Ch 27.2- Protein synthesis

1) Assembling the machinery and overview
   - **Ribosomes**: RNP, structure and properties
   - **Aminoacyl-tRNA**: structure and recognition
   - Basic mechanisms and architecture

2) A stepwise process
   - **Initiation**: steps up to formation of 1st peptide bond
   - **Elongation**: synthesis of the 1st bond to addition of the last amino acid
   - **Termination**: release completed polypeptide chain
   - **Ribosome recycling**: disassembly of the ribosome for next use

Components of bacterial vs eukaryotic ribosome

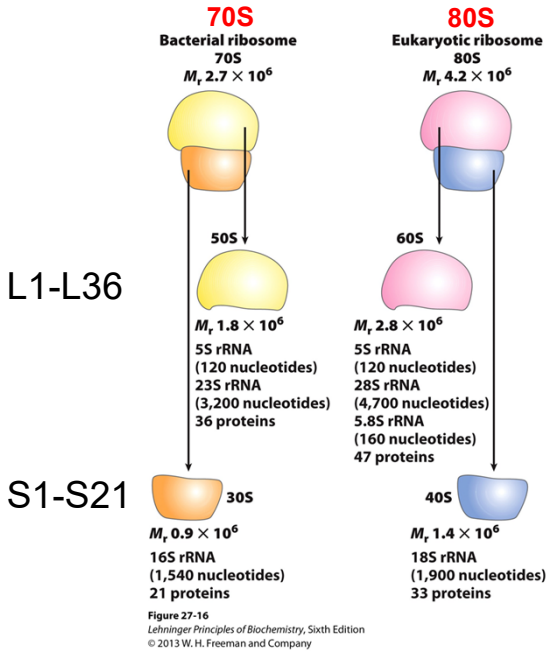

Figure 27-16
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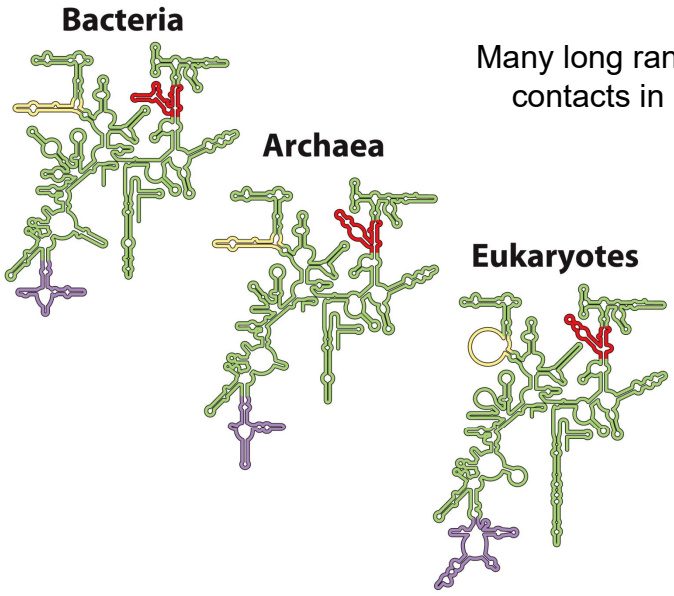
E. coli ribosome
total mass: 2700 kDa
protein: 900 kDa (34%)
RNA: 1700 kDa (66%)

16S rRNA conserved secondary structures



Many long range (3°) contacts in rRNA

Figure 27-15
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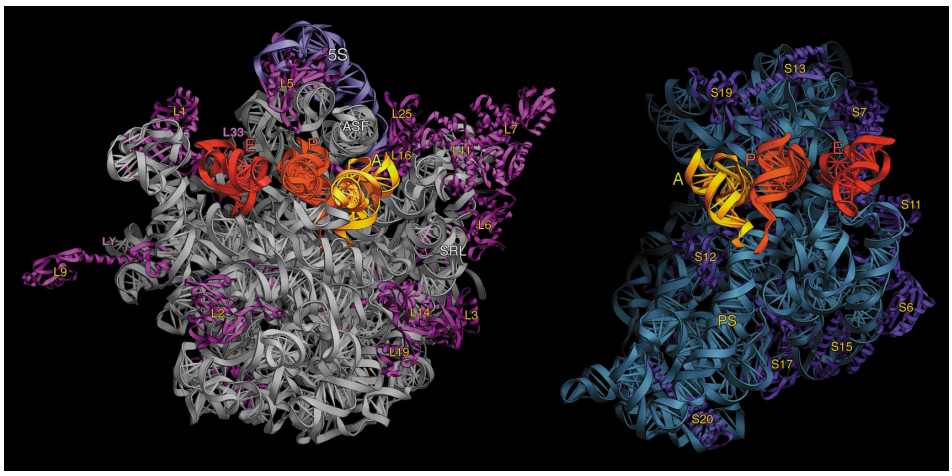
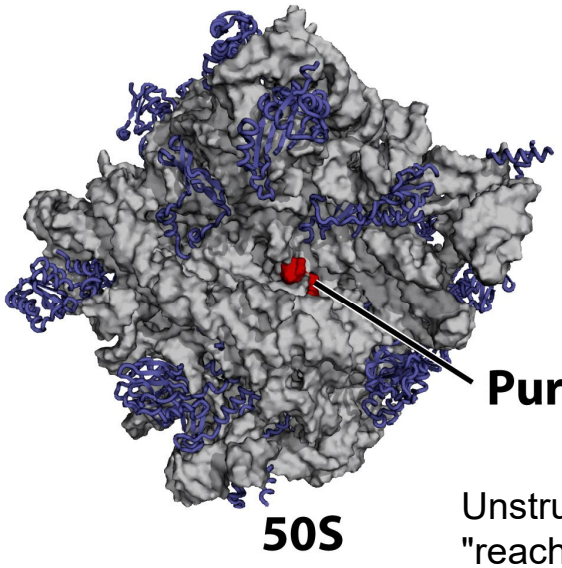
Structure of the *T. thermophilus* 70S ribosome

50S 30S



Yusupov (Noller) Science 2001

RNA-only active site



Unstructured protein tails "reach" into rRNA core

Figure 27-13b
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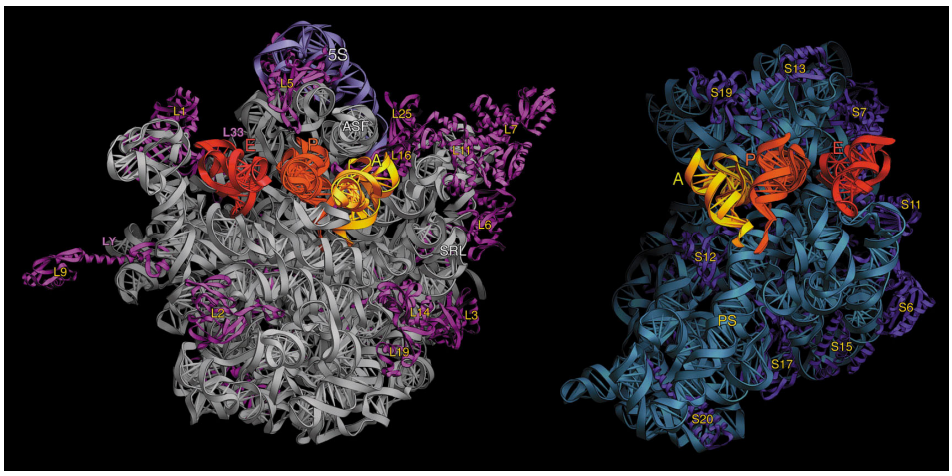
Structure of the *T. thermophilus* 70S ribosome

50S 30S



Yusupov (Noller) Science 2001

General cloverleaf secondary structure of tRNAs

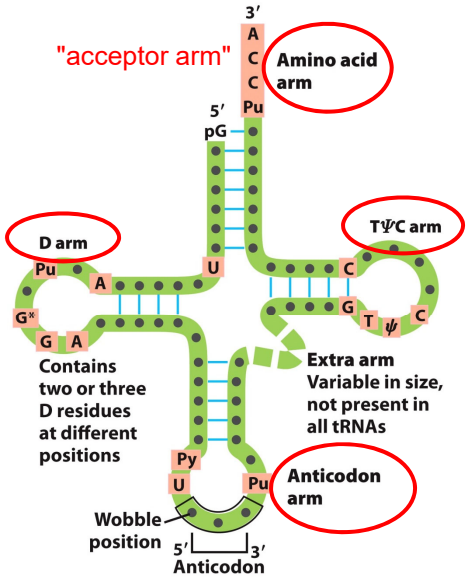


Figure 27-17
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Cytosolic/bacterial tRNA: archaea and organelles can exhibit differences

Extensive tertiary interactions in tRNA structure

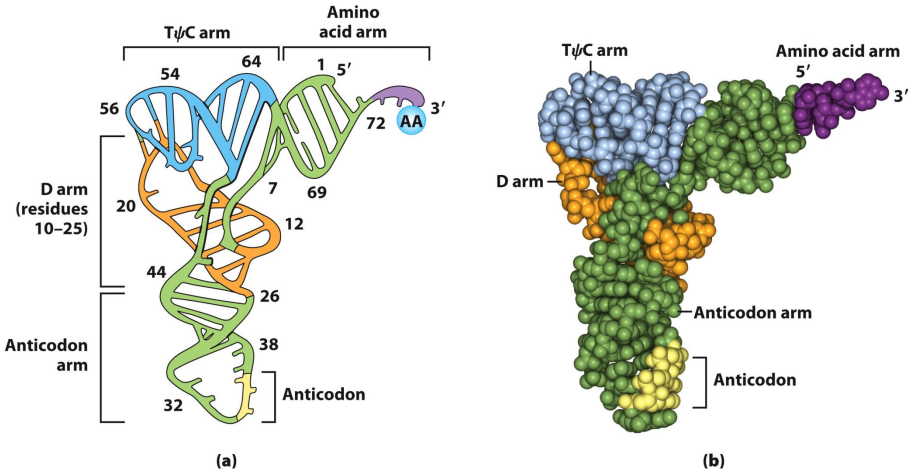

Figure 27-18
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Aminoacyl-tRNA synthetases (aaRS) charge tRNAs

Nomenclature:
- tRNAs: Arg-tRNA^{Arg}, Lys-tRNA^{Val}
- synthetases: ArgRS, GlyRS, etc

Properties:
- Form an ester-linked amino acid at 3'-end of tRNA
- One aaRS per amino acid (mostly)
- Two classes (Class I and Class II)
  structurally distinct but same catalytic activity
  arose independently in evolution

Mechanism:
- Aminoacyl-adenylate
- nucleophilic attack on activated carbonyl

Prototypical aaRS from two different classes

***E. coli* GlnRS (class I)** **Yeast AspRS (Class II)**

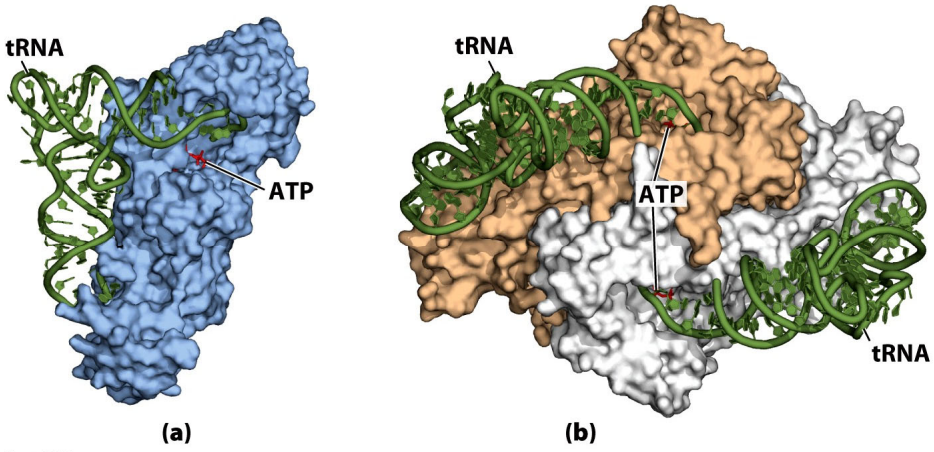

Figure 27-22
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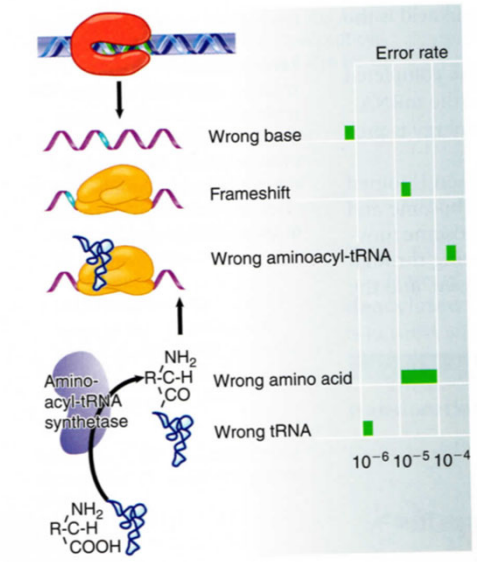
The central dogma: fidelity of gene expression



Overall error rate- $1/10^4$

aaRS recognition and proofreading

Lewin's Essential Genes

Fidelity of aminoacylation by aaRS

The problem: many similar-looking substrates for aaRS

Use of "identity elements": defined sequences that specify a certain amino acid
- determinants and antideterminants
- test: necessary and sufficient for recognition

"A 2nd genetic code"…

$tRNA^{Ala}$ identity elements- conserved G-U base pair

necessary: change and don't aminoacylate

sufficient: transplant to another tRNA and mis-aminoacylate

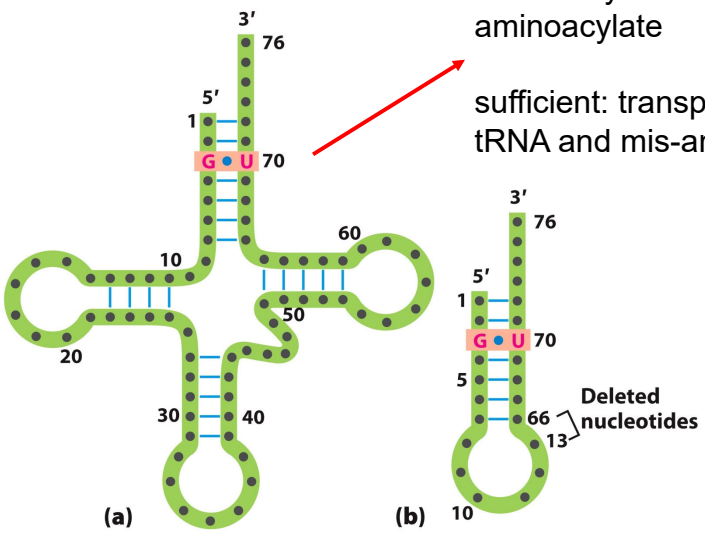


Figure 27-23
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Fidelity of aminoacylation by aaRS

The problem: many similar-looking substrates for aaRS

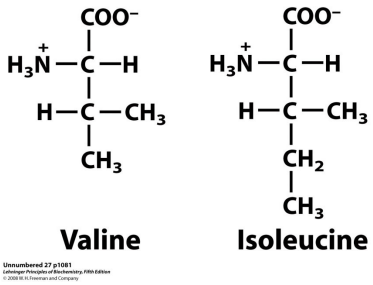
Additional strategies used by some aaRS:

1) Kinetic mechanism:
   - aminoacylation is faster with cognate tRNA than non-cognate

2) Chemical proofreading:
   - hydrolyze incorrect aa-tRNA combinations

Fidelity of aminoacylation by aaRS

Chemical proofreading (editing)



- tRNA not always required

- aaRS-specific

- "Double sieve" mechanism
  Ex. IleRS:
  - 1st: size for aa-AMP formation, Leu won't fit
  - 2nd: size for editing site, Val fits, Ile doesn't

Two types of editing (depending on aaRS):

1) hydrolyze non-cognate aa-AMP (pre-transfer)
2) hydrolyze mischarged aa-tRNA (post-transfer)