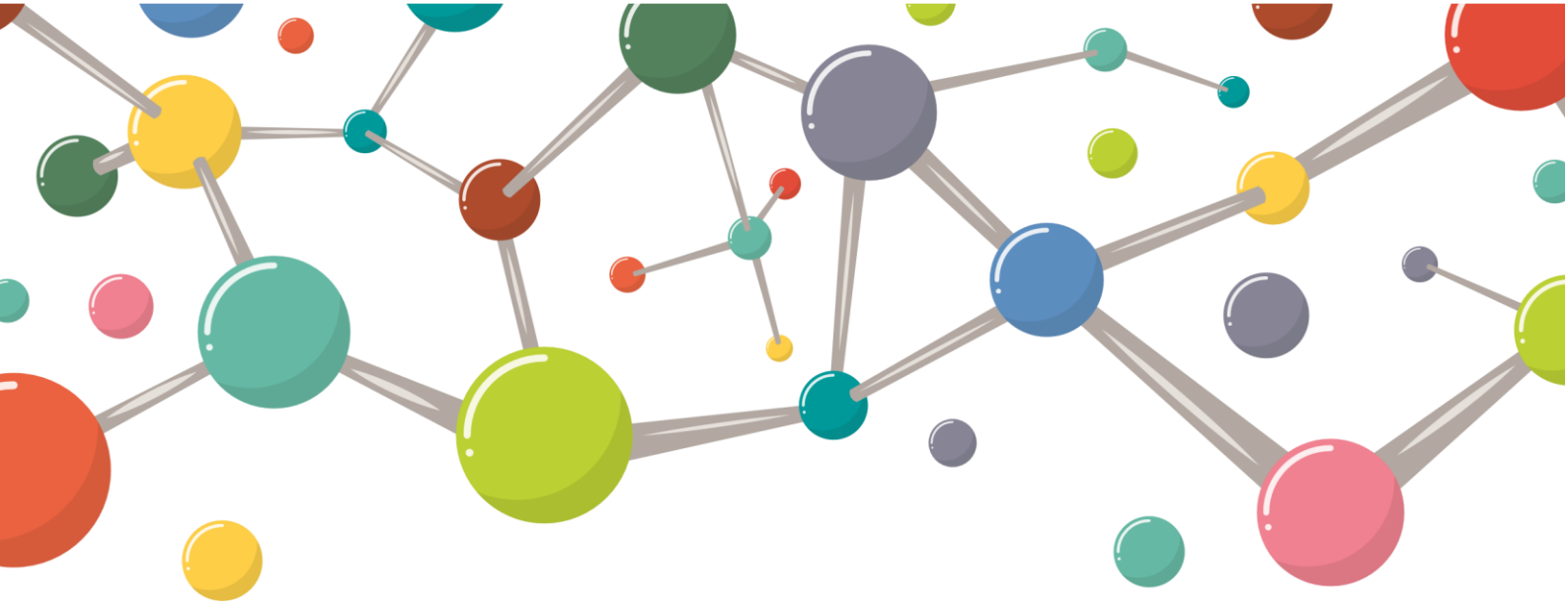

MOLECULAR BIOLOGY

SHEET (9)

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Black color: doctor's explanation, Navy color: slides, Blue color: extra information, Red color: Dr. Khaldun's notes, highlighted sentence: modified, purple color: book notes .

PROTEIN SYNTHESIS

- There are two types of amino acids:
  1) Essential amino acids: are provided directly from the food we eat.
  2) Non-essential amino acid: are built biochemically (through biochemical pathways) from essential amino acids (by chemical modifications).

- Protein synthesis (translation): doesn't mean that amino acids are being synthesized or built from scratch or from precursors. However, protein synthesis actually means the linking and bonding of amino acids together in a specific sequence.

- Proteins differ and vary in their **function** due to their difference in **structure**. Proteins differ and vary in their **structure** due to their difference in their **amino acid sequence**.

- Therefore, through determining the amino acid sequence, we can determine the type, structure, then the function of each protein, then we can determine the **phenotype**.

- Proteins are what determine the phenotype!

- The protein synthesis process is also called translation, but why? First of all, we need to compare the translation process with the transcription process:

  ⇨ Transcription: we use the information stored in the **DNA** (a nucleic acid) to make another type of nucleic acid, which is **RNA** (from DNA to RNA, we stayed at the same level of chemistry).
  Also, we can consider it as photocopying (copying), remember that the produced RNA contains the same base sequence as the coding strand in the DNA, except for the fact that RNA contains uracil instead of thymine and ribose sugar instead of deoxyribose.

  ⇨ Translation: we use the information in the **nucleic acids** (mRNA specifically) to arrange the **amino acids** (different chemistry) in a specific sequence. As if the information had been translated (transferred) from a **nucleic acid** to a sequence of **amino acids** (a polypeptide, which is an entirely different chemical molecule).

- The **sequence** of the amino acids determines the **folding** of the polypeptide (in the secondary and tertiary structures), therefore it determines the **shape** of the protein, which also determines the **function** of the protein, which determines the **phenotype**.

- That's why the **phenotype** is determined by the **genotype**.

  ⇨ Because the **genotype** (the base sequence in the gene) decides the **function** of the protein, how exactly?

  ⇨ The **genotype** decides the shape of the protein, which is decided by the **primary sequence of amino acids**, as this sequence is translated from the base sequence of the **mRNA**, which is transcribed from the **gene**.

  This is how the genotype decides the phenotype, which is why it is a very important and essential process in our cell bodies.

- What are the required components for the translation process?
-
- ⇨ mRNA (mature-mRNA in eukaryotes)

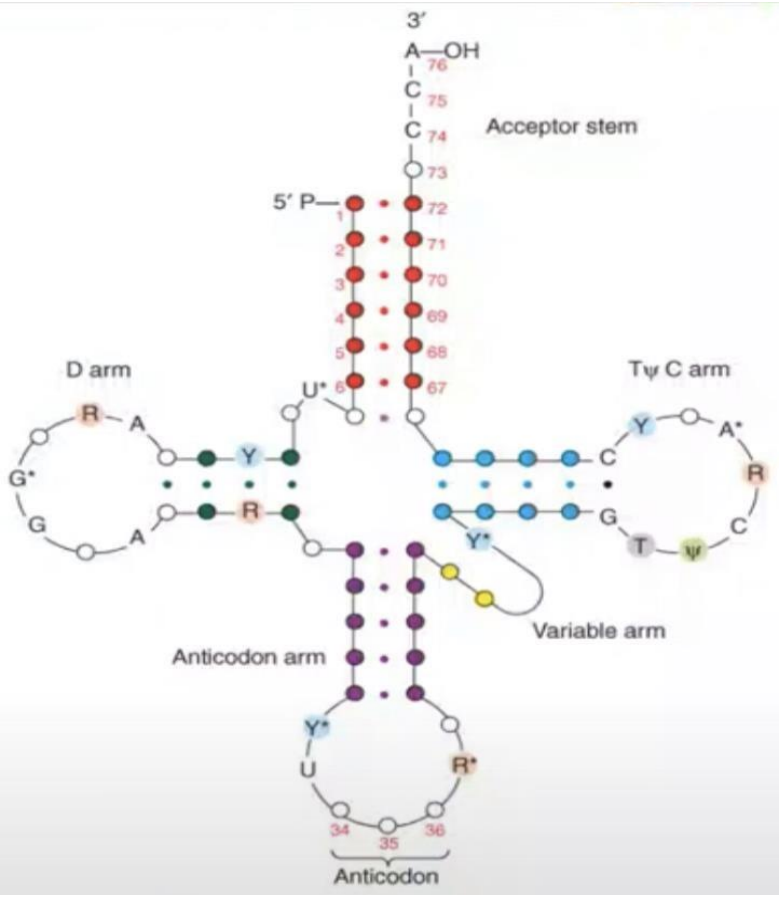
  ⇨ ribosome (large subunit and small subunit which both contain rRNA)

  ⇨ tRNA (the most important component)

————————————————————————————

Now we are going to take a look at the tRNA

- tRNA is a type of the RNA molecules which possess a specific shape.



RNA secondary structure

- tRNA also has a tertiary structure (3d-structure) which looks like an upside-down L shape that has two ends:
  - ⇨ one end contains the **anticodon** (anticodon is the sequence from 3'➒5' that is complementary to the codon on the mRNA which we will talk about later)
  - ⇨ The other end binds to the **amino acid** (the 3'-ACC-5' sequence in this end is found in all tRNA)

- The tRNA has two arms: the D arm and the T arm.

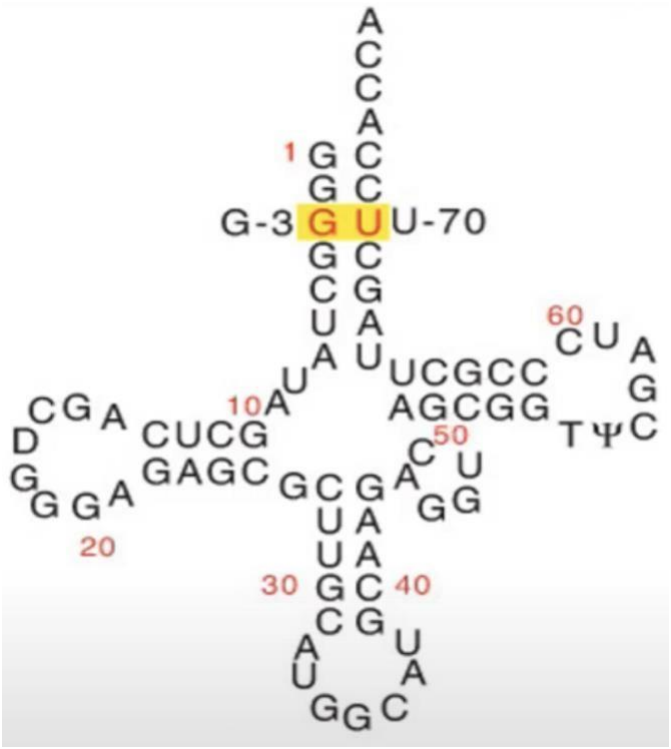
- The best term to describe tRNA is "adaptor", because it connects the **amino acid** it holds to the **mRNA**, as the anticodon on the tRNA complementary to the codon on the mRNA will specifically bind to it (according to the Watson-crick basepair principle).

- The **anticodon** therefore determines which **tRNA** (that holds specific amino acid) will bind, and then determines the **certain amino acid** to be added to the growing polypeptide.

- Of course, we have tRNA specifically for every amino acid as there is no tRNA for other amino acid than it's own.

- The process which ends in the tRNA bound to an amino acid, is called **charging** or **loading**. This chemical reaction which is catalyzed by the enzyme **aminoacyl tRNA synthetase**, is important as it results in **charged tRNA** (a tRNA which has an amino acid).

- The charged (or loaded) tRNA then plays it's important role in translation (which will be discussed later). Then, the tRNA will lose its amino acid and will become uncharged (or unloaded).



The tRNA which is specified for alanine

- It is important to recall that every tRNA is specific for one amino acid only. So, tRNAs which bear different amino acids are composed of different sequences.

- Therefore, the tRNA for alanine (shown above) has a unique composition.

- Every nucleotide within the tRNA has been given a sequential number which depends on its sequential position from 5'➒3' (for example: the first nucleotide on the 5' end is numbered "1" and the 20th from the 5' end is numbered "20" and so on…)

- Sequential numbering of nucleotides of the tRNA helps us distinguish different tRNAs from one another.

- "G-3 with U-70" is a sequence found only in tRNA which bears alanine.

- Even though tRNAs have the same shape, but every type has a specific sequence which makes it specific to bear only one type of amino acids.

MESSENGER-RNA AND GENETIC CODE

The reading frame

- Three adjacent bases in mRNA that specify an amino acid are called **a codon**. .

- In vitro (when dealing with chromosomes and the DNA), we must take into consideration that we have to read the codon correctly just as the cell read it, but how? By beginning reading of the chromosome from its end and divide it into triplets (read it as multiples of triplets, the one triplet is composed of three adjacent bases) so we can read the first three nucleotides (the 1st codon) and then the three nucleotides adjacent to the first one (the 2nd codon) and so on…

- We wouldn't know how to read and distinguish the codons if we began reading the chromosomes from sites other than the end, because we wouldn't be able to distinguish the triplets and read the chromosome as multiples of three bases (for example we can't recognize the triplets in the middle of the chromosome).

- To read the codons correctly like the cell does is called **the reading frame**.

The frameshift mutation

- Sometimes mutations occur such as addition or deletion of a base pair, or two bad pairs which **shift** the reading frame.
  This mutation is called **frameshift mutation**.

- The frameshift mutation is a very dangerous mutation because it changes the whole reading frame (downstream to the mutation). Which means that the cell will read all the codons downstream to the mutation different from the wild-type (the original sequence before the mutation), which does not change 1 amino acid, but a large amount of amino acids depending on the location of the mutation.

- The addition or deletion of **3 nucleotides** (1 triplet) just adds or deletes one codon but does not affect or change the whole reading frame (downstream to the mutation).

How many nucleotides do codons contain?

- Genetic experiments provided strong evidence that a codon contains three nucleotides.

- Put in mind that there are about 20 different amino acids in our biological system which make up our proteins.

- So logically, the number of codons **must** exceed 20 so that every tRNA—with an anticodon complementary to a specific codon in the mRNA—can bear only one specific codon.

- For example: if there were only 4 codons(less than 20), then there will be 4 anticodons at most, which means 4 types of tRNA at most (why at most? Because of the wobbling and the redundancy of codons, which we will discuss later). And that is impossible because how are only 4 types of tRNA going to bear 20 different amino acids when every one type of tRNA can only bear 1 type of amino acid.

- From a mathematical point of view, If the codon was composed of:
  - ⇨ Only one amino acid: then there would be only 4 codons possible, which is impossible because there would be only 4 tRNAs at most
  - ⇨ Two amino acids: then there would be 16 codons possible— which is still less than 20–as a result, there would be only 16 different tRNAs at most. So, 1 codon can't be composed of only 2 amino acids.
  - ⇨ Three amino acids: then there would be 64 different codons— which is more than 20 and also redundant— as a result, we can have 64 different tRNAs **at most**. Therefore, the idea that a codon is composed of 3 amino acids is the most rational and factual one.

The codon is composed of 3 amino acids ➔ there are 64 different codons mathematically ➔ there is more than one codon for a single amino acid

- **So a three nucleotides(a triplet)➔ 1 codon ➔ 1 amino acid**

- mRNA sequence is read (translated) from 5'➈3' direction during translation (the last nucleotide has a free 3'-hydroxyl group).

  ⇨ The **N-terminal** of the first amino acid in the growing polypeptide corresponds to the **5'-end** of the mRNA.

  ⇨ The **C-terminal** (carboxyl group) of the last amino acid in the polypeptide chain corresponds to the **3'-end** of the mRNA.

Transcription & translation in bacteria and eukaryotes

1) In bacteria:

- Because both transcription and translation proceed in the same direction, bacteria can begin to translate an mRNA before it's transcription is complete.

- So, transcription and translation are **simultaneous** in **bacteria**, but not at the beginning of transcription. Translation occurs during transcription in bacteria when the growing mRNA is elongated enough.

- There is no "pre-mRNA" in bacteria because there is no RNA-processing in bacteria. So the bacterial cell does not wait for the transcription process to be completed to translate the mRNA. Instead, it starts translating simultaneously with transcription.

- The growing mRNA binds to the ribosome, because there is **no physical barrier** or separation between the **bacterial DNA** (nucleoid) and the **cytoplasmic ribosomes**.

2) In eukaryotes:

- In **eukaryotic cells**, there is a **physical barrier** or separation between the **eukaryotic DNA** and the **cytoplasmic ribosomes**. This barrier is **the nuclear envelope**.

- **The nuclear envelope** separates the cytoplasm (the site of translation) from the inside of the nucleus (the site of transcription). Therefore, transcription and translation occur in two separate biological compartments.

- So, **in eukaryotes**, the transcription process must be **fully completed**, then the "pre-mRNA" must be processed to become "mature-mRNA", then the "mature-mRNA" must leave the nucleus to reach the cytoplasm—through nuclear pores—for the translation process to take place.

Eukaryotes cannot begin translation before transcription is complete because transcription and translation occur in 2 separate biological compartments (the nucleus and the cytoplasm)

Transcription➒mRNA processing➒mature-mRNA leaves the nucleus➒mRNA binds to the ribosome➒Translation

Stop codons

- Three codons (most common) that are polypeptide chain termination signals (end translation): **UAA, UAG and UGA.** They won't add amino acids to the growing polypeptide chain, but they will stop and end the translation process (there are more "polypeptide chain termination codons" than these three but we won't discuss them).

- The three "polypeptide chain termination codons" are called **stop codons**.

- The three **stop codons** were given different names:

  ⇨ Amber (UAG)
  ⇨ Ochre (UAA)
  ⇨ Opal (UGA)

- Out of the 64 codons, **61** codons will be translated into amino acids, while **3 (UAA, UAG and UGA)** won't be translated and will stop the translation process.

Nonsense mutations:

- Mutations that change a **sense codon** (a codon which is translated into an amino acid) into a **stop codon** (a codon which is not translated into an amino acid and which stops the translation process) are called **nonsense mutations**.

- A nonsense mutation stops the translation in a location or position where it shouldn't stop, which means that all the codons downstream to the nonsense mutation won't be translated. Therefore, the remaining polypeptide won't be synthesized, and the resulting polypeptide will be incomplete.

- The nonsense mutation is quite dangerous, and the severity of the consequences of the nonsense mutation depends on the location of the mutation within the gene. Because the location of the mutation determines the length of the resulting polypeptide and how long is the polypeptide segment which won't be translated.

- For example, the nonsense mutation could occur at the last translated codon, that will result in a polypeptide missing the last amino acid, which is of course less severe than a nonsense mutation occurring in the middle of the codon sequence.

- It is more probable for a nonsense mutation to cause an appreciable segment of the polypeptide chain to be left untranslated.

- So far we have discussed two types of mutations:

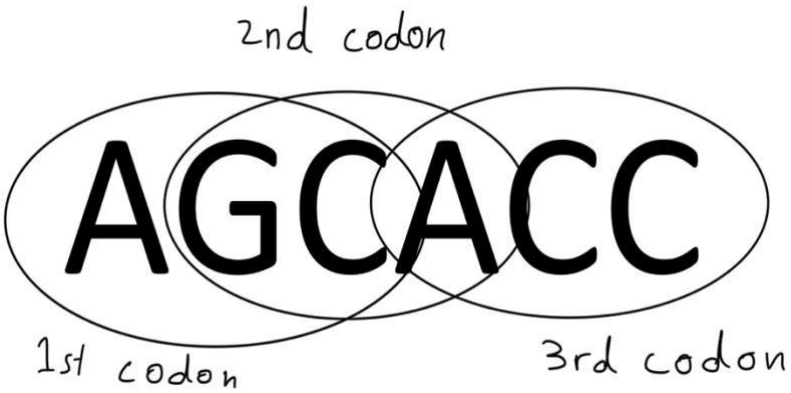
1) **The frameshift mutation**: by adding or deleting one nucleotide or a number of nucleotides that is not a multiple of "3", niches changes the reading frame downstream to the mutation.
2) **The nonsense mutation**: by changing one of the sense codons to a stop codon, which stops the translation process of the downstream sense codons that are supposed to be translated, and the polypeptide synthesized will be incomplete.

Five general statements can be made about the genetic code

Genetic code: every sense codon that codes for (or corresponds to) an amino acid

1) **The genetic code is non-overlapping**: each base is part of **only one codon.**

- Overlapping genetic code means that: the same one nucleotide is found in two codons.

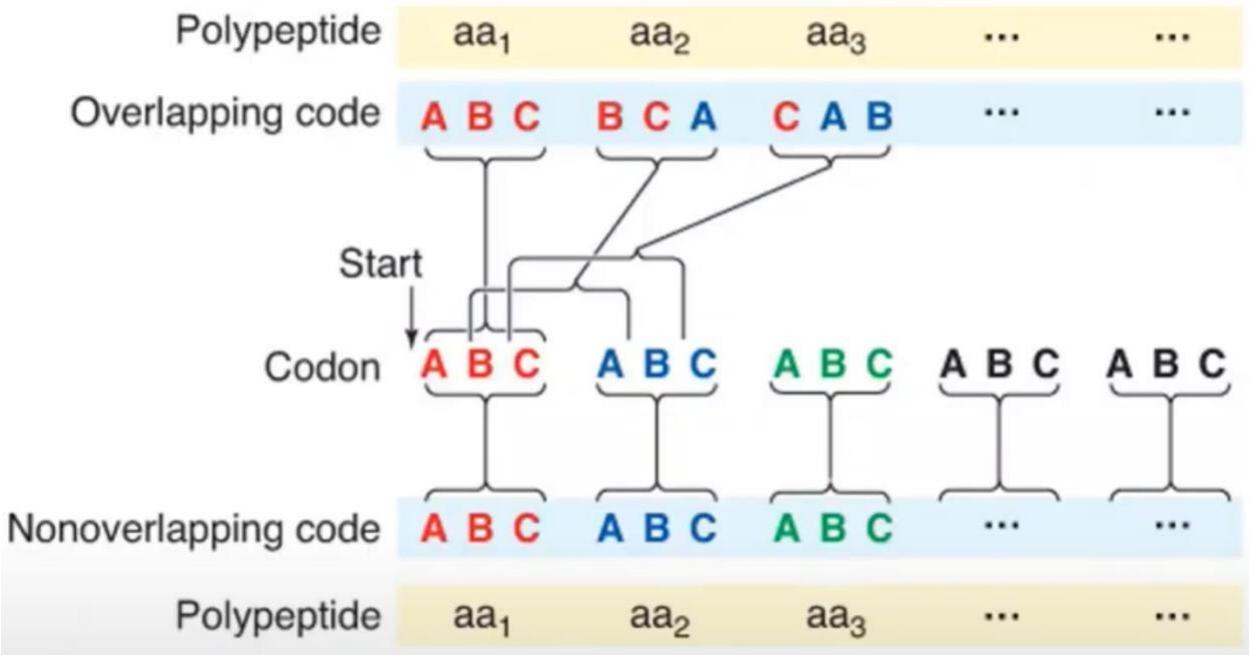


Overlapping codons

Notice how the nucleotides (G,C and A) are found in two codons (more than one)

Nonoverlapping codons

Notice how every nucleotide is found only in one codon



Difference between an overlapping and a nonoverlapping strand

2) **The genetic code is commaless**: there are no intervening distance, barriers or bases between adjacent codons.

3) **The genetic code is almost universal**: mRNA from one species can be correctly translated by another, and the genetic code can be translated into the same amino acids in most species.

- For example: the codon "AUG" is translated to the amino acid "methionine" in most living creatures and species (humans, animals and plants).
- If there was a specific and unique genetic code for each type of species, we wouldn't be able transform genes from an organism to another which is a different species (such as transforming genes to bacteria so it can produce desired proteins or products).
- Of course, there are exceptions to the universality of the genetic code (species that translate the same codons to other amino acids or have different genetic code)

4) **The genetic code is highly degenerate**: most amino acids are specified by two or more codons (4 o perhaps r 6), such as "methionine".

- Different codons that are translated to the same amino acid share the same first two nucleotides while they differ in the 3rd nucleotide.

5) **The genetic code is unambiguous**: each codon specifies **only one** amino acid.

⇨ Each codon can be translated to **only one** amino acid.

⇨ Each amino acid can be added to the growing polypeptide chain during translation by **more than one** codon.

- If there was a codon which specifies two amino acids (for example), then the two different amino acids have equal probability to be added to the growing polypeptide chain at the same location or position, which will result in two different possible polypeptides, one of them may be the one desired by the cell and the other may not.
  In conclusion, the cell may or may not produce the desired protein.
- So, it is inefficient for the cell to have an ambiguous genetic code (that is, to have one codon to specify more than one amino acid) سبحان الله !

ان شاء الله علامتكم بفاينل الموليكيولار أعلى من عدد صفحات شيت ٦

و معدلكوا قد عدد الجوال اللي أكلوها هذولاك الفريقين

Some aminoacyl-tRNA molecules bind to more than one codon because there is some play or wobble in the third base of a codon – the wobble hypothesis

*how to explain that there is more than one codon for each amino acid ?

One tRNA (anticodon 3’ to 5’ , codon 5’ to 3’ = antiparallel) , the 3rd nucleotide may not follow Watson and crick base pairing so maybe (U with A,G) which leads to have more than one codon to each amino acid.

WOBBLE base pairing: The bases that are common to several codons are usually the first and second bases, with more room for variation in the third base, The degeneracy of the genetic codons It acts as a buffer against deleterious mutations

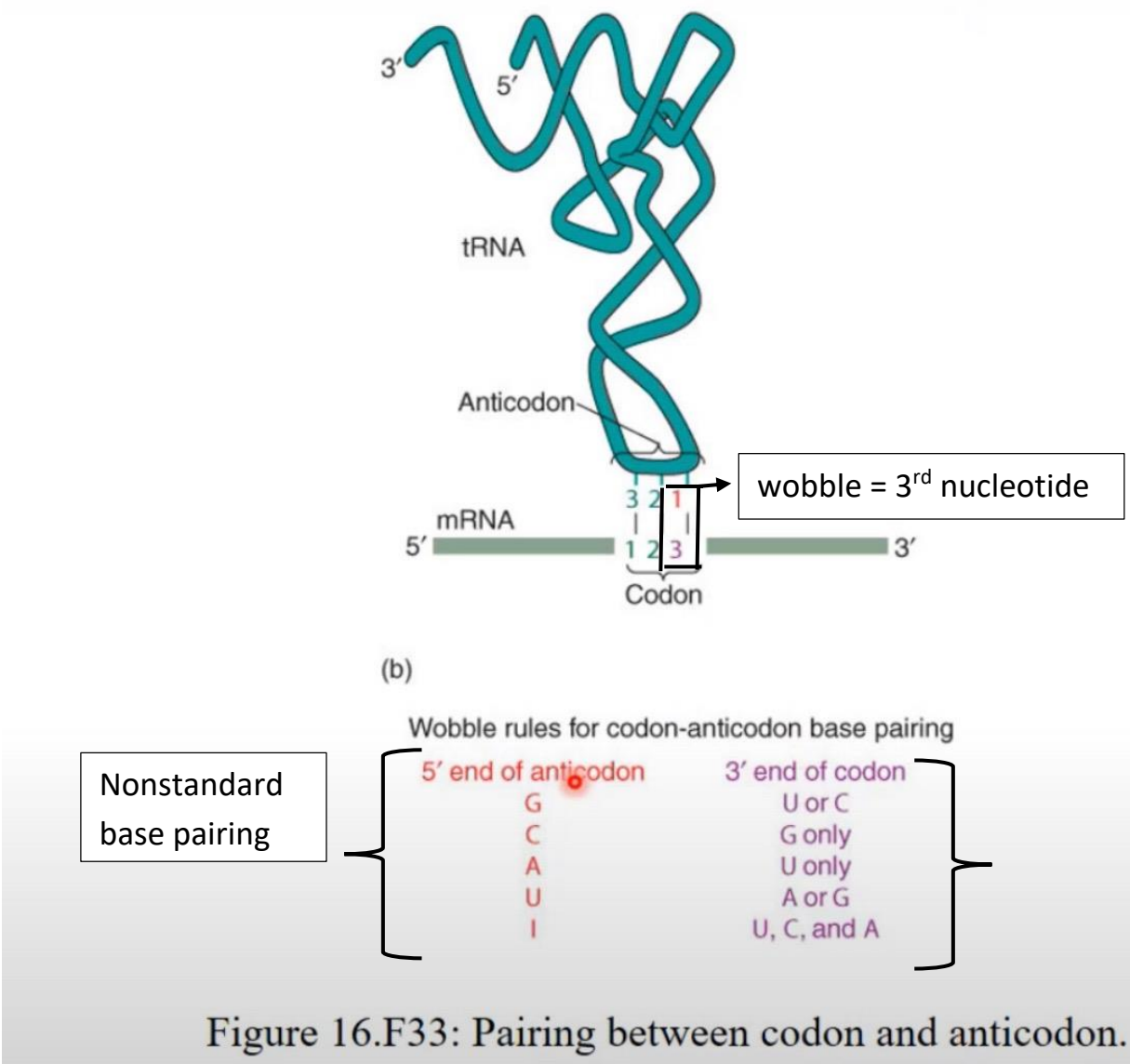


Figure 16.F33: Pairing between codon and anticodon.

In wobble nucleotide it is constant on the anticodon , the changes occur in the mRNA codon

(I) – nucleotide arise from chemical modification for uracil nucleotide

- **To sum up:**
- The codon and anticodon form antiparallel base pairs
- The first two bases in the mRNA codon (5’ to 3’) form standard base pairs with the last two base pairs in the anticodon (3’ to 5’)
- There is a certain amount of play or wobble in base pairing between the first base of the anticodon and the third base of the codon that permits non standard base pairing

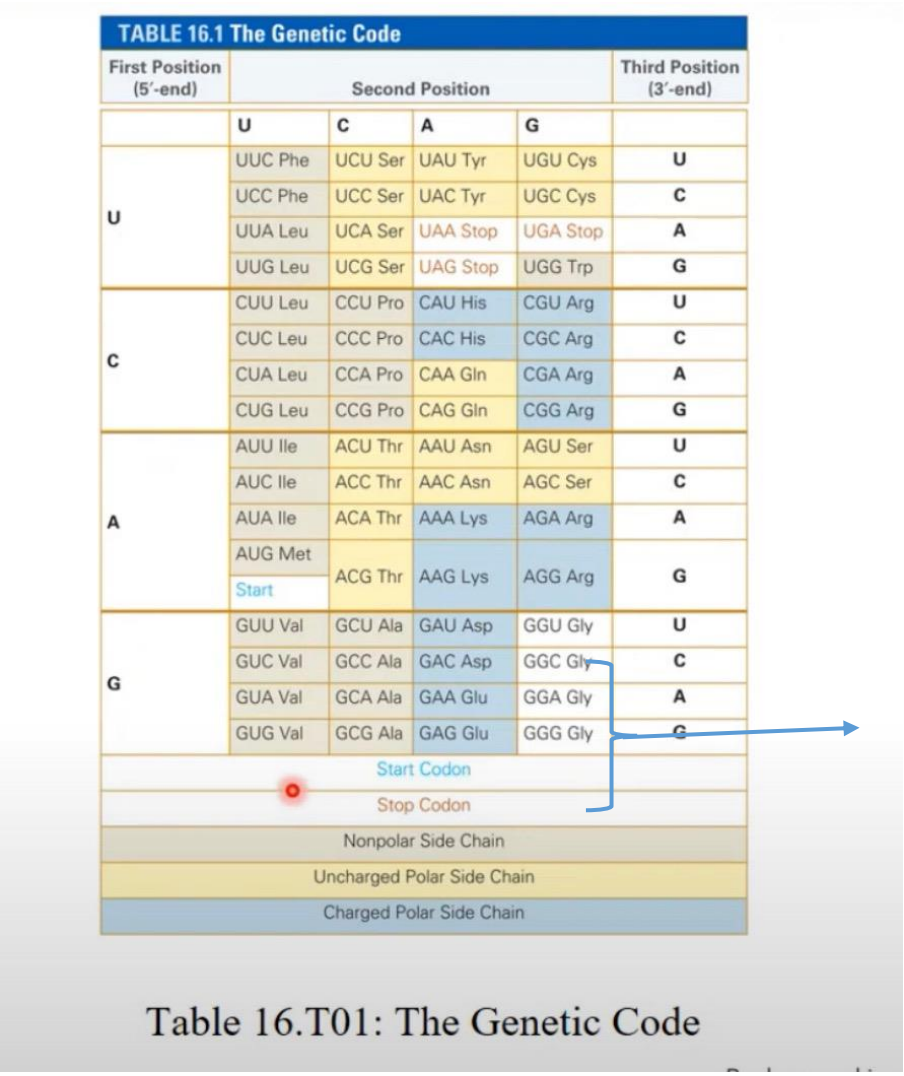


TABLE 16.1 The Genetic Code

First Position (5′-end)	Second Position				Third Position (3′-end)
	U	C	A	G	
U	UUC Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UCC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met Start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Start Codon

Stop Codon

Nonpolar Side Chain

Uncharged Polar Side Chain

Charged Polar Side Chain

Table 16.T01: The Genetic Code

Usually 4 codons for an amino acid
*maybe less than 4
*only (Leu) has 6 as an exception

4 codons for Gly amino acid change only in the 3rd nucleotide (wobble)
*changes are in mRNA because tRNA is constant
Similarities between

sequences lead to similarities between amino acids for example Non polar amino acids (Pro , Val , Phe)

**Relaxed base pairing results from the formation of G-U base pairs

Ribosome structure : to understand the mechanism of translation
There are some differences between bacterial ribosome and eukaryotic's one, it is not an organelle due to the lack of a membrane. We call it a "cellular structure composed of proteins" (the small subunit lies down and the large one lies up)
Bacterial 30S (small) subunits and 50S (large subunits each have unique structures and functions (no. for bacteria , S = sedimentation rate unit)
When the 2 subunits bound, the resulting sedimentation rate does not equal 80S , it equals 70S because when bounded to each other they have different shape so their coefficient of friction changes that means it is not an additive value.

- The ribosome is a complicated (small subunit , large subunit and rRNA) , dynamic (have motion) machine with many moving parts

- Translates 10 to 20 codons per second (رقم محترم)with an error rate usually less than 0.03% (and have a proofreading mechanism to correct mistakes – no need to know more details) it is a very efficient process

- The small subunit (more important in the initiation) performs the ribosome decoding function (discriminating between proper and improper codon/anticodon base pairing) recognition function.

- The large subunit contains the peptidyl transferase (an enzyme(ribozyme not protein)) activity (links an amino acid with the growing polypeptide) (using catalytic RNA (23S in prokaryotes) and (28S in eukaryotes))

Crystal structure analysis of 70S ribosome confirms the existence of three sites:

– ***A-site*** (**aminoacyl-tRNA binding site**) tRNA-charged or loaded , (the bond between an amino acid and a hydroxyl Group on the tRNA (3' end) is an ester bond)

– ***P-site*** (**peptidyl-tRNA binding site**) in the middle , the growing polypeptide as a chain

– ***E-site*** (**exit site**) when the t-RNA is uncharged (no amino acid bound).

Protein synthesis can be divided into four stages :

The initiation stage: The reading frame (read mRNA as sets of three nucleotides) is set by binding initiator tRNAs to ribosomes at the start codons with the assistance of translation initiation factors (IFs) , the translation initiation factors in eukaryotic (eIFs) are important part in this stage to find the first start codon

The elongation stage: Amino acids are added to the growing polypeptide chain as codons on the mRNA are matched with anticodons on the tRNA with the assistance of translation elongation factors (EFs)

***** these factors are proteins***

The chain termination stage: Termination codons are recognized by release factors and completed polypeptide chains are released from the ribosome (end of the process)

The recycling stage: Ribosomal subunits (small, large and rRNA) dissociate from each other under the influence of a ribosomal recycling factor (dissociating also include translational factor)

**** in more details :**

Initiation stage :

Each Bacterial mRNA open reading frame (the sequence in DNA have which has a corresponding sequence in RNA, from the start codon until the stop codon , the stop codon is not included) has its own start codon.

cistron = reading frame + stop codon

gene = cistron + promoter + exons and introns + 5' utr + 3' utr (utr = untranslated region)

- Translation in bacteria can begin shortly after the 5'- end of the mRNA emerges from the RNA polymerase (because as we said previously, there is no physical separation between translation and transcription, so there's synchronization between both processes) translation starts from the 5' end of the mRNA.

  Polycistronic mRNAs have an initiation(start) codon at the start of each open reading frame , polycistronic means that there's more than one cistron under one promoter prevalent in most genes in bacteria so the mRNA will be translated to many individual polypeptides (for each cistron) As mRNA- have start and stop codon for each reading frame , in eukaryotes it's monocistronic which means that each cistron has an independent promoter
- The translation machinery must be able to initiate polypeptide chain synthesis at multiple sites along the mRNA

  If One mRNA have multi cistrons the steps will be as follows:

  the translation machinery will start with a start codon – end with a stop codon – then it will find an untranslated region – then will again start with a start codon – stops with a stop codon and so on….

Translation always starts at a specific initiator codon. In bacteria AUG is the initiation codon about 90% of the time, GUG about 10%, and UUG about 1%. In very rare cases another codon such as AUU may function as an initiation codon. Precise recognition is essential because a phasing error of just one nucleotide would cause translation of the mRNA to be in the wrong reading frame.

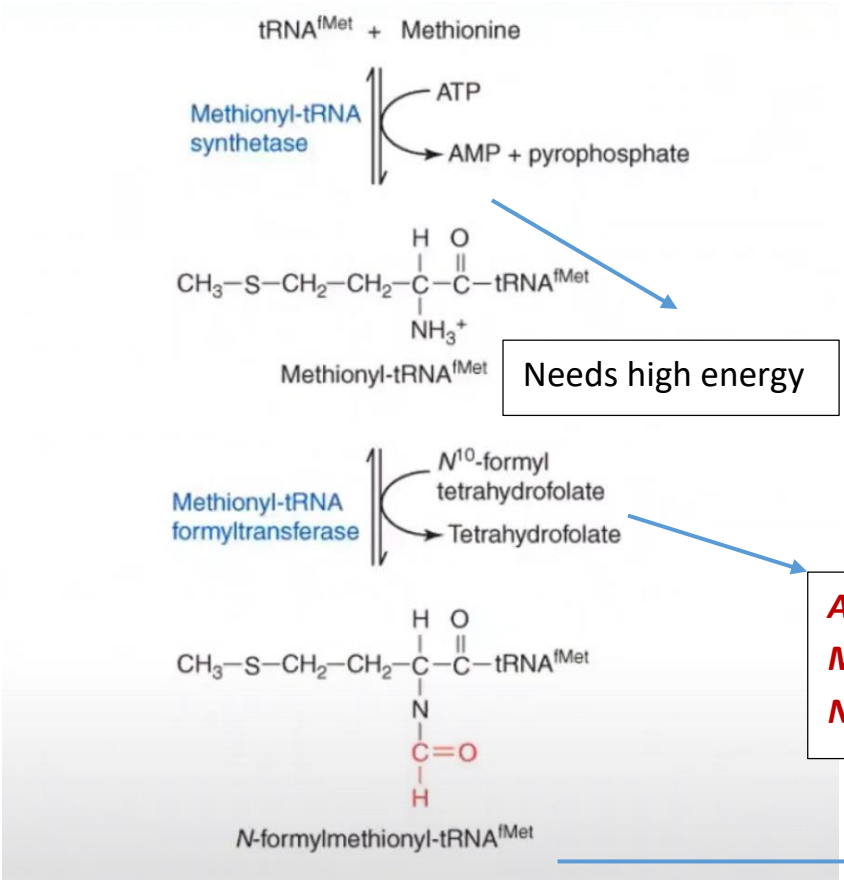
Bacteria have an initiator methionine tRNA and an elongator methionine tRNA.
At initiation it has to recognize the (AUG) which is the initiation codon, but we can have another (AUG) downstream (internally), so how the cell can differentiate between the methionine that must be added at the beggining of the polypeptide and the methionine that has to be added during the elongation (the internal one)?

Bacteria use a methionine derivative: N-formylmethionine (fMet) (عملتلهdifferent chemistry إضافة خلال منFormyl group) **to begin polypeptide synthesis (and by that it labeled the methionine at the beginning of the chain and differentiated it from the remainder methionines)**
***Remember that the polypeptide chain grows from the N terminus to the C terminus.

- ✓ **The Formyl Group Prevents Fmet From Being Incorporated Into A Polypeptide Chain At Any Position Other Than The Amino Terminus (The First)**

(fMet) : the first one , coded by the start codon
(Met): within the elongation process (Met amino acid only , like the remaining amino acids).

- ✓ Has its own tRNA (tRNA fMet) for initiation
- ✓ tRNAMet is used for elongation (unlabeled, no formyl group)
- ✓ Peptide deformylase removes the formyl group the N-terminus ,so the resulting polypeptide doesn't have formyl at its end (was only added for differentiation purposes)



Needs high energy

every tRNA is for a specific amino acid
***Remember we said the tRNA has a specific shape but with some differences in nucleotides sequence between each amino acid's tRNA.*

Addition of formyl group to Methionine after taking it from N-10 formyl tetrahydrofolate

Prokaryotic tr

Bonding of formal group at N-atom

initiation stage

The 30S subunit is an obligatory intermediate in polypeptide chain initiation.

1. **The 70S ribosomes dissociate into 50S (large subunit) and 30S (small subun**

   And by that it is different from the elongator tRNA

2. **fMet-tRNA^{fMet} and mRNA and mRNA bind to the 30S ribosomal subunit to form the 30S initiation complex.**
   Note: 30S Initiation complex = fMet-tRNA^{fMet} + mRNA + 30S
   Remember: fMet-tRNA^{fMet} : initiator tRNA that carries formyl Methionine.

3. **The 50S subunit then combines to form the 70S initiation complex.**

Note: 70S initiation complex = 30S initiation complex +50S.

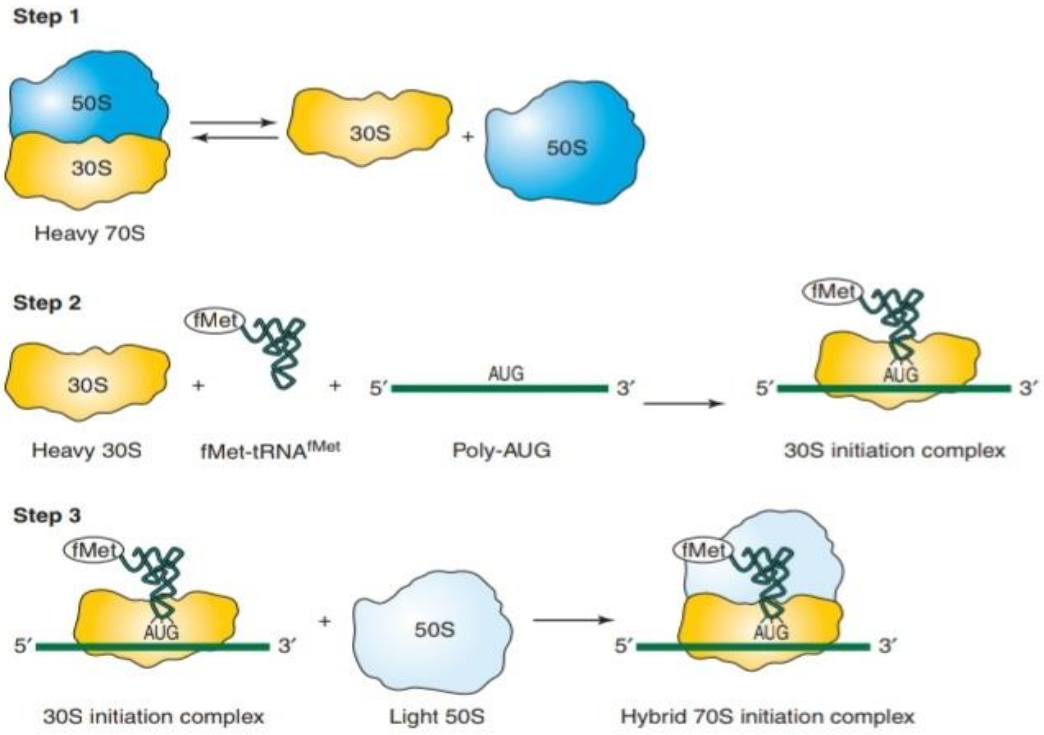


FIGURE 16.39: Outline of Nomura's experiment to test whether the 30S initiation complex is an intermediate in polypeptide chain initiation.

Nomura's model predicts the three-step pathway shown in FIGURE 16.39.

Note: The figure below shows the difference between the elongator tRNA that carries methionine and the initiator tRNA that carries formyl methionine. However, both have the same anticodon.

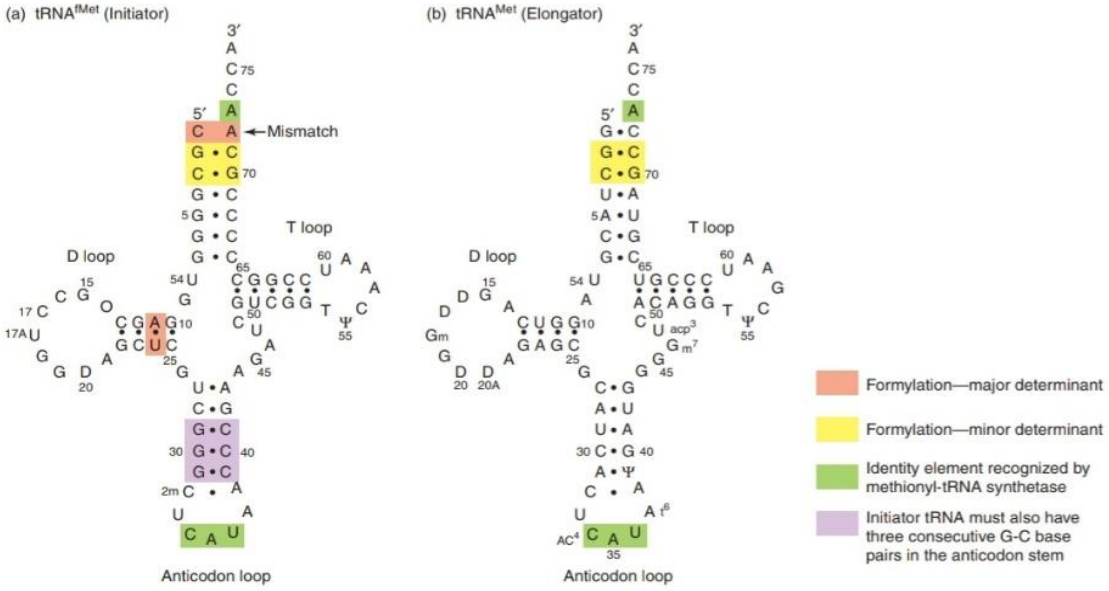


FIGURE 16.38 ***E. coli* tRNA isoacceptors for methionine.** (a) $tRNA^{fMet}$ initiates polypeptide chain synthesis and (b) $tRNA^{Met}$ elongates polypeptide chains. (Adapted from Sperling-Petersen, H. U., Laursen, B. S., and Mortensen, K. K. 2001. *Encyclopedia of Life Sciences.* John Wiley & Sons.)

***Initiation factors participate in the formation of 30S and 70S initiation complexes. (I: initiation, F: factor).**

- **First step:**
  IF3 binds to the 30S subunit after 70S ribosome dissociation and shifts the equilibrium to favor the dissociated subunits.

  ***Explanation*: The IF3 will chemically accelerate and support the dissociation of the 70S, which was formed in previous translation cycle.**

- **Second step:**
  IF1 (the smallest IF) binds at the A-site of the 30S subunit and promotes(يحفز) binding of IF2 and IF3.

Note: IF1 stimulates the activities of IF2 and IF3 by promoting their more efficient binding to the 30S subunit.

Explanation: **The IF3 is already bounded and the IF1 will make it more tightly bounded.**

- **Third step:**
  IF2 (the largest IF) binds and hydrolyzes GTP at its middle domain and a C-terminal domain interacts with fMet-tRNAfMet.
- **In the end:**
  The 50S subunit joins the complex to form a 70S initiation complex. then GTP is immediately hydrolyzed and the IF factors dissociate.

Explanation: **IF2 binds the initiator tRNA (fMet-tRNAfMet) in the middle, then it binds the 50s (large subunit), after that, the GTP is hydrolyzed so all the initiator factors dissociate.**

Note: The resulting 70S initiation complex is now ready to begin the elongation cycle by binding the aminoacyl-tRNA specified by the second codon at the A-site.

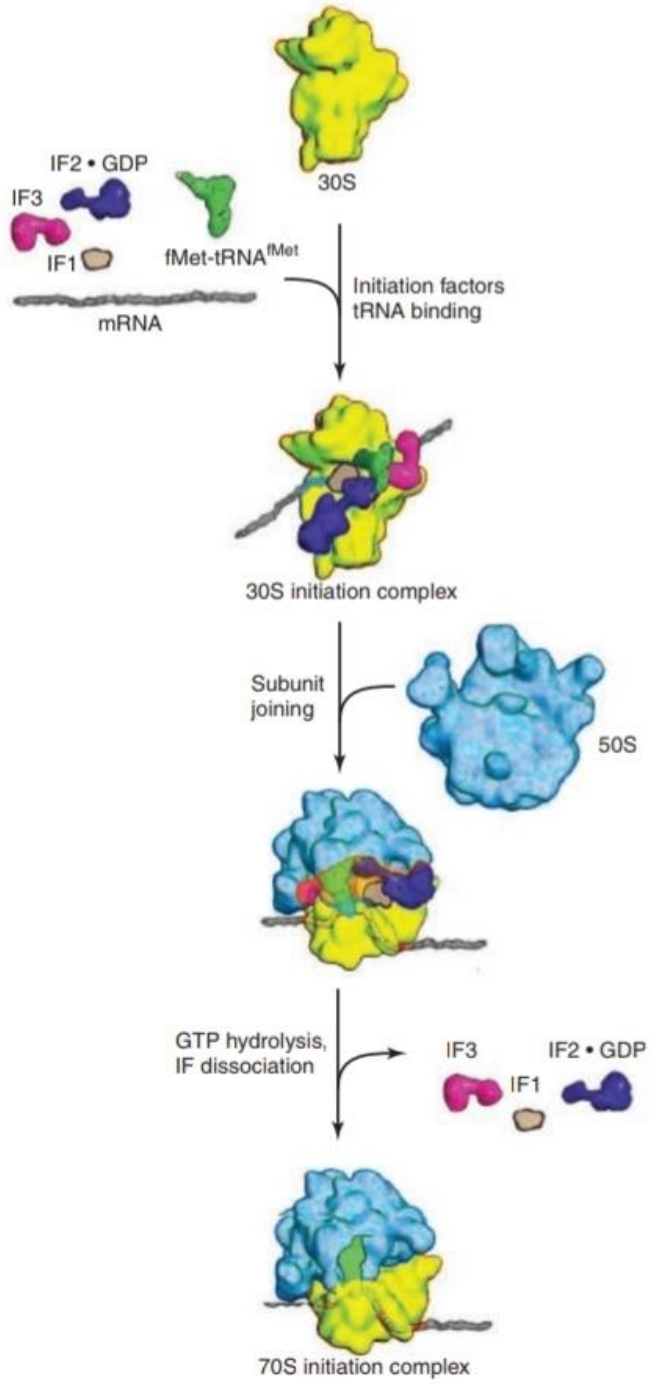


FIGURE 16.40 Translation initiation pathway in bacteria. Components are positioned on the ribosome according to currently available experimental information. (Adapted from Schmeing, T. M., and Ramakrishnan, V. 2009. *Nature* 461:1234–1242.)

Note: IF2 is associated with GTP.

IF1, IF2, and IF3 are required for polypeptide chain initiation at physiological magnesium ion concentrations, estimated to be about 5 mM, but not at higher concentrations. High magnesium ion concentrations stabilize the 70S ribosome and permit mRNA and aminoacyl-tRNA molecules to bind directly to the 70S ribosome in the absence of the initiation factors so the normal physiological pathway for polypeptide chain initiation is bypassed. Nirenberg and Matthaei were able to observe polv(U)-directed polyphenylalanine synthesis because their system contained a high magnesium ion concentration.

The Shine-Dalgarno sequence

Note: The Shine-Dalgarno sequence is named after the two scientists who discovered it, it's also called ribosomal binding sequence.

- **The Shine-Dalgarno sequence in mRNA interacts(يرتبط) with the anti-Shine-Dalgarno sequence in the 16S rRNA.**
- **It helps the bacterial ribosome to distinguish the initiator AUG (that is translated to fMet) from other AUGs that code for internal methionines (Met)** or from AUGs that occur in alternate reading frames.
- **The Shine-Dalgarno sequence is:**
    - **a purine-rich consensus sequence (**5'-UAAGGAGGU-3'**).**
    - **located 5-7 nucleotides upstream from the AUG(initiator codon),** with the highlighted nucleotides being the most frequently present, check figure (16.41)،located upstream means it's a part of the 5` UTR (untranslated region)
- **The 16S rRNA has a complementary** pyrimidine-rich sequence (5'-ACCUCCUUA-3') **near its 3'-end (the anti-Shine-Dalgarno sequence).**

***Check the figure below to understand well. The red sequences represent the Shine-Dalgarno sequence.**

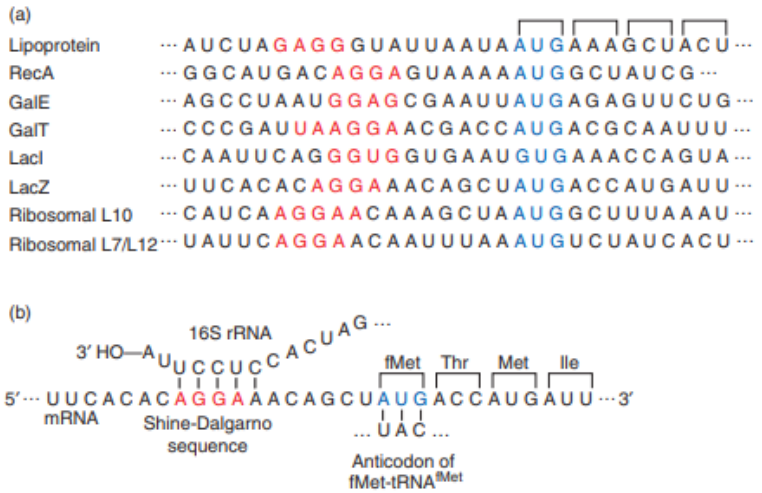


FIGURE 16.41 Shine-Dalgarno sequence. (a) Shine-Dalgarno sequences (ribosome-binding sites), which are located toward the 5′-end of mRNA, are shown for several *E. coli* mRNAs. The initiation (start) codons (blue) are immediately downstream. The optimal spacing between Shine-Dalgarno sequences and initiation sequences is 7–9 nucleotides. (b) Base pairing between the Shine-Dalgarno sequence and the complementary sequence in the 16S rRNA helps to establish the initiation codon and the correct reading frame for translation. (Adapted from Horton, R. H. et al. 2002. *Principles of Biochemistry* (3rd ed). Prentice Hall.)

In 1974 John Shine and Lynn Dalgarno made two important observations that helped to explain how the bacterial protein synthetic machinery performs this task. First, they noticed most cistrons have a purine-rich consensus sequence (5′-UAAGGAGGU-3′) located about five to seven nucleotides upstream from the initiator codon, with the highlighted nucleotides being the most frequently present (**FIGURE 16.41a**). Second, they noticed the 16S rRNA has a complementary pyrimidine-rich sequence (5′-ACCUCCUUA-3′) near its 3′-end. They therefore proposed that the complementary sequences on mRNA and 16S rRNA base pair in an antiparallel fashion (Figure 16.41b), helping the translation machinery to identify the initiator codon. The purine-rich sequence in mRNA is now called the **Shine-Dalgarno sequence** or ribosome binding site and the complementary sequence near the 3′-end of 16S rRNA is called the **anti-Shine-Dalgarno** sequence.

*3 **The mRNA binds with 16S rRNA in the Shine-Dalgarno sequence. Which is important to determine the location of the initiator codon in the middle**

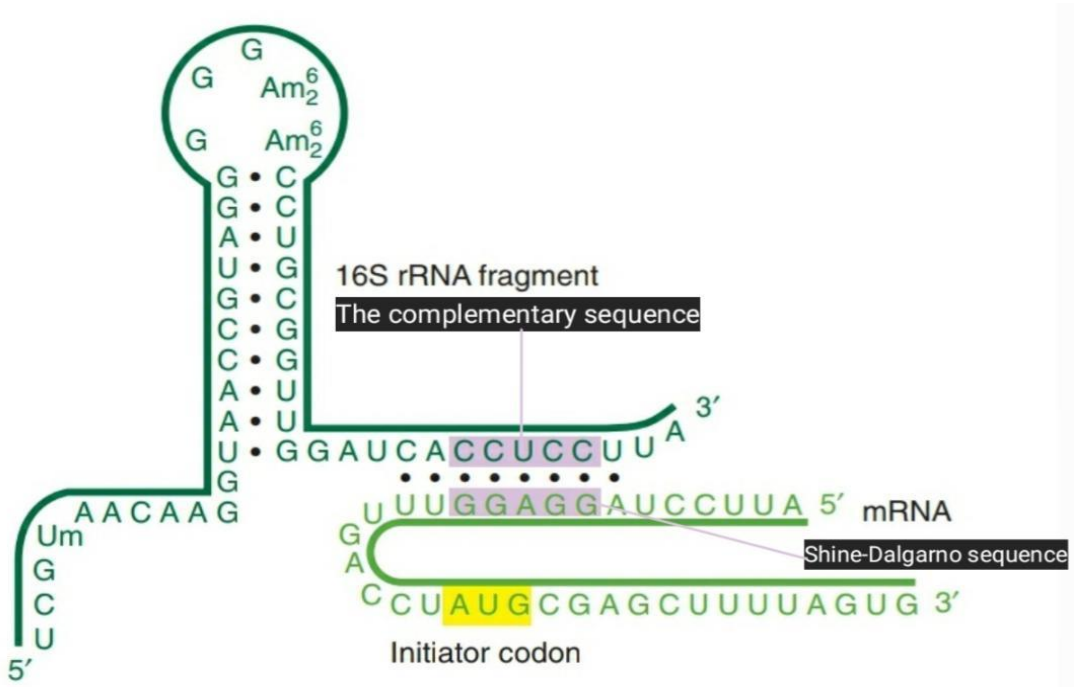


FIGURE 16.42 Binding of mRNA to a complementary sequence in 16S rRNA. The AUG start codon is shadowed in yellow, the Shine-Dalgarno sequence in pink, and the anti-Shine-Dalgarno sequence in tan.

where the large and small ribosomal subunits will bind.

Note: Optimal spacing between the Shine-Dalgarno sequence and the initiation codon is 5 nucleotides, but the Shine-Dalgarno sequence still works if it is up to 13 nucleotides from the initiation codon.

Eukaryotic translation initiation stage

- Eukaryotic initiator tRNA is charged with a methionine that is not formylated
  - However, like bacteria, eukaryotes have an initiator Met-$tRNA_i$ and an elongator tRNA ($tRNA_m^{Met}$). (i: initiator)
  - There are a variety of unique sequence features present in initiator tRNA but not in elongator tRNA, both have the same anticodon, but each one of them binds to a different sequence.

- Eukaryotic translation initiation proceeds through a scanning mechanism

  - Eukaryotic mRNA does not have a Shine-Dalgarno sequence.
  - Instead, the AUG is embedded within a sequence (5`ACCAUGG3`).

    Notes:
    - ACC is a part of 5' UTR (untranslated region).
    - Usually but not always there is G after AUG sequence.

  - Marilyn Kozak in 1978 proposed the SCANNING MODEL.
    – The pre-initiation complex binds to the mRNA 5'-cap and scans 5'→3' until it detects the initiation codon.
    Note: Pre-initiation complex: before the complex finished its composition.
    Note: Initiation complex: after the complex finished its composition.

You will understand!!! don't worry👌👌😁.

Initiation Key stages in eukaryotic:

Note:

- The eukaryotic translation initiation pathway is far more complex than the bacterial pathway.
- Differences between the two pathways arise from the fact that transcription and translation take place in the same compartment in bacteria but in different compartments in eukaryotes. Because eukaryotic mRNA synthesis is completed in the nucleus.

*** Ternary complex-->stage 1 complex --> stage 2 complex-->80S initiation complex**

Note: complex: different components binding to each other.

1) **Ternary complex formation: Ternary means 3 components**
   eIF2 combines with GTP to form an eIF2 · GTP complex, which then binds Met-tRNAi to generate the eIF2 · GTP · Met-tRNAi ternary complex.
   ***NOTE: e: eukaryotic, I: initiation, F: factor, $tRNA_i$: initiator tRNA.**

2) **Stage one complex:**
   Ternary complex binding to 40S (small) subunit: Assisted by translation initiation factors ,interacts with the 40S ribosomal subunit to form a complex.

3) **mRNA activation:**
   Secondary structures and proteins are removed
   ***Note: we are discussing the mature mRNA after modification process.**
   Eukaryotic mRNA passes through the nuclear pore into the cytoplasm as a structured molecule that is coated with polypeptides.Translation initiation factors assist in activating the mRNA by removing many of the polypeptides that coat the mRNA and secondary structures(that was formed due to base pairing between its own regions) to make it linear, therefore, it is now active (ready) to bind with the small subunit.

4) **stage two complex formation:**
   mRNA entry into the complex formed in stage1
5) **5'→3' scanning to detect the initiation codon:**
   complex from stage 2 moves along the mRNA until the AUG codon is aligned with the anticodon on the initiator tRNA . The complex formed in stage 2 moves along the mRNA in a 5' to 3' direction. Translation initiation factors assist in this ATP-dependent movement, which

continues until the AUG initiation codon on the mRNA is aligned with the anticodon on the initiator tRNA.

6) **80S initiation complex formation:**
60S subunit binds to stage2 complex. Translation initiation factors facilitate the joining of the 60S subunit to the complex containing the 40S subunit to form the 80S initiation complex.

*Remember:

In the eukaryotic: 60S + 40S = 80S.

In the prokaryotic: 50S + 30S = 70S.

Initiator tRNA

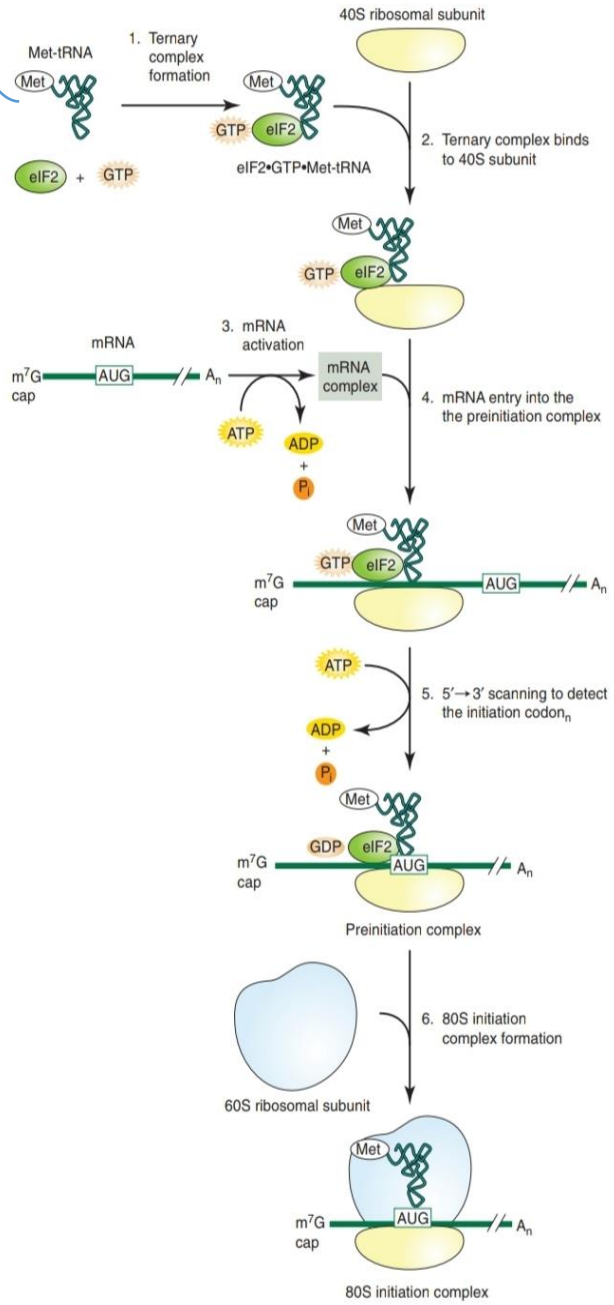


FIGURE 16.45 Eukaryotic translation initiation pathway. This simplified figure does not indicate the contribution(s) that each eukaryotic initiation factor makes to 80S initiation complex formation. See the text for a description of the steps.

Notes:

1. **Activation of mRNA needs ATP.**
2. **The movement of the complex during the scanning needs ATP.**

Quick Recap:

Translation has 4 stages:

1)initiation

2)elongation

3)termination

4)recycling

Elongation stage

Polypeptide chain elongation requires elongation factors (elongation factor: protein that encodes by gene, we have large amounts of them. (Its large quantity is because we need it for translation, and we have continuous translatio))

– In bacteria

• EF-Tu, EF-Ts and EF-G

– In eukaryotes

• eEF1A, eEF1B and eEF2 (e refers to eukaryotes)

• The elongation factors act through a repeating cycle so we need this elongation factors to add amino acids in a polypeptide chain, this chain is also called growing polypeptide and this cycle also explain why we need large amount of these transcription factors.

To understand elongation stage and its mechanisms and processes, you have to know the elongation factors and their functions.

Fritz Lipmann and coworkers showed three translation elongation factors isolated from E. coli extracts partici- pate in poly(U)-directed polyphenylalanine synthesis. The bacterial elongation factors are named EF-Tu, EF-Ts, and EF-G. The terms Tu and Ts indicate that the E. coli proteins are temperature unstable and temperature stable, respectively. The term G indicates the elongation factor has GTPase activity. A new nomenclature system has been suggested for bacterial elongation factors in which EF-Tu, EF-Ts, and EF-G are called EF1A, EF1B, and EF2, respectively. Here we use the old nomenclature because most molecular biologists continue to use the old system.

An EF-TU.GTP. aminoacyl-tRNA ternary complex (this complex is EF-Tu elongation factor linked with GTP (EF-Tu love G "The reason is a chemical") now these EF-Tu.GTP will bind to aminoacyl-tRNA, this 3 components form what we called ternary complex carries the amino-acyl tRNA to the ribosome

FORMING THIS STRUCTURE IS THE FIRST STEP OF ELONGATION PROCESS, it is done in this order:

1)EF.TU is synthesized then bound with GTP to form **EF-TU.GTP**

2) **EF-TU.GTP binds with amino acid to form ternary complex**

amionacyl-tRNA is tRNA loaded with an amino acid by enzyme which is called aminoacyl-tRNA synthetase (aaRS or ARS), also called tRNA-ligase, **and acyl due to the ester bond.**

tRNA moves to ribosomes and it is loded with an amino acids , and returns back without amino acids.Because of that, our cells have to give it an amino acids.

Amino acids sources:

1)essentially from food.

2)non-essentially by biochemical pathways.

Due to the type of amino acid we named this complex, for example if we have tyrosine it's become tyrosinacyl-tRNA, the type of amino acid related to genetic code. (And don't forget that amino acids is the monomers for proteins)

-Our cells have a huge number of elongation factors due to many repeated elongations

• **The tRNA is in an A/T** state

– Anticodon interacting with mRNA in the A site(According to Watson-Crick base pairing in DNA, adenine(A) forms a base pair with thymine (T) using two hydrogen bonds, and guanine (G) forms a base pair with cytosine (C) using three hydrogen bonds)and it tries to bind with mRNA BUT ONLY complementary strand is bound (Anticodon 3'—>5',mRNA codon 5'—>3') ,and it is formed hydrogen bonds.

Now what help the tRNA to come and try to bind? EF-TU help them to make hydrogen bond with mRNA but that will not happen until it is complementary to each other.

Acceptor end remains **bound to the elongation factor (the tRNA Contains two ends one end contain anticodon that will bind to the**

codon and another end loaded amino acid called Acceptor, and this acceptor bind to the elongation factor.)

The ternary complex starts and sets the tRNA in mRNA but if there isn't matching this tRNA will leave and another tRNA comes and tries to bind.Until complementary tRNA comes and binds. (notice that:mRNA that determines which tRNA will bind).

Once again tRNA trying to bind with help of EF-Tu.GTP

If a matching occurs then GTP will hydrolysis-by GTPase which is found in elongation factor-to GDP to produce energy that is important to complete translation

If the codon/anticodon matching is correct, EF- Tu's GTPase activity is

activated that's mean that EF-Tu have GTPase activity and it activated when matching is occurred by hydrolysis GTP.

In another words when ternary structure make hydrogen bonding with mRNA, it Changes the conformation of EF-Tu from inactive to active form in this case GTP will breakdown into GDP+P.

•GTP hydrolysis form Free aminoacyl end of the A-site tRNA moves into the peptidyl transferase center in a process called accommodation

tRNA that loaded with amino acid (Acceptor) It entry the site called Peptidyl transferase center in the large subunit (50s) this place have active site because there is enzyme called peptidyl transferase which is bind amino acid to another one by dehydration reaction(to transfer amino acids in tRNA in P site to tRNA in A site)

the moving of A site tRNA to the peptidyl transferase center called accommodation

Now we have to activate peptidyl transferase by hydrolysis of GTP then we breakdown the bond between tMRN and amino acid in P site then bind this amino acid to tMRN in A site and that what we call growing polypeptide, you can conclude this tRNA in P site is unloaded.

Well, now Another elongation factor comes called EF-G that will hydrolysis GTP to do translocation of each tRNA in each site, tRNA in P

site (with no amino acid) moving to E site and tRNA in A site (have a growing site) moving to P site.
when that happens GDP with EF leaves.

EF-Ts is a GDP-GTP exchange protein

• The EF-Tu.GDP released during the elongation cycle must exchange its GDP for GTP

– Requires EF-Ts to carry out the exchange

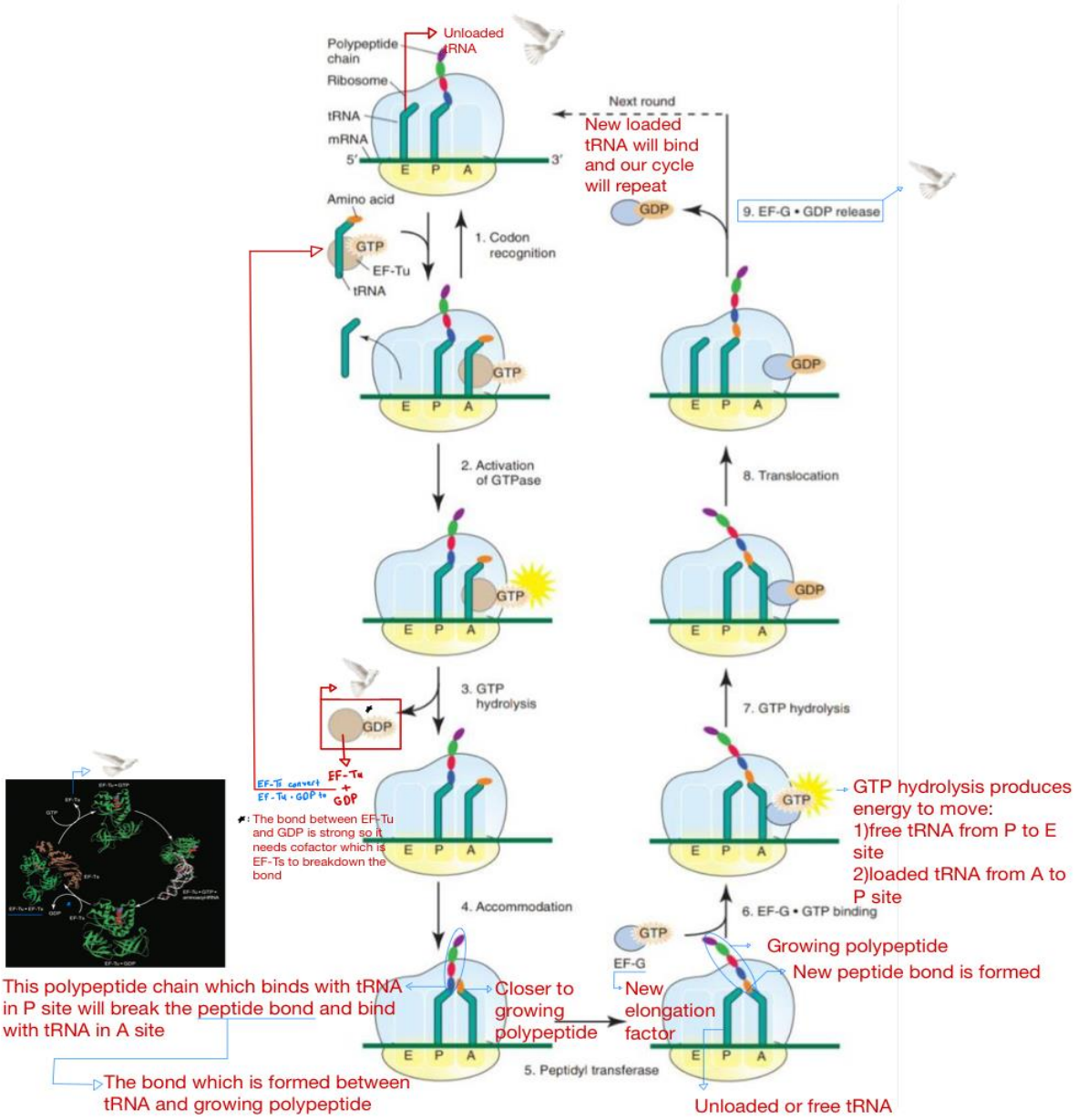
we need to convert that GDP in EF-Tu to GTP to come and repeat the cycle again

So how can we do that? by the last elongation factor which is EF-Ts this factor connect GTP and EF-Tu connect with GDP (bond between GDP and EF-Tu is so strong so we need assistant factor to break them down this assistant factor is EF-TS)

At the first EF-Tu bind with EF-Ts instead of GDP (exchange) this complex called EF-Tu.EF-Ts (2 elongation factors bind to each others).

now the bond between EF-Tu and EF-Ts is easily to breakdown so GTP come and and replace EF-Ts to bind with EF-Tu. Now the cycle is repeated to make this polypeptide longer and longer.

Eukaryotic eEF1B catalyzes the same reaction (same process, different names)



-what are the results of one cycle?

4 🕊️

Termination Stage

Stop codons: UAG,UGA,UAA

Why does the elongation process not continue?

Because we have a stop codons once the cell recognizes them the elongation Stage will stop.

This stop codons is: UAA, UGA and UAG.

Ones the cell recognizes them the release factors will come and bind that leads to breakdown (hydrolysis) the ester bond between tMRN and the last amino acid then the growing polypeptide chain become free.

Polypeptide chain termination requires release factors

• Bacteria have three protein release factors (RFs)

RF1, RF2 and RF3 interact with termination codon

The release factors:

***RF1 and RF2 interact with termination code.**

***RF3: stimulates the rate of peptide release when either RF1 or RF2 is also present but it doesn't act on it's own**

***RF1 and RF2 both recognize UAA**

***RF1 recognize UAG**

***RF2 recognize UGA**

Tow proposed mechanisms:

1) RF1 and RF2 are hydrolases (enzymes) that become activated upon binding to the ribosome.

They themselves do the hydrolases process) at the first they are inactive and don't do they function (hydrolases) until it bind to stop codon it becomes active then it will breakdown the ester bond and polypeptide become free.

2) RFI and RF2 stimulate a ribosomal hydrolases activity (hydrolyses the ester bond linking the complete peptide to the tRNA at the P-site.)

RFI and RF2 triggers conformational change in the ribosomal peptidyl transferase center, which permits water to attack the ester bond in the peptidyl-tRNA at p-site.

In another words in this case RF1 and RF2(they not do the hydrolases directly) they changed the confirmation of the peptidyl transferase center in 50S ribosome which allows water molecule to come and bind on the ester bond between tMRN and polypeptide chain (in last amino acid) and that leads to break this bond and the polypeptide become free.

Now in eukaryotes, the process is much simpler here.

Eukaryotes have two release factors

- eRF1 recognizes all three termination codons (UAA, UGA and UAG)

- eRF3 acts with eRF1 to guarantee efficient termination codon recognition

(eRF2 has no function her).

At the result, free polypeptide is formed.

A mutant tRNA (with an anticodon that recognizes a nonsense codon) can suppress mutations that create termination codons.

That cause to convert normal codon into a stop codon and vice versa.

Recycling Stage

we have also to disassemble the whole structure of ribosomes to be ready to start from the first step(initiation)to do new polypeptide once again.

At the end of the termination stage, the ribosome (small subunit and large subunit)

complex is still associated with mRNA

A new translation factor known as the ribosome

release factor is required for the bacterial ribosomal complex to disassemble

- **EF-G.GTP and IF3 assist (help the bacterial ribosomal Complex)**
- **After ribosome disassembly the small subunit is free to interact with IFs to start a new round of translation**

– Nothing is known about the recycling stage in eukaryotes

-What is the difference between TERMINATION **and RECYCLING:**

TERMINATION is to convert loaded tRNA to free tRNA

RECYCLING is to disassemble ribosomes to small subunits.

For further understanding: https://youtu.be/VhpRtUaO 8 watch this animation

END OF THE STORY ☹.

سبحانك اللهم وبحمدك، نشهد أن لا إله إلا أنت، نستغفرك و نتوب إليك

-إبراءً للذمة:

كان هدفنا -تيم المولي- أنه تكون الشيتات شاملة المادة المطلوبة، و حاولنا و اجتهدنا و بذلنا قصارى جهدنا لنحقق هذا الهدف ، و بما إنه هذا الشيت الأخير و الامتحان كمان أسبوع، فاعذرونا على أي خطأ أو نقص أو سهو بأي شيت من الشيتات و إن شاء الله تكون كافية لهدف العلم و العلامة ، و أي خطأ لاحظتوه خبرونا عنه عشان نعدله،و بالنهاية بنعتذر عن أي خطأ صدر منّا كأعضاء الفريق تجاه أي شخص أو أي خطأ علمي بالشيتات،والسلام عليكم ورحمة الله وبركاته ،بالتوفيق جميعا و فالكم أعلى العلامات!

و من باب نشر الفائدة لا تنسوا تنضموا لمشروع الدفعة القرآني ،مشروع*"لِنُثَبِّتَ بِهِ فُؤَادَكَ" للذكور و الإناث ،تواصوا مع المشرفين و أبشروا بالخير، يعطيكم العافية* ❤

طلع في ايموجيز مش قادرة أطلعهم وأنا بنسق المهم ادعولنا معكم !!!

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