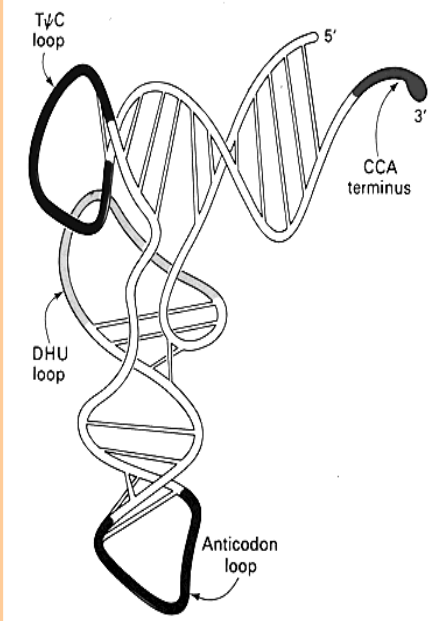
بسم الله الرحمن الرحيم



ليه التعقيد .. ركز معنا ☺

- الطريقة أصبحت معروفة للجميع ☺
- هذه المذكرة اهداء الى جدي الغالي – رحمه الله فلا تنسوه من دعائكم ،،،

RNA Structure & synthesis

Team leader : مجهول

والشكر لجميع من ساهم في اخراجها بالصورة التي هي عليه وأخص بالشكر :

أبو يسرا

عبدالعزيز التركي

Ocean

Blue eye

وجنودنا المجهولين ☺

RNA STRUCTURE & SYNTHESIS

C. Transcription from bacterial operons :

- **structural genes** (that code for the enzymes of a metabolic pathway)
- **regulatory genes** (that determine their transcription as a single long piece of mRNA)

- in bacteria , **structural genes** are often found grouped together on chromosome together with the **regulatory genes**

  ✓ thus , **the genes are coordinately expressed** (MCQ)(هاااااااااام)

  - (this entire package is referred to as an **operon**) & we will speak about the best understood examples –lactose operon of E.coli -- which illustrates both +ve & -ve regulation .

1. the lactose operon (lac operon) : (is coordinated expressed gene)

- It (structural portion)(MCQ) codes for 3 enzymes involved in the catabolism of the sugar lactose :

lacZ genes	*lacy* genes	*lacA* genes
-codes for *β–galactosidase* -(which hydrolyzes lactose to ➔ galactose & glucose	-codes for *permease* -(that facilitates the movement of galactose into the cell	-codes for *thiogalactoside transacetylase* -(its physiologic function is un known)

✓ these enzymes are all produced when **lactose is available** to the cell (but **glucose is not**) (MCQ)

- note : bacteria use glucose as a fuel in preference to any other sugar

- the regulatory portion (MCQ) of the operon consists of : (MCQ)(always come)

I. catabolite gene activator protein (**CAP**, sometimes called : cAMP regulatory portion or CRP) binding site

II. the promoter (P): where RNA polymerase binds

III. the operator site (O)

IV. **additional lac I gene :** codes for the repressor protein

- **lacZ , lacY , lacA genes are expressed when : (MCQ)**

  - ✓ the O site is empty .
  - ✓ CAP binding site (upstream of P region) is bound by a complex of cAMP & CAP protein .

see : ☺

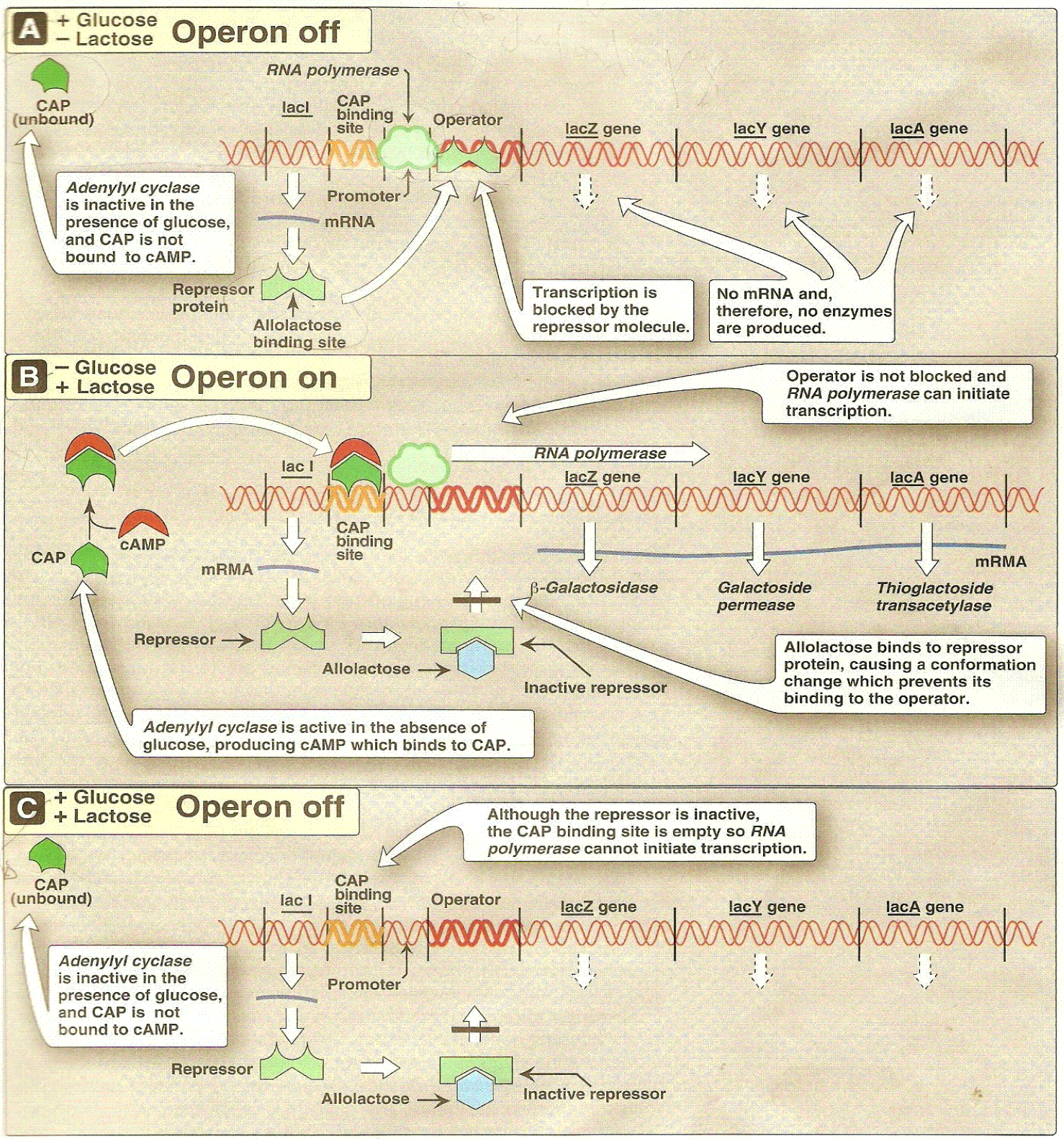


Figure 30.11
The lactose operon of E. coli.

a. when glucose is the only sugar available: (MCQ) هاااااام

1) the repressor protein binds to the operator site (which is down stream of the promoter region)

2) this interferes with the progress of RNA polymerase & blocks transcription from structural gene (negative regulation)

3) a denylyl cyclase is inactive in the presence of glucose , so ➔ no cAMP ➔ no cAMP-CAP complex can form

* So , the final result No mRNA and, therefore , no enzymes are produced .

b. when only lactose is available:

- a small amount of lactose is converted to allolactose . (MCQ)

◙ **what is allolactose & what it is function ?**
- allolactose is inducer
- that binds to repressor protein , causing a conformation change which prevent its binding to the operator

- because no glucose is available ➔ adenylyl cyclase is active ➔ sufficient quantity of cAMP ➔ cAMP-CAP complex can form ➔ cAMP-CAP complex binds to the CAP binding site ➔ allows RNA polymerase to effectively intiate transcription (+ve regulation)

✓ the transcript is a polycistronic mRNA molecule , encoding all 3 enzymes (**β–galactosidase , permease , thiogalactoside transacetylase)**

✓ translation of mRNA is initiated at 3 different start codons , produces the enzymes that allow lactose to be used for energy production by the cell

N.B : eukaryotic cell produce only monocistronic messages . that is , each eukaryotic mRNA moleculeencodes just 1 protein (MCQ)

c. when both glucose & lactose are present :

- a denylyl cyclase is inactive in the presence of glucose , so ➔ no cAMP ➔ no cAMP-CAP complex can form ➔ CAP binding site remains empty ➔ RNA polymerase is unable to effectively intiate transcription, even though the repressor is not bound to the operator region ➔ the 3 genes (**lacZ , lacY , lacA**) are not expressed .

Only glucose	Only lactose	Glucose & lactose
- the repressor protein binds to the operator site	- the repressor protein does not binds to the operator site (cuz of allolactose)	- the repressor protein does not binds to the operator site (cuz of allolactose)
- a denylyl cyclase is inactive in the presence of glucose , so ➔ no cAMP ➔ no cAMP-CAP complex can form	- because no glucose is available ➔ adenylyl cyclase is active ➔ sufficient quantity of cAMP ➔ cAMP-CAP complex can form ➔ cAMP-CAP complex binds to the CAP binding site	- a denylyl cyclase is inactive in the presence of glucose , so ➔ no cAMP ➔ no cAMP-CAP complex can form ➔ CAP binding site remains empty ➔ RNA polymerase is unable to effectively intiate transcription, even though the repressor is not bound to the operator region
- the final result No mRNA and, therefore , no enzymes are produced .	- the transcript is a polycistronic mRNA molecule , encoding all 3 enzymes (β–**galactosidase , permease , thiogalactoside transacetylase)** note : **eukaryotic cell produce only monocistronic messages . that is , each eukaryotic mRNA moleculeencodes just 1 protein**	- the 3 genes (**lacZ , lacY , lacA**) are not expressed

TRANSCRIPTION OF EUKARYOTIC GENES :

- transcription is more complicated in eukaryotes than prokaryotes .

◙ Note : you know that RNA polymerase bind to promoter region and initiate Transcription . (with it , several transcriptin factors bind either to Promoter region or some distance from it)

◙transcriptin factors function : it is protein that determines what genes are to be transcribed .

For all these to happen we should have double helix DNA that assume a loose Conformation and dissociate temporarily from the nucleosome core.(MCQ) (very important)

A - Chromatin structure & gene expression :

-DNA + histone = nucleosome -> affect ability to transcription.

-Regarding DNA transcription : (always come in exams)(MCQ)

Can transcriped	Can not transcriped
Relaxed form of chromatin called euchromatin(active)	Highly condensed form called Heterochromatin(inactive)

◙ Chromatin remodeling : interconversion of active & inactive forms .

* Two major influences on choromosome structure & activity :

1) DNA methylation .
2) histone acetylation .

(MCQ)We notice that the genes that are in permanent inactive form ☹ Have more methylated DNA (5 methylcytosine) هاااااااااااااااااااالمThan the active form .(MCQ)

We take the DNA of one of the X chromosome of a female & we notice that :(see what happen when acetylated or methylated)

Highly methylated	Histone become Highly acetylated
heterochromatin	Euchromatin
Transcription turned off	Actively transcriped .(chromatin become looser. So, the DNA become more Accessible to transcription)

B - RNA polymerase in the nucleus of eukaryotes are :

1) three classes (each class recognize particular type of genes)
2) large enzyme .
3) multiple subunit .

RNA polymerase 1	RNA polymerase 11	RNA polymerase 111
synthesize the precursor of the large RNAs (28S,18S and 5.8 S)	synthesize the precursor of the **messenger RNAs** that Translated to produce Protein .	this enzyme produces the small RNAs, including **tRNA**, the **small 5s riposomal RNA**, & some **snRNAs.**
XXXX	it also synthesize small nuclear **RNA (snRNA)**	See there ↑
in nucleolus (not nucleus)(MCQ)	in nucleoplasm	_____
(note that mRNA & tRNA are synthesized In the nucleoplasm)	(note that it is used to Produce viral DNA by some viruses)	_____

So ,

What come in exams :
1) snRNA (in *polymerase II&III*)
2) is in nucleolus
3) *polymerase I* synthesize large RNAs . but, synthesize small RNAs
4)rRNA in *polymerase III*
Is it easy now ☺ ??

a) promoters for class II genes:

1. Contain 3 box :

a) Hogness box or TATA box: (ATATAAAA)

- a sequence of DNA nucleotides almost identical to **Pribnow box** (TATAAT) (MCQ)
- usually found about 25 nucleotides upstream (- 25) of initial base transcription start sit of mRNA molecule

b) CAAT box :(GGCCAATCT)

- Found between 70 and 80 nucleotide upstream (-70 or -80)

C) GC box :(GGGCGG)

- Many promoter contain this box

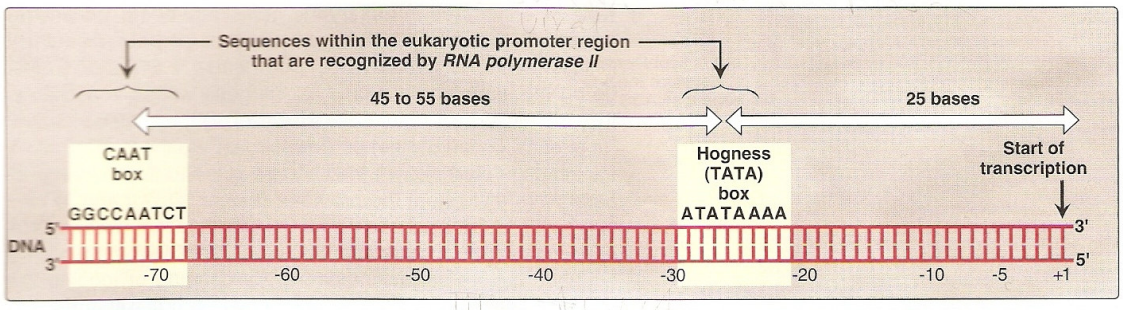


Figure 30.12
Eukaryotic gene promoter concensus sequences.

2. Cis-acting genetic elements : (DNA sequence هاااااام

✓ Because TATA box, CAAT box and GC box are found on the same molecule Of DNA دائما يأتي بالاختبار هام جدا as genes being transcribed they are called cis-acting genetic elements

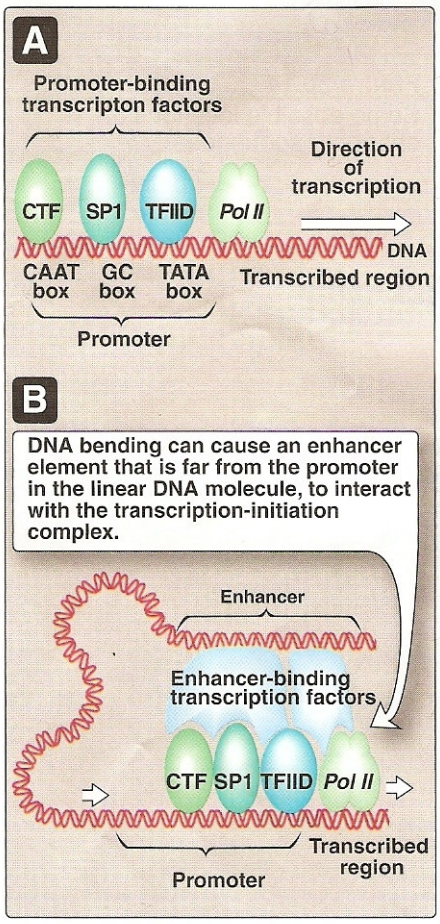


Figure 30.13
A. Eukaryotic general transcription factors bound to the promoter. CTF, SP1, and TFIID are general transcription factors. B. Enhancer stimulation of *RNA polymerase II*.

3. Cis-acting genetic elements serve as binding sites for protein called **general transcription factor** :

- which in turn interact with each other and with RNA polymerase Π
- Transcription factors encode by genes on different chromosomes (not the same gene as cis)
- Because its synthesis in cytosol can diffuse through the cell to their point of action (which may be different chromosome) , they are called trans-acting elements
- (it is **protein** not DNA sequence note the difference (MCQ)) .
- they can either stimulate or inhibit transcription of particular genes
- Note : Promoter- binding transcription factors: *CTF , SP1 , TFIID*

b) Role of enhancer in eukaryotic gene regulation:

◙ Note : What is Enhancers ?? ☹

✓ special **cis- acting** DNA sequence (always come in exams)

And note: because it is Cis so it is DNA sequence not protein (IIIIIIIMP):☺.

✓ **increase** the rate of **initiation** of transcription by *RNA polymerase II*

✓ must be in the **same chromosome** as the gene whose transcription they stimulate .

✓ they can be located "upstream" (to the 5'-side) or "downstream" (to the 3'-side) of the transcription start site .

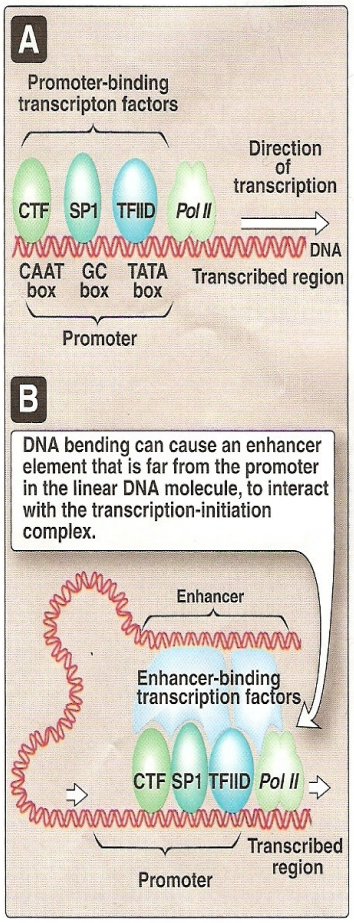
✓ they can be close to or thousand of base-pair away from the promoter .

✓ they can occur on either strand of the DNA .

✓ contain DNA sequences called "**response elements**" that bind specific transcription factor called **activator**

So , By bending or looping the DNA, these enhancer- binding factor can interact with transcription factors bound to a promoter & with *RNA polymerase II*, thereby **stimulating transcription**

Note : **Silencers** act over long distances to **reduce** the level of gene expression.



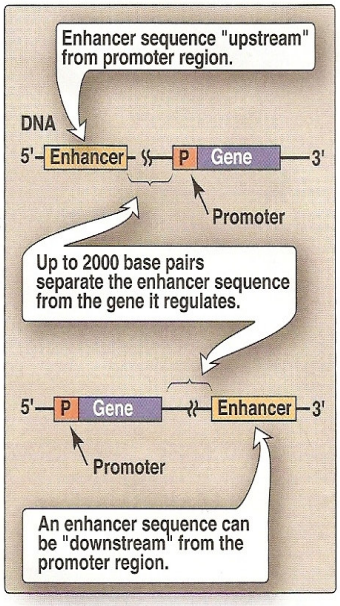


Figure 30.14
Some possible locations of enhancer sequences.

c) Inhibitors of RNA polymerase II:

◙ **α- amanitin :**

- **inhibit RNA polymerase 11 (note 11)(IMP)(MCQ)(always come)**

- a potent toxin produce by the poisonous mushroom Amanita phalloides (sometimes called "death cap" or "destroy angel" –it is said to taste delicious!).

- **because it forms a tight complex with the *polymerase ,SO* *(MCQ):***

1) inhibiting mRNA synthesis .
2) **inhibit protein sunthesis .**

B. Mitochondrial RNA polymerase :

- Mitochondria contain a single *RNA polymerase* that **resembles bacterial *RNA* *polymerase*** more closely than it does the eukaryotic enzyme.

دافعنا نحو كل هذا ما هو ؟؟
ثواب من الله وطمعا في أجره
ومحبة منا لكم ،،

أخوانكم : بيو تيم
Done --------- المحاضرة الثانية