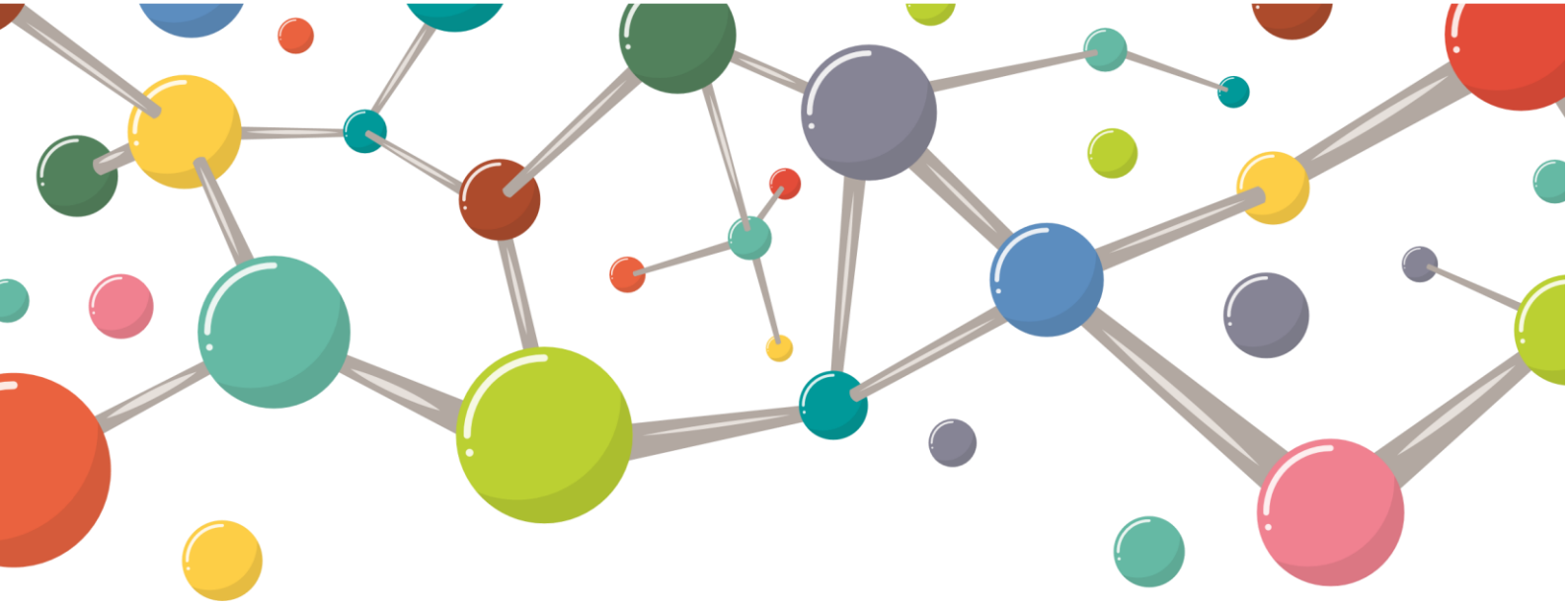

MOLECULAR BIOLOGY

SHEET (7)

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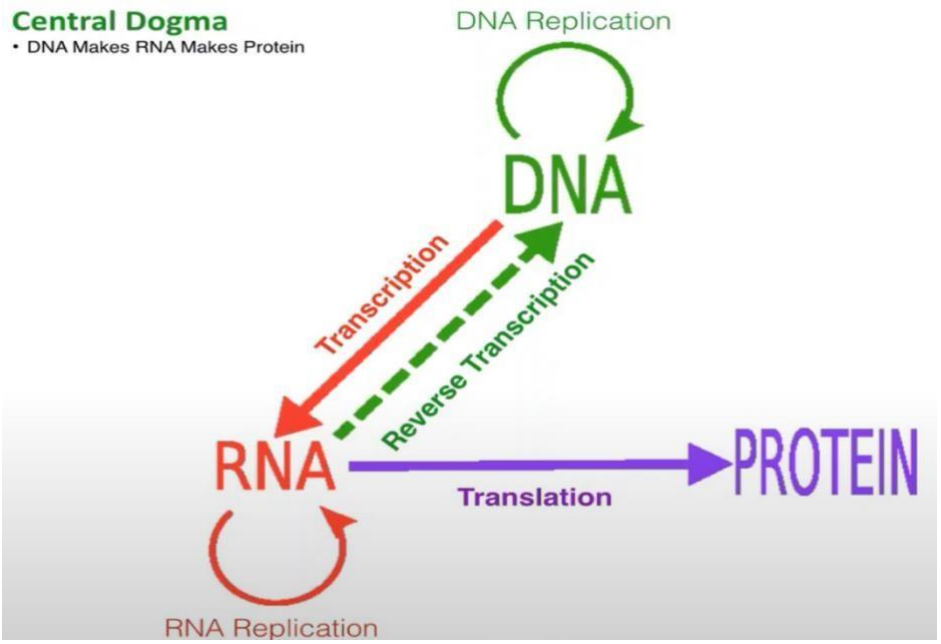
Black color: doctor's explanation, Navy color: slides, Blue color: extra information, Red color: Dr. Khaldun's notes, highlighted sentence: modified, purple color: book notes .

BACTERIAL TRANSCRIPTION AND ITS REGULATION

Before we begin, we must recall **the central dogma**:



In previous lectures, we discussed and studied how the DNA is replicating (how DNA gives another DNA in a process called DNA replication).

And now we're going to study how is the information found in the DNA (base sequence) transcribed into RNA. This process is called the **transcription.**
In simple words, transcription is to make RNA from DNA, which requires tools and materials (specific elements) to make that process.

<u>Generals notes and characteristics of the transcription:</u>

• Transcription requires:

a) A promoter (transcription begins near a promoter)

b) NTP (nucleoside triphosphate) such as ATP, GTP, CTP and UTP (the four of them)

c) An enzyme called RNA polymerase.

⇨ In the **prokaryotic** system: there is **one** RNA polymerase that produces different types of RNA.

⇨ In the **eukaryotic** system: there are **three types** of RNA polymerases which synthesize different types of RNA.

- The RNA synthesis follows the rules of **base-pairing** of DNA (chargaff's rule) ==> A base pairs with U as C base pairs with G.
- Only ribonucleoside 5'-triphosphates participate in RNA synthesis. The first base to be laid down in the initiation event is a triphosphate. Its 3'-OH group is the point of attachment for the second nucleotide and its 5'triphosphate group remains at the S'-end throughout chain elongation.
  •
- Transcription of the DNA to RNA proceeds in 5' to 3' direction, because the RNA polymerase only polymerizes in the 5' to 3' direction as this enzyme also has 3' to 5' exonuclease activity for proofreading.
- There is one strand that is copied from the double stranded DNA, as one of the DNA's two strands is used as a template for the transcription (synthesis of the messenger RNA) ==> for a specific polypeptide chain (which means that the mRNA resulted from the transcription process, will later on be translated into a specific polypeptide chain by a process called translation (which we will study later)).
- Transcription does not require a primer as in DNA replication. It starts with a purine as it uses it's 3' hydroxyl group to make a phosphodiester bond with the first nucleotide which will be inserted according to the base pairing rules.
- SUMMARY

Transcription

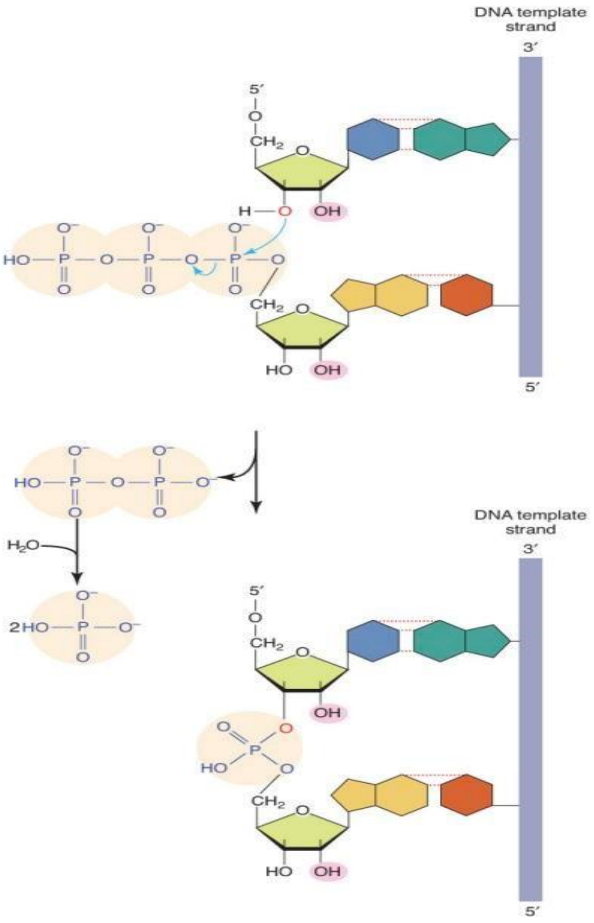
- **DNA Makes RNA**
- **Begins Near a Promoter**
- **Requires ATP, GTP, CT, and UTP**

- **Uses Base-Pairing Rules of DNA**
- **Requires RNA Polymerase**
- **Proceeds in 5' to 3' Direction**
- **Only One Strand is Copied**
  •

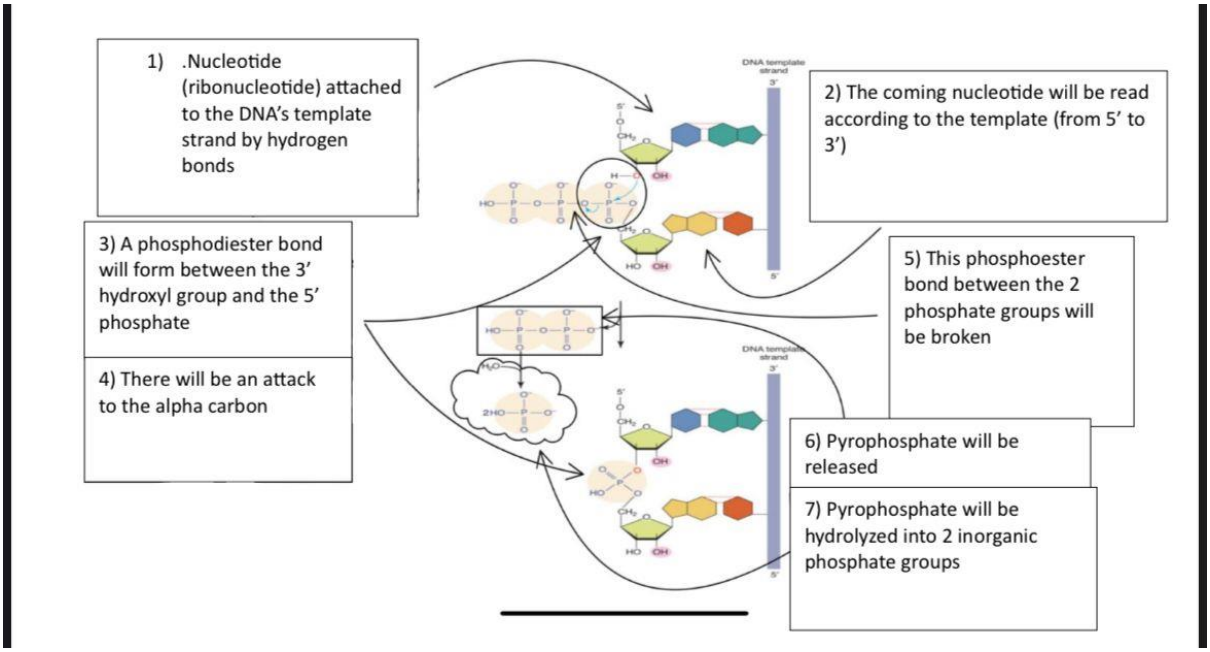
INTRODUCTION TO THE BACTERIAL RNA POLYMERASE CATALYZED REACTION

Now we are going to talk about the RNA polymerase in terms of structure and function.

RNA polymerase will catalyze this type of reaction:



Now the question is: how does this catalyzed reaction occur?



1) Firstly, there is a nucleotide (ribonucleotide actually) attached to the DNA's template strand
2) The coming nucleotide will be read according to the template (from 5' to 3')
3) A phosphodiester bond will be formed between the 3'-hydroxyl group and the 5'-phosphate group
4) There will be an attack to the alpha-carbon
5) The phosphoester bond between the first adjacent phosphate groups in the triphosphate region of the NTP will be broken
6) Pyrophosphate will be released
7) Pyrophosphate will be hydrolyzed into two inorganic phosphate groups.
The energy released by this reaction will keep the direction of polymerization in the 5' to 3' direction, forming phosphodiester bonds which will continue according to the information (base sequence) on the template.

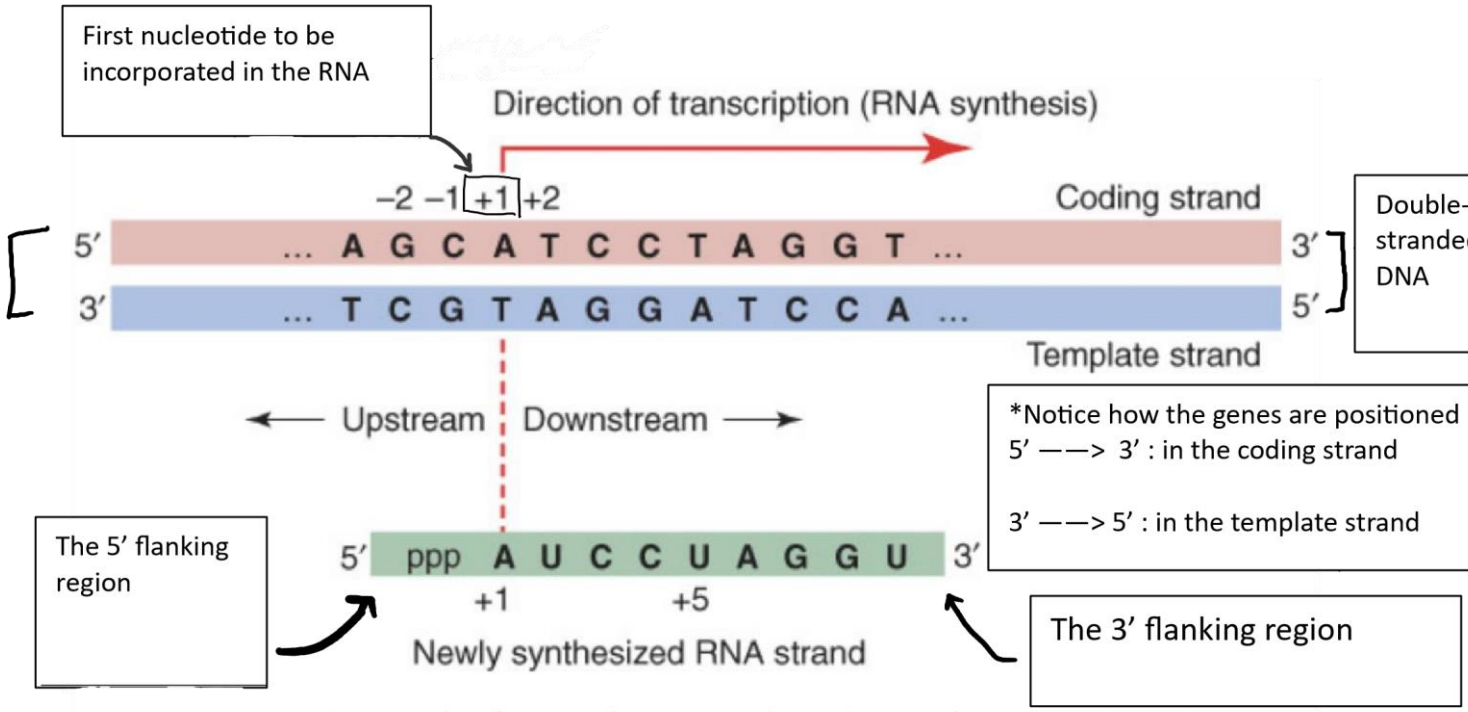


Figure 12.02: Rules for numbering nucleotides on the sense strand.

- The strand that is **used** for transcription is called **the template strand**, **the non-coding strand** or **the anti-sense strand**.
- The strand that is **not used** for transcription is called **the coding strand** or **the sense strand.**
- The transcription could use one strand or the another at a time.
- The polymerization is in the 5′ to 3′ direction.
- So when the genes are positioned in one strand from the 5′ to 3′ direction, that strand will be the coding (or sense) strand and the other strand will be the template (anti-sense or non-coding) strand.
- However, if the genes are positioned in the opposite direction (from the 3′ to 5′) in the strand, that strand will be the template (anti-sense or non-coding) strand and the other strand will be the coding (or sense) strand.

  (Look at the figure above and compare it with the last two paragraphs)

- Now we will discuss how the nucleotides are numbered (the numbers above the nucleotides in the above figure):

⇨ the (+1) number means the first nucleotide in the transcription (the first nucleotide to be incorporated in the RNA or the site where the transcription starts)

⇨ Every nucleotide that comes right (in the figure) to the (+1) nucleotide will take a positive (+) sign. That direction or region of the positive signs of the nucleotides is called the downstream region of the gene (the 5' region of the gene, which is the 5' in the template strand)

⇨ Every nucleotide that comes left (in the figure) to the (+1) nucleotide will take a negative (-) sign. That direction or region of the negative signs of the nucleotides is called the upstream region of the gene. (The 3' region of the gene, which is the 3' in the template strand)

So, the (+) sign means downstream, while the (-) sign means upstream. Furthermore, the positive numbers refer to the sequential number of the nucleotide in the RNA, while the negative numbers refer to the number of the nucleotides upstream to the first nucleotide to be incorporated in the RNA.

Nucleotides with the plus sign (+) indicate where the location of every nucleotide in the gene or in the RNA is, as they are the coding sequences for RNA or proteins.

Nucleotides with the minus sign (-) are very important to indicate some important regulatory sequences that control the gene, but not included in the gene itself.

In the eukaryotes: only the positive-numbered nucleotides will be transcribed and some of them will be translated into polypeptides.
In the prokaryotes: all of the nucleotides carrying positive signs will be within the RNA that is transcribed and translated as all of them are going to be translated into proteins.

Note: some of the nucleotides that carry a negative sign (-) will be included within the RNA (which means they will be transcribed) but they won't be translated.

The plus (+) or minus (-) sign are significant for location determination because there could be some mutations in the gene either upstream or downstream. So in order to determine the location of the mutation, we need a sign that indicate if the mutation is upstream or downstream and we need a number to know where is the mutation exactly and how far it is from the first nucleotide to be transcribed (for example: a mutation in the -500 nucleotide (upstream and 500 nucleotides before the first nucleotide to be transcribed) or in the +1000 nucleotide
(downstream and 1000 nucleotides after the first nucleotide to be transcribed)).

- The newly synthesized RNA could be:

a) Messenger RNA (mRNA)
b) Transfer RNA (tRNA)
c) Ribosomal RNA (rRNA)

- The newly synthesized RNA has the **same** base sequence as in the **coding (or sense) strand**, except for U (Uracil) instead of T (Thymine).

⇨ In prokaryotes: a gene that is transcribed into mRNA, that mRNA is going to be translated into more than one polypeptide

⇨ In eukaryotes: each mRNA will give a single specific polypeptide chain.
(it will be explained why later in this sheet)

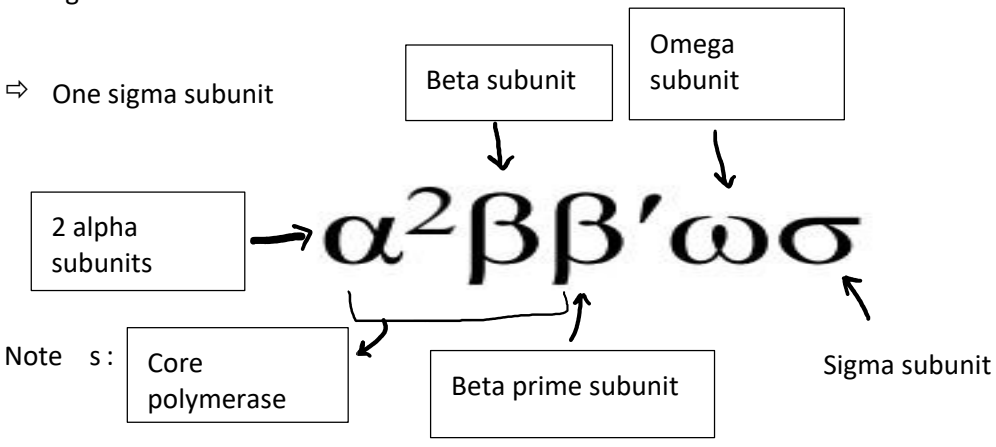
- It is important to know that every gene has boundaries (a starting point and a termination point) as transcription occurs in one gene or some genes, but not all the genes at the same time. Transcription will take place according to the need of the cell. If the cell requires a function or some functions that are produced by some gene or a group of genes, it has a mechanism to transcribe only that gene or that specific group of genes.

Now we are going to talk about the structure of RNA polymerase:

RNA polymerase is not a simple structure as it is composed of many subunits (a subunit is a polypeptide chain interacting with another polypeptide chain non-covalently, for example: when we say that an enzyme is composed of 3 subunits. That means that it is composed of 3 polypeptide chains non-covalently interacting with each other through hydrogen bonding or electrostatic (ionic) bonds).

- RNA polymerase is composed of 6 total subunits:

⇨ Two of them are alpha-subunits
⇨ One beta subunit
⇨ One beta prime (Beta') subunit
⇨ One omega subunit
⇨ One sigma subunit



a) the alpha and beta subunits are names for these subunits and are different from alpha-helices and beta-pleated sheets of the secondary structure of the polypeptides or proteins.

b) Each subunit has its own function.

Bacterial RNA polymerase consists of a core enzyme and sigma factor

$$\underset{\text{RNA polymerase holoenzyme}}{\alpha_2\beta\beta'\sigma} \rightleftharpoons \underset{\text{core polymerase}}{\alpha_2\beta\beta'} + \underset{\text{sigma factor}}{\sigma}$$

Early studies with holoenzyme without omega showed that a2BB'o can dissociate to form:
core polymerase ($\alpha_2\beta\beta'$)
Sigma factor (σ)

a2BB' core polymerase can synthesize RNA using single-stranded DNA or nicked double-stranded DNA as template, but it cannot use intact double-stranded
DNA as a template
Neither sigma nor omega is required for phosphodiester bond. formation

We are going to concentrate on the function of the sigma subunit later on.

- Prokaryotic RNA polymerase can recognize and transcribe about 4300 genes, signaled by about 1000 different binding sites. These binding sites are located at the upstream region very close to (+1) and RNA polymerase will be able to recognize these regulatory sequences (there are about 1000 of them) to transcribe about 4300 genes (the number of genes is roughly about 4-folds the number of binding sites). So, it is concluded that in prokaryotic systems, a set or group of genes will be regulated or controlled by one regulatory sequence (one binding site for RNA polymerase). This regulatory sequence or binding site for RNA polymerase for a set of genes is called a **promoter**.

A transcription unit must have an initiation signal called a promoter for accurate and efficient transcription to take place
In contrast to the core polymerase (a2ßß'), the holoenzyme (a2BB'б) can use intact double-stranded
DNA as a template
Transcription begins when the holoenzyme recognizes a specific transcription initiation sequence called a promoter
Sigma factor is essential for promoter DNA recognition
The primary E. coli б factor is б70

because its molecular mass is about 70 kDa,

*

Sigma factor is essential for promoter DNA recognition but does not bind to promoter DNA on its own

⇨ In **eukaryotic** systems, for every **gene** there is **a promoter**

⇨ In **prokaryotic** systems, for every **group of genes** (which have a common function or the gene group are transcribed and then translated to form integrating polypeptides with common function such as lactose operon which we will talk about later) there is **one promoter.**

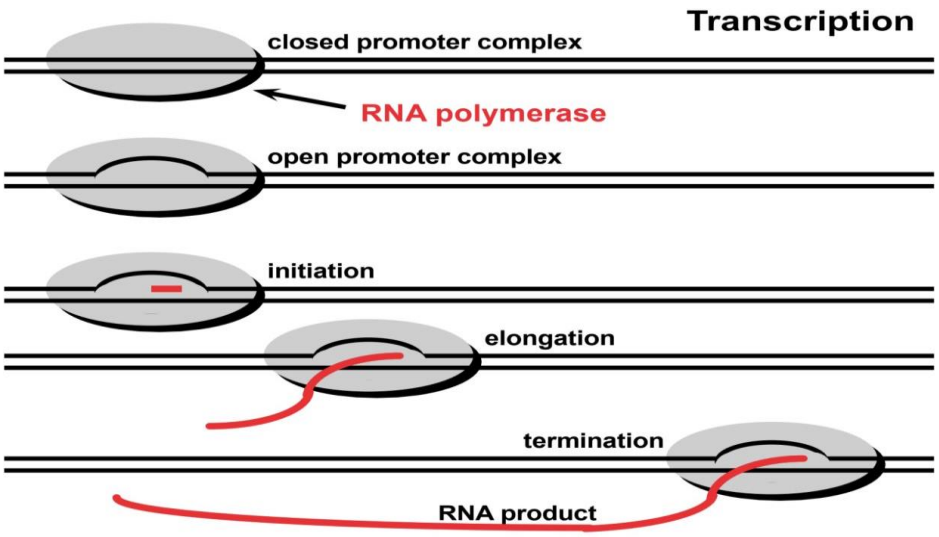
This is why the number of binding sites (or promoters) does not equal the number of genes in the prokaryotic system (roughly every 3 or 4 genes will have one regulatory (or binding site) for RNA polymerase to transcribe them.

————————————————————————————————

an overview of the transcrIptIon mechanIsm

This is an overall description of the transcription process

(The transcription is summarized in these stages)



- The RNA polymerase will recognize its binding site (or its promoter) to form what is called the **closed promoter complex.** (Identification of the binding site (promoter) for RNA polymerase is a process which we will talk about later).

- After the RNA polymerase binds to its specific binding site (or the promoter), it will partially unwind the double stranded DNA forming what is called the **open promoter complex**.
- After doing this, the first step or stage of transcription (which is initiation) will start, and in this process about 9-10 nucleotides will be polymerized according to the complementary bases on the DNA template strand by RNA polymerase.
- Then, RNA polymerase will leave the promoter and will start moving in the 5'➒3' direction entering the elongation stage of transcription (elongation: incorporating more and more of nucleotides complementary to the base pairs in the DNA template strand).
- As the RNA polymerase incorporates more nucleotides and elongates the RNA, the 5' region of the RNA will leave the transcriptional bubble and the 3' region will still be base pairing (or hybridizing) between the RNA and DNA template strand with RNA polymerase, which will continue until it reaches the termination signal.
- Once it reaches the terminal signal, it will stop and release the RNA, as the RNA polymerase will be released from the double stranded DNA (the assembled structure made by RNA polymerase, RNA and DNA will be dissociated as they fall apart from each other when reaching the termination signal).

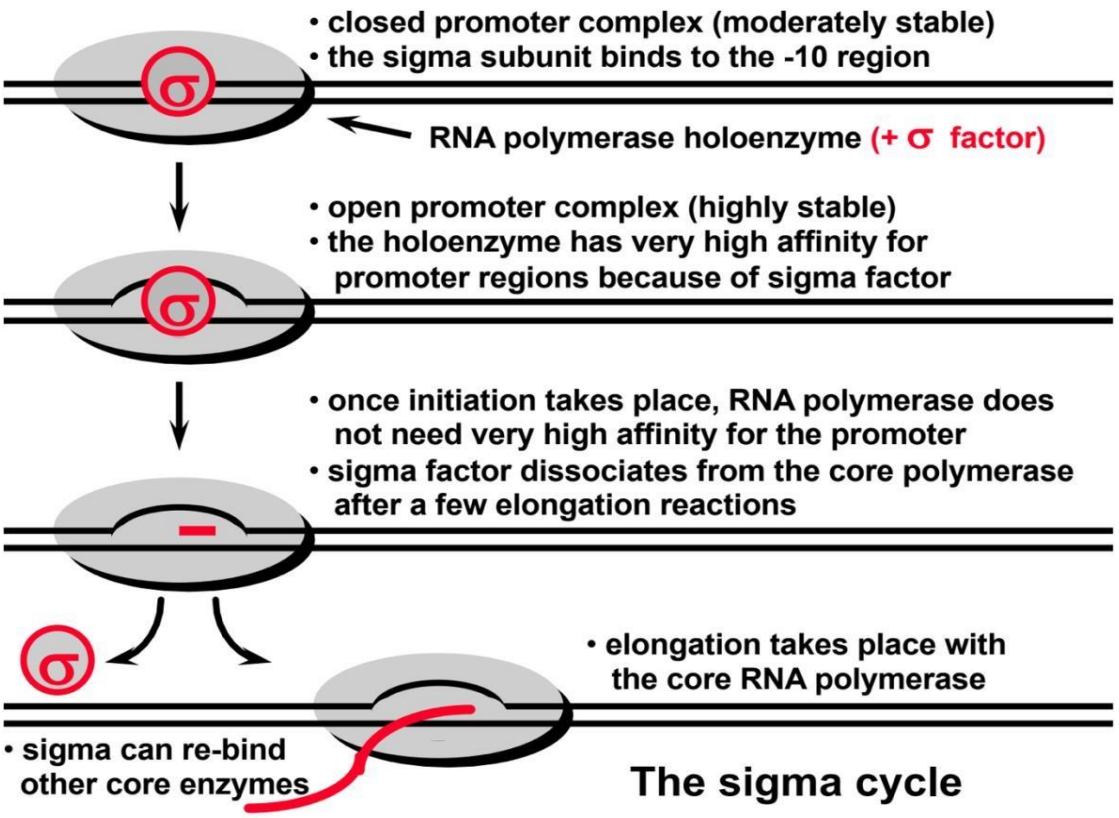
(Initiation➒➒Elongation➒➒Termination)

Initiation then elongation then termination, just as we saw in DNA replication

Now we will examine the first step of transcription, **the initiation stage**.

————————————————————————————

INITIATION STAGE



• What do we mean by RNA polymerase holoenzyme?

All the subunits in the RNA polymerase including (Sigma) subunit.

(Omega) subunit is not important here, but (Sigma) subunit with the two (Alpha) subunits, (Beta) and (Beta-prime) subunits, all of these are called **holoenzyme**.

If (Sigma) subunit leaves the holoenzyme, we are left with what is called the **core enzyme**.

In simple words,

The Core Enzyme= Holoenzyme — (Sigma) Subunit

- The holoenzyme will start looking for the specific binding site for RNA polymerase (the promoter), which is assisted by the (Sigma) subunit.
- (Sigma) is the principle subunit, looking for the recognition binding site of RNA polymerase (the promoter)
- So the holoenzyme will start scanning the sequences randomly around the gene, until (Sigma) finds the recognition binding site of RNA polymerase. Once it finds it, they will bind (at the (-10) region) and form the **closed promoter complex**.
  (Did you now figure out the importance of the sign (- or +) and the numbers of the nucleotides such as (-10)??)
- The promoter ((-10) region) has a regulatory sequence and one of the functions of them is to bind to the RNA polymerase. If the RNA polymerase doesn't bind at a specific site (or at a promoter), transcription will not happen.
- So, in order to initiate transcription (or to begin transcription),the RNA polymerase must find the promoter of the gene and bind to it to form the **closed promoter complex**.
- Then, in the presence of sigma, the RNA polymerase will unwind partially the double stranded DNA and start doing some polymerization in that region (polymerizing about (9-10) nucleotides) .
- Once that happens, the (Sigma) subunit will leave, and once (Sigma) leaves, there is no need for RNA polymerase to stay bound on the promoter, because it finished the initiation and must enter the elongation stage.
- In order to start the elongation stage, the (Sigma) subunit must leave from the holoenzyme, which will decrease the binding affinity of the RNA polymerase to the promoter and let the RNA polymerase **core enzyme** to start the process of elongation process.

Nature of promoter:

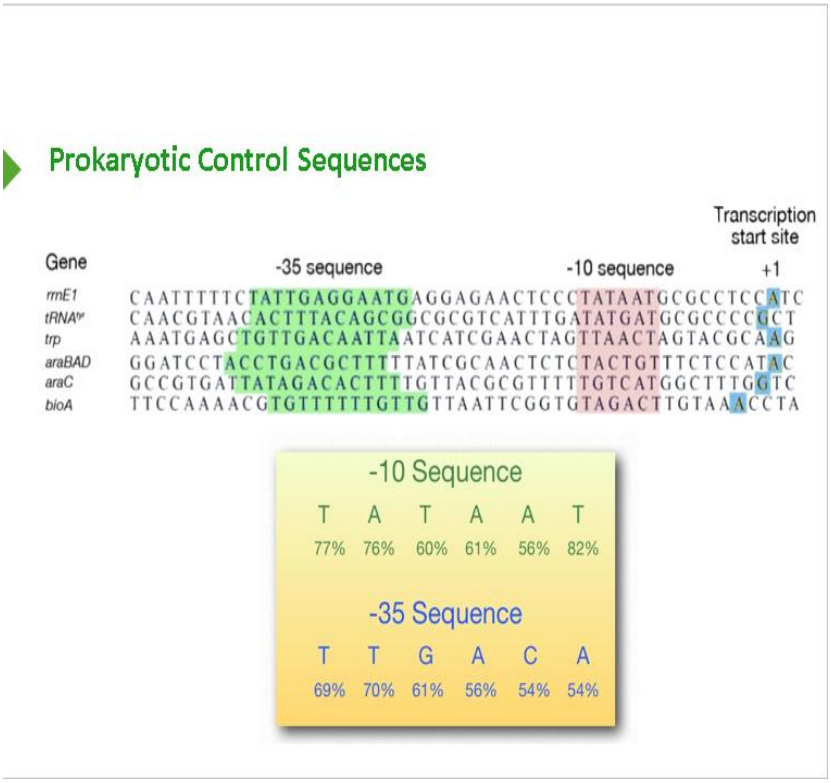
<u>Promoter</u> of a gene is a specific sequence of nucleotides located mainly on the upper stream of a gene (towards the 5' end of the gene).

The following picture shows the position of the promoter regions and their sequences.

- **Idealized sequence is termed a consensus sequence.**

The promoter regions of various different genes (of σ70 genes in E. coli) present common features. It was observed that a 6 base pair sequence about 10 pairs upstream from the start sites was similar and conserved in all of the promoter regions of different σ70 genes. This 6bp sequence was later known as the -10 box or the "Pribnow box" referring to the scientist who discovered it. Another 6bp sequence was later found to also be present in the promoter of many genes and was termed the -35 box. The negative sign refers to the sequence being upstream, and the number refers to the number of base pairs the boxes are located away from the transcription starting site of the gene.

The promoter sequence is not the same for all genes but has some similarities such as these boxes. 300 E.coli genes were looked into and their promoter sequences were compared with each other, this picture shows the percentage of each nucleotide in the promoter boxes of different genes.



The two boxes are present in all σ70 genes, and the sequence of the nucleotides in these boxes are similar, but not exactly the same. And because the sequence of these boxes isn't exactly the same in all promoters, an idealized sequence for these boxes is necessary. These idealized sequences indicate the most frequently found base in each position of many (300) actual sequences, and idealized sequences are known as consensus sequences.

The consensus sequence for the -10 box is TATAAT, and the one for the -35 box is TTGACA.

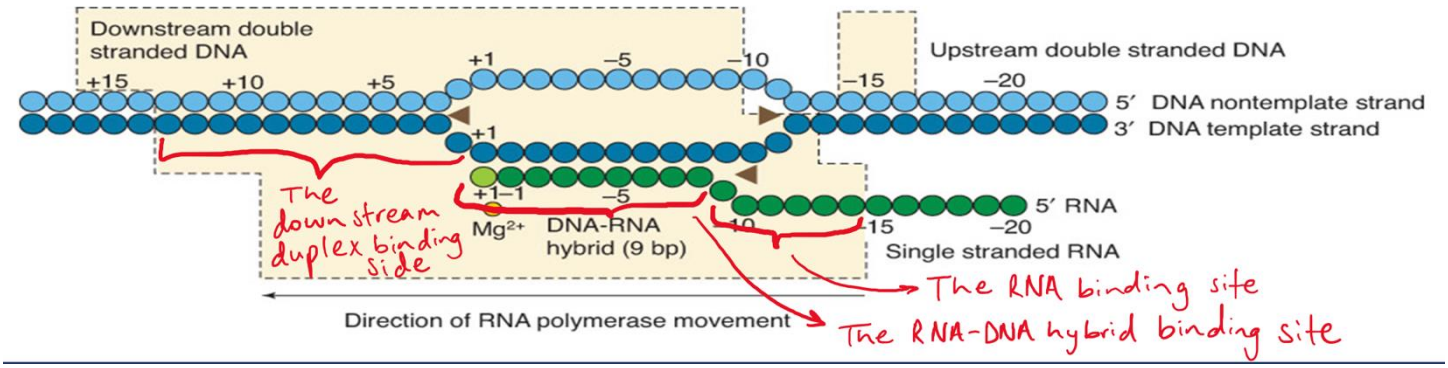
Promoters that have boxes with sequences similar to their consensus sequence or close to it, are considered Strong Promoters, because having a sequence close to the consensus sequence raises the binding affinity of RNA Polymerase, and thus raises the transcription activity (this called gene is transcribed frequently).

The Transcription Elongation Complex

- The transcription elongation complex consists of core RNA polymerase, template DNA and a growing RNA chain
- RNA chain elongation catalytic cycle

1. NTPs **(Nucleosides triphosphates)** move through the secondary channel of the transcription elongation complex to reach the catalytic site (this is the rate limiting step because only a 1 in 4 chance the correct nucleotide will move through).
2. 3'-hydroxyl group at the growing end of the RNA strand makes a nucleophilic attack on the α-phosphoryl group of the incoming NTP to form the phosphodiester bond.
3. Polymerase moves one Nucleotide downstream at a rate of 30 nucleotides/sec. Incoming NTPs provide driving force energy.

A model of the elongation complex shows that it has three adjacent nucleic acid-binding sites:
-The downstream duplex-binding site,
-The RNA–DNA hybrid binding site, and
- The RNA-binding site.
These three nucleic acid binding sites hold the transcription elongation complex together.



Pauses influence the overall transcription elongation rate

- RNA polymerase does not move along the DNA template at a constant rate.

– RNA polymerase spends more time at transcriptional pause sites. Pausing, which is the temporary delay in chain elongation, helps in:

- **Synchronizing transcription and translation.**
- **Slowing the movement of RNA polymerase to allow regulatory proteins to interact with the complex.**
- **And probably, and probably leads to both transcription arrest (the complete halt of transcription without complex dissociation) and transcription termination (complex dissociation).**

In prokaryotic cells, transcription and translation, occurs at the same time and so transcription pauses to synchronize with translation.

- **RNA polymerase can detect and remove incorrectly incorporated nucleotides.**
- **Pausing is an important step in proofreading.**
- **When a nucleotide mismatch is present in the DNA-RNA hybrid region, RNA polymerase pauses then backtracks.**
- **The mismatch can be removed by RNA polymerase but at a very slow rate. Mismatch removal takes place much more rapidly when the transcription elongation factor GreA or GreB is present. GreA hastens the removal of fragments that are 2 to 3 nucleotides long, while GreB does the same for fragments as large as 18 nucleotides long. Furthermore, GreA can only prevent the formation of an arrested complex, whereas GreB can rescue an already arrested complex.**

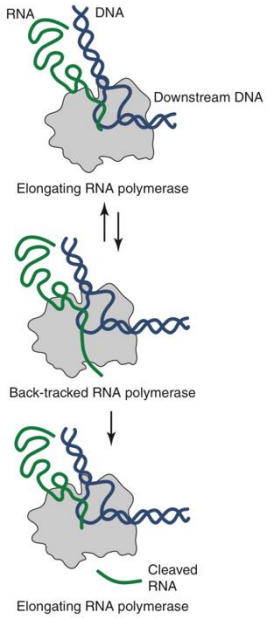


FIGURE 12.18 RNA polymerase transcription and proofreading. Under normal conditions RNA polymerase (gray) elongates the nascent RNA chain (green) as the enzyme moves downstream on the DNA (blue). When a nucleotide mismatch is present in the DNA–RNA hybrid region, RNA polymerase pauses and then backtracks so that the 3′-end of the RNA chain is displaced from the enzyme's active site. The backtracked RNA polymerase can either slide forward again, returning to its previous elongating state (top) or cleave the nascent RNA (bottom) and then resume transcriptional elongation. (Adapted from Shaevitz, J. W., et al. 2003. *Nature* 426:684–687.)

Transcription Termination:

Bacterial transcription machinery releases RNA strands at Intrinsic and Rho-dependent terminators.

- **Intrinsic termination (Rho-Independent).**

- **Utilizes a secondary structure that is formed in the 3' end of the nascent RNA strand.**

When the termination signal is translated into RNA molecule, it will form a secondary structure stem and loop (that forms from a sequence rich in G-C repeats) that lifts up RNA Polymerase from DNA with the help of a sequence of consecutive Uridines that have weak hydrogen bonding and consequently make the binding of RNA to DNA weak. Intrinsic terminators appear to act by first causing the transcription elongation complex to pause

FIGURE 12.19 Transcription termination signal for the intrinsic termination pathway. (Adapted from Mooney, R. A., Artsimovitch, I., and Landick, R. 1998. *J Bacteriol* 180:3265–3275.)

and then to release the nascent RNA chain.

- **Rho-dependent termination.**

- **Requires the Rho factor.**

Rho is a ring shaped helicase with 6 identical subunits that loads onto the nascent RNA at a cytosine-rich region that contains 40 or more nucleotides known as the Rho utilization (rut) site. Then the Rho factor moves in a 5'→3' direction (downstream) until it reaches the area of RNA that is winded with DNA and unwind them and therefore separating them.

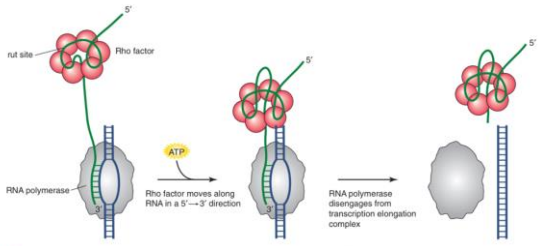


FIGURE 12.20 Rho factor transcription termination. Rho factor hydrolyzes ATP, driving itself along the mRNA in a 5′ to 3′ direction and then somehow causes RNA polymerase to disengage from the transcription elongation complex. (Modified from *Curr. Biol.*, vol. 13, D. L. Kaplan and M. O'Donnell, Rho Factor: Transcription Termination in Four Steps, pp. R714–R716, copyright 2003, with permission from Elsevier [http://www.sciencedirect.com/science/journal/09609822].)

Messenger RNA

Messenger RNA comes as a product of transcription in prokaryotic system.

Bacterial gene regulation has several features that eukaryotic systems don't have.

1- Transcription and translation occur in the same compartment.

Translation of the 5' end of the mRNA can occur before the 3' end is completely transcribed.

That means that mRNA will be translated into protein without any processing at all, which is not what happens in eukaryotic systems. In eukaryotes, mRNA must be processed and modified in order to be ready for translation.

In prokaryotes however, mRNA molecules are ready for translation as soon as the mRNA's 5' end emerges out of the bubble (because transcription and translation happen simultaneously in the same cellular compartment, the nucleoid).

2- Bacterial mRNA molecules often contain two or more cistrons (meaning that one mRNA molecule translates into more than one polypeptide).

An Open Reading Frame: The segment of mRNA that codes for a polypeptide chain.

A Cistron: A DNA segment corresponding to an open reading frame plus the translational start and stop signals for protein synthesis (without the promoter region).

If an mRNA molecule encodes one only polypeptide, it is monocistronic.

If it encodes several or different polypeptides from ONE mRNA molecule, it is polycistronic.

Most mRNA molecules in prokaryotic systems have many open reading frames (are polycistronic).

In bacterial systems in which several enzymes act together in a single metabolic pathway, it is often the case that either all these enzymes are present or all are absent. This phenomenon is called coordinate regulation, and results from the synthesis of a single polycistronic mRNA that encodes all the enzymes.

A Gene: is similar to a cistron but has a broader meaning because it also includes the

promoter region and applies to DNA segments that code for RNA molecules such as tRNA and rRNA that are not translated (so it doesn't only transcribe mRNA).

3- Each cistron in a polycistronic mRNA codes a specific polypeptide chain.

- This is a way to regulate the synthesis of several related proteins with only one signal.

4- Bacterial mRNA usually has a short lifetime compared with other kinds of bacterial RNA

- The half-life of a typical bacterial mRNA is only a few minutes, and this has an important regulatory function.

- A cell can turn off the synthesis of a protein that is no longer needed by turning off synthesis of the mRNA that encodes the protein, and soon after, synthesis of the protein ceases, allowing bacterial cells to save energy. .

Bacterial cells can control gene expression by regulating the rate of gene transcription.
Many factors influence a gene's rate of transcription, one of them being the strength of the gene's promoter (which was previously mentioned). Other factors may also play a role in regulating transcription, the bacteria utilizes these factors to turn on the transcription of specific genes when the gene products are needed, and turning off transcription when the gene products are not needed.

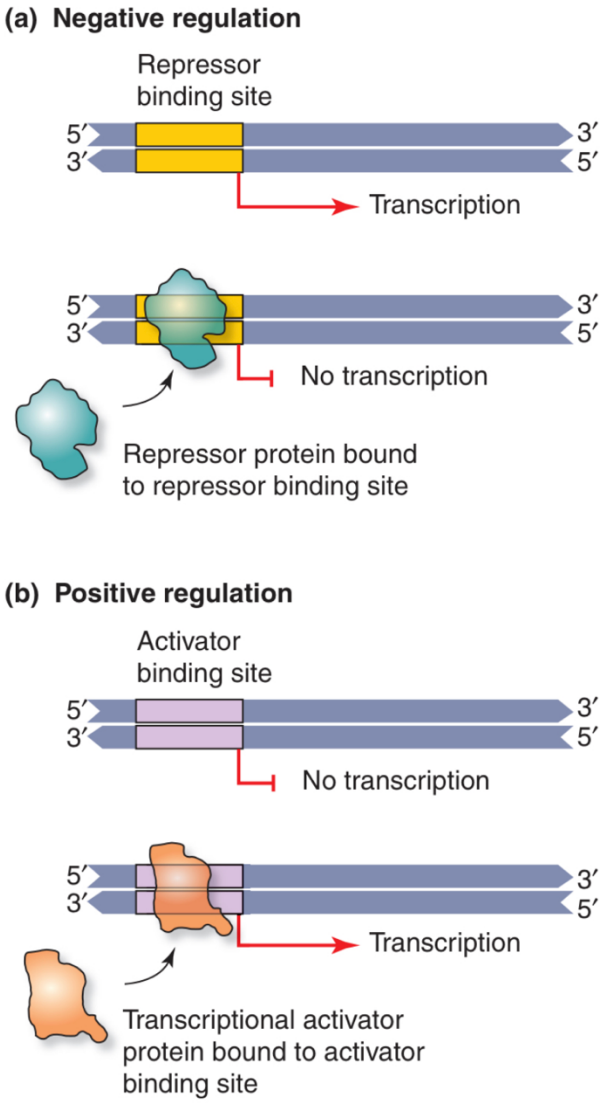
The molecular mechanism of mRNA synthesis regulation can be divided into two major categories:

1-Negative regulation:

A repressor turns off transcription (major inhibition for specific genes and the process is known as repression).

2- Positive regulation:

An activator turns on transcription (activation of specific genes).



Lactose Operon

Lactose operon is an example how bacteria regulate expression of their genes. This operon is a model of the mechanism used in bacteria to metabolize lactose.

Remember**

Lactose is a disaccharide made up of two monosaccharides, glucose and galactose.

The model suggests that a group of cistrons under the control of one promoter, will result in metabolizing lactose inside the bacteria (once they get transcribed).

In E. coli, two proteins are necessary for lactose metabolism. These proteins are the enzyme β-galactosidase, which cleaves lactose to yield galactose and glucose, and a carrier molecule, lactose permease, which transports lactose into the cell. The "*lacZ*" gene is the structural gene for β-galactosidase, while "*lacY*" is the gene that produces lactose permease.

A third enzyme, β-galactoside transacetylase, translated from a third gene known as "*lacA*", functions in transferring an acetyl group from acetyl-CoA to lactose. The function of β-galactoside transacetylase isn't required however, for lactose catabolism.

These enzymes are important to metabolize lactose when lactose is found in bacteria but if bacteria has another source of energy other than the lactose such as glucose, it will not activate translation of this Lac operon.

The *lac* structural genes are regulated.

When E. coli capable of metabolizing lactose is placed in a lactose free environment, the intracellular concentrations of the *lac* enzymes (β-galactosidase, permease, and transacetylase) are exceedingly low, almost one to two molecules of each proteins in each cell. When lactose is added to the growth medium, however, the concentration of these proteins increases for each of these proteins to about 10^5 molecules per cell.

These enzymes are said to be inducible enzymes because their rate of synthesis increases in response to the addition of a small molecule (a derivative of lactose), known as the inducer, to the medium.

Other enzymes, called repressible enzymes, exhibit a decreased rate of synthesis in response to the addition of a small molecule in the medium.

Still other enzymes, called constitutive enzymes, are synthesized at fixed rates under all growth conditions. Constitutive enzymes usually perform basic cellular "housekeeping" functions (that are done at constant rates all the time) needed for normal cell maintenance.

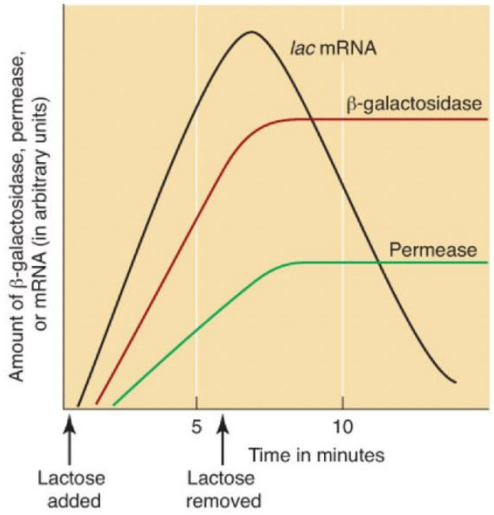
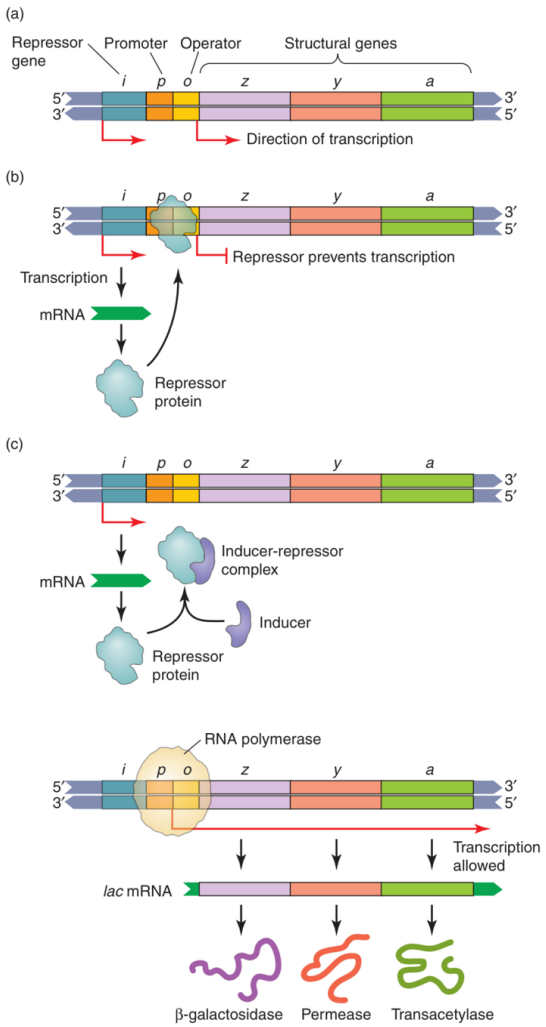


Figure 12.25: The on-off nature of the lac system.

The operon model explains how the *lac* system is regulated. The five major features of the model are as follows:

1. The products of the *lacZ*, *lacY*, and *lacA* genes are encoded in a single polycistronic *lac* mRNA molecule.
2. The promoter for this mRNA molecule is immediately adjacent to the "*lacO"* region. The promoter is located between "*lacI"* and "*lacO"*.
3. The operator "*lacO*" is a sequence of bases (in the DNA) to which the repressor protein binds. The repressor protein that binds to the promoter and operator is synthesized from the "*lacI*" gene that lies before the promoter (check the figure).
4. When the repressor protein is bound to the operator, *lac* mRNA transcription cannot take place.

5. Inducers stimulate *lac* mRNA synthesis by binding to the repressor. In case of lac operon, this inducer is a derivative of lactose known as allolactose. This binding alters the repressor's conformation so it cannot bind to the operator. In the



presence of an inducer, therefore, the operator is unoccupied and the promoter is available for initiation of mRNA synthesis. This state is called derepression.

Two problems were left unanswered by the operon model.

The first being that because the inducer for the transcription of the *lac* genes is a derivative of lactose (allolactose), that means that this inducer must first enter the cell in order to stimulate *lac* mRNA synthesis (by binding to the repressor to remove it from the promoter region of the gene). But how will the inducer be able to enter cell if the protein lactose permease hasn't yet been synthesized (because its gene is still repressed) to allow lactose to enter?

The second problem is related to the first one. The inducer of the *lac* genes is allolactose, which is a derivative of lactose that is produced as a secondary product when β-galactosidase catalyzes the hydrolysis of lactose. This means that β-galactosidase converts a small proportion of lactose to allolactose. But how is the enzyme β-galactosidase present if the gene responsible for its synthesis hasn't been stimulated by the inducer allolactose?

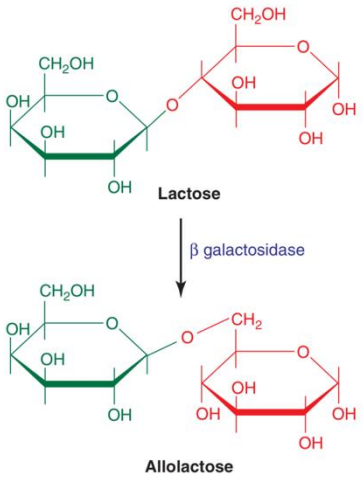


FIGURE 12.28 ***Lac* operon model.** Synthesis of allolactose, the actual inducer of the *lac* operon.

These two problems share the same explanation:

Even when the *lac* genes are still in the repressed state, a small amount of *lac* mRNA is able to get synthesized. This synthesis is known as the basal synthesis, and it occurs because a repressed gene doesn't turn off its ability to express 100%. This basal synthesis is the reason for the presence of one to two molecules of each of the *lac* enzymes even when the E. coli cells were placed in a lactose free environment (an environment where the inducer is absent).

The presence of these small amounts of *lac* enzymes allows for a small number of lactose to enter the cell (via permease) and then get converted to allolactose (via β-galactosidase) to bind to the repressor removing it from the promoter of the *lac* genes.

This removal of the repressor allows for the synthesis of much more *lac* enzymes (10^5) and therefore much more lactose can enter and get metabolized.

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