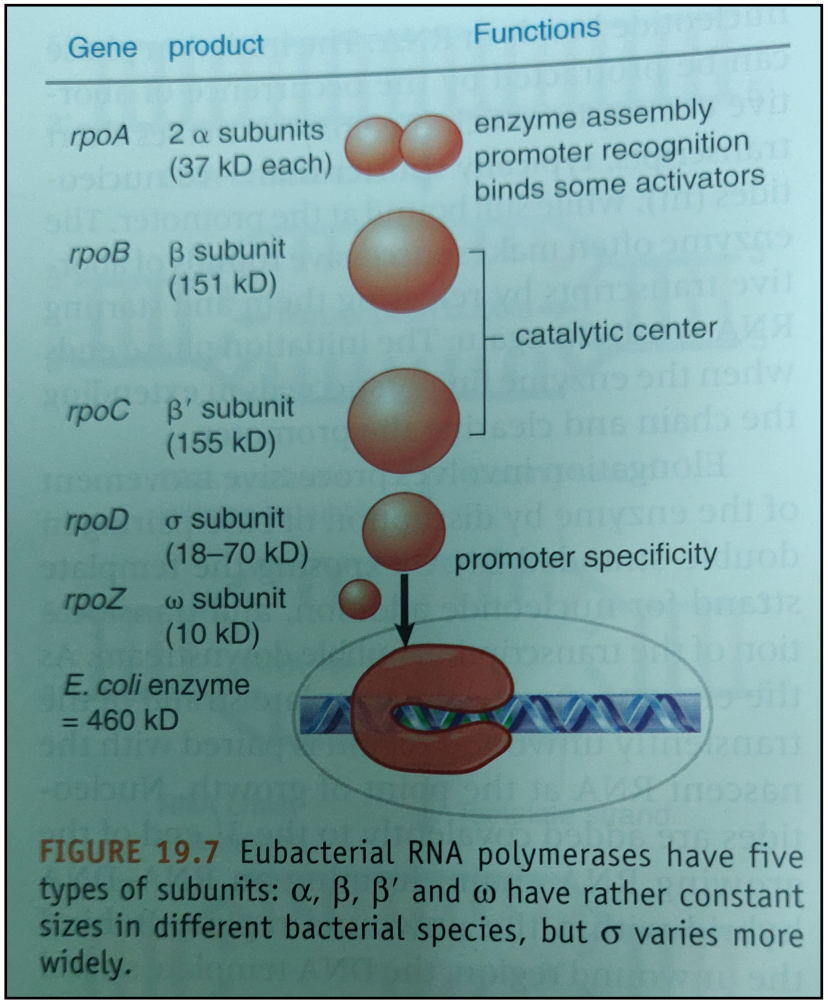


FIGURE 19.7 Eubacterial RNA polymerases have five types of subunits: α, β, β′ and ω have rather constant sizes in different bacterial species, but σ varies more widely.

There is a wide variation in the rate at which the holoenzyme binds to different promoter sequences, and thus this is an important parameter in determining "promoter strength". The promoter strength is an efficiency of an individual promoter in initiating transcription.

Prokaryotic promoter

A promoter can be defined as ***cis* acting, position dependent DNA sequence necessary for accurately and efficiently initiating transcription of the gene.** The DNA sequence of the promoter is recognized by the **RNA polymerase**. The best characterized prokaryotic promoters are those of the bacterium *E. coli* that is recognized by σ^{70} of RNA pol. The promoter contains two 6-base pairs of consensus sequences (-10 and -35 sequence). The -10 sequence is also known as **Pribnow box.** A consensus sequence is the one that reflects the most common base or amino acid at each position when a series of related nucleic acid or protein sequences are compared.

The -10 sequence has the consensus TATAAT and the -35 sequence has consensus TTGACA. The -10 and -35 regions are designated to reflect their approximate distances in nucleotides from the start site. Some strong promoters have an AT- rich sequence in the upstream region. This is called the *UP element* (Upstream Promoter element). It interacts with the α-subunit of the RNA pol.

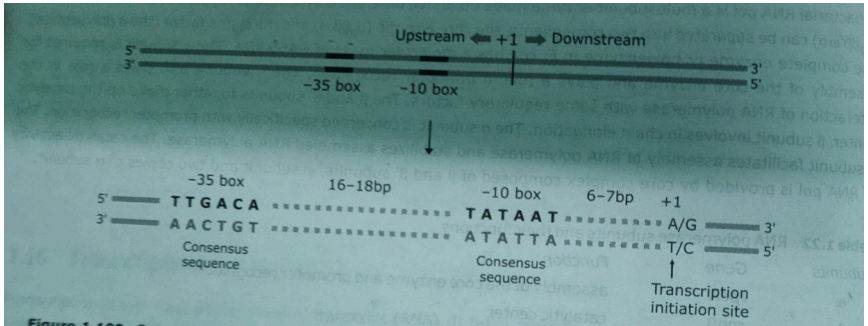


Figure 1.100 Bacterial promoters, such as that from *E. coli* shown here, share two regions of consensus nucleotide sequence. These regions are located 35 and 10 bp upstream from the start site of transcription, which is indicated as +1. By convention, all nucleotides upstream of the transcription initiation site (at +1) are numbered in a negative sense. These elements function only in double-stranded DNA.

Table 1.24 Alternative σ factors and their consensus sequences

Name	*Upstream (–35) consensus*	*Function*
σ^{54}	TTGGCACA	Nitrogen assimilation
σ^{38}	CCGGCG	Major sigma factor during stationary phase, also for genes involved in oxidative and osmotic responses
σ^{32}	TNTCNCCTTGAA	Heat shock response
σ^{28}	TAAA	For genes involved in flagella synthesis

N – any nucleotide

Steps of transcription-

The transcription can be divided into three stages- **initiation, elongation** and **termination.**

Initiation

- Initiation begins with template recognition. Template recognition begins with binding of RNA polymerase to the double-stranded DNA at the promoter. The enzyme first forms a **closed complex** in which the DNA remains double-stranded. The sigma factor confers the ability to recognize the promoter.
- Next the enzyme locally **unwinds the section of promoter DNA that includes the transcription start site to form the open complex**. Separation of the DNA double strands makes the template strand available for base pairing with incoming ribonucleotides and synthesis of the first nucleotide bonds in RNA.
- The next step is to incorporate the **first two nucleotides**; then a **phosphodiester bond** forms between them. This generates a ternary complex that contains **RNA, DNA and enzyme**.
- Only the holoenzyme can initiate transcription. The sigma factor is essential for recognition. The sigma factor is usually released when the RNA reaches 8-9 bases, leaving the core enzyme to undertake elongation.
- RNA synthesis is frequently aborted after usually 2 or 3, but up to 10 nucleotides have been joined. This phenomenon is known as **abortive initiation.** Once a polymerase manages to make a **RNA longer than 10bp**, a stable ternary complex is formed. The initiation phase end when the enzyme succeeds in extending the chain and clears the promoter.

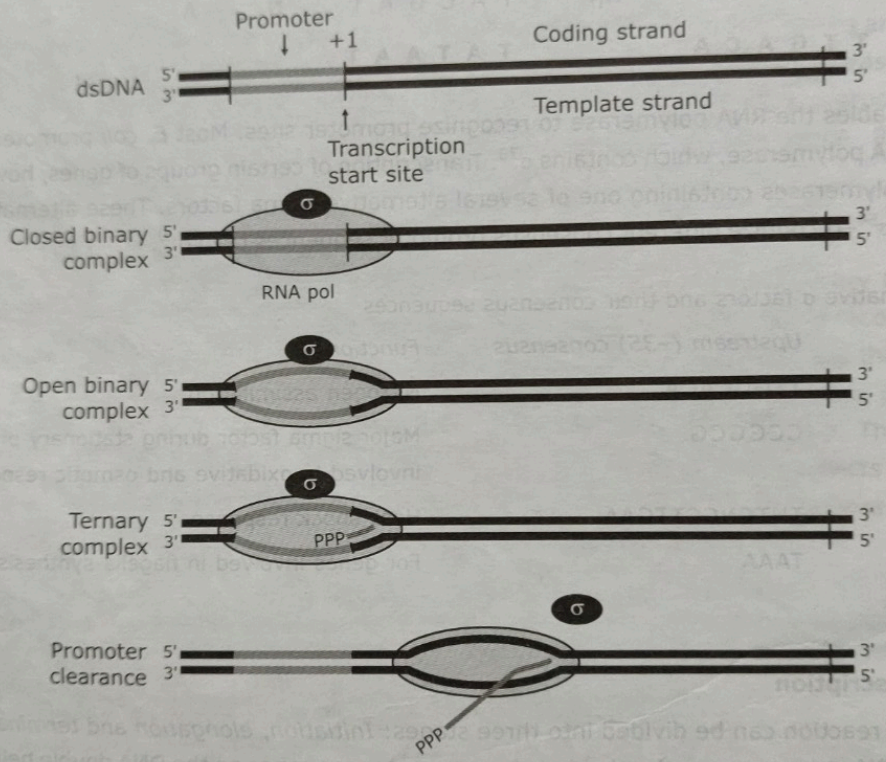


Figure 1.101 Transcription initiation and elongation by *E. coli* RNA polymerase. The enzyme first forms a closed complex in which the two DNA strands remain fully base-paired. In the next step the enzyme catalyzes the opening of the DNA helix to form an open complex, in which the template DNA strand is exposed for the initiation of an RNA chain. The polymerase contains the bound σ-subunit. The conversion to an actively elongating polymerase requires the release of initiation factors (the σ-subunit).

Elongation-

Elongation involves the movement of the transcription bubble by a disruption of DNA structure. The enzyme moves along the DNA and extends the growing RNA chain. As the enzyme moves, it unwinds the DNA helix to expose a new segment of the template in single-stranded condition. Nucleotides are covalently added to the 3'-end of the growing RNA chain, forming a RNA-DNA hybrid. The length od RNA-DNA hybrid within open complex is 8-9 bp.

During each nucleotide addition, the beta and gamma phosphates are removed from the incoming nucleotides and the hydroxyl group is removed from the 3'-carbon of the nucleotide present at the end of the chain. The overall reaction rate is 40 nucleotides/ second at 37 degrees temperature.

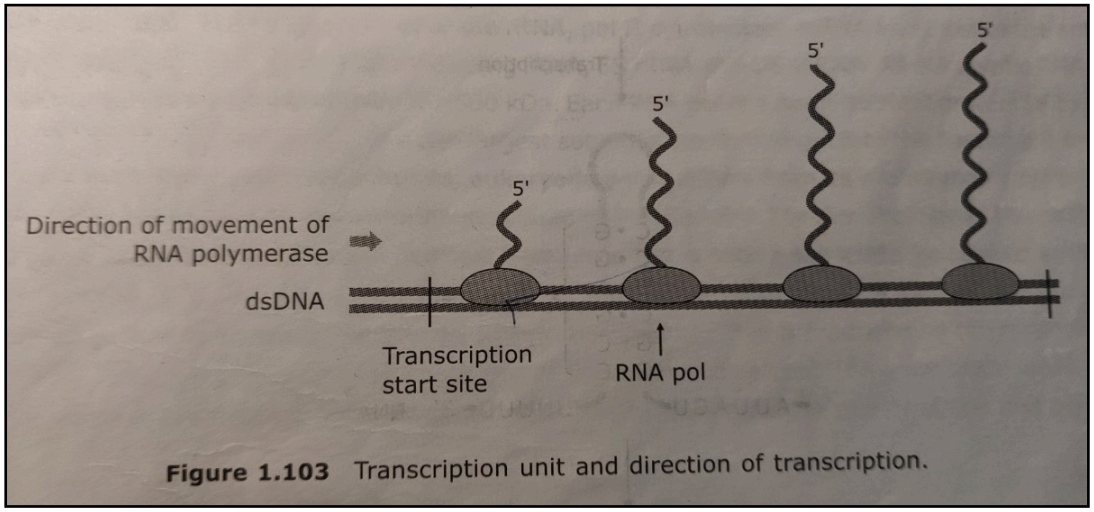


Figure 1.103 Transcription unit and direction of transcription.

During elongation when RNA polymerase transcribes DNA, unwinding and rewinding occurs. As RNA polymerase moves forward along the double helix, it generates positive supercoils ahead and leaves negative supercoils behind. Enzymes gyrase and topoisomerase I participate during this process. Gyrase removes positive supercoil and introduces negative supercoils whereas topoisomerase I removes negative supercoils that develop behind.

Cordycepin , an adenosine analog that lacks a 3-OH group, inhibits elongation. Its addition to the 3' end of RNA prevents the RNA chain's further elongation. It is readily phosphorylated to its mono, di and triphosphate intracellularly. Triphosphate cordycepin can be incorporated into RNA and inhibits transcription elongation.

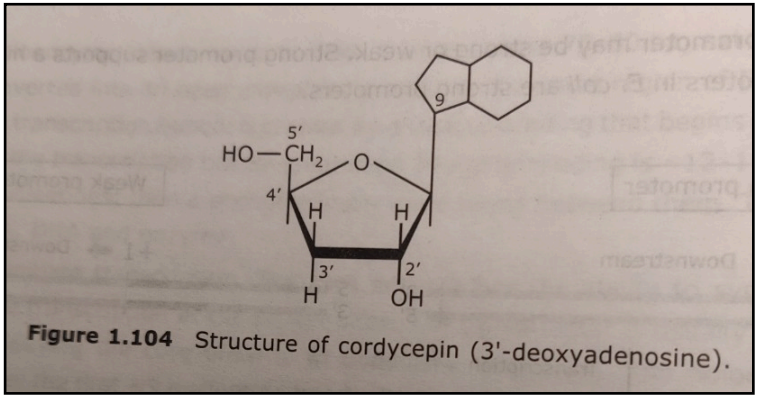


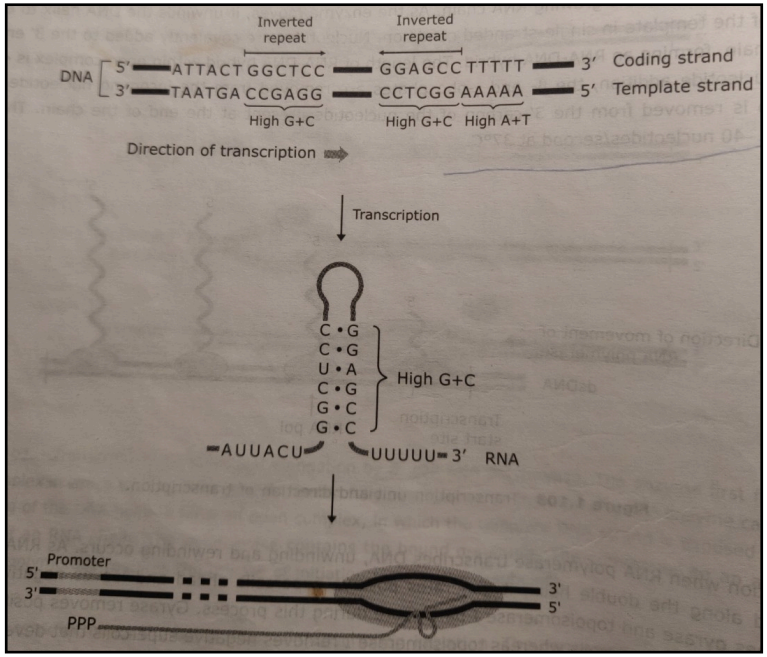
Figure 1.104 Structure of cordycepin (3'-deoxyadenosine).

Termination-

It involves recognition of the point at which no further bases should be added to the chain. The sequence of DNA required for these reactions is called the **terminator.** At this point, the enzyme stops adding nucleotides to the growing RNA chain, releases the completed product and dissociates from the DNA template. Exactly how transcription occurs is not entirely clear.

Bacteria appear to use two distinct strategies for transcription termination- **intrinsic termination** and **Rho- dependent termination**.

Intrinsic terminators- have been thought to promote dissociation of the polymerase by destabilizing the attachment of the growing transcript to the template. In this case, transcript forms a hairpin structure by forming a complementary base pairing. Intrinsic terminators include palindromic sequences that form hairpins varying in length from 7 to 20 bp. The stem loop structure includes a GC rich region and is followed by a U-rich region. The typical distance between the hairpin and the U-rich region is 7-9 bases.



The second type of termination is **Rho dependent.** This termination requires the activity of a protein called **Rho.** Rho is an ATP-dependent RNA- stimulated helicase that disrupts the nascent RNA-DNA complex. It is a 275 kDA hexamer of identical subunits. It binds to RNA at *rut* site is rich in C residues and poor in G residues.

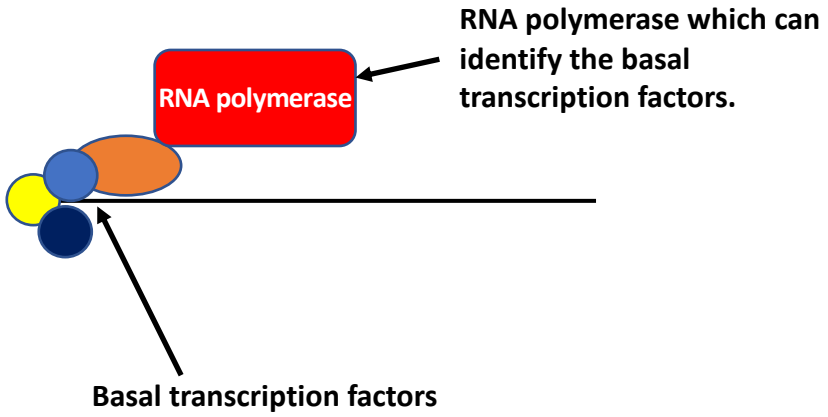
Eukaryotic transcription

- A **single RNA polymerase** is responsible for transcription of all different types of RNAs in **prokaryote**s. However, eukaryotes have three different RNA polymerases: **RNA PolI, RNA PolII, RNA PolIII**.

  RNA poll transcribes 18S/28S rRNA
  RNA PolII transcribes mRNA and a few small RNAs.
  RNA pollII transcribes tRNA, 5S ribosomal RNA , and other small RNAs.

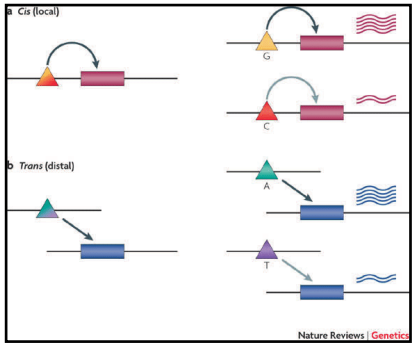
- A significant difference between the transcription of eukaryotic and prokaryotic RNAs is that in **bacteria**, **transcription takes place on a DNA template**, whereas in **eukaryotes, transcription takes place on a chromatin template.**

- Another major difference is that the **bacterial RNA polymerase, with its sigma factor subunit can read the DNA sequence to find and bind to the promoter**. **A eukaryotic RNA polymerase cannot read the DNA**. Initiation at eukaryotic promoters therefore involves a large number of factors that must prebind to a variety of *cis*-acting elements before the RNA polymerase can bind. These factors are called **basal transcription factors..** The RNA polymerase then binds to this basal transcription factor/DNA complex. This binding region is called **Core promoter,** the region containing all the binding sites necessary for RNA polymerase to bind and function.



Cis and trans acting elements-

Cis-regulatory elements are present on the same molecule of DNA as the gene they regulate whereas **trans-regulatory elements** can regulate genes distant from the gene from which they were transcribed. **Cis-regulatory elements** are often binding sites for one or more **trans-acting factors**.



- For all the eukaryotic RNA polymerase, **the basal factors create a structure at the promoter to provide the target that is recognized by RNA polymerase. The basal factors together with RNA polymerase constitute the basal transcription apparatus.**

- Basal transcription factors are needed for initiation , but most are not required after initiation. The transcription factors are responsible for recognizing promoter in eukaryotic transcription.

- Promoters recognized by RNA polymerase II show much more variation in sequence. All the **RNA polymerase promoters have sequence elements close to the startpoint** that are bound by the basal apparatus where the polymerase binds to form the site of initiation.

Enhancers and silencers-

- The other sequences farther upstream or downstream called **Enhancer sequences,** determine whether the promoter is expressed, and if expressed, whether this occurs in all cell types or is cell type-specific. An **enhancer** is another type of site involved in transcription, but are located a variable distance from the core promoter. Enhancer elements are often targets for tissue specific or temporal regulation.
- A regulatory site that binds more negative regulators than positive regulators to control transcription is called a **silencer**.

Promoters that are constitutively expressed and needed in all cells have upstream sequence elements that are recognized by ubiquitous activators. These genes are sometimes called **housekeeping genes.**

The components of an enhancer or silencer resemble those of the promoter. They can bind positive regulators or negative regulators. Eukaryotic transcription is most often under positive regulation. A transcription factor is provided under tissue-specific control to activate a promoter or set of promoters that contain a common target sequence.

RNA polII-

Eukaryotic RNA polII consists if 12 subunits. The two largest subunits are homologous to the bacterial β and β ‘ subunits. In addition to the increased number of subunits, eukaryotic RNA polII differs from its prokaryotic counterpart in that it has a series of heptad repeats with the consensus sequence *Tyr- Ser-Pro-Thr-Ser-Pro-Ser* at the carboxyl terminal of the largest polII subunit.

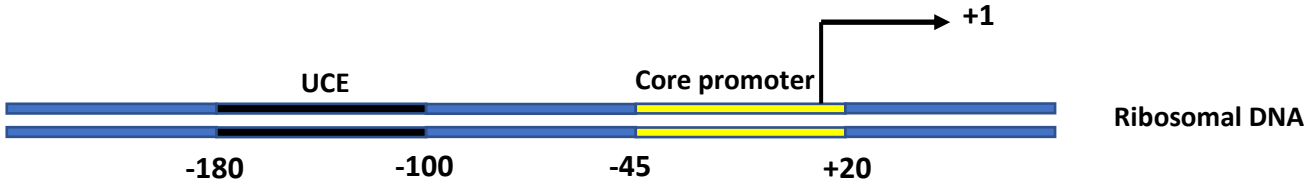
This **Carboxyl Terminal Domain (CTD)** is both a substrate for several kinases, including the kinase component of TFIIH, and a binding site for a wide array of proteins.

Eukaryotic promoters-

In eukaryotes, the term promoter is used to describe all the sequences that are important in the initiation of transcription of a gene . The initiation of transcription in eukaryotes requires the enzyme RNA polymerase and transcription factors. The transcription factors are responsible for recognition of the promoter.

RNA pol I promoter-

RNA polymerase I promoter consists of a **core promoter** spanning the transcription start point, between nucleotides -45 and +20, and an **Upstream Control Element (UCE)** about 100 bp further further upstream. RNA polI binds to the core promoter.



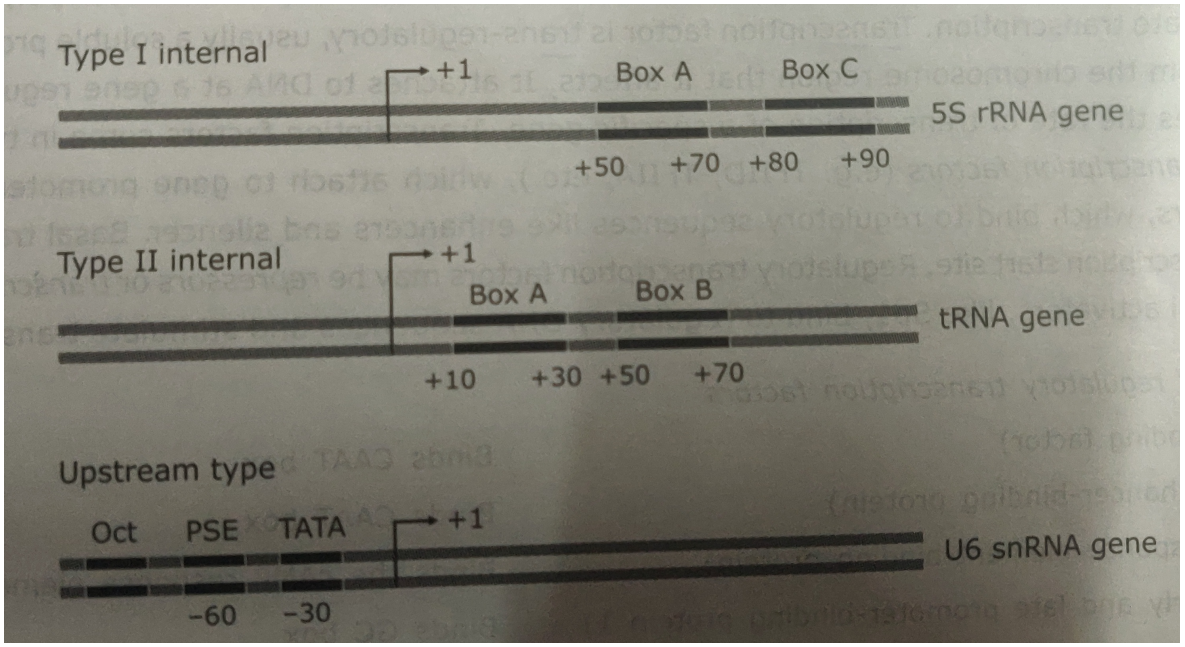
RNA pol III promoter-

The most striking feature of the promoters used by polIII is that important sequence elements downstream of the transcribed start site that means within the transcribed region.

Type I promoter- (found in the 5S rRNA genes) requires two internal elements for efficient transcription- an A-block (located between +50 and +70), and a C-block (from +80 and +90).

Type II promoter- (found in the tRNA genes) consists of two highly conserved sequence blocks, A- and B-, located within the transcribed region.

Type III promoter- (found in the U6 snRNA genes) consists of a TATA box, located between -30 and -25, a Proximal Sequence Element (PSE) between -66 and 46, and a Distal Sequence Element (DSE) between -244 and -214.

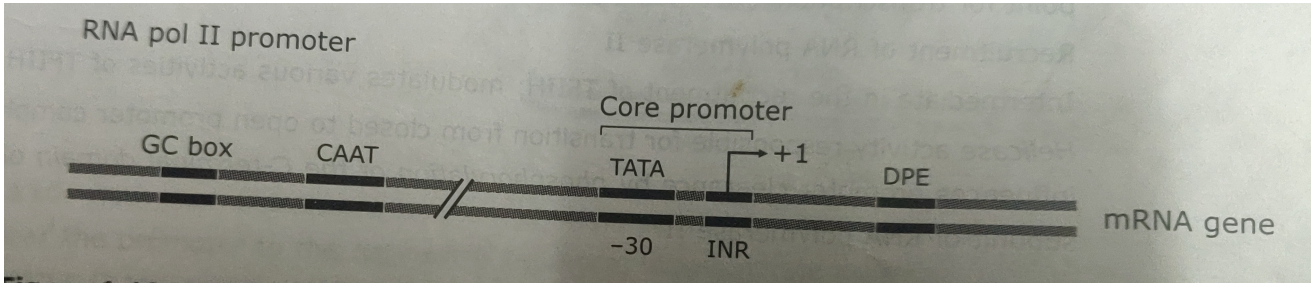


RNA pol II promoter

The core promoter consists of two segments: the -30 or **TATA box** (consensus 5'-TATAXAX-3', where X is A or T) and **initiator (Inr) sequence** located around nucleotide +1. The core promoter elements serve as specific binding sites for the transcription factors.

Other than these, there are other *cis*-acting sequences serve as binding site of several regulatory factors that control the expression of individual genes. Two such regulatory sequences found in eukaryotes are *CAAT* BOX and *GGGCGG* (called G box). These two sequences are recognized by several activators.

A few genes have a Downstream Promoter elements (DPE) located at position +28 to +32. DPE has a variable sequence and binds TFIID.



Transcription factors-

Transcription of eukaryotic genes requires many **protein factors called transcription factors apart from RNA polymerase** to **initiate and also to regulate the transcription**. Transcription factor is trans-regulatory protein produced by a gene distant from the chromosome region that it affects. It attaches to DNA at a regulatory site and by doing so it influences the rate of transcription of a specific gene.

Transcription factors are of two types-

1. Basal transcription factors- eg.- TFIID, TFIIA etc, which attach to gene promoters. They determine the transcription start sites.

2. Regulatory transcription factors- which bind to regulatory sequences like enhancers and silencer. These may be repressors or transcriptional activators.

Examples of basal transcription factors-

CBF	CAAT binding factor	Binds CAAT box
C/ EBP	CAAT/ enhancer-binding protein	Binds CAAT box
CREB	cAMP response element-binding protein	Binds the cAMP response element
Sp1	SV40 early and late promoter-binding protein 1	Binds GC box.

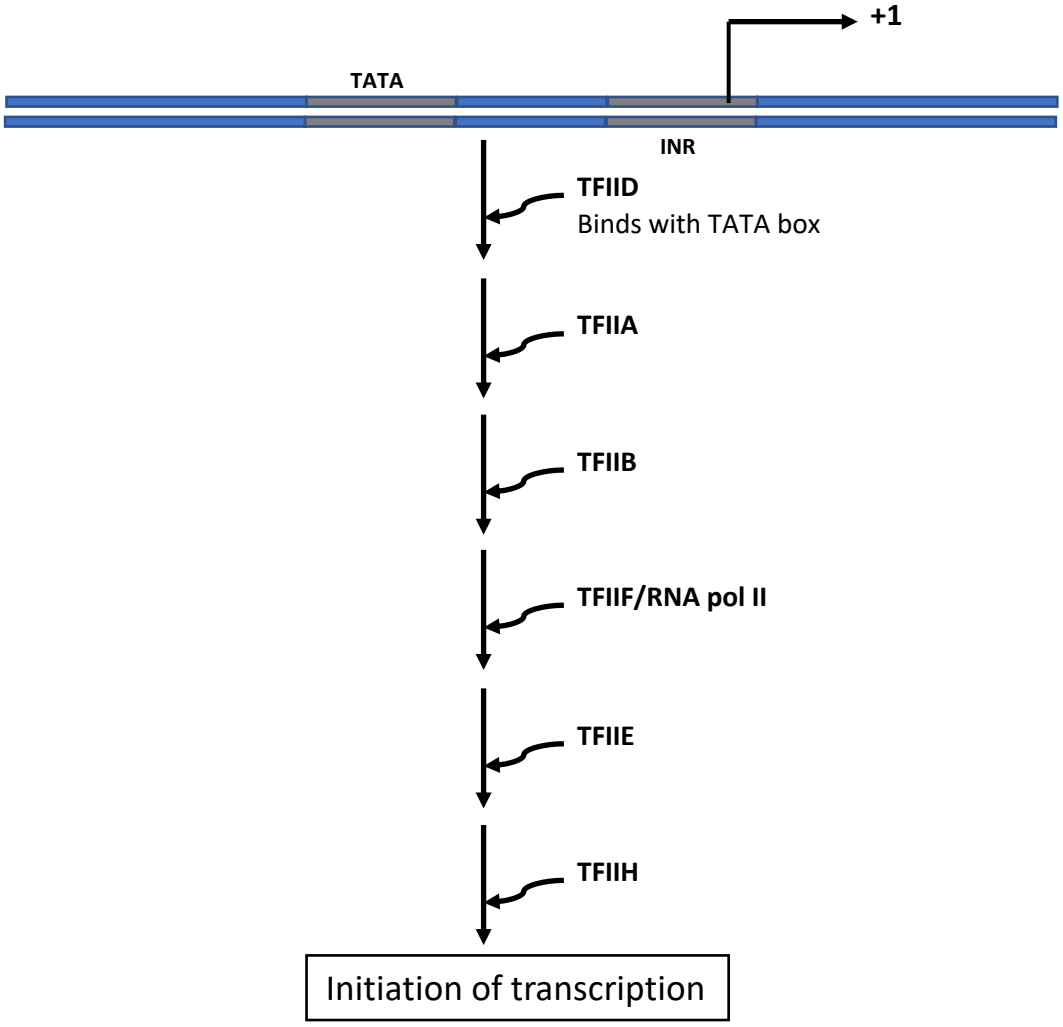
Transcription initiation by RNA polymerase II

- Genes first bind with the basal transcriptional factor like TFIID, which is a complex made up of the TATA- Binding Protein (TBP) and at least 12 TBP-Associated Factors (TAFs).

- After TFIID has attached to the core promoter, the *pre-initiation complex* (PIC) is formed by the attachment of the remaining Transcription factors. These factors bind to the complex in the order TFIIA, TFIIB, TFIIF/RNA pol II, TFIIE, TFIIH.

- The final step in the assembly of the initiation complex is the addition of phosphate groups to the *C-terminal domain* (CTD) of the largest subunit of the RNA polymerase II. The CTD contains a stretch of seven amino acids (Tyr-Ser-Pro-Thr-Ser-Pro-Ser) that is repeated multiple times.

- Once phosphorylated, the polymerase is able to leave the pre-initiation complex and begin synthesizing RNA. Phosphorylation might be carried out by TFIIH. Phosphorylation of CTD tail is needed to release RNA polymerase II from the promoter and transcription factors so that it can make the transition to elongation form



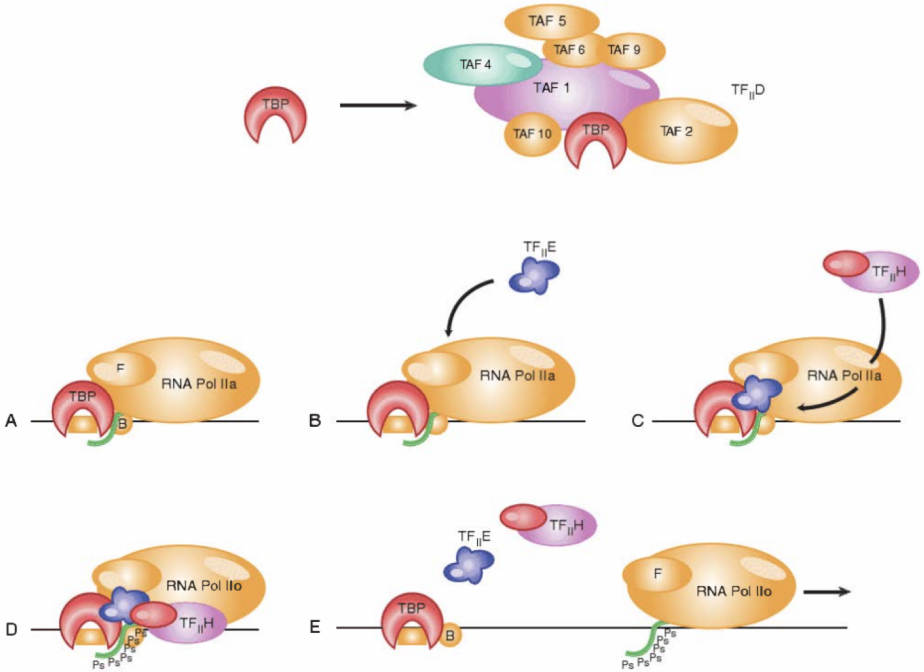


FIGURE 20.11 An initiation complex assembles at promoters for RNA polymerase II by an ordered sequence of association with transcription factors. $TF_{II}D$ consists of TBP plus its associated TAFs as shown in the top panel; TBP alone, rather than $TF_{II}D$, is shown in the remaining panels for simplicity. Adapted from M. E. Maxon, J. A. Goodrich, and R. Tijan, *Genes Dev.* 8 (1994): 515–524.

Table 1.26 Functions of human general or basal transcription factors (GTF)

GTF	*Function*
TFIID (TBP component)	Recognition of the TATA box and possibly *Inr* sequence; forms a platform for TFIIB binding
TFIID (TAFs)	Recognition of the core promoter; regulation of TBP binding
TFIIA	Stabilizes TBP and TAF binding
TFIIB	Intermediate in the recruitment of RNA polymerase II; influences selection of the start point for transcription
TFIIF	Recruitment of RNA polymerase II
TFIIE	Intermediate in the recruitment of TFIIH; modulates various activities of TFIIH
TFIIH	*Helicase* activity responsible for transition from closed to open promoter complex; possibly influences promoter clearance by *phosphorylation of the C-terminal domain* of the largest subunit of RNA polymerase II.

- Transcription initiation in a eukaryotic cell is a more complex process. It requires the presence of transcriptional activator proteins , which bind to specific sequences in DNA. A typical eukaryotic gene has many activators proteins that bind to this region and this together determine its rate and pattern of transcription.

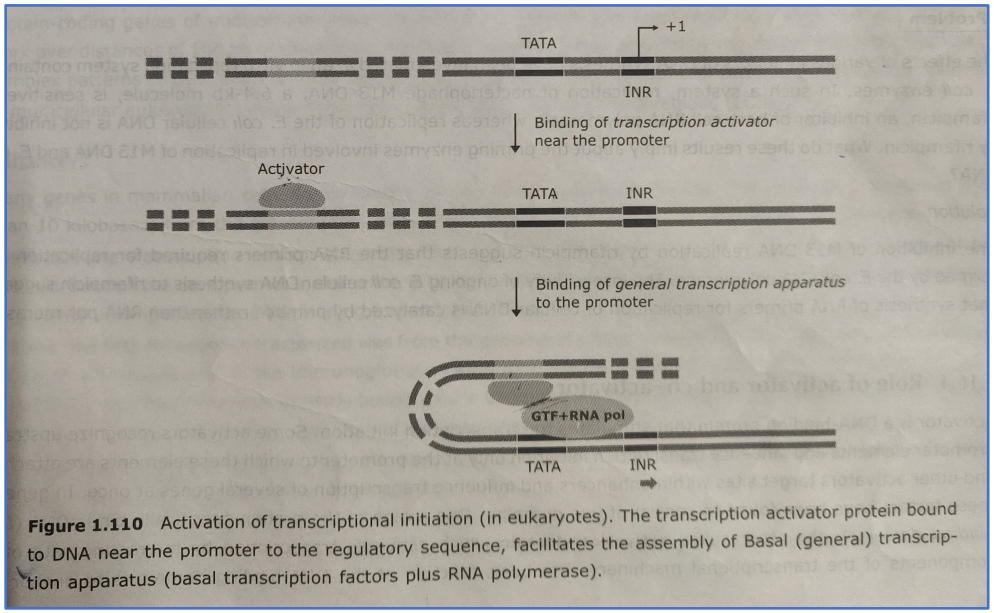


Figure 1.110 Activation of transcriptional initiation (in eukaryotes). The transcription activator protein bound to DNA near the promoter to the regulatory sequence, facilitates the assembly of Basal (general) transcription apparatus (basal transcription factors plus RNA polymerase).

- Sometimes, it acts from a distance of several thousand nucleotides pairs. **These gene regulatory proteins help RNA polymerase, the general transcription factors, chromatin remodeling complex and mediator all to assemble at the promoter.**

- The mediator is a protein complex consisting of about 20 protein subunits and allows the activator proteins to communicate properly with the polymerase II and with the general transcription factors. Mediator is a general coactivator of RNA Pol II mediated transcription.

Transcription termination

In eukaryotes, transcription occurs through different processes, depending on the polymerases utilized.

- For Pol I , transcription is stopped using a termination factor, through a mechanism similar to rho-dependent termination in bacteria.

- Transcription of pol III genes ends after transcribing a termination sequence that includes a polyuracil stretch, by a mechanism similar Rho-independent prokaryotic transcription.

- Transcription of Pol II transcripts is complex. Pol II termination of most protein-coding genes is functionally coupled with an RNA processing event in which the 3’ end of the nascent transcript undergoes cleavage and plyadenylation.

Antibiotic inhibitors of transcription

Rifampicin and actinomycin are two antibiotics that inhibit transcription.

- **Rifampicin** binds in a pocket of the B-subunit and blocks the path of the elongating RNA. By preventing the RNA chain from extending beyond 2-3 nucleotides, this blocks transcription.

- **Actinomycin D** binds tightly and specifically to double-helical DNA and, thereby, prevents it from being an effective template for RNA synthesis. It does not bind to single-stranded DNA or RNA, double-stranded RNA or RNA-DNA hybrids. The deformation of the helix caused by the intercalated actinomycin D prevents the movement of RNA polymerase along the DNA template and thus inhibits RNA synthesis. It is extensively used as a transcription inhibitor for the formation of new RNA in both prokaryotic and eukaryotic cells.

Activators and co-activators-

- Activator is a **DNA-binding protein that stimulates the transcription initiation.**
- Some activators recognize **upstream promoter elements** and influence transcription initiation only at the promoter
- Other activators target sites within **enhancers** and influence transcription of several genes at once.
- In general, these factors have been found to consist of two domains: One region of the protein specifically binds DNA (**DNA binding domains**); the other called **activation domain** which activates transcription by interacting with other components of the transcriptional machinery.
- Gene regulatory proteins that do not themselves bind DNA but assemble on DNA bound activator proteins are often termed coactivator.
- Coactivators, in the broadest sense, can be divided into two main classes: Chromatin modification complexes and chromatin remodeling complexes. These factors enhances the accessibility of the template DNA to general transcription factors or specific activators.

One common example of eukaryotic activator is Gal4. It activates transcription of GAL1 gene in the yeast *S. cerevisiae*.

GAL1 gene codes for the enzyme that convert galactose to glucose. Gal4 binds as a dimer to a 17 bp site on UAS located 275 bp upstream of GAL1. The Gal4 protein, bound to UAS in the vicinity of the promoter, facilitates the addition of TFIIB to the nascent complex of general transcription factors. When bound activates transcription of the GAL1 gene many fold

References-

1. Lewin's Gene XI- Lewin, B., Krebs, J. E., Goldstein, E. S., & Kilpatrick, S. T. (2014).

2. Pathfinder study material part 2