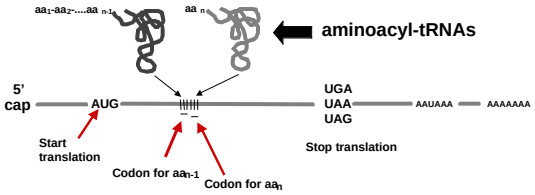
Components needed for **Translation**

tRNAs
Aminoacyl-tRNA synthetases
Ribosomes

Transfer RNAs = tRNAs serve as adapters

- Align the appropriate amino acids with the mRNA templates



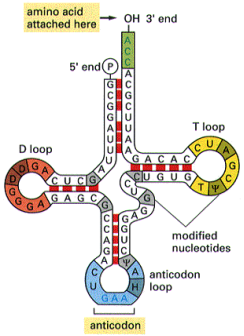
Primary structure of tRNAs

- Short: 73 to 93 nucleotides long
- Have a CCA at their 3' end
  - A **charged** tRNA has an amino acid attached to its 3' end.
- Have a large number of modified bases
  - Reduction of a double bond in uridine gives dihydrouridine ("D")
    - leads to the name D-loop in tRNA
  - In pseudouridine, carbon at position 5 is replaced by a nitrogen, abbreviated ψ .
    - The nucleotide triplet TψC is characteristic of another loop in tRNA.
  - All 4 bases can be methylated

Secondary structure of tRNA

- Cloverleaf: 4 arms (duplex RNA) and 3 loops
- Amino acid acceptor arm: duplex between the 5' segment and 3' segment, but terminal CCA is not base paired
- D arm ends in **D loop**
- Anticodon arm ends in **anticodon loop**: anticodon is in the center of the loop
- TψC arm ends in **TψC loop**.
- Variable loop just before TψC arm

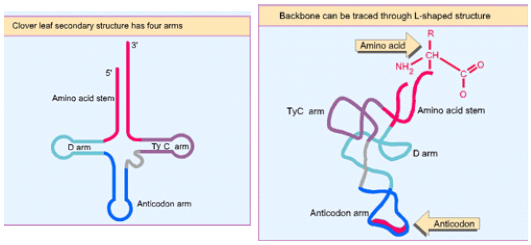
Secondary structure of tRNA



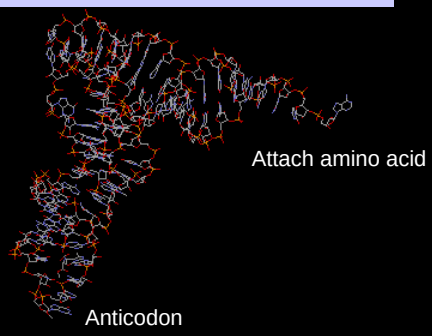
Tertiary structure of tRNA

- "Fat L"
  - Base pairing between nucleotides in the D loop and the TψC loop, plus other interactions pull the tRNA cloverleaf into a pseudoknot.
- Two RNA duplexes predominate in the "fat L" structure
  - TψC stem is continuous with the amino acid acceptor stem = one arm of the "L"
  - D stem is continuous with the anticodon arm = other arm of the "L"
- Amino acid acceptor site is maximally separated from the anticodon.

Folding of cloverleaf into L-shaped tRNA



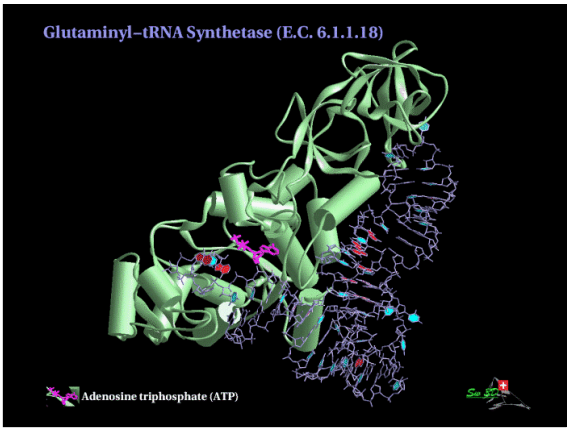
3-D structure of tRNA



Attachment of amino acids to tRNA

Aminoacyl-tRNA synthetases

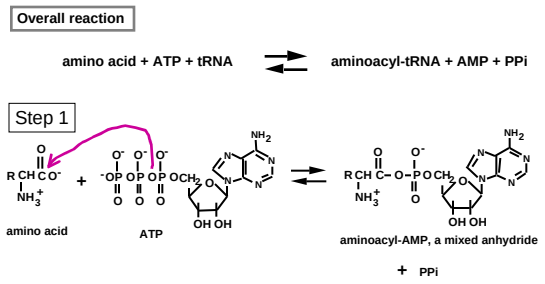
- 20 enzymes, 1 per amino acid
- Highly specific on BOTH business ends of the tRNA:
  - Each must recognize several cognate tRNAs
    - Recognize several or all the tRNAs whose anticodons complement the codons specifying a particular amino acid
  - Must recognize the correct amino acid
- Two different classes of aminoacyl-tRNA synthetases, based on 3D structure



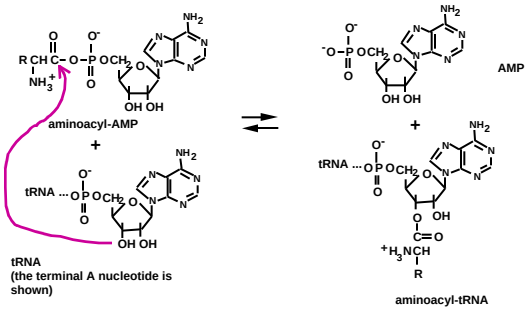
Mechanism of aminoacyl-tRNA synthetases

- 2-step reaction
  - 1st: Amino acid is activated by adenylylation
  - 2nd Amino acid is transferred to to the 3' or 2' OH of the ribose of the terminal A on tRNA
- Product **retains a high-energy bond** joining the amino acid to the tRNA
  - Unusual in that this is an ester linkage
  - Provides the thermodynamic energy to drive protein synthesis
- Hydrolysis of PPi to 2 Pi can drive the synthesis of aminoacyl-tRNA

Addition of amino acids to tRNAs: Step 1



Addition of amino acids to tRNAs: Step 2

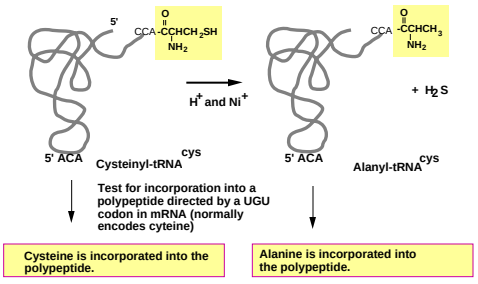


Proofreading by aminoacyl-tRNA synthetases

- In addition to precision in the initial recognition of substrate amino acids, the aa-tRNA synthetases catalyze proofreading reactions.
- If an incorrect amino acid is used in the synthetase reaction, it can be removed.
  - Some enzymes "check" the amino acid at the aminoacyl-adenylate intermediate. If incorrect, this intermediate is hydrolyzed.
  - Other enzymes "check" the aminoacyl-tRNA product, and cleave off incorrect amino acids.

Anticodon determines specificity

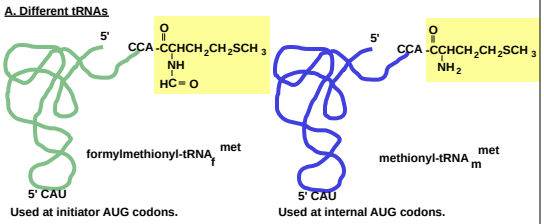
- Does a ribosome recognize the anticodon on the tRNA or the amino acid?



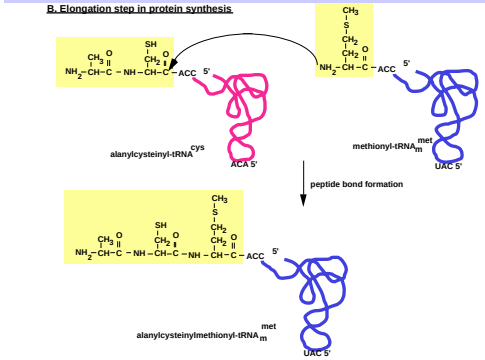
Special tRNA for initiation of translation

- fmet-tRNA$_f^{met}$ is used at initiation codons (AUG, GUG, UUG …)
  - Carries formylmethionine, or fmet (blocks the amino terminus)
  - fmet is the initiating amino acids in bacteria, but methionine is used in eukaryotes
  - In both cases, a special initiating tRNA is used.
- met-tRNA$_m^{met}$ is used at internal codons.
- Different amino acids are used, depending on the context.

Different methionyl-tRNAs for initiation vs. elongation



Use free amino group for elongation during translation



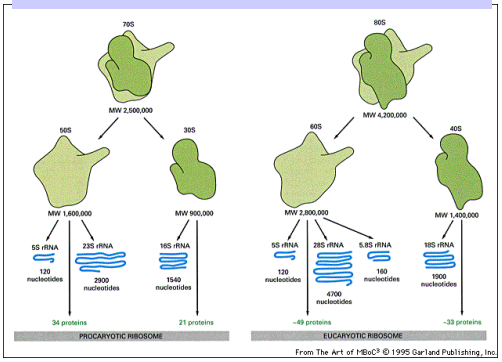
Ribosomes

Molecular machines that catalyze peptide bond formation directed by information in mRNA

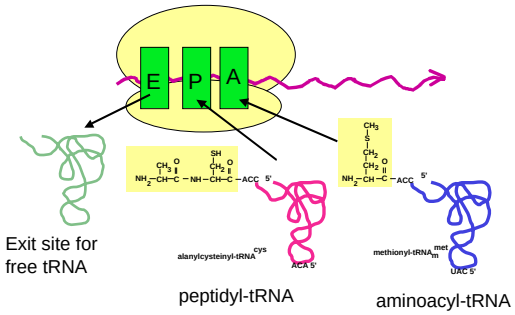
Composition of ribosomes

- 2 subunits, each composed of about **60% RNA** and about **40% protein** (by mass).
- Small ribosomal subunit
  - 16S rRNA (bacteria), 18S rRNA (eukaryotes)
  - 21 (bacteria) to 33 (eukaryote) proteins
  - Initial binding of mRNA and initiator met-tRNA
- Large ribosomal subunit
  - 23S & 5S rRNA (bacteria), 28S, 5.8S, & 5S rRNA (eukaryotes)
  - 31 (bacteria) to 49 (eukaryote) proteins
  - Forms complete, catalytic ribosome.

Diagram of ribosomes



3 sites on ribosome for interaction with tRNAs



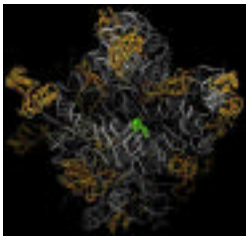
Exit site for free tRNA

peptidyl-tRNA

aminoacyl-tRNA

Images of a ribosome

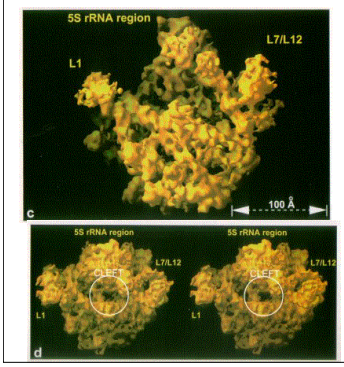
Large subunit is gray, small subunit is blue, and three tRNAs are red, blue and green.



RNA is silver ribbons, protein is gold coils, and a green tRNA is at the peptidyl transferase active site.

http://currents.ucsc.edu/99-00/09-27/ribosome.art.html
http://www.npaci.edu/features/01/05/05_03_01.html

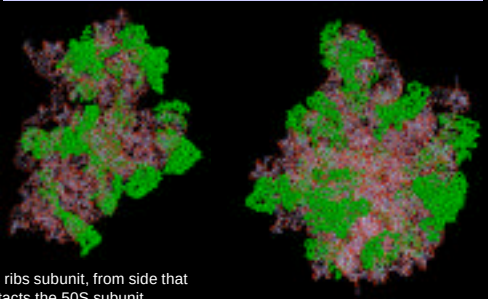
Large ribosomal subunit, 9 A resolution



Ban, ..Moore, Steitz,
Cell, Vol. 93, 1105-1115
1998

http://www.life.nthu.edu.tw
/~b830356/ribosome.html

3-D views of ribosome subunits



30S ribs subunit, from side that contacts the 50S subunit

http://chem-faculty.ucsd.edu/joseph/Ribosome_Structure_Gallery.html
Schluenzen et al. Cell (2000) 102, 615-623
Ban, ...P. Moore, T. Steitz; Science (2000) 289 905-920

50S ribs subunit, from side that contacts the 30S subunit