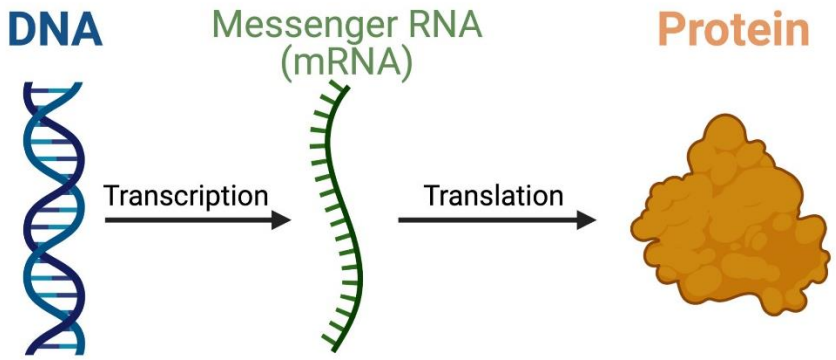
Translation

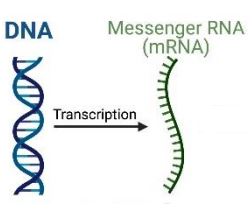


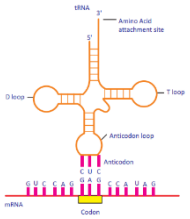
first position (5′ end)	second position: U	second position: C	second position: A	second position: G	third position (3′ end)
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA* stop	UGA* stop	A
	UUG Leu	UCG Ser	UAG* stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG† Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

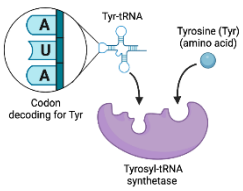
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

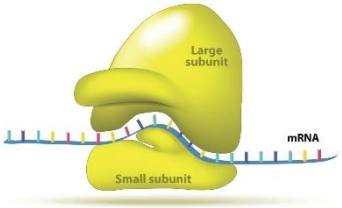
- **Translation machinery is composed of four primary components:**

✓ **mRNAs**
✓ **tRNAs**
✓ **aminoacyl-tRNA synthetases**
✓ **ribosome**



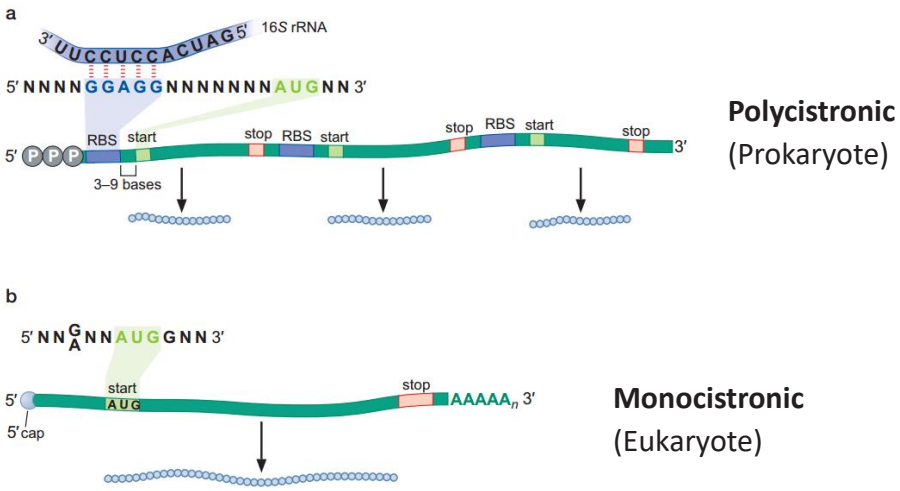






Messenger RNA (mRNA)

- The protein-coding region(s) of each mRNA is composed of a contiguous, non-overlapping string of codons called an open reading frame (ORF).
- The first and last codons of an ORF are known as the start and stop codons.
- In bacteria, the start codon is usually 5′-AUG-3′, sometimes 5′-GUG-3′ and 5′-UUG-3′.
- Eukaryotic cells always use 5′-AUG-3′ as the start codon.
- The start codon has two important functions. First, it specifies the first amino acid to be incorporated into the growing polypeptide chain. Second, it defines the reading frame for all subsequent codons.
- mRNAs containing multiple ORFs are known as polycistronic mRNAs, and those encoding a single ORF are known as monocistronic mRNAs.



Ribosome binding to ORF

- To facilitate binding by a ribosome, prokaryotic ORFs contain a short sequence upstream (on the 5′ side) of the start codon called the ribosome binding site (RBS), also known as Shine–Dalgarno sequence.
- The RBS sequence is complementary to a sequence located near the 3′ end of the 16S rRNA. The RBS base-pairs with this RNA, thereby aligning the ribosome with the beginning of the ORF.

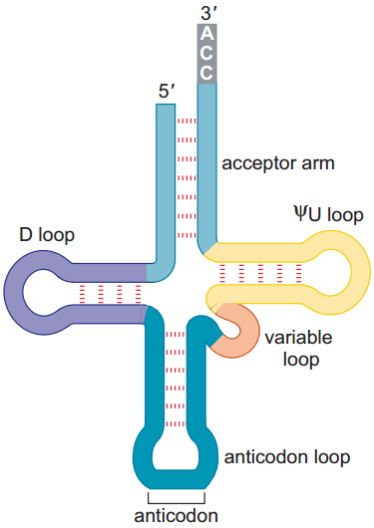
high complementarity- active translation
limited complementarity- lower levels of translation

Translational coupling:
The start codon of the downstream ORF often overlaps the 3′ end of the upstream ORF (5′-AUGA-3′). Thus, a ribosome that has just completed translating the upstream ORF is positioned to begin translating from the start codon for the downstream ORF. This arrangement circumvents the need for an RBS to recruit the ribosome. This phenomenon of linked translation between overlapping ORFs is known as Translational coupling.

Scanning: The 5′ cap of mRNA is required to recruit the ribosome to the mRNA. Once bound to the mRNA, the ribosome moves in a 5′ - 3′ direction until it finds a 5′-AUG-3′ start codon. The process is called scanning.

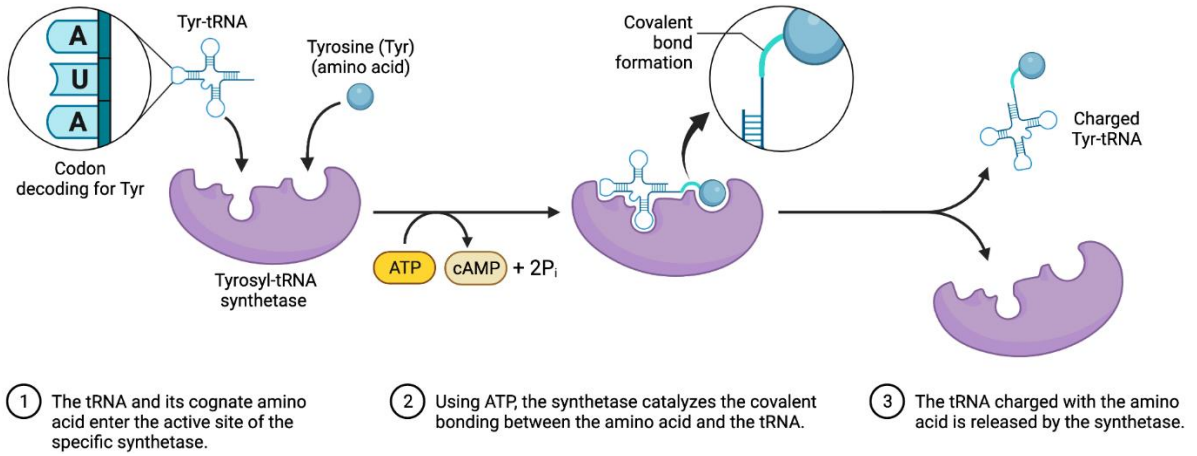
Transfer RNA (tRNA)

- tRNAs are adaptors between Codons and Amino Acids.
- tRNA molecules are between 75 and 95 ribonucleotides in length.
- all tRNAs end at the 3′ terminus with the sequence 5′-CCA-3′.
- tRNAs share a common secondary structure that resembles a Cloverleaf.



- The acceptor stem: it is the site of attachment of the amino acid, is formed by pairing between the 5′ and 3′ ends of the tRNA molecule.
- T loop or ΨU: it is so-named because of the presence of pseudouridine (an unusual base) in the loop. The ΨU is often found within the sequence 5′-TΨUCG-3′.
- The D loop: dihydrouridines (an unusual base) is present in the loop.
- The anticodon loop: contains the anticodon, a three-nucleotide-long sequence, which recognizes the codon in the mRNA.
- The variable loop sits between the anticodon loop and the ΨU loop and, as its name implies, varies in size from 3 to 21 bases.

Aminoacyl-tRNA Synthetases in tRNA Charging



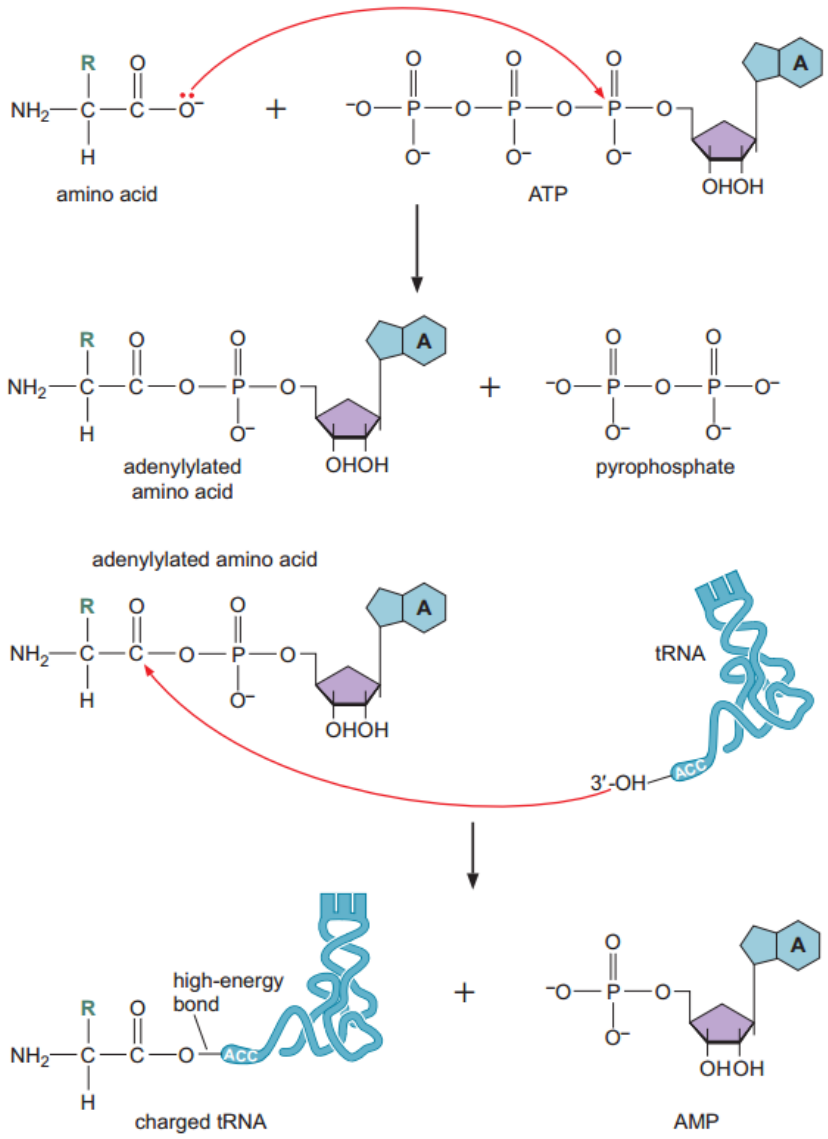
ATTACHMENT OF AMINO ACIDS TO tRNA

- tRNAs are Charged by the Attachment of an Amino Acid to the 3′-Terminal Adenosine Nucleotide via Acyl Linkage
- tRNA molecules to which an amino acid is attached are said to be charged, and tRNAs that lack an amino acid are said to be uncharged.

- Aminoacyl-tRNA Synthetases Charge tRNAs in Two Steps

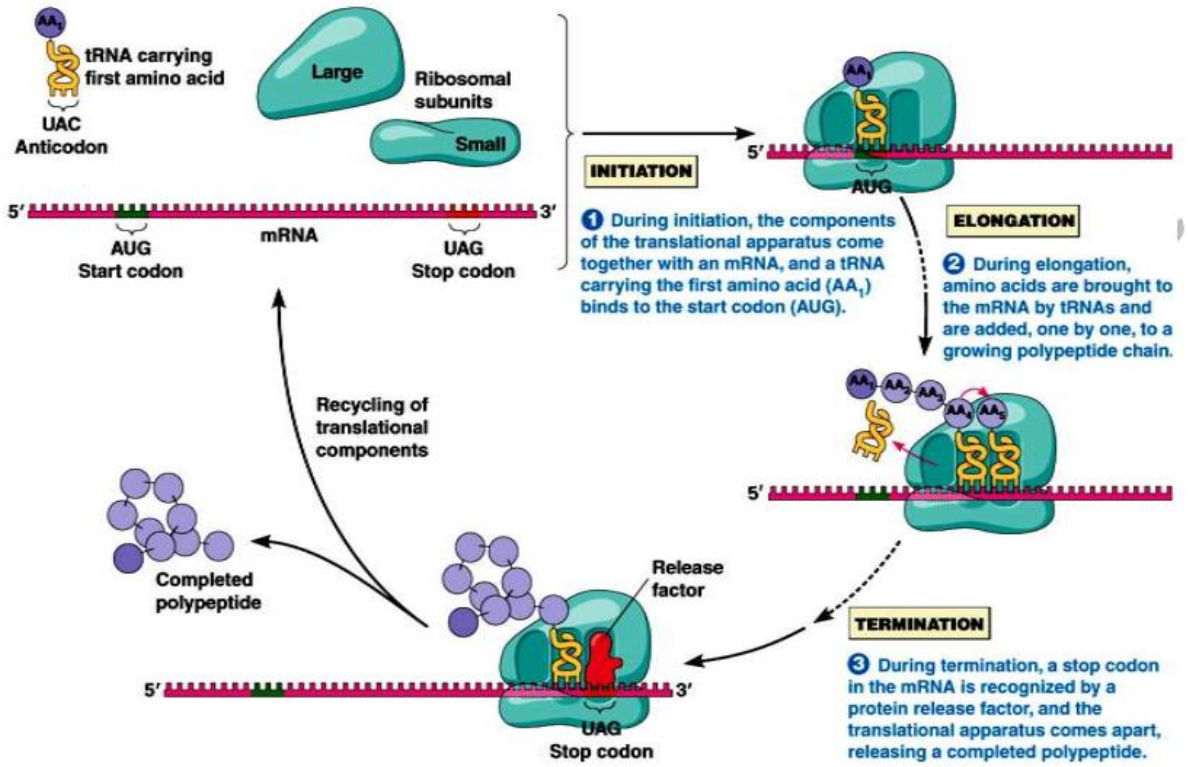
    1. Step one is adenylylation in which the amino acid reacts with ATP to become adenylylated with the concomitant release of pyrophosphate.

    2. Step two is tRNA charging in which the adenylylated amino acid, which remains tightly bound to the synthetase, reacts with tRNA. This reaction results in the transfer of the amino acid to the 3′ end of the tRNA via the 2′- or 3′-hydroxyl and the release of AMP.



Translation

Translation is the process where the mRNA is decoded to build a polypeptide chain (protein).



Steps:

a. Initiation

- **mRNA Binding**: The mRNA binds to the small subunit of the ribosome.
- **Start Codon Recognition**: The ribosome identifies the start codon (AUG) on the mRNA.
- **tRNA Binding**: A transfer RNA (tRNA) molecule with the complementary anticodon (UAC) and the amino acid methionine binds to the start codon.

b. Elongation

- **Codon Recognition**: The ribosome reads the next codon on the mRNA.
- **tRNA Binding**: A tRNA molecule with the complementary anticodon and corresponding amino acid binds to the mRNA codon.
- **Peptide Bond Formation**: The ribosome forms a peptide bond between the amino acids, creating a growing polypeptide chain.
- **Ribosome Movement**: The ribosome moves along the mRNA, allowing the next tRNA to bind.

c. Termination

- **Stop Codon Recognition**: The ribosome reaches a stop codon (UAA, UAG, or UGA) on the mRNA.
- **Release Factors**: Release factors bind to the ribosome, prompting the release of the polypeptide chain.
- **Disassembly**: The ribosome subunits disassemble, and the mRNA and tRNA are released.

Post-Translational Modifications:

After translation, the newly synthesized polypeptide may undergo several modifications:

- **Folding**: The polypeptide folds into its three-dimensional structure, assisted by chaperone proteins.
- **Cleavage**: Some proteins are cleaved to become active.
- **Chemical Modifications**: Additions such as phosphorylation, glycosylation, or methylation may occur to regulate protein function.

Fidelity of translation:
The fidelity of translation refers to the accuracy with which a cell's ribosomes synthesize proteins according to the genetic instructions encoded in mRNA. High fidelity is crucial for proper cellular function, as errors in translation can lead to malfunctioning proteins and potentially harmful consequences for the cell and organism. Several mechanisms ensure the high fidelity of translation:

- **Specificity of aminoacyl-tRNA synthetases**: Ensuring correct tRNA charging.
- **Accurate codon-anticodon matching**: Ensuring correct tRNA selection during translation.
- **Ribosomal proofreading mechanisms**: Monitoring and correcting errors during translation.
- **Error correction mechanisms**: Detecting and addressing mistakes in mRNA and tRNA.

These mechanisms collectively ensure that proteins are synthesized accurately according to the genetic instructions, maintaining cellular and organismal health.

Proteins involved in Translation:
Protein synthesis involves several key proteins and complexes that facilitate the stages of initiation, elongation, and termination. Here are the main proteins involved in each stage:

Initiation
The initiation phase involves the assembly of the translation machinery at the start codon of the mRNA.

1. **Initiation Factors (IFs) in Prokaryotes**
   - **IF-1**: Binds to the A site of the 30S ribosomal subunit to prevent premature tRNA binding.
   - **IF-2**: GTP-binding protein that assists the initiator tRNA in binding to the P site of the ribosome.
   - **IF-3**: Binds to the 30S subunit to prevent premature association with the 50S subunit and aids in the proper positioning of the start codon.
2. **Initiation Factors (eIFs) in Eukaryotes**
   - **eIF1 and eIF1A**: Aid in scanning the mRNA for the start codon.
   - **eIF2**: GTP-binding protein that delivers the initiator tRNA (Met-tRNAi^Met) to the 40S ribosomal subunit.
   - **eIF3**: Binds to the 40S subunit and prevents premature association with the 60S subunit.
   - **eIF4 Complex**: Includes eIF4E (cap-binding protein), eIF4G (scaffold protein), and eIF4A (RNA helicase) to facilitate mRNA binding to the ribosome.
   - **eIF5**: Promotes GTP hydrolysis on eIF2, aiding in the release of initiation factors.
   - **eIF6**: Prevents premature assembly of the 60S and 40S ribosomal subunits.

Elongation
The elongation phase involves the sequential addition of amino acids to the growing polypeptide chain.

1. **Elongation Factors (EFs) in Prokaryotes**

- **EF-Tu (Elongation Factor Thermo Unstable)**: GTP-binding protein that delivers aminoacyl-tRNA to the A site of the ribosome.
- **EF-Ts (Elongation Factor Thermo Stable)**: Regenerates EF-Tu by exchanging GDP for GTP.
- **EF-G (Elongation Factor G)**: GTP-binding protein that facilitates the translocation of the ribosome along the mRNA.

2. **Elongation Factors (eEFs) in Eukaryotes**
   - **eEF1A**: GTP-binding protein that delivers aminoacyl-tRNA to the A site of the ribosome.
   - **eEF1B**: Regenerates eEF1A by exchanging GDP for GTP.
   - **eEF2**: GTP-binding protein that facilitates the translocation of the ribosome along the mRNA.

Termination

The termination phase involves the release of the newly synthesized polypeptide and the disassembly of the translation machinery.

1. **Release Factors (RFs) in Prokaryotes**
   - **RF1**: Recognizes the stop codons UAG and UAA.
   - **RF2**: Recognizes the stop codons UGA and UAA.
   - **RF3**: GTP-binding protein that promotes the release of RF1 and RF2 from the ribosome after peptide release.
2. **Release Factors (eRFs) in Eukaryotes**
   - **eRF1**: Recognizes all three stop codons (UAA, UAG, UGA) and promotes the release of the polypeptide from the ribosome.
   - **eRF3**: GTP-binding protein that associates with eRF1 to facilitate the termination process.
3. **Ribosome Recycling Factors**
   - **RRF (Ribosome Recycling Factor)**: Works with EF-G to disassemble the ribosome-mRNA complex in prokaryotes.
   - **ABCE1**: A protein involved in ribosome recycling in eukaryotes, facilitating the disassembly of post-termination complexes.

These factors work together to ensure the accurate and efficient synthesis of proteins, allowing for proper cellular function and regulation.

Inhibitors of Protein synthesis

Protein synthesis inhibitors can be classified into several categories based on their targets and mechanisms of action:

1. Aminoglycosides
- **Examples**: Gentamicin, Neomycin, Streptomycin
- **Target**: 30S ribosomal subunit (prokaryotes)
- **Mechanism**: Causes misreading of mRNA and premature termination.

2. Tetracyclines
- **Examples**: Tetracycline, Doxycycline
- **Target**: 30S ribosomal subunit (prokaryotes)
- **Mechanism**: Blocks attachment of aminoacyl-tRNA to the A site.

3. Macrolides
- **Examples**: Erythromycin, Azithromycin
- **Target**: 50S ribosomal subunit (prokaryotes)
- **Mechanism**: Blocks translocation by binding to the exit tunnel.

4. Chloramphenicol
- **Target**: 50S ribosomal subunit (prokaryotes)
- **Mechanism**: Inhibits peptidyl transferase activity.

5. Lincosamides

- **Examples**: Clindamycin
- **Target**: 50S ribosomal subunit (prokaryotes)
- **Mechanism**: Inhibits peptide bond formation and translocation.

6. Oxazolidinones

- **Examples**: Linezolid
- **Target**: 50S ribosomal subunit (prokaryotes)
- **Mechanism**: Prevents formation of the initiation complex.

7. Streptogramins

- **Examples**: Quinupristin/Dalfopristin
- **Target**: 50S ribosomal subunit (prokaryotes)
- **Mechanism**: Inhibit elongation by binding to different sites on the ribosome.

8. Fusidic Acid

- **Target**: Elongation factor G (EF-G) (prokaryotes)
- **Mechanism**: Inhibits translocation by preventing EF-G from dissociating.

9. Puromycin

- **Target**: Both prokaryotic and eukaryotic ribosomes
- **Mechanism**: Mimics aminoacyl-tRNA, causing premature chain termination.

10. Cycloheximide

- **Target**: 80S ribosome (eukaryotes)
- **Mechanism**: Inhibits translocation during elongation.

11. Diphtheria Toxin

- **Target**: Eukaryotic elongation factor 2 (eEF2)
- **Mechanism**: ADP-ribosylates eEF2, inhibiting protein synthesis.

These inhibitors are used in various clinical settings to treat infections, study protein synthesis, and develop new therapeutic strategies.