

## Chem 431c-Lecture 9b

### Lehninger Principles of Biochemistry Fourth Edition

#### Chapter 27: Protein Metabolism

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#### Last Friday: test #2

Mean = 63/90 (70%) highest = 88/90

Cut off: A ≥80, B ≥ 70, C ≥55, D ≥ 45

----- future graded activities -----

Quiz# 7 on Monday, Nov 30 - on Translation

Research paper due Monday, Nov. 30

penalty for lateness: 10 pts/day late

Quiz #8 on Fri., Dec 4 - on gene regulation

(No more furloughs next week for our class!

Final exam Dec 9, 8-1030 am)

### Lecture Topics:

- 1) the genetic code : triplet codons; degeneracy; wobble rules (\*know these)
- 2) Open reading frames (ORFs)
- 3) Transfer RNA -

#### Today:

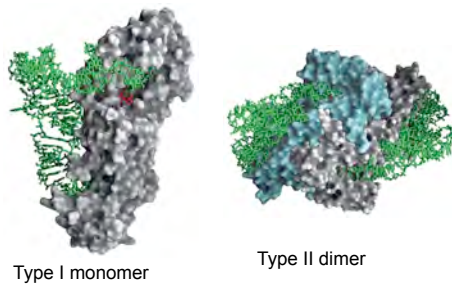
- 1) tRNA (structure:4 common arms)
- 2) aminoacyl-tRNA synthetases-steps
- 3) Steps of translation:
- 4) Protein processing post translation

### Aminoacyl-tRNA synthetases

- **Aminoacyl-tRNA synthetase** attaches aa to their tRNAs :  
 $aa + tRNA + ATP \rightarrow aa-tRNA + AMP + PP_i$  (ester linkage; 2 high energy bonds broken; highly exergonic, irrev)
- how is accuracy ensured? 3 steps:
  - (a) selective binding of aa to enzyme to form aa-AMP,
  - (b) binding of incorrect aa-AMP products to hydrolysis separate site
  - (c) inherent hydrolysis activity of synthetase enhanced by wrong combos of aa and tRNA.
- How does the synthetase recognize the right tRNA?
  - Mostly thru the amino acyl arm and anticodon arms.

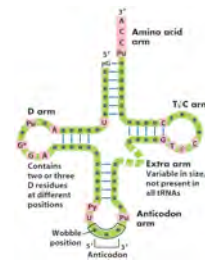
aa =amino acid

### 2 types of aa-tRNA synthetases



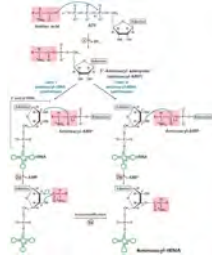
### tRNA structure: at least 4 arms

- tRNA has cloverleaf 2<sup>nd</sup> struc with 4 or 5 arms. 73-93 nts.
- Amino acid arm (CCA-3')
- D arm (contains 2-3 D residues=5,6 dihydrouridine)
- TΨC arm (contains this conserved seq; Ψ = pseudouridine; T=ribothymidine)
- Anticodon arm (contains anti codon)
- each aa's carboxyl group is attached to its corresp tRNA in the cytosol. tRNA "charged".



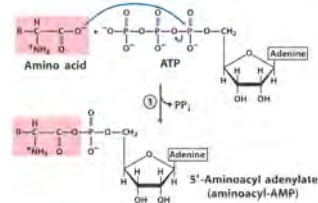
## Stage 1 :Charging the tRNA

- First aminoacyl adenylate formed
- Second, transfer to 3' terminal A residue of tRNA

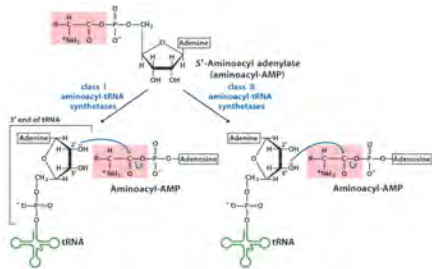


Charging the tRNA with an amino acid takes energy (occurs in active site of aa-tRNA synthetase)

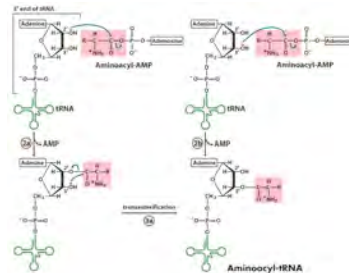
- 1) Amino acid + ATP  $\rightarrow$  5'-amino acyl adenylate + PPi



5' amino acyl AMP is transferred to 2' OH in case of type I synthetase or 3' OH for type II synthetase



5' amino acyl AMP eventually transferred to 3' OH in case of type I (already there for type II).

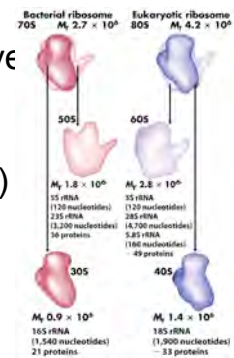


## Ribosomes: giant ribozyme

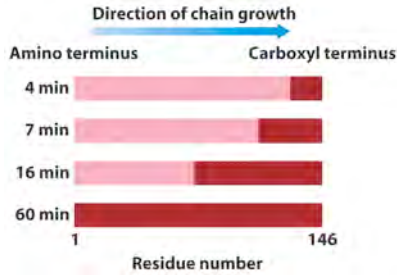
15,000 ribosomes/E coli; 25% of dry wt  
65% RNA,35% protein  
180 Å diameter  
2 subunits:30S, 50S  
Self assembly in vitro

Ribosomes have 2 subunits:  
50s & 30s (prk)  
60s & 40s (euk)

Note:  
50s+30s = 70s  
60s+40s=80s



Dintzis Experiment: showed polypeptides growth direction: from N-terminal to C-terminal direction.



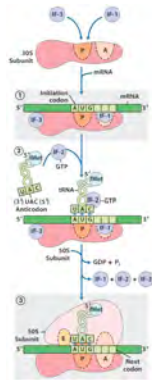
## Stages of protein synthesis

- Stage 1 :activation of amino acids
- Stage 2: initiation (using f-Met)
- Stage 3: Elongation
- Stage 4: Termination and ribosome recycling
- Stage 5: Folding and Posttranslational processing

## Stage 2: initiation

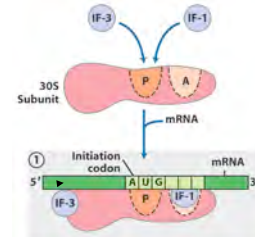
3 steps of initiation complex formation:

- a) 30S subunit binds IF-1,IF-3
- b) fMet-tRNA+IF-2,GTP binds AUG
- c) 50 S subunit added, IF's removed



## Initiation, step 1

- 30s subunit-IF-3,IF-1 complex recruited by mRNA for initiation by binding to special Shine-Dalgarno sequence



## Shine-Dalgarno sequence

In prokaryotes. Helps recruit ribosomes to start translation

```

E. coli rpsA (5') A G C A C G A G G A A A U C U G A U G A A C C U A C T (3')
E. coli rpsL  U U U G U A G G A G U A A C G A U G G C G A U U C C A
S. coli rpsL  T A A U U C A G G G U G G A A U G U A A C C A U A
S. cerevisiae A A U C U G A G G C U U U U A U G U U C U U C U
S. pombe rpsL A U G U A C G A G G A G G U G U G A A C A A C C G C
  
```

Shine-Dalgarno sequences:  
pairs with 16S rRNA

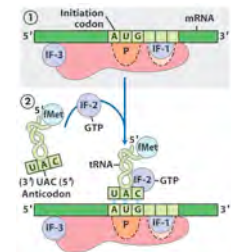
Initiation codon:  
pairs with fMet-tRNA<sup>fMet</sup>

```

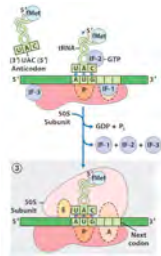
Prokaryotic mRNA 3' End of 16S rRNA
Shine-Dalgarno sequence 5'-G A M V C C A A G G A G G-3'
3' End of 16S rRNA 3'-U A C U G A C-5'
  
```

N.b. In addition to AUG, alternative start codons, ( GUG and UUG) used in prokaryotes. E.g. E. coli uses 83% AUG, 14% GUG, 3% UUG. When an alternative start codon used, it's translated as Met - even if codon normally encodes different amino acid. Due to separate tRNA used for initiation.

## Initiation, step 2



### Initiation, step 3



### Stage 3: Elongation

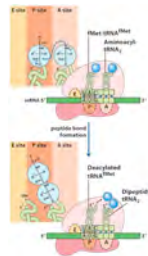
Elongation steps:

- Binding incoming aa-tRNA (needs EFs)
- Peptide bond forms
- Translocation



### Elongation: step 2

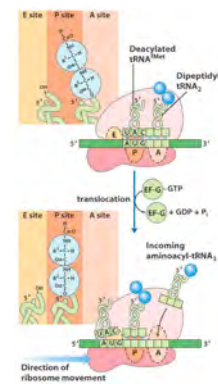
- Peptide bond formation:



Elongation step 3:

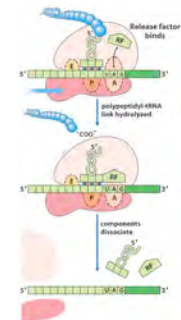
Translocation

- ribosome moves 1 codon toward 3' end of the mRNA.



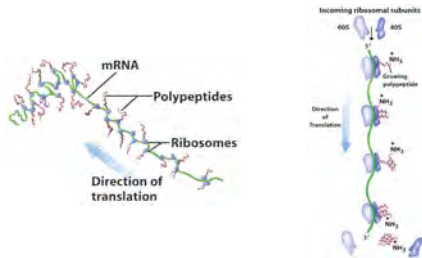
### Stage 4: Termination

- Signaled by condons: UAA, UAG or UGA
- RF binds nonsense codon
- Nonsense mutation can cause premature release

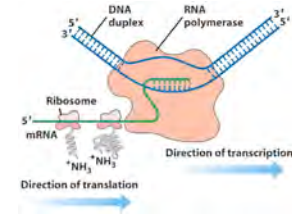


Energy cost per amino acid added : 4 high energy bonds on the average.

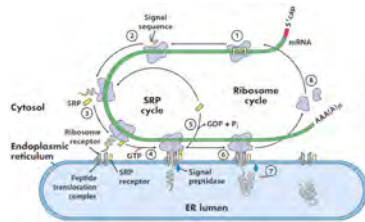
### Stage 5: protein folding



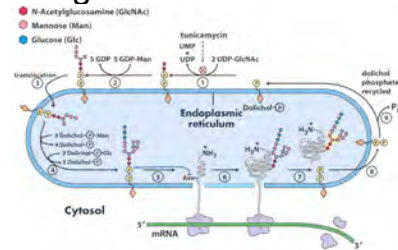
### Coupling transcription and translation



### Protein targeting



### Synthesis of Core Oligosaccharides



Proteins destined for lysosomes, plasma membrane or section



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