



بسم الله الرحمن الرحيم



كل هالبروتين وين يروح

هذه المذكرة كافية تماما وهي اتمام لما بدأت قبل سنتين

هي اهداء لدفعة ٤٢٨ الراعين

واهداء لأغلى انسان لي في هذه الدفعة خاصة

اهداء الى كل الدفعات القادمة . . .

أحمد العقيل ..

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Overview

- Protein Synthesis called "**Translation**"
- Translation needs **Genetic Code**.
- **Genetic Information** :
 - Stored In Chromosomes .
 - Transmitted to daughter cells by DNA replication .
- **Genetic Information** is expressed through Transcription to RNA → mRNA -
→ Subsequent translation into proteins .

Note :

- Any alter in the nucleic acid sequence may result in an improper Amino Acid being inserted into protein chain ,potentially causing Disease or even death
- **Many Proteins are covalently modified to :**
 - Activate them
 - Alter their activities
 - Target them to extracellular or intracellular destination

The Genetic Code

- * Each Individual word in the code is composed of three Nucleotide bases .
- * These Genetic words are called " *Codons* "

A - Codons

- Present In mRNA .
- They are presented by the language of : C, G , A , U
- Their nucleotides should be written from 5'-end to 3'-end
- there are 64 different combinations of bases
- كل ثلاثة منهم تترتب بترتيب معين ويسوا كودن جديد

Adenine (A)

Guanine (G)

Cytosine (C)

Uracil (U)

NO T (thymine) (MCO) J >>>



How To Translate a Codon

you can translate the codons to Amino acid .

- For Example , 5'-AUG-3' codes for methionine .
- 61 of 64 are coded for the 20 common amino acid

Termination (or " stop " or " nonsense ") codons :

هاالم { UAG - UGA - UAA }

If One of these amino acid appears in mRNA : termination will occur and they do not code for Amino acid

à

sending signals that the process is complete

5' - BASE	MIDDLE BASE				3' - BASE
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

1 These four rows show sixteen amino acids whose codons begin (5') with A.

2 This column shows sixteen amino acids whose codons have the middle base U.

3 These four, separated rows show sixteen amino acids whose codons end (3') with G.

4 The codon 5'-AUG-3' designates methionine (Met).

Figure 31.2 Use of the genetic code table to translate the codon AUG.

من الجدول اعرف التالي :

- 1) Met & Trp is the only amino acids that form by only one codon
- 2) others amino acids formed by more than one codon as u see
- 3) Not in every protein we find Met



any mutation that altered the manner in which the code was translated would have caused the alteration of most, if not all, protein sequences, which would certainly have been lethal

Characteristics of the genetic code

Specificity

- The genetic code is specific
- a particular codon always codes for the same amino acid

Universality

في أي مكان من الجسم أي ترتيب للبيس يعطي نفس الامينو اسيد بأي مكان ثاني بالجسم
ولهذا استثناء الميتوكوندريا J

- the specificity of the genetic code has been conserved from very early stages of evolution [Note: An exception occurs in mitochondria, in which a few codons have different meanings like , UGA codes for trp.]

Degeneracy (Redundancy)

- a given amino acid may have more than one triplet coding for it. For example, arginine is specified by six different codons.

كل امين ممكن يكونه اكثر من كودون بس كل كودون يكون أمين واحد

لا تنسوا ال Met يكونه كودن واحد تتذكروه ؟

Nonoverlapping and commaless

- The genetic code is nonoverlapping and commaless, that is, the code is read from a fixed starting point as a continuous sequence of bases, taken three at a time. For example, ABCDEFGHIJKL is read as ABC/DEF/GHI/JKL without any "punctuation" between the codons.



C. Consequences of altering the nucleotide sequence:

- Alteration in single nucleotide base (point mutation):

1. *silent mutation* :

the codon containing changed base may code for the same amino acid
(e.g. UCA \rightleftharpoons UCU = serine)

2. *missense mutation* :

the codon containing changed base may code for different amino acid
(e.g. UCA =serine \rightleftharpoons CCA = proline)

3. *Nonsense mutation* :

the codon containing changed base may become termination codon
(e.g. UCA = serine \rightleftharpoons UAA = termination codon)

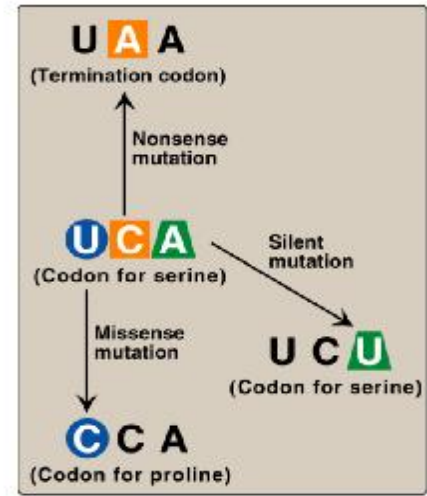


Figure 31.3
Possible effects of changing a single nucleotide base in the coding region of an mRNA chain.

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ي مربوط وقتہ ل



Alteration in amount or structure of protein produced by translation :

A. Trinucleotide repeat expansion :(MCQ)

Occasionally, a sequence of three bases that is repeated in tandem will become amplified in number, so that too many copies of the triplet occur.

If this occurs:

1) Within coding region of gene :

the protein will contain many extra copies of one amino acid .

- I. Huntington protein (11-34 CAG = 11-34 glutamine) è huntington disease (MCQ) (neurodegenerative).

* N.B : Huntington protein is unstable

2) In untranslated portion of gene è decrease in produced protein :

- I. Fragile X syndrome (7-50 CGG)
- II. Myotonic dystrophy (5-35 CTG)

B. Splice site mutations:

- Mutation at splice site è no removal of introns from pre-mRNA è apparent protein production

C. Frame – shift mutations :

- *frame – shift mutations :*

- ü Addition or deletion of 1 or 2 nucleotides to interior of message sequence (not 3) (MCQ) هالم
- ü Reading frame of mRNA is altered
- ü Resulting amino acid sequence may become different from this point on

- *Addition or deletion of amino acid:*

- ü Addition or deletion of 3 nucleotides (amino acid)
- ü Not frame– shift mutations
- ü Reading frame of mRNA is not altered

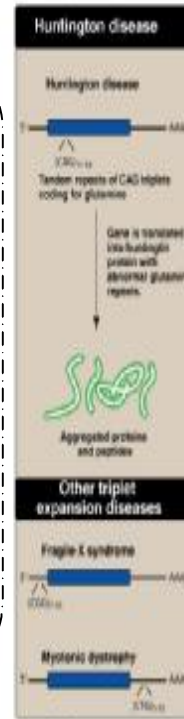


Figure 31.4 Role of tandem triplet repeats in mRNA causing Huntington disease and other gene expansion diseases.

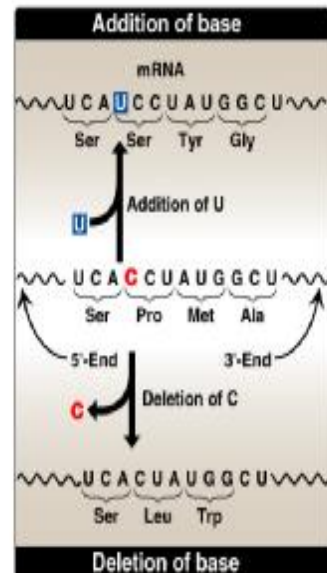


Figure 31.5 Frame-shift mutations as a result of addition or deletion of a base can cause an alteration in the reading frame of mRNA.



Loss of three nucleotides maintains the reading frame, but can result in serious pathology.

For example, cystic fibrosis (CF):

- a *hereditary disease*
- primarily affects the *pulmonary and digestive systems*
- is most commonly caused by deletion of three nucleotides from the coding region of a gene, resulting in the loss of phenylalanine at the 508th position ($\Delta F508$) in the protein encoded by that gene. This $\Delta F508$ mutation prevents normal folding of the CF transmembrane conductance regulator (CFTR) protein, leading to its destruction by the proteasome .

Note :

CFTR normally functions as a chloride channel in epithelial cells, and its loss results in the production of thick, sticky secretions in the lungs and pancreas, leading to lung damage and digestive deficiencies . In over seventy percent of patients with CF, the $\Delta F508$ mutation is the cause of the disease.



III. Components Required for Translation:

- 1- all the amino acids that are found in the finished product
- 2- the mRNA (to be translated)
- 3- transfer RNA (tRNA) .
- 4- functional ribosomes .
- 5- energy sources .
- 6- enzymes
- 7- protein factors

Amino acids

All the amino acids must be present at the time of protein synthesis

(If one amino acid is missing (for example, if the diet does not contain an essential amino acid), translation stops at the codon specifying that amino acid.)



Transfer RNA

- At least one specific type of tRNA is required per amino acid
- In humans, there are at least fifty species of tRNA
- some amino acids have more than one specific tRNA molecule.
- But every single tRNA is directed for one amino acid

Amino acid attachment site

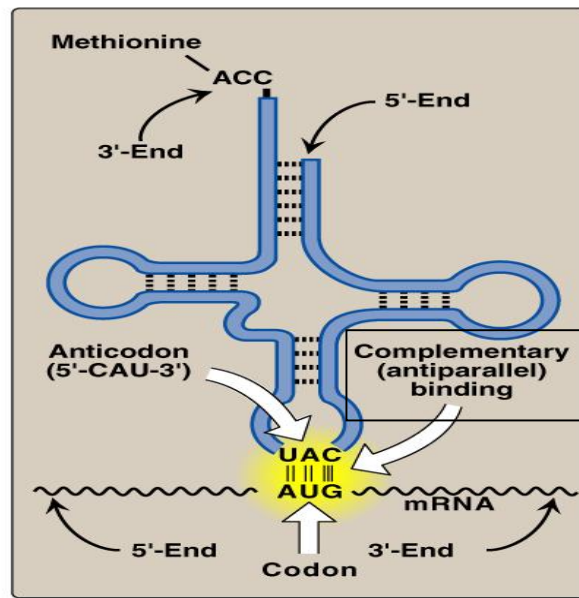
- Each tRNA molecule has an attachment site for a specific (cognate) amino acid at its 3'-end.
- charged : tRNA has a covalently attached amino acid
- Uncharged :tRNA is not bound to an amino acid

When an amino acid become activated ?
 - when it attached to t- RNA

Anticodon:

Each tRNA molecule also contains a three-base nucleotide sequence—the anticodon—that recognizes a specific codon on the mRNA

tRNA are able to carry a specific amino acid and to recognize the codon for that amino acid. tRNA, therefore, function as adaptor molecules. (MCQ)



هاااااام

Figure 31.6
 Complementary, antiparallel binding of the anticodon for methionyl-tRNA (CAU) to the mRNA codon for methionine (AUG).



Aminoacyl-tRNA synthetases:

- This family of enzymes is required for attachment of amino acids to their corresponding tRNA .
- Each member of this family recognizes a specific amino acid and the tRNA that correspond to that amino acid (isoaccepting tRNA).

Aminoacyl-tRNA synthetases:

- catalyzes a two-step reaction
- The overall reaction requires adenosine triphosphate (ATP), which is cleaved to adenosine monophosphate (AMP) and inorganic pyrophosphate (PP_i)
- The extreme specificity contributes to the high fidelity of translation of the genetic message .
- have a "proofreading" or "editing" activity that can remove mischarged amino acids from the enzyme or the tRNA molecule.

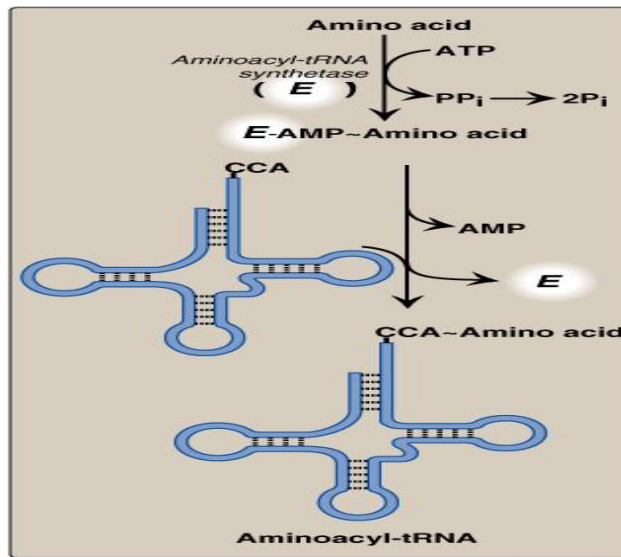


Figure 31.7
Attachment of a specific amino acid to its corresponding tRNA by *aminoacyl-tRNA synthetase (E)*.

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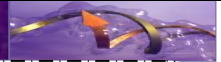
Messenger RNA

The specific mRNA required as a template for the synthesis of the desired polypeptide chain must be present

Functionally competent ribosome

- Ribosomes are large complexes of protein and ribosomal RNA (rRNA)
- They consist of two subunits —one large and one small— .
- Their relative sizes are generally given in terms of their sedimentation coefficients, or S (Svedberg) values.
- Because the S values are determined both by shape as well as molecular mass, their numeric values are not strictly additive. For example, the prokaryotic 50S and 30S ribosomal subunits together form a 70S ribosome. The eukaryotic 60S and 40S subunits form an 80S ribosome.] (MCQ)(IMP)
- Prokaryotic and eukaryotic ribosomes are similar in structure, and serve the same function, namely, as the “factories” in which the synthesis of proteins occurs.

The large ribosomal subunit catalyzes formation of the peptide bonds that link amino acid residues in a protein. The small subunit binds mRNA and is responsible for the accuracy of translation by ensuring correct base-pairing between the codon in the mRNA and the anticodon of the tRNA



Ribosomal RNA :

prokaryotic ribosomes contain three molecules of rRNA, whereas eukaryotic ribosomes contain four molecules of rRNA

Ribosomal proteins :

Ribosomal proteins are present in considerably greater numbers in eukaryotic ribosomes than in prokaryotic ribosomes

A, P, and E sites on the ribosome :

The ribosome has three binding sites for tRNA molecules—the A, P, and E sites—each of which extends over both subunits. Together, they cover three neighboring codons. During translation :

the A site binds an incoming aminoacyl-tRNA as directed by the codon currently occupying this site. This codon specifies the next amino acid to be added to the growing peptide chain. (MCQ)

The P-site codon is occupied by peptidyl-tRNA. This tRNA carries the chain of amino acids that has already been synthesized. (MCQ)

The E site is occupied by the empty tRNA as it is about to exit the ribosome. (MCQ)

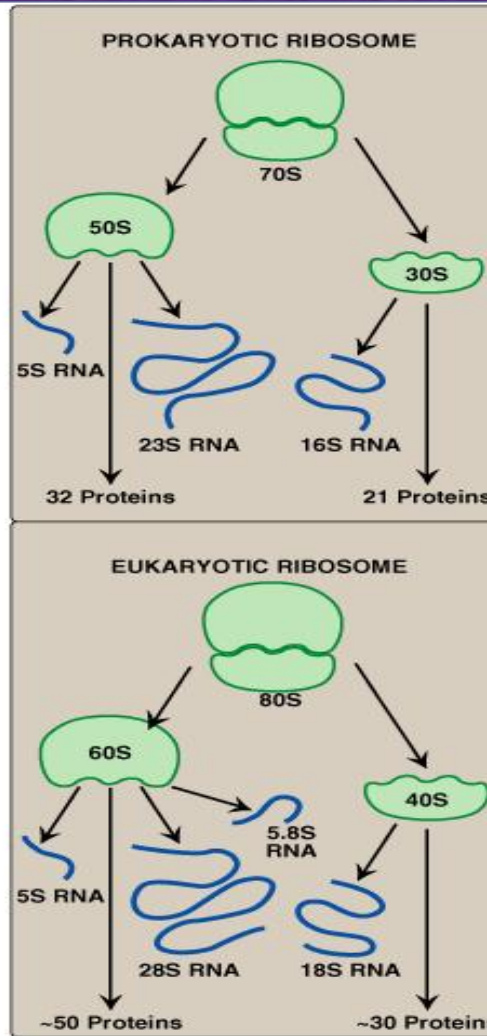


Figure 31.8 Ribosomal composition. (The number of proteins in the eukaryotic ribosomal subunits varies somewhat from species to species.)

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Cellular location of ribosomes :

- In eukaryotic cells the ribosomes are:

Free (cytosolic) (MCQ)

Cytosolic ribosomes synthesize proteins required in the cytosol itself, or destined for the nucleus, mitochondria, and peroxisomes

Associated with RER :

The RER-associated ribosomes are responsible for synthesizing proteins that are:

1- to be exported from the cell .

2- destined to become integrated into plasma, endoplasmic reticulum, or Golgi membranes, or incorporated into lysosomes.

Note : Mitochondria contain their own set of ribosomes and their own unique, circular DNA.



تخیلو اور فیہ شی بالحیاء اسمہ بروتین کان مدرستواہالب



Protein factors

Initiation, elongation, and termination (or release) factors are required for peptide synthesis

ATP and GTP are required as sources of energy

Cleavage of **four** high-energy bonds (MCQ) is required for the addition of one amino acid to the growing polypeptide chain:

1- **two** from ATP in the *aminoacyl-tRNA synthetase* reaction:

- one in the removal of PP_i
- one in the subsequent hydrolysis of the PP_i to inorganic phosphate by *pyrophosphatase*.

2- two from GTP :

- one for binding the aminoacyl-tRNA to the A site .
- one for the translocation step .

[Note: Additional ATP and GTP molecules are required for initiation in eukaryotes, whereas an additional GTP molecule is required for termination in both eukaryotes and prokaryotes.]

لا تحقیق للطموحات دون معاناة J



بسم الله الرحمن الرحيم



كل هالبروتين وين يروح L

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هي اهداء لدفعة ٤٢٨ الراعين J

واهداء لأغلى انسان لي في هذه الدفعة خاصة J

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Team Leader :

أحمد العقيل

Team members :

Yahya Al-Aseeri
Metallic O Mind

أماني علي البجادي
غرك زمتك



Codon recognition by tRNA

Antiparallel binding between codon & anticodon :

Read :

Anticodon (3→5) read mRNA codon (5→3)

Write :

(both always 5→3)

Wobble hypothesis :

One amino acid may have more than one tRNA & one tRNA may have more than one codon for a specific amino acid

Wobble hypothesis decrease mutation rate

First end of the anticodon allows non traditional base pairing with the 3-base of The codon (the last pair)

In non traditional :

A-----U
C----- G

نحفظها زين بعدها جيب اللي عاليه ووصل بين زاويتين

U -----A,G
G-----U,C

أما ال

I----U,C,A

ركزوا عالرسمه ل وطريقة حفظها عرفتها

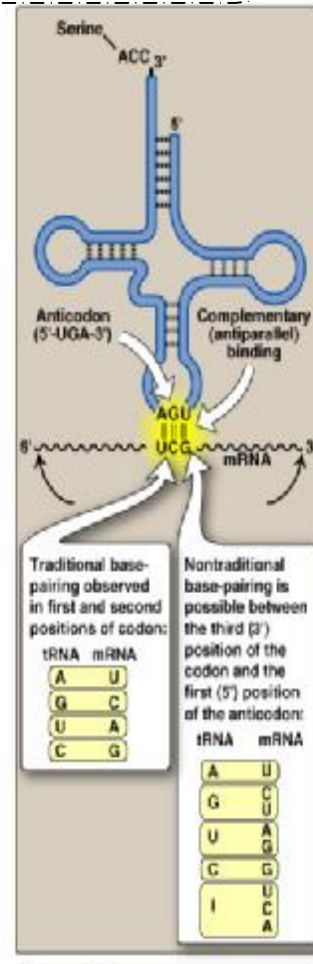


Figure 31.9

Wobble: Nontraditional base-pairing between the 5'-nucleotide (first nucleotide) of the anticodon with the 3'-nucleotide (last nucleotide) of the codon. I = inosine.



Steps in Protein Synthesis

Prokaryotic mRNA often have several coding regions, that is, they are polycistronic Each coding region has its own initiation and termination codon and produces a separate species of polypeptide.

In contrast, each eukaryotic mRNA has only one coding region, that is, it is monocistronic. (MCQ)(Not polycistronic)

One important difference is that translation and transcription are coupled in prokaryotes, with translation starting before transcription is ended.

The process of translation is divided into three separate steps: initiation, elongation, and termination.

A. Initiation

من هالمكان جيب الكتاب وافتح ٤٤٠ - ٤٤١ اجباري

Need :

- two ribosomal subunits – mRNA - aminoacyl-tRNA – GTP and initiation factors .

In prokaryotes, three initiation factors are known (IF-1, IF-2, and IF-3),

Whereas

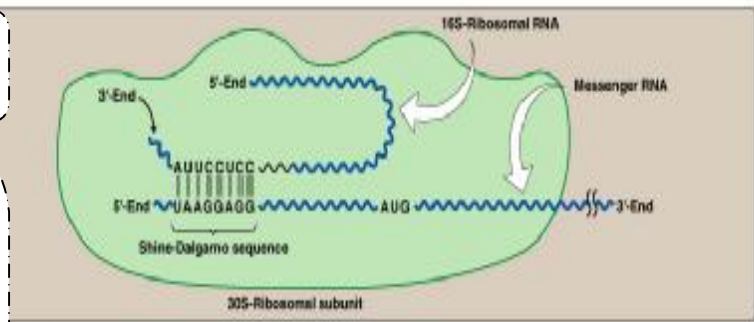
in eukaryotes, there are over ten (designated eIF to indicate eukaryotic origin). Eukaryotes also require ATP for initiation.]

Shine-Dalgarno sequence

- purine-rich sequence of nucleotide bases (for example, 5'-UAAGGAGG-3') in 5'-end .

- The 16S ribosomal RNA component of the 30S ribosomal subunit has a nucleotide sequence near its 3'-end that is complementary to all or part of the SD sequence , facilitating the binding and positioning of the 30S ribosomal subunit on the mRNA, in close proximity to the initiating AUG codon

هذا الشيء فقط بال prokaryotes



In eukaryotes, (**No Shine-Dalgarno sequence**) the 40S ribosomal subunit (aided by members of the eIF-4 family of proteins) binds to the cap structure at the 5'-end of the mRNA and moves down the mRNA until it encounters the initiator AUG. This “scanning” process requires ATP .



إذا م ذكرته بالسابق مبني على :
 كيف نتعرف على كود البداية AUG
 والطريقة مختلفة بين eukaryotes & prokaryotes
 كما ذكرت ،،

Initiation codon:

- **AUG is recognized by a special initiator tRNA :**
 - 1) in prokaryotes : IF-2 (bound to GTP)
 - 2) in eukaryotes : eIF2-GTP (plus additional eIF)
- The amino acid-charged initiator tRNA enters the ribosomal P site
- GTP is hydrolyzed to GDP

Always come in exams (MCQ)

The initiator tRNA is the only tRNA recognized by eIF-2, and the only tRNA to go directly to the P site

لازم تجيك بالاختبار ان شاء الله

- transformylase :

- In : : bacteria and in mitochondria
 Function : The formyl group is added by this enzyme to the methionine after that amino acid is attached to the initiator tRNA
- uses N10-formyl tetrahydrofolate as the carbon donor
 - this N-terminal methionine is usually removed before translation is completed.]

In eukaryotes, the initiator tRNA carries a methionine that is not formylated.

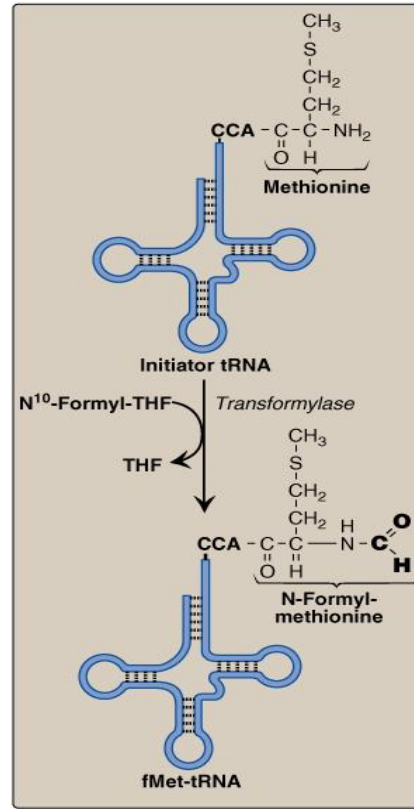


Figure 31.11 Generation of the initiator N-formyl-methionyl-tRNA (fMet-tRNA).

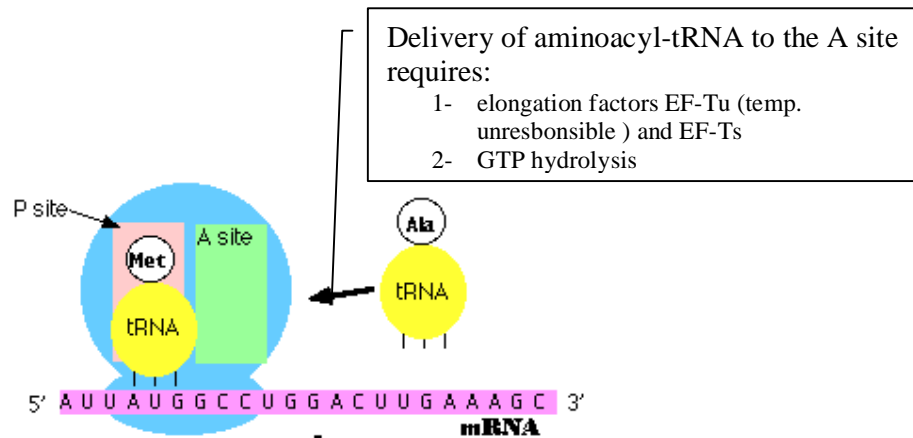
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B. Elongation:

- It's the addition of amino acid on to the carboxyl end (MCQ) of the growing chain.
- Ribosomes move from the 5'-end to the 3'-end of the mRNA.

Prokaryotes



- **Tetracyclines:** block the access of aminoacyl-tRNA to the A site by interacting with 30S ribosomal subunits. (inhibit elongation)(MCQ)

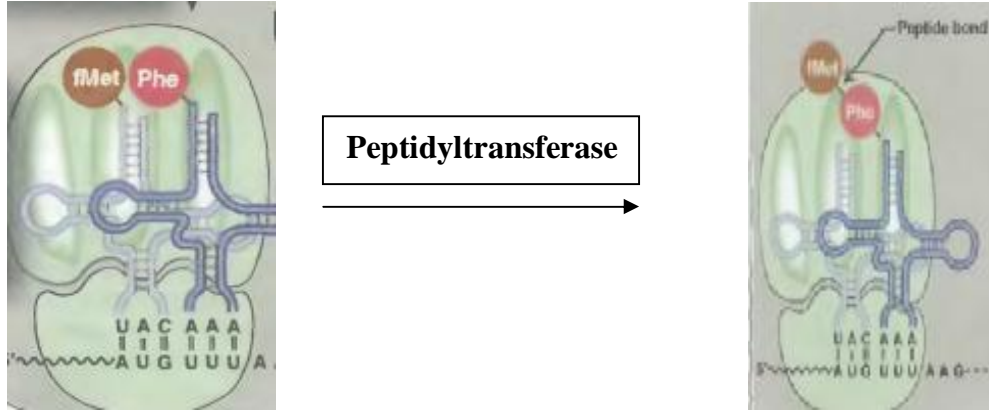
Eukaryotes

- Delivery of aminoacyl-tRNA to the A site requires :
 - 1- Factors EF-1 α and EF-1 β
 - 2- GTP hydrolysis.
- EF-1 β has the same function of EF-Ts.

دائما يجي بالاختبار هاهنا امجد

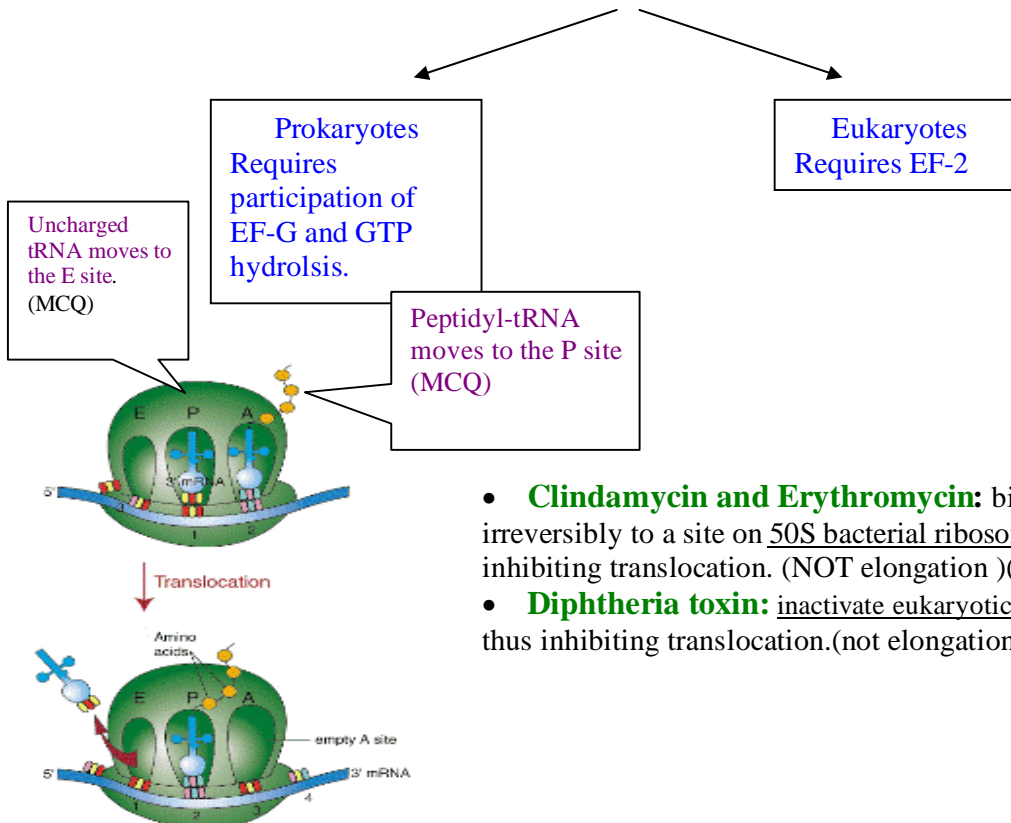
Peptidyltransferase: catalyze the formation of the peptide bond .

- **23S rRNA (ribozyme) and GTP.** (MCQ)
- Referred to as **ribozyme** because it contains only RNA. (MCQ)



- **Chloramphenicol:** inhibit prokaryotic **peptidyltransferase**, high level may also inhibit mitochondrial protein synthesis(act on large subunit & inhibit elongation only in prokaryotes) (MCQ)
- **Puromycin:** resembles to aminoacyl-tRNA and becomes incorporated thus, inhibit elongation in prokaryotes and eukaryotes.

Translocation: ribosomes advances three nucleotides toward the 3'-end of the mRNA.



- **Clindamycin and Erythromycin:** binding irreversibly to a site on 50S bacterial ribosome thus inhibiting translocation. (NOT elongation) (MCQ)
- **Diphtheria toxin:** inactivate eukaryotic factor eEF-2 thus inhibiting translocation.(not elongation)(MCQ)



C. Termination:

- **Occur when one of these termination codons move to the A site:**
 1. **UAA** (recognized in E. coli by factors **RF-1** and **RF-2**)
 2. **UAG** (recognized by **RF-1**)
 3. **UGA** (recognized by **RF-2**)

من هذا المربع دائما يجي سؤال

- Eukaryotes have single factors recognizing them known as **eRF**.
- Binding of these factors cause **peptidyltransferase** to hydrolyze the bond between the peptide chain and t-RNA on the **P site**.
- Third factor **RF-3** (bound to GTP) hydrolyze its GTP and cause the release of **RF-1** and **RF-2** (MCQ)
- Eukaryotes have factor **eRF-3** that has the same function of **RF-3**

Ricin (from castor beans): potent toxin that removes **adenine** from 28S ribosomal RNA thus inhibiting eukaryotic ribosomes. (MCQ) ههنا م بتجي بالاختبار

D- polysomes

- Several ribosomes and one mRNA
- this many ribosomes act on 3-end mRNA is called polysome (polyribosome)

E- protein targeting

- 1\ most protein synthesis occurs in the cytoplasm of eukaryotic cell
- 2\ many proteins are destined to perform their functions within specific cellular organelles such proteins usually contain AA sequences that direct the proteins to their final location

For example :

- proteins destined for secretion from the cell are targeted to RER-associated ribosomes by presence of an N-terminal hydrophobic signal sequence
- nuclear proteins contain a " **nuclear localization signal** ", whereas mitochondrial proteins have a " **mitochondrial entry sequence** "



F- Regulation of translation

- 1\ gene expression is most commonly regulated at the transcriptional level
- 2\ the rate of proteins synthesis is also sometimes regulated by
 - a\ important mechanism by which this is accomplished in eukaryotes is by covalent modification of eIF-2 (phosphorylated eIF-2 is inactive)
(e.g : heme) (MCQ)
 - b\ in both eukaryotes and prokaryotes, regulation can also be achieved through proteins that bind mRNA and either inhibit its use by blocking translation or extend its use by protecting it from degradation
(e.g: ferritin mRNA) (increase ferritin \Rightarrow decrease transferring receptor synthesis)

روح وراجع كل م كتبت عليه MCQ مرة ثانية لأن أغلب الأسئلة تجي
بالجزء المذكور في هذا الجزء من المذكرة J

بالاختبار راح يجيك أدوية كثير ..
بيسألك وين تشتغل ب eukaryote or prokaryote or both
على 30 s or 50 s
تنشط initiation or elongation or translocation or termination
موفقين ،،

تعلم قول لا أدري .. فإنك إن قلت لا أدري

علموك حتى تدري ...

وإن قلت أدري سألوك حتى لا تدري

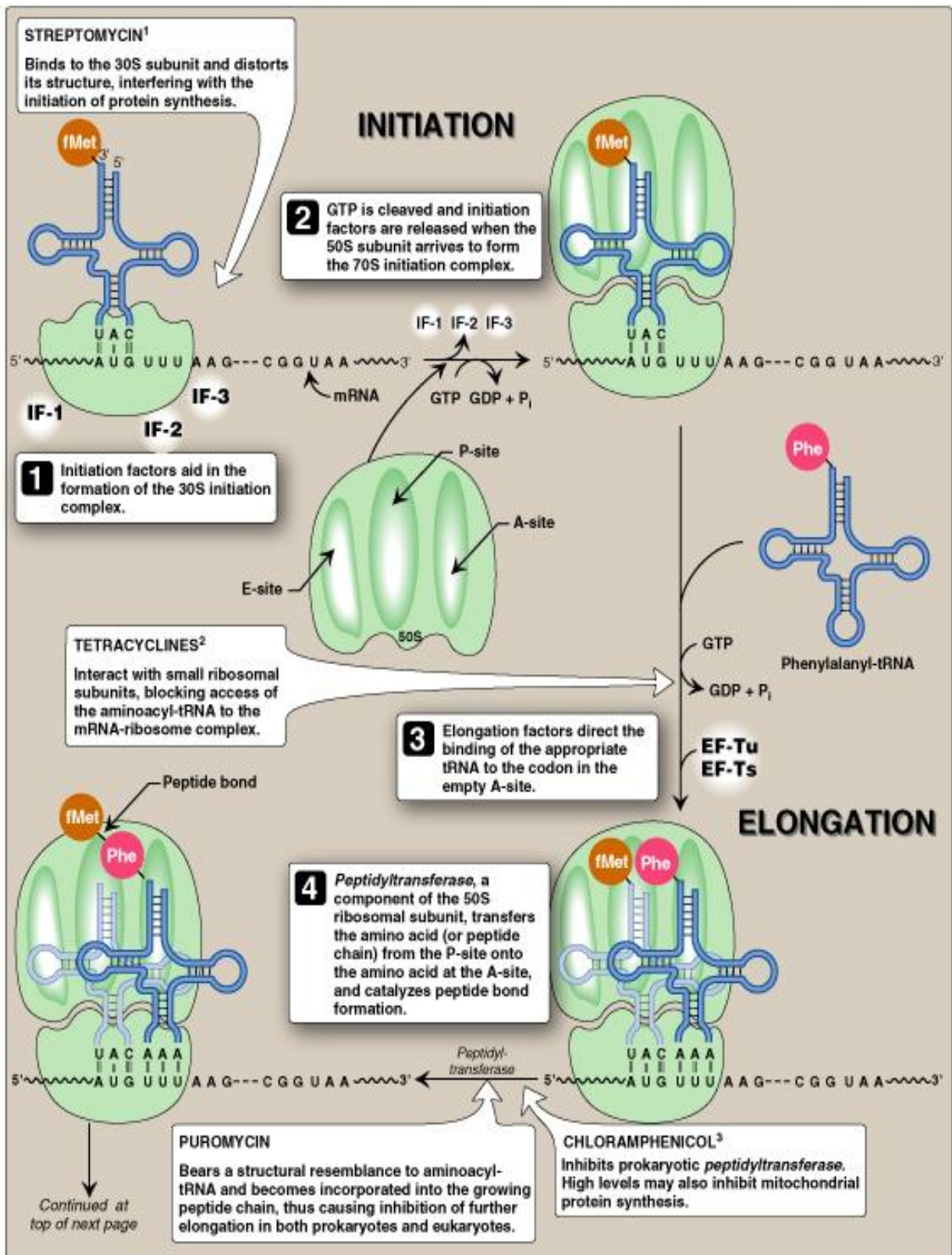


Figure 31.13
 Steps in prokaryotic protein synthesis (translation). (*Continued on the next page*)

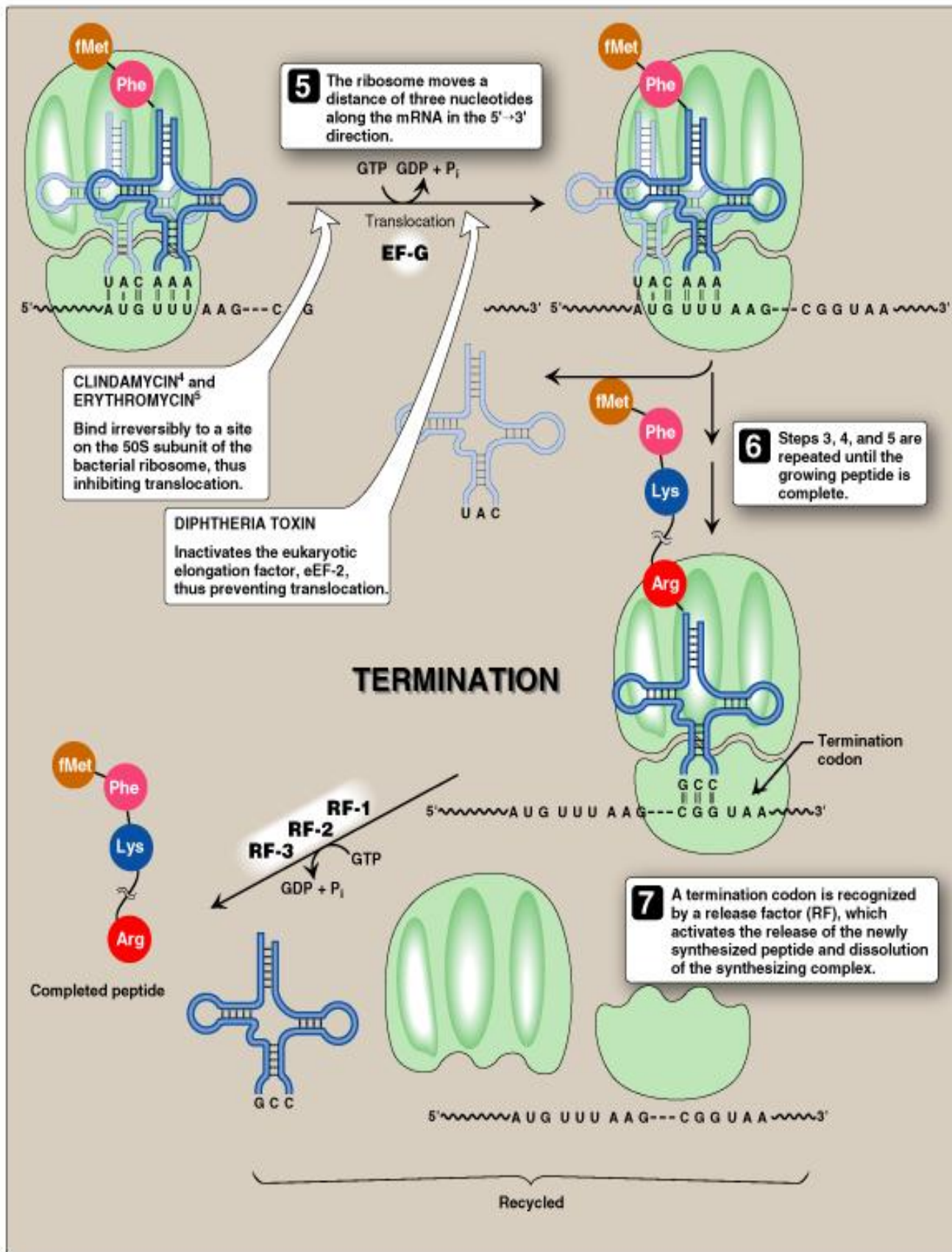


Figure 31.13 (Continued)



كل العمليات السابقة تحتاج بالمجمل 4 ATP (MCQ)

هذه مراجعه لأهميتها ..

J

Initiation Process is inhibited by :

1- streptomycin (bind 30S unit interfering with initiation of protein syn.)

- Elongation Process is inhibited by :

1- Puromycin (resemble aminoacyl-tRNA structure inhibiting elongation)

2- Chloramphenicol (inhibit peptidyltransferase)

- Translocation Process is inhibited by :

1- Clindamycin and Erythromycin (bind irreversibly to 50S unit in bacteria)

2- Diphtheria Toxin (inactivate eukaryotic elongation factor eEF-2)

- also you should know:

- tetracyclines (block access of aminoacyl-tRNA to the mRNA-ribosomal complex)

- ricin (from castor beans , remove adenine from rRNA 28S in eukaryotic cells

العواصف الشديدة تحطم الأشجار الضخمة

ولكنها لا تؤثر في العيدان

الخضراء التي تنحني لها

J