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

The translation cycle

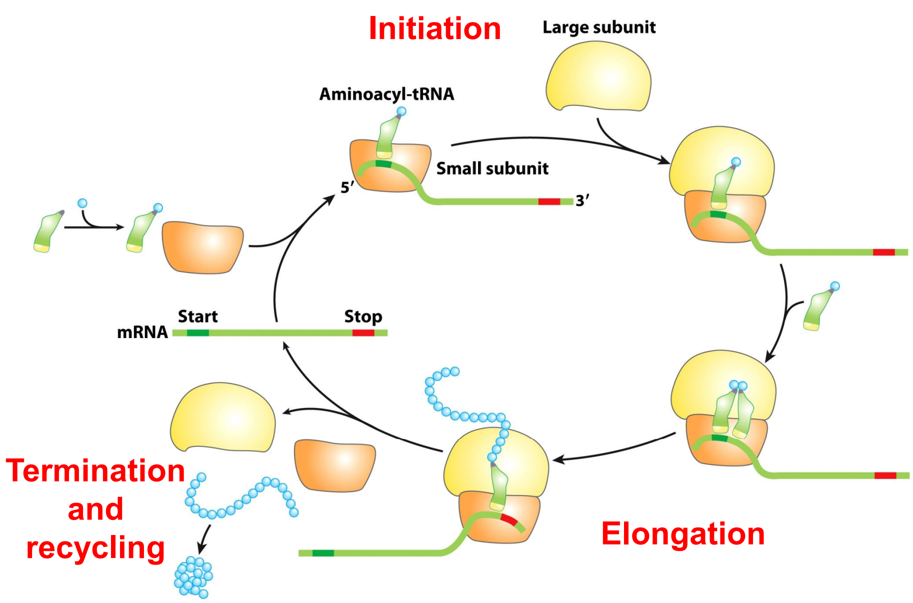

Figure 27-13 part 1
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Stage I: Initiation

<u>Steps in **bacterial** **initiation**</u>

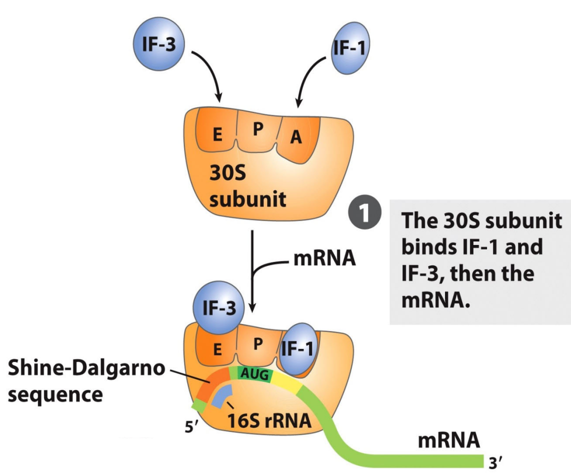
1) Binary complex formation (30S/mRNA)
   30S subunit binds **IF1** and **IF3**
   Shine-Dalgarno (SD) sequence guides ribosome to AUG

2) Ternary complex formation (30S/mRNA/init-tRNA)
   GTP-IF2 brings in fMet-tRNA^{fMet}
   selection of initiator tRNA by initiation factors

3) Formation of the initiation complex
   Hydrolysis of GTP
   50S subunit binds
   Initiation factors exit

Stage I: Initiation

Formation of the binary complex:



Bacteria: Shine-Dalgarno recognized by 16S rRNA

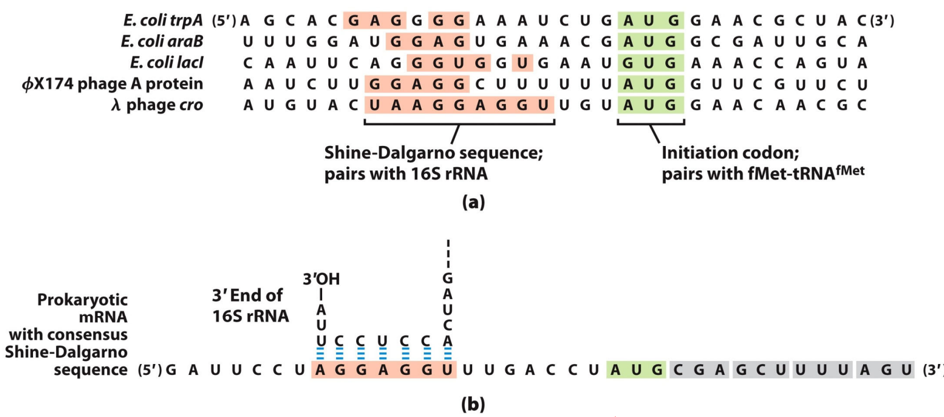


Figure 27-26
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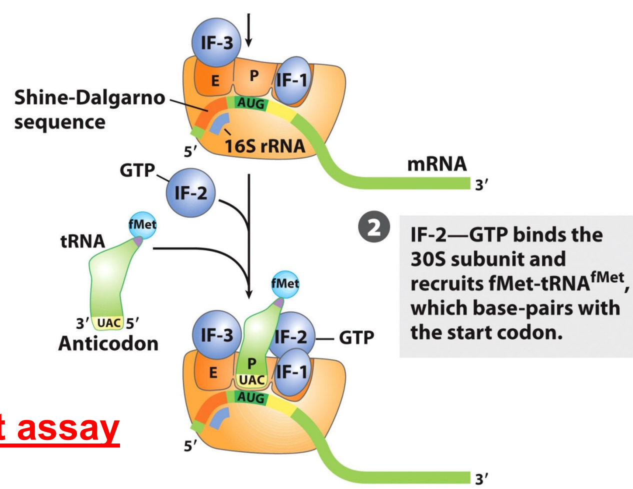
variable distance: how to select AUG??

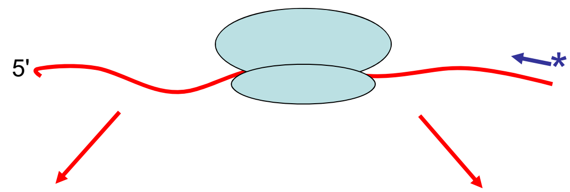
Stage I: Initiation

Formation of the ternary complex:
How is the initiator tRNA selected?



Toeprint assay

Toeprint vs. Footprint



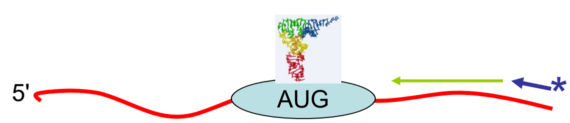
Footprint:

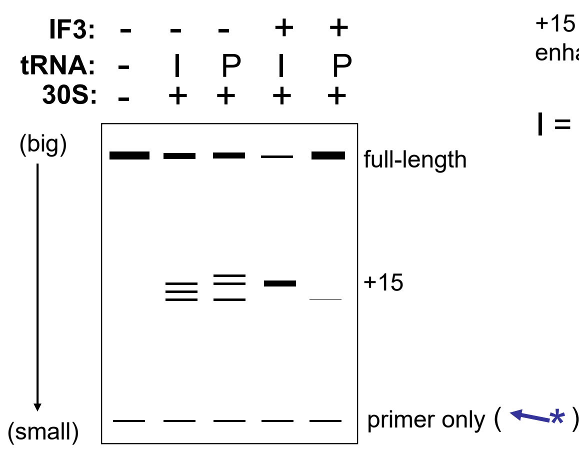
- labeled mRNA
- add nuclease/chemical modifier and probe for protected regions
- directly visualize RNA cleavage events

Toeprint:

- labeled primer annealed to unlabeled mRNA
- add reverse transcriptase and make cDNA copy of mRNA
- pause sites when transcriptase runs into ribosome (30S subunit)

Toeprint assay





+15 from site of AUG selectively enhanced in the presence of IF3

I = initiator tRNA (fMet)

P = $tRNA^{Phe}$

Stage I: Initiation

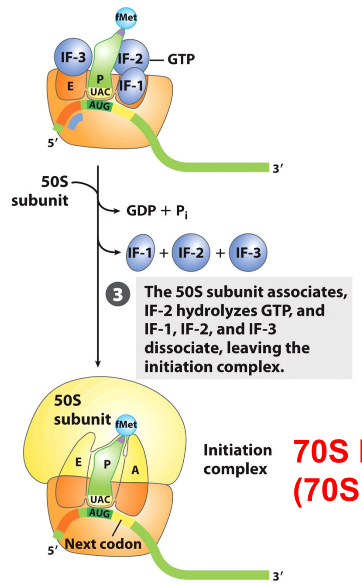


Figure 27-25 part 2
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GTP hydrolysis (conformational changes at multiple steps of translation cycle)

70S Initiation Complex (70S IC)

Eukaryotic initiation: similar, but more complex

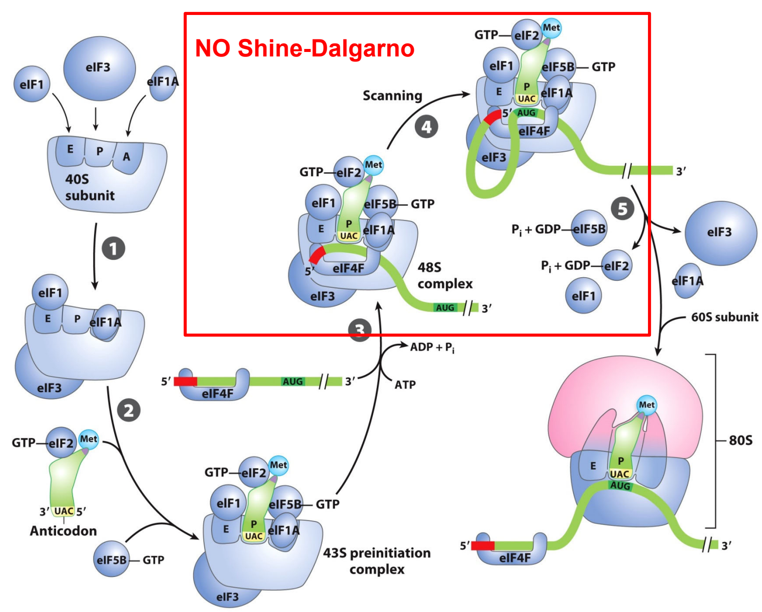

Figure 27-27
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Stage 2: Elongation

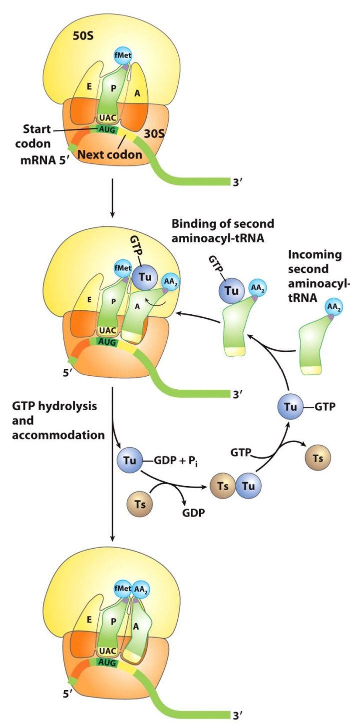
Steps in **bacterial** elongation

1) Binding incoming aa-tRNA into the A-site
   - any aa-tRNA but fMet-tRNA^{fMet}
   - aa-tRNA brought in by **EF-Tu●GTP**
   - **hydrolysis of GTP**

2) Peptidyl transfer reaction
   - NH_2 of A-site tRNA attacks ester-linked peptidyl-tRNA
   - left with uncharged tRNA in P-site, pept-tRNA in A-site
   - formation of **hybrid states**

3) Translocation
   - Requires **EF-G**: molecular mimicry

Binding of the incoming aa-tRNA



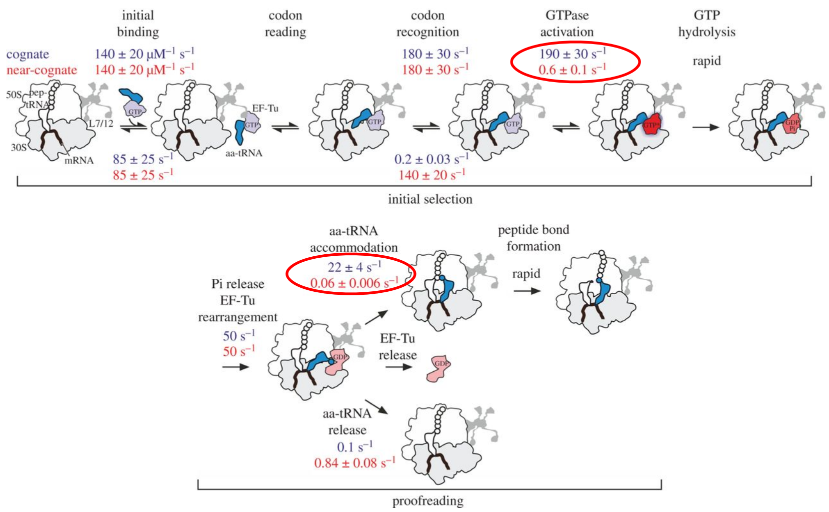
1) Anticodon end of tRNA enters A-site
   - if correct: conformational change, triggers fast **GTP hydrolysis**

2) **Accommodation**: acceptor end enters A-site

Both steps: **kinetic basis** for fidelity

3) Recycling of **EF-Tu*GDP**
   - **EF-Ts** is a GTP exchange factor

Kinetic steps involved in tRNA selection



Marina V. Rodnina et al. Phil. Trans. R. Soc. B 2017;372:20160182

Stage 2: Elongation

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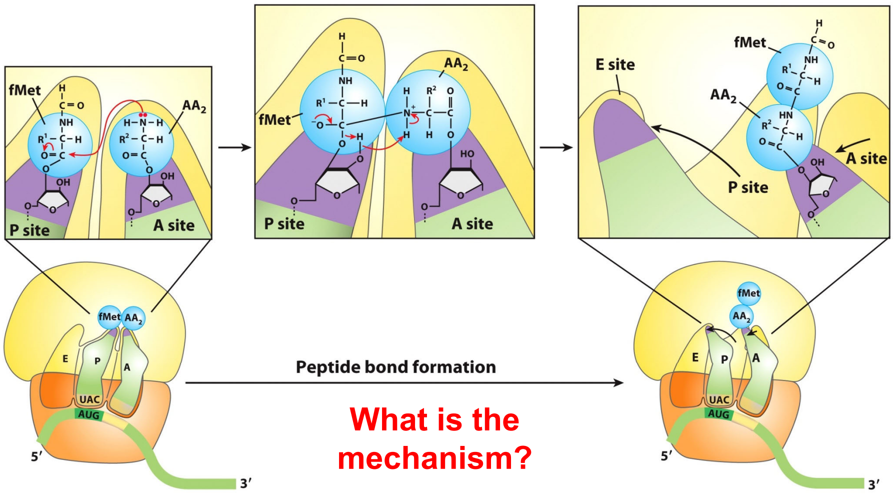
Peptidyl transfer reaction



What is the mechanism?

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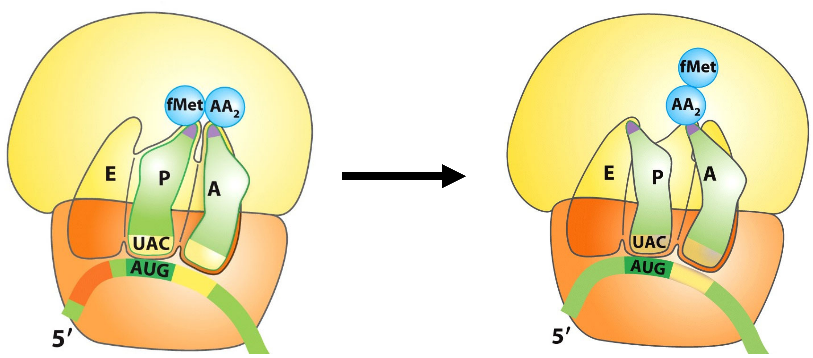
<u>Possibilities considered:</u>
- Acid/base catalysis
- Metal ions
- Ribosome as an entropy trap, role of tRNA 2'-OH

Peptidyl transfer reaction

Formation of hybrid states:

<u>Before</u> peptidyl transfer | <u>After</u> peptidyl transfer



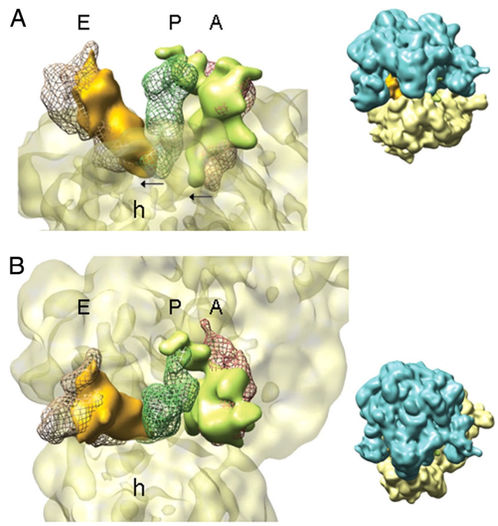
"Standard states"
P/P, A/A

"Hybrid states"
E/P, P/A

Hybrid states visualized by cryo-EM

mesh= standard states
(A/A, P/P, E/E)

solid= hybrid states
(E/P, P/A)

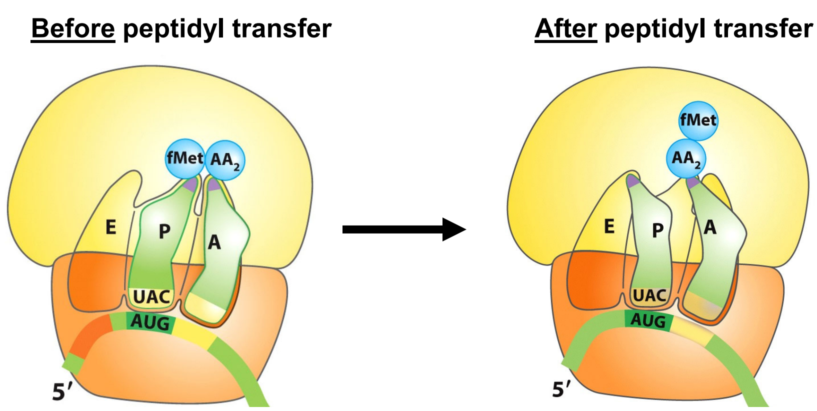


Julián P. et.al. PNAS 2008;105:16924-16927

PNAS

Peptidyl transfer reaction

Formation of hybrid states:



Problem: How to bring in next aa-tRNA?

Stage 2: Elongation

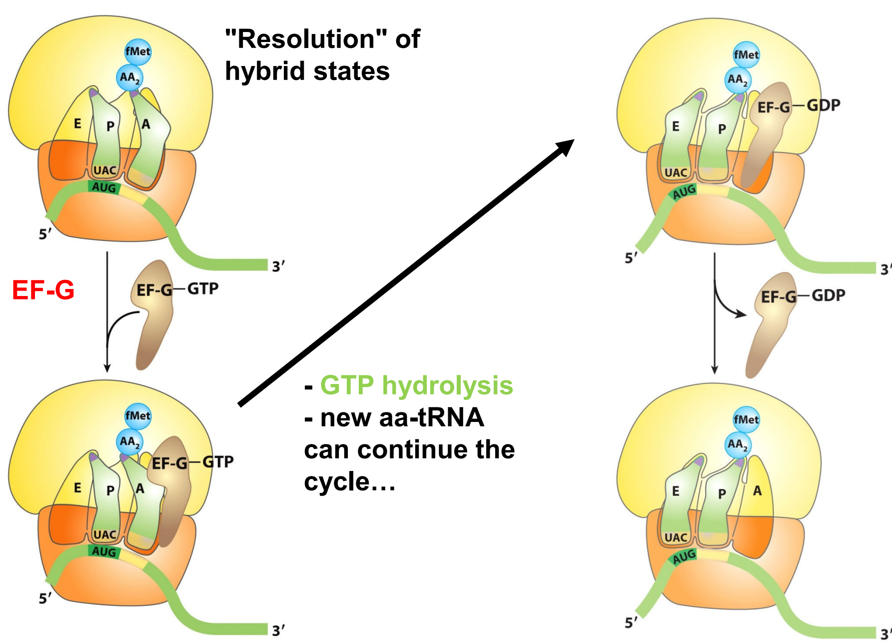
<u>Steps in **bacterial** elongation</u>

1) Binding incoming aa-tRNA into the A-site
   - any aa-tRNA but fMet-$tRNA^{fMet}$
   - aa-tRNA brought in by **EF-Tu●GTP**
   - **hydrolysis of GTP**

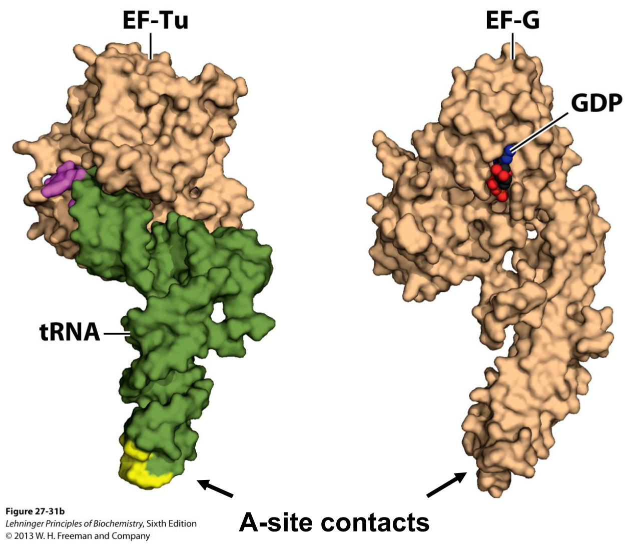
2) Peptidyl transfer reaction
   - NH_2 of A-site tRNA attacks ester-linked peptidyl-tRNA
   - left with uncharged tRNA in P-site, pept-tRNA in A-site
   - formation of **hybrid states**

3) Translocation
   - Requires **EF-G**: molecular mimicry

Translocation



Molecular mimicry Part I: EF-G



EF-G binds to A-site and displaces rest of peptidyl-tRNA into P-site

Pulls mRNA along with it so next codon is in A-site

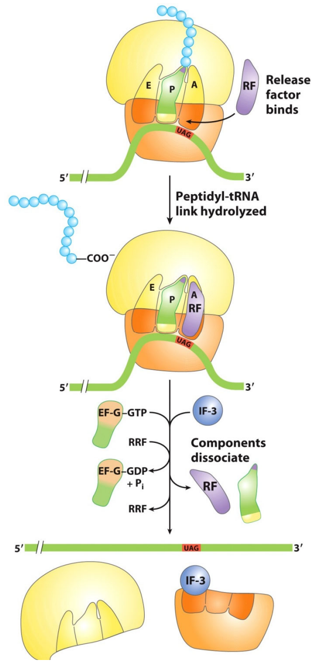
Stage 3: Termination… and beyond

1) Release factors
   - Bacteria: **RF1 and RF2** (RF3 non-essential)
   - **RF1**: UAG, UAA; **RF2**: UGA, UAA
   - Eukaryotes have a single eRF for all 3 stop codons
   - Bind and induce peptidyl transfer to OH_2 instead of NH_2
   - Molecular mimicry again

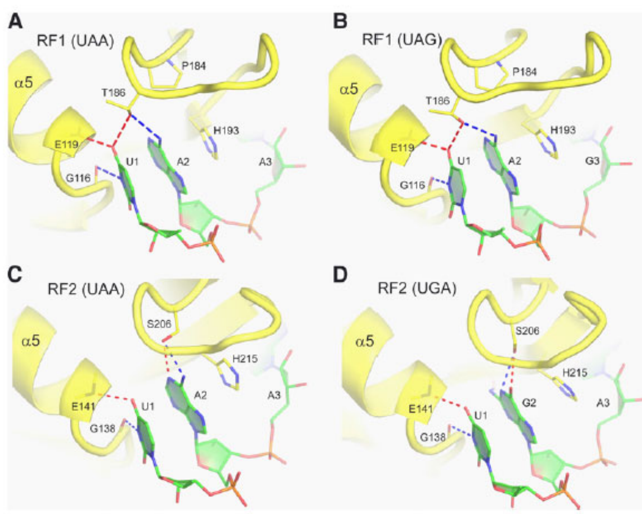
2) Dissociation and recycling
   - **RRF** (Ribosome recycling factor) and **EF-G*GTP** (**hydrolysis of GTP**)
   - 50S and 30S dissociate: **IF3** rebinds 30S to restart cycle

3) Folding and processing of final protein products

Termination and ribosome recycling

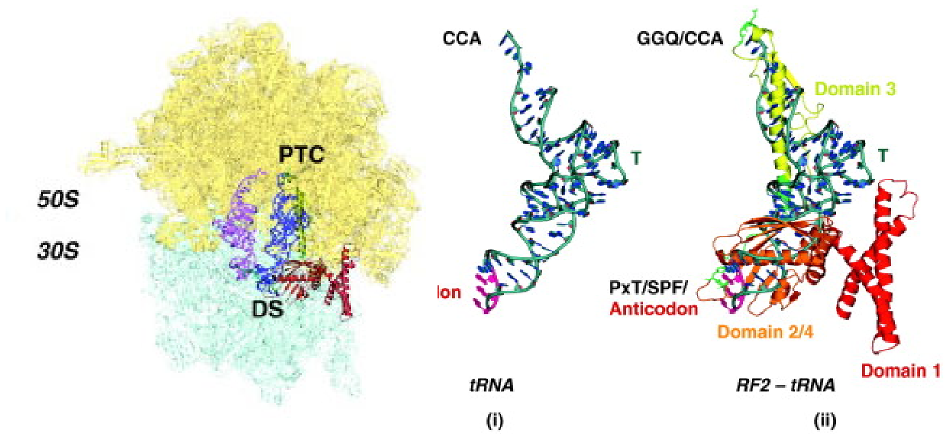


Recognition of stop codons by RFs



Korostelev RNA 2011

Molcular mimicry Part II: release factors

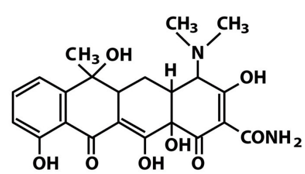


Klaholz BP *Trends Biochem Sci* 2011

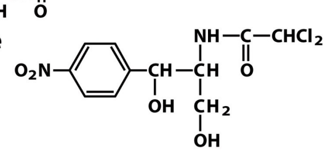
Inhibitors of protein synthesis

Tet: blocks A-site (no aa-tRNA, but still hydrolyzes GTP)

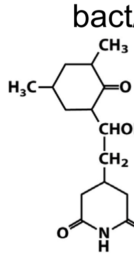
Cam/CHX: block peptidyltransfer, bact/euk respectively



Tetracycline



Chloramphenicol



Cycloheximide

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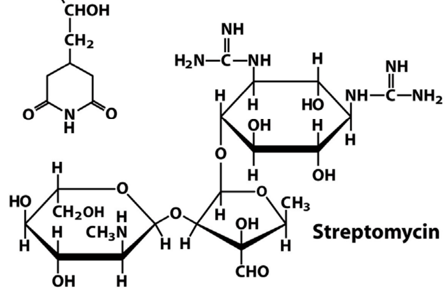
man and Company



Streptomycin

Unnumbered 27 p1098b
Lehninger Principles of Biochemistry, Fifth Edition

Strep: aminoglycosides interfere with decoding of mRNA on 30S-characteristic misreading events

Disruption of elongation by puromycin

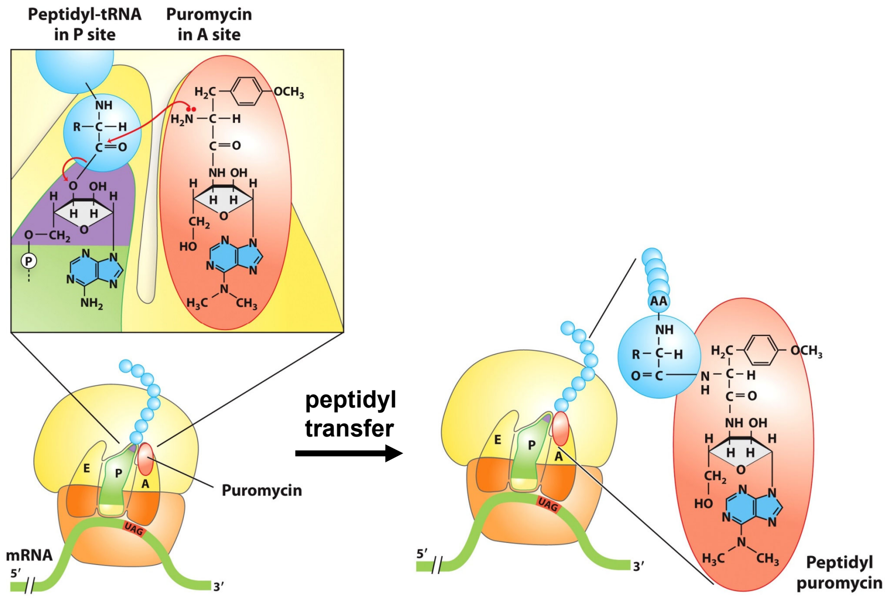


Figure 27-36 part 1
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Figure 27-36 part 2
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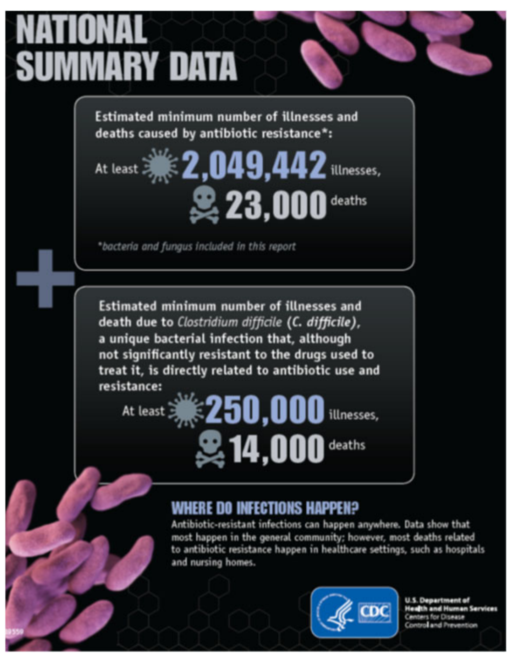
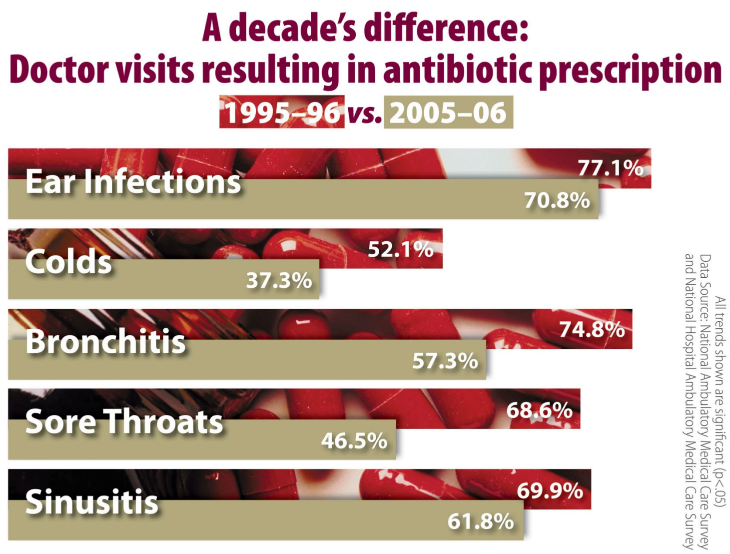


cdc.gov/drugresistance/threat-report-2013



Ch 27.2- Protein synthesis: Part II

A stepwise process

Stage 1: **Initiation-** steps up to formation of 1st peptide bond

Stage 2: **Elongation-** synthesis of the 1st bond to addition of the last amino acid

Stage 3: **Termination-** release completed polypeptide chain and ribosome recycling: disassembly of the ribosome for next use

Questions:

- How is fidelity achieved?
- What are roles for energy consumption?
- What are differences/similarities eukarya vs. bacteria?