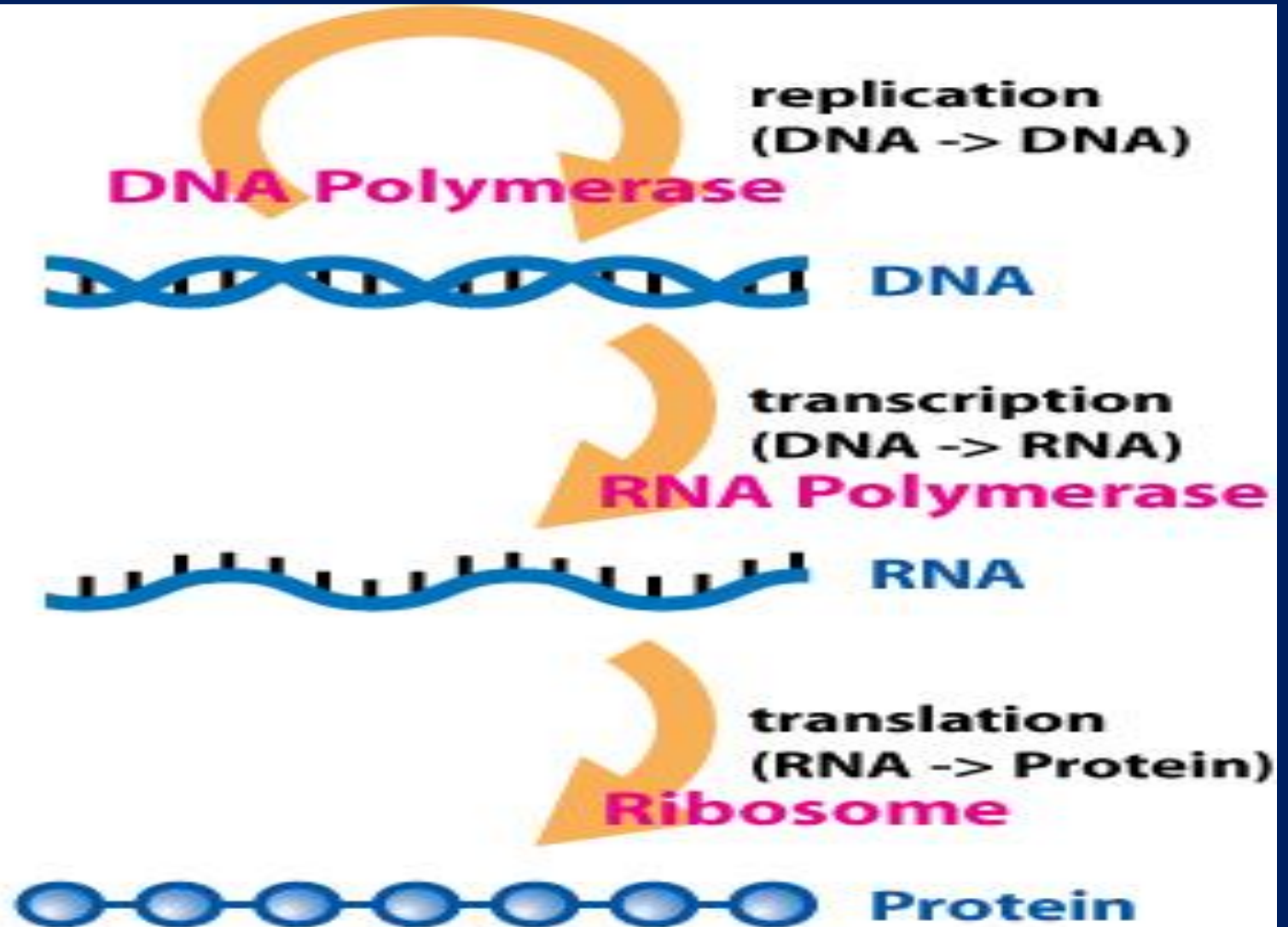

TRANSLATION

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INTRODUCTION

- The genetic information contained within the order of nucleotides in **messenger RNA(mRNA)** is interpreted to generate the linear sequence of amino acids in proteins.
- First in 1950 **Paul Zamecnik** designed a set of experiment to investigate where in the cell proteins are synthesized.
- **Mahlon Hoagland & Zamecnik** found AA were activated when incubated with ATP.

CENTRAL DOGMA



CELLULAR LOCATION

- Translation occurs in **cytosol** on ribosomes.
- In eukaryotic cells, the ribosomes are either free in the cytosol or in close association with endoplasmic reticulum (rough E.R./RER).
- **RER**- associated ribosomes are responsible for synthesizing proteins that are to be exported from the cell.

BASIC REQUIREMENTS OF TRANSLATION

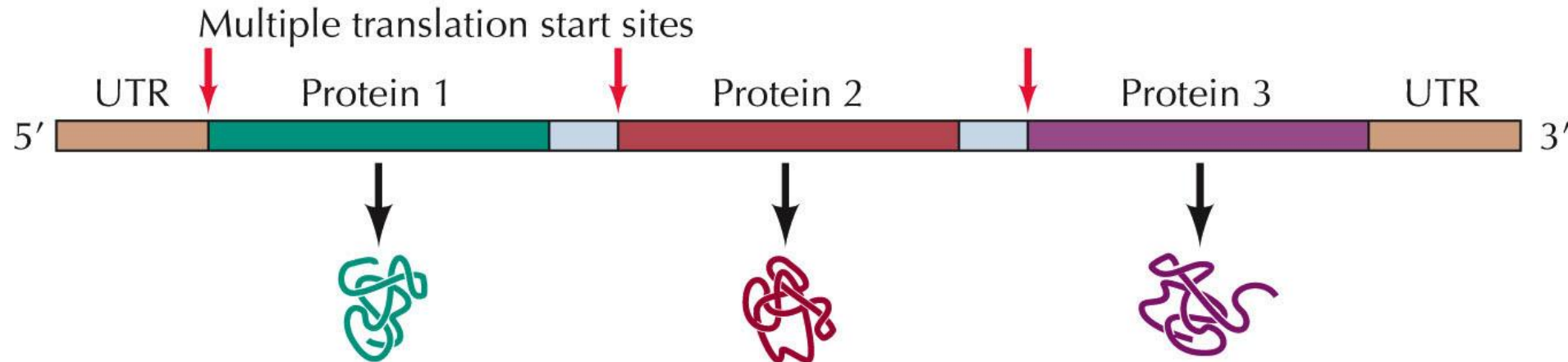
- mRNA
- tRNA
- Ribosomes
- Energy in the form of ATP & GTP.
- Protein factors

MESSENGER RNA

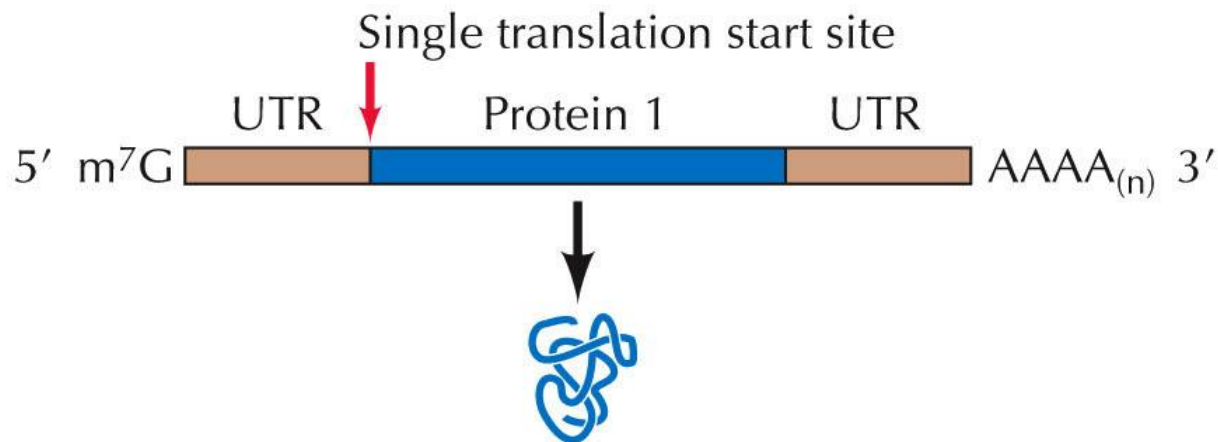
- mRNA acts as a template for protein synthesis
- mRNA is translated from 5' end to 3' end resulting polypeptide chain synthesized
- Eukaryotic mRNA is **monocistronic**
- Prokaryotic mRNA is **polycistronic**
- The initiating codon AUG is present closer to 5' end

- The protein coding regions of each mRNA is composed of **strings of codons** called an **OPEN READING FRAME (ORF)**
- Translation **starts at 5' end** of the ORF and proceeds one codon at a time to the 3' end.
- The **first** and the **last codons** of an ORF are known as **start** and **stop codons**.
- In bacteria, the start codon is usually 5'-AUG-3' but 5'-GUG-3' and 5'-UUG-3' are also used.

Prokaryotic mRNA

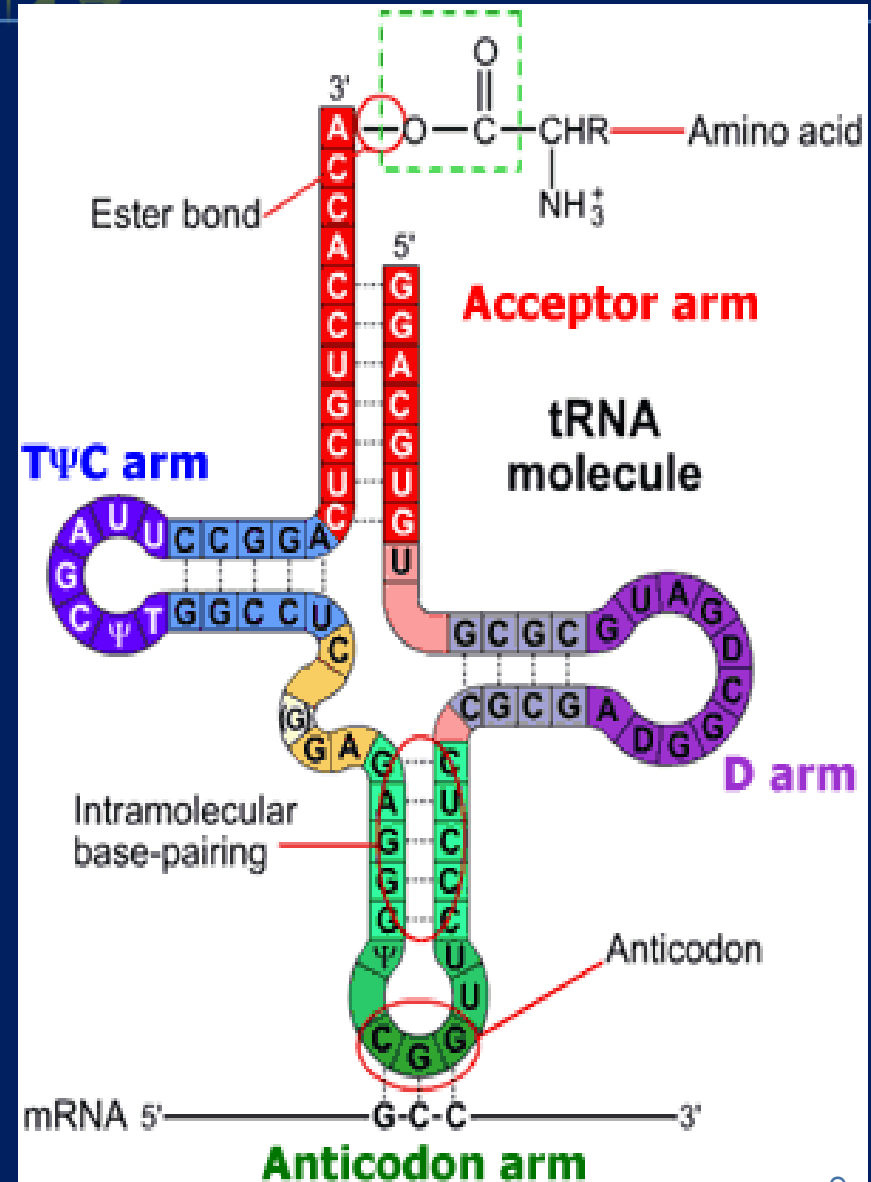


Eukaryotic mRNA



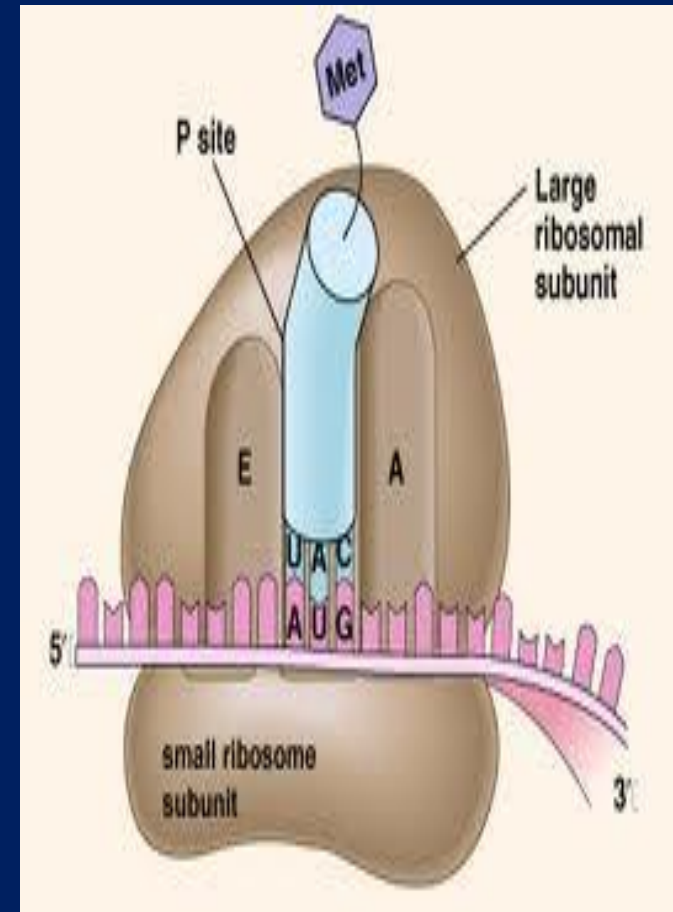
tRNA

- Act as adaptors between AA and codons.
- Many types, each attached to a specific AA and each recognizes a particular codon, or codons, in the mRNA.
- Unusual bases eg.
Pseudouridine, dihydrouridine, hypoxanthine



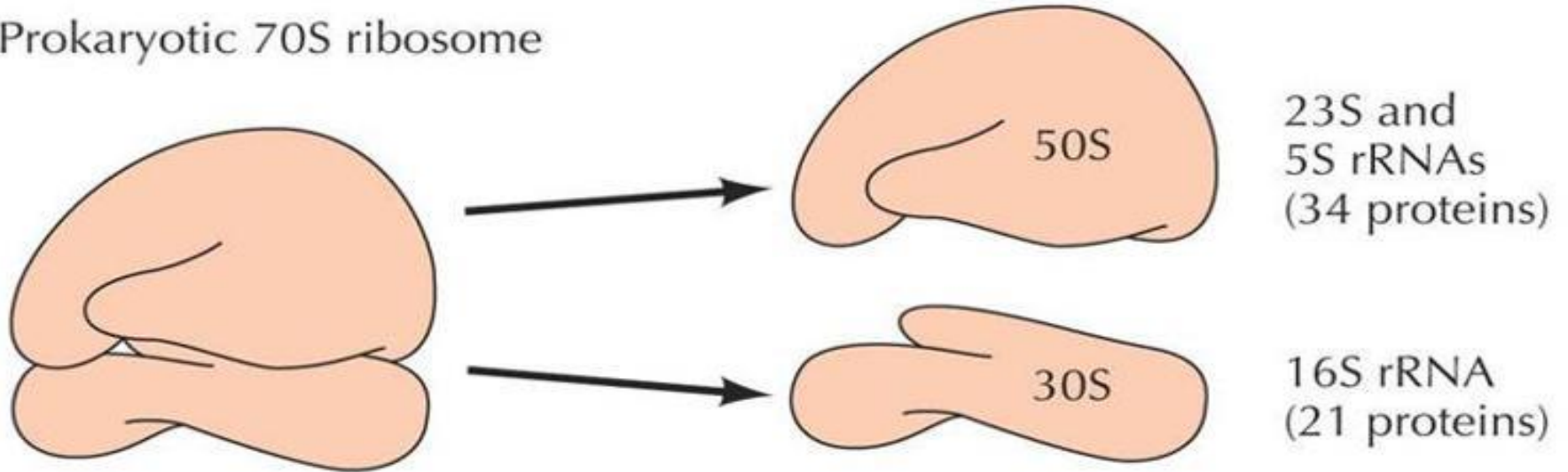
RIBOSOMES

- Molecular machines for making polypeptides chains
- Large complex of **protein and rRNA**
- Composed of 2 subunits one large and one small
- **Eukaryotic ribosomes** are 80S particles of 40S & 60S
- **Prokaryotic ribosomes** are 70S particles of 30S & 50S
- It has 3 binding sites for tRNA molecules i.e. **A (aminoacyl)** **P (peptidyl)** and **E (empty)**.

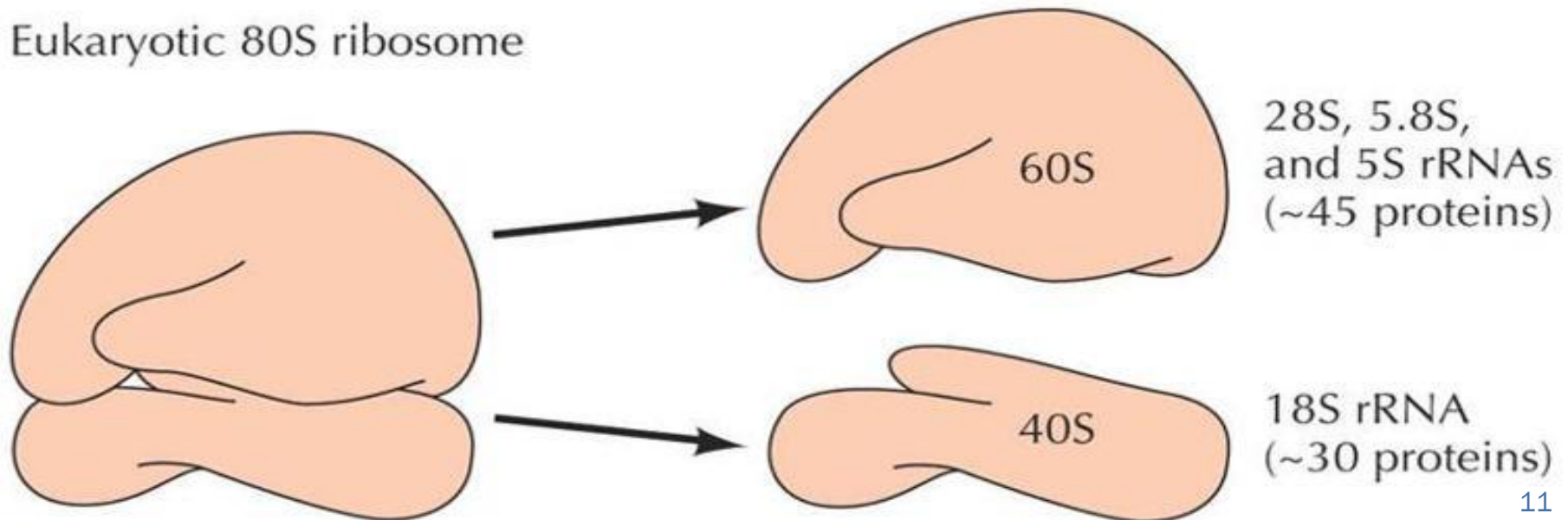


(A)

Prokaryotic 70S ribosome



Eukaryotic 80S ribosome

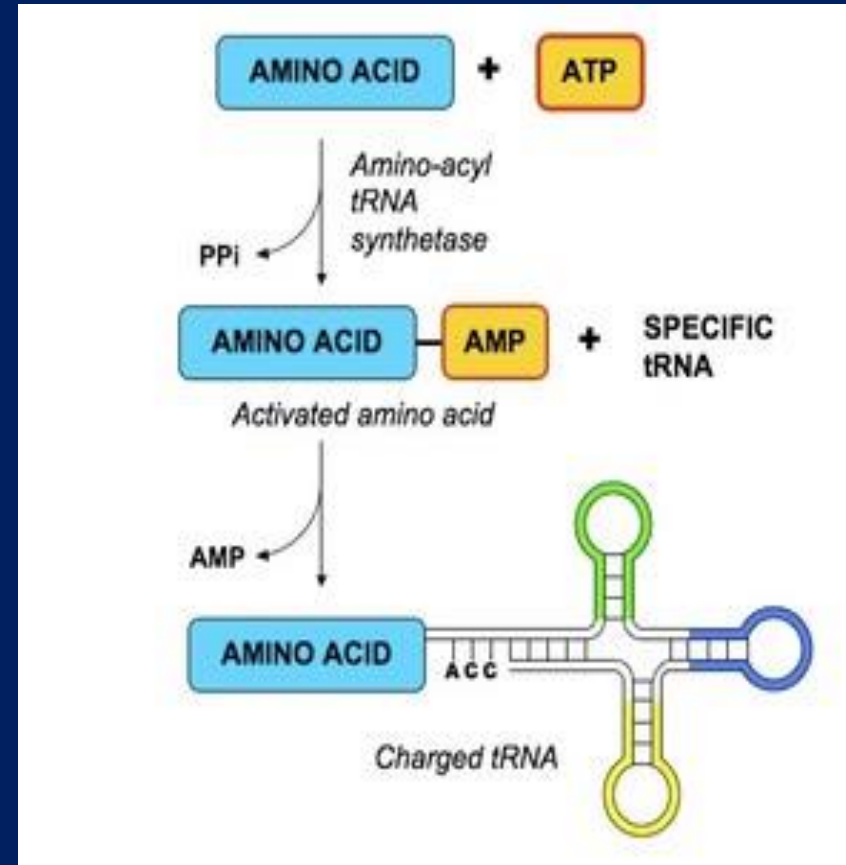


SOURCE OF ENERGY

- ATP & GTP are required as sources of energy.
- Cleavage of 4 high energy bonds is required for addition of one AA to growing PP chain.
- 2 ATPs in aminoacyl-tRNA synthetase rxn.
- 2GTPs , one for binding aminoacyl-tRNA to A site & one for translocation step.
- Additional ATP & GTP are required for initiation & termination of PP chain synthesis.

ACTIVATION OF AMINO ACIDS

- tRNA molecule + AA $\rightarrow$ charged
- 20 amino acids, is covalently attached to their respective tRNA
- After the formation of aminoacyl tRNA, latter recognizes a codon on mRNA



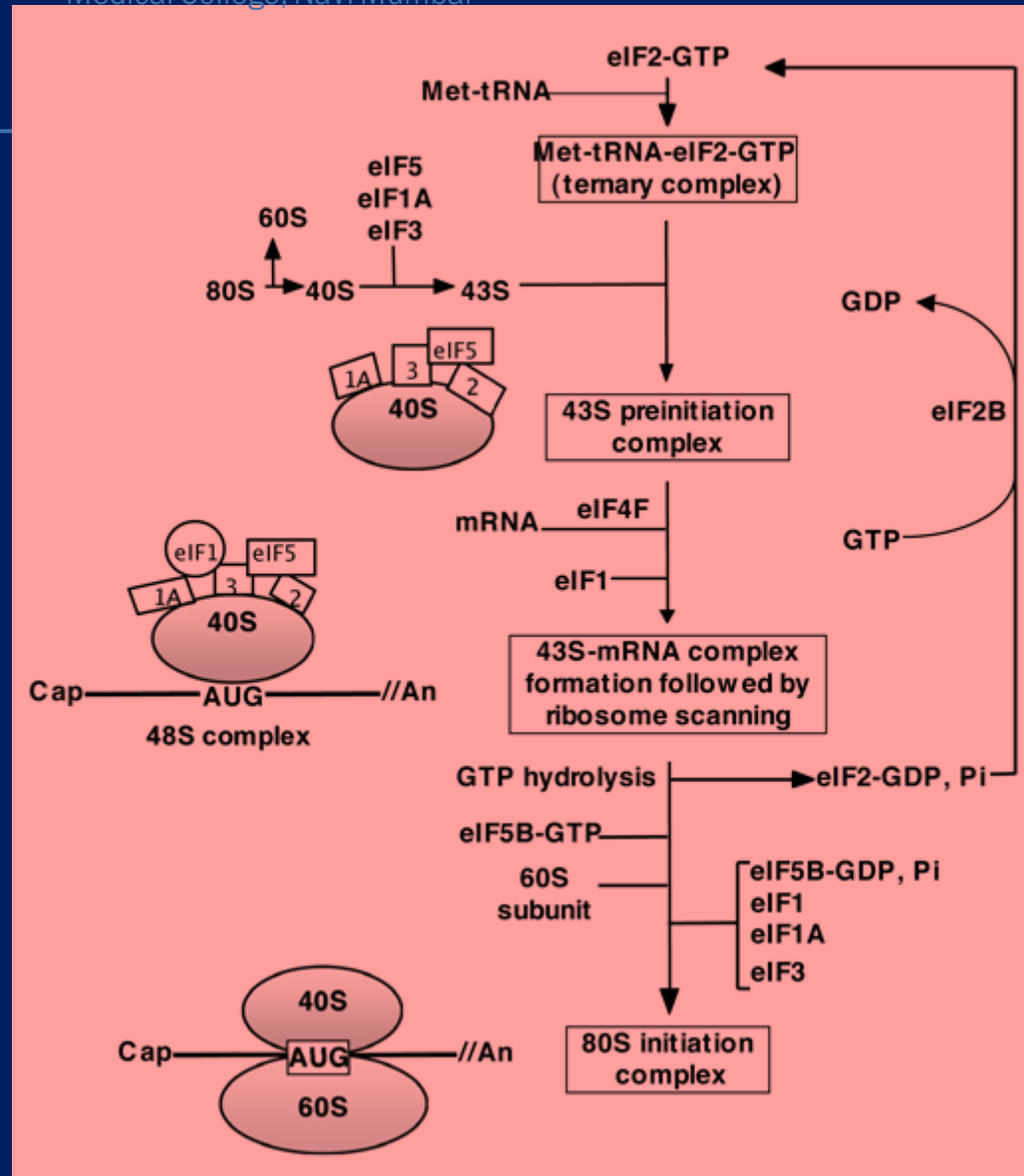
STAGES OF TRANSLATION

- **INITIATION**
- **ELONGATION**
- **TERMINATION**

INITIATION

- **Ribosomal dissociation**
 - 40S subunit binds eIF2 and eIF-1A and prevents reassociation
- **Formation of 43S pre-IC**
- **Formation of 48S IC**
 - Binding of mRNA to 43S PIC
 - eIF-4F complex is formed

*IC- Initiation complex



RECOGNITION OF INITIATION CODON

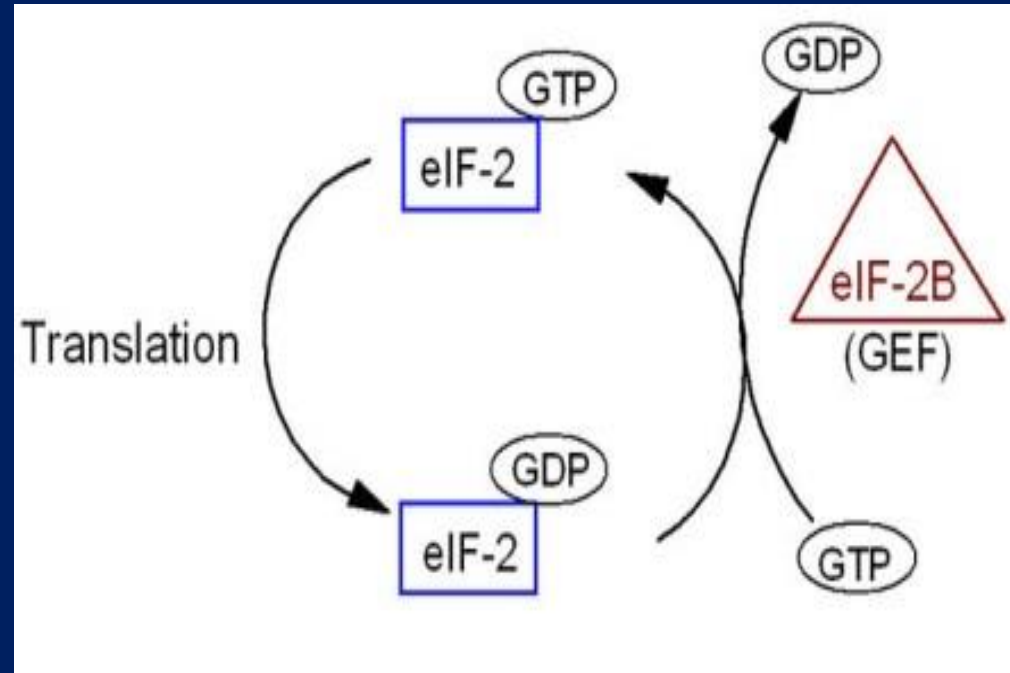
- The ribosomal complex scans the mRNA for the identification of initiation codon.
- 5'AUG is the initiation codon and its recognition is facilitated by specific sequence of nucleotides surrounding it.
- The **marker sequence** for the identification of AUG is called as **KOZAK CONSENSUS SEQUENCE**
- In **prokaryotes** the recognition sequence is called **SHINE-DALGARNO SEQUENCE**.
- **Formation of 80S IC**

Initiation Factor	Activity
eIF-1	repositioning of met-tRNA to facilitate mRNA binding
eIF-2	heterotrimeric G-protein composed of α , β , and γ subunits; formation of ternary complex consisting of eIF2-GTP + initiator methionine tRNA (met-tRNA _i ^{met}); AUG-dependent met-tRNA _i ^{met} binding to 40S ribosome
eIF-2B (also called GEF) composed of 5 subunits: α , β , γ , δ , ϵ	GTP/GDP exchange during eIF-2 recycling; genes encoding the subunits identified as EIF2B1–EIF2B5,
eIF-3, composed of 13 subunits	ribosome subunit antiassociation by binding to 40S subunit
Initiation factor complex often referred to as eIF-4F	mRNA binding to 40S subunit, ATPase-dependent RNA helicase activity, interaction between polyA tail and cap structure

PABP: polyA-binding protein	binds to the polyA tail of mRNAs and provides a link to eIF-4G
eIF-4A	ATPase-dependent RNA helicase
4E-BP (also called PHAS) 3 known forms	when de-phosphorylated 4E-BP binds eIF-4E and represses its' activity, phosphorylation of 4E-BP occurs in response to many growth stimuli leading to release of eIF-4E and increased translational initiation
eIF-4G	acts as a scaffold for the assembly of eIF-4E and -4A in the eIF-4F complex, interaction with PABP allows 5'-end and 3'-ends of mRNAs to interact
eIF-4B	stimulates helicase, binds simultaneously with eIF-4F
eIF-5	release of eIF-2 and eIF-3, ribosome-dependent GTPase
eIF-6	ribosome subunit antiassociation

REGULATION OF TRANSLATION INITIATION: THE EIF2A KINASES

- Regulation of initiation in eukaryotes is primarily effected by **phosphorylation of Ser (S) residues** in the α -subunit of eIF-2.
- However, when **eIF-2 is phosphorylated the GDP-bound complex is stabilized and exchange for GTP is inhibited.**
- The **exchange** of GDP for GTP is **mediated by eIF-2B**.
- **REGULATION OF eIF-4F ACTIVITY**



ELONGATION

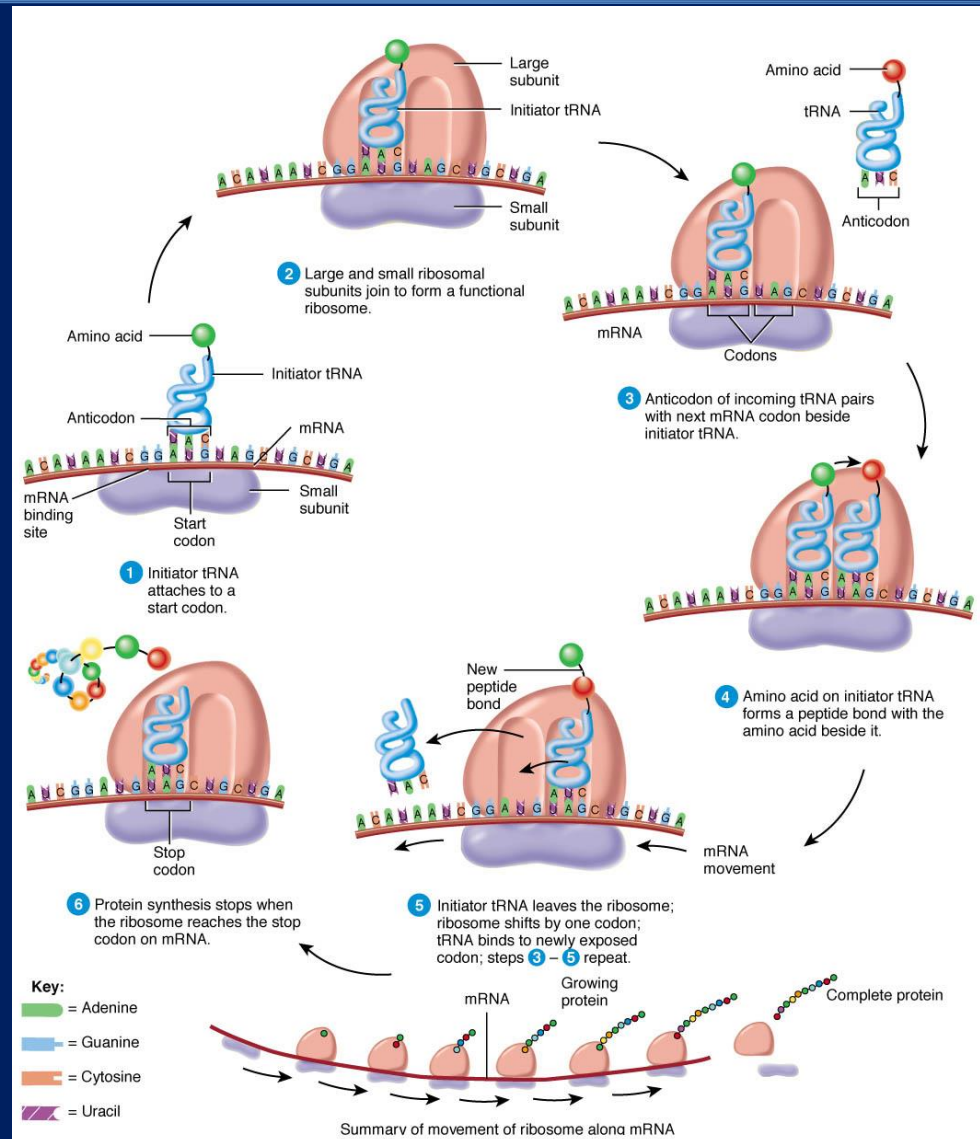
1) Binding of aminoacyl tRNA to A site

2) Formation of peptide bond

- This step is catalysed by **peptidyltransferase** (ribozyme), a component of 28S rRNA of the 60S subunit
- As a result of this reaction, a dipeptide tRNA is formed on the A site.

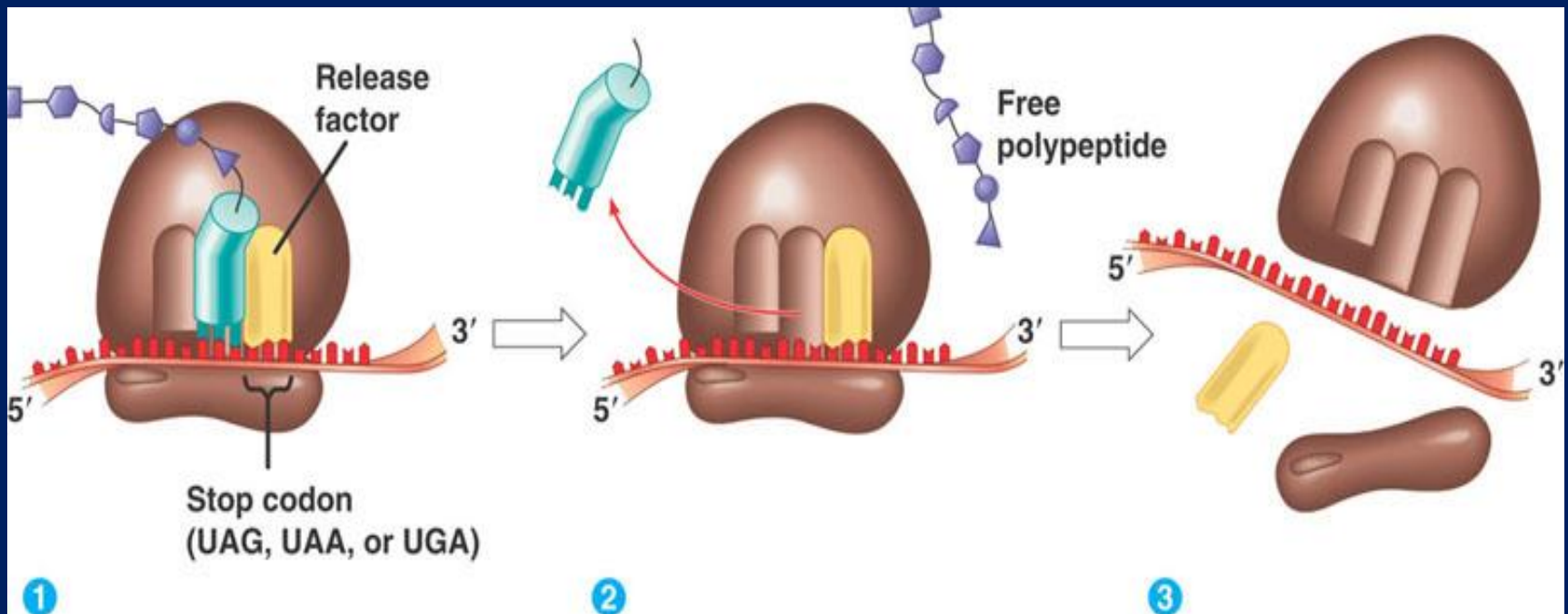
3) Translocation

- Ribosome moves along the mRNA



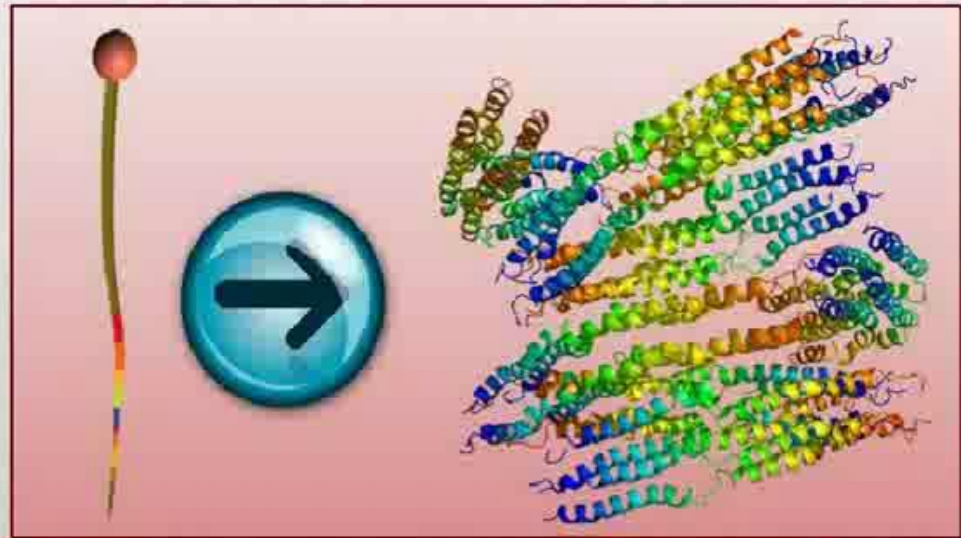
TERMINATION

- Elongation proceeds until one of the three termination codon of mRNA appears on A site.
- Releasing factors recognize the termination signal.
- RF in conjugation with GTP and peptidyl transferase hydrolyse the peptidyl tRNA bond.



Introducing: Translation

Translation is the synthesis of a protein from mRNA template.



This process involves several key molecules...

POST TRANSLATIONAL MODIFICATION

1. Folding into three dimensional conformation and may undergo processing by enzymatic action
2. *Types of PTM:-*
 - **A. Amino terminal and carboxy terminal modification:-**
 - Polypeptide begins with formyl methionine(in prokaryotes) and methionine (in eukaryotes).
 - The formyl group, the amino terminal methionine residue may be removed.

➤ B. Covalent alterations:-

- **Phosphorylation:-** e.g. serine, threonine, and tyrosine residues to yield phosphoserine etc
- **Carboxylation:-** Amino acids like glutamic acid e.g. blood clotting protein prothrombin contain a number of gamma-carboxylation residues.
- **Methylation:-** lysine residues present in muscle proteins and in Cytochrome C
- **Hydroxylation**

C. Proteolytic processing

- Insulin, and some viral proteins and proteases are initially synthesized as larger precursor proteins
- Trimmed to produce their final, active forms.

D)Additional prosthetic group

Attached to the polypeptide after it leaves the ribosome
E.g. Covalently bound biotin molecule in acetyl Co-A,
Heme group of cytochrome C

POST –TRANSTIONAL MODIFICATION OF PROTEINS THROUGH THEIR AMINO ACIDS

AMINO ACIDS	MODIFICATIONS
Amino terminal AA	Glycosylation, acetylation
Carobxy terminal AA	Methylation, ADP-ribosylation
Arginine	Methylation
Aspartic Acid	Phosphorylation, hydroxylation
Methionine	Sulfoxide formation
Serine	Phosphorylation, glycosylation
Tryptophan	Hydroxylation
Tyrosine	Phosphorylation, Hydroxylation

DIFFERENCES BETWEEN EUKARYOTIC & PROKARYOTIC PROTEIN SYNTHESIS

Characters	Eukaryotes	Prokaryotes
Ribosome	Larger 80S consists of 60S & 40S.	Smaller 70S consists of 50S & 30S.
Initiator RNA	Met-tRNA _i ^{Met}	f-Met-tRNA _i ^{f Met}
Start signal	Initiating AUG closest to the 5' end of an mRNA is preceded methylguanosyl triphosphate.	Initiating AUG of mRNA is preceded by a purine rich sequence
Initiation factor	Contains more than prokaryotes. 9 are known & several consists of multiple subunits. Prefix eIF.	Contain three i.e. IF ₁ , IF ₂ ,&IF ₃
Termination factor	Termination is carried out by a single release factor eRF.	Termination is carried by 3 releasing factors RF ₁ , RF ₂ & RF ₃ .

INHIBITORS OF TRANSLATION

Inhibitors	Action
Streptomycin	Inhibits initiation , binds to 30S at A site & prevent chain elongation.
Tetracycline	Binds to 30S & inhibits binding of aminoacyl tRNA to mRNA in prokaryotes
Chloramphenicol	Binds to 50S & blocks peptidyl transferase rxn in prokaryotes.
Erythromycin	Binds to 50S & inhibits translocation rxn in prokaryotes.
Lincomycin & Clindamycin	Binds to 50S & inhibits peptidyl transferase
Puromycin	Causes premature chain termination by acting as an analog of aminoacyl tRNA in both prokaryotes & eukaryotes.
Cycloheximide	Inhibits peptidyl transferase activity of 60S ribosomal subunit in eukaryotes.

THANK YOU
