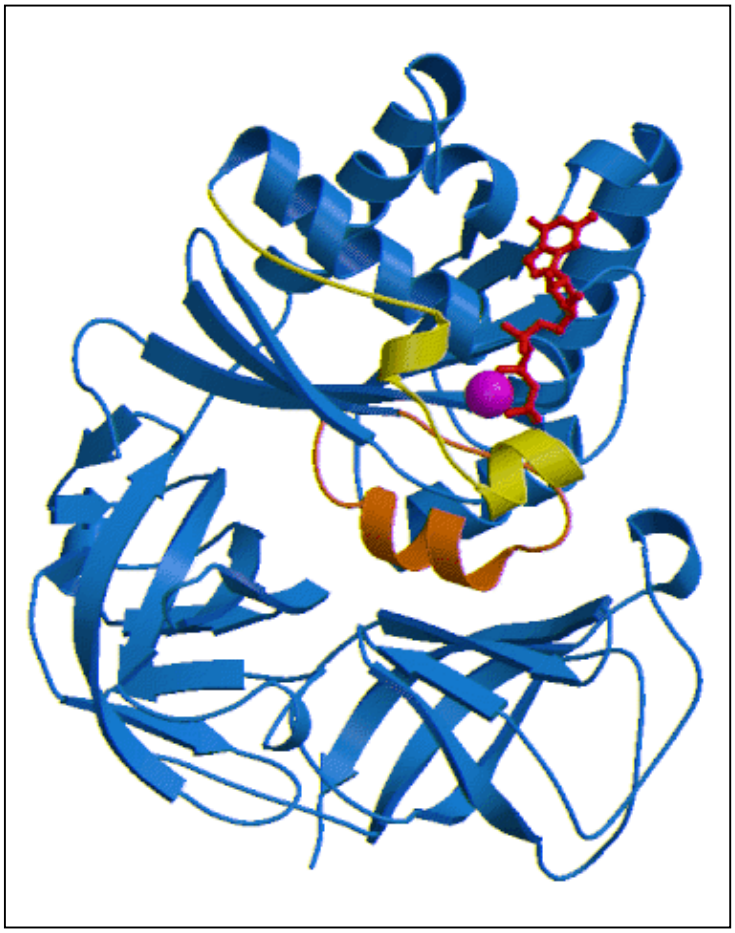
Lecture 6

Regulation of Protein Synthesis at the Translational Level

Comparison of EF-Tu-GDP and EF-Tu-GTP conformations

EF-Tu-**GDP**



EF-Tu-**GTP**

Next: Comparison of GDP and GTP binding region in EF-Tu

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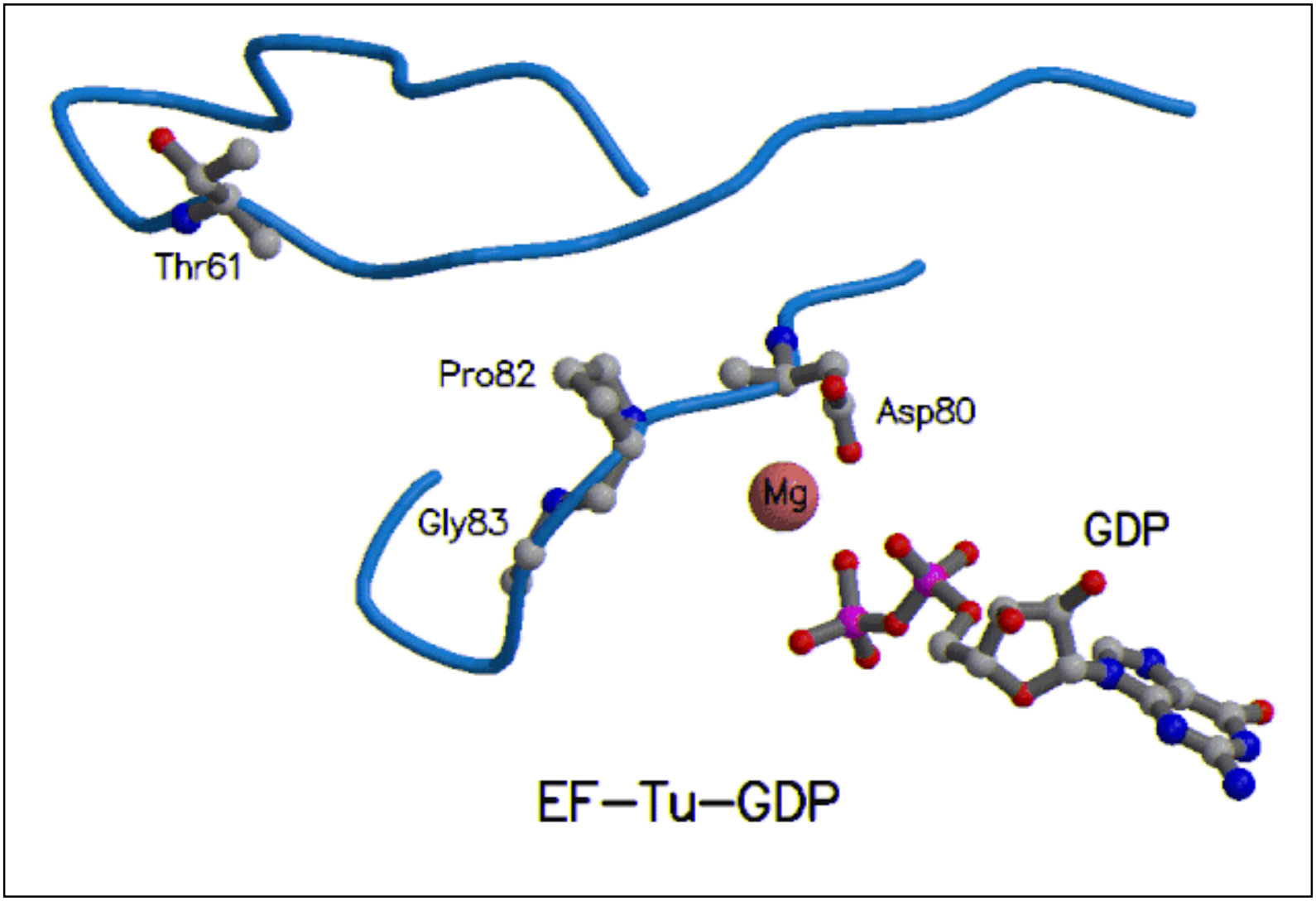
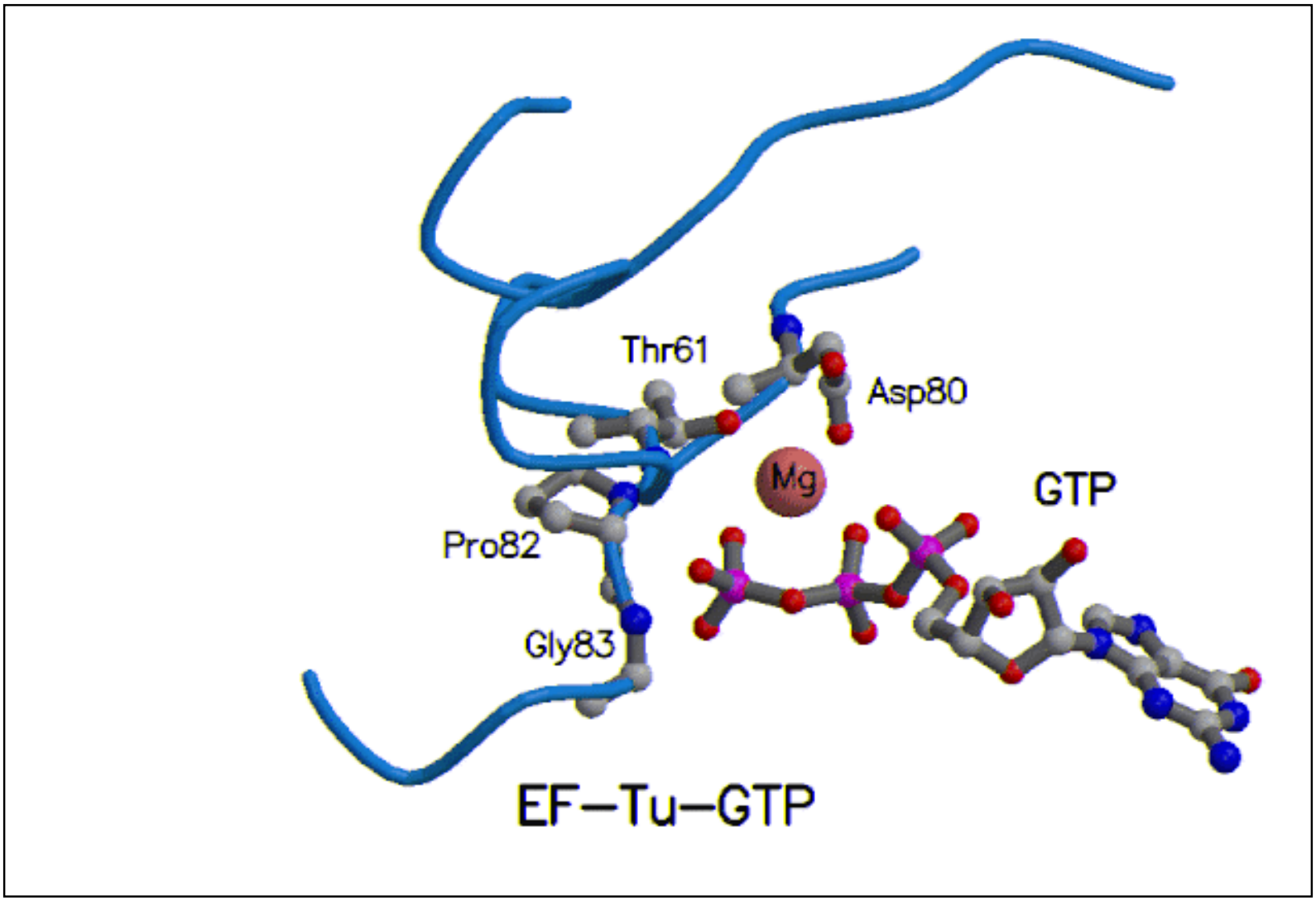
Comparison of GDP and GTP binding region of EF-Tu





Next: Molecular Mimicry

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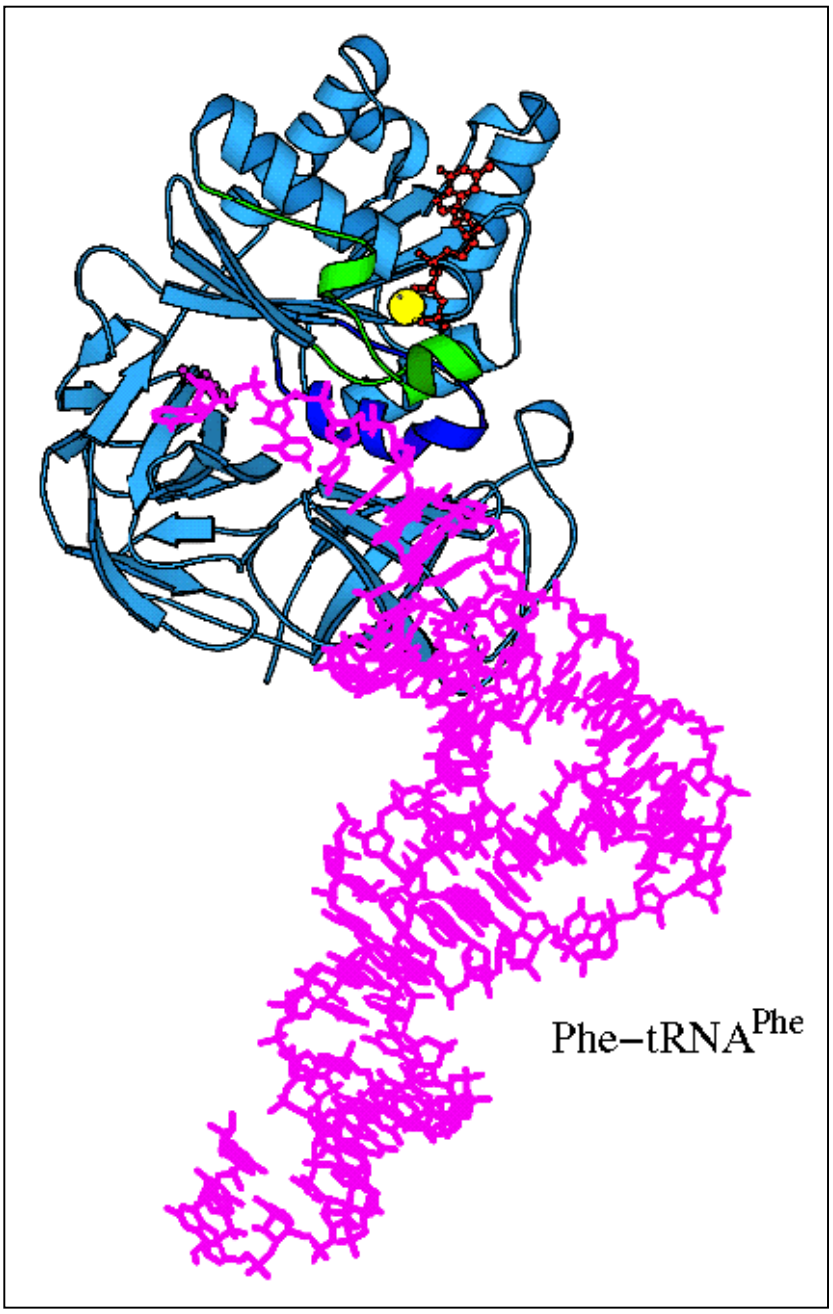
Molecular mimicry between EF-G and EF-Tu-GTP-aminoacyl-tRNA

EF-G



EF-Tu-GTP-Aminoacyl-tRNA

Next: Rates and Energetics of Translation

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Rates and energetics of translation

At 37° C, the rate of translation in *E. coli* is about 15 amino acids per second.

The translational rate is equivalent to the transcriptional rate which is ~45 nucleotides per second.

Energy cost for synthesis of a protein with N amino acids:

2N	ATPs to charge tRNA (ATP -> AMP + PP -> AMP + $2P_i$)
1	GTP for initiation (IF2)
N-1	GTPs to position tRNA for N-1 peptide bonds (EF-Tu)
N-1	GTPs for N-1 translocation steps (EF-G)
1	GTP for termination (RF-3)
====	
4N	

Total of 4 high-energy phosphate bonds cleaved per amino acid

Each ATP or GTP cleavage generates ~40 kJ/mol

Each peptide bond costs ~160 kJ/mol in the cell, yet an uncatalyzed chemical reaction to form a peptide bond costs only ~20 kJ/mol.

Why is it so costly to make a peptide bond on a ribosome?

The excess energy is used for generating an accurate, defined polypeptide sequence, not a random one or a combination of multiple possibilities.

Two sources of errors during translation:

- Attachment of an incorrect amino acid to a tRNA
- Mispairing of the tRNA anticodon with the mRNA codon

Two proofreading mechanisms exist to prevent these errors:

- Proofreading ***before*** aminoacyl adenylate intermediate is attached to tRNA.

- Kinetic proofreading ***before*** peptide bond formation: A delay is introduced between the binding of an aminoacyl-tRNA to the codon and the formation of the peptide bond to allow errors to be corrected:
  - EF-Tu-GTP binds an aminoacyl-tRNA and bring it into the A-site.
  - EF-Tu allows the anticodon to interact with the codon but prevents peptide bond formation.
  - An incorrect tRNA will bind weakly to the codon and will dissociate from the codon before an incorrect amino acid is incorporated into the polypeptide.
  - Correct codon-anticodon matching triggers hydrolysis of GTP by the EF-Tu, after which EF-Tu-GDP dissociates.
  - Peptide bond formation proceeds.

Each major step in protein synthesis, except peptide-bond formation itself, involves hydrolysis of GTP to GDP.

Regulation of protein synthesis

PROKARYOTES

Short-lived mRNA (few minutes), so little need for complicated translational regulation. In prokaryotes, most of the regulation is at the transcriptional level.

Rates vary only by a factor of 100. Variance is due to differences in Shine-Dalgarno sequences and how strongly a particular sequence base-pairs with the 16S rRNA of the 30S ribosomal subunit.

EUKARYOTES

Long-lived mRNA (hours to days) and thus a greater need to regulate the rate of protein synthesis.

Several known mechanisms:

- **mRNA masking:** mRNA is bound to a variety of proteins that prevent association with ribosomes. When appropriate signal is received, the proteins dissociate from mRNA, leaving the transcript free to associate with the ribosome. The signal is usually in the form of phosphorylation/ dephosphorylation. mRNA masking is a major form of regulation in *early embryonic development*.

- **antisense RNA:** short segment of RNA, complementary to mRNA, that forms double stranded RNA which cannot be translated by ribosome. Two known examples:

    - blockage of protein synthesis of fruit-ripening enzyme in tomatoes
    - the c-myb gene product which promotes smooth muscle development and blockage in injured arteries.

- **Heme Control of Globin Synthesis:** Red blood cells are programmed to synthesize large amounts of globin. The globin chains, subsequent to translation, are assembled with heme into hemoglobin. If there is an insufficient supply of heme to insert into the newly synthesized globin chains, then translation is turned off. The lack of heme triggers the accumulation of a heme-controlled inhibitor (HCI) protein. This protein is a kinase which phosphorylates eIF2-GTP. The phosphorylation blocks the dissociation of eIF2 and eIF2-beta that normally occurs in the initiation cycle. Thus, the cell becomes rapidly *depleted of unphosphorylated eIF2* which is normally recycled for initiation of additonal mRNA. Either the

addition of heme, which represses the production of HCI, or the addition of lots of unphosphorylated eIF2, which bypasses the HCI effect, can restart initiation again.

- **Interferon:** Interferons are glycoproteins that are secreted by virus-infected cells. Interferons prevent additional infection by other types of viruses by inhibiting protein synthesis in infected cells.

Two mechanisms of action:

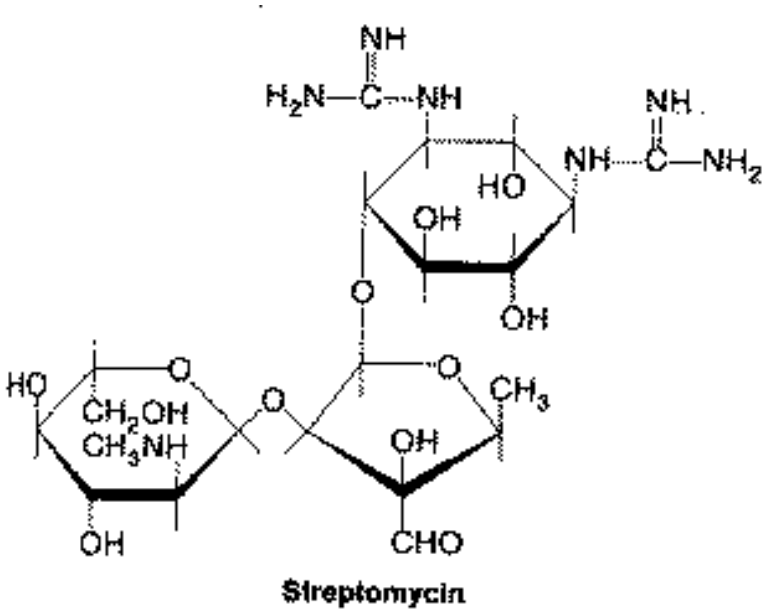
  - induces production of protein kinase, DAI (double-stranded RNA-activated inhibitor) which in the presence of dsRNA, phosphorylates eIF2-alpha and stabilizes the eIF2-alpha-eIF2-beta complex in a manner similar to the heme-controlled inhibitor (HCI).
  - induces a cascade effect which ultimately activates an endonuclease, RNase L, that rapidly *degrades mRNA*.

Inhibition of protein synthesis by antibiotics

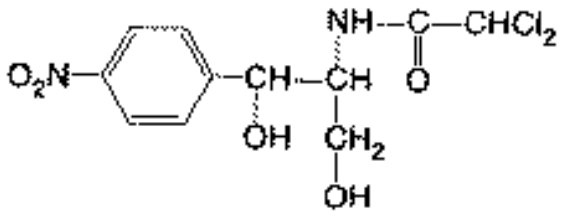
Antibiotics are bacterially or fungally produced substances that inhibit the growth of other organisms. Antibiotics target a wide spectrum of vital processes: they block DNA replication, transcription and bacterial cell wall synthesis. A large number of antibiotics, including medically useful substances, **block protein translation**.

Blocking **protein translation** is very effective for two reasons:

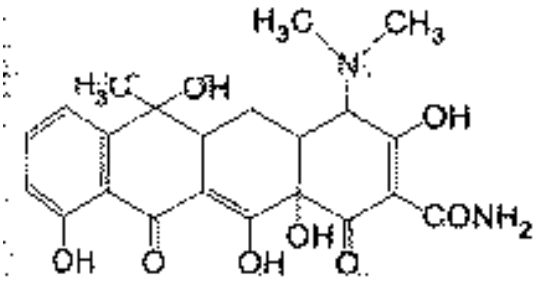
- Protein translation plays a central role in overall metabolism
- The structural differences between prokaryotic and eukaroytic ribosomes and associated factors (IFs/EFs/RFs) allow specific targeting.



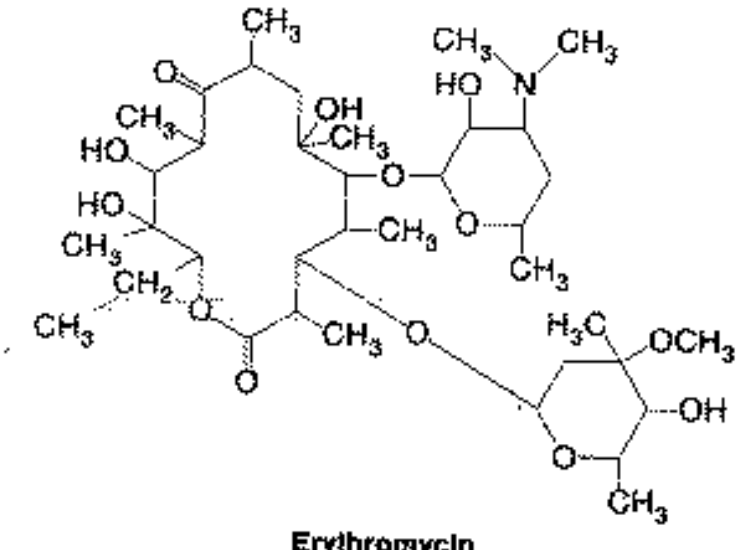
Streptomycin



Chloramphenicol



Tetracycline



Erythromycin

Figure 25-14
The structures of some antibiotic inhibitors of protein synthesis.

Prokaryotic Inhibitors

- Chloramphenicol - inhibits peptidyl transferase on 50S subunit.
- Erythromycin - inhibits translocation by 50S subunit.
- Fusidic acid - inhibits translocation by preventing the dissociation of EF-G-GDP from ribosome.
- Puromycin - an aminoacyl-tRNA analog that causes premature chain termination.
- Streptomycin - causes mRNA misreading and inhibits chain initiation.
- Tetracycline - inhibits binding of aminoacyl-tRNA to ribosomal A-site.

Eukaryotic Inhibitors

- Puromycin & Tetracycline (see prokaryotes above).
- Cycloheximide - inhibits peptidyl transferase on 60S subunit.
- Diphtheria Toxin - inactivates eEF-2 by ADP ribosylation.

Summary

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Summary

- **GTP-binding often causes major conformational changes in proteins**
- **Each major step in protein synthesis, except peptide-bond formation itself, involves hydrolysis of GTP to GDP.**
- **Four high-energy phosphate-bonds are required for each amino acid added**
- **The rate of protein synthesis is well matched to the rate of transcription**
- **EF-G is shaped like EF-Tu-GTP-aminoacyl-tRNA (molecular mimicry)**
- **mRNA in prokaryotes is short-lived (minutes)**
- **mRNA in eukaryotes is long-lived (hours/days), requiring additional control**
- **A large number of antibiotics inhibit protein synthesis, many specifically in prokaryotes**

Next lecture: Post-Translational Processing

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