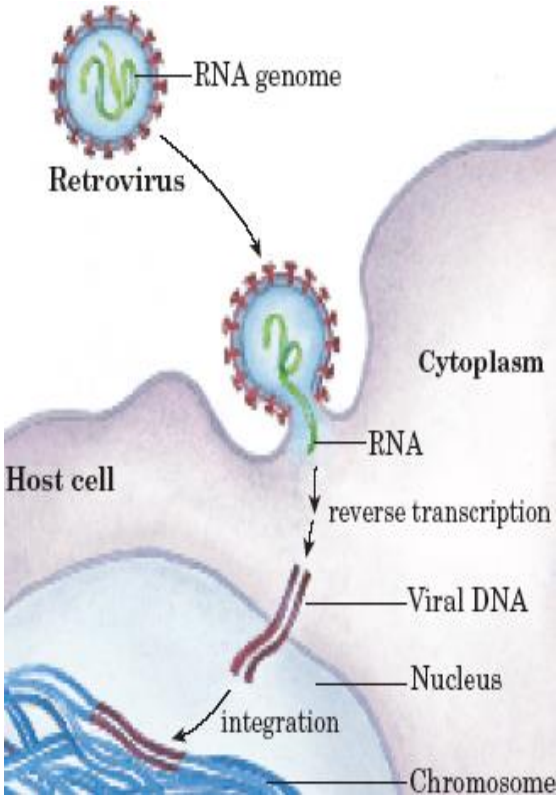
Reverse transcription: synthesizing DNA using RNA template.



- ✓ The genetic information carrier of some biological systems is **ssRNA (single stranded RNA)** instead of **dsDNA (double stranded DNA)** (such as ssRNA viruses).
- ✓ SsRNA viruses called retroviruses because they have their genome in the form of single stranded RNA, (and the viruses with a DNA form of the genome called adenoviruses such as the common cold.)
- ✓ The information flow is from RNA to DNA, opposite to the normal process.
- ✓ This special replication mode is called **reverse transcription**.
- ✓ HIV has an **RNA genome** that is duplicated into DNA.
- ✓ The resulting DNA can be merged with the DNA genome of the host cell.
- ✓ The main enzyme responsible for synthesis of DNA from an RNA template is called **reverse transcriptase (RT)**.
- ✓ **الفايرس عشان يصيب الخلية الهدف بيشبك على غشاء الخلية وبحقن ال genetic material تبعته داخل ال host cell شوف الصورة**
- ✓ The next step depends on the form of the genetic material, so if it is a dsDNA there will be integration of the virus DNA within the genome of the host cell .
  (رح تندمج المادة الوراثية تبعت الفايرس مع المادة الوراثة الخاصة بالخلية المصابة)
- ✓ Which results controlling of all of the host cell activities and it will start synthesizing the viral protein
  (بالتالي الفايرس بهيمن على الخلية المصابة وبسيطر على كل شيء وبخليها تصنع البروتينات الخاصة فيه لحتى يتكاثر ويخرج ليعدى باقي الخلايا)
- ✓ But if the genetic material is in the form of ssRNA, retroviruses, an enzymes called reverse transcriptase will make a DNA molecule from the viral RNA so the integration can occur.

The reverse transcriptase (RT) has the following activities:

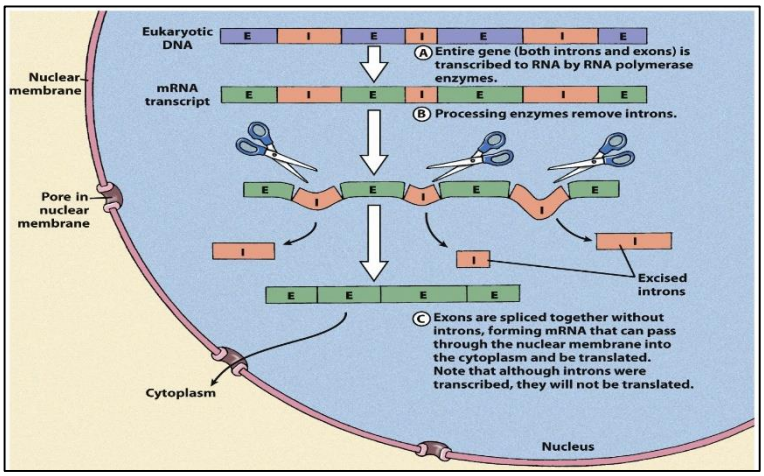
1- RNA-dependent DNA polymerase: using the viral RNA template to synthesize **the first strand of DNA which we call (cDNA = complementary DNA)**.
2- RNase (**ribonuclease H**): degrades the viral ssRNA to ribonucleotides.
3- DNA-dependent DNA polymerase: using the cDNA molecule as a template for synthesizing the second strand of DNA.

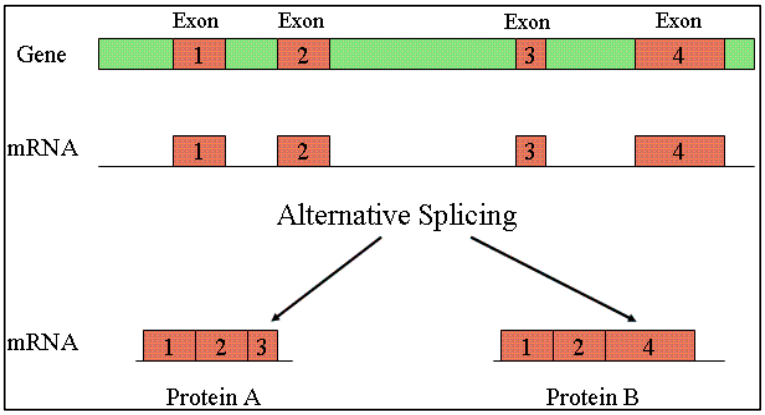
- ✓ In the case of HIV, **reverse transcriptase** is responsible for synthesizing a complementary DNA strand (cDNA) to the viral RNA genome.
- ✓ An associated enzyme, **ribonuclease H**, digests the RNA strand, and **reverse transcriptase** synthesizes DNA complementary strand to form a double helix DNA structure.
- ✓ This DNA is integrated into the host cell's genome by **integrase** enzyme causing the host cell to generate viral proteins that reassemble into new viral particles.
- ✓ However, in retroviruses, the host cell remains intact as the virus buds out of the cell but in the case of HIV, the host cell undergoes apoptosis.

- ✓ اعتماد هذه الفايروسات مثل ال HIV,HCV,HBV على عمل الانزيم **reverse transcriptase** يعد مصيبه وكارثة والسبب انه هالانزيم ما في proofreading يعني ما بيتحقق من أخطاءه، وناتج هاي الاخطاء بالجينوم بينتج انواع مختلفه من الفايرس نفسه والي بخلي عمليه ايجاد علاج قاطع شي صعب، مثلا فايرس الانفولونزا بيتكون من ثلاث فصائل رئيسية، في حال انك أصبت بالانفلونزا كأنك أصبت بما يقارب 159,000 نوع من فايرس الانفلونزا وهذا يعود الى الانزايم الي بعمل أخطاء لا تصحح، فبينتج أمراض استحاله نجد علاج قاطع الها، فايرس الايدز والمصاب به كأنه مصاب بما يقارب 14 مليون نوع من فايرس الايدز نتيجه للاخطاء.
- ✓ So the **reverse transcriptase** doesn't have proofreading (not checking its mistakes) and because of that it makes non-correctable mistakes which makes finding a final cure for these diseases impossible
- ✓ Some eukaryotic cells contain an enzyme with reverse transcription activity called **telomerase**.
- ✓ Telomerase is a reverse transcriptase that lengthens the ends of linear chromosomes.
- ✓ every time a linear chromosome is duplicated, it is shortened in length because of the **Exonucleases** that cut a portion from the newly synthesized DNA (this portion is from the repeating sequence)
- ✓ Telomerase carries **an RNA template** from which it synthesizes **DNA repeating sequence**, or "**junk**" DNA. (it has a short portion of RNA that uses it to synthesize the repeated sequences (بيتكون من6 قواعد) at the 3' end)
- ✓ This non-coding repeated sequence of DNA is important because, the Exonucleases cuts from them and if the telomerase activity is stopped with time the Exonucleases will start to cut from the coding sequence which cause losing important genes and the telomerase activity deceases with age so that for example old people become unable to see or hear properly
- ✓ **Telomerase** is often activated in cancer cells (uncontrolled activity of telomerase) to enable cancer cells to duplicate their genomes indefinitely without losing important **protein-coding DNA sequence** (the discovery of **RT** enriches the understanding about **the cancer-causing theory of viruses**, where cancer genes in RT viruses, and HIV having RT function).
- ✓ Activation of telomerase could be part of the process that allows cancer cells to become immortal.

Alternative splicing (eukaryotes only)

- ✓ Exons are “coding” regions
- ✓ Introns are removed
- ✓ Different combinations of exons form different mRNA resulting in multiple proteins from the same gene
- ✓ Humans have 30 to 50 thousand genes but are capable of producing 100,000 proteins.
- ✓ Intron splicing= intron removal + exon joining
- ✓ الي بصير انه بالاول بينشال ال introns من الجين مثل ما في الرسمة، وبضل عنا ال exons لكن غير مرتبطين سوا، هون في عنا اشي اسمه تأثير ال Alternative splicing حيث انه ال exons ما برتبط كلها سوا، بكون في احتمالات كثير لارتباطها زي الي فالصورة وكل مجموعه بيرتبطو سوا بيعملو mRNA مسؤول عن تكوين بروتين معين، ف من جين واحد ممكن ينتج اكثر من mRNA وبالتالي اكثر من بروتين.

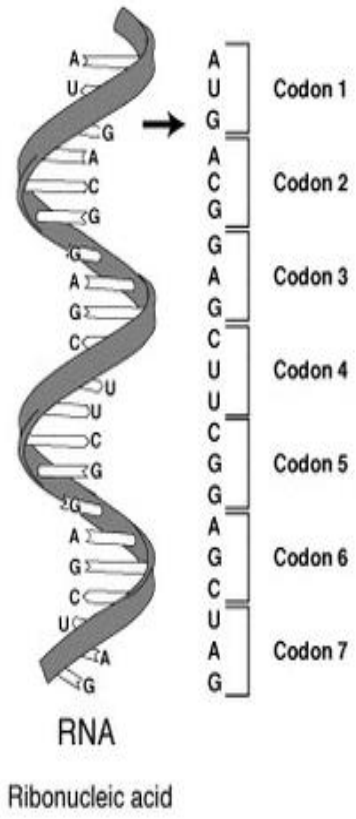




Seconed Position

First Position	U		C		A		G		Third Position
	code	Amino Acid	code	Amino Acid	code	Amino Acid	code	Amino Acid	
U	UUU	phe	UCU	ser	UAU	tyr	UGU	cys	U
	UUC		UCC		UAC		UGC		C
	UUA	leu	UCA		UAA	STOP	UGA	STOP	A
	UUG		UCG		UAG	STOP	UGG	trp	G
C	CUU	leu	CCU	pro	CAU	his	CGU	arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	ile	ACU	thr	AAU	asn	AGU	ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	lys	AGA	arg	A
	AUG	met	ACG		AAG		AGG		G
G	GUU	val	GCU	ala	GAU	asp	GGU	gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	glu	GGA		A
	GUG		GCG		GAG		GGG		G

- ✓ It is the set of rules by which information encoded in genetic material (DNA or mRNA sequences) is translated into proteins (amino acid sequences) by living cells.
- ✓ With some exceptions, a triplet codon in a nucleic acid sequence specifies a single amino acid.
- ✓ Because the majority of genes are encoded with exactly the same code, this particular code is often referred to as standard genetic code, though in fact there are many variant codes.
- ✓ For example, protein synthesis in human mitochondria relies on a genetic code that differs from the standard genetic code.



Characters of genetic code:

1- The genetic code is composed of nucleotide triplets. In other words, three nucleotides in mRNA (a codon) specify one amino acid in a protein.

2- The code is non-overlapping. This means that successive triplets are read in order and each nucleotide is part of only one triplet codon.

**The genetic code is read in groups (or "words") of three nucleotides. After reading one triplet, the "reading frame" shifts over three letters, not just one or two. In the following example, the code would not be read GAC, ACU, CUG, UGA...

GACUGACUGACU

Rather, **the code would be read GAC, UGA, CUG, ACU

3- The genetic code is **degenerate (redundant,وفرة)**. some amino acids can be specified by more than one codon.

- There are 64 different triplet codons, and only 20 amino acids.
- **there three stop codon UAA,UAG,UGA and one staring codon AUG which represent the methionine (the amino acid found in the beginning of every protien)**
- some redundancy is built into the system: some amino acids are coded by multiple codons.
- In some cases, the redundant codons are related to each other by sequence; e.g., leucine is specified by CUU, CUA, CUC, and CUG.
- The codons are the same except for the 3rd nucleotide position.
- This third position is known as the "wobble" position of the codon.(wobble effect or phenomena, causes flexibility)
- This property allows some protection against mutation - if a mutation occurs at the third position of a codon, there is a good chance that the amino acid specified in the encoded protein won't change.
- This is because in a number of cases, the identity of the base at the third position can wobble, and the same amino acid will still be specified.

Wobble Positions in Anticodon and Codon Interactions

anticodon	C	A	G	U	I
mRNA codon	G	U	C	A	C
			U	G	U
					A

Wobble Positions in Codon and Anticodon Interactions

mRNA codon	C	A	G	U
anticodon	G	U	C	A
	I	I	U	G
				I

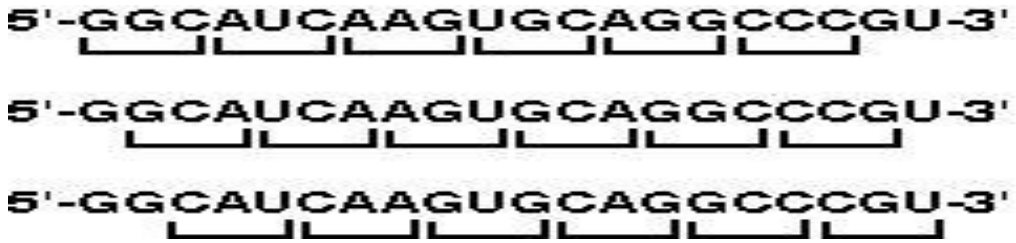
- ✓ The photo above in the first case, shows the flexibility in the 3rd nucleotide position in the codon but in the first and the second there isn't , the C from the anticodon base pairing with the G from the mRNA and the A base pairing with the U BUT the 3rd position in the anticodon have three possibilities G,U,I (INOSINE) and each one can base pair with more than one nucleotide so for example if the anticodon was CAG and the codon should be GUC BUT a mutation happened and becomes GUU because of wobble the mutation was silent mutation (doesn't affect the amino acid),regarding the second case, we see the flexibility in first and the second position as well.
  هذا الي فهمته من كلامه): الي بحب يرجع للريكورد موجود بالدقيقة 29

4- The genetic code is **unambiguous** (مفهومه وواضحه) Each codon specifies a particular amino acid, and only one amino acid. In other words, the codon ACG codes for the amino acid threonine, and only threonine.

5- The code is **nearly universal**. Almost all organisms in nature (from bacteria to humans) use exactly the same genetic code. The rare exceptions include some changes in the code in mitochondria, and in a few protozoan species.

Reading frames:

- If you think about it, because the genetic code is triplet based, there are three possible ways a particular message can be read, as shown in the following figure:



- Clearly, each of these would yield completely different results.
- Genetic messages work much the same way: there is one reading frame that makes sense, and two reading frames that are nonsense.
- The code contains signals for starting and stopping translation of the code.
- The **start codon is AUG**, which codes for methionine and encountered signals for translation to begin and subsequent triplets are read in the same reading frame.
- Translation continues until a stop codon is encountered.
- There are three stop codons: **UAA, UAG, and UGA.**
- **To be recognized as a stop codon**
  (الشرط الوحيد عشان يتم الاعتراف بكودون التوقف انه يكون داخل الفريم يعني جاي بعد كودون البداية غير هيك لا),
  the triplet must be in the same reading frame as the start codon.
- A reading frame between a **start codon** and an **in-frame stop codon** is called an **open reading frame**.
- Translation can take place considering the following sequence:
  5'-GUCCCGUG**AUGCCGAGUUGGAGUCGAUAA**CUCAGAAU-3'
- First, the code is read in a 5' to 3' direction.
- The **first AUG** read in that direction sets the reading frame, and subsequent codons are read in frame, until the stop codon**, UAA, is encountered**.

- In this sequence, there are nucleotides at either end that are outside of the open reading frame. Because they are outside of the open reading frame, these nucleotides are not used to code for amino acids.
- This is a common situation in mRNA molecules, where the region at the 5' end that is not translated is called **5' untranslated region (5' UTR)** and at the 3' end is called the **3' untranslated region (3' UTR)**.

- *If I told you that you have a mRNA with a 37 ribonucleotides, knowing that the 5'UTR = 8 ribonucleotides and the 3'UTR also 8 ribonucleotides, how many amino acids are in the protein formed by this mRNA?*
- *To know that you must find how many ribonucleotides in the open reading frame* ***which = 37 -8-8=21***
- *So 21 ribonucleotides, that includes the stop codon , so without the stop codon we have 18 ribonucleotides that actually translated into amino acids so finally our protein contains 6 amino acid, and it is the same example above .*

- These sequences (5'UTR and 3'UTR), even though they do not encode any polypeptide, are not wasted: in eukaryotes these regions typically contain **regulatory sequences** that can affect when a message gets translated, where in a cell an mRNA is localized, and how long an mRNA lasts in a cell before it is destroyed.

- The position of a ribonucleotide in a codon can be classified according to how many ribonucleotides can be in this position without affecting the amino acid formed into four types:

- A position of a codon is said to be a **fourfold degenerate site** (four genetic codons) if any nucleotide at this position specifies the same amino acid, e.g. the third position of the glycine codons (GGA, GGG, GGC, GGU) is a fourfold degenerate site, because **all nucleotide substitutions at this site are [synonymous]**; i.e., they do not change the amino acid. So, only the third positions of some codons may be fourfold degenerate.
- There is only one **threefold degenerate site** where changing to three of the four nucleotides may have no effect on the amino acid (depending on what it is changed to), while changing to the fourth possible nucleotide **always results in an amino acid substitution.**
- **This is the third position of an isoleucine codon: AUU, AUC, or AUA all encode isoleucine, but AUG encodes methionine.** The possibility of mutation in the 3rd position here that can affect the amino acid formed =25%
- A position of a codon is said to be a **twofold degenerate site** if only two of four possible nucleotides at this position specify the same amino acid. For example, the third position of the glutamic acid codons (GAA, GAG) is a twofold degenerate site. The possibility of mutation in the 3rd position here that can affect the amino acid formed =50%
- A position of a codon is said to be **a non-degenerate site** if any mutation at this position results in amino acid substitution.

Protein synthesis:

- It is the process in which cells build proteins (a multi-step process, beginning with amino acid synthesis and transcription of nuclear DNA into messenger RNA, which then decoded by the ribosome to produce proteins).
- When a protein must be available on short notice or in large quantities, a protein precursor is produced (proprotein).
- A proprotein is an inactive protein containing one or more inhibitory peptides that can be activated when the inhibitory sequence is *removed* by proteolysis during posttranslational modification (**trimming**).
- A preproprotein is a form that contains a **signal sequence** (an N-terminal signal peptide) that specifies its insertion into or through membranes, i.e., targets them for secretion.
- The signal peptide is cleaved off in the endoplasmic reticulum. (after it passes through membranes) ➔ preproprotein → proprotein
- Preproproteins have both sequences (**inhibitory and signal**) still present.
- For synthesis of protein, a succession of tRNA molecules charged with appropriate amino acids have to be brought together with a mRNA molecule and matched up by base-pairing through their anti- codons with each of its successive codons.
- The amino acids then have to be linked together to extend the growing protein chain, and the tRNAs, that are relieved of their burdens, have to be released.
- These whole complexes of processes are carried out by **the ribosome**, formed of: two main chains of rRNA, and more than 50 different proteins.

Removal of signal sequence
As it passes through the membrane

By endopeptidase
Before reaching Golgi,
Inside the Golgi, or
After secretion

Gene expression

Transcription is synthesis of an RNA that is complementary to one of the strands of DNA.

Translation **when** ribosomes read a messenger RNA and make protein according to its instruction.

Gene encoding region (ORF)

↓ transcription

mRNA

↓ Translation

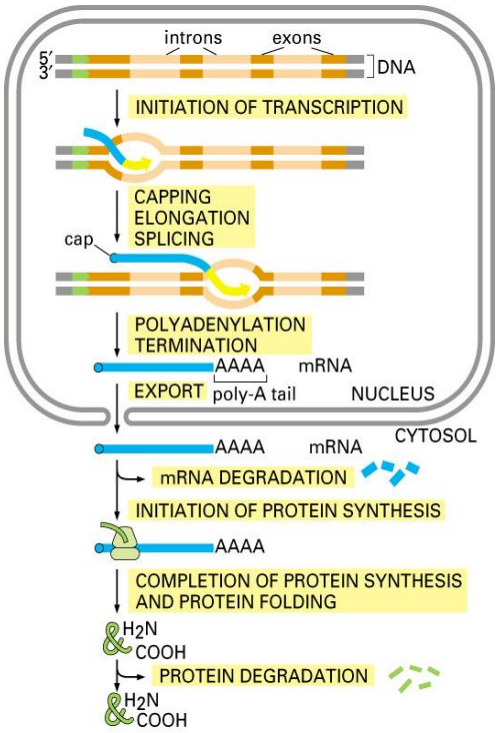
Precursor protein

↓ Post – translational Modifications

Mature protein

↓ folding

Biologically active protein



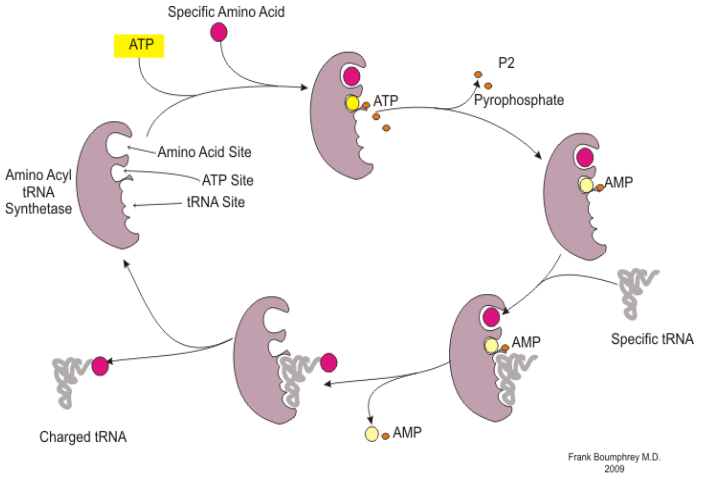
Charging the tRNA

tRNA acts as a translator between mRNA and protein

Each tRNA has a specific **anticodon** and an amino acid **acceptor site**.

Each tRNA also has a specific charger protein (aminoacyl tRNA synthetases) which can only bind to that particular tRNA and attach the correct amino acid to the acceptor site.

The energy to make this bond comes from ATP



Loading a tRNA molecule

Aminoacyl tRNA synthetases:

- There are 20 different synthetases one for each amino acid that can catalyze the covalent bond between the amino acid and tRNA
- A single synthetase may recognize multiple tRNAs for the same amino acid specified by the mRNA codon to which the tRNA anticodon binds (Amino acids might have more than one tRNA thus the number of tRNA is more than 20 (between 31-41))
- Class I - monomeric, acylates the 2'OH on the terminal ribose
  Arg, Cys , Gln, Glu, Ile, Leu, Met, Trp Tyr, Val
- Class II - dimeric, acylates the 3'OH on the terminal ribose
  Ala, Asn, Asp, Gly, His, Lys, Phe, Ser, Pro, Thr

- The process of charging tRNA needs high energy and is irreversible thus the enzyme needs to check if the anticodon of the tRNA matches the amino acid that to it its going to be attached ,Ex a tRNA with CAA anticodon attach to the enzyme, the enzyme knows that this anticodon is specific to valine so it insures that the amino acid that bind to the tRNA is valine.

Wobble

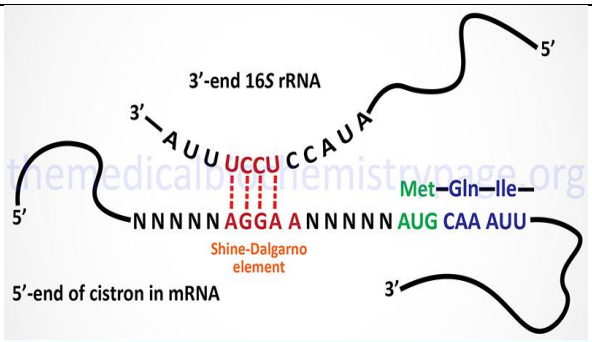
- If there was one tRNA for each mRNA codon, there would be 61 different tRNAs but there are fewer
- Some tRNAs have anticodons that recognize 2 or more different codons
- Base pairing rules between the third base of a codon and its tRNA anticodon are not a rigid as DNA to mRNA pairing
  Example: U in tRNA can pair with either A or G in the third position of an mRNA codon
- This flexibility is called **wobble**

Note: the codon has three nitrogen bases the first two are rigid while the last one is flexible (wobble), and as if some amino acids have different codons that encode them which differ between each other in the first two nitrogen bases (example: leucine is encoded by both UUA and CUU in which the differ in the first nitrogen base) this result in that some amino acids is translated by more than one tRNA, thus there is more than 20 tRNA (as we compare them from the anticodon side but if we compare them from the AAs binding site by then we will have only 20 tRNA)

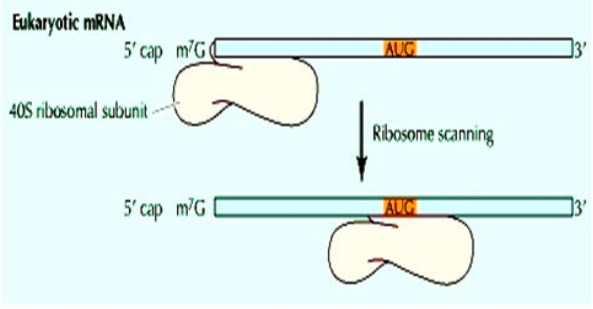
There are two levels of control to ensure that the proper amino acid is incorporated into protein through:

1. The reaction of amino acyl tRNA synthetase for charging the proper tRNA
2. Matching the specific tRNA to a particular codon of mRNA

In **prokaryotes**, specific sequences in the mRNA around the AUG codon, called **Shine – Delgarno** sequences, are recognized by an initiation complex consisting of a Met amino-acyl tRNA, initiation factors (IFs) and the small ribosomal subunit.



In eukaryotes, there is a process called ribosome **scanning**, where mRNA is moving along the small subunit of ribosome till finding the codon of initiation (AUG) of methionine to be located in the P site.



Note: charging tRNA uses ATP as energy whereas initiation, elongation, and termination use GTP as energy.

➢ Prokaryotic protein synthesis

Initiation

- This phase of protein synthesis results in the assembly of a functionally competent ribosome in which an mRNA has been positioned correctly so that its start codon is positioned in the P (peptidyl) site and is paired with the initiator tRNA.
- The following ingredients are needed for this phase of protein synthesis:

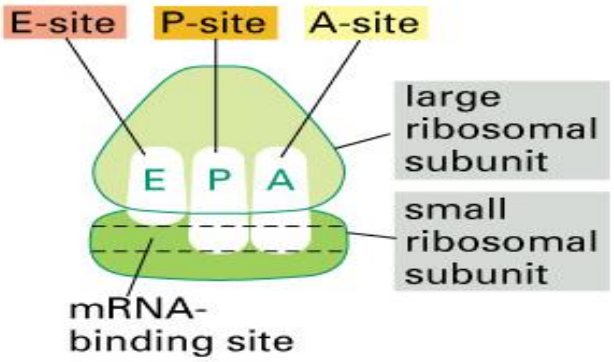
1- Two ribosome subunits - 30S and 50S
2- The mRNA
3- Three Initiation Factors - IF1, IF2 (GTP) and IF3
4- The initiator fMet-tRNA$_f^{Met}$

The following steps take place:

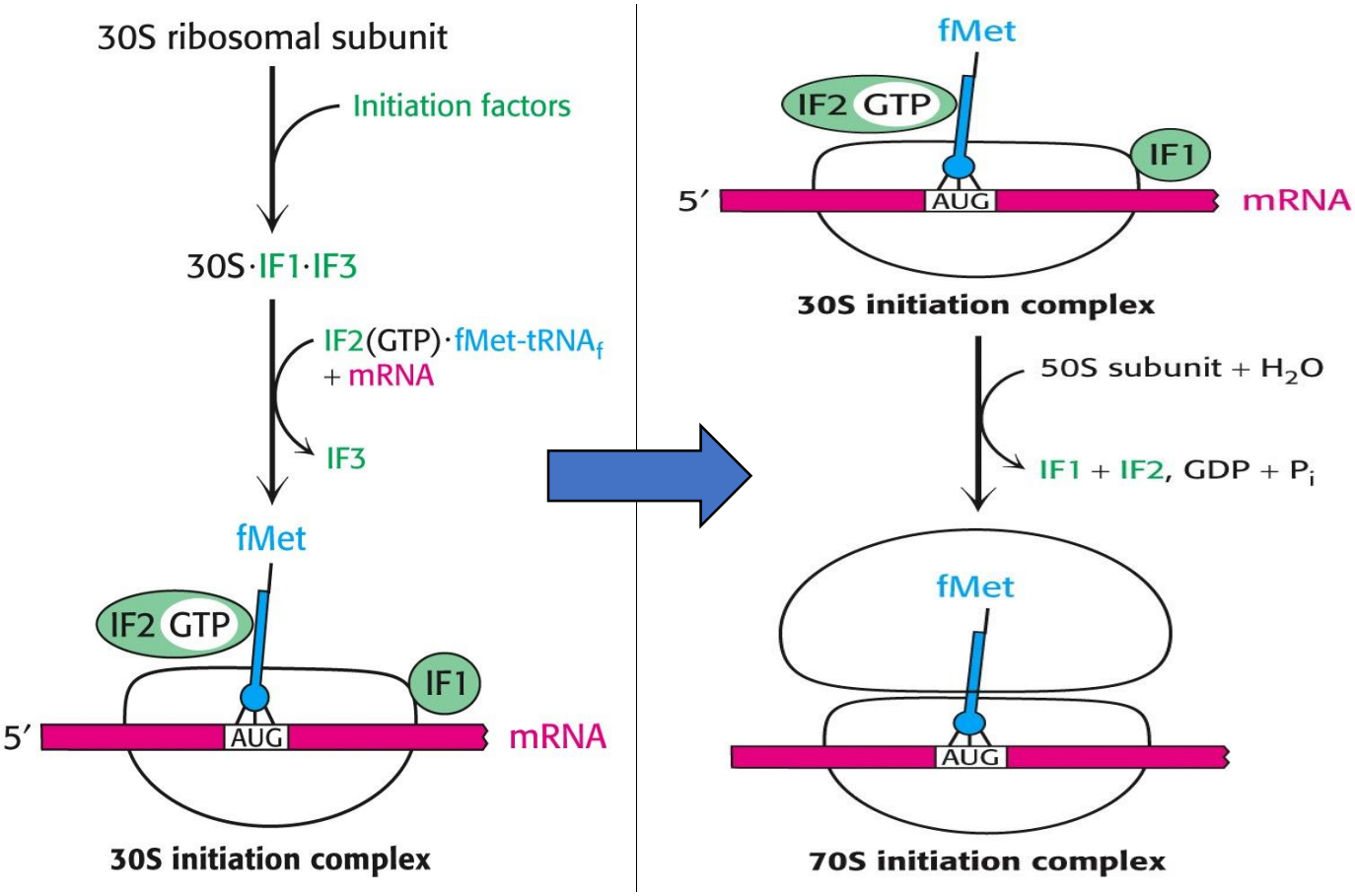
A. Binding of the ribosome 30S subunit with initiation factor (IF3) ➔ promotes the dissociation of the ribosome into its two components subunits.
B. The presence of IF3 permits the assembly of the initiation complex and prevents binding of the 50S subunit prematurely, IF1 assists IF3 in some way, perhaps by increasing the dissociation rate of the 30S and 50S subunits of the ribosome
C. Binding of the mRNA and the fMet-tRNA$_f^{Met}$
   - ❖ IF3 assists the mRNA to bind with the 30S subunit of the ribosome so that the start codon is correctly positioned at the peptidyl site of the ribosome.
   - ❖ The mRNA is positioned by means of base-pairing between the 3' end of the 16S rRNA with the Shine- Dalgarno sequence immediately upstream of the start codon.
   - ❖ IF2(GTP) assists the fMet-tRNA$_f^{Met}$ to bind to the 30S subunit in the correct site - the P site.
   - ❖ At this stage of assembly, the 30S initiation complex is complete and IF3 can dissociate.
D. Binding of the ribosome 50S subunit and release of Initiation Factors
   Three events now happen "simultaneously".
   - ❖ As the 50S subunit of the ribosome associates with the 30S initiation complex, GTP hydrolysis occurs on IF2.
   - ❖ The energy released from the hydrolysis of GTP:
     1. Dissociate the initiation factors
     2. Associate the large subunit
   - ❖ This hydrolysis may be helped by the L7/L12 ribosomal proteins rather than by IF2 itself.

In addition to the APE sites there is an **mRNA binding groove** that holds onto the message being translated

- **The A site** binds an aminoacyl-tRNA (a tRNA bound to an amino acid);
- **The P site** binds a peptidyl-tRNA (a tRNA bound to the peptide being synthesized).
- **The E site** binds a free tRNA before it exits the ribosome.



The A and P sites are located on both subunits but the E subunit is located only on the large subunit



- **<u>Eukaryotes</u>** use a scanning mechanism to initiate translation.
- Recognition of the AUG triggers GTP hydrolysis by eIF-2
- GTP hydrolysis by eIF2 is a signal for binding of the large subunit and beginning of translation

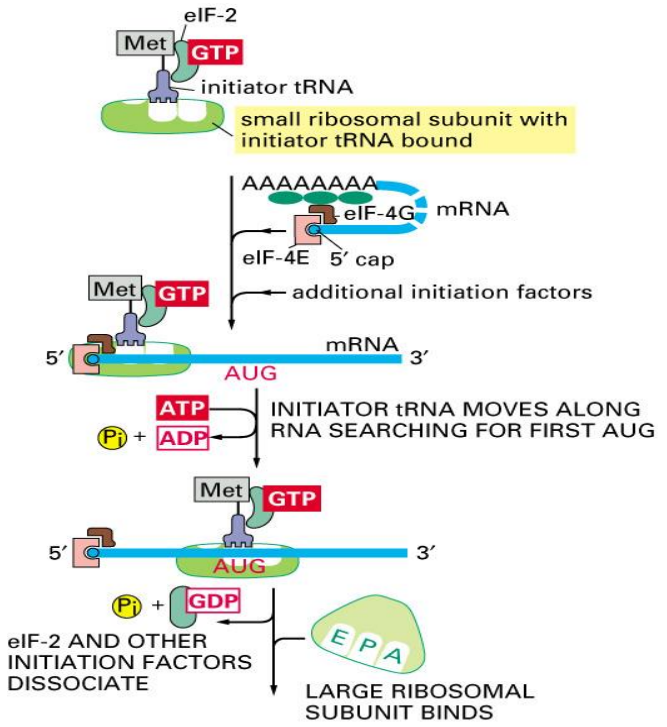


Figure 6–71 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

<u>Elongation</u>

- Three special Elongation Factors are required for this phase of protein synthesis:

EF- Tu (GTP), EF-Ts and EF-G (GTP).

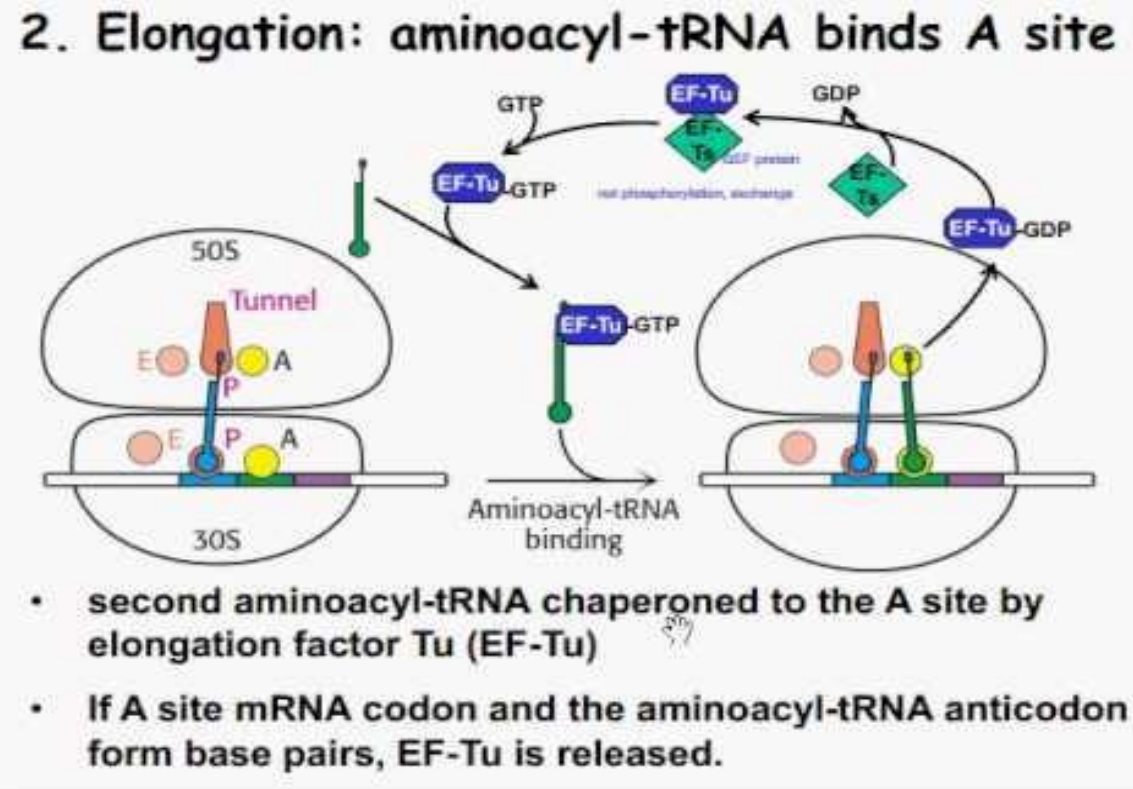
- The Elongation phase of protein synthesis consists of <u>a cyclic process</u> where by a new aminoacyl-tRNA is positioned in the ribosome, the amino acid is transferred to the C-terminus of the growing polypeptide chain, and the whole assembly moves one position along the ribosome.
- A new codon is now positioned at the A site and awaits a new aminoacyl-tRNA.
- Binding of a new aminoacyl-tRNA at the A site

<u>At the start of each cycle:</u>

1. The A (aminoacyl) site on the ribosome is empty
2. The P (peptidyl) site contains a peptidyl-tRNA, and the E (exit) site contains an uncharged tRNA.
3. The elongation factor, EF- Tu (GTP) binds with an aminoacyl-tRNA and brings it to the ribosome.
4. Once the correct aminoacyl-tRNA is positioned in the ribosome, GTP is hydrolyzed, EF- Tu (GDP) undergoes a conformational change and then dissociates away from the ribosome.

There are two ways that EF- Tu functions to ensure that the correct aminoacyl-tRNA is in place:

1. EF- Tu prevents the aminoacyl end of the charged tRNA from entering the A site on the ribosome.
   - This ensures that codon- anticodon pairing is checked first before the charged tRNA is irreversibly bound in the A site and a new, potentially incorrect, peptide bond is made.
2. GTP hydrolysis is SLOW and EF- Tu cannot dissociate from the ribosome until it occurs.
   - The amount of time prior to GTP hydrolysis allows the final fidelity check to take place.
   - Hydrolysis is associated with a conformational change in EF-Tu.



Termination

- The final phase of protein synthesis requires that the finished polypeptide chain be detached from a tRNA.
- This can only happen in response to the signal that a stop codon has (UAA, UAG, UGA) been reached.

Binding of Release factors

- There are no tRNAs that recognize the stop codons.
- Rather stop codons are recognized by release factor RF1 (which recognizes the UAA and UAG stop codons) or RF2 (which recognizes the UAA and UGA stop codons).
- These release factors act at the A site of the ribosome.
- A third release factor, RF3 (GTP), stimulates the binding of RF1 and RF2.

Hydrolysis of the peptidyl-tRNA

- Binding of the release factors alters the peptidyl transferase activity with a nucleophilic effect.
- The result is hydrolysis of the peptidyl-tRNA and release of the completed polypeptide chain.
- The uncharged tRNA in the E site can dissociate as can the release factors.
- GTP is hydrolyzed without dissociating tRNA in the P site

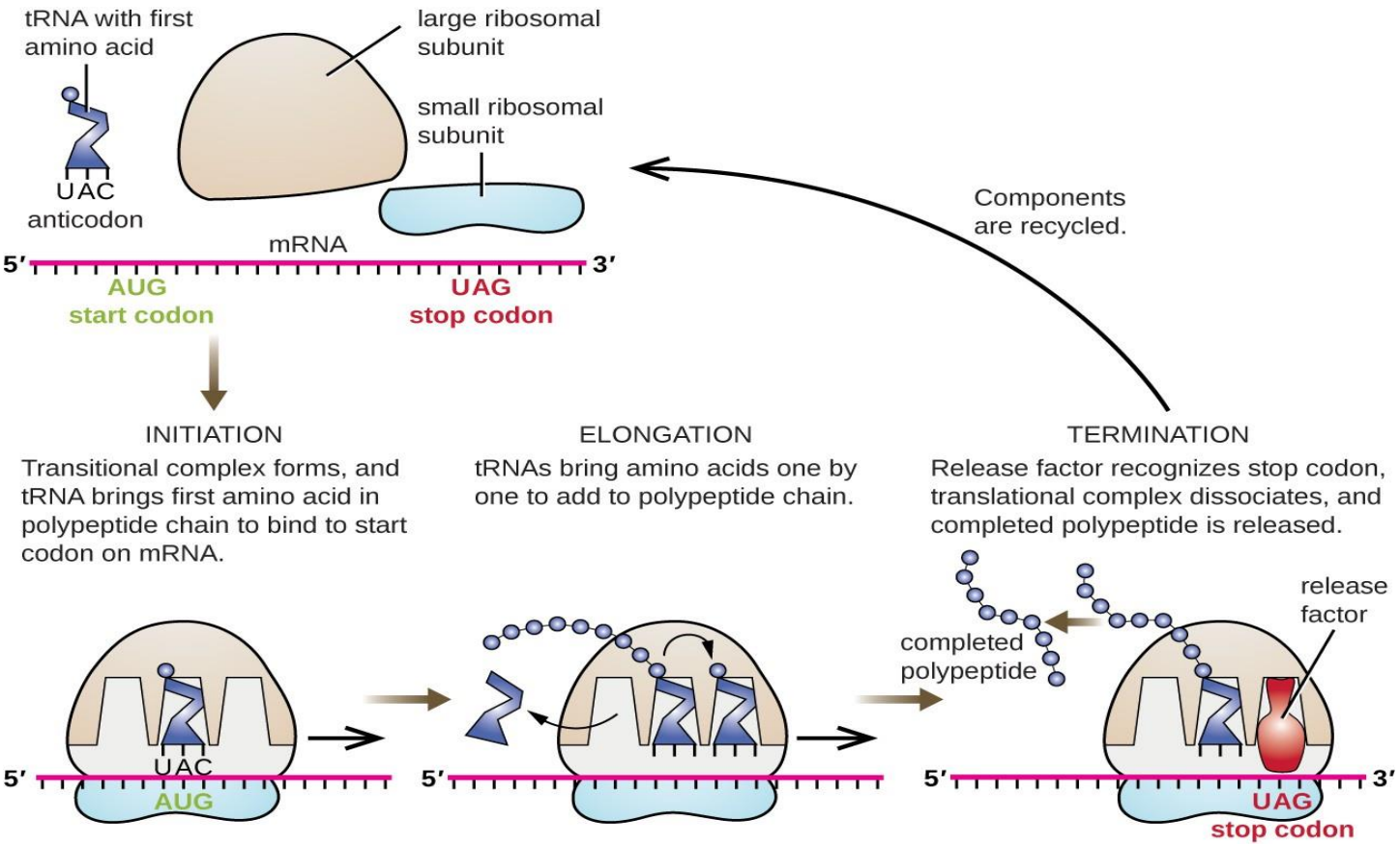


TABLE 29.4 Antibiotic inhibitors of protein synthesis

Antibiotic	Action
Streptomycin and other aminoglycosides	Inhibit initiation and cause misreading of mRNA (prokaryotes)
Tetracycline	Binds to the 30S subunit and inhibits binding of aminoacyl-tRNAs (prokaryotes)
Chloramphenicol	Inhibits the peptidyl transferase activity of the 50S ribosomal subunit (prokaryotes)
Cycloheximide	Inhibits the peptidyl transferase activity of the 60S ribosomal subunit (eukaryotes)
Erythromycin	Binds to the 50S subunit and inhibits translocation (prokaryotes)
Puromycin	Causes premature chain termination by acting as an analog of aminoacyl-tRNA (prokaryotes and eukaryotes)

Diphtheria toxin **Inhibits eEF-2 by ADP-ribosylation of modified histidine in the factor**

Antibiotics inhibiting translation:

The bacterial ribosomal structure and the accessory functions differ in many respects from its eukaryotic equivalent. The translation reaction itself can be subdivided into three parts:

1.Formation of the initiation complex, blocked by Streptomycin and Tetracyclins (the latter inhibiting binding of aminoacyl-tRNA to the ribosomal A- site at the 30S ribosomal subunit.
2.Introduction of aminoacyl-tRNA and synthesis of a peptide bond, inhibited by puromycin (leading to premature termination) and chloramphenicol (probably inhibiting the peptidyltransferase).
3.Translocation of the mRNA relative to the ribosome blocked by erythromycin and fusidic acid (the latter preventing release of EF-G/GDP.

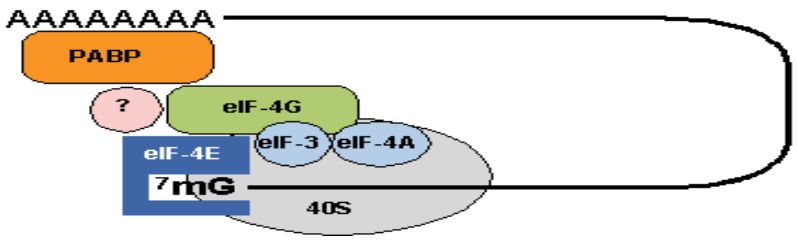
Protein synthesis in eukaryotes

- A major difference between eukaryotes and prokaryotes is that, in a typical eukaryotic cell, protein synthesis takes place in the cytoplasm while transcription and RNA processing take place in the nucleus.
- In bacteria, these two processes can be coupled so that protein synthesis can start even before transcription has finished.
- The steps of protein synthesis are basically the same in eukaryotic cells as in prokaryotes.
- The ingredients, however, can be different:

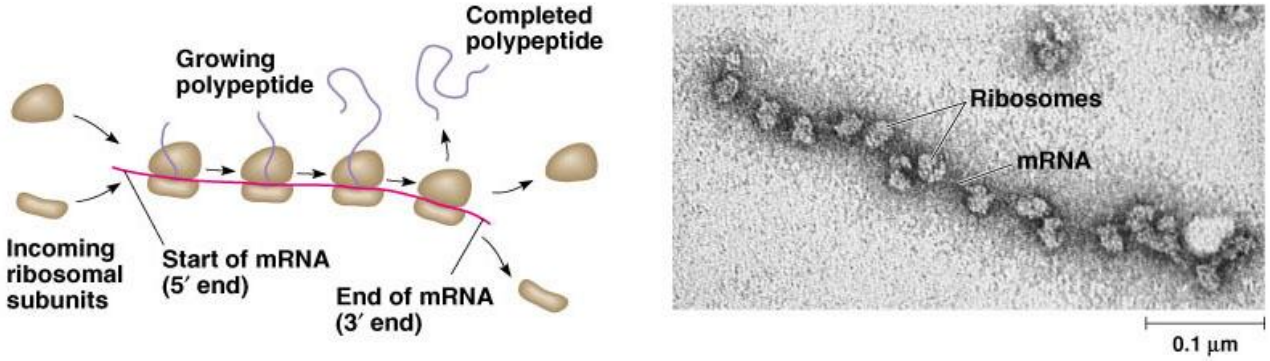
1- Ribosomes are larger. 60s and 40s subunits combine to give 80s ribosomes which contain 4 RNAs: 28S,5.8S and 5S in the 60S subunit; 18S in the 40S subunit.
2- While the initiating amino acid in eukaryotic protein synthesis is still methionine, it is not formylated.
3- Eukaryotic mRNA is capped. This is used as the recognition feature for ribosome binding -- not the 18S rRNA.
4- The initiation phase of protein synthesis requires over 10 eukaryotic initiation factors (eIFs) one of which the cap binding protein.
5- The eukaryotic elongation phase closely resembles that in prokaryotes. - The corresponding elongation factors are eEF-1a (EF-Tu), eEF-1bg (EF-Ts) and eEF-2 (EF-G).
6- Eukaryotes require just a single release factor, eRF.

Coordinating protein synthesis with mRNA synthesis

- It has recently been found that the eukaryotic initiation factor eEF- 4G binds not only with other factors in the initiation complex but also with PABP (poly A binding protein) which binds to the poly A tail of mRNA. and this to ensure that the translation will start from the 5' to 3'
- It is thought that the binding of eEF-4G to PABP serves as a critical recruitment step for driving downstream translation.
- In another sense, however, the binding of eEF-4G to PABP represents a mechanism to ensure that only mature intact mRNAs are translated.



- Most mRNA are translated by more than one ribosome at a time; the result, a structure in which many ribosomes translate a mRNA in tandem, is called a polysomes.



(a) An mRNA molecule is generally translated simultaneously by several ribosomes in clusters called polyribosomes.

(b) This micrograph shows a large polyribosome in a prokaryotic cell (TEM).

Post-translational modifications:

- They are the chemical modifications of a protein after its translation Characterized by:
  1- Being numerous and diverse
  2- Able to change the charge, conformation or size of protein molecule Effects:الاهداف
  1. Stability of protein
  2. Biochemical activity (activity regulation)
  3. Protein targeting (protein localization)
  4. Protein signaling (protein - protein interaction).

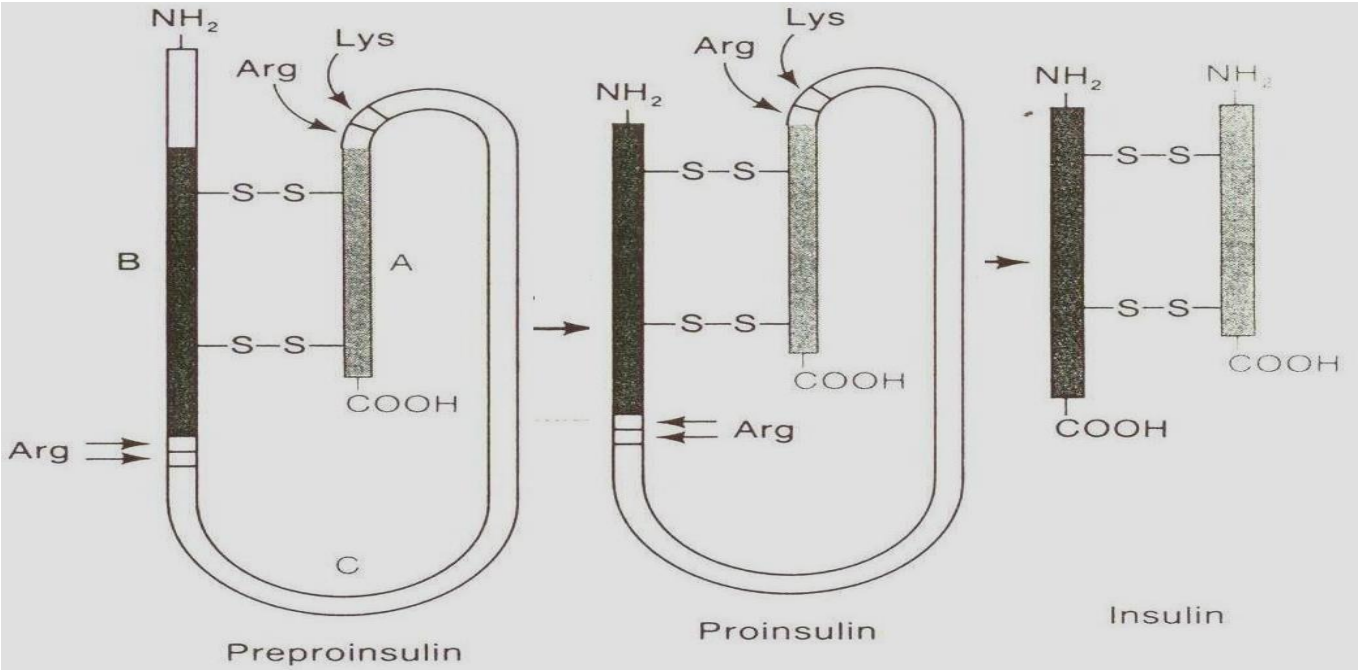


Figure 20-22
Conversion *in vivo* of preproinsulin to proinsulin and then to insulin.

1- the preproinsulin=109 amino acids and it's modified to be the active insulin = 51 amino acids in two steps, first one ,happens by signal peptidase that remove 23 amino acids from the amino terminal end, in the lumen of the endoplasmic reticulum resulting proinsulin=86 amino acids which will be stored ,the second step , happens by endoprotease that cut 35 amino acids in the form of c resulting two chains of polypeptide having 20,31 amino acids which are conceted to each other by interchain and intrachain disulfide bridges. In other words …

Insulin, which is a low-molecular-weight protein having two polypeptide chains which fold to allow interchain and intrachain disulfide bridges. A specific protease then clips out the segment that connects the two chains which form the functional insulin molecule, where a propeptide is removed from the middle of the chain; the resulting protein consists of two polypeptide chains connected by disulfide bonds.

2- Also, most nascent polypeptides, the initial methionine is usually taken off during post-translational modification by specific aminopeptidases.
3- Other modifications, like phosphorylation, are part of common mechanisms for controlling the behavior of a protein, for instance activating or inactivating an enzyme.
4- Some animal viruses, as poliovirus and hepatitis A virus, synthesize long polycistronic proteins from one long mRNA molecule, these proteins must be cleaved at specific sites to provide the several specific proteins required for viral function.
5- Collagen, an abundant protein in the extracellular spaces of higher eukaryotes, is synthesized as procollagen three polypeptide molecules, that align themselves in a particular way that is dependent upon the existence of specific amino terminal peptides.
6- Specific enzymes then carry out hydroxylations and oxidations of specific amino acid residues within the procollagen molecules to provide cross-links for greater stability with cleavage of the NH2 terminal end to form a strong, insoluble collagen molecule.

Types of posttranslational modifications:

A- Trimming:

- Many proteins secretion from the cell are made as: large precursor molecules but functionally inactive
- Change of protein from non-active for active molecule by removing portions of the protein chain (signal peptide, inhibitor sequences) by specialized endoproteases

Sites of the cleavage reaction:

- Endoplasmic reticulum
- Golgi apparatus
- Secretory vesicles

- N.B. zymogens which are inactive enzymes, become activated through cleavage when they reach their proper sites of action.

B- Covalent alterations:

- Proteins may be activated or inactivated by the covalent attachment of a variety of chemical groups

1- Phosphorylation :

- By adding phosphate group to the hydroxyl groups of (serine, threonine, tyrosine residues in a protein) which is catalyzed by protein kinases and reversed by protein phosphatases
- The phosphorylation may ↓ or ↑ the functional activity of protein

2- Glycosylation:

- Many of proteins → become part of a plasma membrane, lysosomes or secreted from the cell have **carbohydrate** chains attached to serine or threonine hydroxyl groups (O-linked) or the amide nitrogen of asparagine (N-linked)
- Occurs: in the endoplasmic reticulum and golgi apparatus
- Used to : target protein to specific organelles (receptors on the plasma membrane)

3- Hydroxylation :
As proline and lysine in endoplasmic reticulum by prolyl or lysyl hydroxylases (e.g. in collagen).

4- Other covalent modifications :
a-Carboxylation:
- Carboxyl groups can be added to glutamate residues by vitamin K
- The resulting carboxyglutamate residues are essential for the activity of several of the blood-clotting proteins

b- Biotinylated enzyme:
- Biotin is covalently bound to the amino groups of lysine residues of biotin-dependent enzymes that catalyze carboxylation reactions
- Such as: pyruvate carboxylase

c- Farnesylated protein:
- Help anchor proteins in membranes.
- Note : many proteins are acetylated.

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أاهم معلومه الى اللقاء الى العام القادم الفصل الثاني