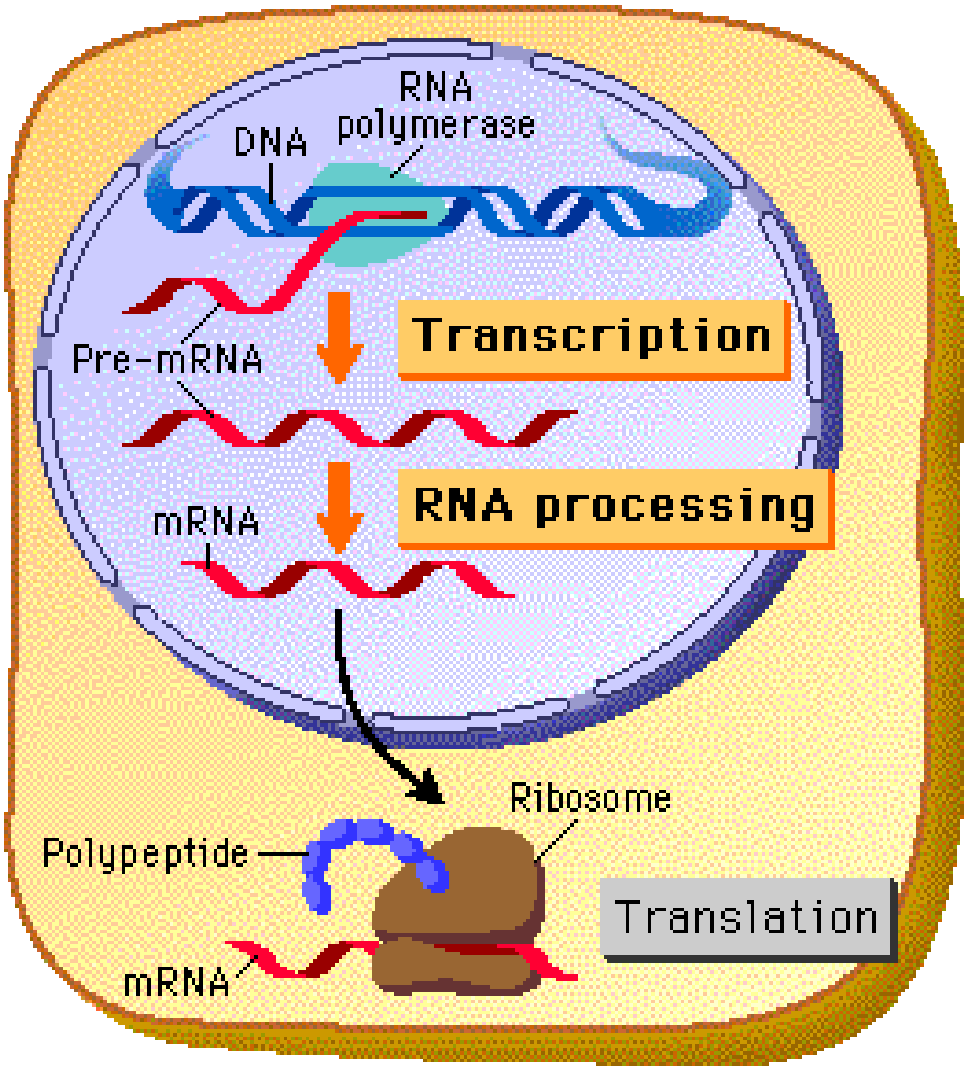
Mechanism of Translation in Prokaryotes

INTRODUCTION

- A well-conserved process among prokaryotes and eukaryotes.
- Protein synthesis is the final stage of gene expression .
- The genetic message transcribed to mRNA is translated into protein by a complex cellular machinery. Additional processing and assembly often required to modify the proteins.
- Occurs in 3 stages:
  initiation, elongation & termination

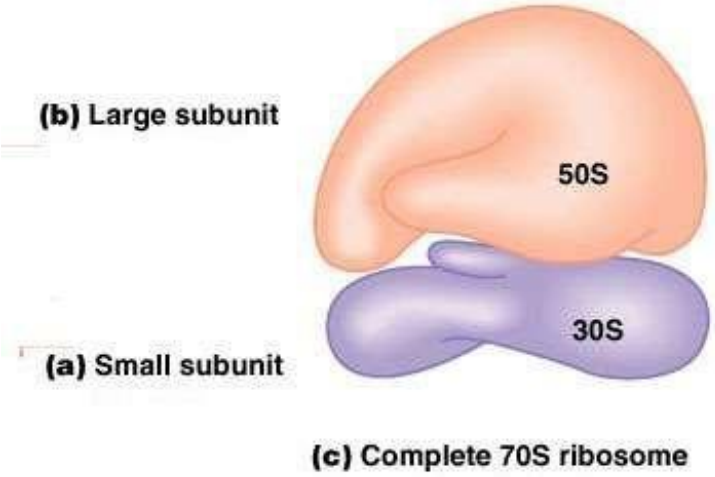

RNA
polymerase
DNA
Pre-mRNA
Transcription
mRNA
RNA processing
Ribosome
Polypeptide
Translation
mRNA

PROCESS OF TRANSLATION

- A.A is activated by Rn with RNA(ATP) to form activated A.A.
- The activated A.A joined to the 3 prime terminus of tRNA to form amino acyl tRNA (catalyzed by aminoacyltRNA synthetase)
- Messenger RNA brings polypeptide-coding information to the ribosome.

INITIATION

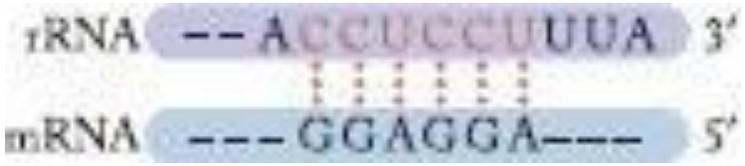
- In prokaryotes, 70S ribosome.



- Initiation starts with interaction of 30S subunit with an mRNA mol & 3 IFs

- In prokaryotes, protein synthesis initiated with a modified methionine residue i.e.. N-formylmethionine-tRNA
- Chain initiation begins with the formation of 2 complexes:
- 1.IF-2 & N-formylmet.TRNA
- mRNA,30s subunit,IF-3
- Prokaryotic mRNAs contains a consensus seq -ShineDalgarno Sequence.

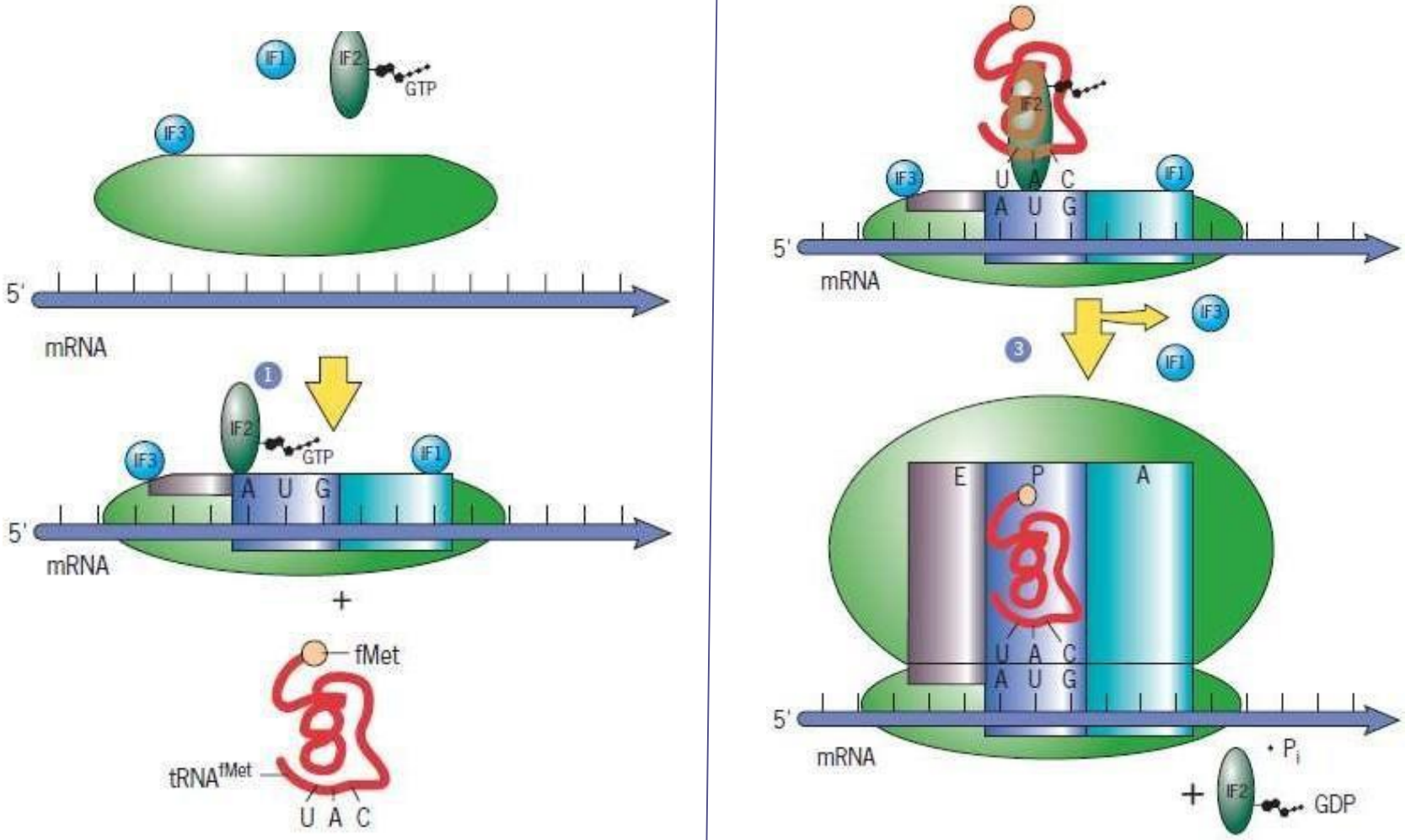
- Shine- dalgarno seq is complementary to a seq of nucleotides at 3prime end of ribosomal rna of the 30 S subunit.



- Interaction b/w these complementary seq enhances the attachment of the 30 S subunit to the AUG initiator codon.

- Both the complexes combine with each other with IF-1 and 1 mol of GTP
- 50 S subunit gets added to the complex structure. IF-3 is released. The addition of50S subunit utilizes GTP which in turn triggers the release of IF-1 and IF-2

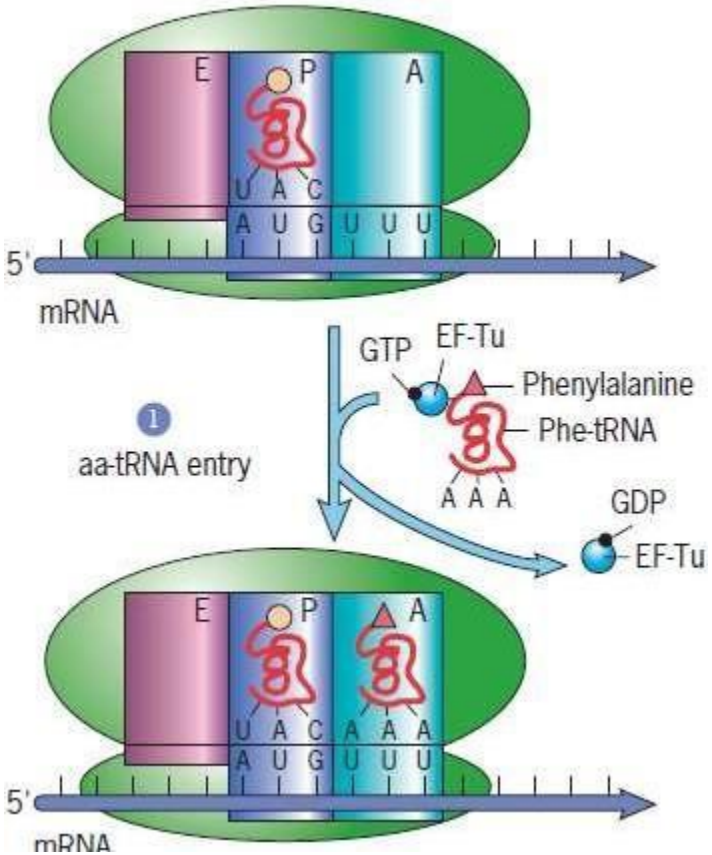
- Addition of 50S to the complex,positions N-formyl met tRNA in the P site directly with the anticodon of the tRNA aligned with AUG codon of mRNA
- With the AUG initiatior codon positioned in the P site, 2d codon in the mRNA positions in such a way that it corresponds to the incoming aminoacyl-tRNA in the A -site

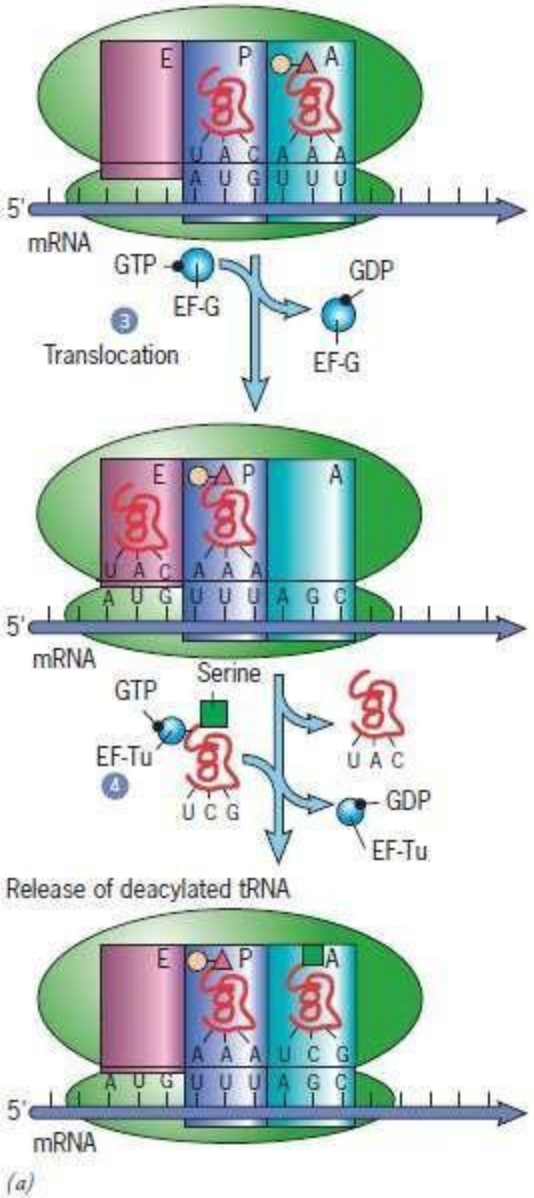

IF1
IF2
GTP
IF3
5'
mRNA
1
IF2
GTP
IF3
IF1
A U G
5'
mRNA
+
fMet
tRNA fMet
U A C
U A C
IF3
IF1
3
E
P
A
U A C
A U G
5'
mRNA
+ Pi
IF2
GDP

CHAIN ELONGATION

- Elongation begins with the binding of the second aminoacyl tRNA at the ribosomal aminoacyl (A) site.
- The tRNA is escorted to the A site by the elongation factor EF-Tu, which also carries bound GTPs. As the tRNA binds, the GTPs are hydrolyzed and EF-Tu is released.
- A peptide bond formed b/w the N-f-met-Trna at the P site& 2nd amino acyl tRNA at the A site; catalyzed by the peptidyl transferase .
-

- Transfer of N-f-met. to aminoacyl-tRNA at A site forming a peptidyl tRNA at that position and leaving an uncharged tRNA at the P site
- Peptidyl trna translocated to the P site & uncharged Trna is translocated to E domain.
- A site unoccupied ; new aminoacyl-TRNA bind to A site &process continues


E
P
A
U A C
A U G U U U
5'
mRNA
GTP
EF-Tu
Phenylalanine
Phe-tRNA
A A A
1
aa-tRNA entry
GDP
EF-Tu
E
P
A
U A C A A A
A U G U U U
5'
mRNA


E
P
A
U A C A A A
A U G U U U
5'
mRNA
GTP
EF-G
GDP
3
Translocation
EF-G
E
P
A
U A C A A A
A U G U U U A G C
5'
mRNA
GTP
Serine
EF-Tu
4
U C G
U A C
GDP
EF-Tu
Release of deacylated tRNA
E
P
A
A A A U C G
A U G U U U A G C
5'
mRNA

(a)

CHAIN TERMINATION

- When a stop codon (UAG, UAA, or UGA) arrives at the A site, it is recognized and bound by a protein release factor. (RFs).2 classes of RFs: ClassI & Class II. In E.coli 2 Class I RFs are seen- RF-1 ,RF-2
- RFs bind to the termination codon at A site & stimulate hydrolysis of bond b/w tRNA&polypeptide chain at P site , resulting in release of complete polypeptide from ribosome.
- Termination is completed by release of mRNA mol from ribosome & dissociation of ribosomes into its subunits