

University of Jordan Faculty of Medicine Batch 2013-2019



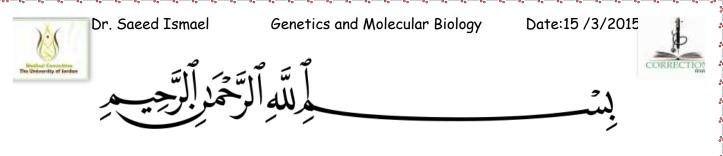
GENETICS &

MOLECULAR BIOLOGY

O Slides O Sheet O Handout O other.....

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DESIGNED BY NADEEN AL-FREIHAT



اللمو لا سمل إلا ما جعلته سملا و أنبت تجعل المزن اذا شئبت سملا , اللمو اشرح لنا حدورنا و يسر لنا أمورنا ...

The concepts that we're going to discuss in this lecture.

1 – A quick revision for the previous 2 lectures (the main points only)

2 – What is the meaning of "TRANSCRIPTION"?

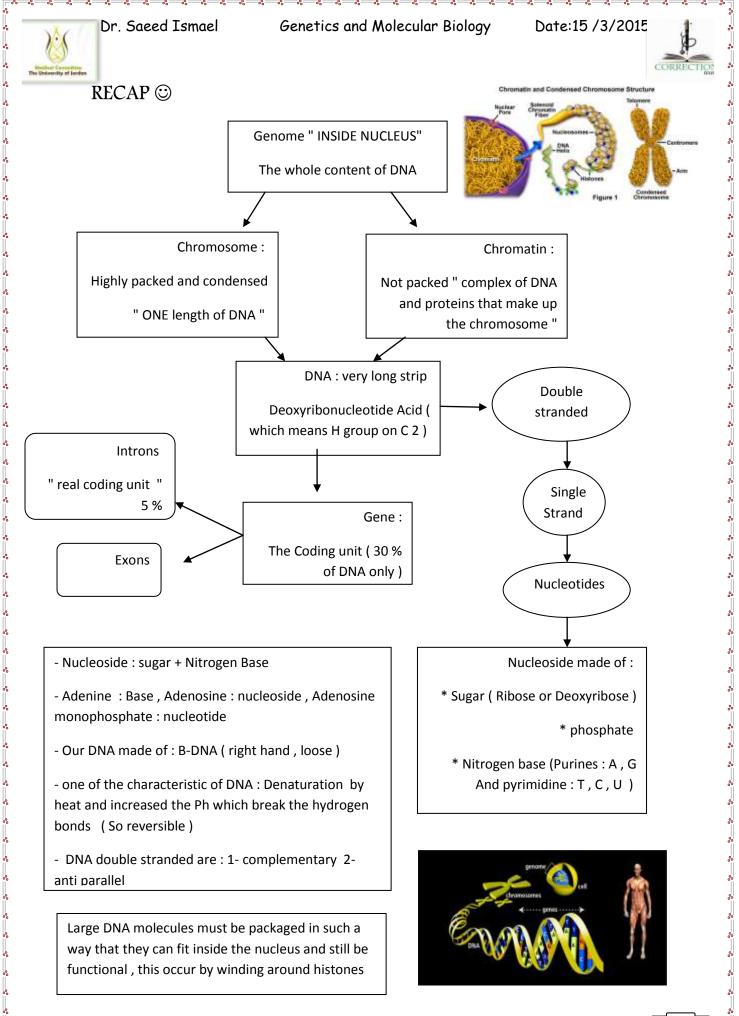
3 – the types of RNA

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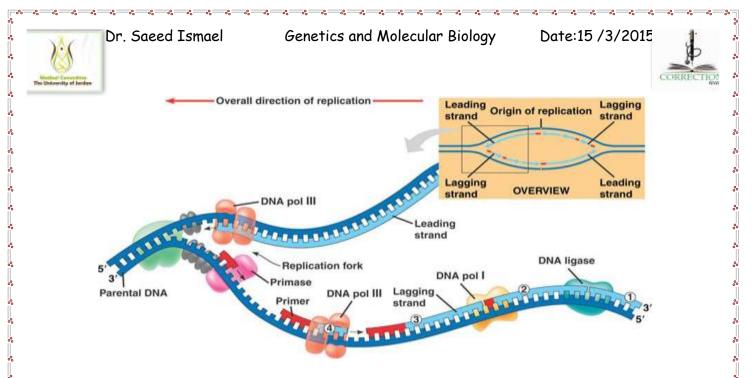
4- RNA polymerase, the difference between DNA polymerase and RNA polymerase, and the types of RNA polymerase in prokaryotes and eukaryotes.

5- Transcription of Genes

It's an easy lecture , I hope that you'll enjoy it P.S : I re-arranged the information according to subjects , so please don't be confused when you listen to the record ©



Lecture # 16



- DNA synthesis occur in S phase

- DNA replication begins at (**origin of replication**) bidirectional, so there is 2 forks for replication

- Helicase . separate dsDNA in front of DNA polymerase (denaturate)

- DNA polymerase : copy the DNA so the new strand made from 5' to 3'

It requires a primer (short RNA sequence complementary to a specific DNA sequence at the start of replication point) which created by Primase

" during this lecture we talked about the main characteristic of DNA pol."

- **Topoisomerases** : upstream of helicase , to identify the stress points which occur when DNA is overwound ahead of the fork , they relief the tension by cutting and rejoining the DNA .

- **RNase** : removes the primer

- DNA ligase : joining the OKAZAKI fragments (intermittent synthesis of the new strand) by phoshphodiester bond

- SSB : prevent renaturation of DNA

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RNA Transcription

Now, we'll start with chapter 14 which talks about " RNA transcription " which is the same as " Synthesis of RNA ".

1 – what is **the TRANSCRIPTION**?

Transcription : it's basically copying part of the genetic code found in the nucleus to be translated in the cytoplasm partially and temporally

- or you can say it's . Copying a gene into RNA to be translated into protein

DNA (gene) \rightarrow RNA \rightarrow protein

- RNA is synthesized in nucleus and then transferred to cytoplasm

To understand the definition very well, you can always imagine this example : You have such a valuable, very old reference, it's one thousand years old, in the reference library in that museum or university.

If you want to borrow that reference, there's no way that they will give you it out of the library

The maximum they can do for you is to allow you to copy the part you want to study on microfilament or any other method, so you can take the photocopies not the original reference, and once you finish what you want to study, you can just throw it away.

By that way you preserve that valuable reference in the reference room, it will stay in the room.



Line Understanding

The same thing goes for the DNA, which is the reference of our genetic material.

It mustn't go out of the nucleus every time we want to copy it or translate a gene out of 25,000 genes

So it should be preserved in the nucleus , and once you want to express a gene out of 25,000 genes , you just send a copy of that gene that you want to be translated (so you don't send the actual gene) . For this reason , we have translation , RNA : **to preserve** the main reference of the cell (the DNA) which will stay with it along its life .

2- Types of RNA :

We have 5 types : 3 of them are the main types and 2 newly

discovered

All types are naturally occurring.

MAIN TYPES :

A) m RNA B) t RNA c) r RNA

The new ones :

D) snRNA (small nuclear RNA) E) micro RNA * OR siRNA (small interfering RNA) *

- we will talk about micro RNA in chapter 16 and 17.

For the purpose of this chapter , we are going to discuss only the first 3 main types \odot



- RNA of eukaryotes is different from RNA of prokaryotes, there are **3** main differences we will talk about them in details in this lecture :

1) capping at 5' end of RNA

2) poly (A) tail on 3' end of RNA

3) the procedure of spilling or removal of the non-coding regions which is known as " INTRONS ".

Let's Start 😳

1) m RNA

From it's name, you can know it's function, it's a "message".

 $\underline{\text{Function}}$: the transfer of the code in the nucleus into cytoplasm where the protein is synthesized .

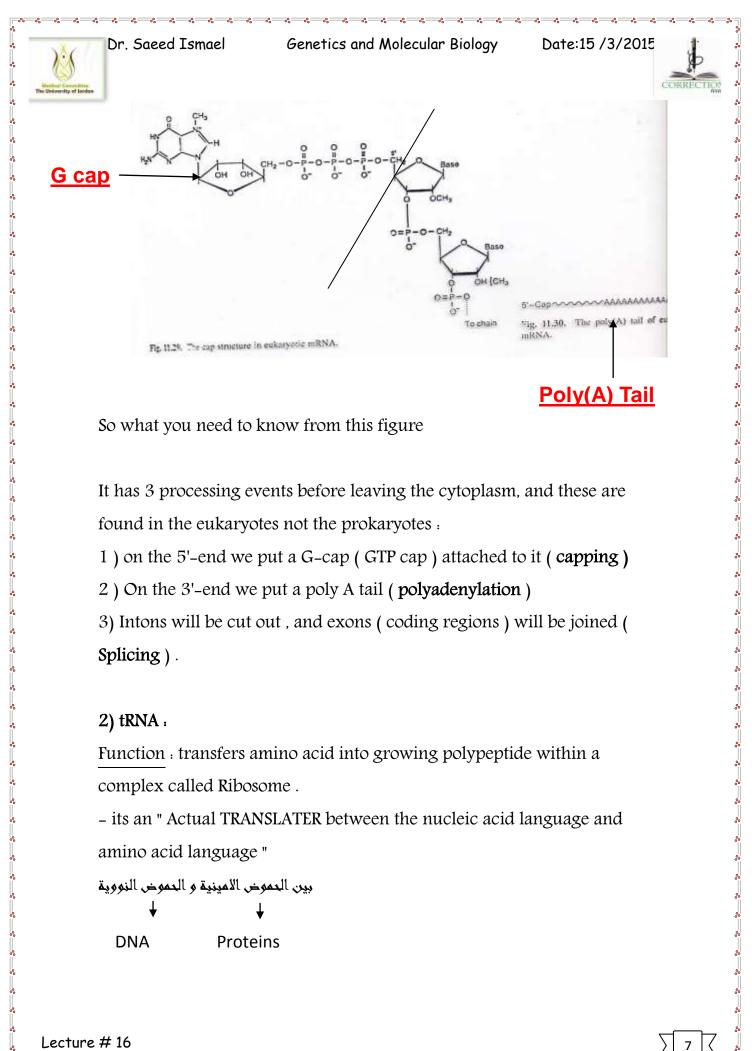
" As it's written in the slides : Copy of the genetic code , read by ribosome and translated into proteins "

- synthesized in the nucleus and then transferred to the cytoplasm .

– m RNA has :

• 5' cap : a methylated GTP attached to 5'-OH group of ribose at the 5'end of mRNA

• Poly (A) tail : a series of adenosine nucleotides linked together by a 3' to 5' phosphodiester bonds and added to the 3' end of mRNA molecule.



So what you need to know from this figure

It has 3 processing events before leaving the cytoplasm, and these are found in the eukaryotes not the prokaryotes :

1) on the 5'-end we put a G-cap (GTP cap) attached to it (capping)

2) On the 3'-end we put a poly A tail (polyadenylation)

3) Intons will be cut out, and exons (coding regions) will be joined (Splicing).

2) tRNA :

Function : transfers amino acid into growing polypeptide within a complex called Ribosome.

- its an " Actual TRANSLATER between the nucleic acid language and amino acid language "

بين الحموض الامينية و الحموض النووية

DNA **Proteins**

Lecture # 16



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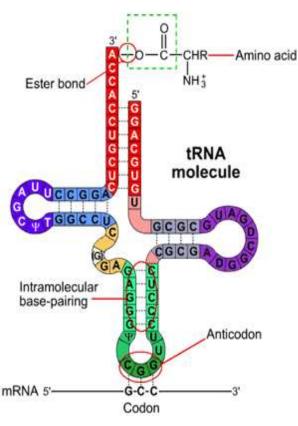
So , it's the tRNA that can read the codon at RNA level and then tells this codon that it should be translated into Lysine , Glycine , Alanine , or whatever amino acid .

"Identifies the codon on mRNA and brings the appropriate amino acid" - We can say that " Ribosomes " responsible for the translation , but actually it uses the tRNA for this purpose

- tRNA is a single stranded RNA, short (only about 80 bp long) (All RNAs are single stranded **except**. the micro RNA which is firstly produced as Double stranded then converted into single stranded RNA)

Now look at the figure below to understand the structure of tRNA

If you go from 5' end to 3' end You will notice 3 loops , And at 3' end there is an amino acid linked to it by an ester bond . The question is : Why these loops occur !? Because of <u>intramolecular</u> Homologues sequences Which means : It folds on itself by hydrogen bonding of complementary sequences. This specific folding is essential for it's function . For example :



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Here we have CCC and on the other side there is GGG

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So what happen is that GGG and CCC will join by hydrogen bonding because they're COMPLEMENTRY

So if there is intra complementary sequences it will form the "Stem" And when there are No intra-complementarities it will form the "loop" Therefore, the final structure will be **: Stem and loop** " hair-pin loop " . Intra-complementary : within the same molecule

* pay attention to loop no. 2 :

It exposes 3 nucleotides making what is known as " **ANTI-CODON** " This strip is what will bind the Anti-codon to mRNA.

" so it has an anti-codon at one end and the related amino acid at the other end " .

- we said that the ribosome by itself can't translate, and what make the transcription is the (tRNA).

But that's not true, it's not made by cleverness of tRNA,

It's all about the cleverness of a unique enzyme that reads the anticodon and brings the related amino acid that should be put here © These enzymes called (Amino-Acyl tRNA synthetase)

The name will tell you the story \bigcirc

Amino : Amino acid : P #WOW : P

tRNA : we want to put the a.a on tRNA

synthetase : to synthesize the ester bond

there're 50 different types of this enzyme from this family , each one binds to the tRNA and reads the loop no. 2 then reads the anti-codon and put the related a.a for it O

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Now , From where these enzyme know ! how they do it ! We don't know actually .

But , the doctor said that they have their own small brains , so they think and communicate by their language with each other :D

From Molecular point view : it's all about STRUCTURES (the pocket of that enzyme will only fit that sequence and because of the structural motif, it will bring only that amino acid)

A student asked :

If it was a structural based, so we must have more than one enzyme? The doctor said that sometimes, structural complementary does explain what happens and in so many times they don't.

Take an example :

Enzymes that read DNA, like helicase, it only opens at the site of origin of replication, although it bind to DNA and read million of nucleotides and all of them G T C A, with a specific sequence and certain length.

But It has a memory , after it read all of it , it remembers and opens at certain place , so they're very SMART

3) r RNA .

– Ribosomal RNA 🕲

- most abundant RNA type (75 % of cellular RNA)

- it's a structural and functional component of ribosomes .

* **Ribosome** : huge complex made of proteins and rRNA which is the site of protein synthesis

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Each subunit of ribosome has a large no. of enzymes and rRNA We will see it in the next chapter Inshallaa ③ * Structural : means that the ribosome made of proteins and rRNA . Functional : one of the rRNA found in the <u>large subunit</u> has an enzymatic activity This enzyme known as " **peptidyl transferase** " .

If you remember, Ribosomes will be linking the amino acid together to make a polypeptide by a (peptide bond)

So who exactly links the 2 a.a together with the peptide bond :

An ezyme within the ribosome is " peptidyl transferase "

→This enzyme is an RNA component at the large subunit of ribosome " not protein component " has an enzymatic activity .

As you see, this is **the first exception** for the rule that said (All enzymes are proteins).

So Actually , what makes a protein is an enzyme , not the protein itself , it's an rRNA O

Now, we will talk about the Story of (18 S, 20 S, 5 S)

* don't care about the numbers "

These numbers basically means and indicate the RATE OF

SEDIMENTATION

In the past, before they were able to know the sequences of DNA and

RNA, they would just set them down In " centrifuge ".

So they put RNA and rotate it, the heaviest one will participate first,

the lighter it becomes, it takes more time to precipitate.

So the ones that sediment faster at certain time, they give it a number which indicates indirectly : the size of rRNA through measuring the speed of sedimentation

But it's so specific for each species.

If you take all bacteria from one species, you will find that the rRNA has the same number and sedimentation rate, Also all the relative living things have the same rRNA.

Based on this, they re-classified some types of bacteria then according

to same "Biochemical Activity " they found that they have the same

rRNA $_$ so they re-study the bacteria.

Now, they classify according to rRNA ©

3) RNA polymerase ..

It's the enzyme that's responsible for transcribing (copying) DNA into RNA

Now , let's compare between the DNA polymerase and RNA polymerase

We'll start with the similarities between them :

1) both of them will synthesize nucleotides the in (5' to 3' direction)

2) both will use nucleotides (but different types) as precursors

These were the main similarities between them , let's discuss the main **differences** between the 2 polymerases :

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| J | DNA polymerase | RNA polymerase |
|------------|--------------------------|--------------------------------|
| | | |
| Precursors | deoxyribo-nucleosides | Ribo-nucleosides |
| | dNTPs , where N | NTPs (UTP , ATP , CTP |
| | stands for one of the | GTP) |
| | bases (A,G,C,T) | |
| | | |
| Primer | need a primer to | doesn't need a primer |
| | Initiate the synthesis | it can initiate RNA |
| | | synthesis alone . |
| Proof | has 3' to 5' exonuclease | doesn't have it so→ the |
| reading | Activity (proofreading) | error rate is higher |
| | Both DNA strands | only one copy of |
| | Used as templates | the DNA is copied |
| | Because DNA is a | Which is " the template strand |
| | semi – conservative " | |

- we said that the error rate in the RNA polymerase is higher , because it doesn't have proof reading , it goes so fast , so any mistake it does , it cannot go back , erase it , then re-write it and go back again, IT CAN'T \otimes

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But , why it's not such a big deal to not have exonucleases for the RNA polymerase ?!! For 3 reasons :

1) the mistake in RNA is a temporal mistake, because RNA gives many copies, each one will give one protein or more, so after a while it will break the mRNA and proteins in the cytoplasm.

Then it's will again re-synthesize proteins

If this copy of RNA give us an error protein , the second copy will give us the correct protein[©]

While the mistake in the DNA is a permanent mistake, once it's happened during cell division. the cell will take this error with it permanently.

So , in short " m RNA is a very short lived messenger , degrades immediately "

2) when the cell divides, it only gives us 2 copies of the DNA : the original one and the new one.

While copying a gene, will give us million copies, so one or two mistakes will not affect especially that, as we said, it will be broken after a while.

What we care about in the RNA polymerase is " THE SPEED "

I want to copy as much RNA as possible even if I make some mistakes which doesn't really matter :D

In Short : " we have million copies of mRNA "

3) Here, I'm copying only one or few genes which is a small area in the DNA



So the chance of getting mistakes is small ; If the error rate of RNA

polymerase = 1 of 10,000, most of our genes under this number (It's

just few thousands)

So it will NOT make an error $\textcircled{\odot}$

While copying the DNA, we copy millions and millions, so the chance to get error is bigger.

In short : " we copy a small area of DNA to RNA"

- Another thing we want to explain is :

" in RNA synthesis, only one copy of the DNA is copied Which is " the template strand "

What we mean by (TEMPLATE STRAND) !

The 2 strands in the DNA are known as :

A) sense and Anti-sense (at DNA level)

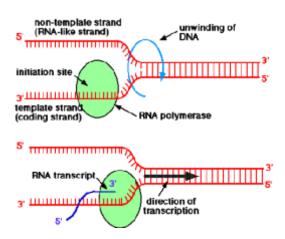
B) template and coding strands.

قالب Template =

Coding = strand that have the codes for

reading the genes from it from 5' to 3'.

- So we use the template to copy the DNA



into RNA which will be same as coding, when RNA is transcribed from the template, it will be similar to coding, complementary to the template.

So the DNA TEMPLATE is complementary and anti-parallel both to :

- coding DNA strand - RNA transcript

* From this information , now we exactly know why the DNA is double stranded :D

" because we will not translate the DNA into protein immediately, it will send copies which is RNA.



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So if I copied RNA from the coding strand, we will get a strand similar to template which we don't need, it will give a different protein So I have a complementary strand (template) to use it to synthesize the RNA which will be similar to coding strand (except that there is a U instead of T) and the coding one will go to cytoplasm and translated to protein ©

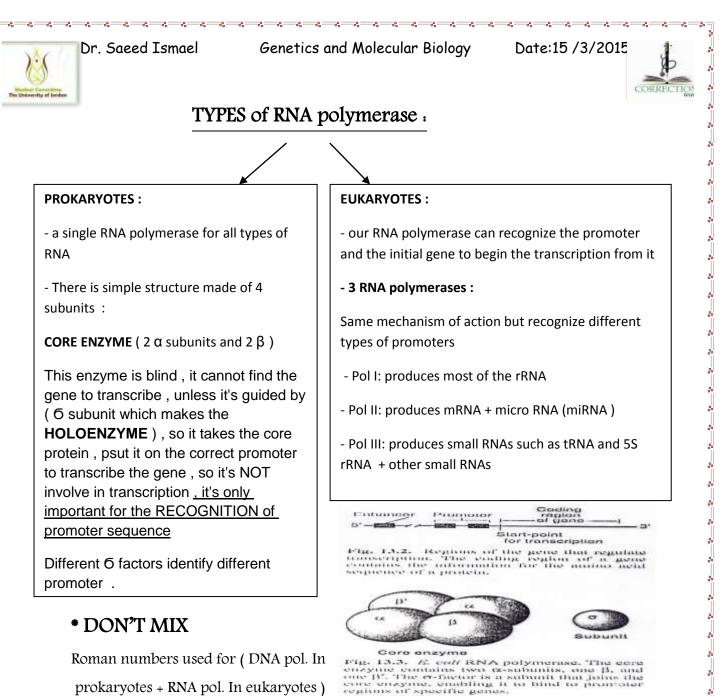
" If you want to know which strand is template and which one is coding, just look at the RNA, the opposite of RNA is the template "

Some Notes about RNA polymerase " in slides " :

- Energy needed for RNA synthesis is derived from release of

pyrophosphate

- ESTER BONDS are made between successive nucleotides



prokaryotes + RNA pol. In eukaryotes)

While Greek letters used for DNA pol. In eukaryotes .

4) Transcription of GENES !

We will start with EUKARYOTES " more complex " .

As you remember, we said that our DNA is a very long stretch that

consists of many non-coding regions

So when RNA polymerase want to transcribe the genes, it will cross

long distance of non-coding regions until it reach relatively as a small



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area of coding genes , so how it will know \rightarrow that from here to there \rightarrow it must transcribe ?

There must be a mark at the start and end points of gene for RNA polymerase to know where to start and where to stop . These markings at the beginning of gene called as **" PROMOTER** ",

while at the end of gene, they called as " TERMINATERS ".

Promoter means in Arabic " يبدأ

يروج، يبدأ

so it marks the beginning of the gene and promotes the gene by attaching the RNA polymerase

which means that , if the promoter is on \rightarrow RNA pol. Will transcribe and if it was off \rightarrow RNA pol. Will not even see the gene to transcribe it

So , we can define **PROMOTER** as <u>"</u> DNA sequence located upstream to the coding start site where the transcription begins , it's the key for starting the transcription "

Or as it's written in the slides : " cis – acting (sequence inside DNA not a regulator from outside like a proetin) specific sequences at the start of the gene that specifies the start point of transcription and the frequency of gene expression "

- terminators : it's the stop signal , telling the RNA pol. Where to stop the transcription

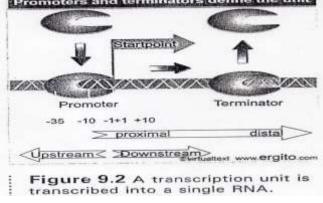
| Dr. Saeed Ismael | Genetics and Molecular Biology | Date:15 /3/2015 |
|-----------------------------|---------------------------------|------------------|
| Some directions we must | know : | |
| | - | |
| - This indicate the " upstr | ceam " – this will indic | ate |
| Direction | the " downstream | L " |
| -Towards 5' end | - Towards 3' end | 1 |
| - We symbolize it by a ne | egative – we symbolize | it by - positive |
| number | number | |
| - before the promoter | – after the promo | ter to 3' |

-the downstream could be proximal or distal but for more accurate, you can give numbers such as " + 1 , + 20 , ...)

• (+1): means the first nucleotide to be transcribed by RNA pol. (we call it TRANSCRIPTION START SITE)

As you can see the **promoter sequence will not be transcribed**, it's a docking sequence for RNA pol. To settle on and then start transcribing afterwards

* (-47), what this numb
er indicates for you ?
* upstream * 5'end * it's 47 away
from transcription start site +1
For example :



The main promoter element which is the TATA Box is found between – 20 and – 30.

Meaning that this sequence of promoter found between -30 and -20, r=, related to (+1) from where the transcription begins.

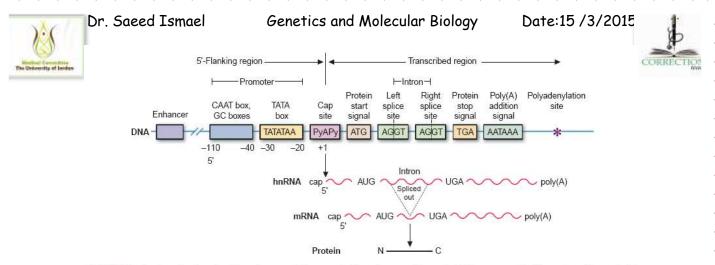


FIG. 14.4. A schematic view of a eukaryotic gene and steps required to produce a protein product. The gene consists of promoter and transcribed regions. The transcribed region contains introns, which do not contain coding sequences for proteins, and exons, which do carry coding sequences for proteins. The first RNA form produced is heterogeneous nuclear RNA (hnRNA), which contains both intronic and exonic sequences. The hnRNA is modified such that a cap is added at the 5'-end (cap site), and a poly(A) tail is added to the 3'-end. The introns are removed (a process called *splicing*) to produce the mature mRNA, which leaves the nucleus to direct protein synthesis in the cytoplasm. Py is pyrimidine (C or T). Although the *TATA box* is still included in this figure for historical reasons, only 12.5% of eukaryotic promoters contain this sequence.

As you see in the above figure, it's the "typical ANATOMY" of the gene

This (ATG) codon resemble the start signal, from it the RNA

polymerase will start transcription and continue downstream .

Before this " protein start signal" we have (5' or the upstream) which

has the most important thing " PROMOTER ".

There's several components of promoter, the most important one is the

" TATA Box " which is found between -20 and -30

• TATA Box :

- Hexamer A and T rich sequence - TATAAT

- the closest to the transcription start site

- in E.coli \cdot found in approx \cdot -10 (recognized by the sigma factor **6**

70)

- in eukaryotes : found in position -25

And there's other components which is :

• CAAT Boxes and GC rich sequences between -40 and -110 in

eukaryotes

• TTGACA box at -35 in Bacteria

And as we said , the function of promoter simply is to \rightarrow determine the start site of transcription and promotes it to transcribe the gene that's

located below it by RNA pol. Actually , there's no direct interaction between promoter and RNA polymerase , there's " MEDIATORS " known as

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transcription factor (TF's) these proteins can identify the promoter then they will recruit RNA pol. To transcribe the DNA .

TRANSCRIPTION FACTOR : trans-acting element (comes from outside as a protein, not a sequence inside DNA) involving in regulating the binding and efficiency of RNA pol.

Also , you can see that RNA pol. Really didn't read the TATA box Over the TATA box , we have those TRANSCRIPTION FACTORS , those can read TATA box and then once they bind to promoter , they bring RNA pol. And tell each other (protein to protein) copy from here , and how many copies they want to copy. When it reaches the last copy , they disappear to stop the function of RNA pol.

So RNA pol. see that the promoter doesn't have transcription factors, so it will go away :P

So In one word : (Promoters bind proteins which can activate or inhibit the binding of RNA pol. And thus determine the rate of

transcription)

- we will talk about the transcription factor in chapter 16 \odot The last thing about promoter is :

It needs a help by an sequence known as (**ENHANCERS**) to attract the RNA pol.

From the name of this sequence we can expect it's function \rightarrow it enhances the function of promoter

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So it's a very strong enhancer, without it the promoter function will be so weak and will be capable for copying only a few mRNA copies, while with it \rightarrow the RNA polymerase can make millions of copies \bigcirc So the transcription frequency is determined by other DNA sequences called (ENHANCERS).

Look at the same figure above (Anatomy of gene)

They put the enhancer like a (break) without any number

What does this mean?

This implies that it doesn't have a constant sequence or certain location, it may be located from tens to thousands of nucleotides far away from the promoter.

Now , if we want to compare between promoter in prokaryotes and eukaryotes

We will find the following :

In our cells each gene has its own promoter, but in Bacteria, it

COULD have several genes under the control of one promoter (Group regulation).

This means :

Bacteria have 3000 genes, it may have only 2000 promoters WHY ! Because, it says, I have 4 genes which will give me 4 proteins used in the same pathway, So put a one promoter for them

 \rightarrow Turn it on and you will get a one large RNA called

(POLYCISTRONIC RNA)

Then the ribosome will translate it into different proteins and they will work together \odot

While in our cells, we have 25,000 genes with 25,000 promoter

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So if you want 10 proteins from 10 different genes, we turn on 10 promoters together, we can do this D So eukaryotes have more accurate control more than prokaryotes By this we finish talking about the PROMOTER.

** the beginning and ending of the transcription is determined by the promoter and the terminator , while those of the translation are determined by the start and stop codons **

Now, we have what is known as **TERMINETORS** to terminate the transcription. In eukaryotes it's know as: **POLYADENYLATION SIGNAL**

RNA pol. Keeps transcribing until it reaches a sequence which tells him" STOP", this sequence goes like AAUAA But what happen is, because the momentum of RNA pol., it's going so fast, so it reads this sequence (AAUAA) and continues a little bit forward so it transcribes around 30-40 (maximum to 50) nucleotides after the termination signal then it stops Another enzyme called " CLEAVAGE " enzyme will come and cut these extra genes, but 20 of them will remain The **"POLY A POLYMERASE "** enzyme will come and add 200-250 adenine at 3' end. What's the FUNCTION of this poly A tail? 1) to protect RNA from degradation Because RNA is short lived in the cytoplasm, because there are enzymes in the cytoplasm that break it, called RNAse As RNA released, they want to break it, they start at the 3' end So when you put a non coding poly A tail, RNase will waste time breaking these non coding regions (they're not part of the codon)



At the same time , Ribosomes translate the codons at 5' end and produce proteins before RNase reach and break the coding region so it (ELONGATES the life of RNA in cytoplasm).

2) It is used to increase the rate of export of prepared RNA out of nucleus to cytoplasm

What we mean by prepared RNA, it has a cap, poly A tail and composed of exons only.

There are proteins known as CHAPERONS (shuttle protein) they enter the nucleus and carry the prepared RNA to cytoplasm, so any RNA that has this poly A tail will be caught and moved outside nucleus. That's the first thing that happens at 3' end

What will happen to 5' end !

Another enzyme will put a G-cap (GTP cap) at 5' end

The function of this cap is :

Quick identification of the 5' end of mRNA by ribosome .

(Ribosomes want to translate the RNA from 5' to 3', but it will translate million copies quickly, so it needs something relatively large to identify the 5' end quickly to start translation O)

If you remember, when we talked about DNA synthesis we use dNTP

(Deoxynucleoside triphosphate)

But when I incorporate them , it's incorporated as monophosphate , so

we remove 2 phosphates as pyrophoshphate for energy.

GTP that we put at 5' end is a triphosphate.

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Until now, what I'm saying is that We copy all the gene but before it go to cytoplasm, some processes will be done on it.

- 1) capping at 5' end
- 2) poly A tail at 3' end
- 3) splicing of introns

** note : these processes only happen in eukaryotes **

We have discussed the first 2 in details, now we will start with "

SPLICING PROCESS ":

RNA splicing is a very very complicated process , we remove the introns and join the exons .

For example :

Take one gene like (β glubin gene) which has 10 introns in its mRNA , we copy it into one million copy in one cell. So we need to cut 10 millions introns.

While we are removing them, we should be very accurate not to make any mistake. If we say that they are arranged in triples of nucleotide (not necessary but for clarification).

And we remove theses 3 nucleoides , we still have the appropriate sequence of proteins (Alanine – glycine for example) but if we remove one extra nucleotide or minus one , the whole sequence will be changed.

We will have (Alanine – another a.a)

This known as (FRAME SHIFTing mutation)

So introns should be removed with " Super accuracy "

For this purpose to be achieved :

For each intron border, there is 45 proteins at least that decide the beginning and the end of introns

°°°°°°°

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So they translocate, some of them stand before of introns, some after, and some will be in the middle to ensure that there is an intron sequence.

Then the intron will be cut quickly

Here are some videos may help you in understanding this lecture https://www.youtube.com/watch?v=WsofH466lqk https://www.youtube.com/watch?v=6YqPLgNjR4Q

That's it .D

I hope that you understand the subject , I tried my best \odot

I'm sorry for any mistake 😊

Written by: Nadya Baklizi

Special million thanks to the most awesome Friends . Mai ziad and Sally Awad ©

الشيء الذي نتومّع أننا لن نستطيع أن نفعله مو الشيء الذي لا نريد ان نفعله .. إن القرار الذي يريد أن يتخذه الإنسان في شأنٍ، قل ما تنقضه الأيام إذا كان حادراً حقاً عن إرادة وإيمان.

بالتوفيق بامتدان السبت و الله يباركلكم بوقتكم و يرزقكم أعلى العلامات 😳