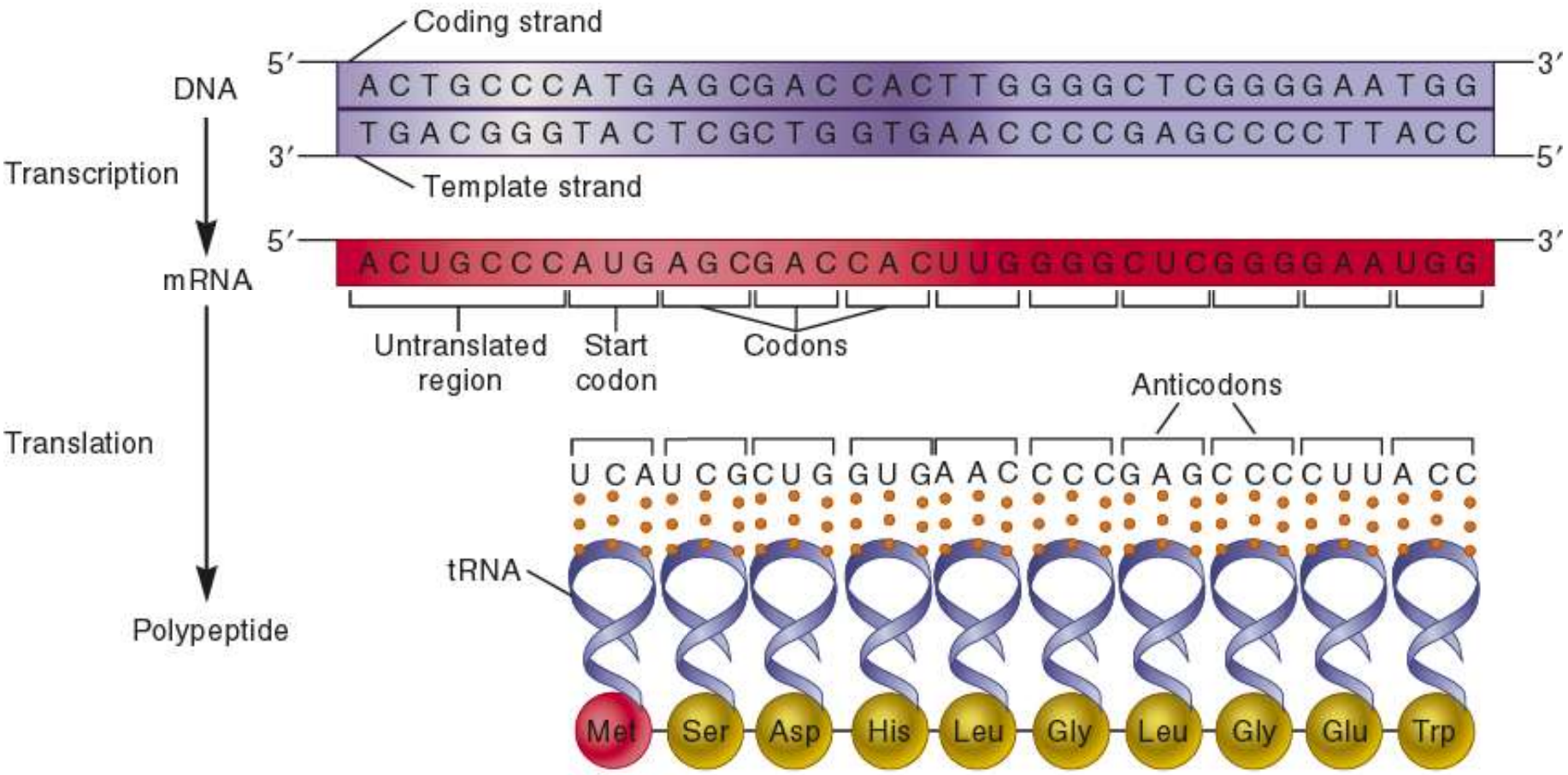
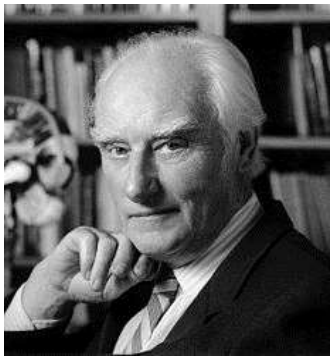
SOS in Biochemistry, Jiwaji University, Gwalior
M.Sc. II Semester (2019-20)
Paper BCH 201: Fundamentals of Molecular Biology (Unit IV)

Translational Adaptors

An Overview of Gene Expression



Crick in 1958



Formally formulated following TWO Hypotheses in the review "On Protein Synthesis" to explain 'how information present in the genetic material of an organism may get transferred to proteins':

1. **Sequence Hypothesis**
2. **Adaptor Hypothesis**

Sequence Hypothesis

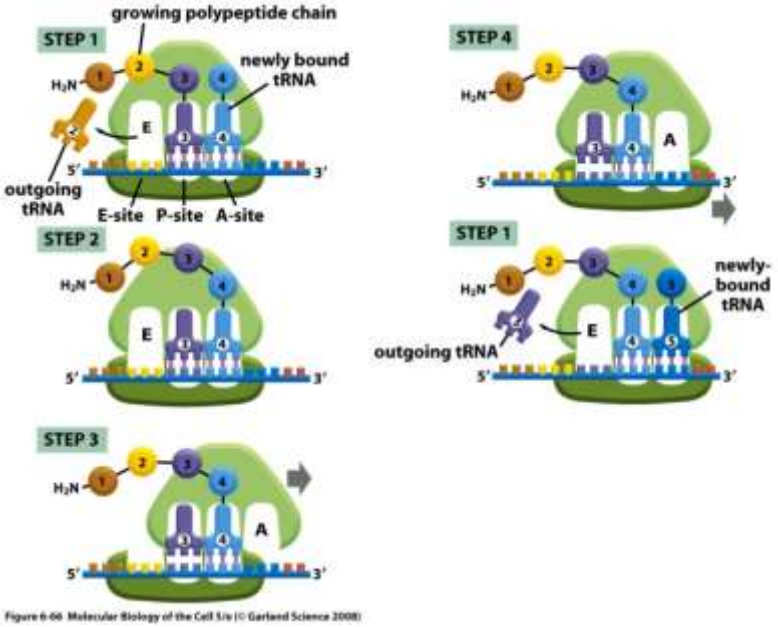
- **It states that the sequence of bases in the genetic material (DNA or RNA) determines the sequence of amino acids for which that segment of nucleic acid codes, and this amino acid sequence determines the three-dimensional structure into which the protein folds.**
- **The three-dimensional structure of a protein is required for a protein to be functional.**
- **This hypothesis then lays the essential link between information stored and inherited in nucleic acids to the chemical processes which enable life to exist.**

Adaptor Hypothesis

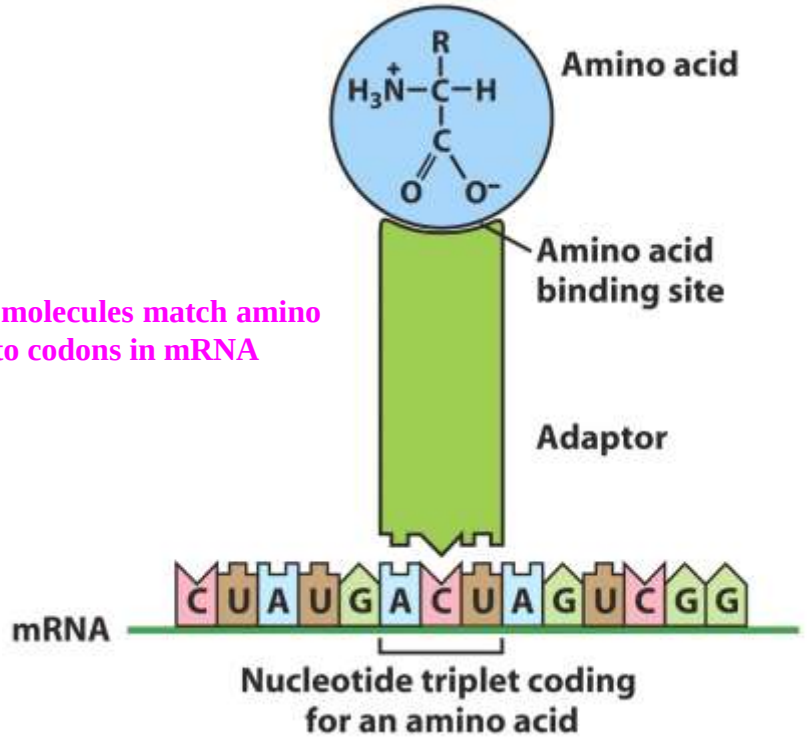
➢**The ‘Adaptor Hypothesis’ is part of a scheme to explain how information encoded in DNA is used to specify the amino acid sequence of proteins.**

Crick's Adaptor Hypothesis (1958)

- Translational adaptors are tRNA molecules
- They read the genetic code by recognizing a particular codon on mRNA
- And carry (or adopts) a specific amino acid in a growing polypeptide chain during translation as the codons appear on the mRNA.



tRNA molecules match amino acids to codons in mRNA



tRNAs share a common secondary structure that resembles a cloverleaf

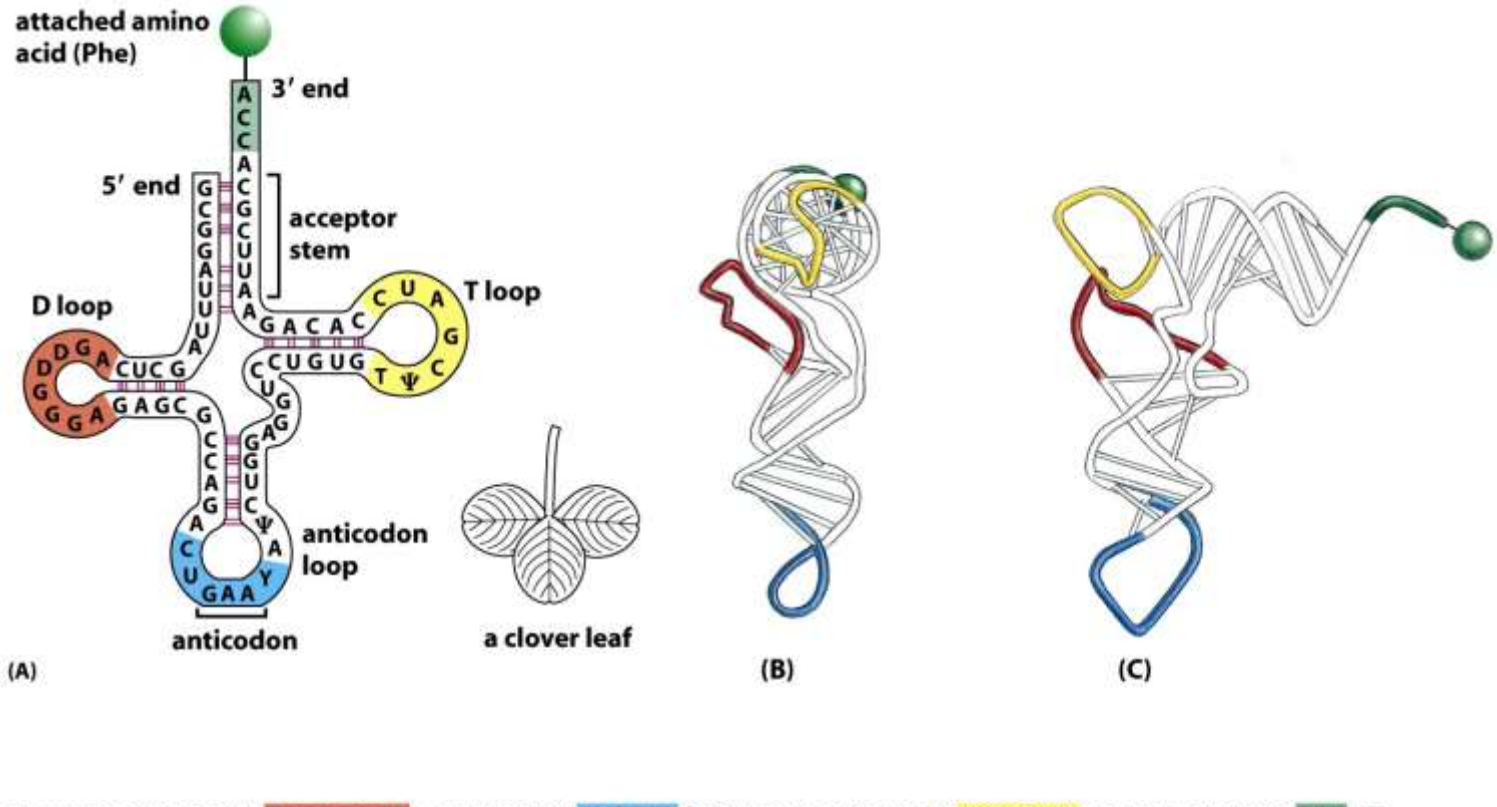
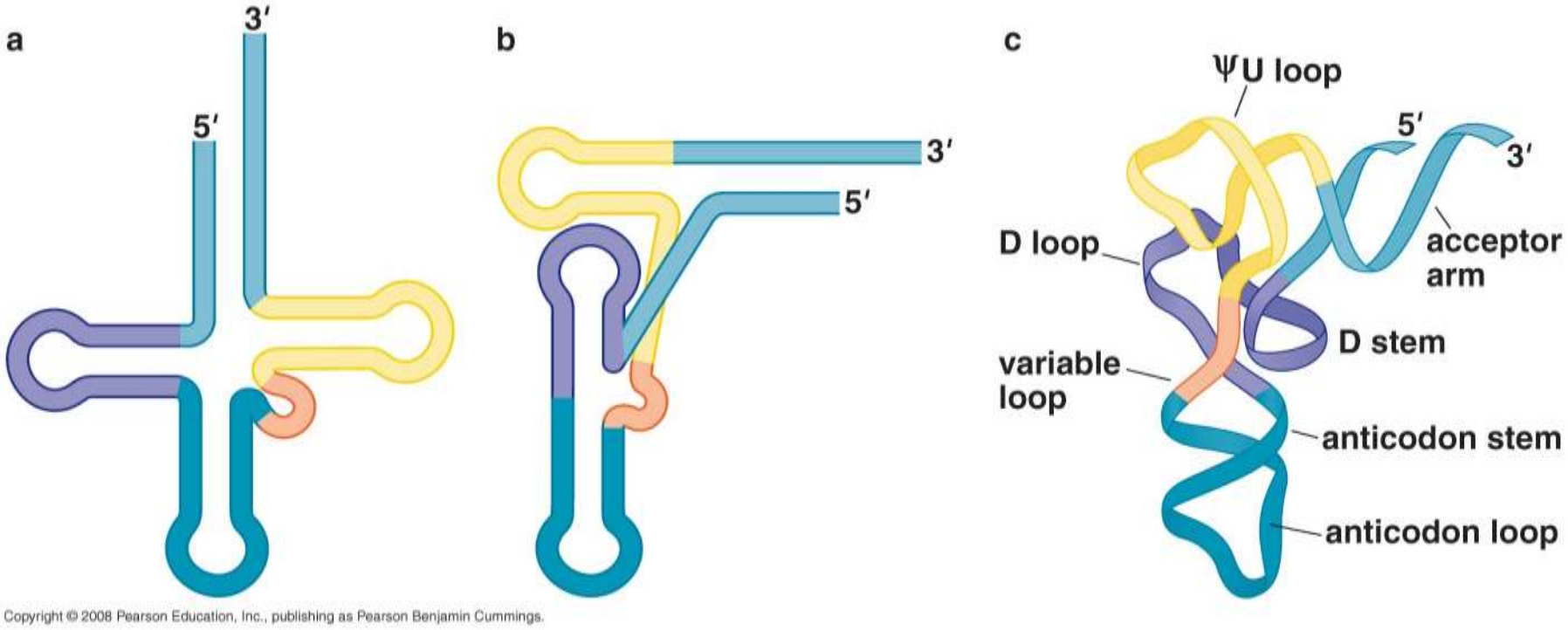
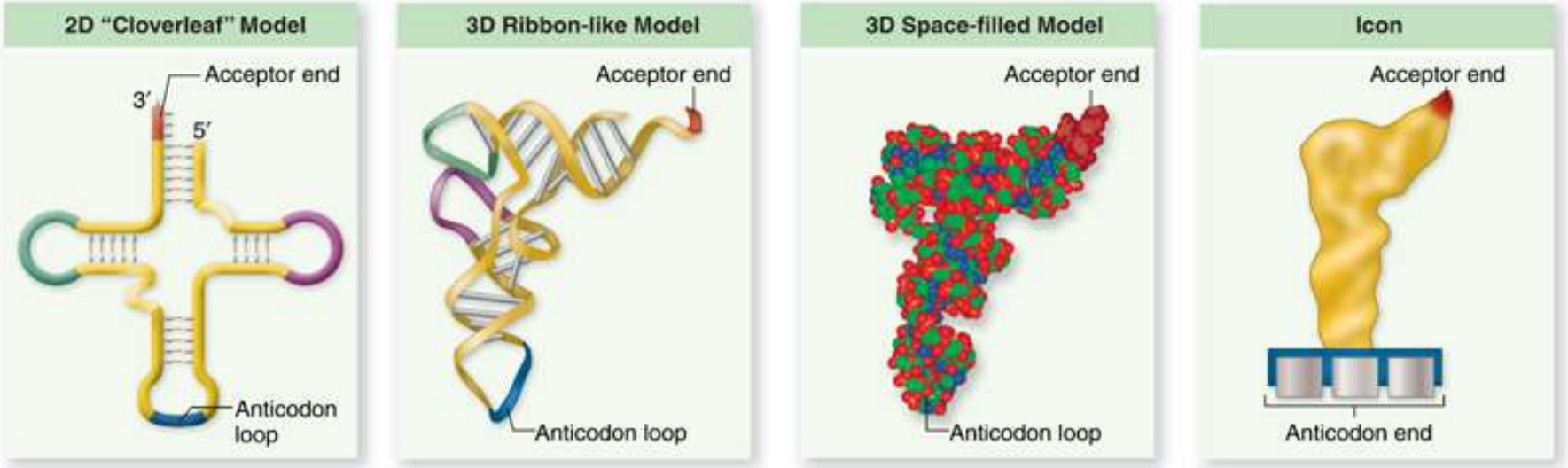


Figure 6-52 Molecular Biology of the Cell 5/e (© Garland Science 2008)

The anticodon is the sequence of three nucleotides that base-pairs with a codon in mRNA. The amino acid matching the codon/anticodon pair is attached at the 3' end of the tRNA. tRNAs contain some unusual bases, which are produced by chemical modification after the tRNA has been synthesized. For example, the bases denoted y and D are derived from uracil. (B and C) Views of the actual L-shaped molecule, based on x-ray diffraction analysis. Although a particular tRNA, that for the amino acid phenylalanine, is depicted, all other tRNAs have very similar structures. (D) The linear nucleotide sequence of the molecule, color-coded to match A, B, and C.

tRNAs have an L-shaped 3 dimensional structures



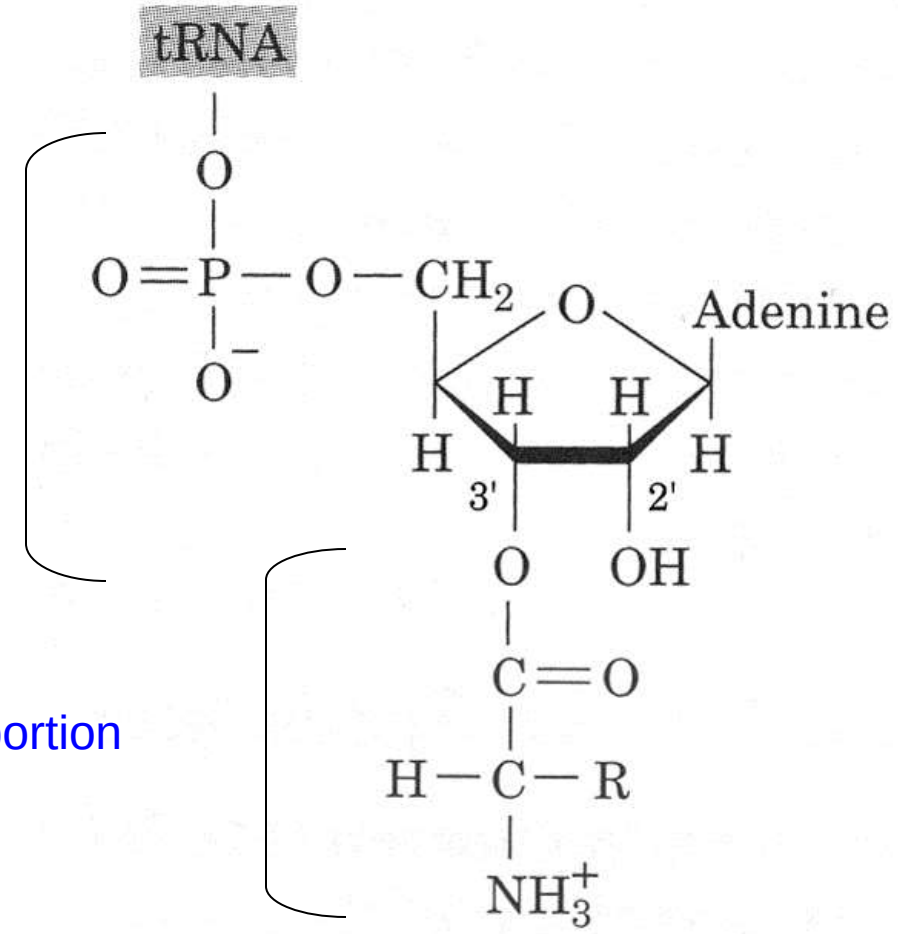


© Created by John Beaver using ProteinWorkshop, a product of the RCSB PDB, and built using the Molecular Biology Toolkit developed by John Moreland and Apostol Gramada (mbt.sdsc.edu). The MBT is financed by grant GM63208

Attachment of AA to tRNA

(tRNA Charging)

Amino acids are attached to the 3’ terminal nt of tRNAs (adenosine), via the 3’ **or** 2’ OH group.



Terminologies

Aminoacyl tRNA/Charged tRNA	**When an amino acid is attached to tRNA e.g., Seryl-tRNA^{Ser} or Seryl-tRNA**
Mischarged tRNA	**When a wrong amino acid is attached to tRNA e.g., Seryl-tRNA^{Leu} or Seryl-tRNA**
Uncharged tRNA	**When no amino acid is attached to tRNA e.g., tRNA^{Ser}, tRNA^{Leu}**

How?

ATTACHMENT OF AMINO ACIDS TO tRNA

tRNAs are charged by attachment of an AA to its 3' terminal

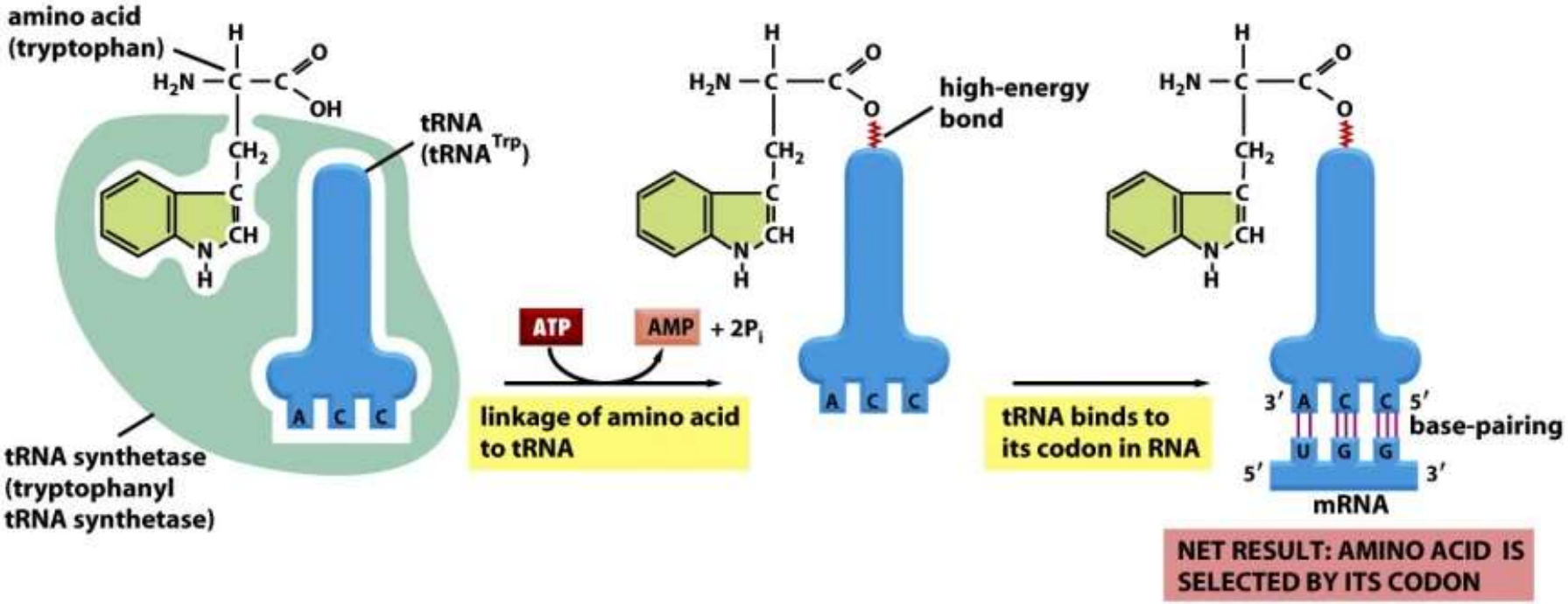


Figure 6-58 Molecular Biology of the Cell 5/e (© Garland Science 2008)

tRNA Charging

- **Occurs in two steps:**

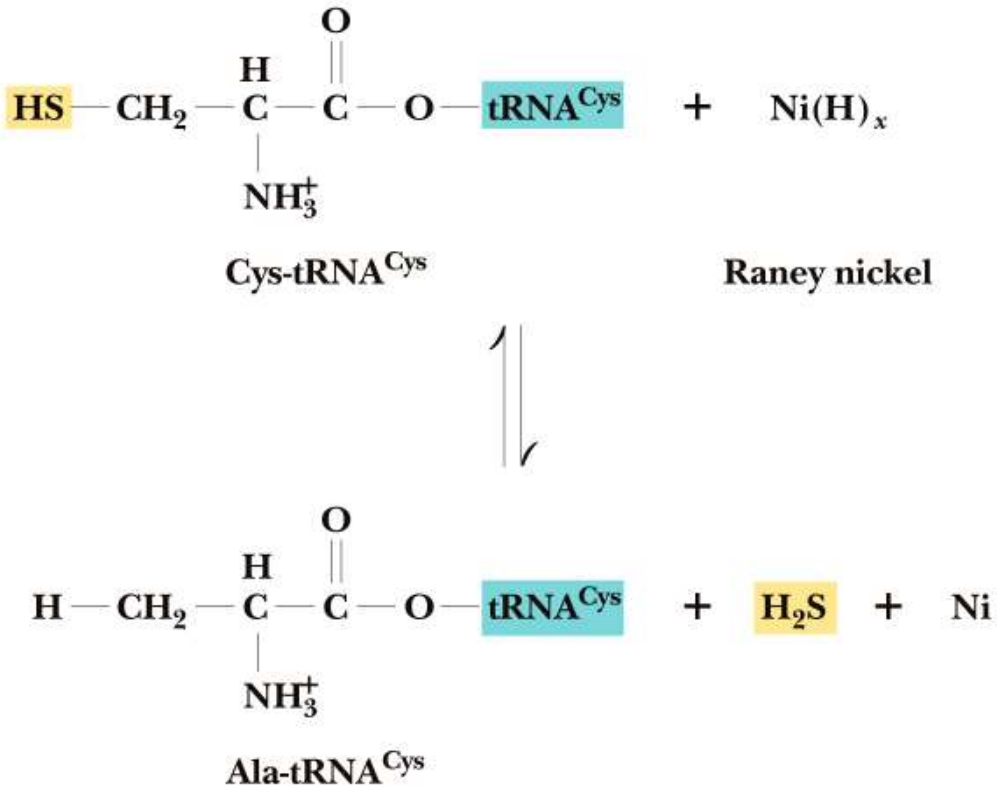
1. **AA + ATP → Aminoacyl-AMP + PP**
2. **Aminoacyl-AMP + tRNA → Aminoacyl-tRNA + AMP**

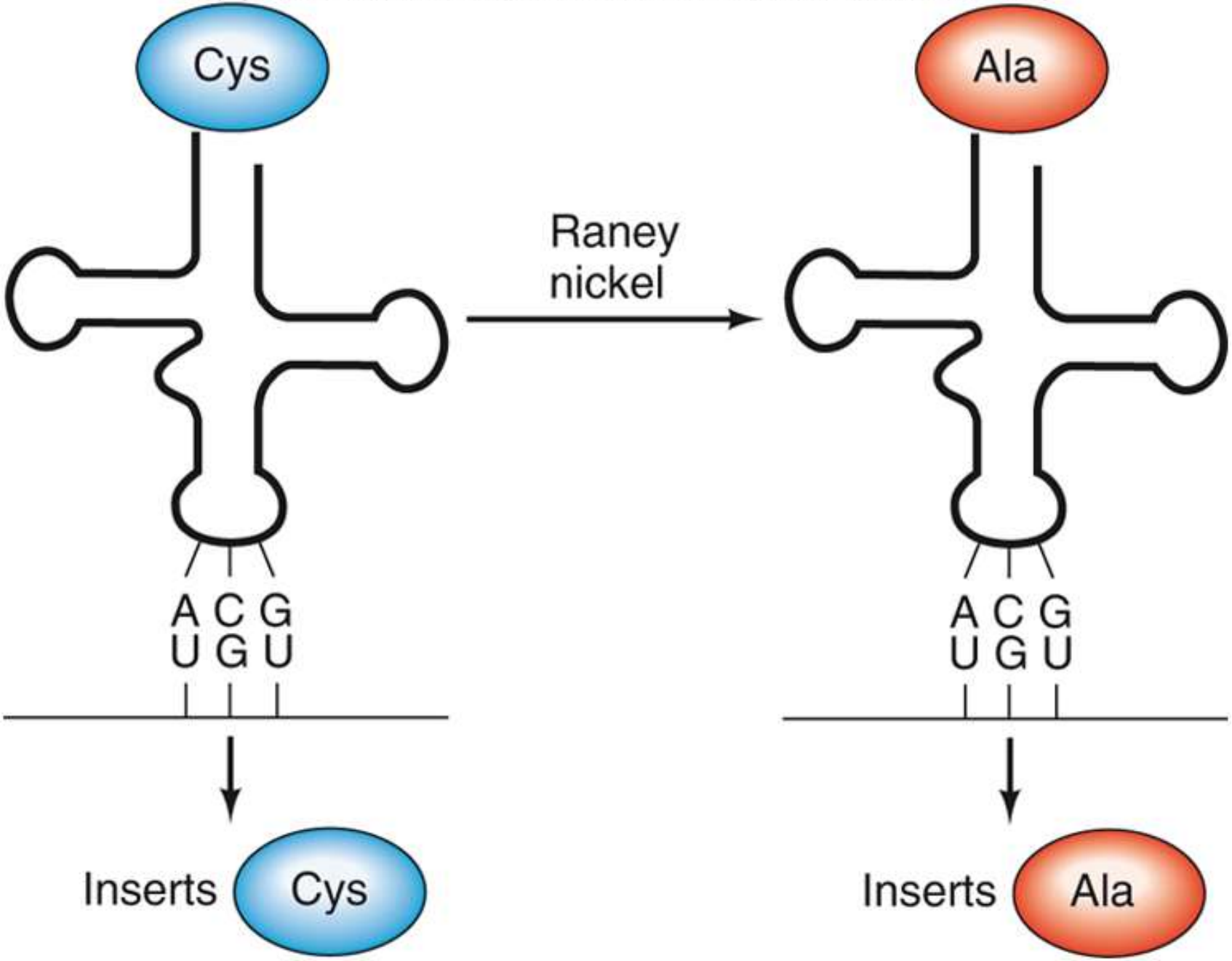
- **Catalyzed by Aminoacyl-tRNA synthetases**
- **Cells must have at least 20 aminoacyl-tRNA synthetases, one for each amino acid**

Codon Recognition

- **Codons are recognized by aminoacyl-tRNAs**
- **Base pairing must allow the tRNA to bring its particular amino acid to the ribosome**

Experiment of Lipmann & Benzer (1962)





Aminoacyl-tRNA Synthetases

- **But aminoacyl-tRNAs do something else: activate the amino acid for transfer to peptide**
- **Aminoacyl-tRNA synthetases do the critical job - linking the right amino acid with "cognate" tRNA**
- **Two levels of specificity - one in forming the aminoacyl adenylate and one in linking to tRNA**

tRNAs Are Charged with Amino Acids by Aminoacyl-tRNA Synthetases

- **Aminoacyl-tRNA synthetases are a family of enzymes that attach amino acid to tRNA, generating aminoacyl-tRNA in a two-step reaction that uses energy from ATP.**

- **Each tRNA synthetase aminoacylates all the tRNAs in an isoaccepting (or cognate) group, representing a particular amino acid.**

- **Each type of amino acid is activated by a different amino acyl tRNA synthetase.**
- **Two high-energy bonds from an ATP are required.**
- **The aminoacyl tRNA synthetase transfers the activated amino acid to the 3' end of the correct tRNA.**
- **The amino acid is linked to its cognate tRNA with an energy-rich bond.**
- **This bond will later supply energy to make a peptide bond linking the amino acid into a protein.**

Aminoacyl-tRNA Synthetases

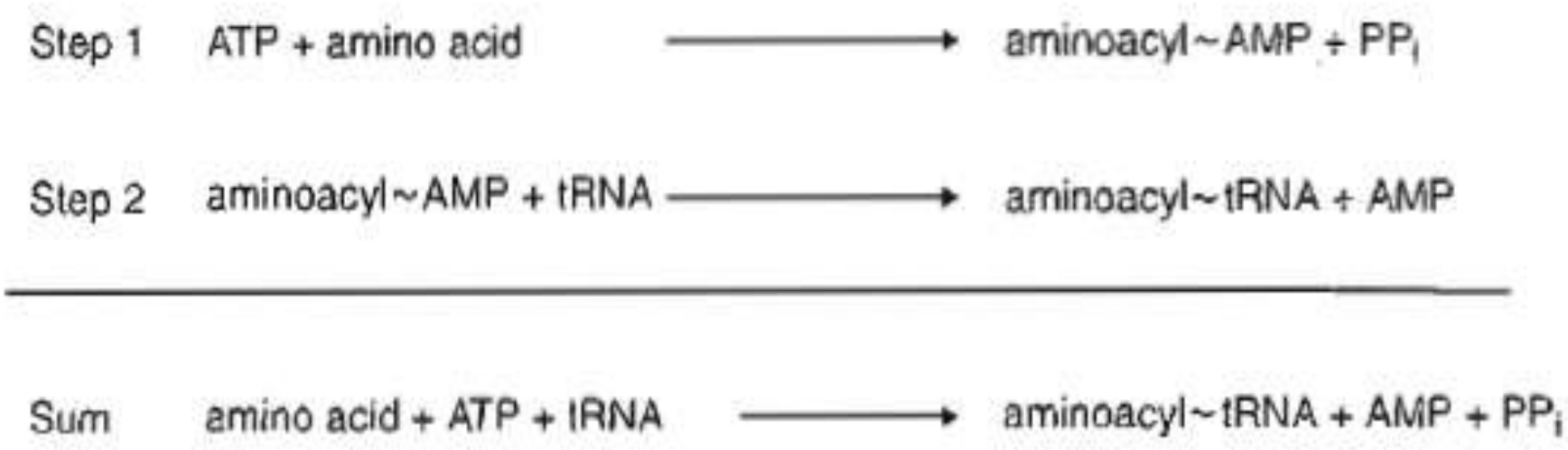
Mechanism and Specificity

- **Deacylase activity "edits" and hydrolyzes misacylated aminoacyl-tRNAs**
- **Despite common function, the synthetases are a diverse collection of enzymes**
- **Four different quaternary structures: α, α_2, α_4 and $\alpha_2\beta_2$**
- **Subunits from 334 to more than 1000 residues**
- **Amino acid acylation reaction occurs in two different steps:**

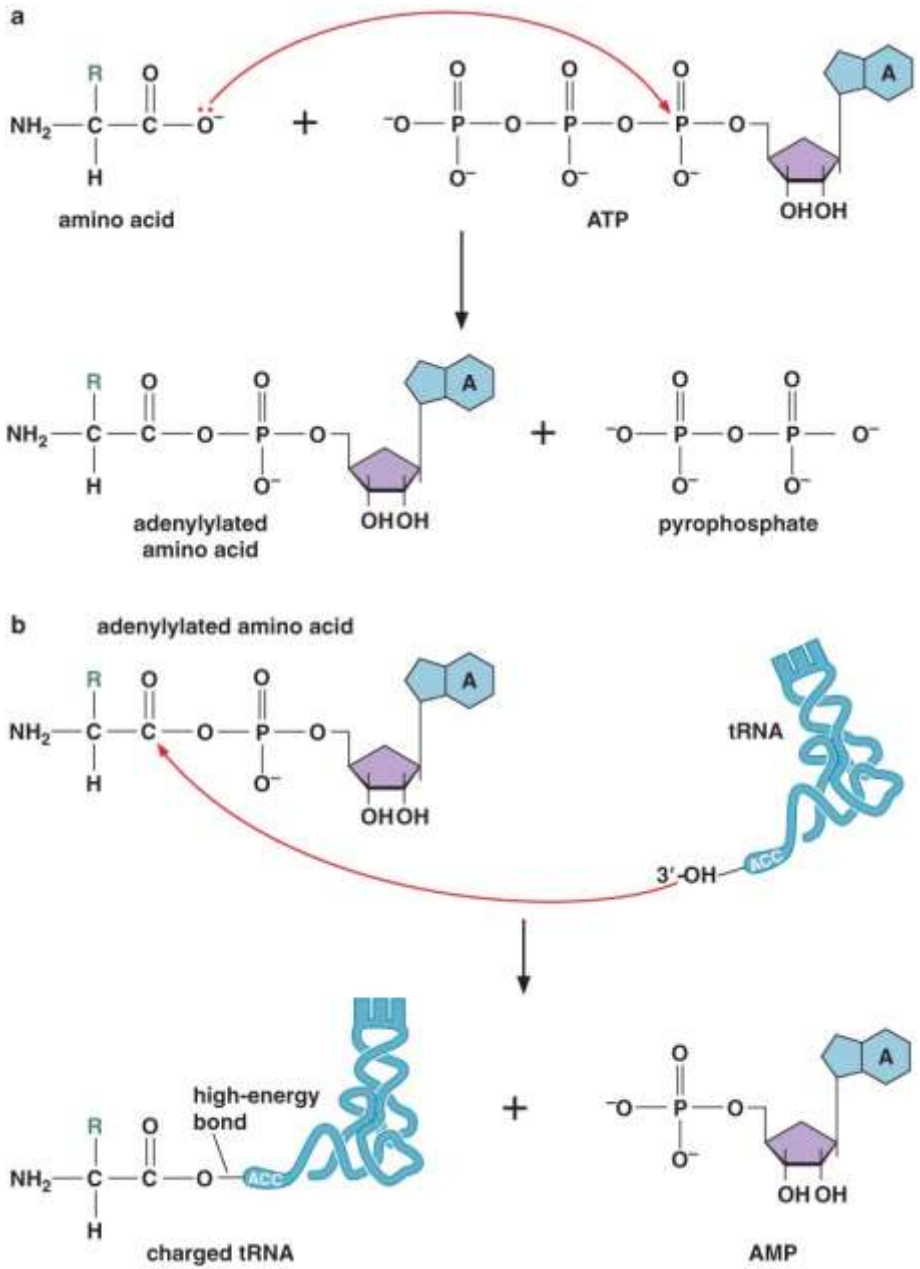
  a) Activation Step

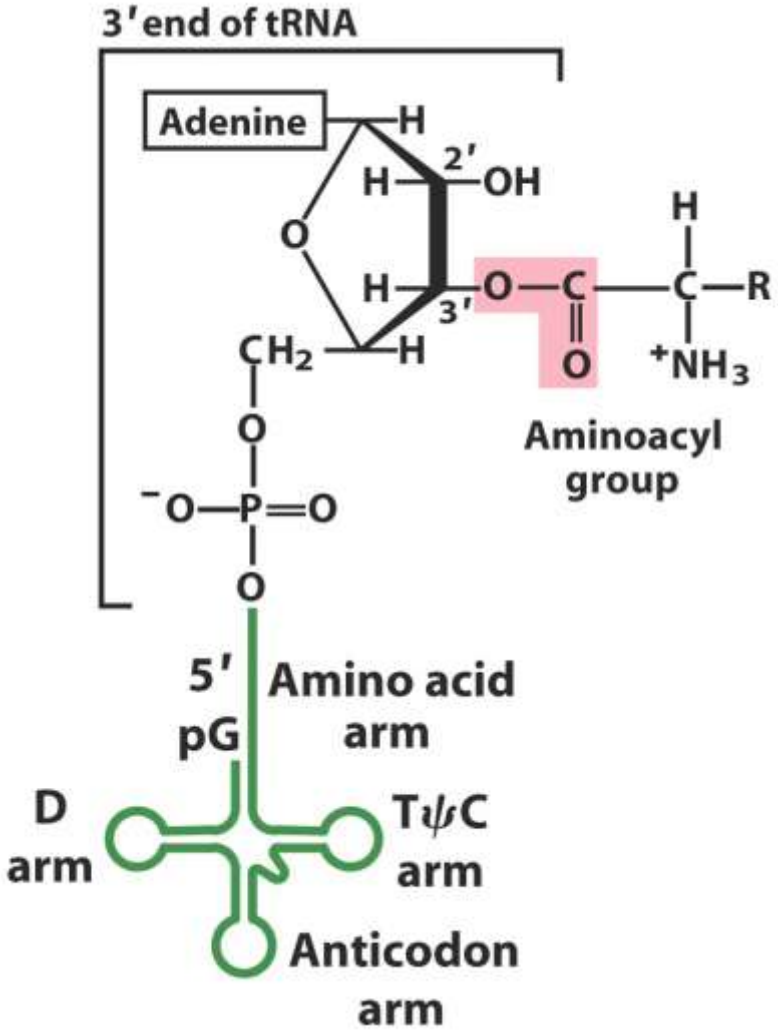
  b) Transfer Step

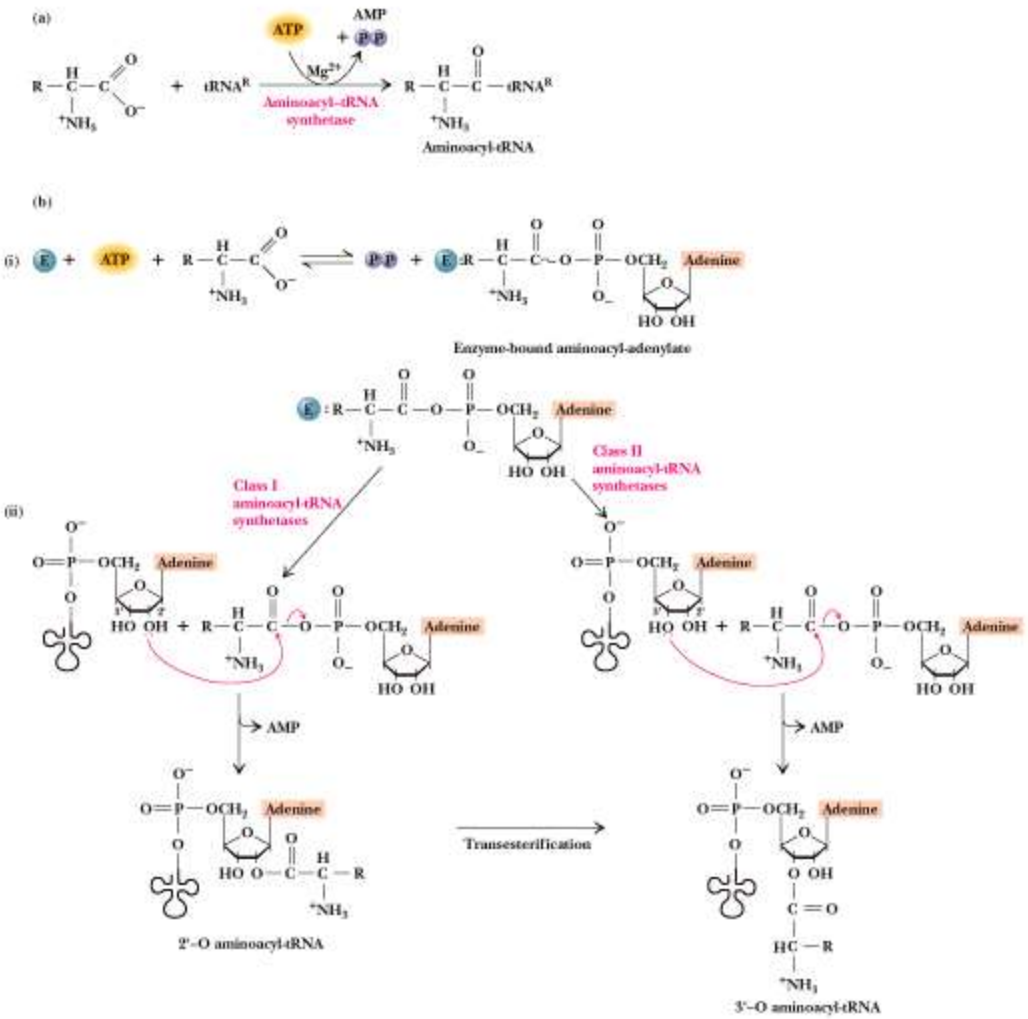
Formation of Aminoacyl tRNA



Aminoacyl-tRNA synthetases charge tRNAs in two steps





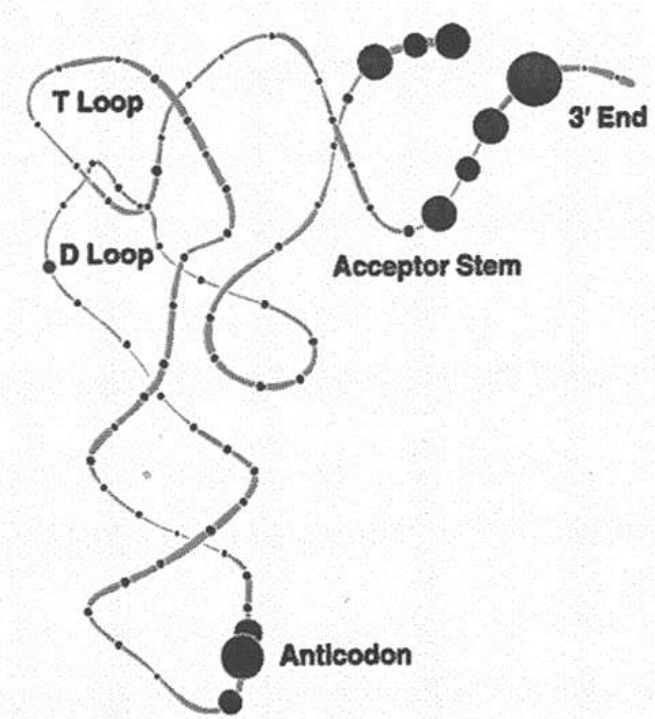

(a)
ATP
AMP
+ P P
Mg2+
Aminoacyl-tRNA synthetase
tRNAR
Aminoacyl-tRNA
(b)
(i)
Enzyme-bound aminoacyl-adenylate
Class I aminoacyl-tRNA synthetases
Class II aminoacyl-tRNA synthetases
(ii)
AMP
Transesterification
2'–O aminoacyl-tRNA
3'–O aminoacyl-tRNA
Adenine

- **Aminoacyl tRNA synthetases have self-checking functions to prevent incorrectly paired amino acyl tRNAs from forming.**

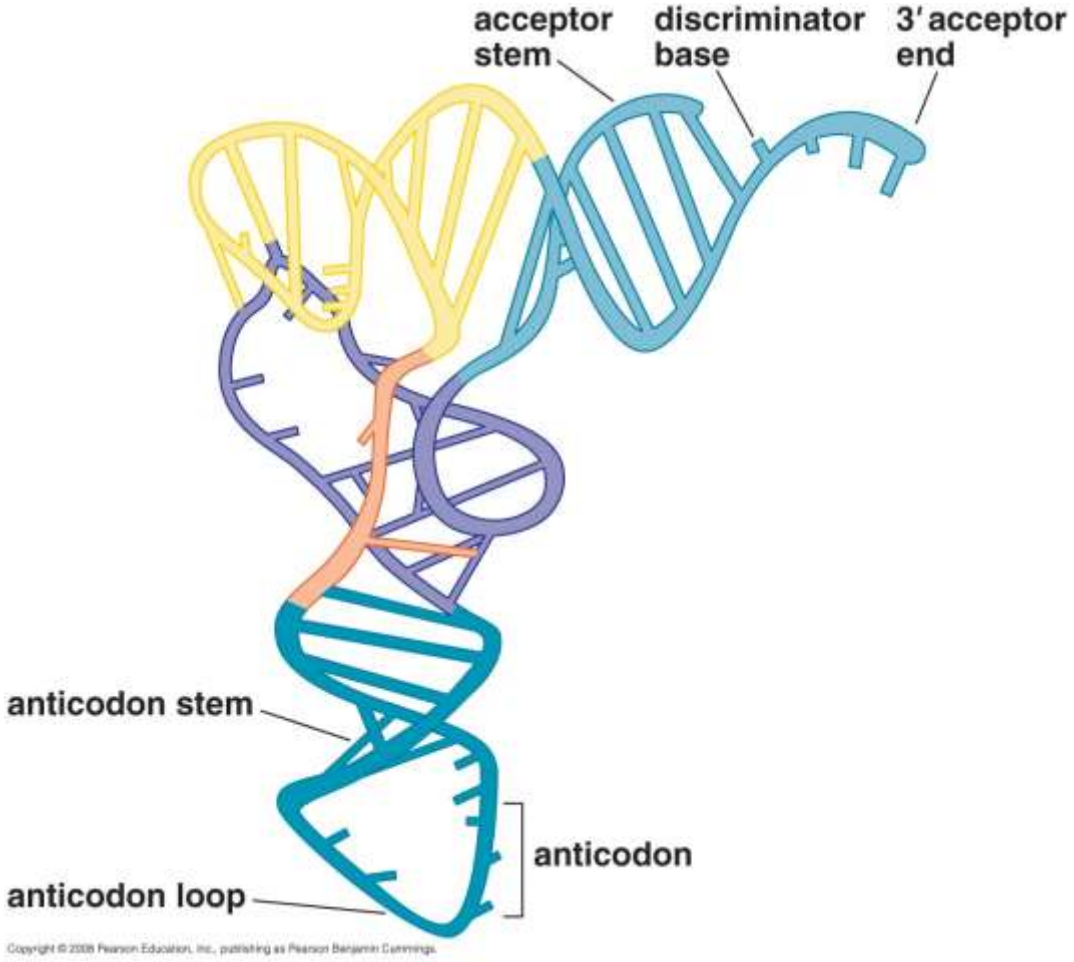
- **If, however, an aminoacyl tRNA synthetase does release an incorrectly paired product (ala-tRNASer), there is no mechanism during translation to detect the error, and an incorrect amino acid will be introduced into some protein.**

Recognition of tRNAs by Aminoacyl-tRNA synthetases

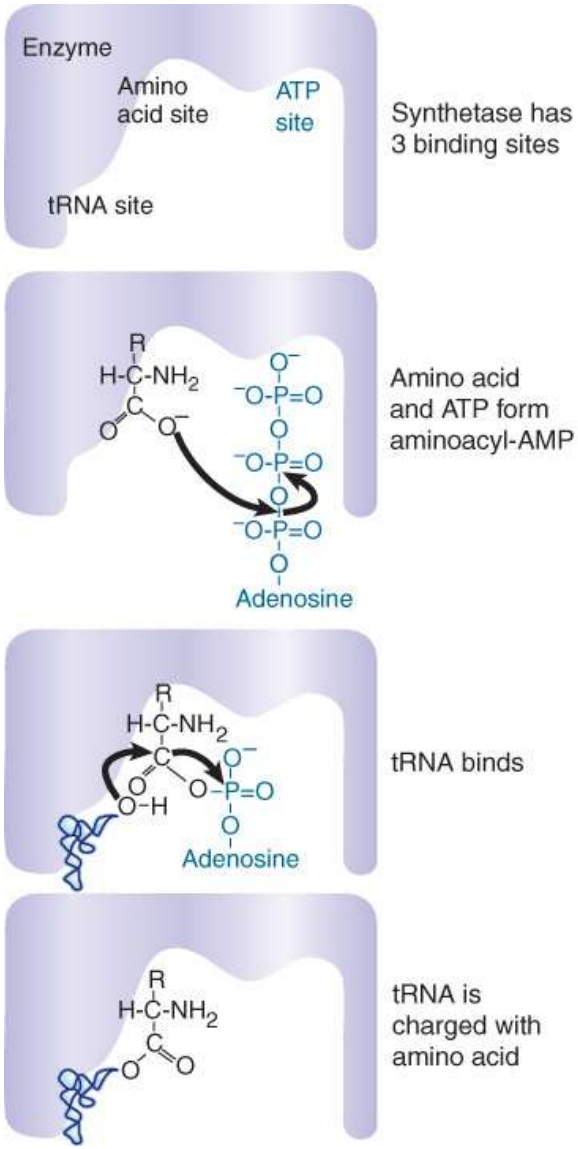
Aminoacyl-tRNA synthetases recognize mainly the acceptor stem and the anticodon loop region of tRNA that is also called as 'Identity Set'.



Positions of observed nucleotides that determine tRNA identity.

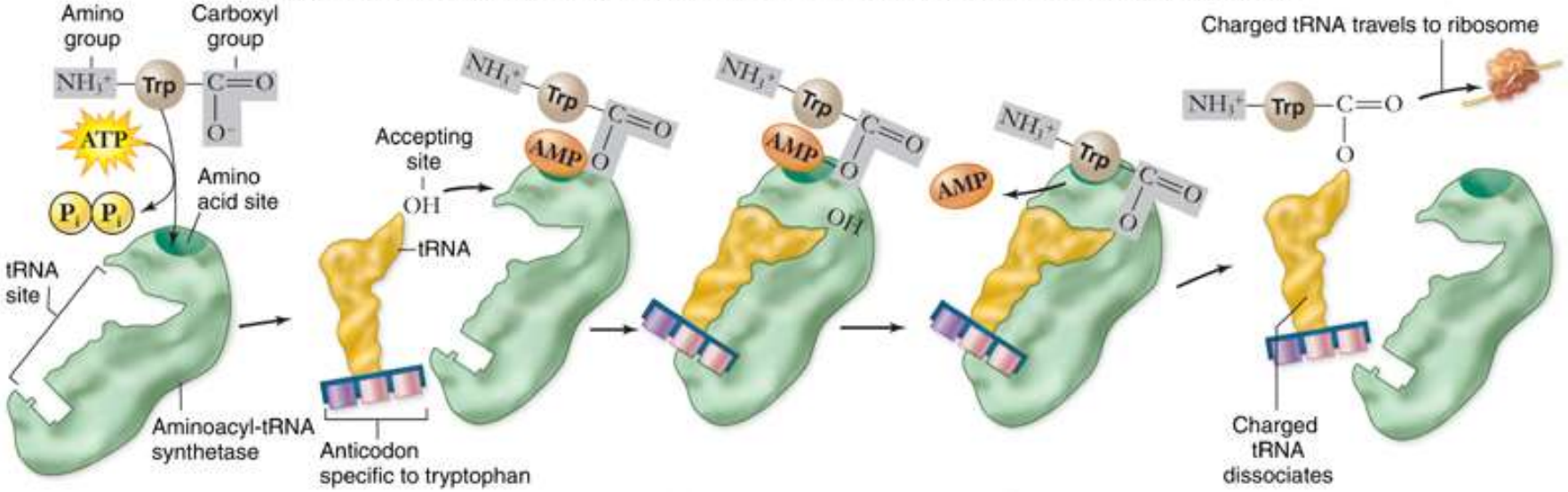


tRNAs Are Charged with Amino Acids by Aminoacyl-tRNA Synthetases



Recognition of tRNA by tRNA synthetases is based on a particular set of nucleotides, the tRNA "identity set," that often are concentrated in the acceptor stem and anticodon loop regions of the molecule.

Figure: An aminoacyl-tRNA synthetase charges tRNA with an amino acid.



1. In the first step of the reaction, the amino acid is activated. The amino acid reacts with ATP to produce an intermediate with the carboxyl end of the amino acid attached to AMP. The two terminal phosphates (pyrophosphates) are cleaved from ATP in this reaction.

2. The amino acid-AMP complex remains bound to the enzyme. The tRNA next binds to the enzyme.

3. The second step of the reaction transfers the amino acid from AMP to the tRNA, producing a charged tRNA and AMP. The charged tRNA consists of a specific amino acid attached to the 3′ acceptor stem of its RNA.

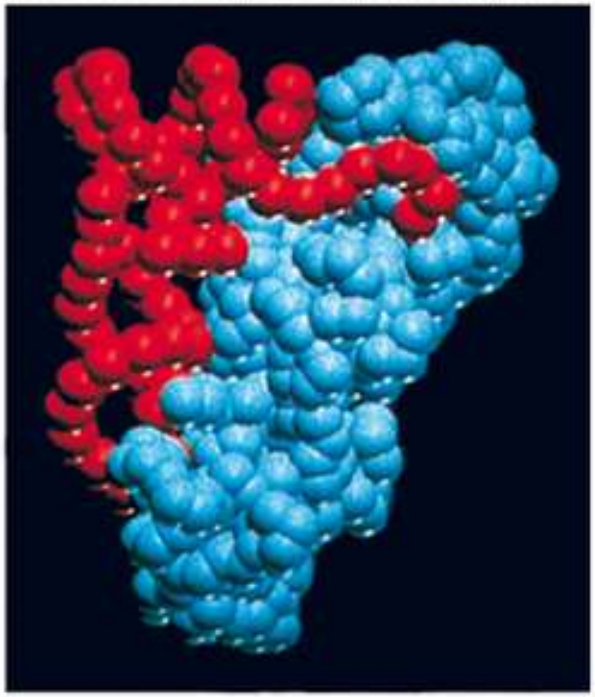
Types of Aminoacyl-tRNA Synthetases

Aminoacyl-tRNA synthetases (cont.)

- **Diverse group of enzymes despite recognizing fairly similar substrates**
- **Not well conserved, however there are 2 main classes**
  - **Class I (aminoacylate the 2' OH)**
  - **Class II (aminoacylate the 3' OH)**
- **Each class has the same 10 members in all organisms**
- **The classes bind tRNA somewhat differently, but both bind to the acceptor stem and the anticodon loop**

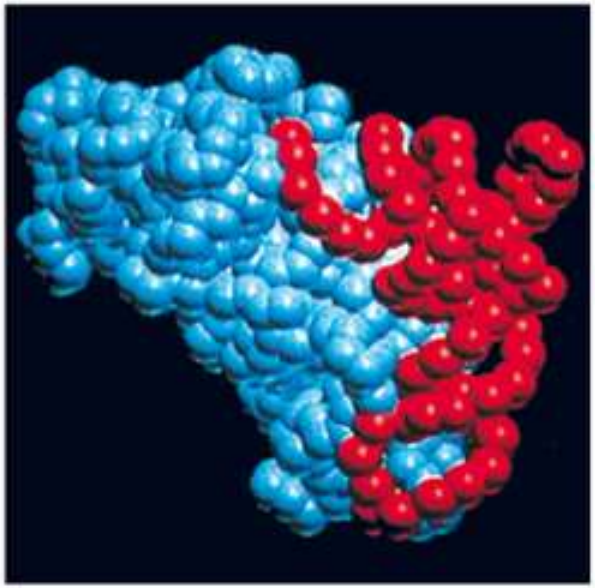
Class I - binds from the D loop side

(a)



GlnRS – $tRNA^{Gln}$ (Class I)

(b)



Class II – binds from the Variable loop side

AspRS-$tRNA^{Asp}$ (Class II)

© Ruff, M., S. Krishnaswamy, M. Boeglin, A. Poterszman, A. Mitschler, A. Podjarny, B. Rees, J.C. Thierry, and D. Moras, Class II aminoacyl transfer RNA synthetases: Crystal structure of yeast aspartyl-tRNA synthetase complexed with tRNA\Asp/. *Science/xi

Two Classes of Aminoacyl-tRNA Synthetases

Basis of classification in two families:

Mutually exclusive sets of sequence motifs and structural domains.

Aminoacyl-tRNA synthetases

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

Each aminoacyl-tRNA synthetase attaches a single amino acid to one or more tRNAs

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.

Aminoacyl-tRNA formation is very accurate

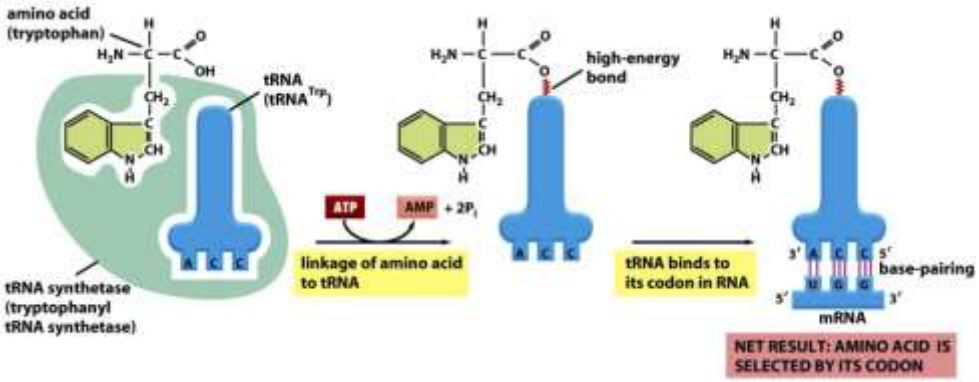
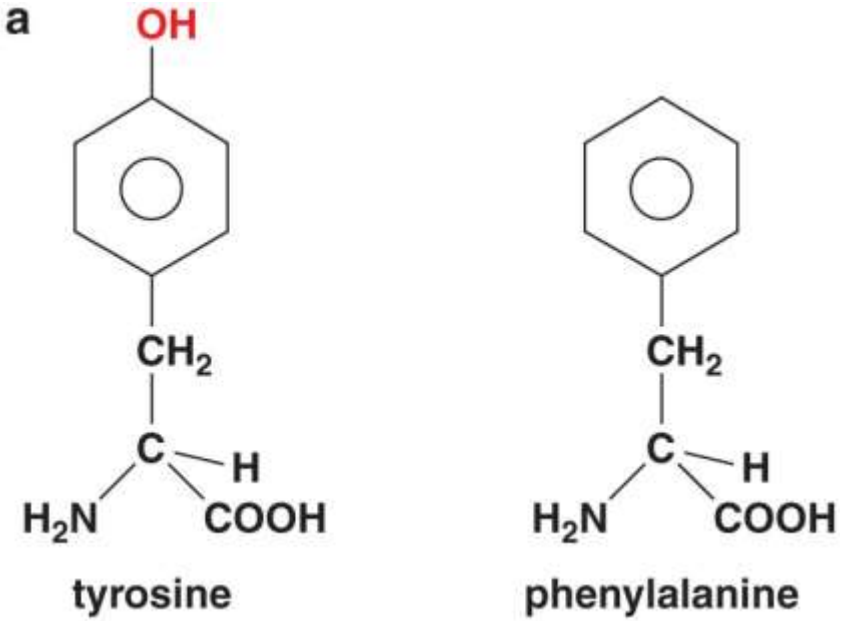
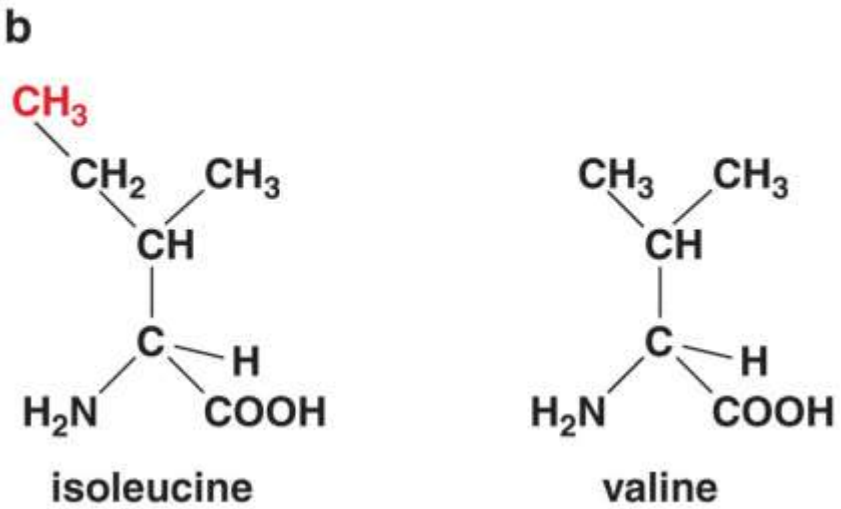


Figure 6-58 Molecular Biology of the Cell 5/e (© Garland Science 2008)





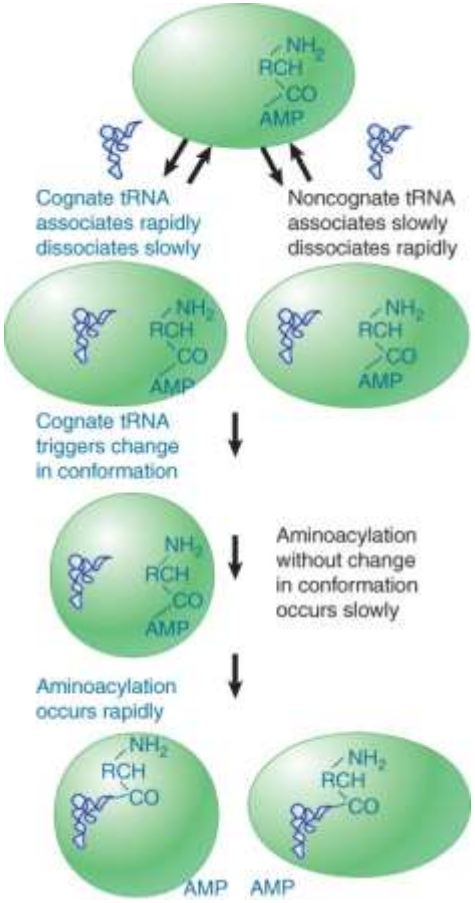
Synthetases Use Proofreading to Improve Accuracy of Charging Activity

- **Specificity of amino acid-tRNA pairing is controlled by proofreading reactions that hydrolyze incorrectly formed aminoacyl adenylates and aminoacyl-tRNAs.**

- **Kinetic Proofreading - A proofreading mechanism that depends on incorrect events proceeding more slowly than correct events, so that incorrect events are reversed before a subunit is added to a polymeric chain.**

- **Chemical Proofreading - A proofreading mechanism in which the correction event occurs after the addition of an incorrect subunit to a polymeric chain, by means of reversing the addition reaction.**

Synthetases Use Proofreading to Improve Accuracy of Charging Activity

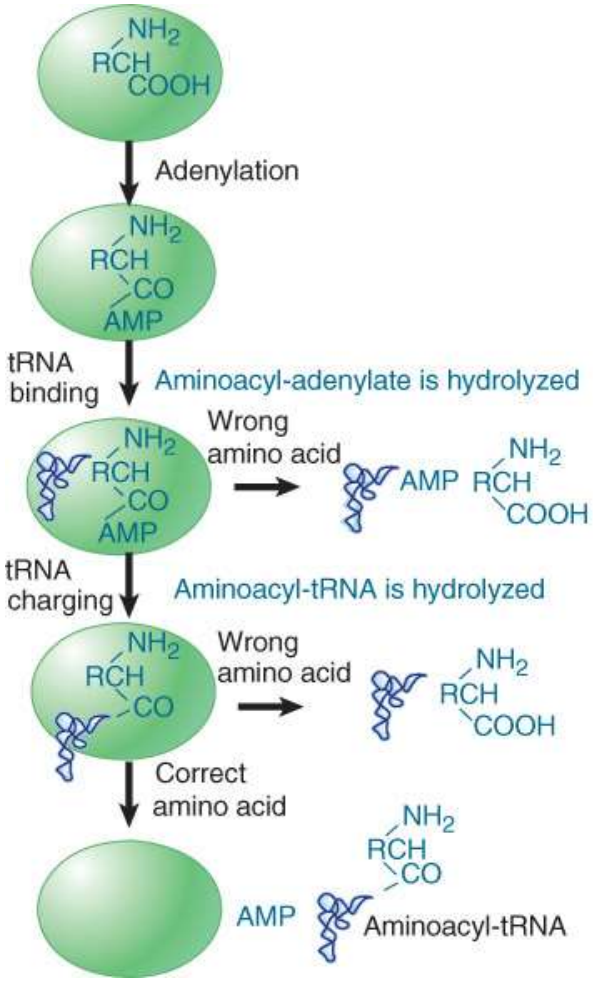


Kinetic Proofreading by aminoacyl-tRNA synthetases.

Kinetic Proofreading

A proofreading mechanism that depends on incorrect events proceeding more slowly than correct events, so that incorrect events are reversed before a subunit is added to s polymeric chain.

Synthetases Use Proofreading to Improve Accuracy of Charging Activity



Chemical Proofreading by aminoacyl-tRNA synthetases.

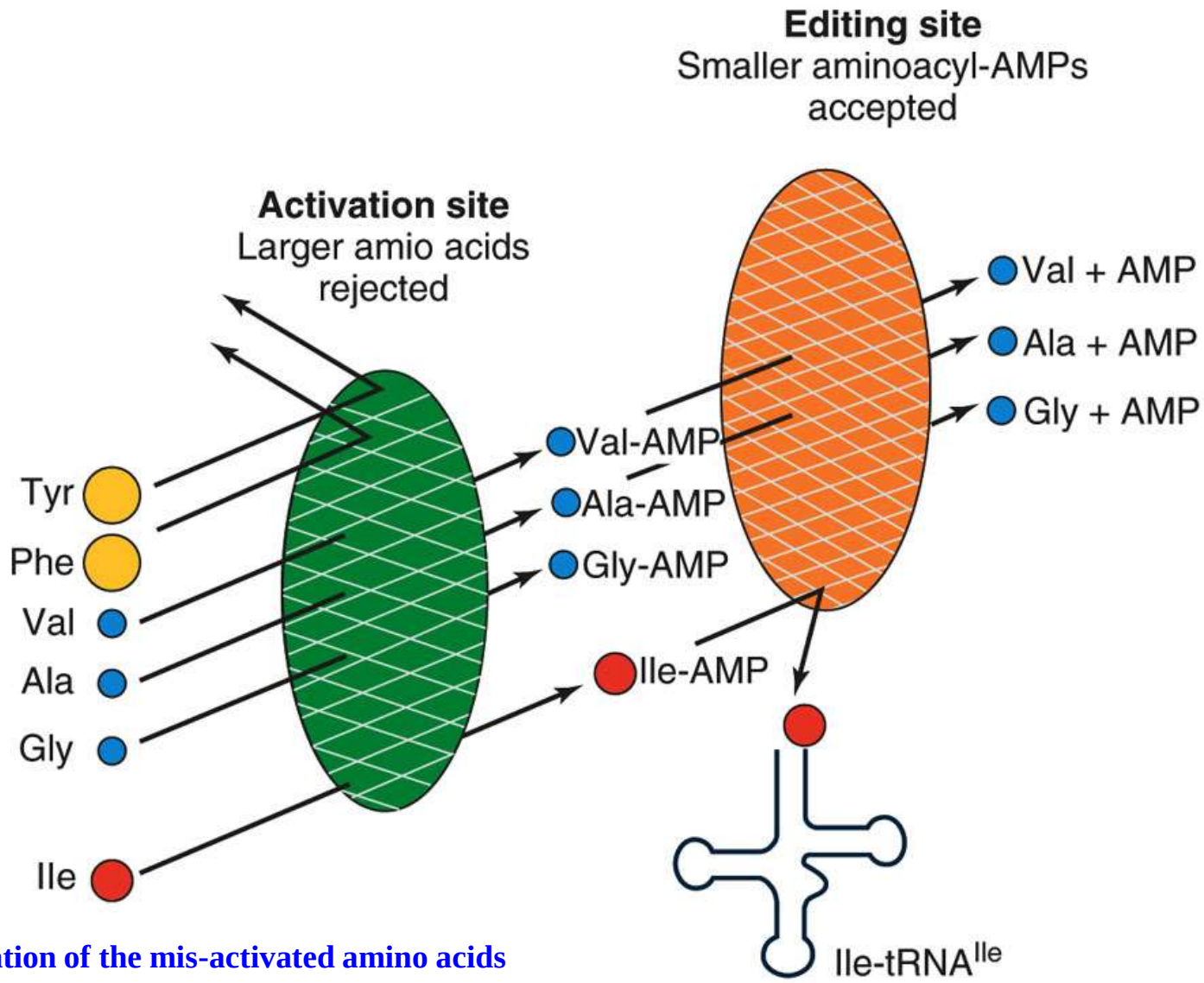
Chemical Proofreading

A proofreading mechanism in which the correction event occurs after the addition of an incorrect subunit to a polymeric chain, by means of reversing the addition reaction.

How is charging accuracy achieved?
(given the structure of amino acids)

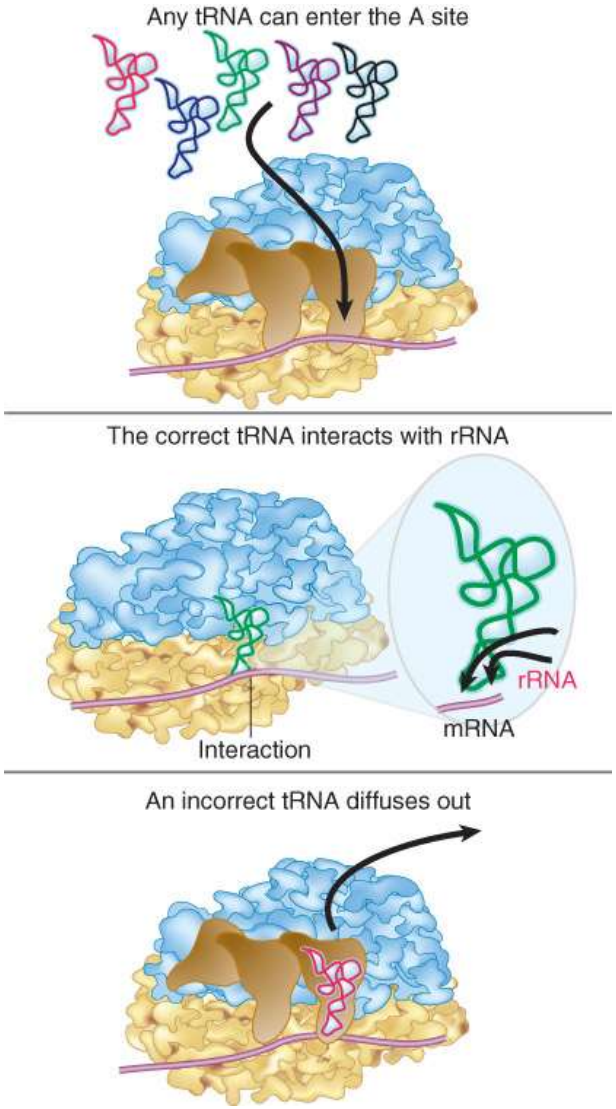
- **Isoleucine tRNA synthetase (IleRS is Class I) discriminates > 50,000-fold for Ile over valine**
- **Ile and Val differ by only one methylene group (Isoleucine has 1 more)**
- **Accuracy achieved by the IleRS having 2 active sites: 1st one activates most small amino acids (to aa-AMP) and the 2nd one hydrolyzes the aa-AMPs smaller than Isoleucine (the editing site)**

The **double-sieve** model for IleRS



Also, translocation of the mis-activated amino acids between the 2 active sites on the protein requires the tRNA to be bound. Protein also changes conformation. Similar mechanism for other class I tRNA synthetases.

The Ribosome Influences the Accuracy of Translation



The structure of the 16S rRNA at the P and A sites of the ribosome influences the accuracy of translation.

Figure: Any aminoacyl-tRNA can be placed in the A site, but only one that pairs with the anticodon can make stabilizing contacts with rRNA.

The Nobel Prize in Chemistry 2009

"for studies of the structure and function of the ribosome"

Venkatraman Ramakrishnan

1/3 of the prize

United Kingdom

MRC Laboratory of Molecular Biology Cambridge, United Kingdom

b. 1952 (in Chidambaram, Tamil Nadu, India)

Thomas A. Steitz

1/3 of the prize

USA

Yale University New Haven, CT, USA; Howard Hughes Medical Institute

b. 1940

Ada E. Yonath

1/3 of the prize

Israel

Weizmann Institute of Science Rehovot, Israel

b. 1939