Genetics & molecular biology

◉ Sheet ○ Slide

Number:		9
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FROM THE E. COLI PROMOTER>>>>>

If the promoter is older than 17, this leads to weakness because it is a special size. The typical thing to have is between 15-17 and is a filling that exists between the two consensus sequences.

There are three important areas in the promoter area:

1. -10

2. -35

3. Up element

The carboxyl terminal domain (CTD) comes out of the Alpha Sub Unit and plays a very important role in the regulatory process.

RNA polymerase needs to be regulated, which makes it on phosphorylation, which is carried out by the regulatory protein, which are proteins that act on the regulation transcription.

Phosphorylation is very important for two processes:

1. Regulation of transcription

2. Regulation of mRNA

The coordinating process for processing operations is phosphorylation on the CTD domain.

CTD Domain binds to UP element through a protein that stimulates the transcription process tremendously and thus if we remove the UP element; RNA polymerase activity can be reduced by 50 times. Instead of being very active, it is not active, because on the UP element there is an element that works through the CTD domain.

Sigma Factors:

Scientists have found that there are different Sigma Factors, so sigma is not one type. Each Sigma has a different sequence from another. Each sigma comes out at a different time.

Consensus Sequence has certain variations because there is a different possible sigma attached to the promoter.

S54=modulation of cellular levels

If the nitrogen on the bacteria differs its concentration, then this is an abnormal pressure situation, and the bacteria must react to it, so the bacteria produces proteins that help them adapt to the stress situation.

When nitrogen decreases, the cell concentrates on the production of S54 and leaves the rest

It’s a miracle that the presence of ten genes that adapt to bacteria and all ten genes that work on nitrogen starvation have the same sequence, which is estimated to bind to it S54.

Its function is Heat Shock Genes, because bacteria live at 37 ° C. If the temperature changes, this puts pressure on the bacteria to produce proteins to live at that pressure, so it produces S32, which specializes in finding promoters for genes that work to skip the temperature.

This is called Alternative Sigma, which works in the regulation of genes who work in the same pathway.

After the RNA polymerase is taken, the elongation process begins where the process is performed without the presence of a sigma. After the promoter is removed from Sigma, it goes to another promoter. The elongation process is slower than DNA polymerase because the transcription process only occurs to a gene of any nucleotide, whereas DNA polymerase occurs for each genome. The transcription process is slow in areas rich in G and C.

At each stage, there is a hybrid, which is a cross section between the RNA section and the original DNA section. This hybrid is called RNA and it is eight nucleotides. As the RNA polymerase moves, its position changes according to the RNA polymerase.

There are bubbles called transcription bubbles and they are never the size of 17 bp.

Helicase plays a role in the transcription process, as the double strand needs to be opened. Topoisomerase decompresses if it occurs, which is less than the doubling.

RNA manufacturing process

RNA polymerase comes at the active site and needs magnesium factors, without it, ALA does not work and DNA polymerase needs magnesium factors.

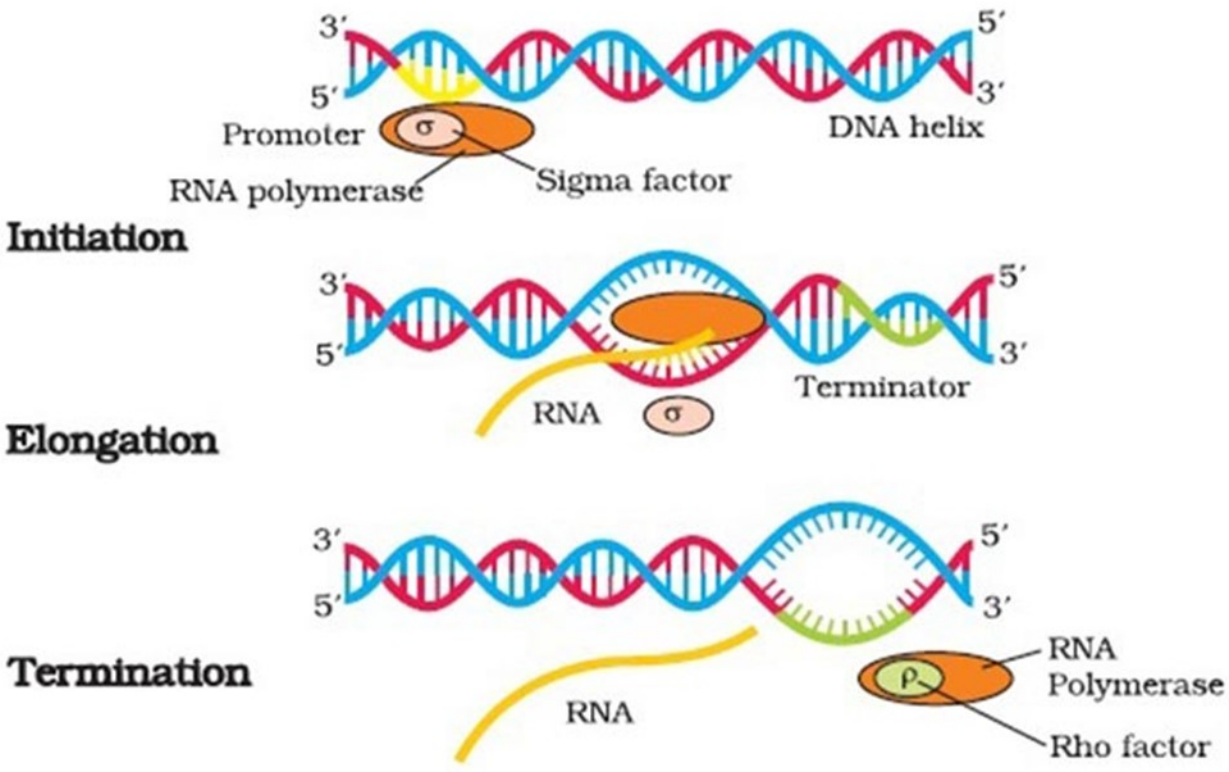
Incoming nucleotide is an energy compound. Nucleotide building blocks are all energy compounds in the DNA repositioning process. Adenine building stone enters the ATP form. These energy compounds where Tri phosphate. This is called a nucleotide bond (when the hydroxyl group on carbon 3 makes a binding to the phosphate group in the nucleotide). It is used by RNA polymerase in the construction process.

This bonding is called esterliphication reaction, because there is a hydroxyl group and phosphoric acid bonding, including ester bond.

The end of transcription is no less important than its beginning, because of mRNA.

Lecture 9

One of the similarities between DNA Replication and RNA Transcription is that both have: Initiation, Elongation and Termination. But a big difference is in the synthesis .
DNA deoxyribonucleotide.
RNA ribonucleotide.
ملاحظة: أحجار البناء تاعون النيوكليوتايد كلها مركبات طاقة.



The are 2 pathways for termination:

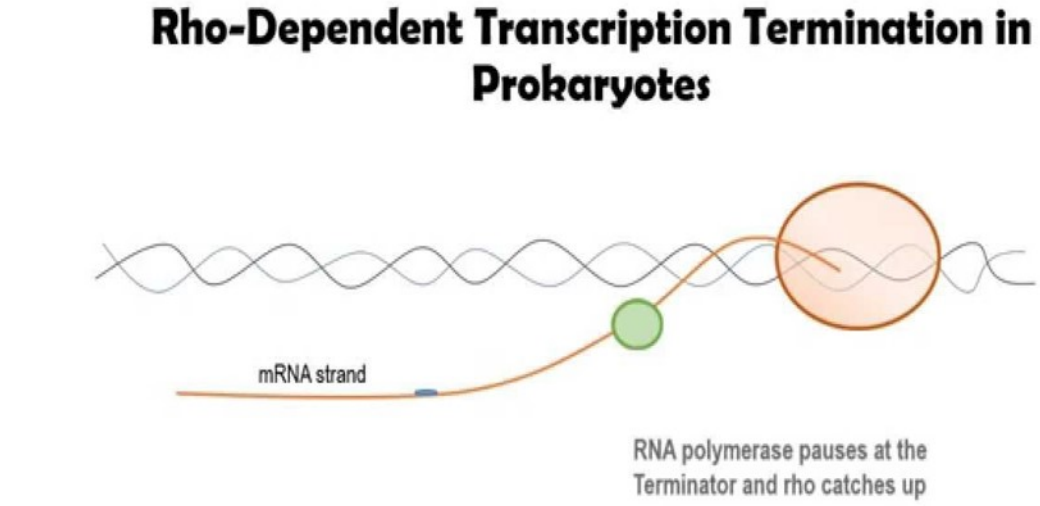
1- Rho Factor independent: there's no Rho proteins to help the transcript to dissociate from the template (stem and looping structure.)

2- Rho Factor dependent: there's a termination site and the protein Rho.
بتكون هاي المنطقة يلي بصير فيها هاي الطريقة غنية بروابط C-G وبالتالي بكون مشي ال البولامريز بطيئ عندها فبسمح يصير اصطدام مع Rho يلي سرعته بتبقى كما هي.

Rho protein has a recognition (specific) site on the transcript to bind with. Rho speed is 50 or 60 N\S such as RNA pol. Rho protein can move on the transcript (migration) = Follow the RNA polymerase, so it consumes ATP. Its function is the unbinding of DNA-RNA hybrid, because in this termination the termination site doesn't have the same structure as in the termination site of the Rho independent. So it has the helicase activity for the unwinding of DNA-RNA hybrid. "But it is a cute helicase". It has self-complementary sequences (hair pin structure) but has differences in the As and Us sequences.

*If there's a recognition site in the transcript then the Rho protein is needed, (no recognition site no protein).

*If there's Rho protein there's no As and Us sequences in the end of the termination site.



In Prokaryotes there's one RNA polymerase and in Eukaryotes there's 3 types of RNA polymerase:

1. RNA polymerase 1: Synthesis of Pre-rRNA, there's different types of rRNA (e.g. 18s, 5.8s and 28s) except 5s rRNA. (Pre= Immature).
2. RNA polymerase 2: Synthesis of mRNA& snRNA "small nuclear RNA".
3. RNA polymerase 3: Synthesis of tRNA& snRNA and 5s RNA.

Genes are defined as a sequence that codes for polypeptides or RNA, (rRNA and tRNA are RNA products –final product-, but mRNA is translated to protein, whereas tRNA and rRNA are not. Not all of the mRNA will be translated.)

Promoters in eukaryotes are composed of: Initiator, TATA box and various regulatory sequences.

معلومة:" الكثافة الجينية لدى البكتيريا أكثر منا وذلك لاحتوائها على جينوم صغير، بينما نحن لدينا كثافة أقل لاحتوائنا على جينوم كبير جدا. فتكون عملية ايجاد ال promoter لدينا أصعب. أكثر عدد من الجينات أكثر عدد من التعقيدات."

* Regulatory sequences, high regulation for transcription (turning on or off of genes).

* TATA box is conserved in all eukaryotes (the same in all of them).

* Initiator is not very conserved in all the eukaryotes, (not the same in all the promoters).

* +1: is the first nucleotide to be added in the transcript.

* +1 is within the initiator.

*RNA pol 2 is a huge enzyme that's composed of 12 subunits: RBP1, RBP2, RBP3….RBP11, and the CTD (12^{th} subunit). (CTD: carboxyl terminal domain.)

* In Eukaryotes we don't have "sigma" but "GTF" which is: General Transcription Factor.

تعمل كبنية تحتية على ال promoter بعد أن تجده عشان يقدر يجي ويربط ال RNA pol.

* TBP: TATA box binding protein and it is the first from GTF, binds with TATA box first with TF2A.

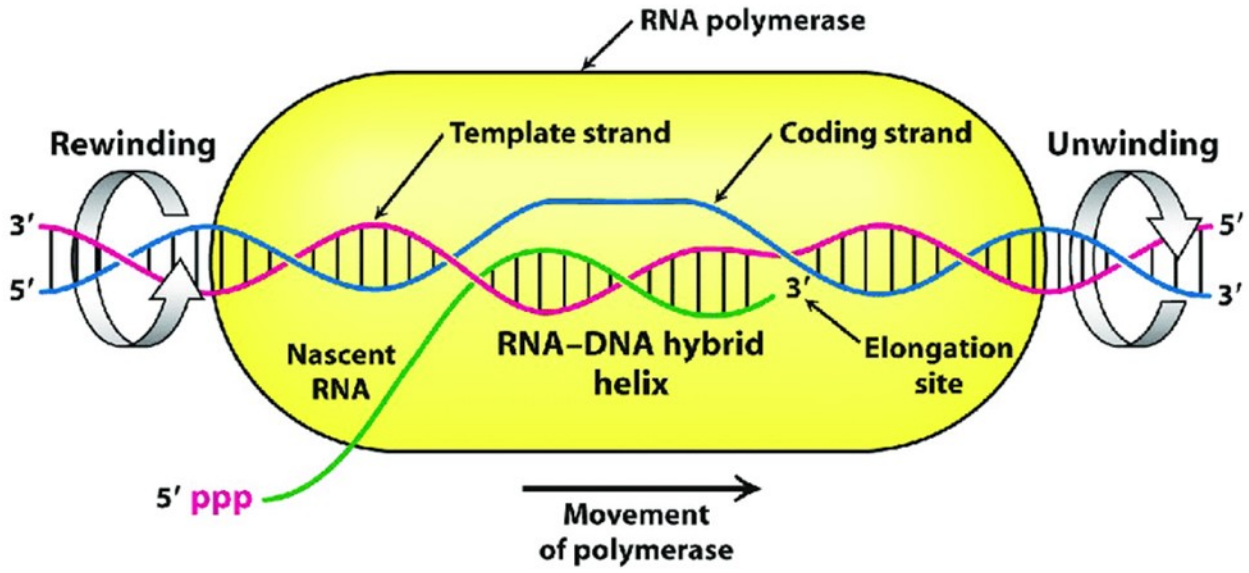
* TF2B: transcription factor, 2= RNA pol.2 and B (F, E, H) = the type of the transcription factor.

*The attachment of the TBP recruits the binding of the other transcription factors.

*Closed complex= All the transcription factors including TBP + RNA pol.2 + templet. (Closed: Closed strands).

*Unwinding happens in the initiator region because it has the +1.

*TF2H, for unwinding. “works as a helicase”



*Phosphorylation of CTD means that this complex is committed to elongation (release from promoter).
وتعتبر نقطة التشغيل لبدء العمل.
There are many patterns for phosphorylation happening in CTD amino acids and each one motivates a process by motivating a special factor.

* TF2H is responsible for phosphorylation of CTD.

Functions of TF2H:

1- Unwinding of the DNA strands (helicase activity).

2- Phosphorylation of the CTD (Kinase activity).

*In the end of initiation and the beginning of elongation, all transcription factors are released, and elongation factors bind.

*When it reaches the termination site, RNA pol.2 releases and dephosphorylation of the CTD occurs; so the RNA pol.2 is recycled and ready to initiate another transcript.

***Slides: Table 26-2: only the underlined + TBP + TF2A are required.**

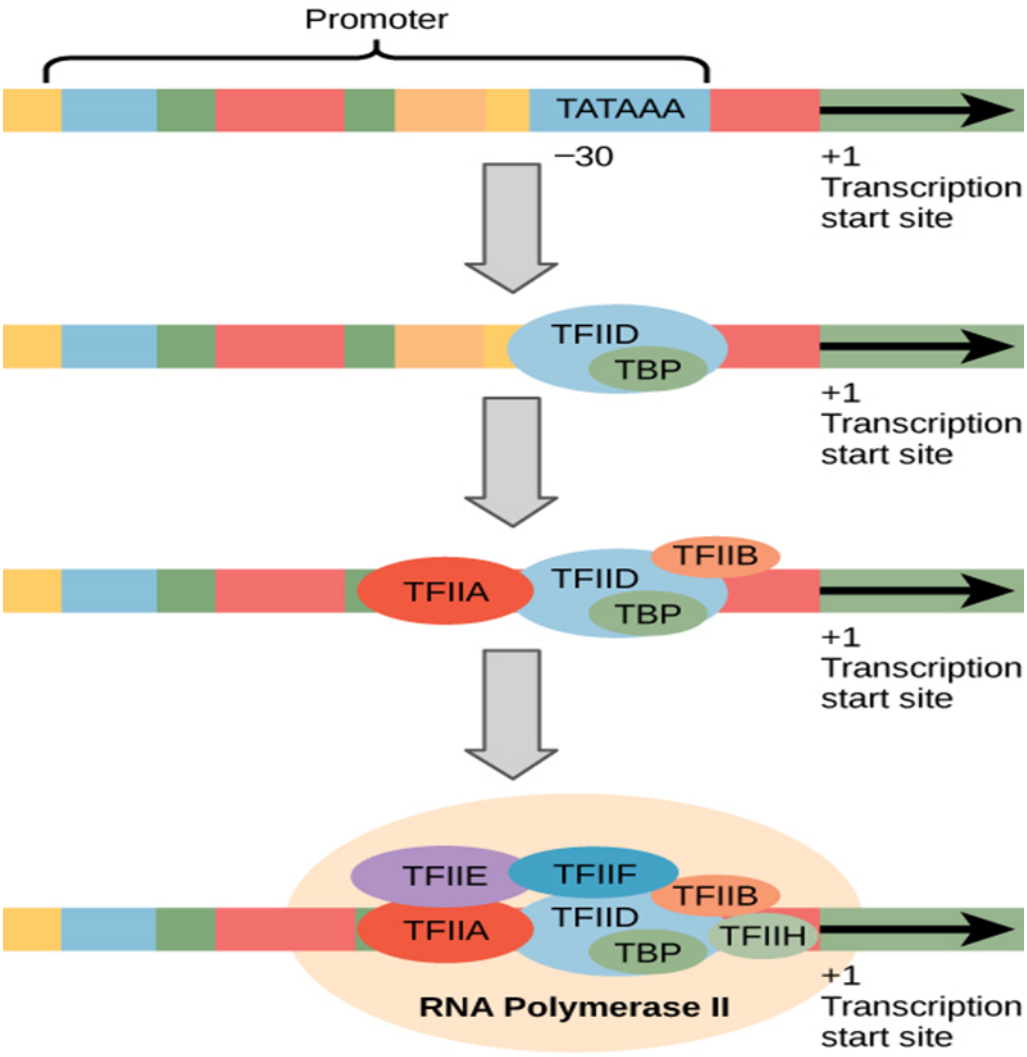
*TF2H: Recruits nucleotide-excision repair because RNA pol.2 can’t do proofreading from 3’ to 5’.

* Not all the transcripts are translated into proteins, (so any mistake in the transcript leads to degradation of it).

* After the TF2H is released, any mistakes can't be repaired.

* The helicase activity of the TF2H is in the beginning only, then it opens automatically.

* Post transcriptional modification occurs in the nucleus.



mRNA Modification in Eukaryotes:

- Capping, to prevent the degradation or the exonuclease of the mRNA because it's a single strand (protection), and also to help the mRNA bind with the ribosome after getting out of the nucleus.

* Capping occurs during transcription on the 5' end.

- Splicing, removal of introns and rejoining of exons. Alternative splicing has regulatory significance: Regulation of the coding genes and its activity.

- Polyadenylation: adding of adenines on the 3' end.

* The mature mRNA is much smaller than the transcript.

* After completion of primary transcript, cleavage of introns happens. (Some books say that cleavage of introns happens during transcription).

* Intercalating agents like some medications, inhibit the RNA polymerase.

* Intercalating agents on DNA prevent the opening of the 2 strands so there's no transcription. (initiation might happen but it inhibits elongation).

* Actinomycin is quite similar to the nitrogen bases so it forms H-bond or covalent bond, and prevents the opening of the strands.

* Actinomycin D, Acridine and Rifampicin are examples of these medications.

* Each medication has a mechanism, Rifampicin, for example binds with B-subunits of the bacterial RNA and prevents the promoter clearance step of transcription (releasing of sigma factor). And others make intercalating agents.

*DNA footprinting: to identify the DNA sequence bound by a particular protein (e.g. RNA polymerase).

*** One strand of DNA molecule is radio actively labeled. (so it can be recognized on gel).**

- 2 tubes with DNA strands labeled with radioactive material have DNAse (makes a cut one time in the strand) and a protein (in one of the tubes only).

--> Protein prevents the cut on its site of binding by DNAse (covers the site so no cut in this site).

Chapter 6 RNA Processing, Post transcriptional processing.

* Cap synthesis enzyme (In the beginning of the transcript) binds to the 5' end of the transcript and the CTD → Cap synthesis complex.

* The adding of the cap is 5' to 5' (addition of Guanine to the 5' end).

* The 5' end starts with triphosphate group bound to the nitrogenous base (pppNp).

* Phosphohydrolase: Removal of the phosphate group of the triphosphate group (ppNp).

* Guanylytransferase: Transfer of pG (from GTP, pppG) to the 5' end (GpppNp).

* Methylation of Guanine for more protection from degradation.

* Guanine-7-methyltransferase adds Methyl group to the Guanine (m7GpppNp).

* For more protection: Methylation for the first base nucleotide by –O-methyltransferase (m7GpppmNp) and the second base sometimes "in us specially".