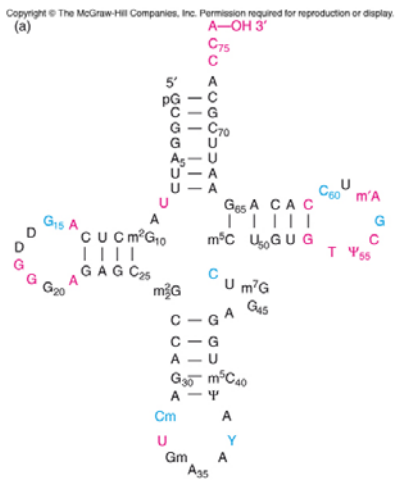
tRNA

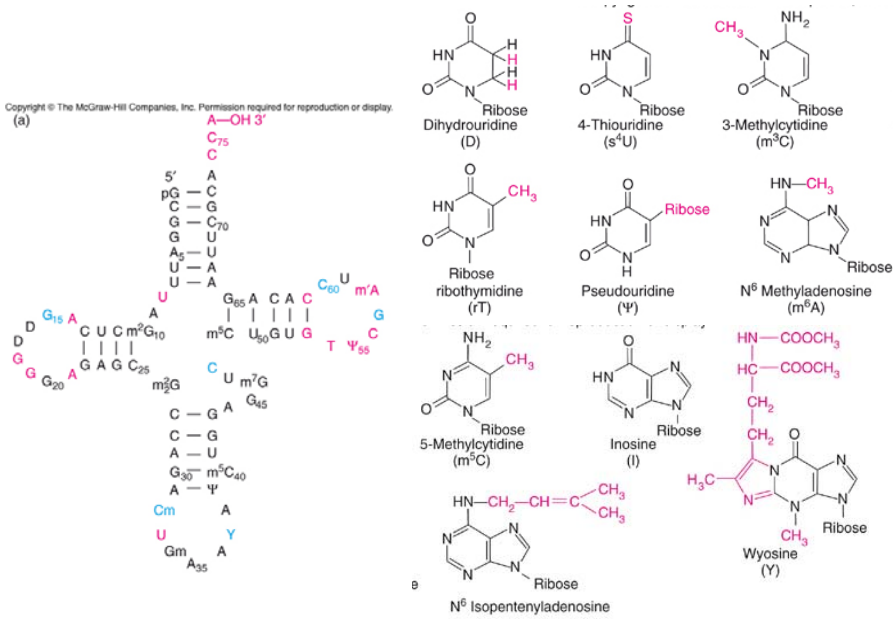
- Structure
  - Conserved Structure
  - Variant tRNA's
  - Anticodon-Codon Interactions
- Deviations from Universal Genetic Code
- Synthetase Recognition
  - tRNA Recognition
  - Amino Acid Recognition and Proofreading
- Suppressor Mutations

Conserved Elements among tRNA's

- Size 70-90nt long
- Five Arms
  - Acceptor Arm
    - 7bp stem
    - Invariant CCA extension 3'
  - Anticodon Arm
  - D Arm
    - Dihydrouracil
  - Variable arm
    - 3-5nt Class I tRNA
    - 12-13nt Class II tRNA
  - TUC arm
    - Pseudouracil
- Tertiary Structure



Clover Leaf Structure



Tertiary Structure

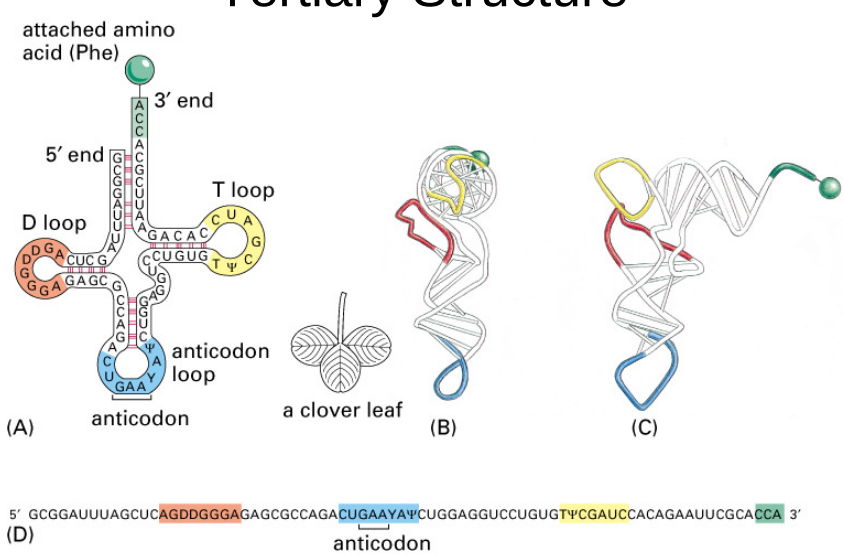
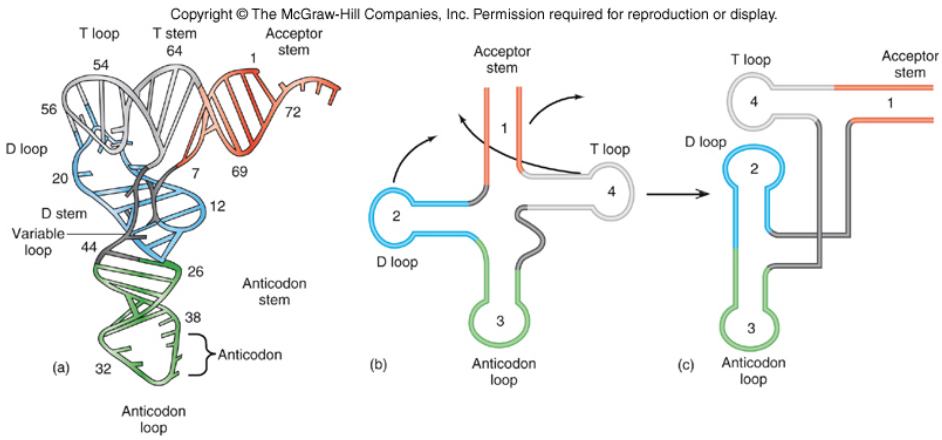


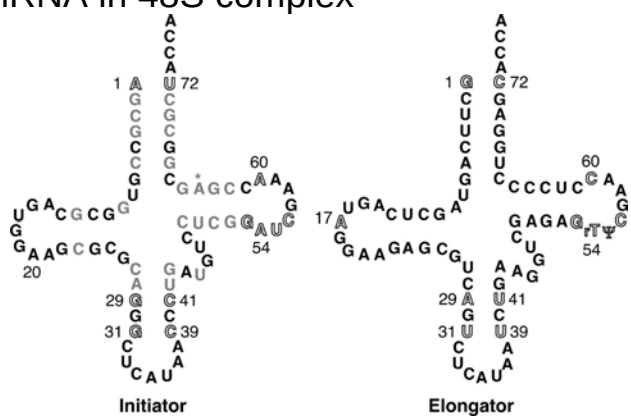
Figure 6–52. Molecular Biology of the Cell, 4th Edition.

tRNA Folding



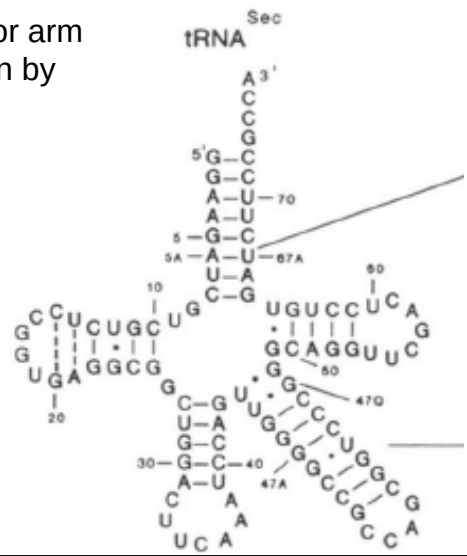
Variant tRNA's

- tRNAi
  - AU in acceptor arm recognized by eIF2
  - GGG/CCC in anticodon loop – tight Binding of mRNA in 48S complex

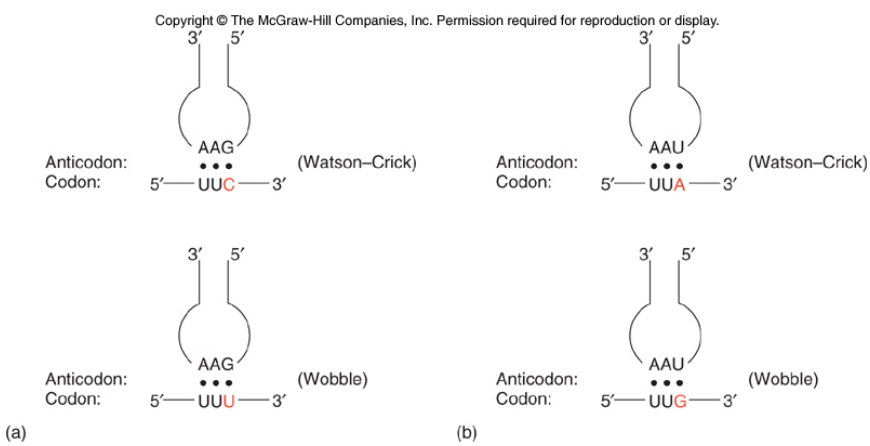


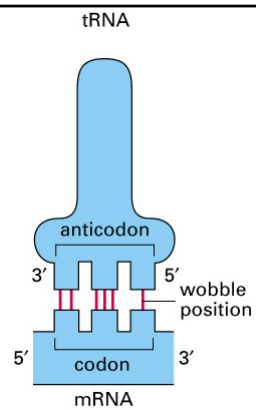
Special $tRNA_{sec}$

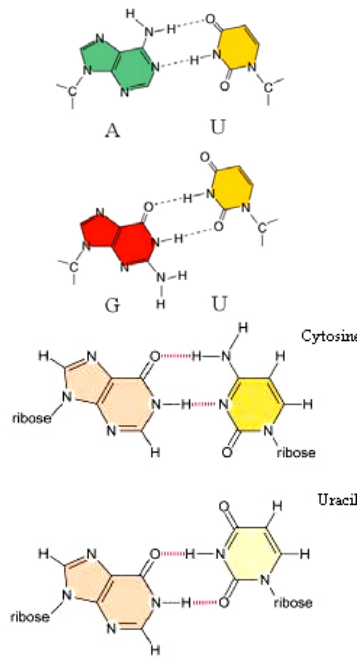
$tRNA_{sec}$ – 8 bp acceptor arm
Required for recognition by
eEF_{sec}



Anticodon-Codon Interactions Wobble







eucaryotes

wobble codon base	possible anticodon bases
U	G or I
C	G or I
A	U
G	C

Humans have 497 tRNA genes but only use 48 anticodons

eucaryotes

wobble codon base	possible anticodon bases
U	G or I
C	G or I
A	U
G	C

tRNA	Anticodon	Codons
Ser1	AGG	UCU,UCC
Ser2	AGU	UCA,UCG
Ser3	UCG	AGU,AGC

Humans have 497 tRNA genes but only use 48 anticodons

Variants in Genetic Code

Second position

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

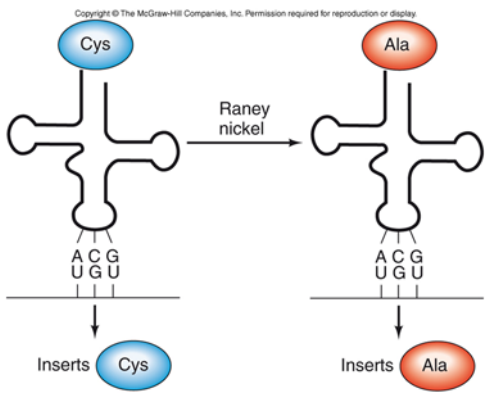
Table 18.1 Deviations from the "Universal" Genetic Code

Source	Codon	Usual Meaning	New Meaning
Fruit fly mitochondria	UGA	Stop	Tryptophan
	AGA & AGG	Arginine	Serine
	AUA	Isoleucine	Methionine
Mammalian mitochondria	AGA & AGG	Arginine	Stop
	AUA	Isoleucine	Methionine
	UGA	Stop	Tryptophan
Yeast mitochondria	CUN*	Leucine	Threonine
	AUA	Isoleucine	Methionine
	UGA	Stop	Tryptophan
Higher plant mitochondria	UGA	Stop	Tryptophan
	CGG	Arginine	Tryptophan
Candida albicans nuclei	CTG	Leucine	Serine
Protozoa nuclei	UAA & UAG	Stop	Glutamine
Mycoplasma	UGA	Stop	Tryptophan

*N = Any base

Amino acids not distinguished by ribosome complex

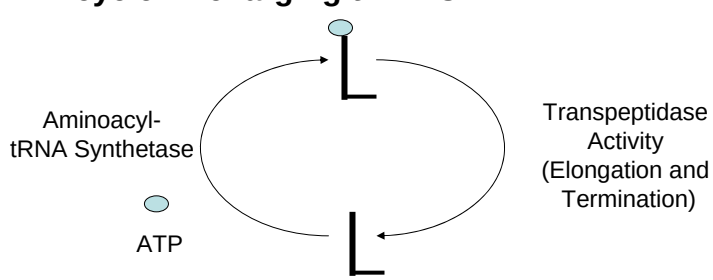
- Lipman Experiment



Second Genetic Code

- 20 Aminoacyl-tRNA synthetases
  - 20 Amino acids
  - 48 or more tRNA's
  - Isoaccepting tRNA's – different tRNA's attached to the same amino acid
  - Cognate tRNA's
  - Noncognate tRNA's

tRNA cycle – "charging tRNA's"



<u>20 Different Aminoacyl-tRNA synthetases</u>

Each synthetase recognizes only one cognate amino acid

Each synthetase recognizes a set of cognate tRNA's

How does the right amino acid get added to the right tRNA?

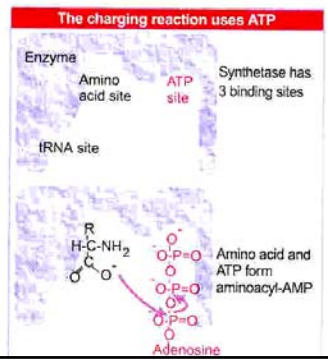
Synthetase Reaction

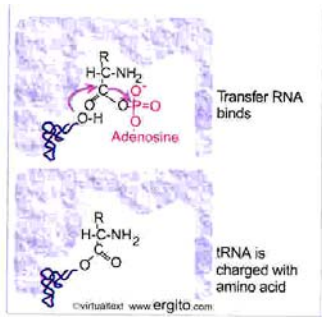
(i) Activation of amino acid;

enzyme + amino acid + ATP $\xrightarrow{Mg^{++}}$ enzyme(amino-acyl-AMP) + PPi

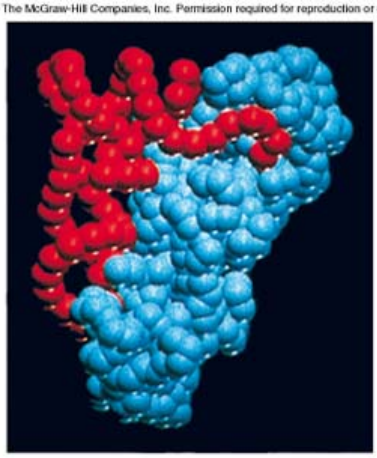
(ii) Transfer of amino-acyl group to tRNA - amino acid is <u>activated</u>

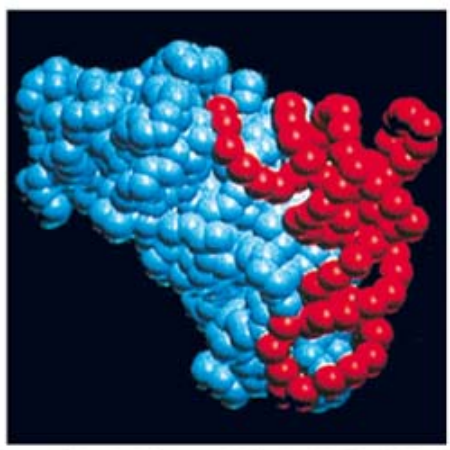
tRNA + enzyme(amino-acyl-AMP) $\longrightarrow$ amino-acyl-tRNA + enzyme

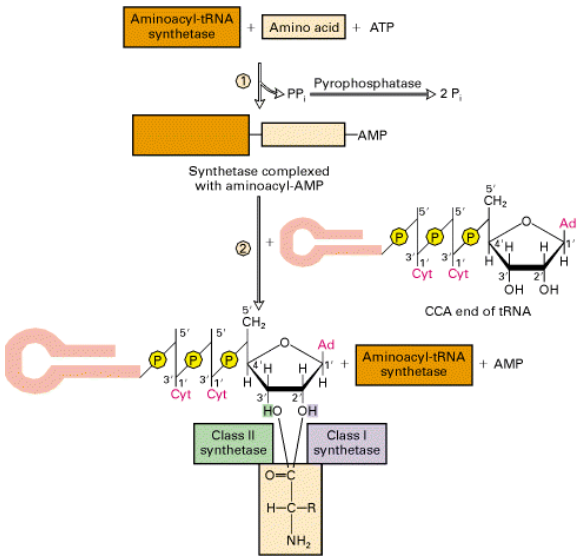




Cognate tRNA Recognition
Two Classes of synthetases







tRNA Recognition

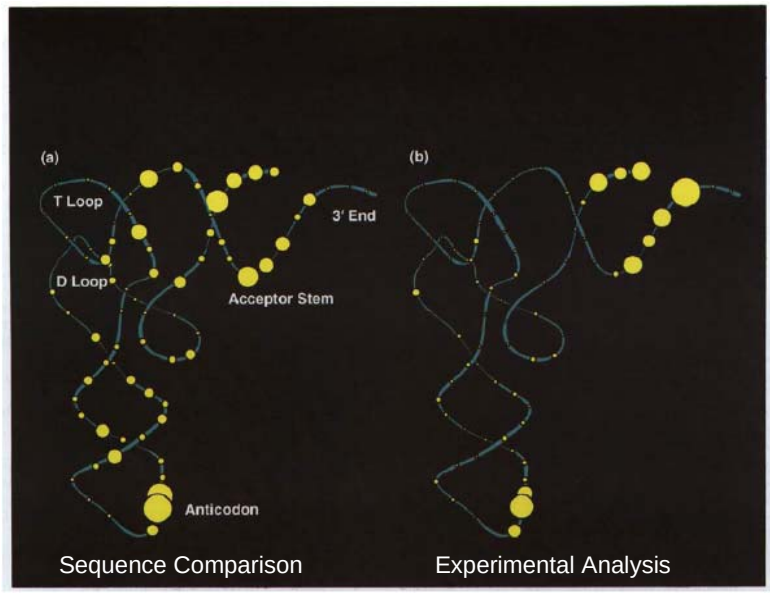
Problem – Synthetases must recognize and charge only cognate tRNA's; therefore they must be able to distinguish cognate from non-cognate tRNA's.

Experimental Approaches

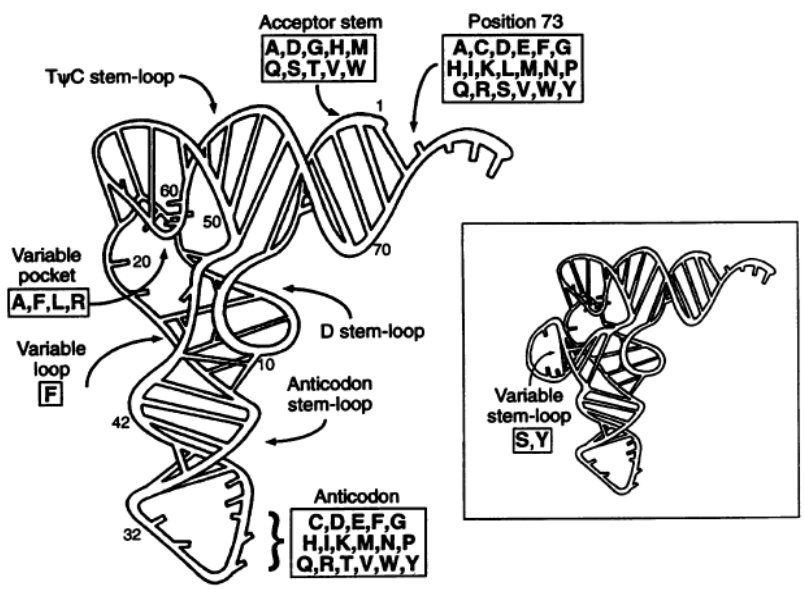
Comparison of tRNA sequences – identification of elements conserved among the cognates but that differ among the non cognate. (limits)

Crosslinking Studies – treat complex with crossinglinking reagents to demonstrate interactions between specific molecules. (limits)

Mutagenesis – Alter non cognate tRNA sequences to mimic cognate tRNA's. (limits – difficulting with synergistic effects of multiple sites)

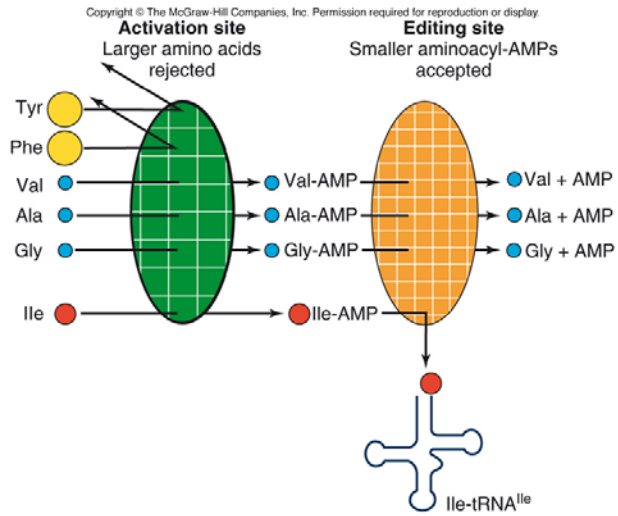


McClain 1993



Saks et al 1994

Cognate Amino Acid Discrimination



Editing Sites

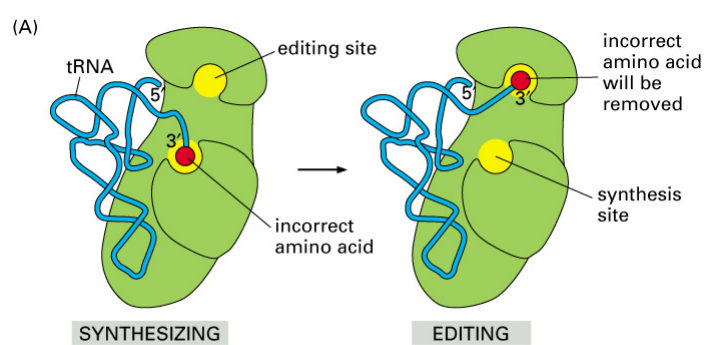
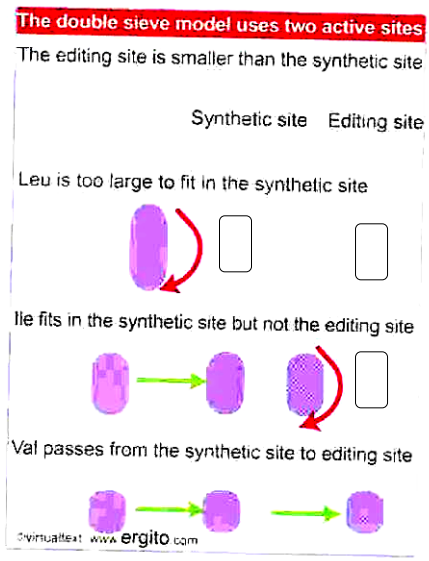
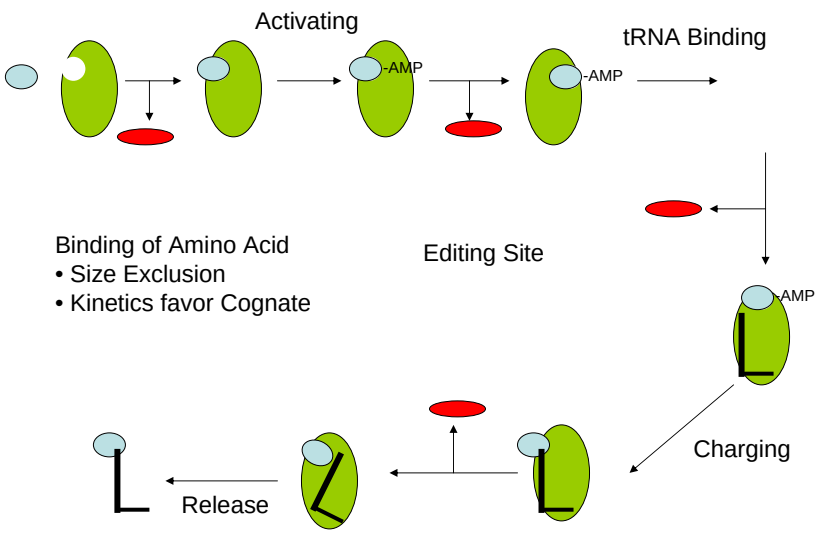


Figure 6–59 part 1 of 2. Molecular Biology of the Cell, 4th Edition.



Proofreading Steps



Suppressor Mutations

- Suppressor tRNA's
  - Suppressors that read new codons
    - Anticodon Mutations
      - Non-Sense Suppressors
      - Mis-Sense Suppressors
    - Non Anticodon Mutation
  - Suppressors that alter interaction with non-cognate synthetase
    - Mis-Sense Suppressors
- Other Suppressors
  - Frameshift Suppressors
  - Mutant Synthetases

Nonsense Suppressors (Mutant Anticodon)

Functional Gene

Met Asn His Arg Gly Lys - - -

XXX XXX AUG AAU CAU AGA GGA AAA XXX

Mutant Gene

Met Asn His (stop)

XXX XXX AUG AAU CAU UGA GGA AAA XXX

WildtypeTrp tRNA ACC anticodon

Nonsense suppressor tRNA has ACU anticodon

Missense Suppressors (Mutant Anticodon)

Functional Gene

Met Asn His Arg Gly Lys - - -

XXX XXX AUG AAU CAU AGA GGA AAA XXX

Mutant Gene

Met Asn His Ser Gly Lys - - -

XXX XXX AUG AAU CAU AGU GGA AAA XXX

Wildtype Arg tRNA has UCU at anticodon

Suppressor Arg tRNA has UCA at anticodon

Missense Suppressors (Mutant Anticodon)

A Trp non-sense suppressor was identified that has a normal anticodon ACC but has a mutation in D loop.

Hypothesis: D loop mutation changes conformation of tRNA in a way that extends wobble rules for last nucleotide so that C can base pair with A.

Missense Suppressors (Synthetase Interaction)

Functional Gene

Met Asn His Arg Gly Lys Phe - - -

XXX XXX AUG AAU CAU AGA GGA AAA UUU XXX

Mutant Gene

Met Asn His Ser Gly Lys Leu - - -

XXX XXX AUG AAU CAU AGU GGA AAA UUA XXX

Leu tRNA has AAU as anticodon.

The Suppressor tRNA is a mutant form of Leu tRNA that is charged by the phenyl acyl tRNA synthesis. This charges the Leu tRNA with phenylalanine with then inserts phenylalanine at UUA

Frameshift Suppressors (Changes Codon Length)

Functional Gene

Met Asn His Pro Gly Lys - - -

XXX XXX AUG AAU CAU CCC GGA ACC XXX

Mutant Gene

Met Asn His Pro Arg Asn - - -

XXX XXX AUG AAU CAU CCC CGG AAC CXX

Wildtype proline tRNA has GGG in anticodon

Mutant suppressor tRNA has extra G inserted into anticodon loop. This tRNA know recognizes CCCC as its codon.

Synthetase Suppressors

- a mutation in an aminoacyl-tRNA synthetase gene might change the enzymes recognition specificity for tRNAs. These aminoacyl-tRNA synthetase suppressor mutations are rare, probably because the mutation must create new recognition sites in the aminoacyl-tRNA synthetase enzyme and these sites are complex compared to 3 bases involved in codon:anticodon interactions.

Suppose:

Mutant Ile Synthetase that lost editing acitivity and failed to remove valines from the Ile tRNA. How might this result in suppression?