Lecture -3-

The Microbial Genetics

Dr. Yasir Alabdali

DNA repair

Mismatch repair

The simplest of replication errors is one that leads to the wrong base being incorporated into the new strand. If this occurs, and is not dealt with by the proof-reading mechanism, it would lead to **mutation**. However, the cell has an effective mechanism for removing such mismatches and replacing them with the correct nucleotide. In order to do this, it has to know which of the two strands contains the correct information. The methylation of the DNA identifies the new and old strands, at least until the new strand becomes methylated – hence the mechanism is referred to as **methyl-directed mismatch repair.** The system recognizes the mismatched bases, removes a short region of the non-methylated strand and fills in the gap.

Excision repair (NER)

Other types of DNA damage, in particular the formation of **pyrimidine dimers by ultraviolet irradiation**, give rise to distortion of the double helix, which can activate a repair mechanism known as excision repair.

The process is initiated by an endonuclease (a complex enzyme, coded for by genes known as uvrA, uvrB and uvrC, since mutations in these genes cause reduced Ultraviolet Resistance). This enzyme cuts the DNA strand on either side of the damage, which exposes a 3′ OH group; this can be used as a primer by DNA polymerase I to replace the short region of DNA between the nicked sites (15–20 bases long). The final step is the joining of the newly repaired strand to the existing DNA by DNA ligase.

Recombination (post-replication) repair

There is another type of mutant that is **abnormally sensitive to UV**, although possessing a functional excision repair system. These bacteria are defective in a gene (recA) that is amongst other things responsible for general recombination (cut off lexA repressor for recA). This indicates that excision repair is not the only mechanism for dealing with UV damage, but that there is a further repair mechanism involving general recombination. The double mutant (uvrA recA) is even more sensitive than either of the single mutants.

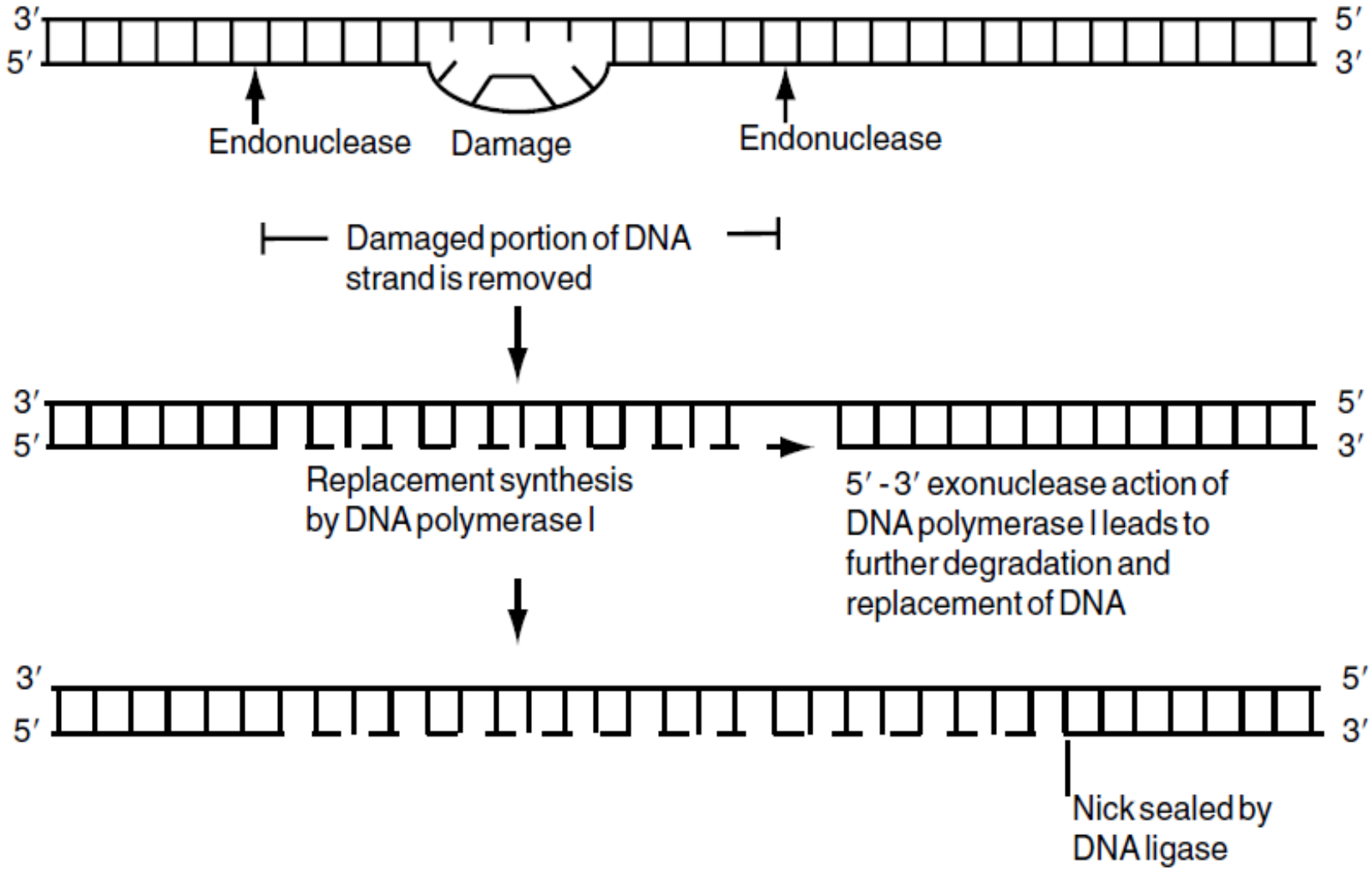


Figure 1.14 Mechanism of excision repair. Endonuclease cleavage removes a portion of the damaged strand. The gap is filled in by DNA polymerase I; the 5′—3′ exonuclease action of DNA polymerase I allows it to remove more DNA and replace it. Finally, the sugar-phosphate backbone is resealed by DNA ligase

Forms of DNA damage that interfere with the base pairing between the strands will normally prevent replication, due in part to the requirement of the DNA polymerase for an accurately paired 3′ end. **The replication fork will therefore pause**. It is possible however for replication to restart beyond the lesion, thus leaving a gap in the newly synthesized strand. This portion of DNA cannot be repaired by excision repair, which requires one intact strand.

The gap can however be filled using a portion of DNA from the other pair of strands by a recombination process (i.e. cutting and rejoining the DNA). Although this merely re-assorts the damage rather than directly repairing it, it does achieve a situation where the damage is repairable. The original damage can now be repaired by excision repair while the gap in the other DNA molecule can be filled in by DNA polymerase I and DNA ligase.

SOS repair

An alternative strategy, when faced with overwhelming levels of DNA damage **preventing normal replication**, is to temporarily modify or abolish the specificity of the DNA polymerase. This enables it to continue making a new DNA strand, **despite the absence of an accurately paired 3′ end.** Although the new strand is produced, it is obviously likely to contain many mistakes and the process is therefore described as '**error-prone**'. SOS repair is the cause of mutations arising from ultraviolet irradiation by using RecA and LexA system which is inducible repair mechanism.

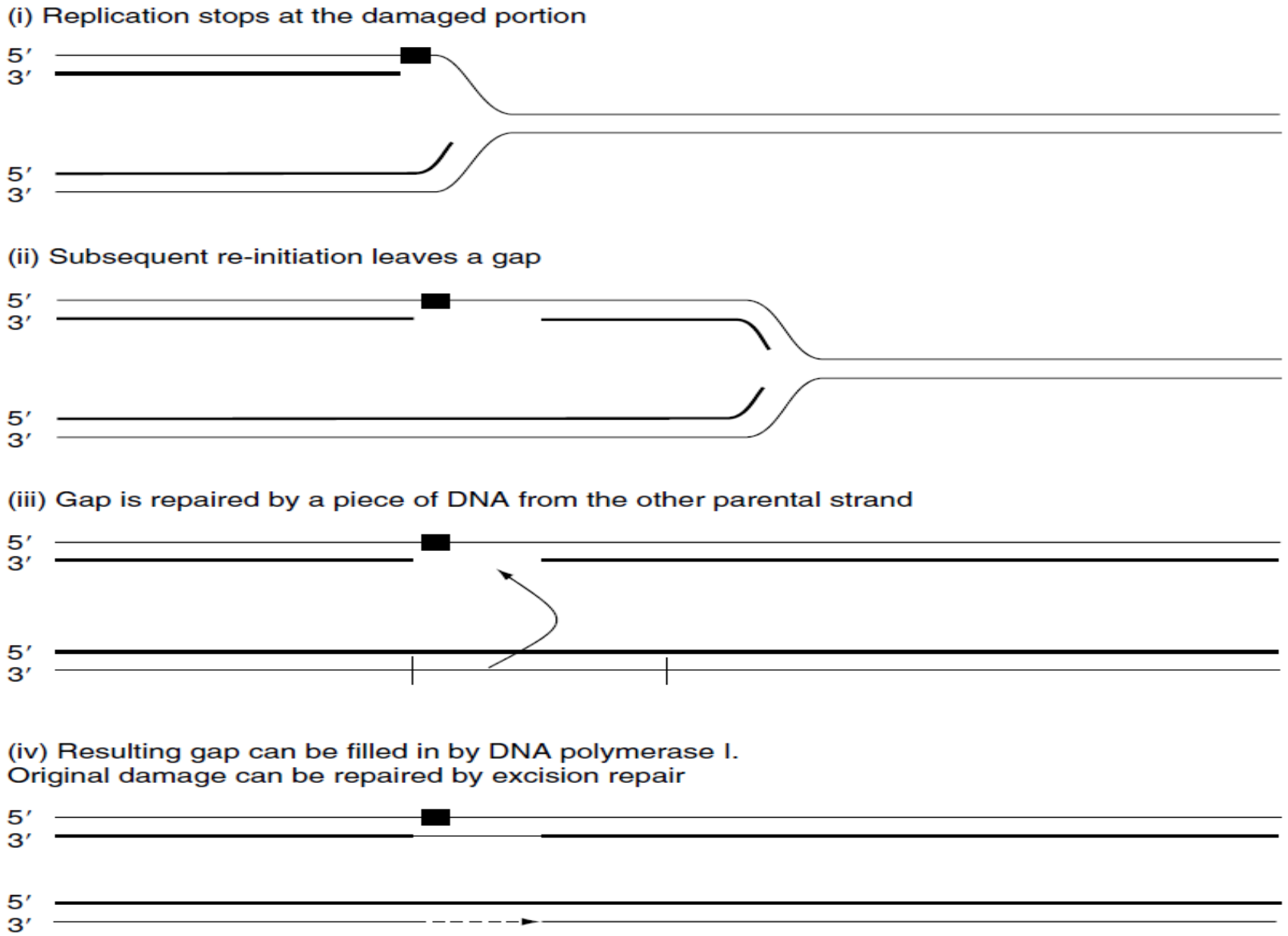


Figure 1.15 Post-replication repair. After replication stops at a damaged site (i), subsequent re-initiation leaves a gap in the new strand (ii). This can be repaired by exchange of DNA (iii), allowing the original lesion to be repaired by excision repair (iv)

Gene expression

The expression of the genetic material occurs for the most part through the production of proteins, involving two consecutive steps in which the information is converted from one form to another: transcription and translation. With those proteins that consist of several different subunits, each one is the product of a distinct region of DNA. The complete protein is thus the product of several different genes, mutation in any of which may lead to the absence of a functional product. The term cistron, meaning that region of the DNA that codes for a single polypeptide chain, is used where it is desirable to emphasize the distinction between a single polypeptide and a multimeric protein.

Transcription

The first step is the conversion of the information into messenger RNA (mRNA). This process (transcription) is carried out by RNA polymerase. As with DNA synthesis, the RNA strand is made in the 5’ to 3’ direction.

There are major differences between transcription and replication.

Firstly, only a comparatively short molecule is produced, and **secondly**, only one of the DNA strands is transcribed. (Some genes use one strand, and some use the other, but in general **any specific region of DNA is only transcribed from one strand**). Since only a single strand is made, it can be produced continuously using a single enzyme; there is no need for lagging strand synthesis.

In addition the production of relatively short single-stranded RNA causes fewer topological problems: the enzyme and the RNA product can essentially rotate around the helix, so there is **no need for the helicases and topoisomerases** that are essential for replication.

Furthermore, RNA polymerase can start synthesis from scratch – no primer is needed.

Transcription is therefore considerably simpler than replication.

Since transcription results in the synthesis of comparatively short mRNA molecules (often just a few kilobases long, corresponding to a defined block of several genes) there must be a large number of signals around the chromosome that direct the RNA polymerase to start transcription at the required place and to stop when the block of genes has been transcribed. The start signals (promoters) also convey the information as to the direction in which transcription should proceed to work.

- **There are three types of enzyme transcription in eukaryotes is:**

1 - RNA polymerase I: found in the nucleolus and the transcription of genes responsible for making rRNA (type 28s, 18s).

2 - RNA polymerase II: the transcription of genes responsible for mRNA.

3 - RNA polymerase III: The transcription of tRNA genes in addition to a section of the rRNA genes of type (5 s).

- Another difference is important in enzyme transcription in the prokaryotic and the eukaryotes is that this enzyme is sensitive to rifampicin in the prokaryotic, where the unit under B in prokaryotic the target site for anti-rifampicin and therefore inhibition the transcription process, either in eukaryotes the enzymes is not sensitive to the rifampicin antibiotic.

- The enzyme molecule RNA transcription building trend 5^{-} - 3^{-} with adding ribonucleotides to the end 3 -OH and polymerize a large number of these nucleotides.
- With the transcription process in three stages: first stage (initiation) and the stage of elongation and the stage of termination.

In E. coli, depending on growth conditions, 2000–5000 copies of RNA polymerase may be engaged on mRNA synthesis at any time. The basic structure of RNA polymerase consists of four polypeptides – two identical a chains plus two other chains (ß and ß′) that are related to one another but are not identical. This structure (α_2ßß′) is referred to as the **core enzyme**. The specificity of promoter binding is due to a fifth subunit, the σ (sigma) factor; the complete structure including the s factor is called the **holoenzyme**.

There are different classes of promoters, recognized by different sigma factors, which allows selective expression of certain groups of genes.

After the **RNA polymerase holoenzyme binds to the promoter region**, the initial structure (the closed complex) is converted to an open complex in which there is localized separation of the two DNA strands. This exposes the bases of the coding strand, allowing base pairing of the ribonucleoside triphosphates for synthesis of the RNA. **The first phosphodiester bond is formed and the σ factor dissociates from the complex**. From now on, the core enzyme alone is required for extension of the RNA strand. A short region of the newly formed RNA remains base-paired to the DNA template, which keeps the DNA strands from re-associating, and therefore permits continued RNA synthesis, until a termination signal is reached when the mRNA and the RNA polymerase are released.

- Associated enzyme transcription template DNA a specific place (the process of organization is very non-random) and close to the desired gene cloned region of the promotor and the mean area of the promotor sequence of nucleotides on the DNA recognized by the enzyme transcription through unity sigma (σ) and associated to start the transcription process and are located short sequences at the beginning of the gene to be cloned).
- Vary the promoter in the prokaryotic than in the eukaryotes, as consisting in the prokaryotic of three regions (-35) and (-10) and(+1) (is the first nucleotide of the gene and which begin transcription).
- Be sequences of the promoter region (-35) is **TTGACA** a region recognize by transcription the enzyme within the promoter region. The area (-10) are called (**Pribnow box**) and be sequences **TATAAT** an area link enzyme transcription with the promoter, including the opening band DNA from each other.
- In eukaryotes there are areas (-25) and (-75) and the region is known (-25) are called (Hogness box). This is known sequences of nitrogen base (Consensus sequence) which determines the strength of the promoter.

- Begin the process of transcription a link enzyme RNA polymerase by promoter to be a complex promoter is called (**Close promoter complex**), which covers (60) double base of the double helix the enzyme recognize the promoter through the area (-35) on the promoter and through under the unit sigma (σ) to open a strip double helix of area (-10) where they are breaking hydrogen bonds of two bands (melting) then consists of a complex promoter open (Open promoter complex).

- Open bands from the area (-10) specifically because rich in nitrogen base T, A, connecting them bond binary be broken easier for the enzyme compared triple bond between nitrogen base of C, G, which abound in the area (-35), after opening bands are nitrogen base is exposed on coding tape and thus begin the enzyme transcription work, building a copy of the RNA according to the nitrogen base located on the tape coding of DNA and the multiplication first ribonucleotide in (+1) then (+2) and linking them bond phosphodiester bond (after that consists of a complex promoter open loses enzyme under unity sigma (σ), because its role is over and back Core enzyme) associated with these nucleotides of DNA tape coding by hydrogen bond consists hybrid (RNA-DNA) and then continue adding ribonucleotides that length of ten ribonucleotides and complete initiation phase of transcription by form primer short of RNA.

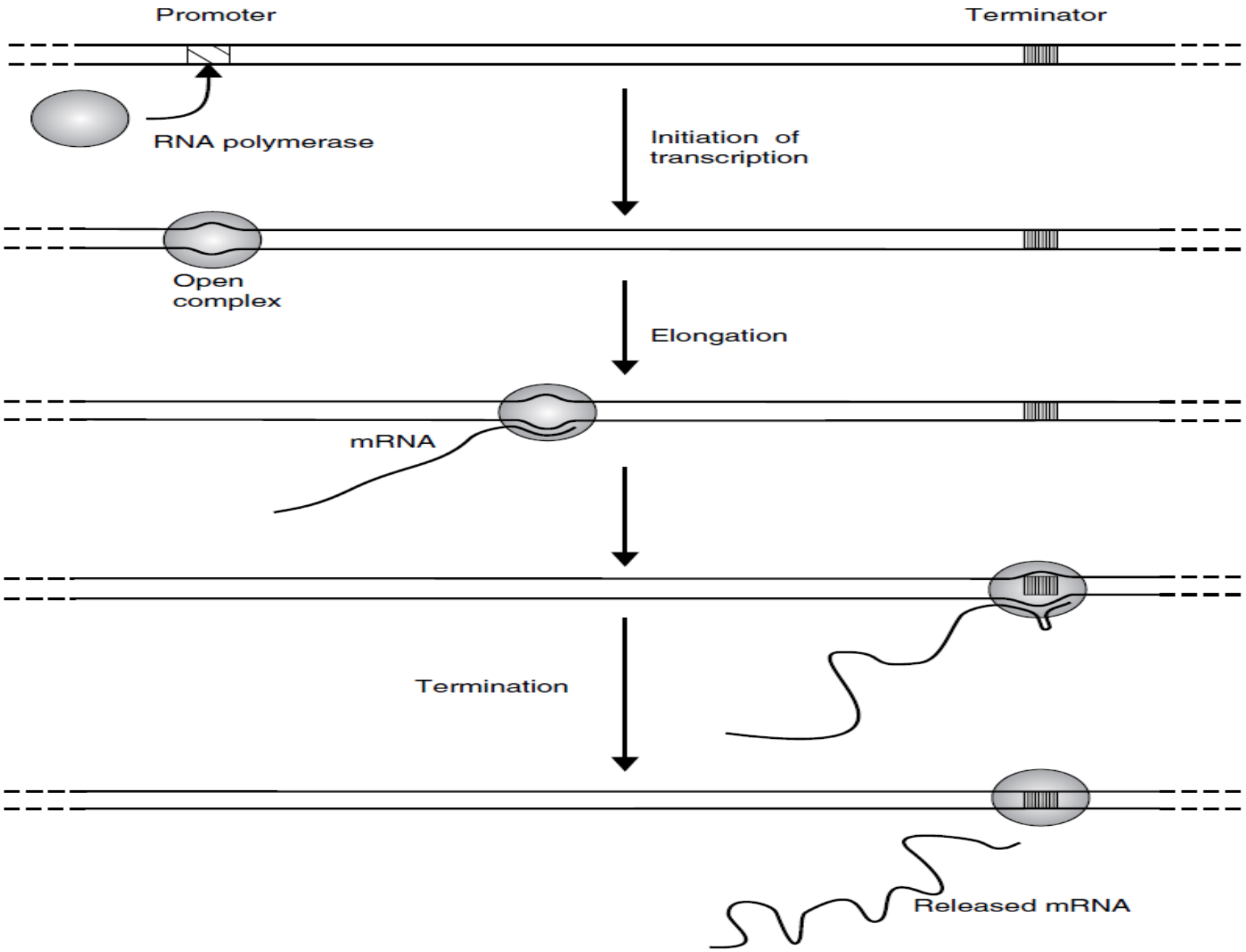
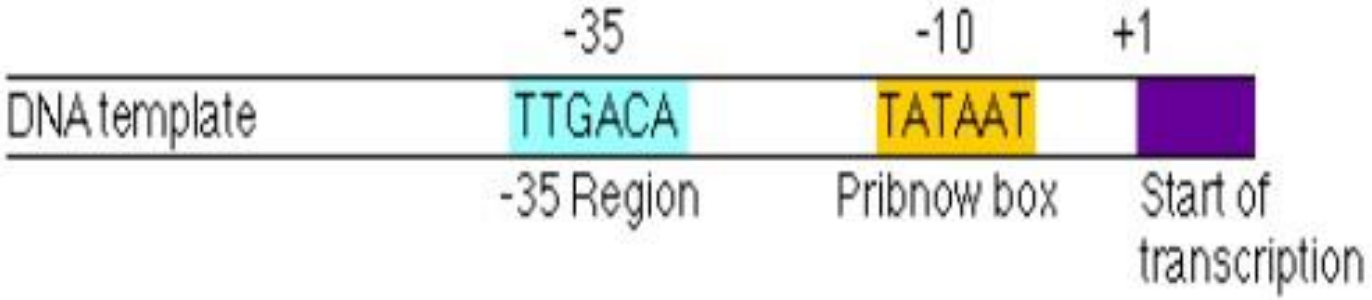
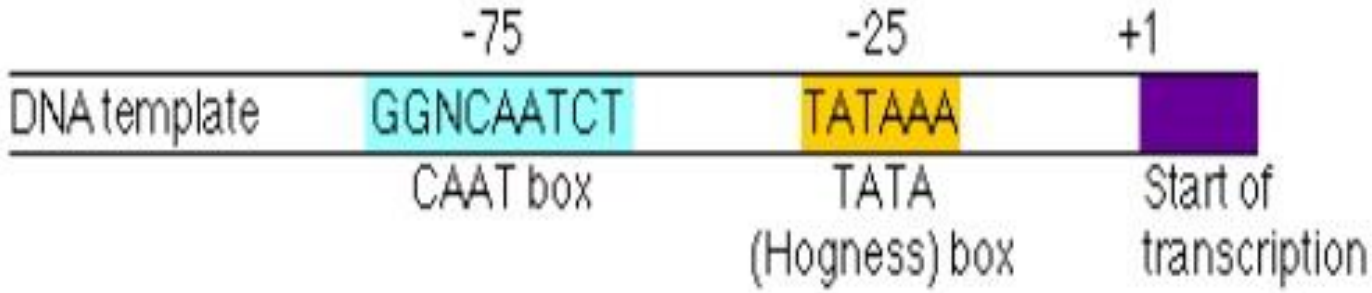


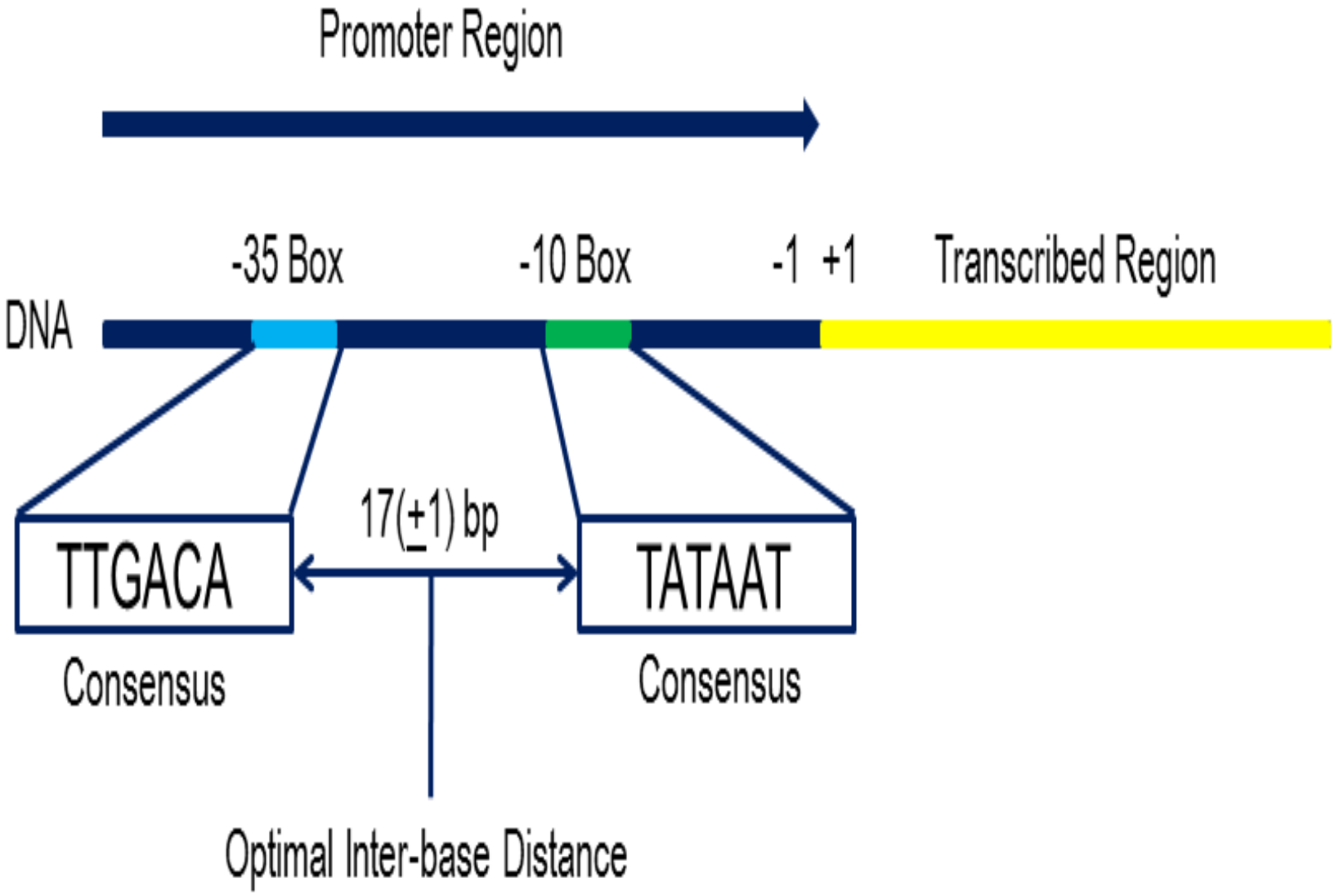
Figure 1.16 Main features of transcription

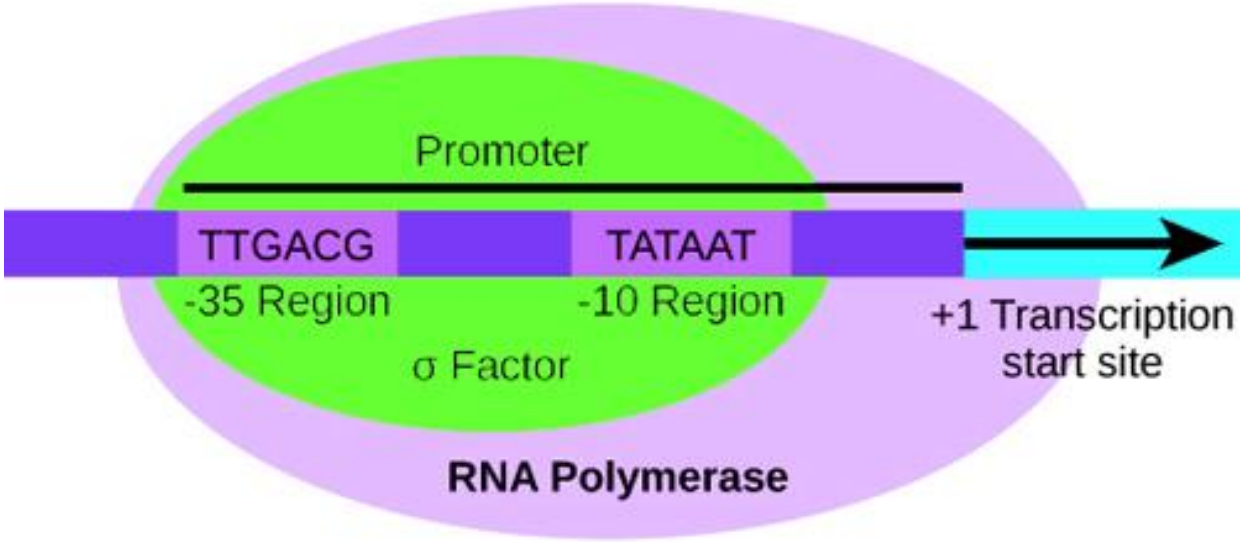
Prokaryotic promoter site:

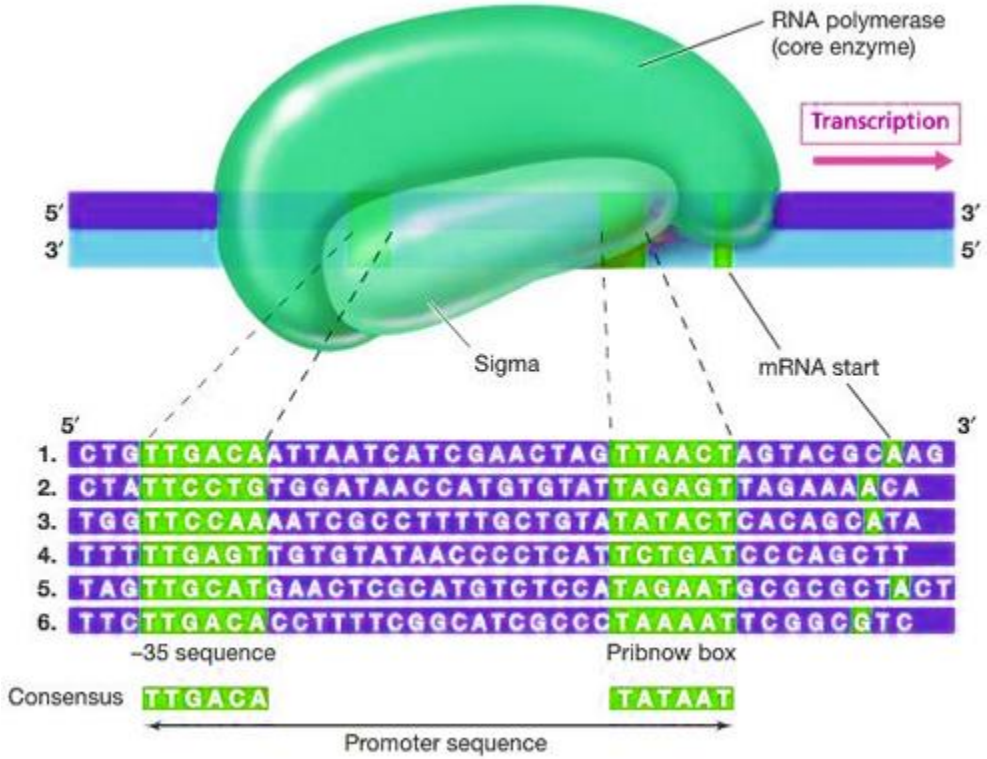


Eukaryotic promoter site:




Promoter Region
-35 Box
-10 Box
-1 +1
Transcribed Region
DNA
17(±1) bp
TTGACA
TATAAT
Consensus
Consensus
Optimal Inter-base Distance


Promoter
TTGACG
TATAAT
-35 Region
-10 Region
σ Factor
+1 Transcription start site
RNA Polymerase


RNA polymerase (core enzyme)
Transcription
5′
3′
3′
5′
Sigma
mRNA start
5′
3′
1. CTGTTGACAATTAATCATCGAACTAGTTAACTAGTACGCAAG
2. CTATTCCTGTGGATAACCATGTGTATTAGAGTTAGAAAACA
3. TGGTTCCAAAATCGCCTTTTGCTGTATATACTCACAGCATA
4. TTTTTGAGTTGTGTATAACCCCTCATTCTGATCCCAGCTT
5. TAGTTGCATGAACTCGCATGTCTCCATAGAATGCGCGCTACT
6. TTCTTGACACCTTTTCGGCATCGCCCTAAAATTCGGCGTC
–35 sequence
Pribnow box
Consensus TTGACA TATAAT
Promoter sequence

Transcriptional terminators

A characteristic feature of a transcriptional terminator is the **presence of a short sequence that is complementary to the sequence just preceding it**. When such a sequence is transcribed, the RNA formed can establish a **stem and loop structure**.

In most terminator sequences, the stem–loop structure is followed by a run of U residues.

RNA polymerase requires a short length of unwound DNA (about 17 bp) in which the two DNA chains are separated. However, the two DNA strands will tend to snap back together unless something prevents them.

It is the mRNA itself that is responsible for keeping the DNA 'bubble' open, by remaining base-paired with the complementary DNA strand for a short while.

Under physiological conditions, **the RNA–DNA hybrid is more stable than the DNA–DNA pairing.** The size of the bubble is limited however by topological constraints. The helix has to be unwound to some extent in order to allow the strands to separate, which causes stress in the molecule, since the two strands can only be separated by increasing the winding on either side. The larger the unwound region, the greater the stress. Beyond this point therefore the remainder of the mRNA molecule is dissociated from the template DNA

When the RNA polymerase encounters a transcriptional terminator sequence, it will transcribe it into RNA, with the consequent formation of a stem–loop structure which includes a portion of RNA that would otherwise be engaged on keeping the DNA bubble open. The bubble will close up, which will hinder the activity of the RNA polymerase. The enzyme will therefore pause at this point, a few bases beyond the stem–loop structure. Since the stem–loop structure in a typical termination sequence is followed by **a string of U residues**, all that keeps the **mRNA attached at this stage is the relatively weak hydrogen bonding of the A-U base pairs**. As a result, the **RNA tends to dissociate from the DNA template, thereby terminating mRNA synthesis.**

With some terminators, the stem–loop structure is not followed by a run of U residues. Although the RNA polymerase may pause at these sites, termination is dependent on the activity of another protein known as the **rho factor.** These are therefore known as **rho-dependent terminators.**

To be completed after transcription the desired gene transcription ends in a specific region of DNA (a process regular very non-random), but how the enzyme known as transcription should be termination at the moment.

Found in *E.coli* bacteria the end transcription depending on:

1. Found a sequence of bases on the DNA stripe at the end of the gene rich bases G and C, followed by a region rich in bases A, T when copying to the RNA copied to the U and A, regions of DNA are called inverted repeat or palindrom read from right to left and reversal, is called dyad symmetry when transcript installation is made up like a pin hair or steam loop structure has a tail of bases uracil (5 to 10) this structure affects the stability of hybrid RNA:DNA and thus separated mRNA and ends with transcription.

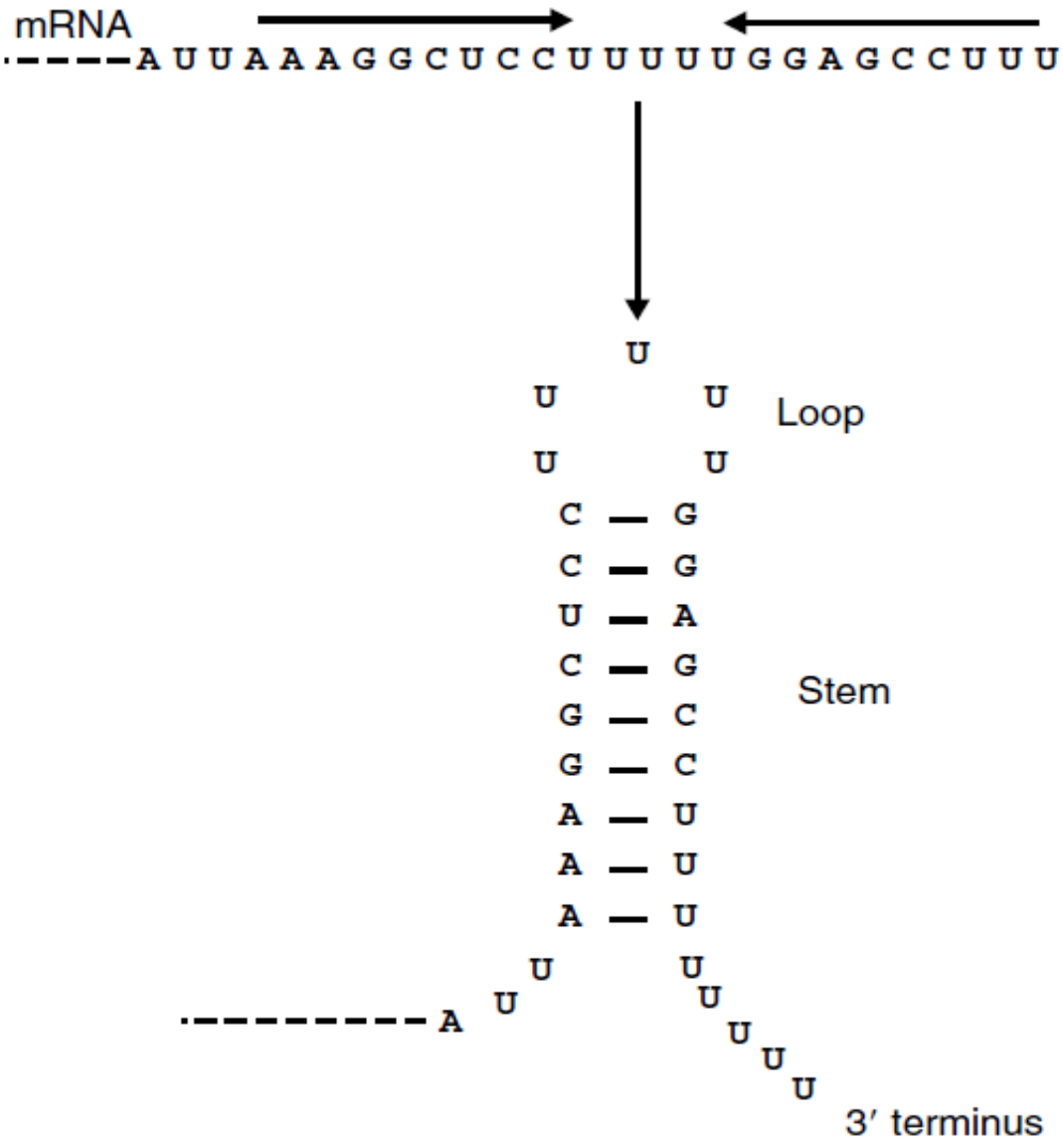


Figure 1.17 Structure of a typical terminator. The regions marked with arrows are complementary, and so can anneal together resulting in the formation of a stem–loop structure

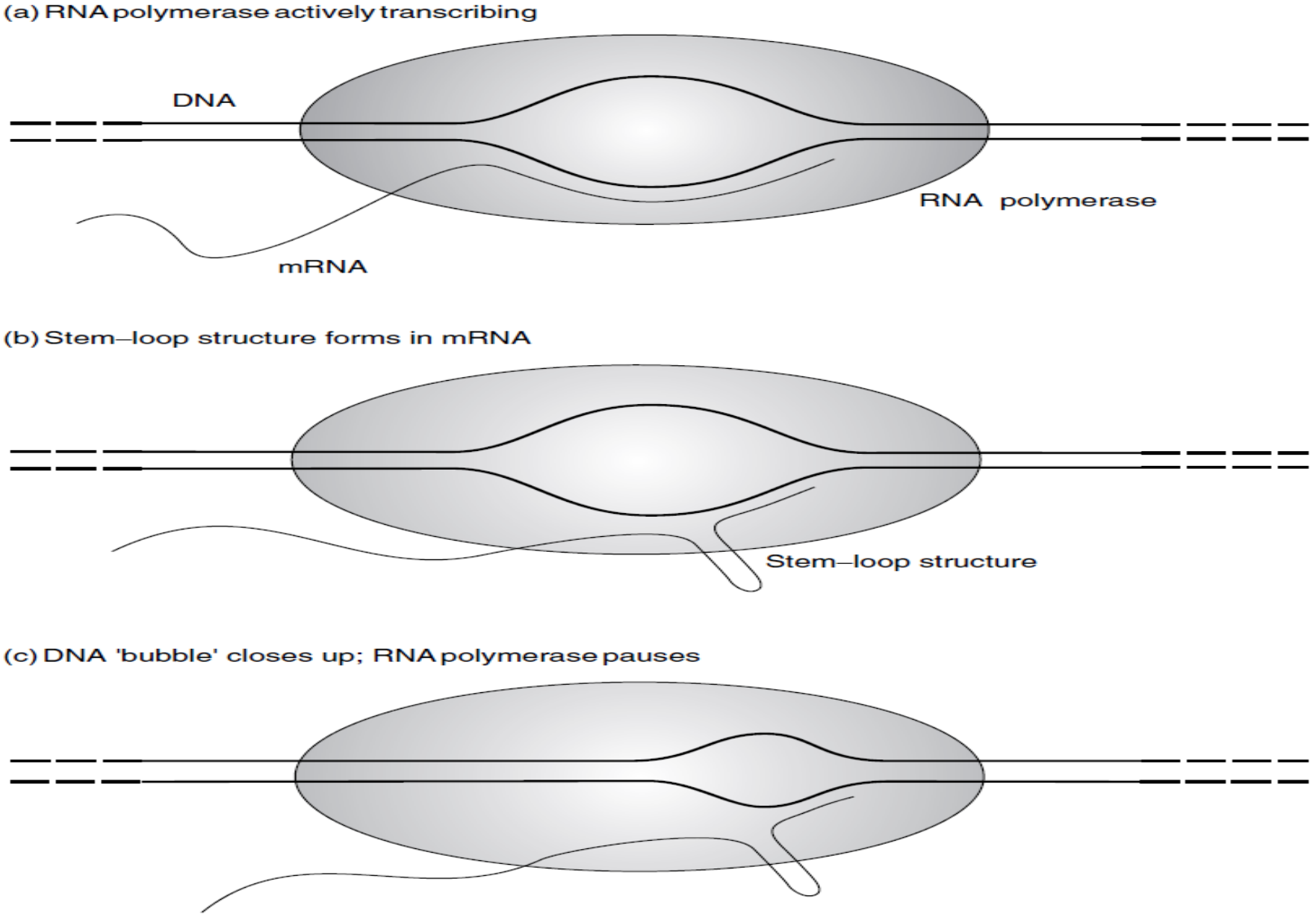
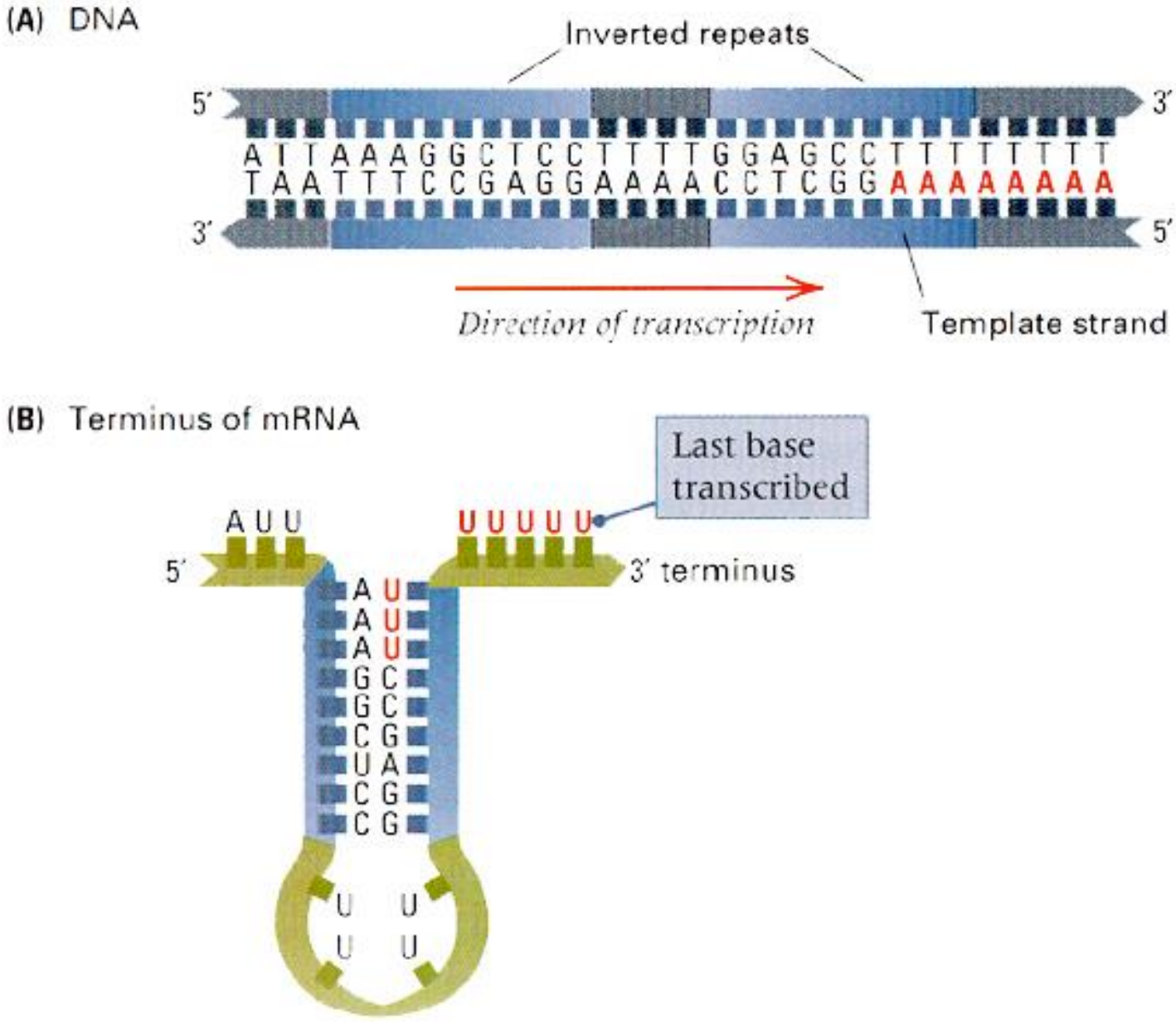


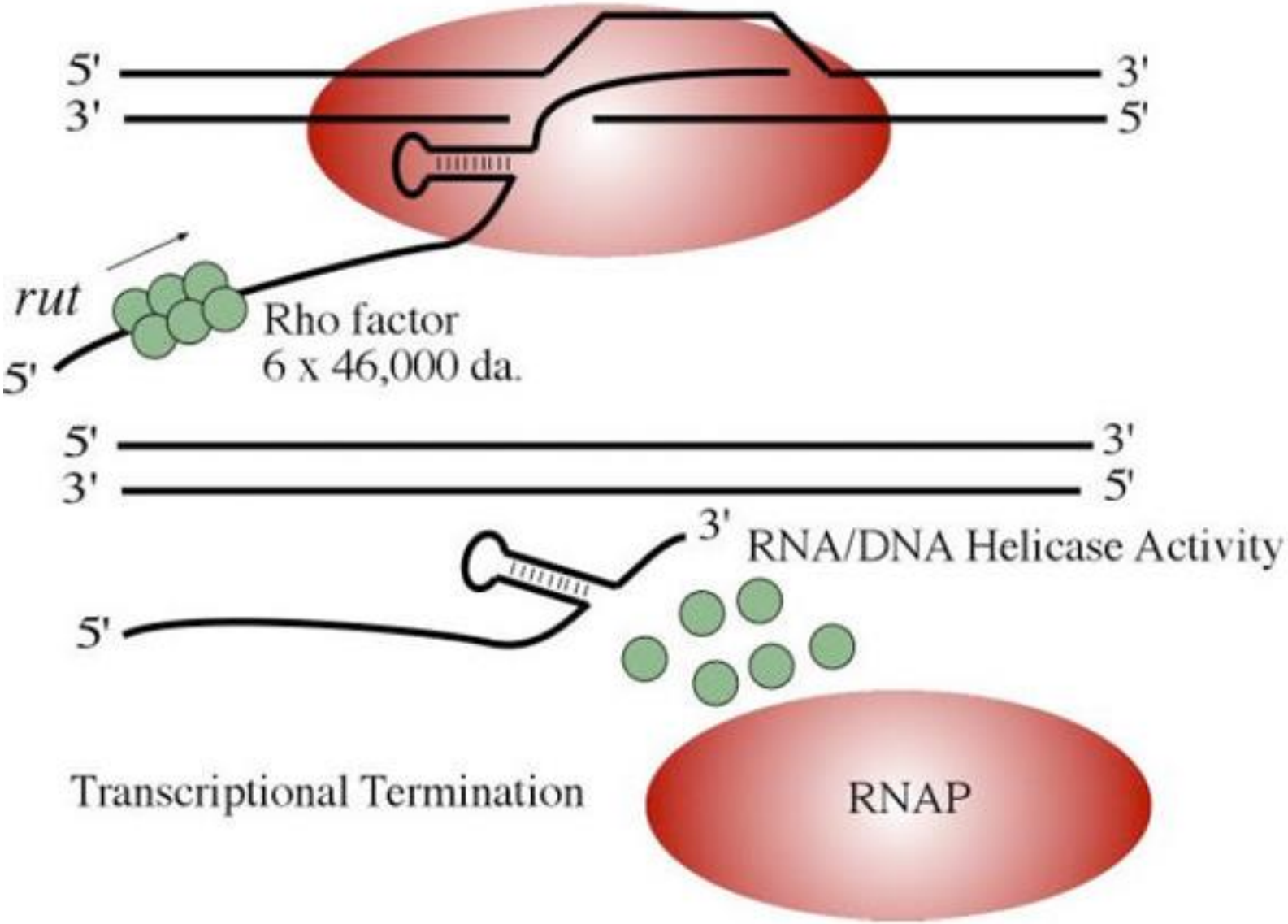
Figure 1.18 Model for transcription termination. (a) During transcription, the presence of the mRNA keeps the DNA strands separated over short region; (b) when the termination site is reached, the mRNA forms a stem–loop structure; (c) this allows the DNA strands to re-associate, leading to a pause in transcription


(A) DNA
Inverted repeats
5′
3′
A T T A A A G G C T C C T T T T G G A G C C T T T T T T T T
T A A T T T C C G A G G A A A A C C T C G G A A A A A A A A
3′
5′
Direction of transcription
Template strand
(B) Terminus of mRNA
Last base transcribed
A U U
U U U U U
5′
3′ terminus
A U
A U
A U
G C
G C
C G
U A
C G
C G
U U
U U

The end-dependent on protein Rho (Rho dependent)

- It was found that there are protein small is known (Rho protein) which is associated with stripe RNA developing when the transcription to the end and separated from the DNA template and stop the transcription process, after the end of the transcription process separated the enzyme RNA polymerase associated with the Unit sigma (σ) of a new return cycle transcription new.

Rho-Dependent Termination



There are modifications obtain to the RNA molecule after the transcription of DNA stripe coding, a process to DNA (RNA processing):

- 1- known to be prokaryotic cells contain connected genes within the coding stripe of the DNA not separate between genes sequence called the (Intron) and thus when transcription you reproduce a copy of the RNA can be translated at the same time, even during the transcription process.
- But it's different in eukaryotes was characterized by the presence (Split gene) genes that contain coded sequences (exons) intermediate with areas of non-coding (Introns).
- When transcript mRNA contains the introns and exons, and after transcription remove (Introns) and still remain areas encoded (exons) that are translated later to the protein by translation process, so the process of transcription separated from the translation process in the eukaryotes and are treated to introns through cutting enzymes by attacking certain sequences in the interval between (Introns) and (exons) and cut and remove (Introns) are then link (exons) together and this process is called (Splicing).
- 2- Is added Cap structure to finish 5 $^{-}$ of mRNA in eukaryotic and the addition of a multi-Adenosine (Poly-A) to finish 3^{-}, either in the prokaryotic not added Cap structure but is added only multi-Adenosine (add CCA to the end 3^{-}) added almost 200-150 adenosine to end (3 $^{-}$) by the enzyme (**Terminal transferase).**

What's Different PROKARYOTES and EUKARYOTES

EUKARYOTES	PROKARYOTES
three different RNA polymerases	one type of RNA polymerase
Transcription takes place in the nucleus; translation occurs in the cytoplasm. The two processes are spatially and temporally separated.	Transcription and translation occur simultaneously.
The mRNA transcript is modified during transcription. Precursor mRNA (a.k.a. "heterogenous nuclear RNA, or hnRNA) is found only in the nucleus. There is only about 25% similarity between hnRNA and the finished mRNA of a eukaryote.	The mRNA transcript is not modified.
Promoter consensus sequences (the most common of which are the TATA ("Goldberg-Hogness Box"), the CAAT box and the GC box), differ from the Pribnow Box.	Promoter contains the Pribnow Box (5'-TATAAT-3')
Initiator factor (IF) proteins localize and stabilize the initiation complex at the start of a gene.	Initiation sites are recognized by sigma factors.
Mitochondrial and chloroplast genes are transcribed (and translated) within the	No mitochondria or chloroplasts

References

Molecular Genetics of Bacteria. By Jeremy W. Dale and Simon F. Park2004 , 4 edition, John Wiley & Sons Ltd.

p.: 19 - 26