Translation in Prokaryotes and Eukaryotes

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Contents

Introduction to Translation

- **Translation:** The biosynthesis of a protein or a polypeptide inside a living cell.

- In process of translation the language of nucleotides sequence on mRNA is translated in to the language of amino acid sequence.

- It occur in cytoplasm where ribosomes are located
- It is a universal process

Cont……

- In translation ,messenger RNA is decoded to produce a specific polypeptide
- This uses mRNA sequence as a template to guide the synthesis of a chain of amino acid that form protein
- Many types of transcribed RNA, such tRNA,rRNA,snRNA are not necessarily translated to amino acid sequence

Steps of Translation

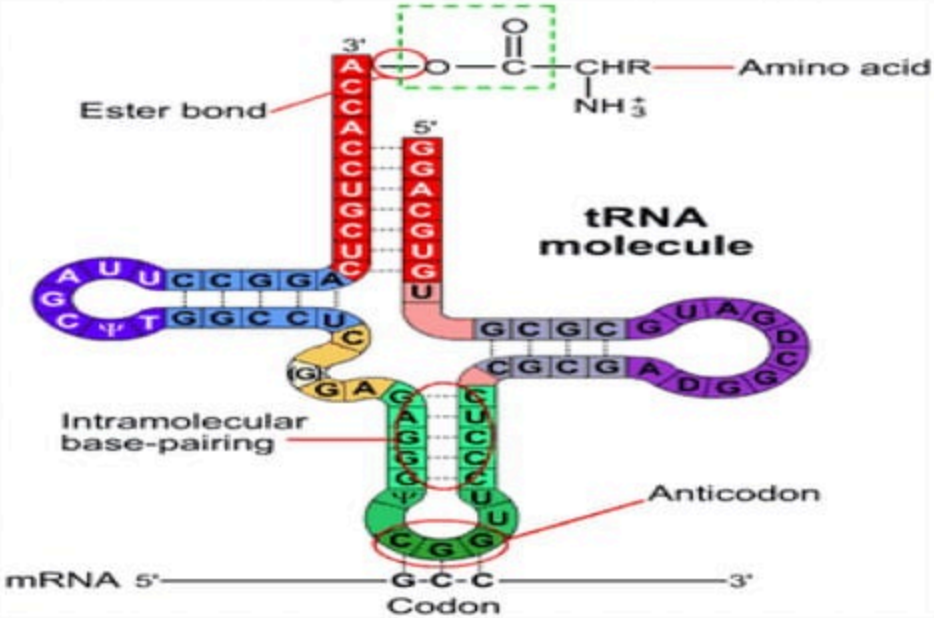
- Translation proceeds in four phases
  - Activation
  - Initiation
  - Elongation and
  - Termination

Most studies do not include activation as a step of translation

Activation

- In activation, the correct amino acid is covalently bonded to the correct tRNA
- While technically this not a step in translation, it is required for translation to proceed
- The amino acid is joined by its carboxyl group to the 3` OH of tRNA by an ester bond with help of ATP
- When tRNA has an amino acid linked to it, it is termed as "charged"

Cont.....


3'
5'
Ester bond
Amino acid
CHR
NH3+
tRNA molecule
Intramolecular base-pairing
Anticodon
mRNA 5'
G-C-C
3'
Codon

Initiation

- Prokaryotes initiation require the large and small subunits, the mRNA, the initiator tRNA and three initiation factors (IF-1, IF-2, IF-3) and GTP.
- IF-3 binds to the free 30s subunit, this help to prevent large subunit binding to it without mRNA and forming an inactive ribosome
- IF-2 complexed with GTP and IF-1 binds to small subunit . It will assist the charged initiator tRNA to bind

Cont.....

- The 30s subunit attached to a mRNA molecule making use of the ribosomal binding site on mRNA
- The initiator tRNA can then bind to the complex by base pairing of its anticodon with AUG codon on mRNA
- At this point,IF3 can be released, as its role in keeping the subunits apart are complete
- This complex is called *30s initiation complex*

Cont....

- The 50s subunit can now bind, which displace IF1 and IF2, and the GTP is hydrolysed in this energy consuming step

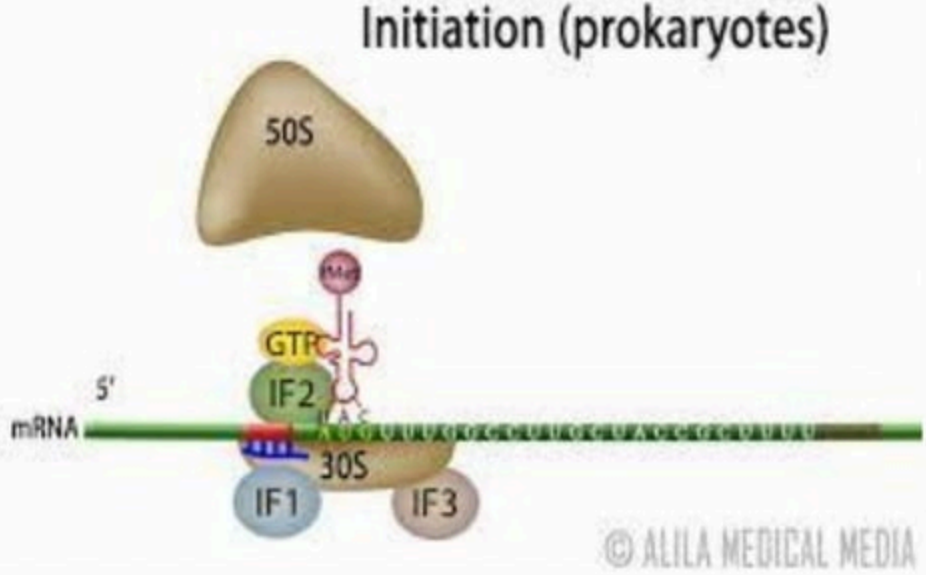
- This complex is called *70s initiation complex*

Cont.....

- The assembled ribosome has two tRNA binding sites
- These are called the A and P sites , for amino acyl and peptidyl sites and one site is E (exit site) for empty tRNA
- The A site is where incoming amino acyl tRNA molecule bind, and the p site is where the growing polypeptide chain is usually found
- The sites are in cleft of small subunit and contain adjacent codon that are being translated

Cont……

- One major of initiation is the placement of initiator tRNA in the P site
- It is the only tRNA that does this , as all other must enter the A site


Initiation (prokaryotes)
50S
GTP
IF2
5'
mRNA
30S
IF1
IF3
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Elongation

- With the formation of 70s initiation complex the elongation cycle can begin

- It involves three elongation factors EF-Tu, EF-Ts and EF-G, GTP, charged tRNA and the 70s initiation complex

Elongation is divided into 3 steps

1. ***Amino acyl tRNA delivery.***

- EF-TU is required to deliver the amino acyl tRNA to A site and energy is consumed in this step by hydrolysis of GTP
- The released EF-Tu GDP complex is regenerated with the help of EF-TS

- In the EF-Tu EF-Ts exchange cycle EF-Ts displaces the GDP and replace itself by GTP
- The resultant EF-Tu.GTP complex is now available to bind another amino acyl tRNA and deliver it to ribosome

Cont....

- All amino acyl tRNA can form this complex with EF-Tu except the initiator tRNA

Cont.....

2 .Peptide bond formation.

- After aminoacyl-tRNA delivery ,the A and P sites are both occupied and the two amino acids that are to be joined are close to each other

- The peptidyl transferase activity of the 50s subunit can now form a peptide bond between the two amino acids

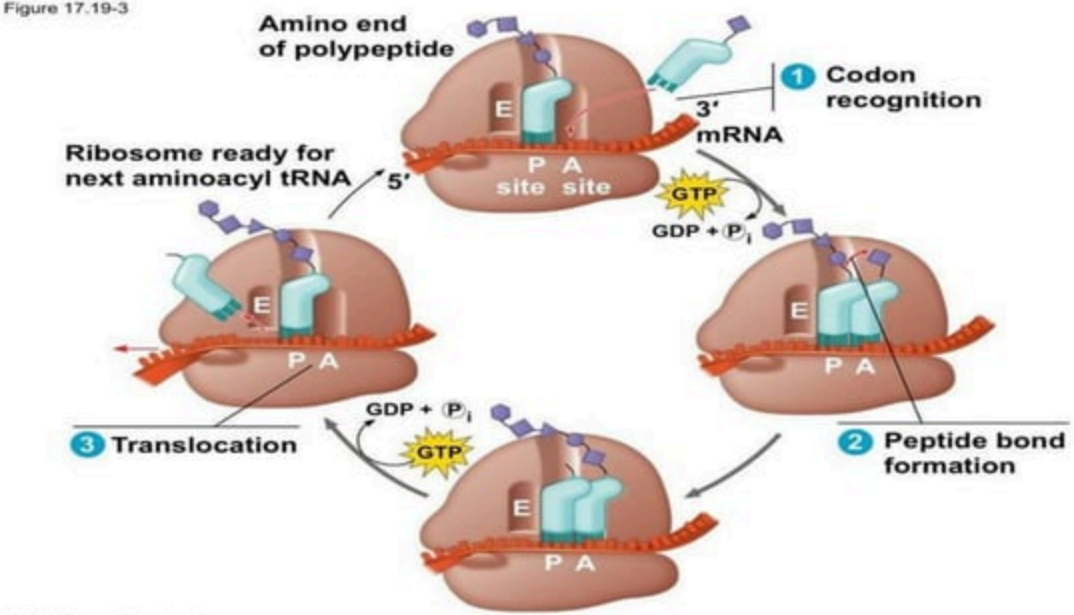
3. ***Translocation***

- A complex of EF-G(translocase) and GTP binds to the ribosome and ,is an energy consuming step, the discharged tRNA is ejected from the P site, the peptidyl-tRNA is moved from A site to P site

- The mRNA moves by one codon relative to one codon to the ribosome

- GDP and EF-G are released . A new codon is now present in the vacant site

Figure 17.19-3



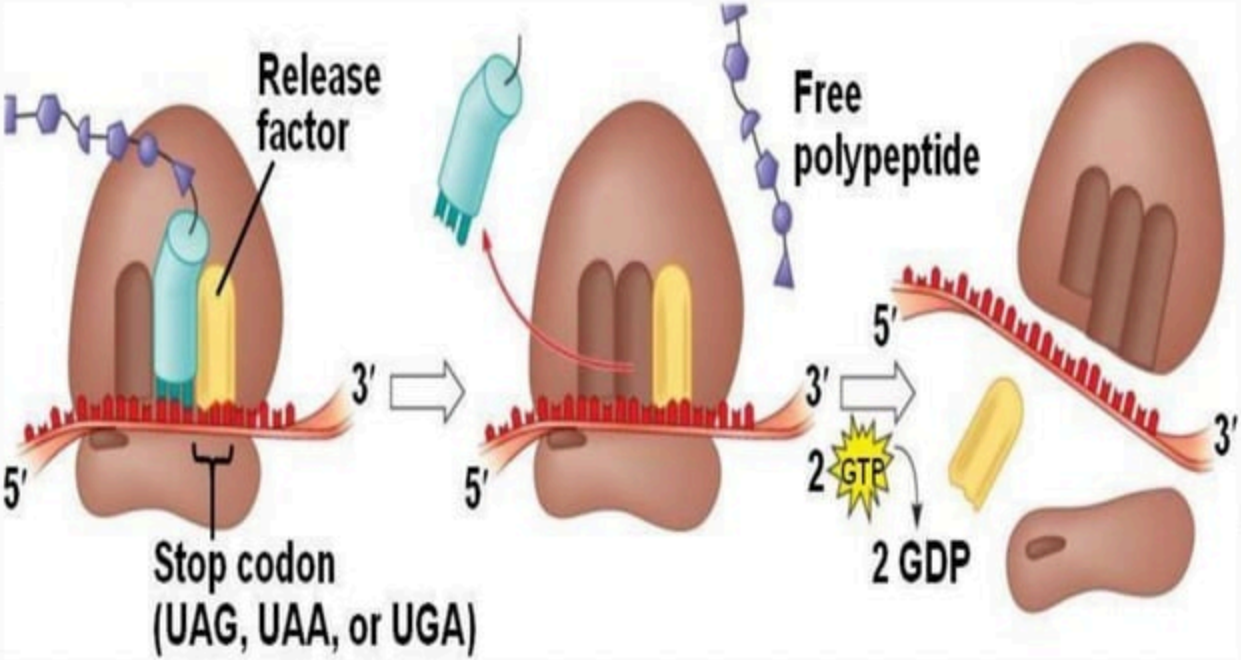
Termination

- Termination of translation happens when the A site of the ribosome faces a stop codon (UUA,UGA or UGA)
- When this happens , no tRNA can recognize it, but a releasing factor can recognize the stop codons and causes the release of polypeptide chain
- In prokaryotes once a stop codon occupies the A site, three termination or release factor (RF_1,RF_2,RF_3) contribute to the hydrolysis of peptidyl-tRNA bond

Cont.....

- Release the free polypeptide and last uncharged tRNA from P site
- The dissociation of the 70 s into 30s and 50s subunits
- RF1 binds A site and release the polypeptide and uncharged tRNA
- RF2 releases the RF1 from A site and release itself as well from translation binding site(present on large subunit)
- RF3 function unknown
- Another factor called Ribosomal releasing factor causes the dissociation of 70s complex

Cont....


Release factor
Free polypeptide
3′
5′
Stop codon (UAG, UAA, or UGA)
2 GTP
2 GDP

Translation in Eukaryotes

Eukaryotic Translation

- In prokaryotic cell, transcription and translation are coupled, that is, translation begins while the mRNA is still being synthesized. In a eukaryotic cell, transcription occur in the nucleus , and translation occur in the cytoplasm.

- Translation process in eukaryotes involve
  - Activation (not essentially the step of translation. This occur the same way as in prokaryotes)
  - Initiation
  - Elongation and
  - termination

1. Initiation

- The initiation of translation in eukaryotes is complex, involving at least 10 initiation factors (eIFs) and is divided into three steps :

a) Formation of 43s preinitiation complex.
b) Formation of 48s initiation complex.
c) Formation of 80s initiation complex.

a. Formation of 43s preinitiation complex

- A ternary complex containing met-tRNA and eIF-2 bound to GTP attaches to 40s ribosomal subunit to form 43s preinitiation complex.

- The presence of eIF-3 and eIF-1A stabilizes this complex.

b.Formation of 48s initiation complex

- The binding of mRNA to 43s preinitiation complex results in formation of 48s initiation complex.
- eIF-4f is formed by the association of eIF-4G, eIF-4A with eIF-4E
- The eIF-4F(referred to as cap binding protein) binds to the cap of mRNA.

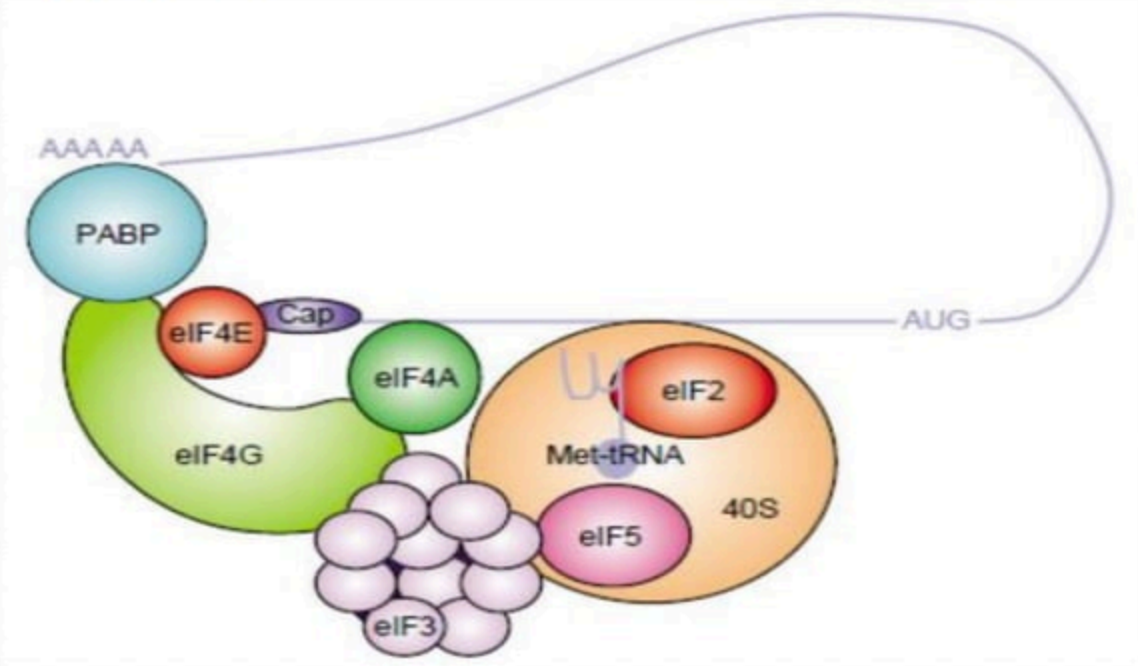
Cont.....

- Then eIF-4A and eIF-B binds to mRNA and reduces its complex structure.
- This mRNA is then transferred to 43s complex
- The ribosomal initiation complex scans the mRNA for identification of appropriate initiation codon
- 5'-AUG is the initiation codon

c.Formation of 80s initiation complex

- 48s initiation complex binds to 60s ribosomal subunit to form 80s initiation complex
- The binding involves hydrolysis of GTP(bound to eIF-2)
- This step is facilitated by the involvement of eIF-5
- As the 80s complex is formed, the initiation factors bound to 43s initiation complex are released and recycled

Cont.....


AAAAA
PABP
eIF4E
Cap
AUG
eIF4A
eIF2
eIF4G
Met-tRNA
40S
eIF5
eIF3

2. Elongation

- Ribosomes elongate the polypeptide chain by sequential addition of amino acids
- The amino acid sequence is determined by the order of the codons in the specific mRNA
- Elongation, a cyclic process involving certain elongation factors(EFs)
- Elongation may be divided into three steps

a. Binding of aminoacyl-tRNA to A-site
b. Peptide bond formation
c. translocation

a.Binding of Aminoacyl t-RNA to A-site

- The 8os initiation complex contains met tRNA in the P-site and A- site is free
- Another aminoacyl-tRNA is placed in the A site
- This require proper codon recognition on mRNA and involvement of EF-1a and supply of energy by GTP
- The aminoacyl –tRNA is placed in the A-site ,EF-1a and GDP are recycled to bring another aminoacyl-tRNA

b. Peptide bond formation

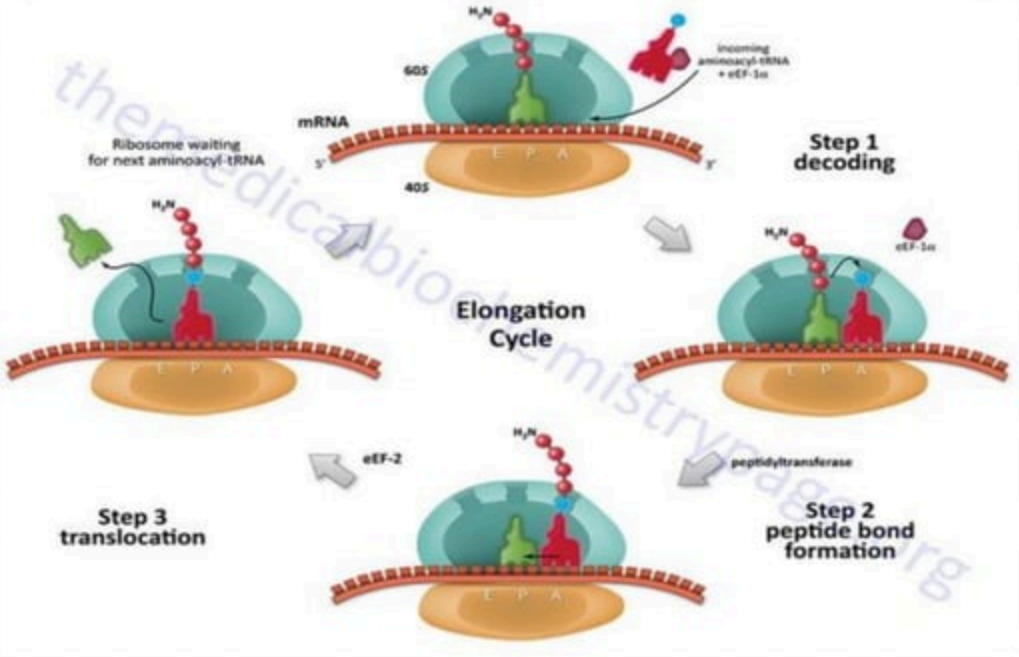
- The peptidyl transferase catalyzes the formation of peptide bond

- Net result of peptide bond formation is the attachment off the growing peptide chain to the tRNA in A-site

c. Translocation

- The ribosome moves to the next codon of mRNA (towards 3' end)
- This process is called translocation, involves the movement of growing peptide chain from A-site to P-site
- Translocation require EF-2 and GTP
- GTP get hydrolyzed and supplies energy to move mRNA
- EF-2 and GTP complex recycles for translocation

Cont....


60S
mRNA
40S
Ribosome waiting
for next aminoacyl-tRNA
incoming
aminoacyl-tRNA
+ eEF-1α
Step 1
decoding
Elongation
Cycle
eEF-1α
peptidyltransferase
Step 2
peptide bond
formation
eEF-2
Step 3
translocation

3. Termination

- One of the stop signals (UGA,UAA and UGA) terminates the growing polypeptide
- When the ribosome encounter s a stop codon, there is no tRNA available to bind to the A site of ribosome
- Instead a release factor binds to it
- In eukaryotes eRF1 recognizes all the three stop codons, and eRF3 stimulate the termination events

Cont…

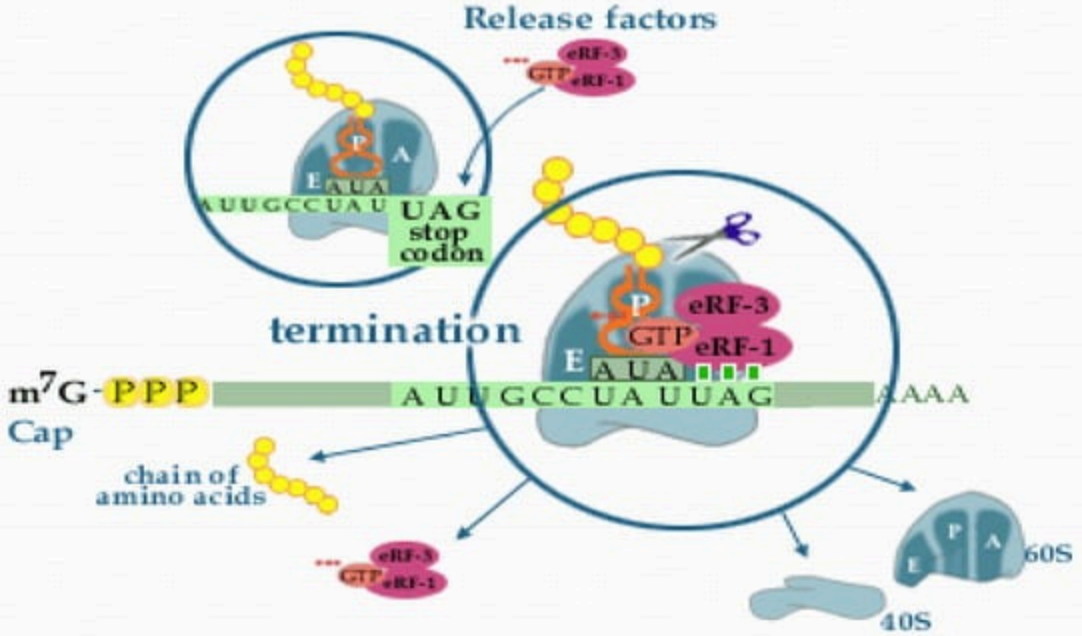
- Once the release factor binds, the ribosome unit falls apart

  - releasing the large and small subunits

  - the tRNA carrying the polypeptide is also released, freeing up the polypeptide product.

  -

Cont....


Release factors
eRF-3
GTP
eRF-1
P
A
E
A U A
A U U G C C U A U
UAG
stop
codon
termination
eRF-3
GTP
eRF-1
m7G-PPP
A U U G C C U A U U A G
AAAA
Cap
chain of
amino acids
60S
40S

References

- www.namrata.com (biochemistry For Medics By Dr.Namrata Chhabra)
- slideshares
- www.easybiologyclass.com
- YouTube lecture by shomou's Biology
- Wikipedia

Questions/suggestions:
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Thank you