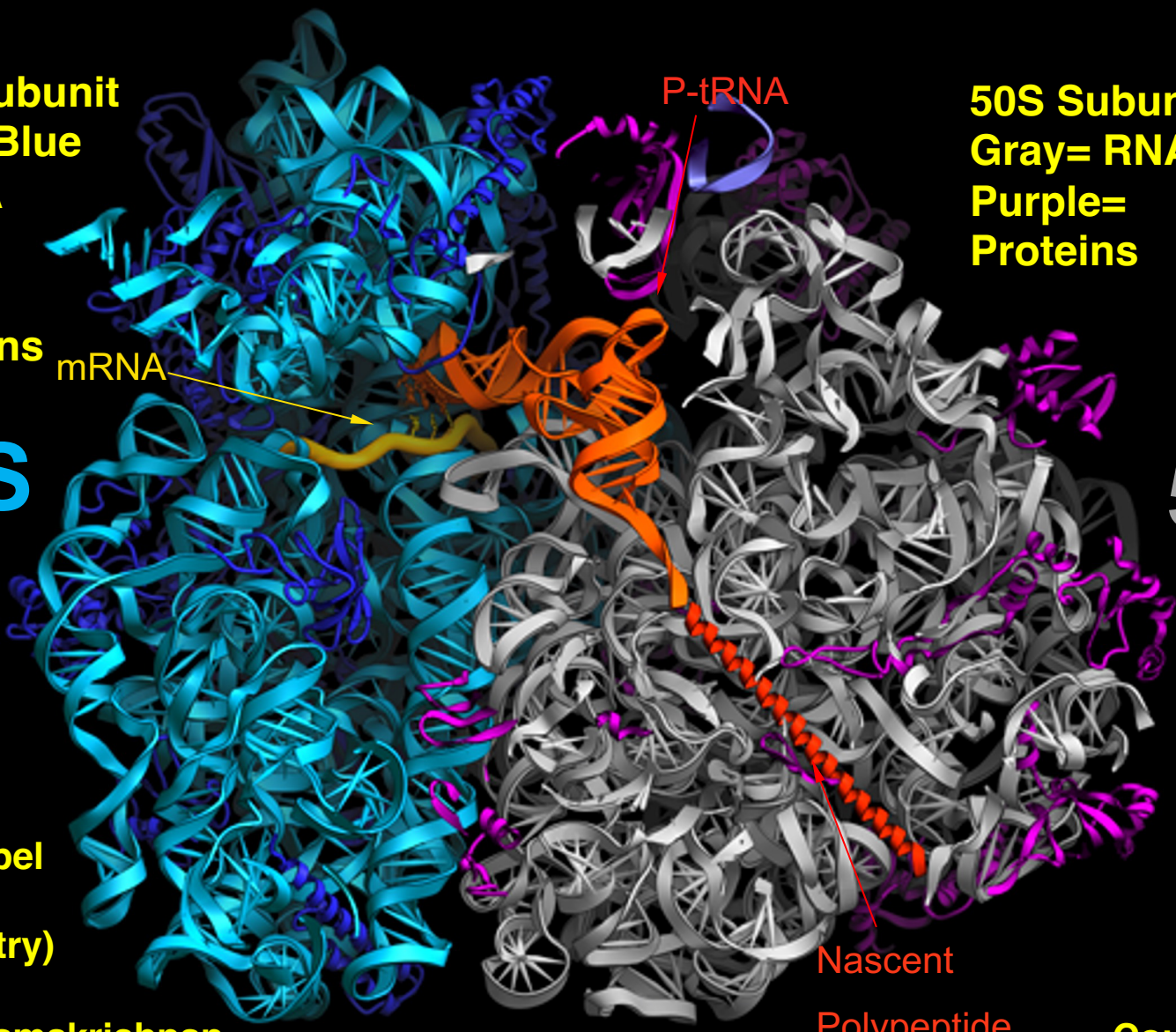
3D structure of a bacterial Ribosome



2009 Nobel Prize (Chemistry)

Venka Ramakrishnan
Tom Steitz
Ada Yonath

Courtesy
Harry Noller

Ribosome subunits functions

Small Subunit

Bacteria: 30S

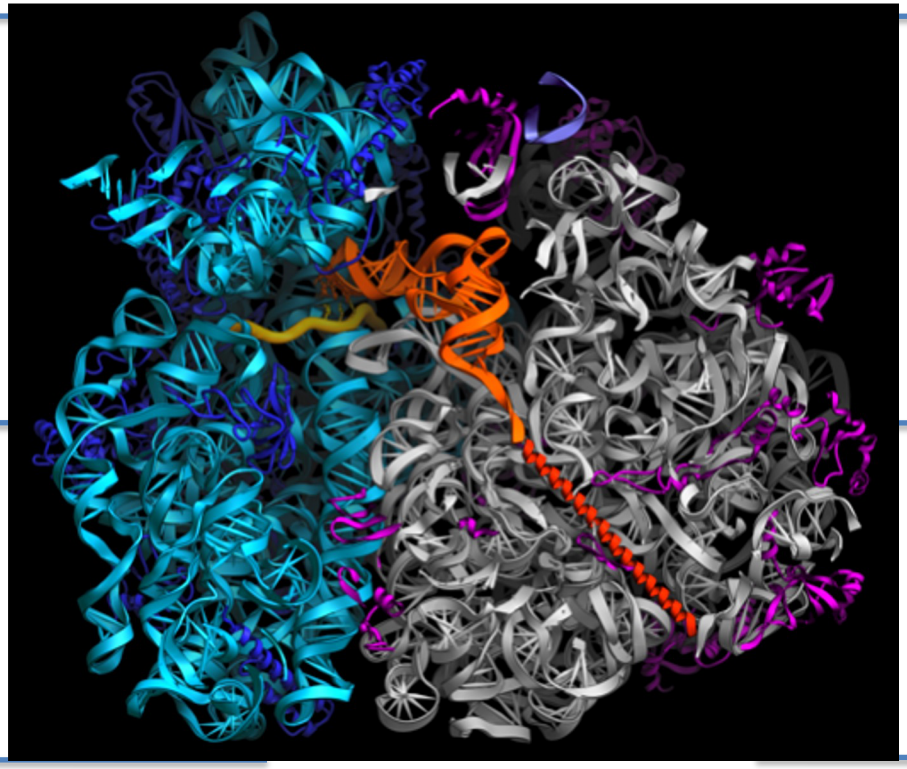
16S rRNA

21 proteins

Eukaryotes: 40S

18S rRNA

33 proteins



Large Subunit

Bacteria: 50S

23S, 5S rRNAs

31 proteins

Eukaryotes: 60S

28S, 5S, 5.8 S rRNAs

49 proteins

Functions

mRNA binding

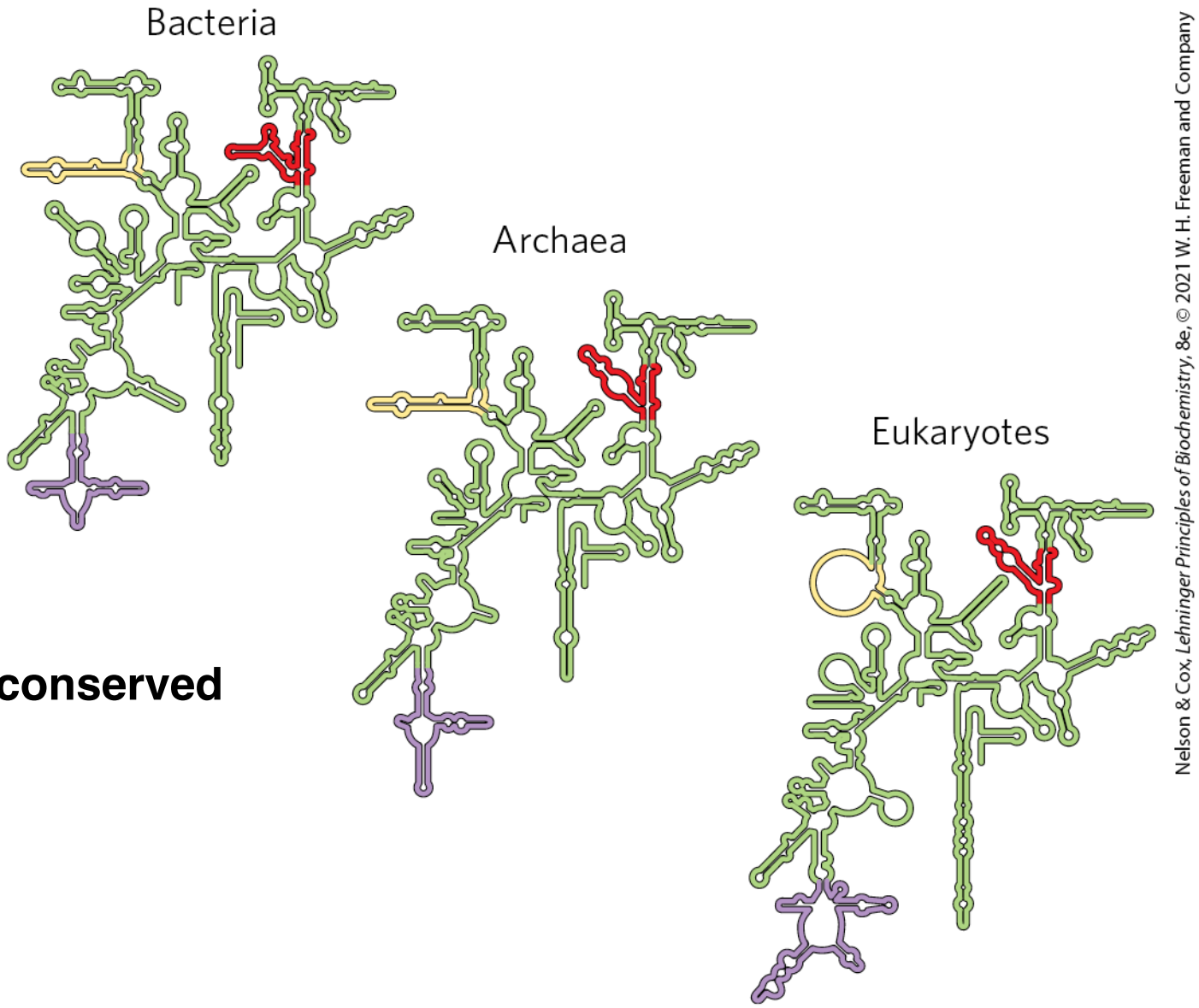
Codon/Anticodon interaction

Functions

Peptidyl transferase

Exit tunnel for proteins

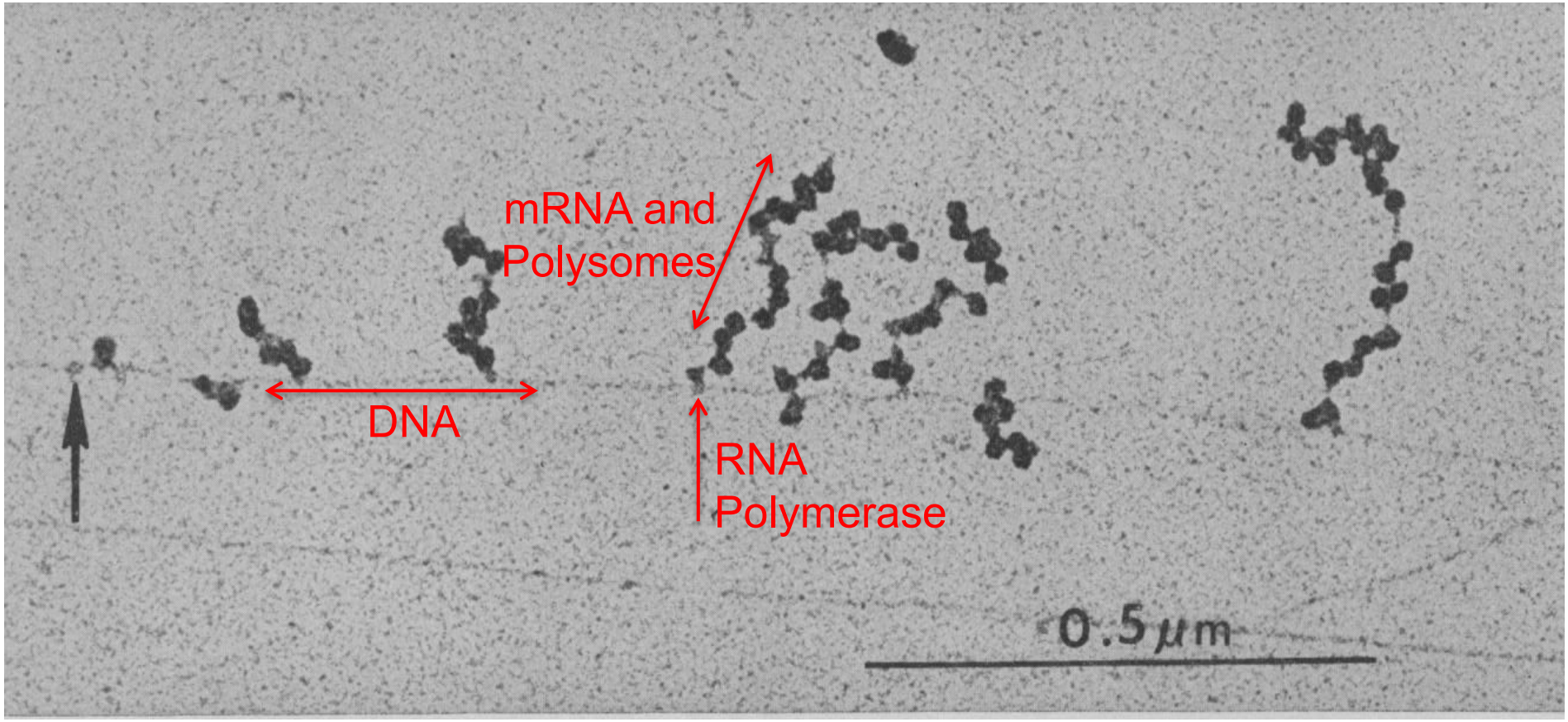
Conservation of rRNA secondary structure across 3 domains of life



Green: conserved regions

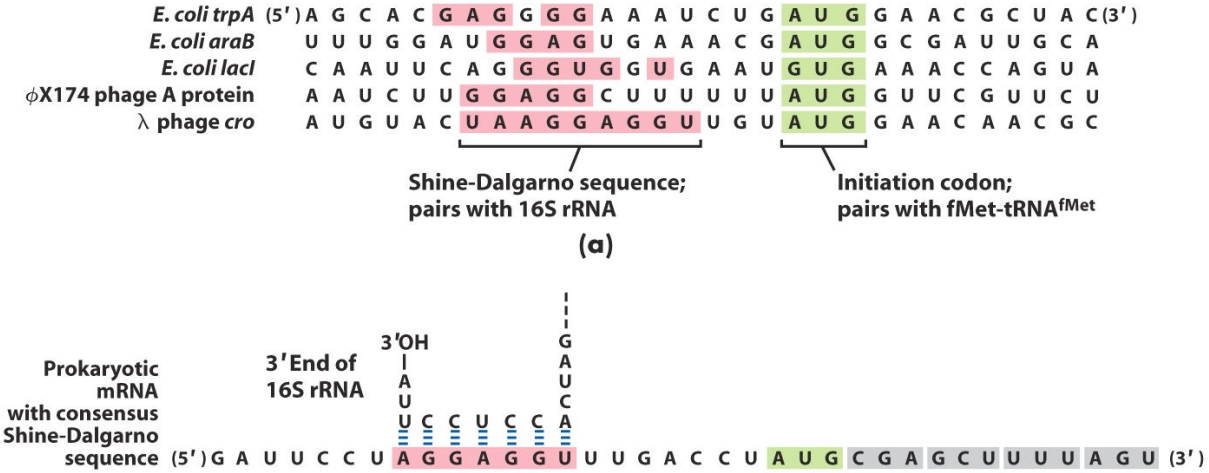
Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2021 W. H. Freeman and Company

Transcription and translation are coupled in bacteria



Miller et al., Science 1970

Bacterial translation initiation



STEP 1: small subunit binds to the mRNA – Shine-Dalgarno sequence pairs with 16S rRNA

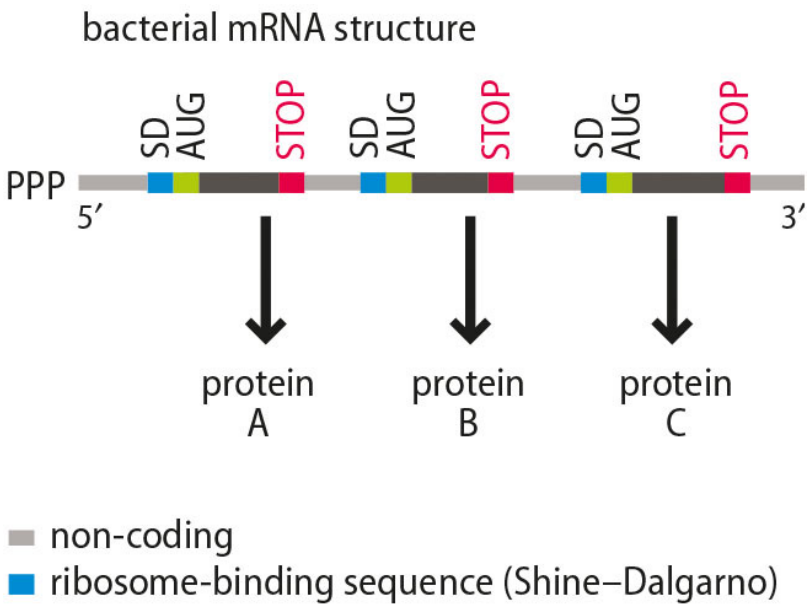
STEP 2: initiator tRNA joins the complex, binds to the P site

STEP 3: large subunit binds, resulting in functional 70S ribosome

Bacterial translation initiation

Translation immediately follows transcription in bacteria (no nuclear/cytoplasmic compartmentalization)

Translation of a polycistronic mRNA with multiple ORFs in bacteria



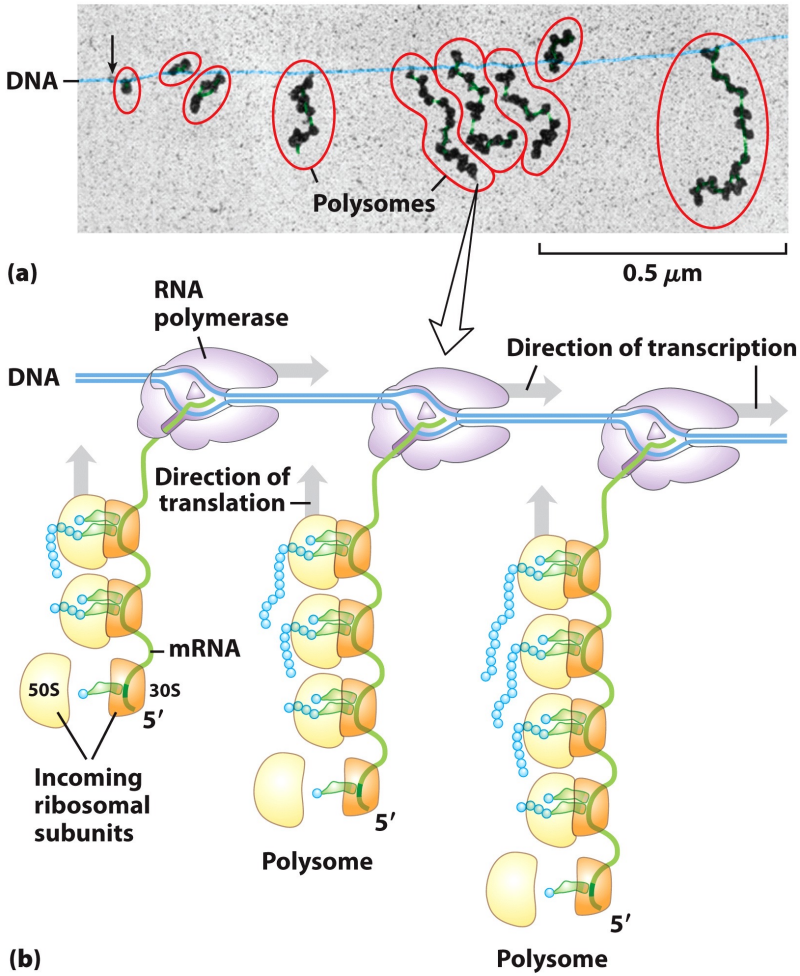


Figure 27-33
Lehninger Principles of Biochemistry, Sixth Edition
© 2013 W. H. Freeman and Company

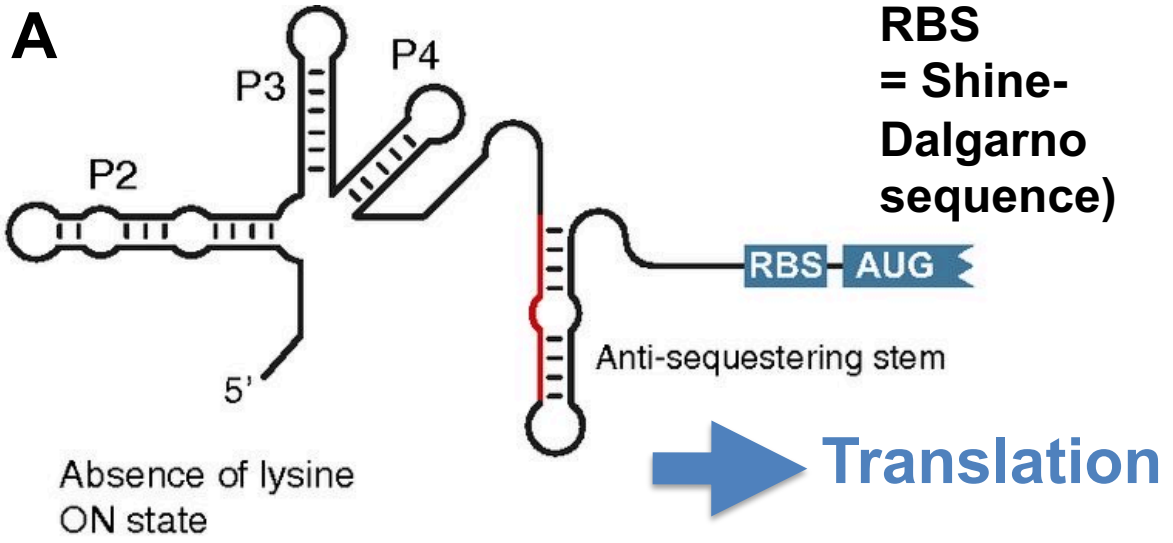
https://www.youtube.com/watch?v=u9dhO0iCLww

Regulation of bacterial translation initiation by riboswitches

Riboswitch: RNA sequence that changes its structure upon metabolite binding

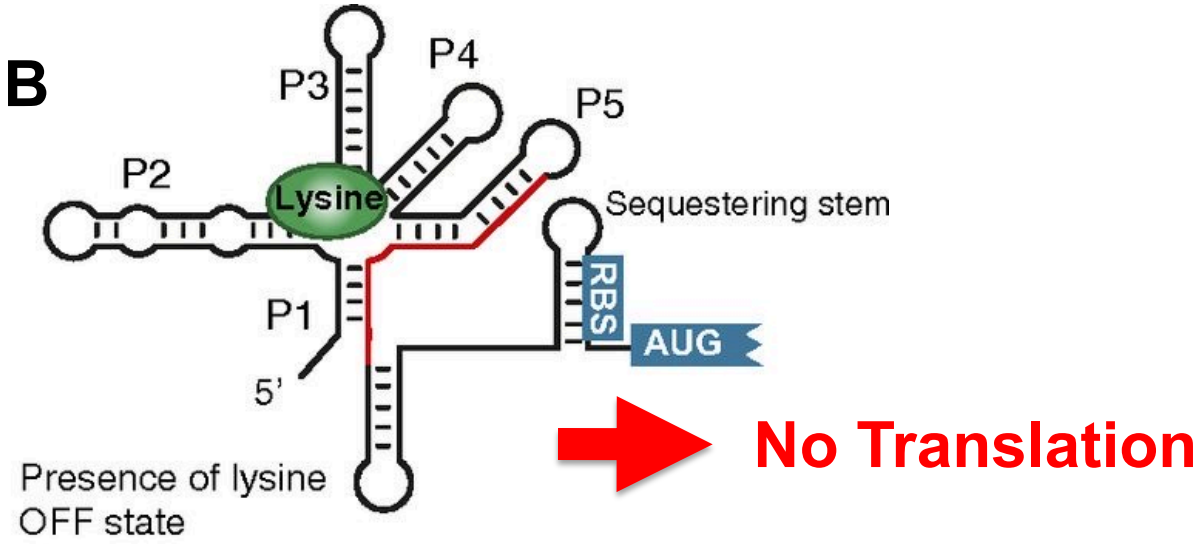
Example: Lysine riboswitch

A – Low cellular Lysine levels → Translation of Lysine metabolic genes because the RBS is available

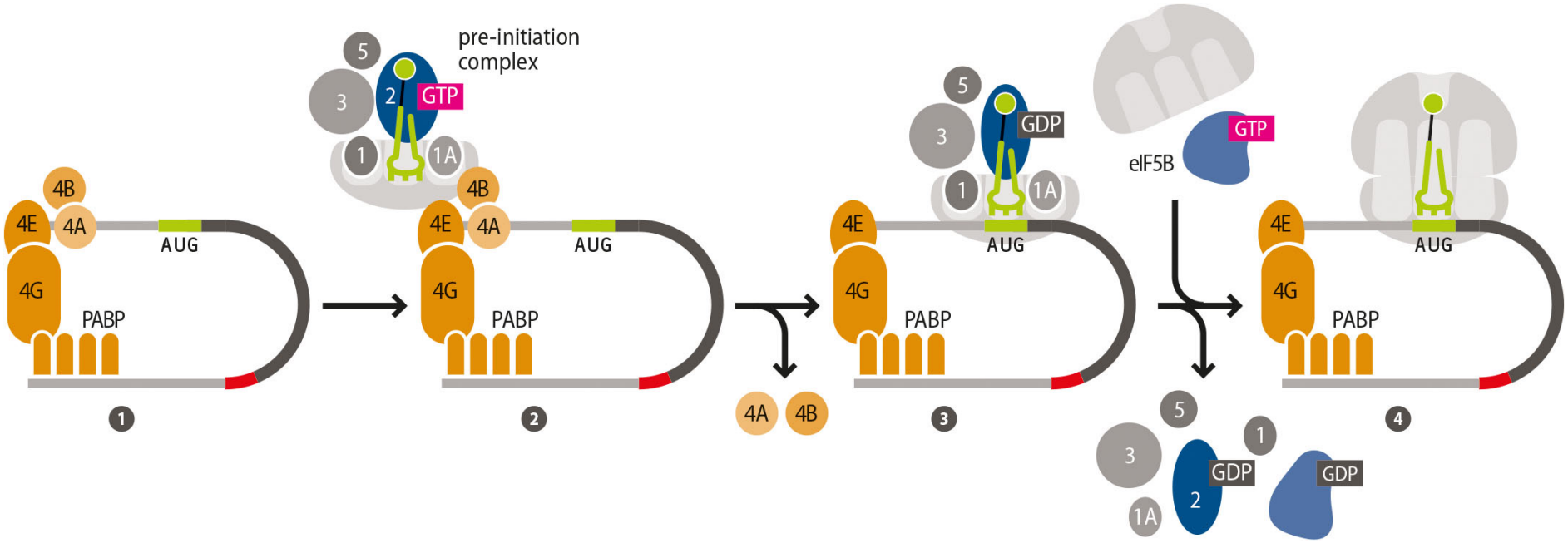


RBS = Shine-Dalgarno sequence)

B – High cellular Lysine levels → Repression of translation of Lysine Metabolic Genes because the RBS is sequestered



Eukaryotic translation initiation: cap-dependent mechanism



1) mRNA <u>preparation</u> by eIF4F components (4A, 4B, 4E, 4G)

2) <u>Loading</u> of pre-initiation complex (40S, initiator tRNA bound to eIF2-GTP, initiation factors)

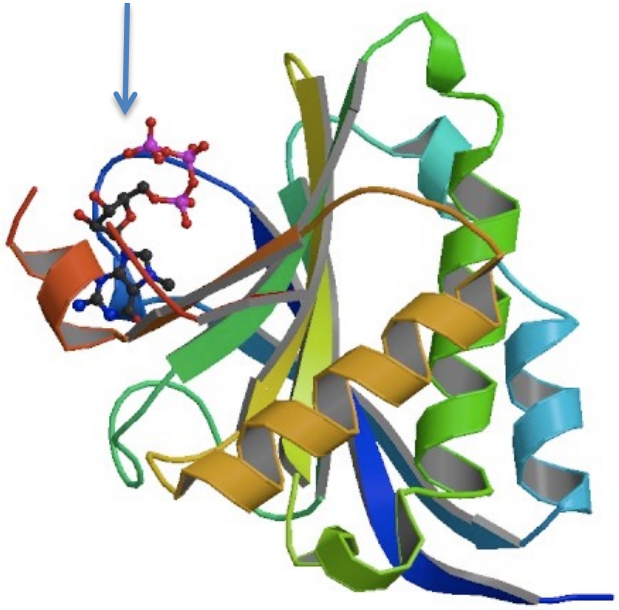
3) <u>Scanning and start codon recognition</u>

4) <u>60S joins</u> to form 80S, most initiation factors released

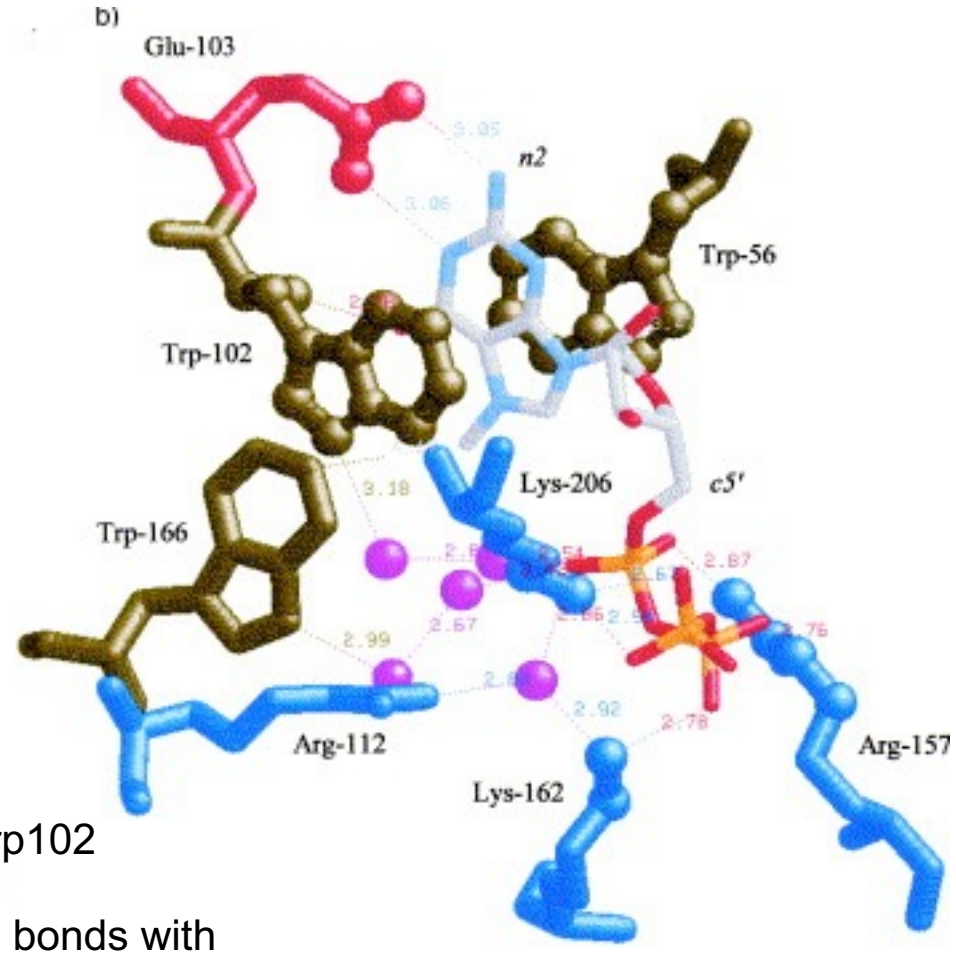
From Molecular Biology, Principles of Genome Function

Eukaryotic translation initiation:
Recognition of the 5´-cap structure of eukaryotic mRNAs by eIF4E (part of the EIF4F complex)

Cap binding pocket is on the concave surface of the protein – allows easy entry of the mRNA 5´-end



PDB ID = 1L8B
Niedzwiecka et al. J.Mol.Biol. 2002

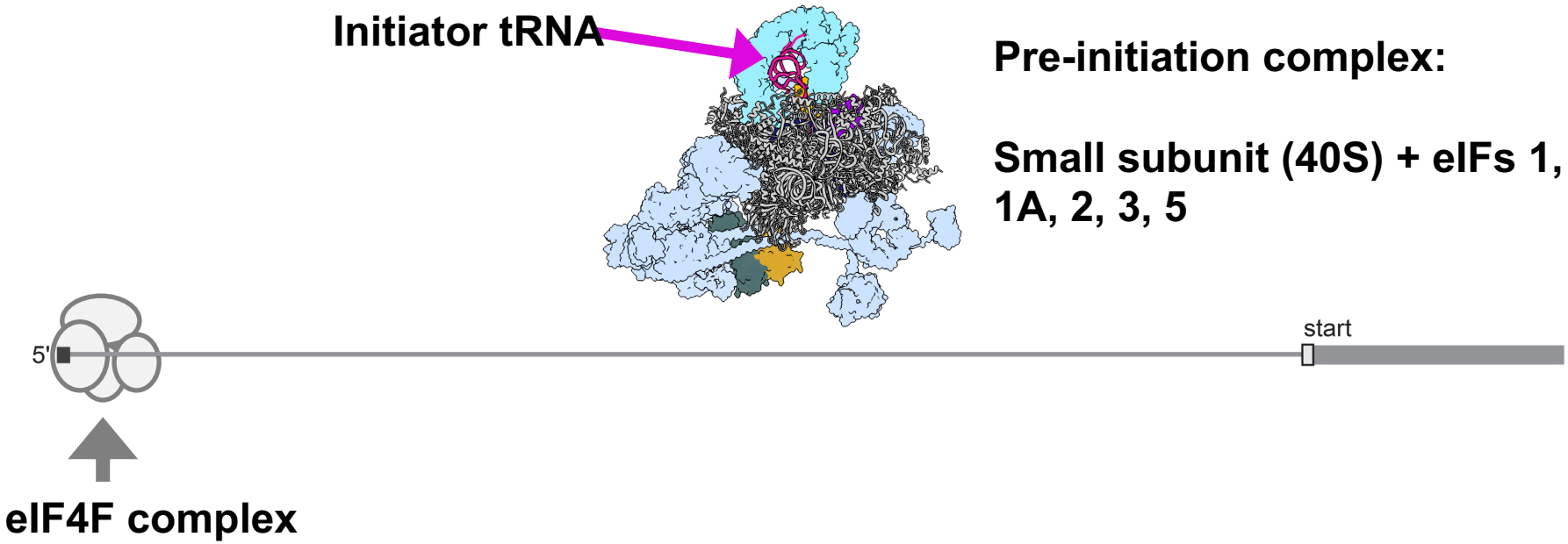


- base sandwich-stacking between Trp56 and Trp102
- formation of three Watson–Crick-like hydrogen bonds with Glu103 and backbone NH of Trp102
- Hydrophobic interaction of the 7-methyl group with Trp166

● = H2O

PyMol: eIF4e_Cap_Complex.pse

Eukaryotic initiation factors (eIFs) and a tRNA choreograph initiation



Slide courtesy of Chris Lapointe, Fred Hutch CRC

40S (small subunit) loading onto an mRNA selects the mRNA template

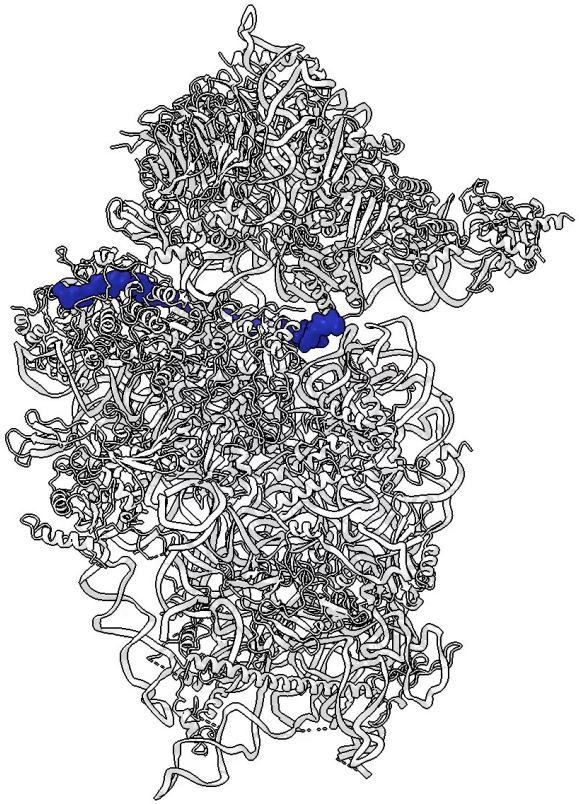
Selected from thousands of mRNAs
Rate limiting

Slide courtesy of Chris Lapointe, Fred Hutch CRC

The mRNA is loaded into the 40S (small) subunit

40S (small) subunit

mRNA



Head

Body

Brito Querido et al. 2020.

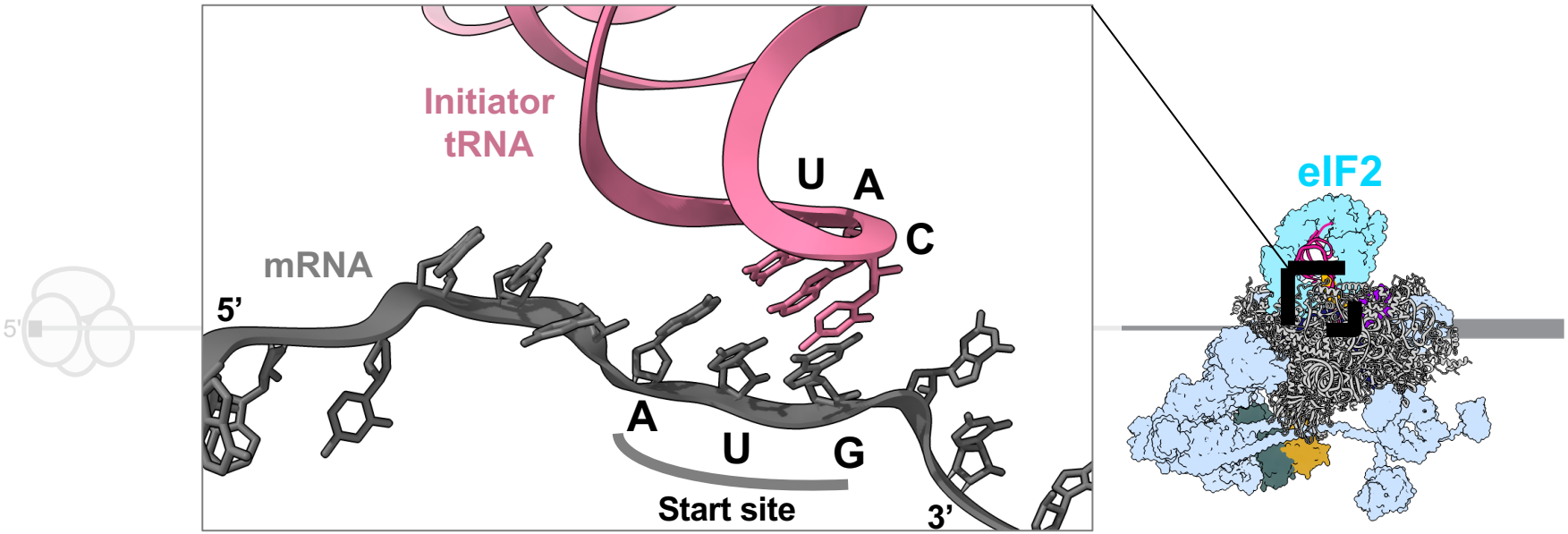
Slide courtesy of Chris Lapointe, Fred Hutch CRC

The initiation complex “scans” the 5’ UTR for a translation start site

Establish reading frame

Slide courtesy of Chris Lapointe, Fred Hutch CRC

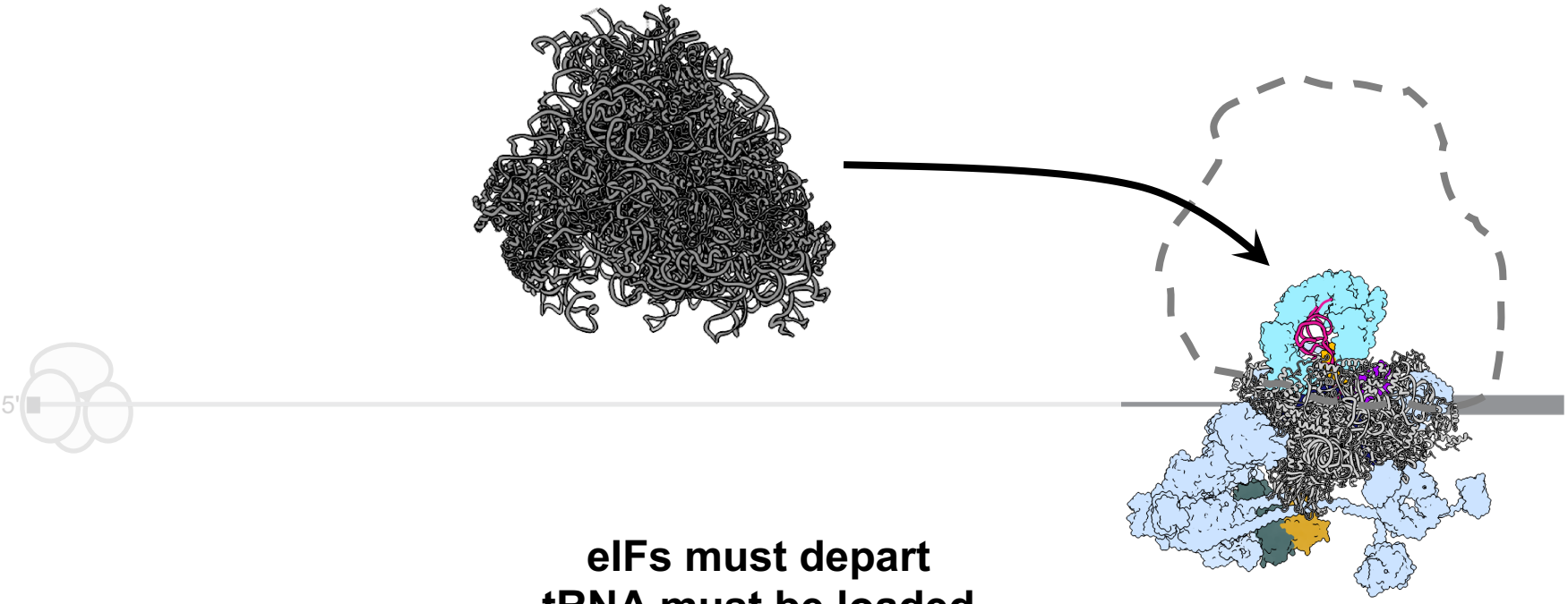
The eIFs and initiator tRNA decode the mRNA to identify the start site



Base by base decoding
Recognition triggers GTP hydrolysis by eIF2

Slide courtesy of Chris Lapointe, Fred Hutch CRC

Large subunit (60S) joins



eIFs must depart
tRNA must be loaded
Inter-subunit contacts formed
Catalyzed by a 2nd GTPase

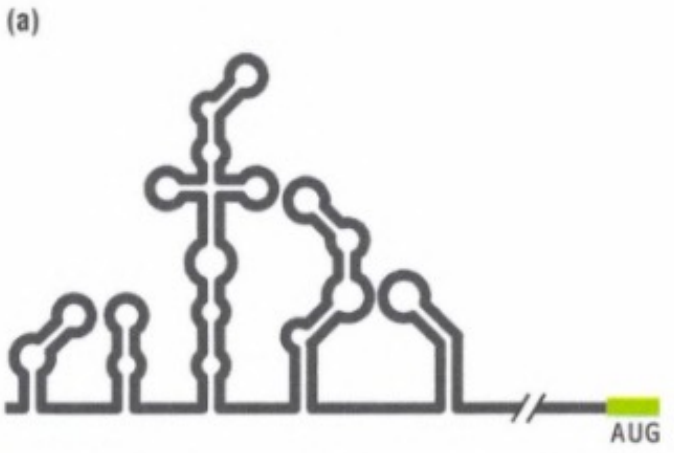
Slide courtesy of Chris Lapointe, Fred Hutch CRC

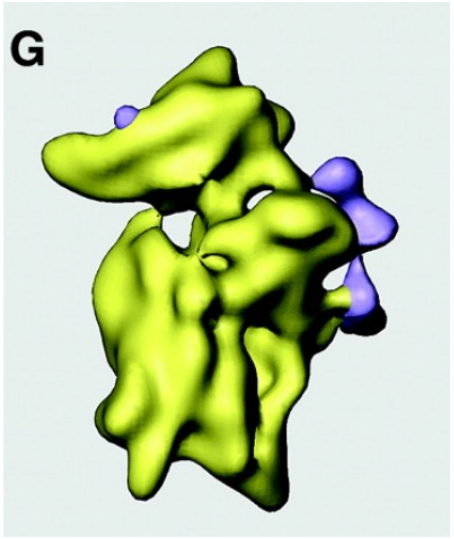
Subunit joining commits the ribosome to synthesize a protein

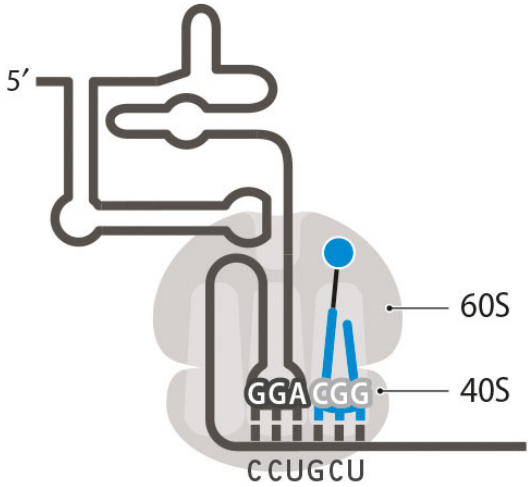
Ready for synthesis

Slide courtesy of Chris Lapointe, Fred Hutch CRC

Internal Ribosome Entry Site (IRES) – common strategy for viruses



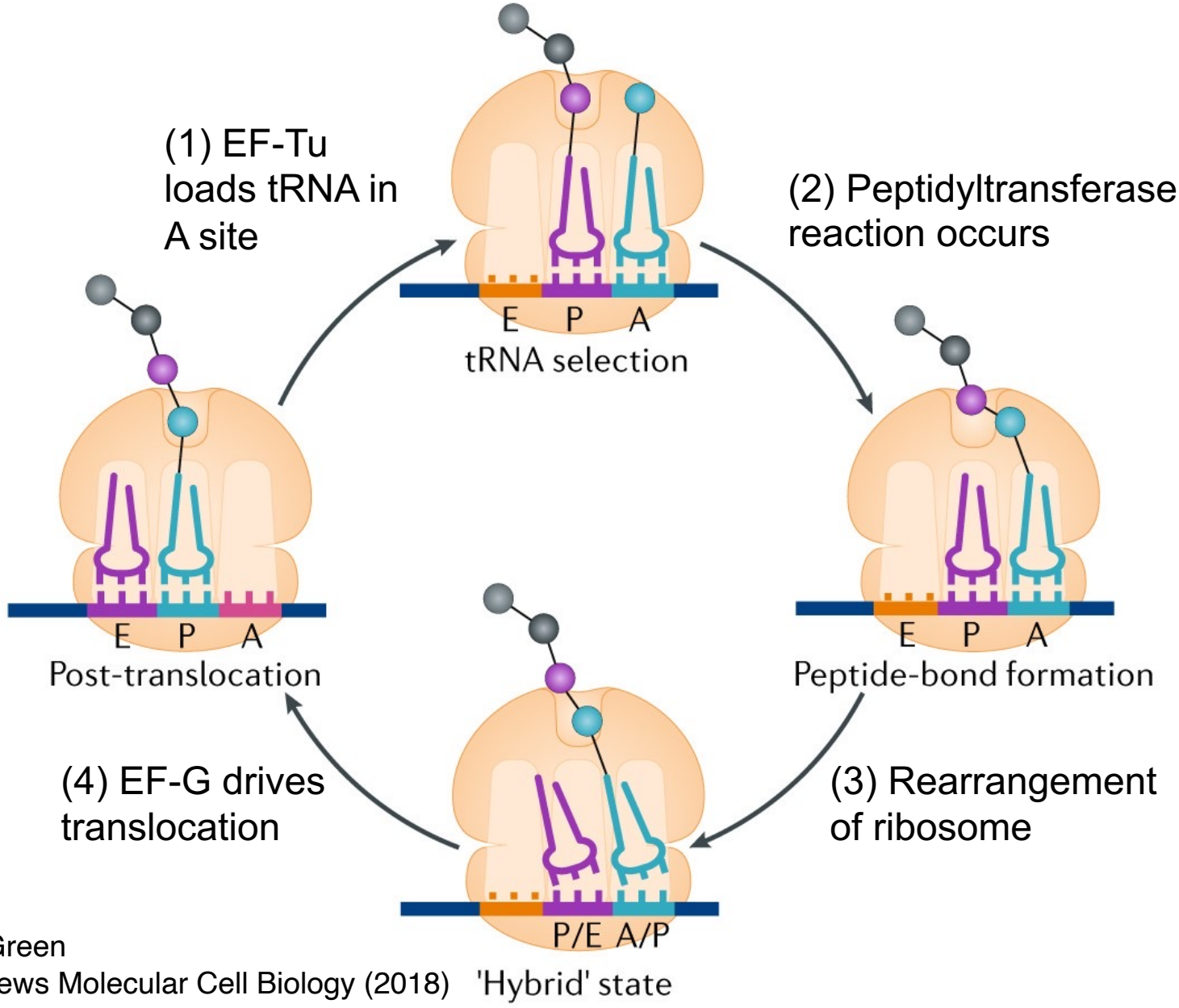




- IRES in 5'UTR of viral RNAs can facilitate translation initiation
- Highly structured elements, direct binding to ribosome
- Dispense with need for some (HCV, top images) or all (CrPV, bottom image)

Diagrams from Molecular Biology, Principles of Genome Function
Figure from Spahn et al., Science 2001

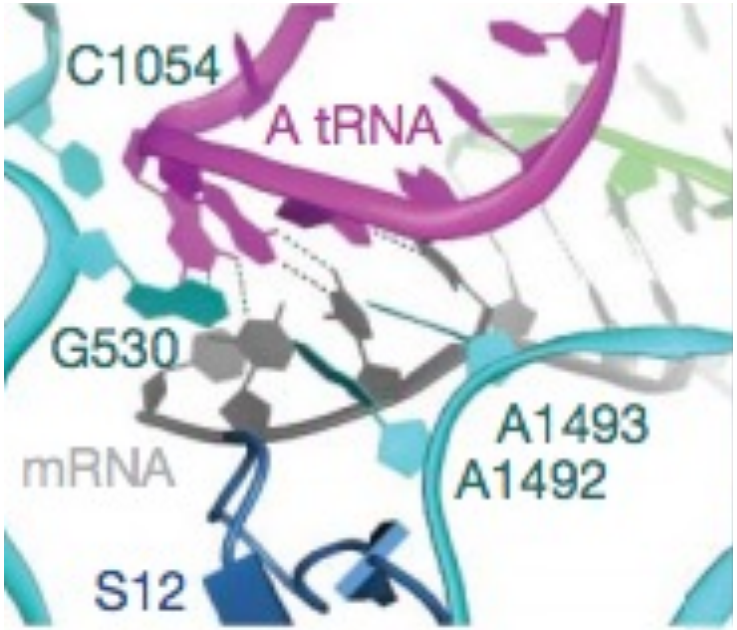
Summary of translation elongation (similar in bacteria and eukaryotes, bacterial names shown)



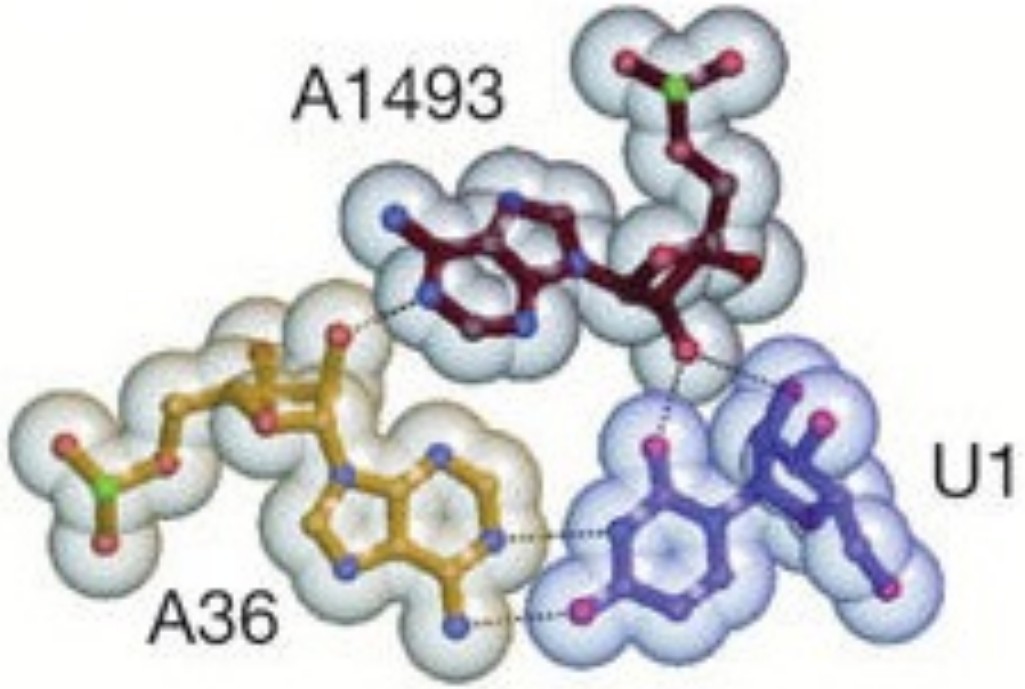
Schuller & Green
Nature Reviews Molecular Cell Biology (2018)

The codon/anticodon interaction is stabilized in the A site through interactions with the 16S rRNA

5´-U1 U2 U3-3´ **codon**
| | •
3´-A_{36} A_{35} G_{34}-5´ **anticodon**



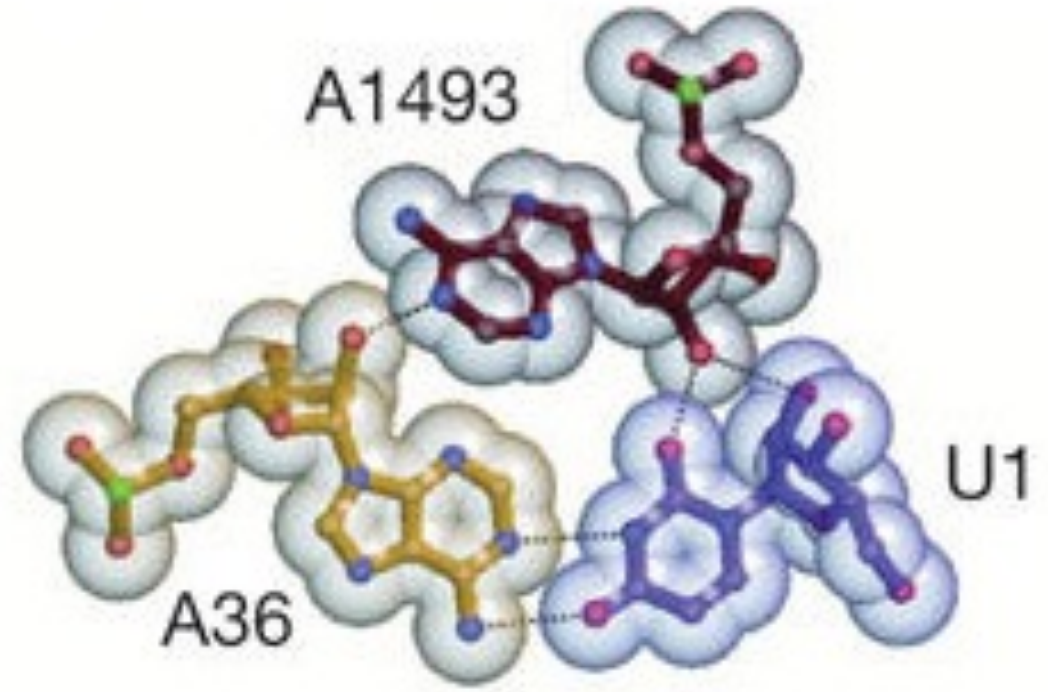
Schmeing & V. Ramakrishnan
Nature 2009



A-Minor interaction between A1493 of 16S rRNA and the first codon/anticodon base-pair

Moore &
Steitz
Nature 2002

Is this interaction specific to the codons containing a U at position 1?



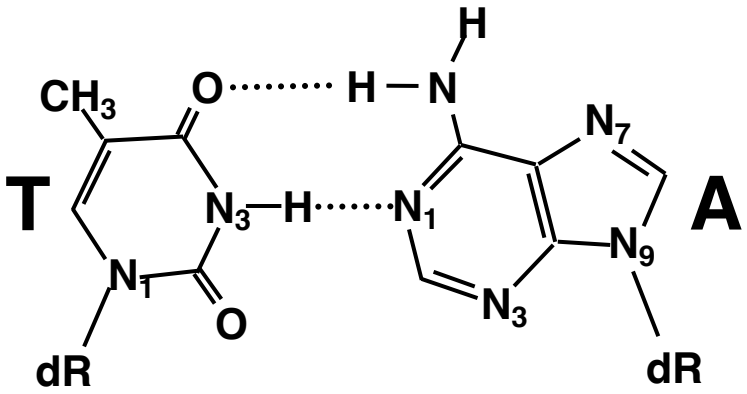
A: Yes because it involves the carbonyl of U in the minor groove

B: No because it involves the 2´OH of both codon and anticodon

C: Yes because a G instead of the U would sterically clash with A1493

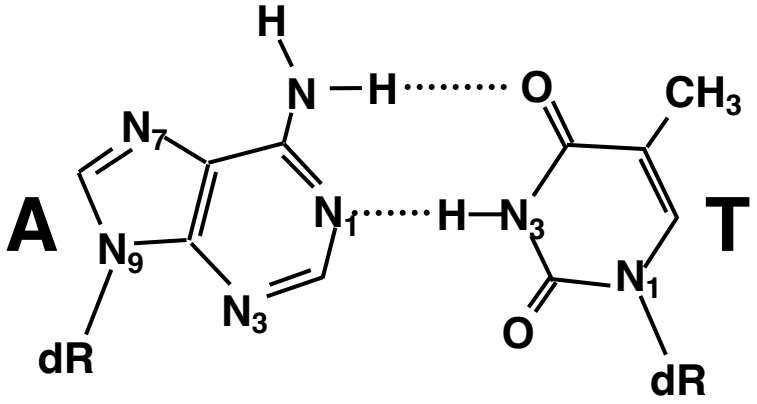
D: No because you could form interactions with the H-bond acceptor in the minor groove and the 2´-OH regardless of the sequence

Recognition of Specific sequences by DNA-binding proteins



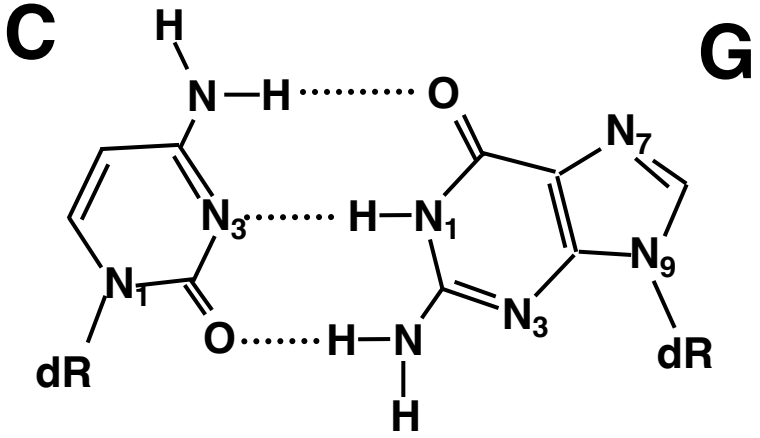
Distribution of H-bonds Donors (D) Acceptors (A) and Hydrophobic groups (H) available for recognition

D
H A A
Major groove
Minor groove
A A



D
A A H
Major groove
Minor groove
A A

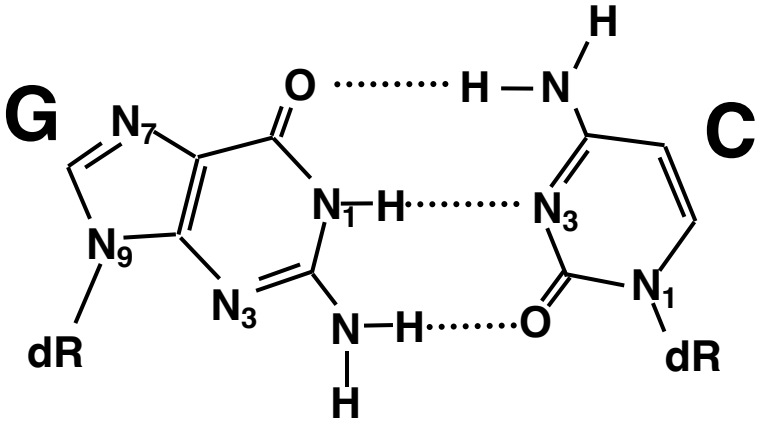
Recognition of Specific sequences by DNA-binding proteins



Patterns of H-bonds Donors (D), Acceptors (A), and Hydrophobic groups (H) available for recognition

D A A — Major groove

A A D — Minor groove



D A A — Major groove

A A D — Minor groove

Fidelity of A-site codon/anticodon interaction is sensed numerous times in elongation (kinetic proofreading)

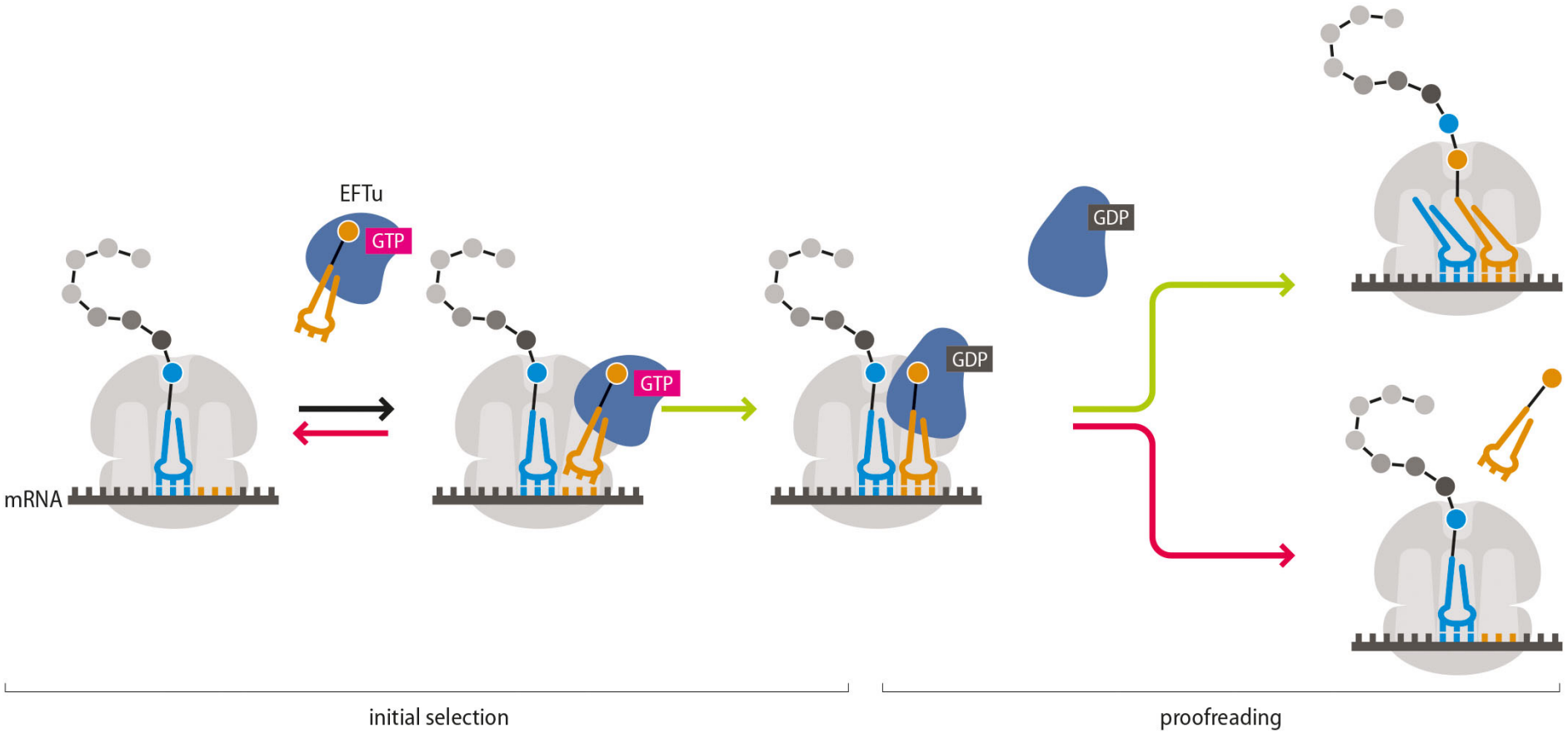
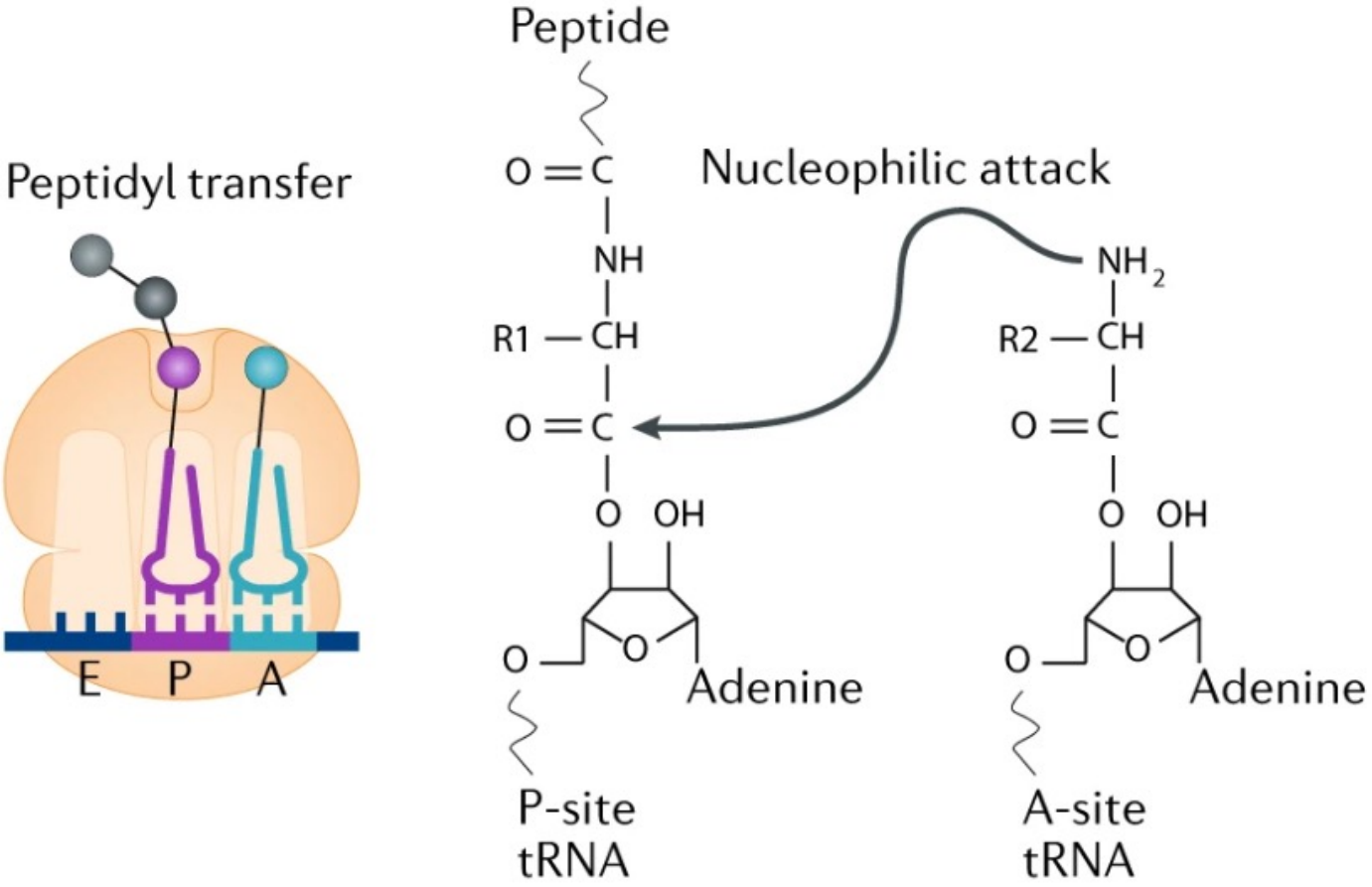


Figure 11.28 Decoding (tRNA selection) by the ribosome and EFTu. The selection of a cognate aminoacyl-tRNA in the A site of the ribosome involves two distinct steps separated by the hydrolysis of GTP by EFTu. In the 'initial selection' phase, an aminoacyl-tRNA complexed with EFTu can be rejected from the ribosome before EFTu hydrolyzes GTP. Following GTP hydrolysis and the departure of EFTu, ribosomes still can reject near-cognate tRNA in a 'proofreading' phase. Both thermodynamic rejection steps are shown with red arrows. Additionally, cognate tRNAs promote more rapid GTPase activation and accommodation (shown in green arrows), allowing for kinetic discrimination mechanisms to contribute to the overall fidelity of tRNA selection.

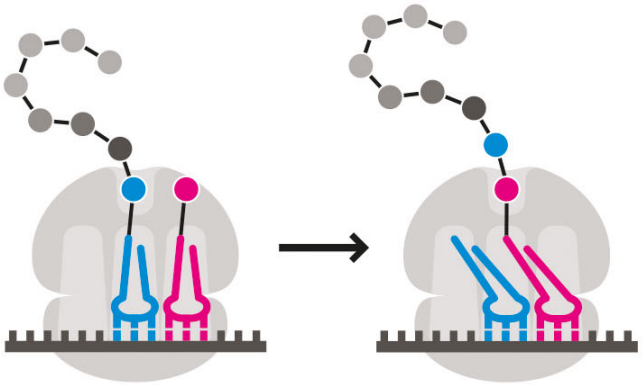
From Molecular Biology, Principles of Genome Function

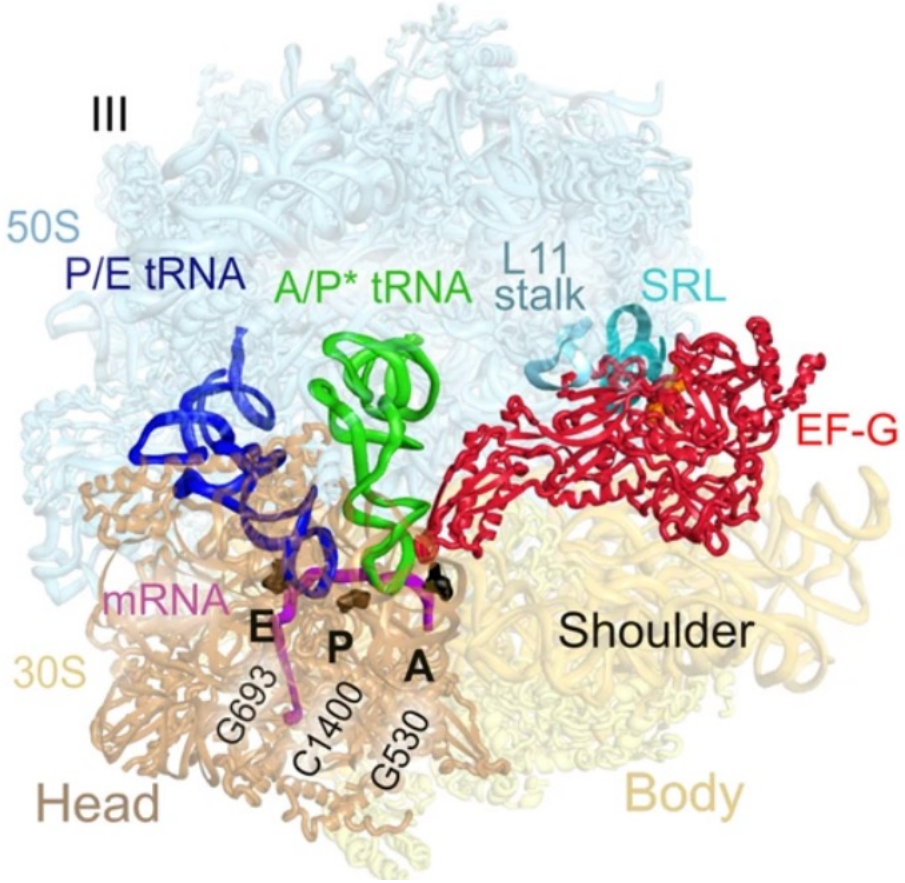
The peptidyl transferase reaction



Schuller & Green
Nature Reviews Molecular Cell Biology (2018)

EF-G drives translocation of tRNAs through the ribosome





- Rearrangement to hybrid state concurrent with peptide bond formation
- EF-G is a GTPase that mimics a tRNA-EfTu complex, drives forward movement of tRNAs through ribosome

Diagram from Molecular Biology, Principles of Genome Function
Structure from Carbone et al., Nat Comm 2021