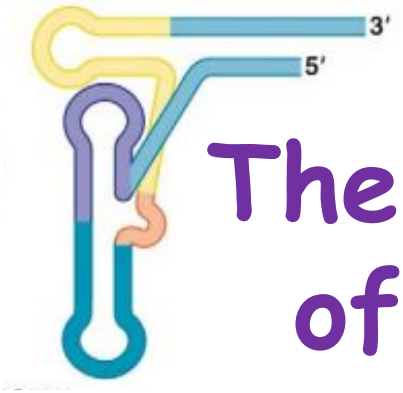


The codon-dependent binding of aminoacyl-tRNA in the elongation cycle.

Done by: Moldakozhayev A.
Naizabayeva D.

Introduction into translation

Four primary components:

1)mRNA: template for translation, codons specify the order of amino acids

2)tRNA: provide physical interface between amino acids and codons in mRNA

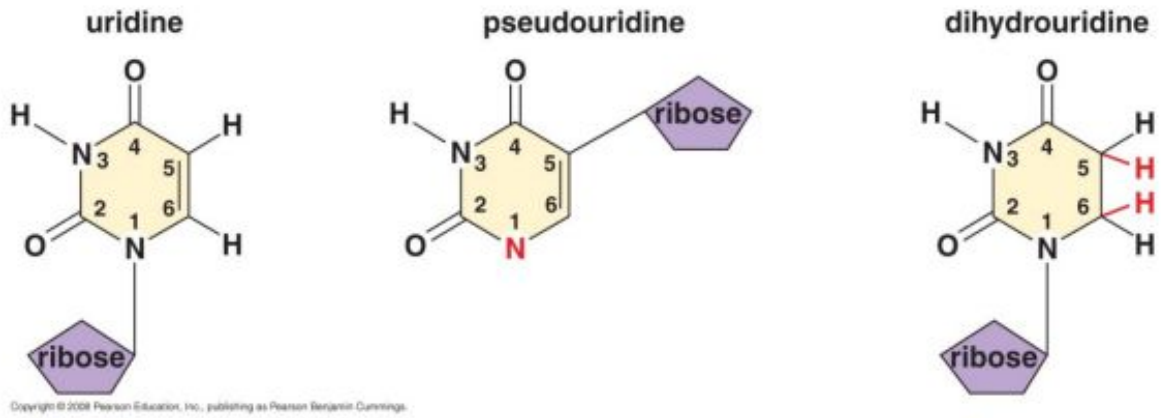
3)aminoacyl-tRNA synthetase: couple amino acids to specific tRNA that recognize the appropriate codon

4)ribosome: multi-megadalton machine composed of both rRNA and protein, coordinate the correct recognition of mRNA by each tRNA and catalyze peptide bond formation

tRNAs are adaptors between codons and amino acids

There are many types of tRNAs, but each is attached to a specific amino acid (AA) and recognize a particular codon(s) -> most tRNA recognize more than one codon, 75~95 ribonucleotides in length, sequences vary->

…but all have certain features in common: 1) all end at 3' terminus with "CCA" at which cognate AA is attached 2) presence of several unusual bases (e.g., pseudouridine, dihydrouridine, hypoxanthine, thymine, methylguanine): created posttranscriptionally by enzymatic modification, these modified bases are not essential for tRNA function, but experimental results suggest that they lead to improved tRNA functi

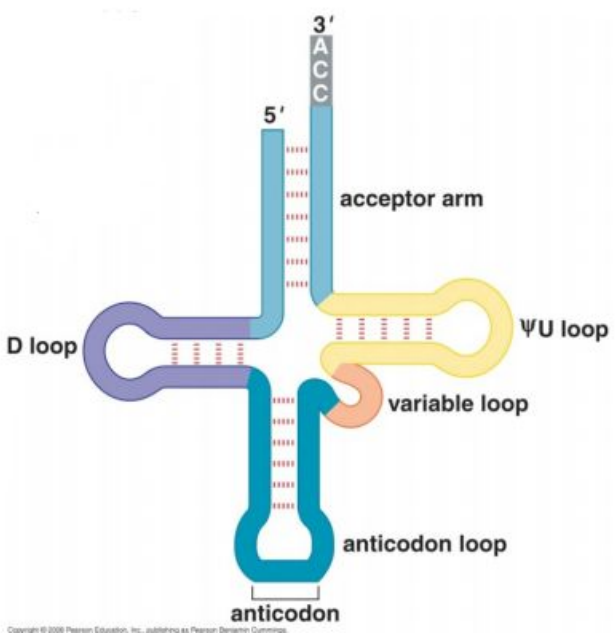


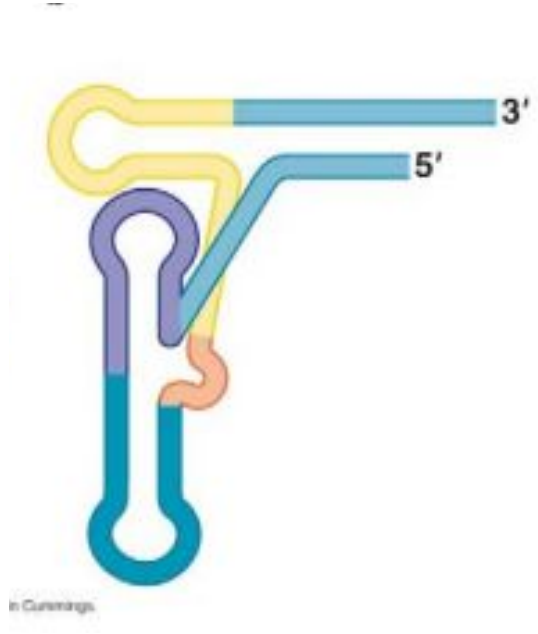
Five characteristic regions:

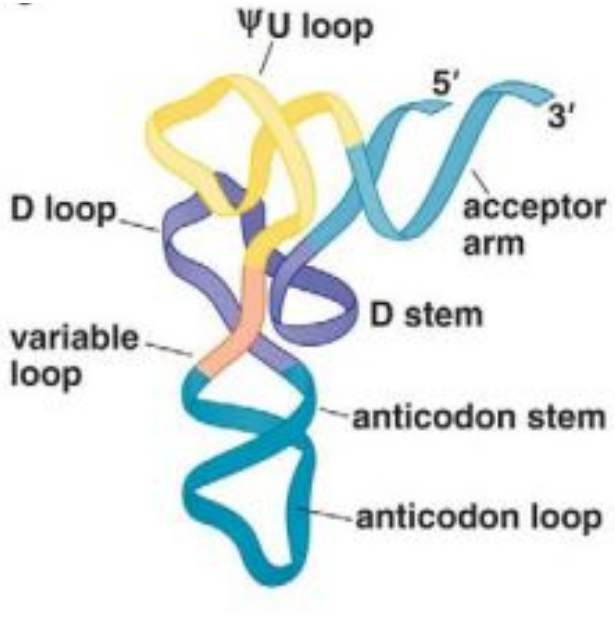
1)acceptor stem: site of attachment of AA, "CCA" at the extreme 3' end is single-stranded and protruded

2) ΨU loop: contains ΨU, often found within the sequence 5'-T ΨUCG-3'

3)D loop: contains dihydrouridine 4)anticodon loop: contains anticodon, responsible for recognizing the codon by base pairing 5)variable loop: sits between anticodon loop and ΨU loop, varies in size from 3 to 21







ATTACHMENT OF AMINO ACIDS TO tRNA

*charged (tRNA-AA) vs uncharged (tRNA)

*charging requires an acyl linkage between –COOH of amino acid and 2'- or 3'-OH of adenosine of "CAA" at the 3' end of tRNA

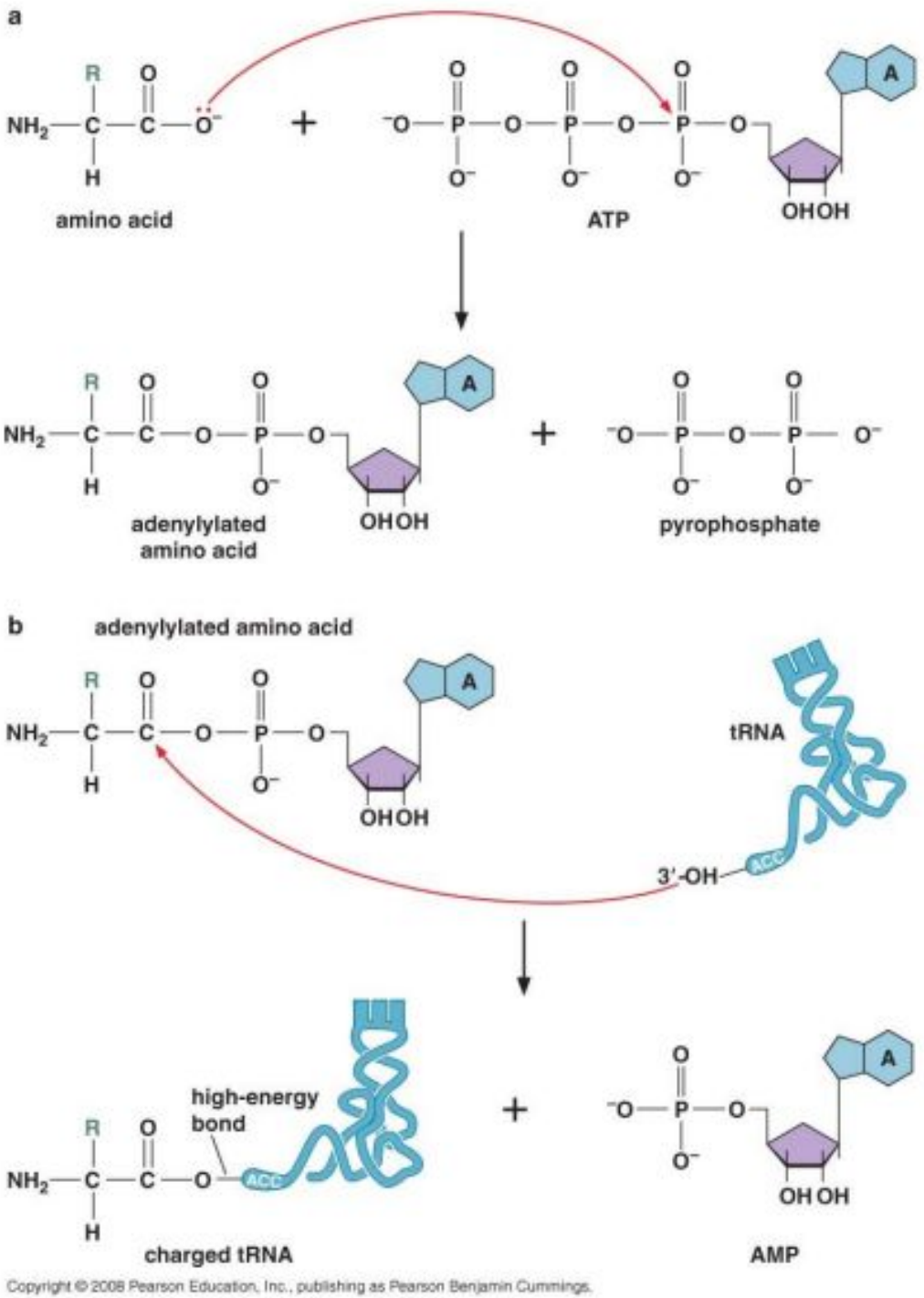
*this is high energy bond -> important for protein synthesis to help drive the formation of peptide bond

Aminoacyl-tRNA formation

Aminoacyl-tRNA synthetases Charge tRNAs in two steps:

1)adenylylation: Amino acid react with ATP and AMP is transferred to amino acids

2)tRNA charging: transfer of aAmino acid to the 3' end of tRNA via 2'- or 3'-OH and release of AMP



Aminoacyl tRNA symthetase

1)class I: attach AA to 2'-OH of the tRNA and generally monomeric 2)class II: attach AA to 3'-OH of the tRNA and typically dimeric or tetrameric

TABLE 14-1 Classes of Aminoacyl-tRNA Synthetases

Class II	Quarternary Structure	Class I	Quarternary Structure
Gly	$(\alpha_2\beta_2)$	Glu	(α)
Ala	(α_4)	Gln	(α)
Pro	(α_2)	Arg	(α)
Ser	(α_2)	Cys	(α_2)
Thr	(α_2)	Met	(α_2)
His	(α_2)	Val	(α)
Asp	(α_2)	Ile	(α)
Asn	(α_2)	Leu	(α)
Lys	(α_2)	Tyr	(α)
Phe	$(\alpha_2\beta_2)$	Trp	(α)

Adapted, with permission, from Delarue M. 1995. *Curr. Opin. Struct. Biol.* 5: 48–55, Table 1. © Elsevier.
Class I enzymes are generally monomeric, whereas class II enzymes are dimeric or tetrameric, with residues from two subunits contributing to the binding site for a single tRNA. α and β refer to subunits of the tRNA synthetases and the subscripts indicate their stoichiometry.

Specificity of binding

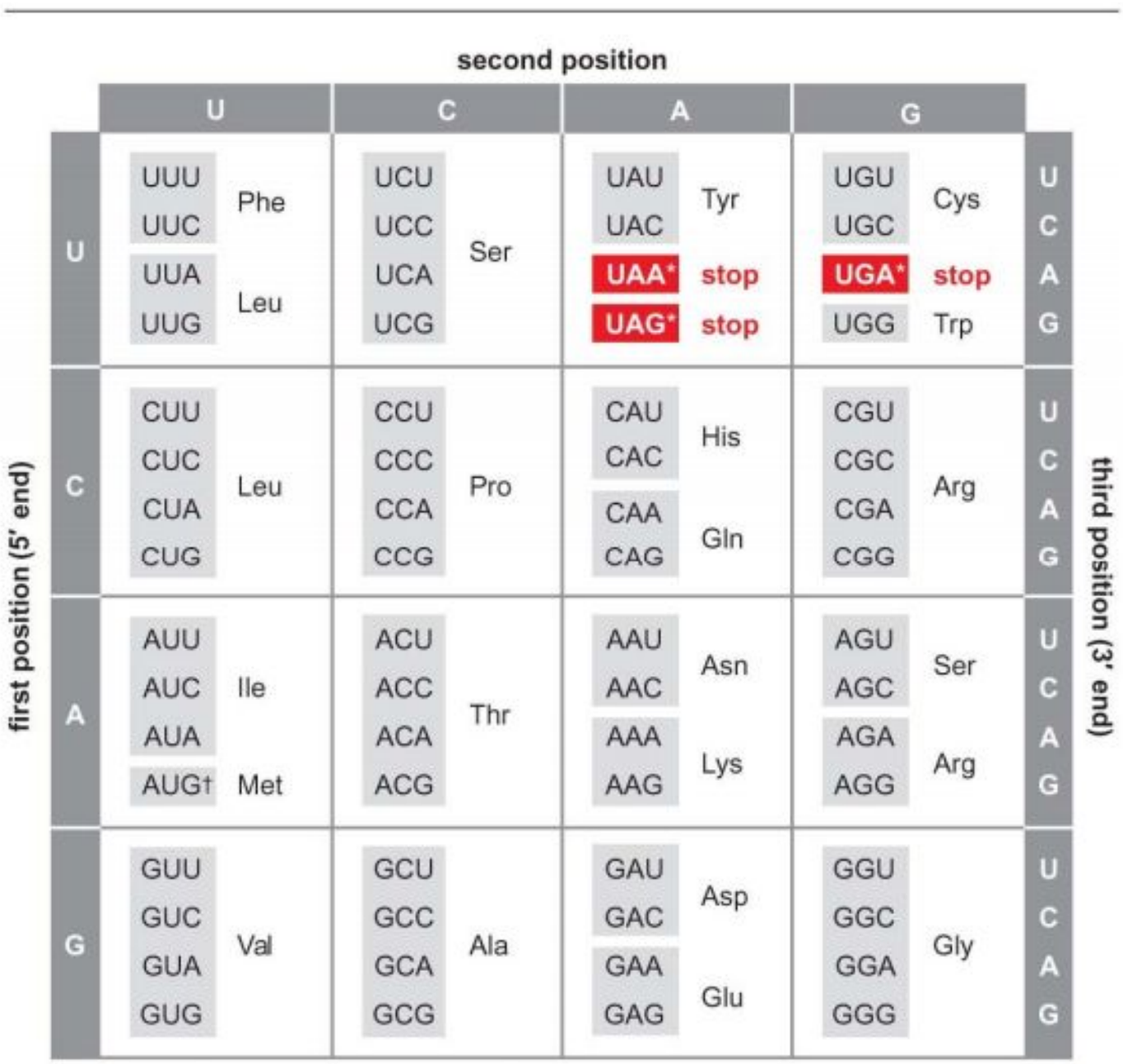
*Each of 20 amino acid is attached to appropriate tRNA by a single, dedicated tRNA synthetase

*Isoaccepting tRNA: because AA is specified by more than one codon, it is not uncommon for one synthetase to recognize and charge more than one tRNA (i.e., single synthetase to multiple tRNA relationship)

*most organisms have 20 different tRNA synthetase but this is not always the case

*an aminoacyl-tRNA synthetase can never attach more than one kind of AA to a given tRNA (i.e., dedicate to only one AA)

Genetic code



first position (5′ end)	second position: U	C	A	G	third position (3′ end)
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA* stop	UGA* stop	A
	UUG Leu	UCG Ser	UAG* stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG† Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

* Chain-terminating or "nonsense" codons.

† Also used in bacteria to specify the initiator formyl-Met-tRNA^{fMet}.

What features of tRNA enable synthetase to discriminate the correct set of tRNA from tRNA for other 19 AAs?

*acceptor stem: discriminator base is sufficient to convert specificity from one synthetase to another -

*anticodon loop: contribute to discrimination as well (see numerous contacts in 3-D structure)

*second genetic code: e.g., note "Ser" has six different codons -> synthetase must rely on determinants that lie outside of the anticodon

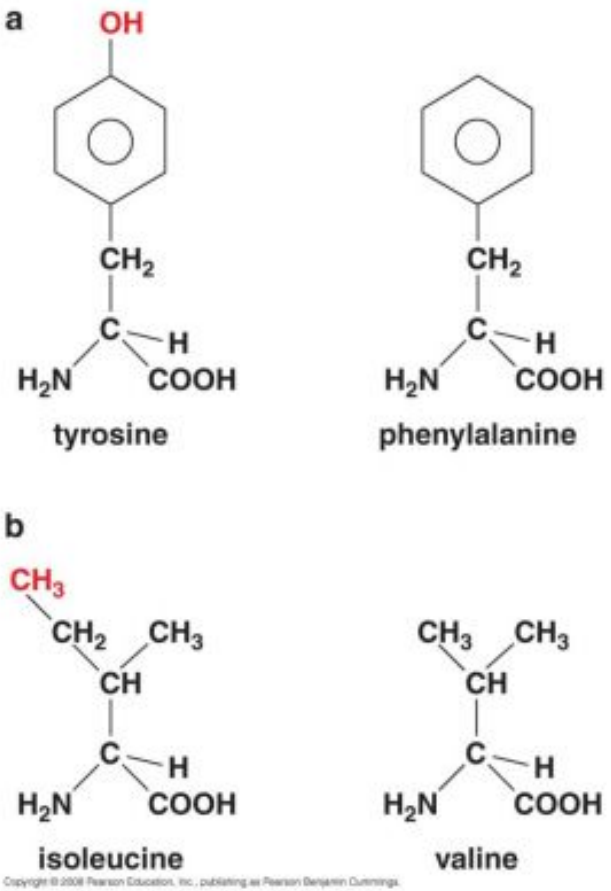
Aminoacyl-tRNA formation is very accurate

Selecting correct AA by the synthetase is dauntingly challenging due to relatively small size of AA and structural similarity

*However, the frequency of mischarging is very low, 1/1000 tRNA

-Tyr vs Phe: -OH of Tyr -> form a strong and energetically favorable H-bonding (discriminate by chemical properties)

-Ile vs Val: valyl-tRNA synthetase can sterically exclude Ile from its catalytic pocket (discriminate by size)



Aminoacyl-tRNA formation is very accurate

Some aminoacyl-tRNA synthetases use an editing pocket to charge tRNA with high accuracy -one additional common mechanism to increase the fidelity is to proofread the products of the charging reaction

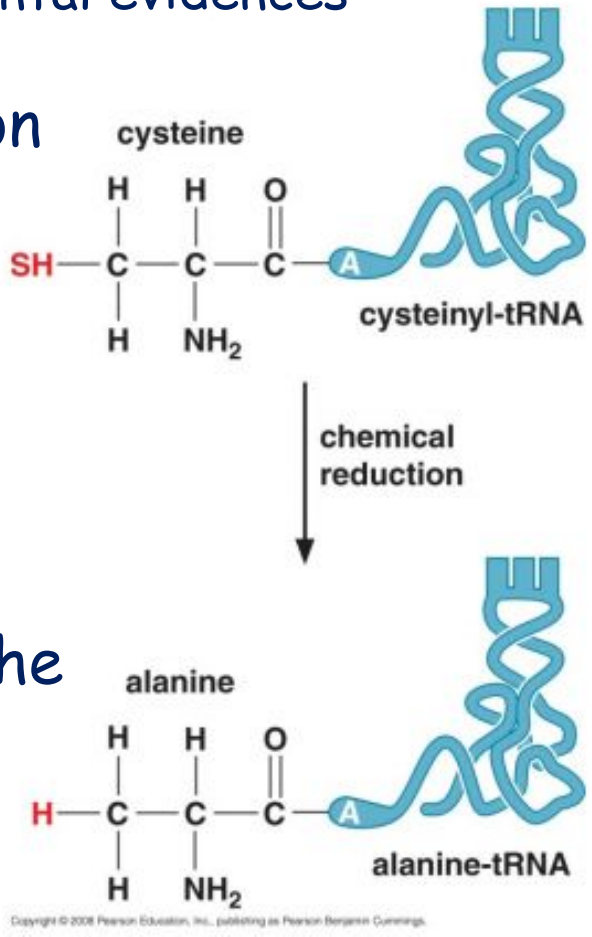
*For example, Ile-tRNA synthetase has a editing pocket near the catalytic pocket: AMP-Val is small and can enter into the pocket and subject to hydrolysis, while AMP-Ile is too large to enter and is therefore not subject to hydrolysis

Aminoacyl-tRNA in the elongation cycle

*Ribosome blindly accepts any charged tRNA that exhibits a proper codon-anticodon interaction, whether or not the tRNA is charged with correct AA -revealed by two kinds of experimental evidences

1)genetic: introduce mutation in anticodon -> results in delivery of its AA to wrong codon

2)biochemical: biochemical modification and cell-free translation system-> introduce wrong AA to its codon



*thus, ribosome recognizes tRNA , not the AA , and translation machinery relies on the high fidelity of aminoacyl-tRNA synthetases to ensure the accurate decoding of mRNA

Aminoacyl-tRNA in the elongation cycle

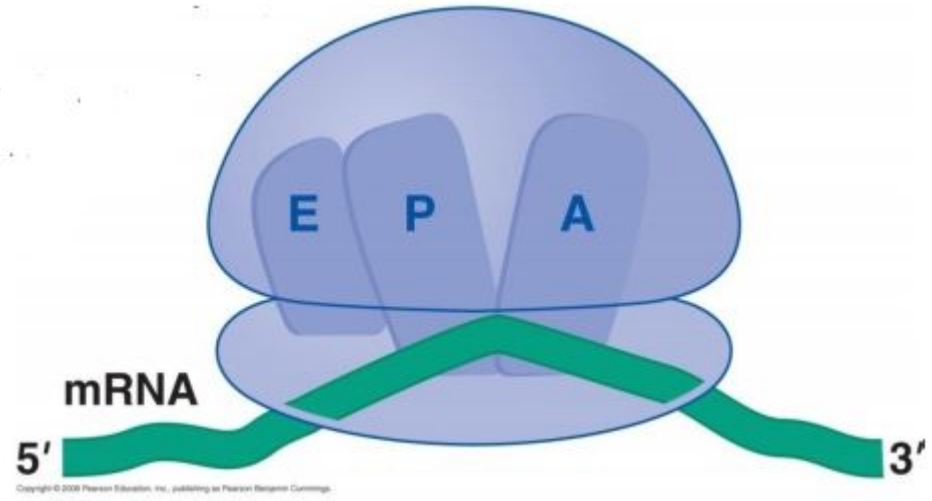
The ribosome has three binding sites for tRNA

1)A site: binding site for aminoacyl-tRNA
2)P site: binding site for peptidyl-tRNA
3)E (denote exit) site: binding site for tRNA released after growing polypeptide chain has been transferred to the aminoacyl-tRNA (i.e., free tRNA)

-each tRNA binding site is formed at the interface between large and small subunits

-> tRNA span the distance between peptidyl transferase center in the large subunit and the decoding center in the small subunit

-3′ end of tRNA is adjacent to the large subunit while anticodon loop to the small subunit

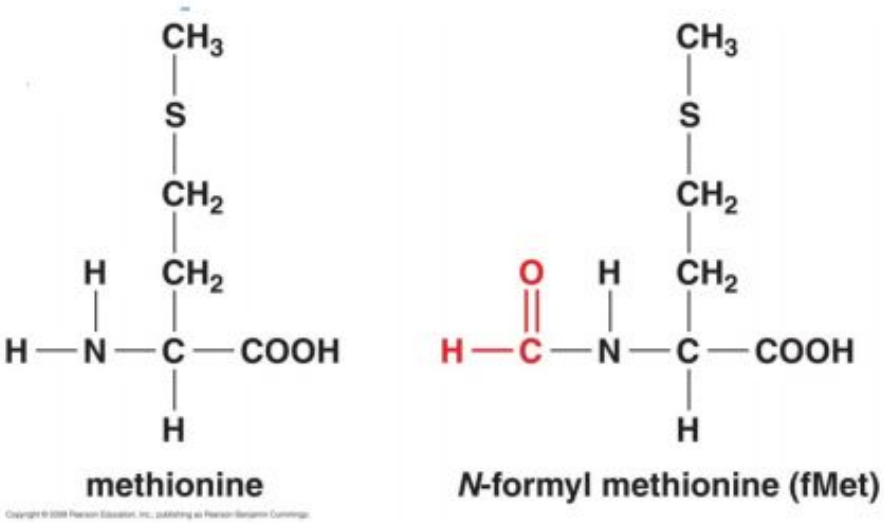


Aminoacyl-tRNA in the elongation cycle

A specialized tRNA charged with a modified methionine binds directly to the prokaryotic small subunit -Initiator tRNA, enter the P site directly, which base-pairs with start codon (AUG or GUG)

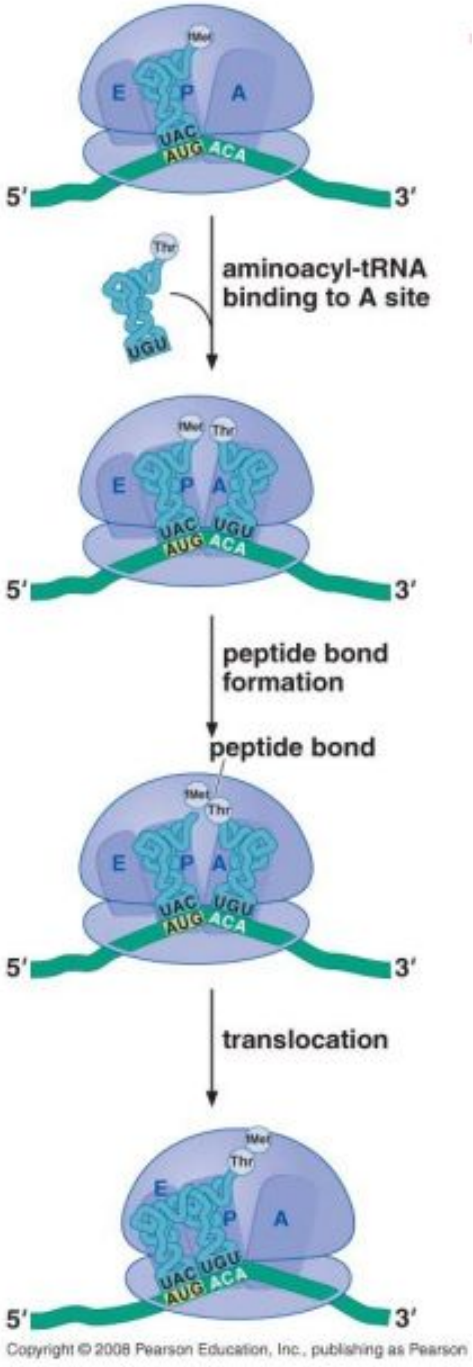
*initiator tRNA is charged with a modified form of methionine, N-formyl methionine

*deformylase removes the formyl group during or after the synthesis of polypeptide chain



*additionally, aminopeptidase often remove the amino terminal Met as well as one or two additional amino acids

Aminoacyl-tRNA in the elongation cycle



Three key events must occur:

1) correct aminoacyl-tRNA is loaded into A site

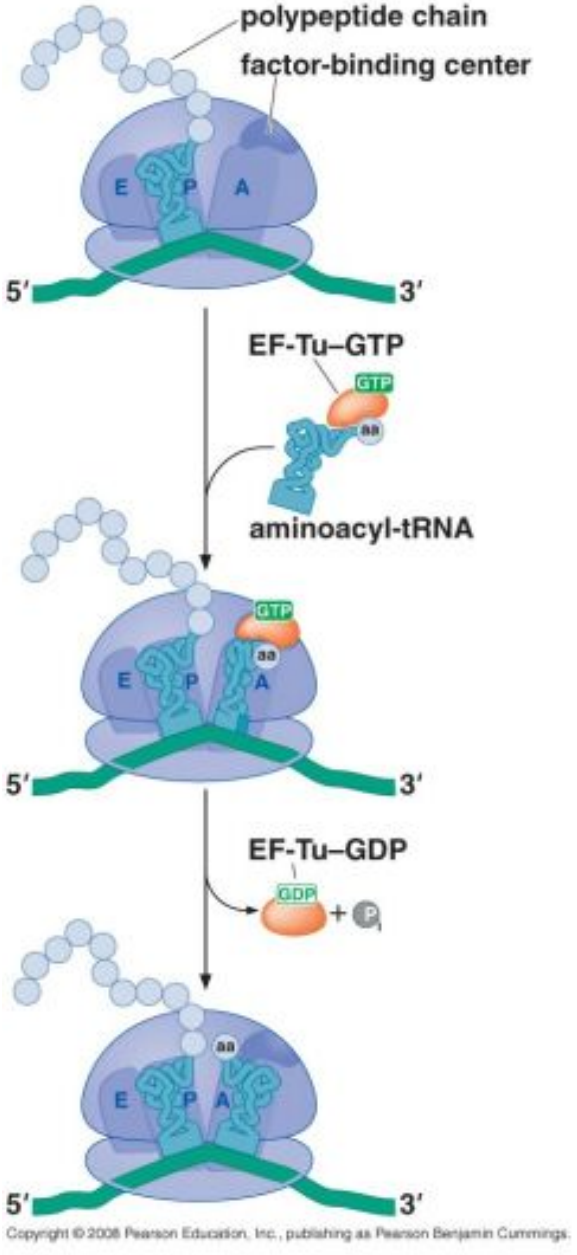
2) peptide bond formation and peptidyl transferase reaction

3) translocation to P site -two elongation factors control these events, which use the energy of GTP

Aminoacyl-tRNA in the elongation cycle

-aminoacyl-tRNA is escorted to the ribosome by elongation factor EF-Tu

-EF-Tu binds to tRNA's 3' end, masking the coupled amino acid ->
*prevent the bound aminoacyl-tRNA from participating in peptide bond formation
*affinity of EF-Tu is regulated by GTP status
*control of GTP hydrolysis by EF-Tu is critical to the specificity of translation



Aminoacyl-tRNA in the elongation cycle

The ribosome uses multiple mechanisms to select against incorrect aminoacyl-tRNAs

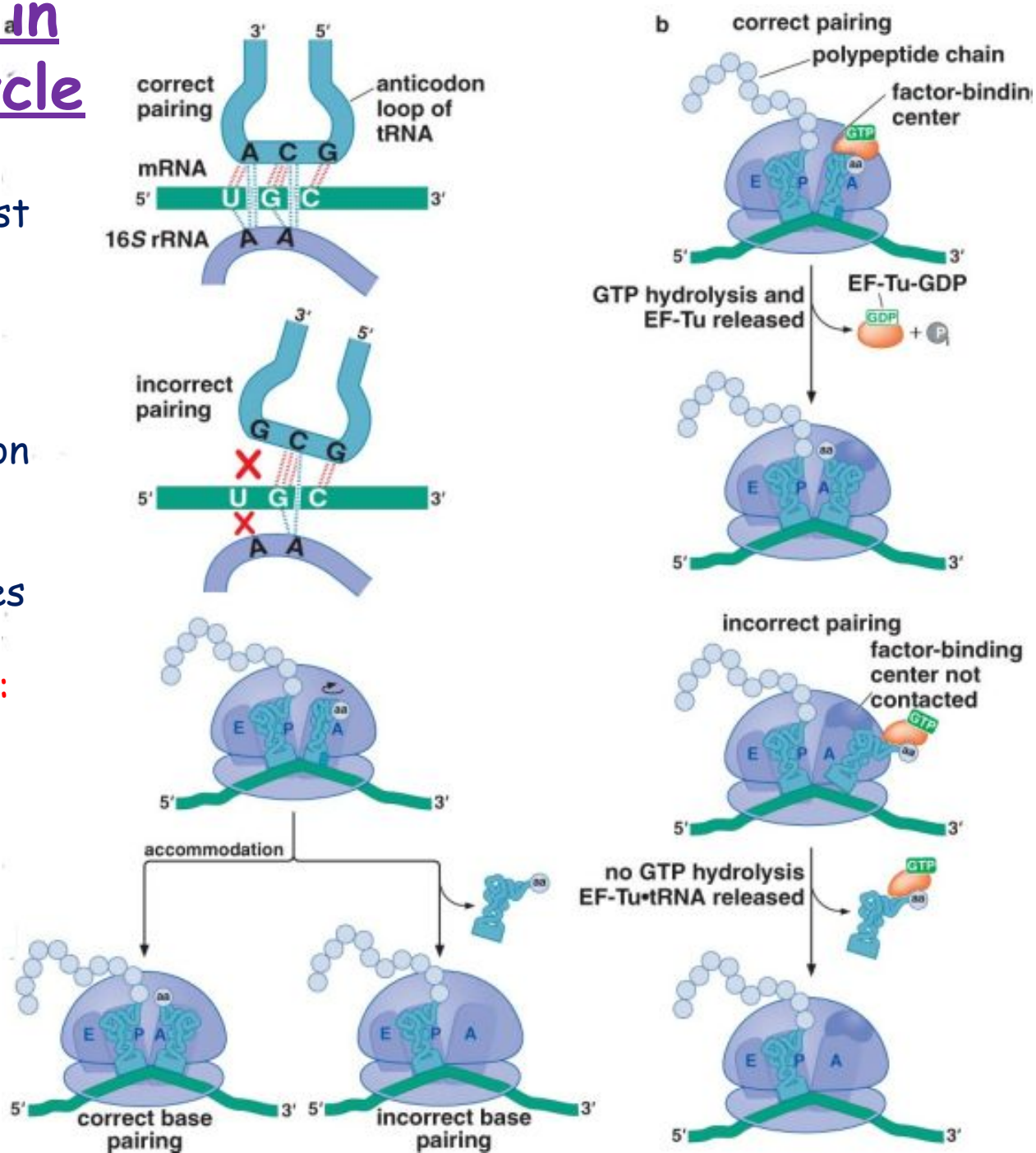
*the error rate of translation is no more than 1/1000 Æ fidelity

*codon-anticodon interaction

*three additional mechanisms 1)additional H-bonding by two A residues in 16S rRNA

2)GTPase activity of EF-Tu: single mismatch alters the position of EF-Tu, reducing interaction with factor-binding center

3)involves accommodation: only correct base-pairing sustain the strain during accommodation

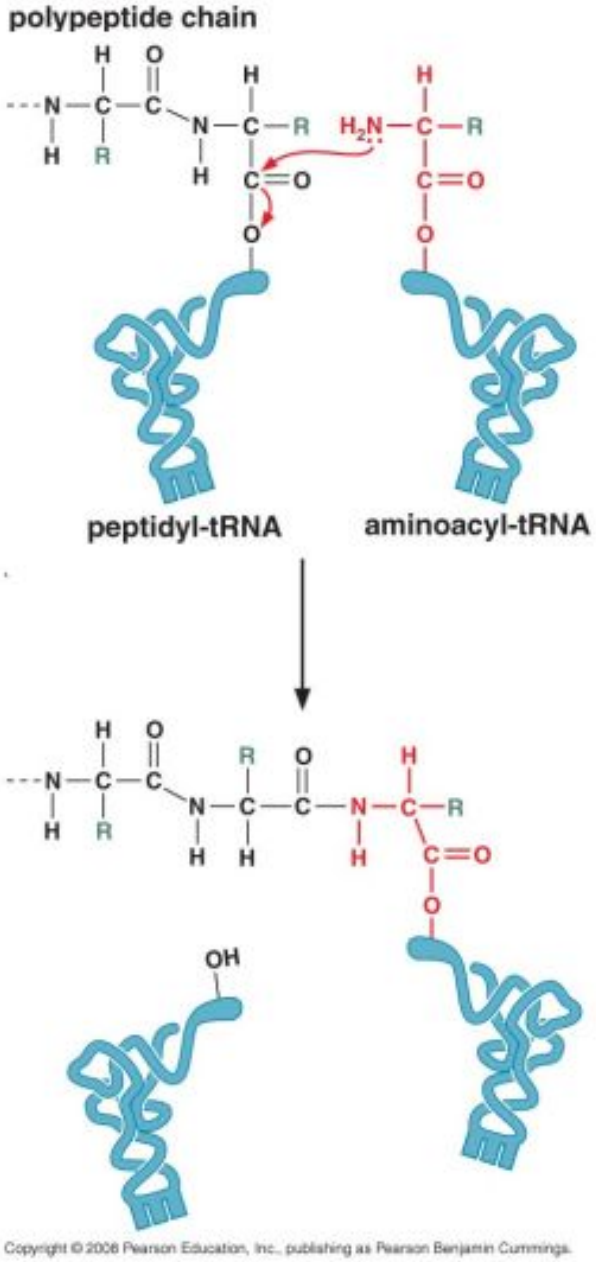


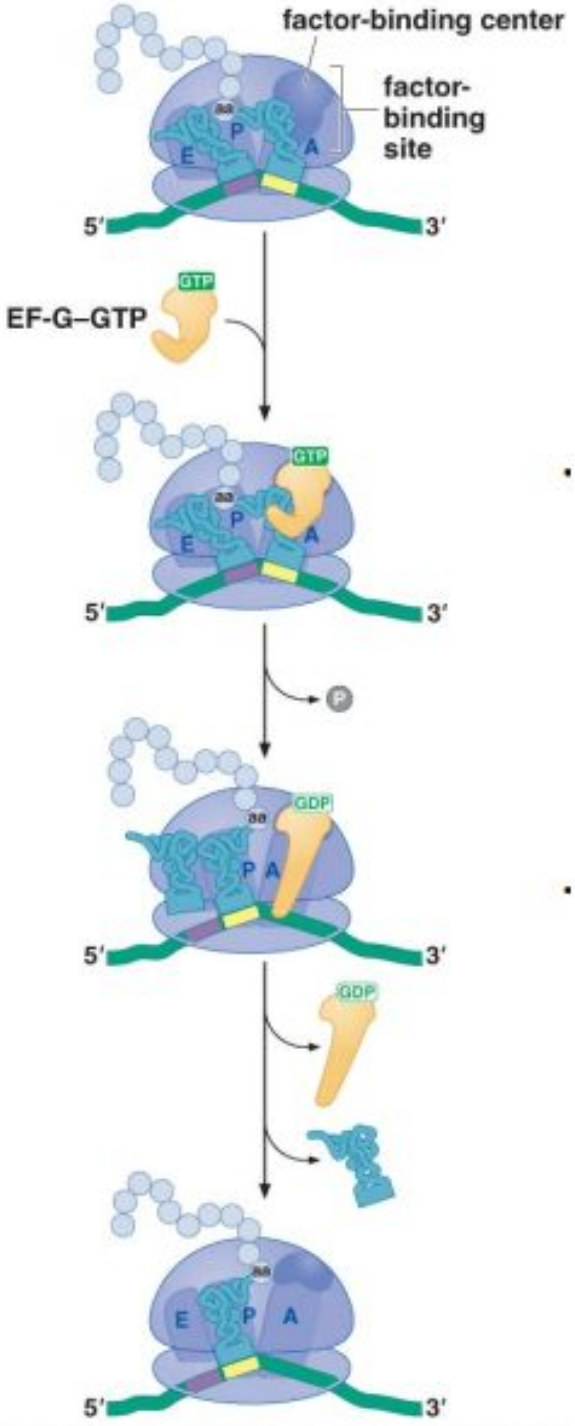
Peptide bonds are formed by transfer of the growing polypeptide chain from one tRNA to another

-intrinsic polarity of translation: 5′ to 3′ -> N to C -substrates for this reaction are two charged species of tRNA: aminoacyl-tRNA and peptidyl-tRNA

-these two substrates are brought into close proximity on the ribosome -> allow the reaction result in that growing polypeptide chain is transferred from the peptidyl-tRNA to the aminoacyl-tRNA -> thus, peptidyl transferase reactioin

-no energy input required because the energy is supplied by high energy acyl bond that are formed by ATP input during tRNA charging





Peptide bond formation and the elongation factor EF-G drive translocation of the tRNA and the mRNA

Three movement during translocation:

1) P-site tRNA -> E-site,
2) A-site tRNA -> P site,
3) mRNA to next codon

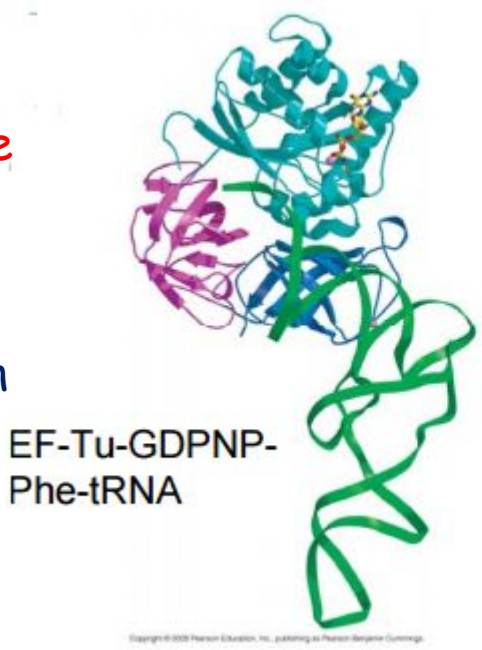
*EF-G-GTP bind to ribosome
-> contacts factor binding center
-> stimulate GTP hydrolysis
-> change conformation of EF-G, allowing it to reach into the small subunit
-> trigger translocation of A site tRNA
-> release EF-G-GDP

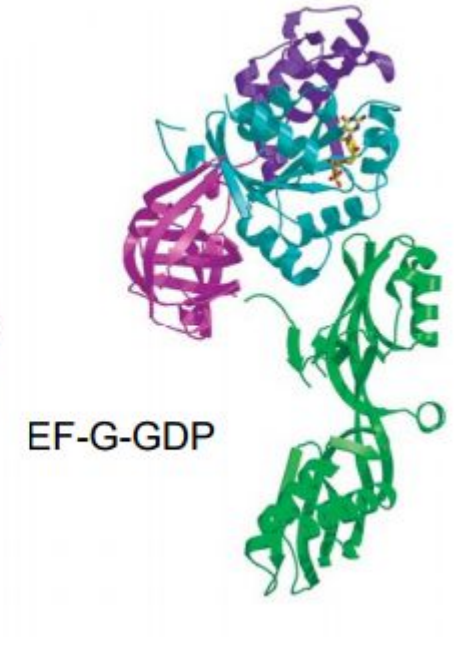
EF-G drives translocation by displacing the tRNA bound to the A site

-the translocation mechanism is not clear but part of mechanism involves the ability of EF-G-GDP to occupy the A site portion of decoding center
-probably domino-like mechanism
-movement of P-site tRNA into E site disrupt base pairing of tRNA with mRNA-> uncharged tRNA in E site is free to dissociate
-another contributor: changes in subunits or counterclockwise rotation of small subunit -> result in "gates " -> gates should open for translocation

-How does EF-G-GDP interact with the A site of the decoding center so effectively?

Molecular mimicry it is likely that such a conformational change is important for the function of EF-G during translocation





Codon-Dependent tRNA Fluctuations Monitored with Fluorescence Polarization

Padmaja P. Mishra, Mohd Tanvir Qureshi, Wenhui Ren, and Tae-Hee Lee*

During protein synthesis dictated by the codon sequence of messenger RNA, the ribosome selects aminoacyltRNA (aa-tRNA) with high accuracy, the exact mechanism of which remains elusive. By using a single-molecule fluorescence resonance energy transfer method coupled with fluorescence emission anisotropy, we provide evidence of random thermal motion of tRNAs within the ribosome in nanosecond timescale that we refer to as fluctuations. Our results indicate that cognate aa-tRNA fluctuates less frequently than near-cognate. This is counterintuitive because cognate aa-tRNA is expected to fluctuate more frequently to reach the ribosomal A-site faster than near-cognate. In addition, cognate aa-tRNA occupies the same position in the ribosome as near-cognate. These results argue for a mechanism which guides cognate aa-tRNA more accurately toward the A-site as compared to near-cognate. We suggest that a basis for this mechanism is the induced fit of the 30S subunit upon cognate aa-tRNA binding. Our single-molecule fluorescence resonance energy transfer time traces also point to a mechanistic model for GTP hydrolysis on elongation factor Tu mediated by aa-tRNA.

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