Genetics & molecular biology

◉ Sheet ○ Slide

Number:	14
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In this lecture, we will be discussing the last step in protein synthesis: Translation.

General Information

After DNA is transcribed into an mRNA molecule "Transcription", the mRNA is translated into amino acid sequences building up a protein "Translation".

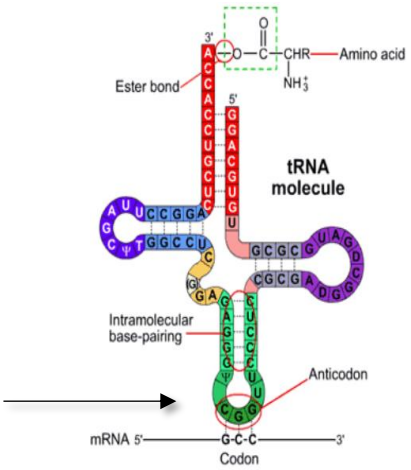
Translation involves interactions between 3 types of RNA molecules:

1- **tRNA**, carrying the amino acids.
2- **rRNA**, as a part of ribosomes.
3- **mRNA**, as a template for translation.

> ***Note:*** *The ribosome is made up of* ***60% rRNA*** *and* ***40% protein*** *by weight.*

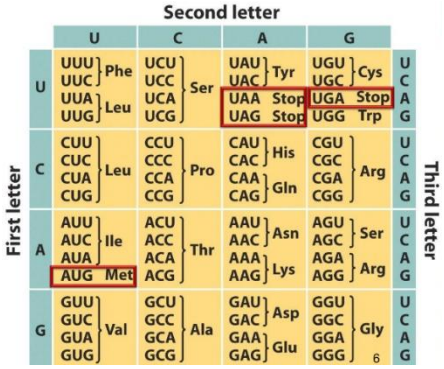
tRNA Structure

- tRNAs are **small, non-coding, RNA** molecules composed of, typically, **80 bases** long. They have the **same** bases present in mRNA, in addition to **inosine** sometimes.
- On the **3' end** of the tRNA, there is a consensus sequence of **CCA**. An amino acid binds **covalently** (ester bond) to the **adenosine** "A" of that sequence, catalyzed by an **Aminoacyl-tRNA synthetase** producing a '**charged/activated**' tRNA molecule.
- For every amino acid, there is a **special** aminoacyl-tRNA synthetase. Thus, there are **20** different aminoacyl-tRNA synthetases.
- Another region on the tRNA is called the **anti-codon region,** which is what determines which amino acid binds to the tRNA. It is **complementary** to the codon on the **mRNA** being translated binding **anti-parallelly** to it.



Codons

- Every **three nucleotides** in mRNA make up **one codon** which codes for a certain **amino acid**. Multiple codons, usually, code for **one** amino acid except for **Methionine** which is coded by **only one** codon.
- In mRNA, we have **64** possible codons (4^3), as shown in this picture to the right.



The codons are not for memorization, the doctor said he will bring the table if we needed it, just learn the following:

1- **AUG:** It is a **start codon** which codes for **Methionine**. This implies that most peptide chains, typically, **start** with **Methionine**.

2- **UAA, UAG, UGA:** They are **stop codons** that do **not** code for any amino acid having **no specific** anti-codons, **terminating** the translation process.

Therefore, we have **61 different tRNA molecules** *(64 - 3 stop codons)* each with a **specific anticodon**. Some organisms have **fewer** than 45 tRNA molecules, therefore, **one tRNA** needs to pair with **more** than one codon *(explained how later in wobble base pairing).*

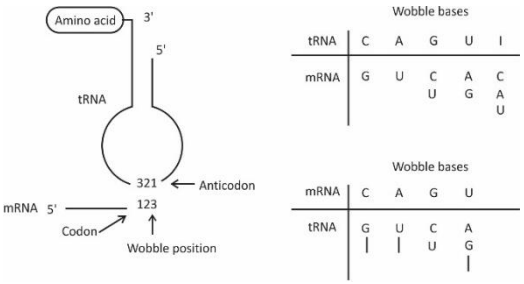
Universality of Codons

- Codons are usually **universal**, meaning that a **code** can be **transcribed** and **translated** into the **same** amino acids in many **different** organisms.
- However, they are **not** always universal. This means that a codon can **differ** between prokaryotes and eukaryotes, the **same organism** can also contain 2 **different** genetic codes.

  For example, **AUA** in the **mitochondrial** genome codes for **Methionine** whereas in the **genomic DNA** it codes for **Isoleucine**.

Wobble Base Pairing

- The **first two bases** in a codon on the mRNA are usually what create the **specificity** for a certain amino acid-charged tRNA, forming strong bonds with the **anticodon** on the **tRNA**.
- However, the **3rd** base usually has a '**relaxed**' bond with the **anticodon** of the **tRNA** *(with the 1st base)* making it **degenerative**/**non-standard**; meaning that it can **avoid** standard base-pairings.
- If the **1st** base on the anticodon is a **C or an A**, pairing is **specific**. However, if the 1st base is **U or G**, the pairing is **less** specific and more flexible and can be **interchangeably** recognized. If the 1st base is **inositol**, it can pair with A, C, or U.



This degeneracy can be useful by:

1- Acting as a **buffer against mutations**. So that if there is a mutation in the wobble base, it **won't** change the amino acid translated.

2- Our bodies have a **limited** amount of tRNAs and the wobble phenomenon **decreases** the required number of different tRNAs.

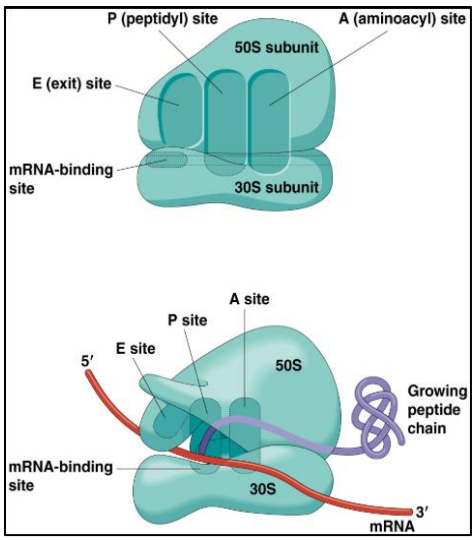
> ***Note:*** *The amino acid attached to the tRNA is specified not only by the **anticodon**, but also by the **identifier sequence** (we are not required to know it in details yet).*

Ribosomes

Ribosomes are **sites of protein synthesis** in both prokaryotic and eukaryotic cells. E. coli contain about 20,000 ribosomes, which account, approximately, for 25% of the dry weight of the cell. While rapidly growing mammalian cells contain about 10 million ribosomes.

They consist of **proteins** and **rRNA**. The function of the proteins is to maintain the **structure**, while the rRNA has **enzymatic activity**.

Ribosomes Structure



Each ribosome is formed of **two** subunits:

1- **Small subunit** *(40s in Eukaryotes, 30s in Prokaryotes):* It is **binds** to the **mRNA** strand.

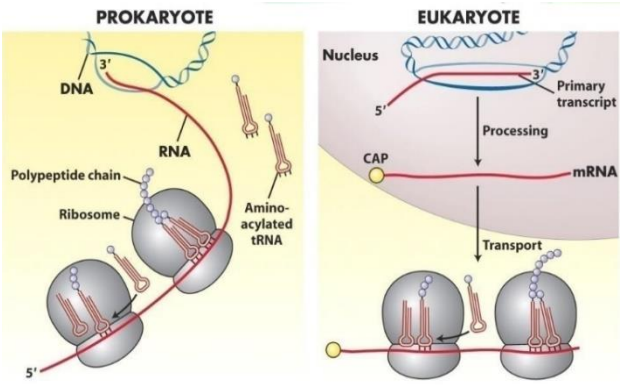
2- **Large subunit** *(60s in Eukaryotes, 50s in Prokaryotes)*: It contains the site of catalysis, the **peptidyl transferase** centre, which forms peptides bond elongating the polypeptide (rRNA portion).

The large subunit has **three chambers**:

1- **A-site,** where the **amino-acyl tRNA binds**.
2- **P-site,** where the **peptidyl transferase** activity takes place.
3- **E-site,** where the **tRNA exits** the ribosome.

Transcription/Translation coupling

- In the ribosome, the **mRNA** is read from the **5' to the 3'** end.
- This **directionality** is important enabling the **coupling** *(in place and time)* of both transcription and translation in **prokaryotes**.
- However, this coupling is not found in the **eukaryotes** because mRNA is **not** transported into the cytosol *(where the translation occurs)* through the **nuclear membrane** before undergoing **post-transcriptional modifications** (capping, splicing and polyadenylation).



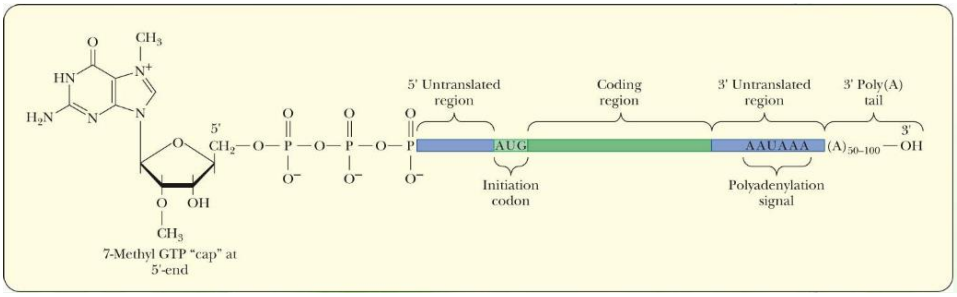
Translation

Translation involves **3 stages**: Initiation, elongation and termination.

In **eukaryotes**, the first amino acid to be added in translation is **Methionine** (AUG). While in **prokaryotes** it is **N-formylmethionine.**

1- Initiation of Translation

In **both** prokaryotes and eukaryotes, translation is initiated at a **specific site** (start codon, typically AUG) located upstream, before, the **first codon** in the **coding region** on the **mRNA**.

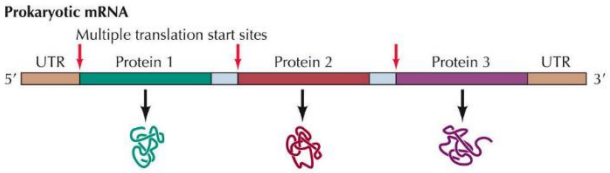


There are **2 untranslated regions** containing **non-coding** sequences on the mRNA of **both** prokaryotes and eukaryotes. One is the **5' terminal** upstream from the initiation sites, referred to as **5' untranslated region** (UTR) and the other is downstream at the **3' terminal**, after the stop codon, referred to as **3' untranslated region.**

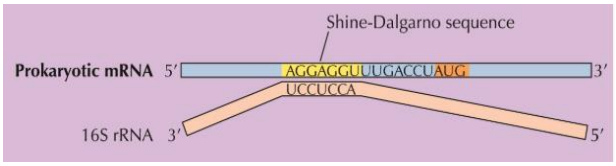
A- In Prokaryotes

- Recall that the **prokaryotic** mRNA is **polycistronic**; meaning that it encodes for **more** than **one** polypeptide within the **same mRNA**.

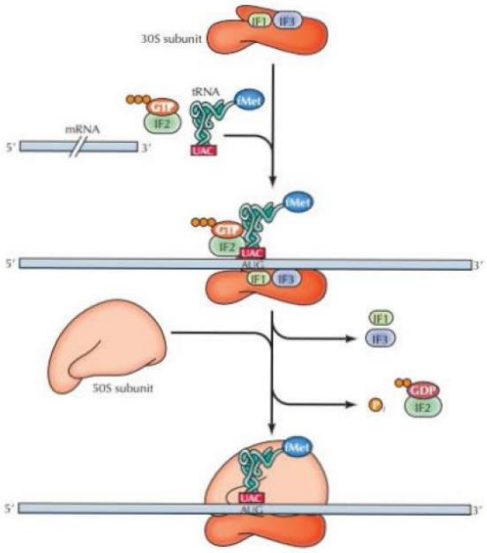
  Before each protein, there is a sequence upstream from the start codon called the **Shine-Dalgarno sequence;** multiple Shine-Dalgarno sequences are found within a single prokaryotic mRNA.



- The 16S rRNA of the small ribosomal subunit has a **complementary** sequence for this Shine-Dalgarno sequence. It **recognizes** it on the mRNA and **binds** to it signaling the ribosome to **scan** for the start codon (AUG) to **initiate** translation.



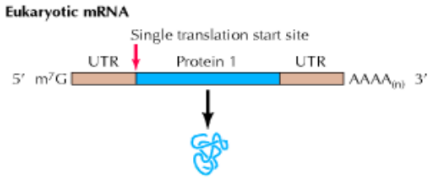
- After the binding of the **small subunit to mRNA**, a **tRNA** molecule carrying an **N-formylmethionine** binds to the **start codon** on the mRNA.
- This tRNA-mRNA-small subunit (30s) is called **the 30S initiation complex**. **GTP** is needed for the formation of this complex in addition to a set of **proteins** who are initiation factors: IF1, IF2, and IF3.
- After the formation of the 30s initiation complex, the **large subunit** (50s) **binds** to the complex having the tRNA in the **P-site**. The complex now is called the **70S initiation complex** and translation will start.



In summary: *Recognition of Shine-Dalgarno sequence → Stable binding between the 30s subunit and mRNA → Formation of the 30s initiation complex → The large subunit binds to the complex → Formation of the 70s initiation complex → Translation begins at the first AUG downstream from the Shine-Dalgarno sequence.*

B- In Eukaryotes

Recall that the **eukaryotic** mRNA is **monocistronic**; meaning that the mRNA encodes only **one** polypeptide for **each** mRNA molecule.

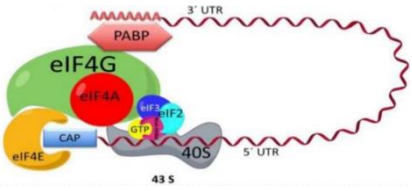


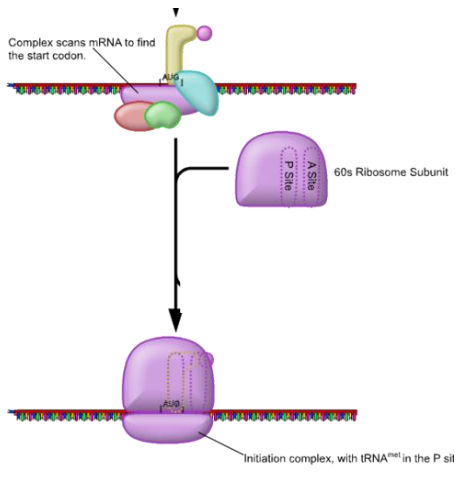
Cap-dependent initiation

- There is **no** Shine-Dalgarno sequence in **eukaryotes** and another process takes place. Important initiation factors of translation in eukaryotes form a **protein complex including** eIF4G, eIF4E and poly-A binding protein (PABP).

"Refer to the figures"

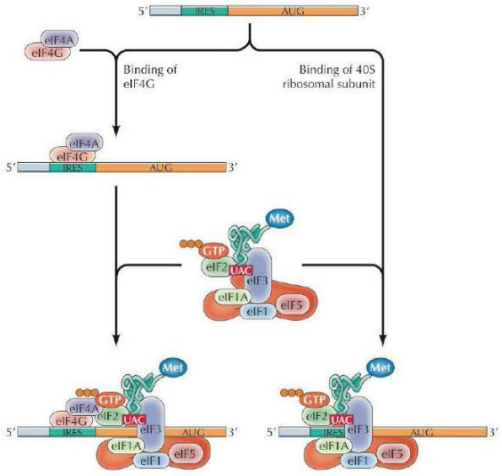
- The **eIF4E** protein binds to the **7-methylguanosine cap,** while the **poly-A binding protein** (PABP) binds to the **poly-A tail**. Then, **eIF4G** links PABP *(linked to poly A tail)* to eIF4E *(bound to the cap)* forming a **complex** at the 5' cap.
- This complex **stabilizes** the small subunit (40s) on the mRNA which then moves along the mRNA '**scanning**' for the **start** codon (AUG).
- Once it finds the start codon (AUG), the large subunit (60s) is **attached** commencing **translation** elongation.





Cap-independent initiation

- In this process, translation **does not require** the 5' cap to **initiate scanning** for the start codon.
- The start codon is **preceded** by a sequence called **Internal Ribosome Entry Site** (IRES).
- The IRES is **recognized** by either the **small subunit** (40s) **directly**, or **indirectly** by the **eIF4G** protein followed by **recruitment** of the small subunit.



From the previous points, we can conclude how **post-transcriptional modification** (capping and polyadenylation) **aid** in translation:

1- **Caps** are recognized by the small ribosomal subunit binding to the eIF4E protein initiating translation.

2- **Poly-A** binds with PABP. Then eIF4G links the poly-A tail to the cap via PABP forming a loop which stabilizes the initiation complex.

> ***Note:*** *In the **last step of initiation** where the **large subunit joins** the small subunit, this complex will have the **P-site occupied** with a charged **initiating tRNA** (typically holding Met) and the **A-site will be empty.***

Re-tracing: The 1st step of translation is initiation.

*In prokaryotes, the Shine-Dalgarno sequence is **recognized** by the **small** subunit (30s) which then moves along the mRNA **scanning** for the start codon (AUG) where the **charged** tRNA can bind to in the presence of **GTP** and initiation factors (IF2 IF3 IF1). Then the **large** subunit (50s) is added forming the 70s complex commencing polypeptide formation and elongation.*

In eukaryotes, initiation is either:

***a- Cap-dependent:** Forming a **complex of proteins** (eIF4G, eIF4E, and PABP) along with the **small subunit** (40s) at the **cap**, which then **scans** for the start codon where then the large subunit is attached **commencing** elongation.*

***b- Cap-independent:** Where an **internal ribosome entry site** (IRES) is either **directly recognized** by the **small** ribosomal subunit or recognized by the **eIF4G** protein which recruits the small subunit **indirectly**.*

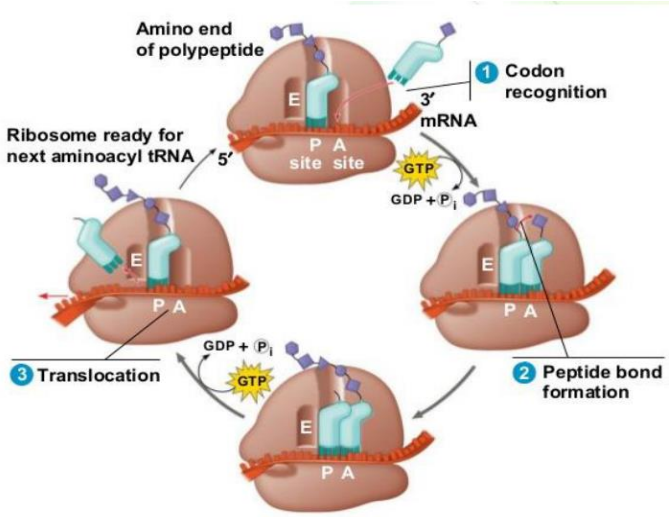
2- Elongation

- During elongation, the amino acids are added to **the C-terminus** of the polypeptide chain as the ribosome moves from the **5'- 3' end** on the mRNA, having the **N-terminus** as the **free** end.
- Recall, in **eukaryotes**, the first amino acid to be added is **Methionine** (AUG). While in **prokaryotes** it is **N-formylmethionine**.
- The elongation consists **of 3 steps** that are repeated continuously until the **stop** codon is reached inducing the **termination** of the process. These steps are:

A- Codon Recognition: Aminoacyl-tRNA, which is **charged** with a specific amino acid according to the **codon recognized**, is **delivered** to the **A-site** facilitated by an elongation factor (**EF-Tu**) requiring **GTP** hydrolysis.

The **P-site** is already **occupied** with the **initiating tRNA**.

After delivering the charged tRNA to the A-site, the **EF-Tu is released** to be **regenerated** again.



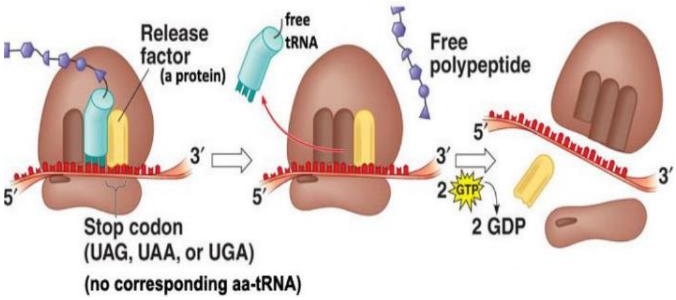
B- Peptide bond Formation: A peptide bond is formed by **peptidyl transferase** between the amino acids, having the **newly synthesized polypeptide** linked to a tRNA in the **A-site**, leaving the tRNA on the P-site **uncharged** (inactive).

C- Translocation: The ribosome then moves by **one codon** (3 nucleotides) towards the **3' end** translocating the first tRNA (the uncharged) from the **P-site to the E-site** causing the tRNA to **exit** the ribosome. The second tRNA (peptidyl-tRNA) is translocated from the **A-site to the P-site** carrying two amino acid chain, leaving an **empty A site**.

Those steps will be repeated over and over until a stop codon is reached.

3- Termination

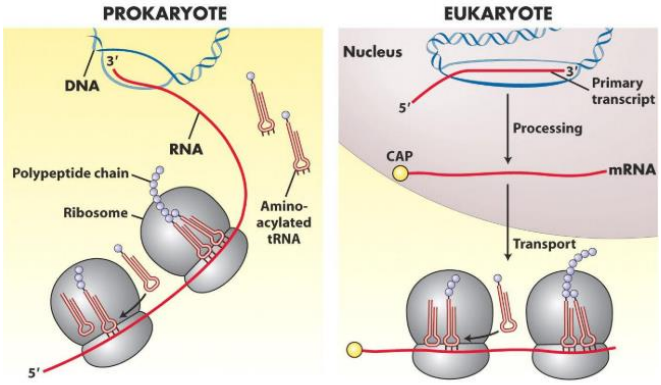
The **stop codons** (UAA, UAG, UGA) are **not** recognized by any **tRNA** molecule. Instead, they are recognized by **Release Factors**. These release factors **block** the binding of a new **aminoacyl-tRNA** and **facilitate** the **hydrolysis** of the bond between the last amino acid added of the peptide (**C-terminus**) and the **tRNA**. Then, the whole complex **dissociates**.



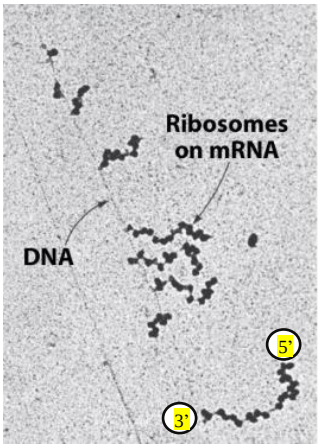
This 'short' video of 3 minutes summarizes all the steps of translation briefly, watch it:

https://www.youtube.com/watch?v=5bLEDd-PSTQ

Polyribosomes (polysomes)



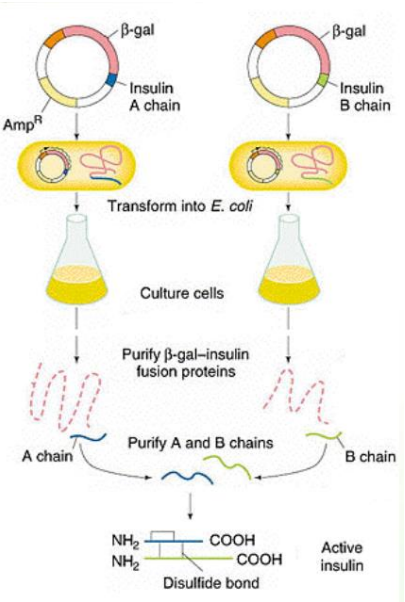
- A **single mRNA** molecule can be **translated** by **several ribosomes** simultaneously (at the same time) forming a '**polyribosome**'.
- This happens in **both** prokaryotes and eukaryotes. Do not mix this with the transcription/translation coupling process which is present in **prokaryotes** only.
- **Each ribosome** produces **one copy** of the polypeptide chain specified by the mRNA. When the protein formation **finishes**, the ribosome **dissociates** into **subunits** that are used in **other** rounds of protein synthesis.



- Recall that the ribosome reads mRNA in the **5' - 3'** direction. This means that the ribosome **near** the **3' end**, having the **longest** polypeptide formed, is the **first** ribosome that got attached and started translation from the 5' end.

Cloning

- **Cloning** is a molecular biology **technique** that makes **many identical** copies of a piece of DNA, such as a **gene**.
- Cloning is used for many purposes such as **synthesizing** eukaryotic **proteins** in **bacteria**, e.g. **insulin**. By adding the promoter along with the gene of interest into the bacterial plasmid where transcription and translation takes place forming our protein of interest.
- **Challenges:** Insulin is a **dimer** linked by **disulfide bonds** and produced from genes containing **introns** which are not found in bacteria.
- **Solution:** **Synthetic** DNA is made for **each** polypeptide, making up the insulin protein, and are **inserted** into bacteria **separately**. The bacteria are then **cultured**, producing many cells. Polypeptides are **purified** from each bacterial batch and are **mixed** to form the mature insulin protein.



Inhibitors of Translation

Some **antibiotics** work by **inhibiting** the process of translation in prokaryotes such as:

1- **Tetracycline**: Blocks binding of aminoacyl-tRNA to A-site of the ribosome.

2- **Streptomycin**: Induces binding of wrong tRNA-AA complexes causing mRNA misreading resulting in false proteins.

3- **Chloramphenicol**: Binds to the 50s subunit and inhibits the peptidyl transferase action.

4- **Erythromycin**: Binds to the 50s subunit and inhibits translocation.

In eukaryotes, **diphtheria** toxin is a protein that interferes with protein synthesis by **decreasing** the activity of the elongation factor **eEF2**.

The doctor said to pay attention to the differences between prokaryotes and eukaryotes, the following video neatly discusses certain differences, I highly recommend watching it:

https://www.khanacademy.org/test-prep/mcat/biomolecules/dna/v/differences-in-translation-between-prokaryotes-and-eukaryotes

Good Luck ♥