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\textsubscript{sec}:

tRNA\textsubscript{sec} – 8 bp acceptor arm
Required for recognition by eEF\textsubscript{sec}

Anticodon-Codon Interactions
Wobble

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Humans have 497 tRNA genes but only use 48 anticodons.
Variants in Genetic Code

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

20 Different Aminoacyl-tRNA synthetases

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:

\[ \text{enzyme + amino acid + ATP} \quad \text{Mg}^{++} \quad \rightarrow \quad \text{enzyme(amiino-acyl-AMP) + PPi} \]

(ii) Transfer of amino-acyl group to tRNA - amino acid is **activated**

\[ \text{tRNA + enzyme(amiino-acyl-AMP)} \quad \rightarrow \quad \text{amiino-acyl-tRNA + enzyme} \]

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 crosslinking 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)
Cognate Amino Acid Discrimination

Editing Sites

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

- Activating
- tRNA Binding

Binding of Amino Acid
- Size Exclusion
- Kinetics favor Cognate

Editing Site

Release

Charging
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**

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**Mutant Gene**

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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 activity and failed to remove valines from the Ile tRNA. How might this result in suppression?