Genetics & molecular biology

◉ Sheet ○ Slide

Number:		8
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Recombination:

Recombination means something hybrid or recombinant.
Types of recombination:

1. Homologous Recombination: it is used for two functions:
A. Crossing over exchange of genetic material for diversity (it occurs in prophase one meiosis) between two homologous chromosomes.
Humans have 46 chromosomes and each one of them has both maternal and paternal which are quite similar with the same order of genes, but differ in sequences and non-identical maternal and paternal. (high similarity and may differ in alleles)
B. Repair double stranded break

2. Site specific recombination: Recombinase, a tetramer enzyme composed of four subunits which make it available to link with the two chromosomes. Its active site contains Tyr and its OH deals with the nucleophilic attack → breakage of the DNA back bone (phosphodiester bond). Recombination site is essential for the enzyme to link with chromosomes.

3. Transpositional recombination:
a process in which a transposable element is inserted into a target DNA.
It has two types:
1. Simple Transposons: which have only the transposition gene which will be translated into the transposes enzyme.
2. Complex Transposons: which have the transposition gene and other extra genes that give the cell new features.
It has two classes:
1. Retro Transposon (copy-paste): firstly, a transcription happens (copying) which transcribes DNA into RNA then a reverse transcription occurs by an enzyme which transforms the RNA to DNA intermediate, and then finally recombination. This product invades the DNA (the target DNA).
2. DNA transposons (cut-paste): a cut is made by a transposase enzyme and then recombination occurs.

RNA metabolism:
Q: What is the central dogma of molecular biology?
DNA is replicated to DNA, or transcribed to RNA and then translated to proteins.

Transcription (RNA synthesis):

DNA transcription..

- Information must be transcribed from DNA in order to function further.
- DNA→RNA→Protein

DNA replication occurs in the nucleus for eukaryotes and in the cytoplasm for prokaryotes.

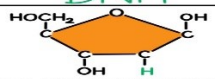
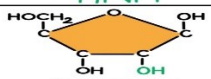
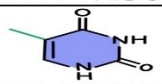
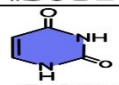
Translation always occurs in the cytoplasm.

All our cells have the same genome, despite their different functions. Because of the regulatory genes. (Genes are not always working.)

Humans have 25-30 thousand genes.

The transcription process is responsible for cell differentiation.

DNA differs with RNA in Sugar type and bases (T&U)

	DNA	RNA
SUGAR	 DEOXYRIBOSE	 RIBOSE
BASE	 THYMINE	 URACIL

Replication occurs completely on all genome parts, while transcription can occur partially (a single part can be transcribed).

RNA Polymerase mistakes are less effective/important than DNA Polymerase, because DNA is the origin.

أي يعني أن أخطاء ال RNA polymerase تعوض رغم كثرتها لأن الأصل وهو ال DNA موجود ويمكن عمل نسخة صحيحة أخرى. أخطاء الDNA يتم توريثها.

Half life time of RNA is very variable.

RNA is a single strand, while DNA is double stranded … which means RNA is more flexible.

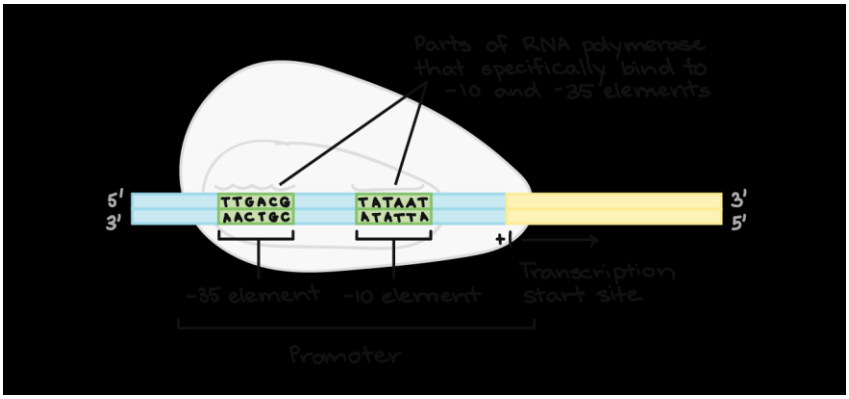
Most RNA is not translated.

A ribozyme is a ribonucleic acid (RNA) enzyme that catalyzes a chemical reaction.

t-RNA and r-RNA are not translated.

The coding strand is the DNA strand whose base sequence corresponds to the base sequence of the RNA transcript produced (thymine replaced by uracil). It is this strand which contains codons.

Transcription initiates from +1 to the end of the gene.

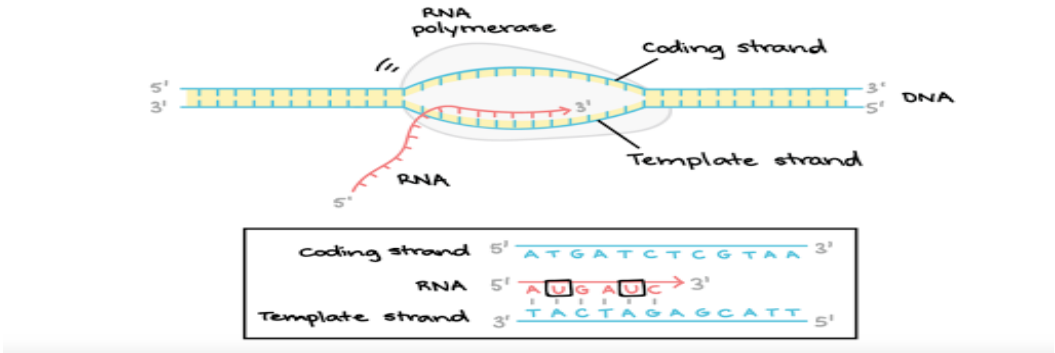


Translation starts from (AUG), ends in stopping codon (translation isn't for the whole gene structure).

Start and end codons are untranslated regions (UTR).

Template strand (Antisense strand)

Coding strand (Sense strand).



RNA Pol is less complex than DNA pol (though both are complex)

RNA POLYMERASE SUBUNITS

Subunit	M_r/kDa	Gene	Function
α	36511	*rpoA*	required for assembly of the enzyme; interacts with some regulatory proteins; involved in catalysis (*16*)
β	150616	*rpoB*	involved in catalysis, chain initiation and elongation (*17*)
β'	155159	*rpoC*	binds to the DNA template (*18*)
σ^D	70263	*rpoD*	directs enzyme to the promoter of the genes expressed in the exponential growth phase (*19*)
ω	10237	*rpoZ*	required to restore denatured RNA polymerase *in vitro* to its fully functional form (*20*)

(Complex is built on promoter region).

Sigma subunit connects DNA in 2 regions (-10 and -35).

Types of RNA:

1. Messenger RNAs (mRNAs): transport genetic information from DNA in nucleus to the ribosomes in the cytoplasm where translation occurs. (eukaryotes)

Q. In prokaryotes there is no nucleus. Why can't DNA be translated directly to proteins? Or why is mRNA important for the translation process? mRNA: encodes the amino acid sequence. ((كأنه المركب القادر يقرأ لغتين (لغة الجين ولغة الأمينو أسيد) .

2. Transfer RNAs (tRNAs): read the information encoded in the mRNA (codons) and transfer the appropriate amino acid to a growing polypeptide chain during protein synthesis.

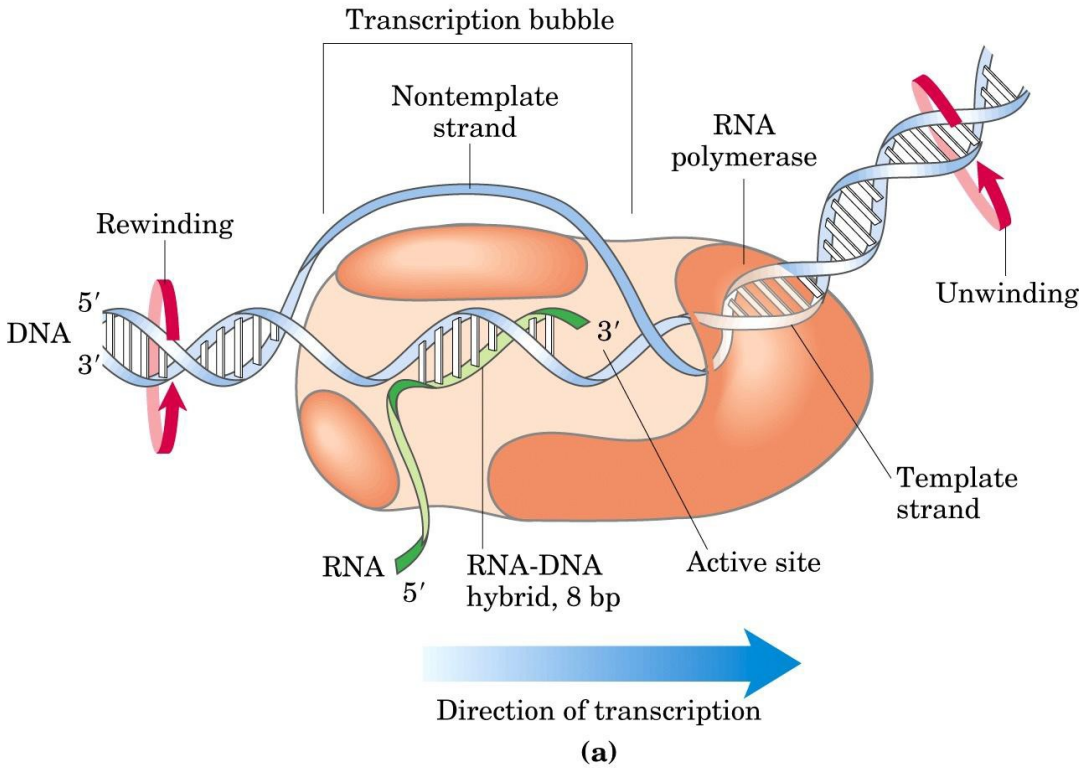
3. Ribosomal RNAs (rRNAs) serve as components of ribosomes.

Overview of RNA transcription, comparing DNA replication with RNA transcription.

- RNA polymerase: An enzyme that synthesizes the RNA sequence… RNA is synthesized by DNA dependent RNA polymerase (DNA template→RNA).
- As in DNA replication; RNA transcription includes three steps: initiation, elongation, and termination.
- A promoter: is a specific sequence of DNA where RNA polymerase links to start RNA synthesis (as well as the origin (oriC) in DNA replication).
  Each gene has a region which is called promoter.

- Transcription bubble: is the place the double strand of DNA unwinds to start transcription in the promoter sequence (like the replication bubble in DNA replication).
- Template: RNA polymerase also needs a template (DNA) for RNA synthesis.
- In DNA replication the whole DNA molecule unwinds but in RNA transcription part of the DNA molecule (a specific sequence which contains the specific gene) unwinds.
- DNA replication needs a primer but RNA transcription doesn't need a primer, which means that DNA polymerase doesn't start from scratch but RNA starts from scratch. Note that the first nucleotide contains triphosphate and other added nucleotides contain monophosphate (release of pyrophosphate).
- DNA-RNA hybrid 8 nucleotides → movement of RNA polymerase → adding of nucleotides from 5' to 3'. RNA transcription is 5' >>> 3'

- Topoisomerase removes the supercoiling
- DNA has two strands and one of them is used in transcription (template strand) and the other one is a non-template strand. Which strand is used? The one that contains the genome.
- No proofreading in RNA transcription but there is proofreading in DNA replication.
- Transcription resembles replication in its fundamental chemical mechanism which means:
  1. There's new nucleotides that need to link to other new nucleotides (oxynecloetide in transcription)
  2. Forming a phosphodiester bond → release of PPI.
- 3. Direction of synthesis in both process from 5' to 3'.
- 3' side is the side where RNA polymerase will add nucleotides
  adding of nucleotides → elongation → release of PPI.
  due to the winding and the unwinding of DNA strands a negative and positive supercoiling results → need of topoisomerase to remove the supercoiling.
- Speed of RNA polymerase → 50-90N/s
- A transcription "bubble." (about 17 bp unwound)



(a)

Transcript RNA is similar to the non-template strand because transcription is a complimentary process (T → U instead)
tRNA reads the transcript which is similar to the non-template strand (or coding strand)

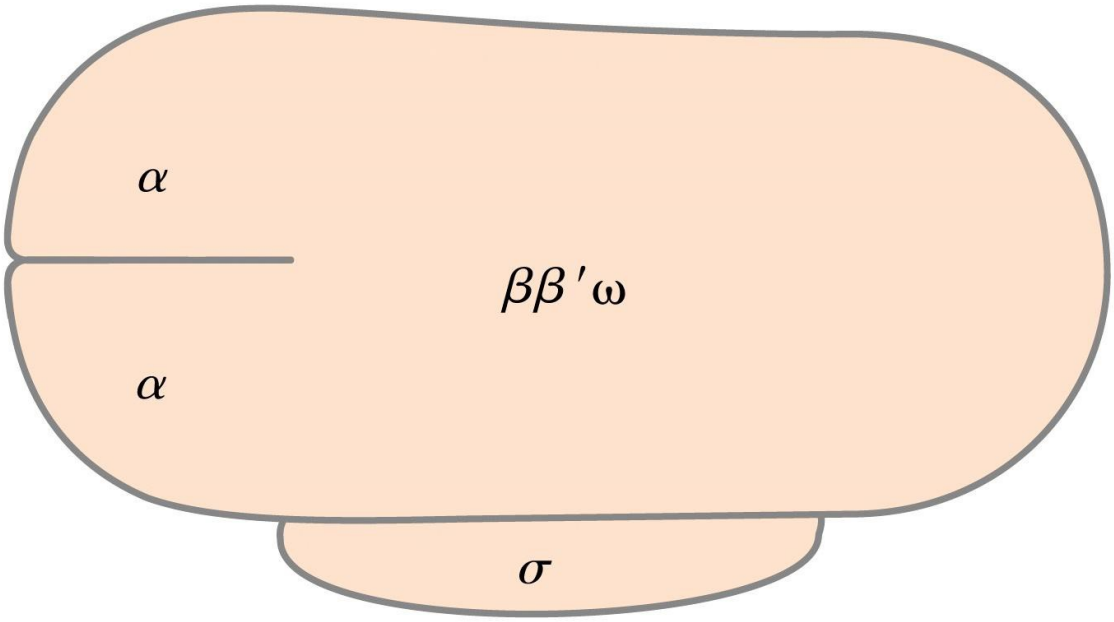
(5′) CGCTATAGCGTTT(3′)	DNA nontemplate (coding) strand
(3′) GCGATATCGCAAA(5′)	DNA template strand
(5′) CGCUAUAGCGUUU(3′)	RNA transcript

RNA polymerase structure:

Holoenzyme = components of the Enzyme + the co-factors

- RNA polymerase is the major subunit with the sigma co factor which helps RNA polymerase to function.
  5 subunits are constant in all types of RNA polymerase, sigma is the variable subunit.
  Sigma changes with the gene. Why does the sigma subunit differ from one polymerase to another?
  sigma → directs the enzyme to specific binding sites on the DNA within the promoter region.
  EX: housekeeping genes: involved in basic cell maintenance and, therefore, are expected to maintain constant expression (transcription & translation) levels in all cells and

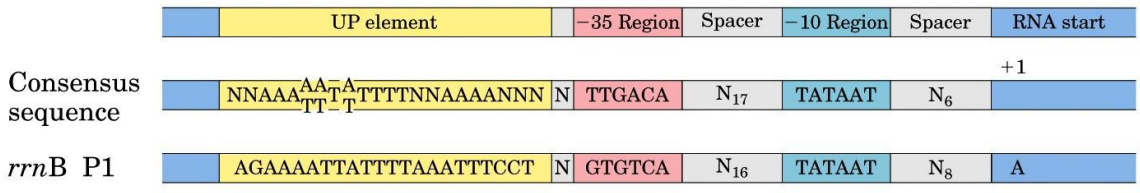
conditions.
70 = housekeeping genes
32 = heat shock protein



Promoter region:
Promoters have three important regions :
1. -10 region.
2. -35 region.
These two sequences are conserved sequences (consensus) → existed in most types of the bacteria.
What does (-) mean?

- the first nucleotide coding the RNA transcript is at position +1.
- any nucleotide that precedes the +1 nucleotide (upstream nucleotide) is donated by a (-).

(+) downstream
(-) upstream
3. UP element. Not present in all bacteria, (UP) upstream promoter region



Initiation:

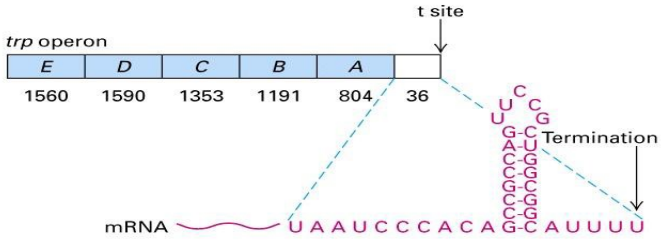
RNA polymerase with 5 subunits builds the core and sigma subunit.
Sigma subunit recognizes the -10 and -35, then directs the RNA polymerase to bind to promoter region.
The binding of DNA (template/promoter) and RNA polymerase (holoenzyme) is called closed complex formation.
In closed complex, DNA molecule is still closed.
Open complex is when the 12 to 15 bp unwind within the -10 region (approximately within the -10 region to position +2/+3/+5)
After unwinding of DNA molecules RNA synthesis begins → synthesis of RNA hybrid (8 Bp) from the +1 region → transcription is initiated when the hybrid is formed
Notice that the unwinding starts from -10 but adding of nucleotides starts from +1

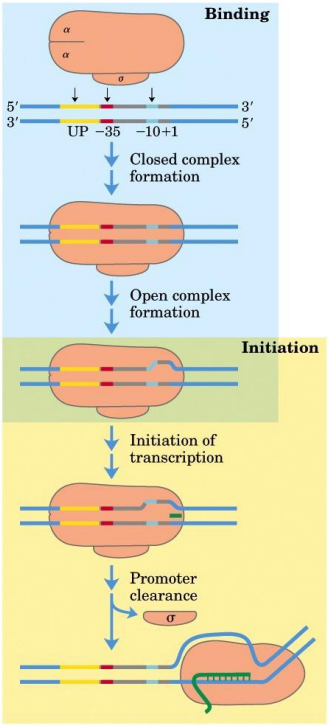
Elongation:

* RNA polymerase leaves the promoter region
* starts by the removal of sigma subunit
→ Once the subunit is released and the polymerase leaves the promoter = becomes committed to the elongation of the RNA.

Termination:

Elongation stops in a termination region. There are two ways to stop elongation:
1. Rho dependent: depends on a protein.
2. Rho independent: depends on something in the transcript itself (something in the DNA molecule but is recognized by the transcript) (termination site in the DNA).
→ t site (DNA) rich with C and G in the middle and in the ends with A. (a lot of adenine)
stem and loop or hairpin structure transcript RNA contains complementary parts linked together
→ in the end of this region (RNA polymerase stops) then the strand is separated by itself because the connection in the hybrid between A and U is unusual and unstable.




Binding
α
α
σ
5′
3′
3′
5′
UP −35 −10+1
Closed complex formation
Open complex formation
Initiation
Initiation of transcription
Promoter clearance
σ