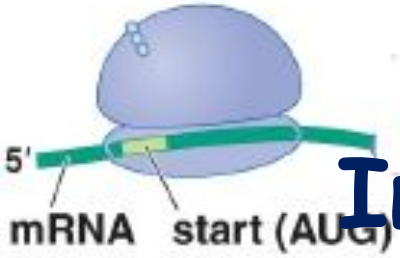
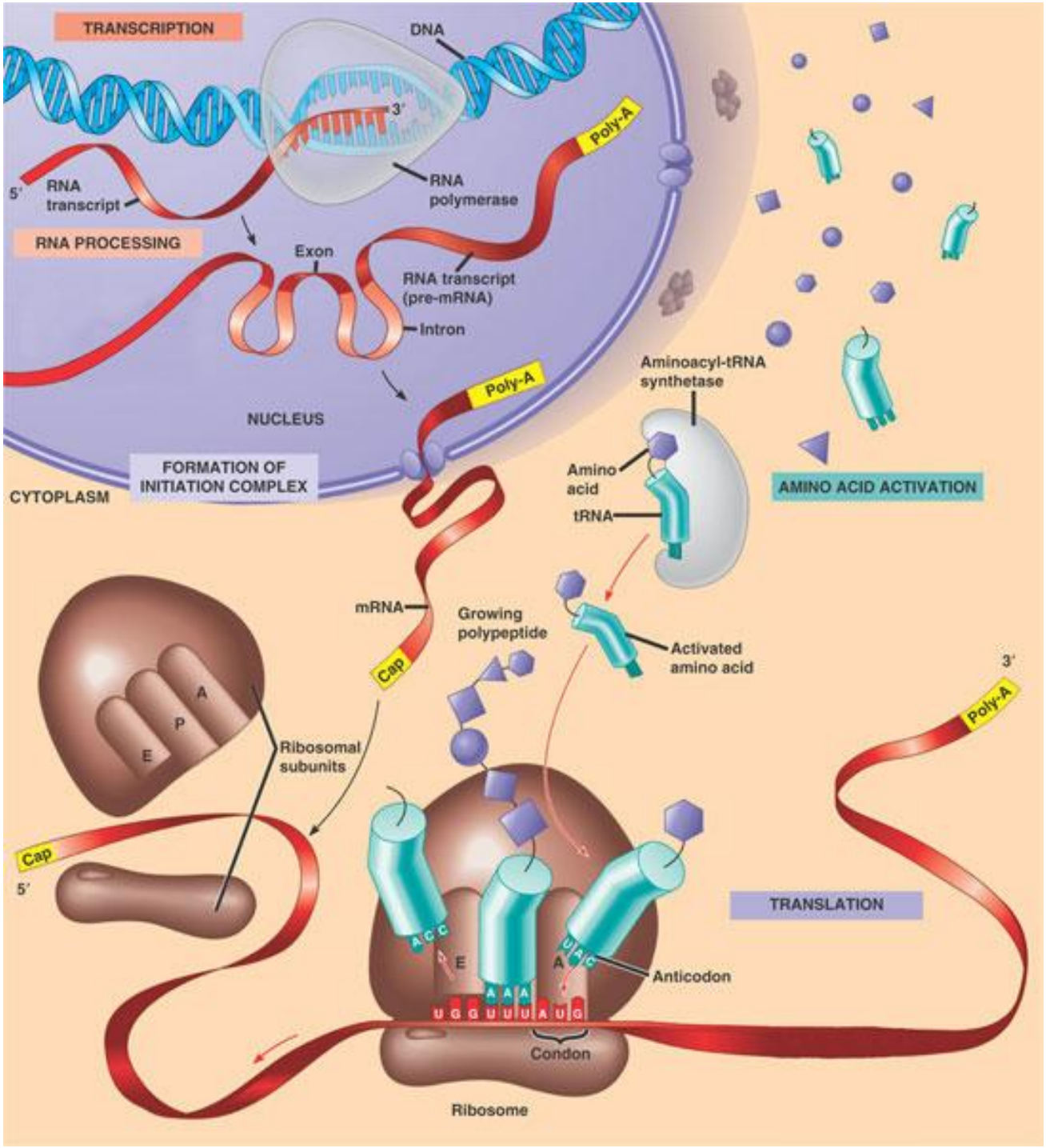


Initiation of translation in prokaryotes: initiation factors, initiator codons, 3'end of RNA small ribosomal subunit and the Shine-Dalgarno sequence in mRNA»

Done by: Maulenova R.
Moldakozhayev A.
Naizabayeva D.



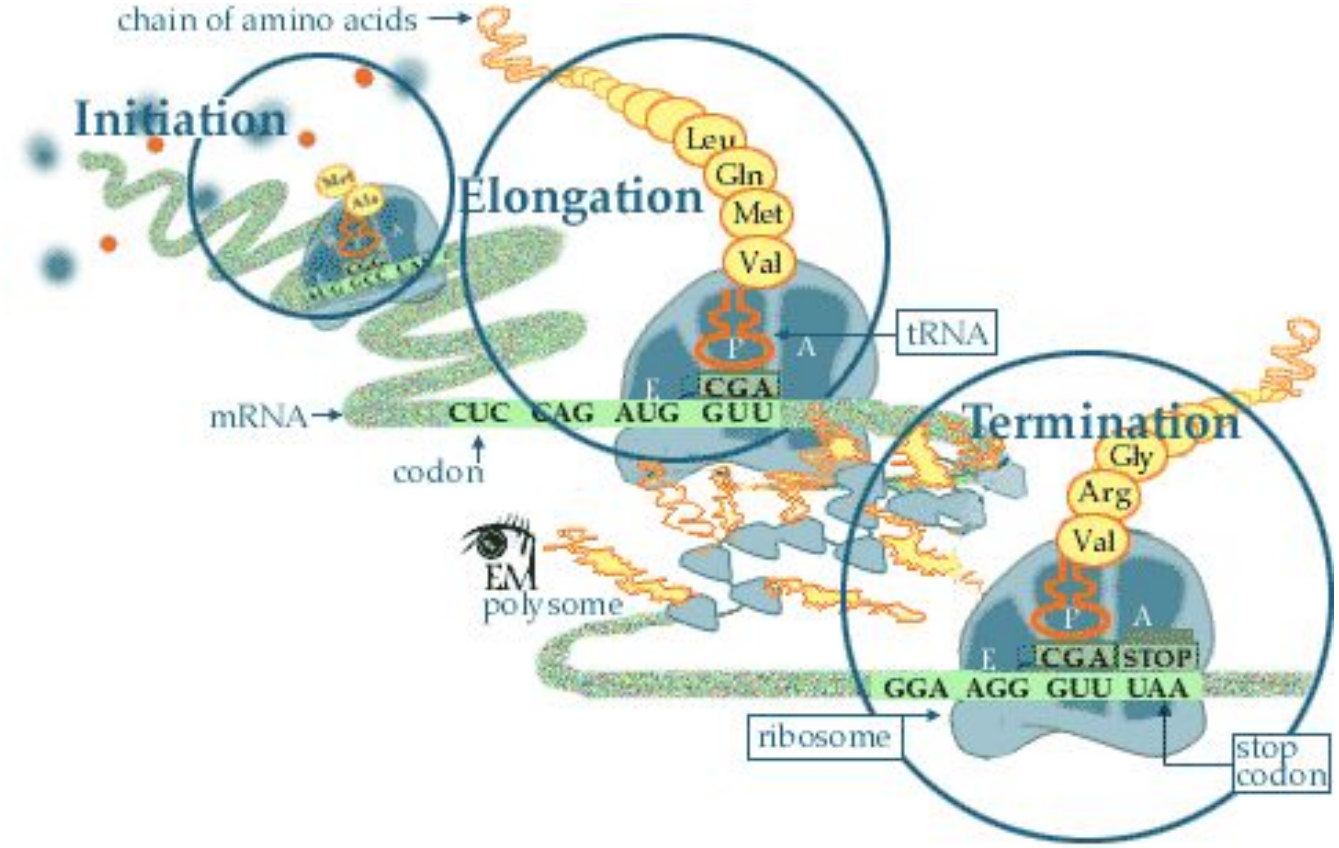
In molecular biology and genetic, translation is the process in which ribosomes in a cell's cytoplasm create proteins, following transcrip-tion of DNA to RNA in the cell's nucleus. The entire process is a part of gene expression.

Translation proceeds in three phases:

Initiation: The ribosome assembles around the target mRNA. The first tRNA is attached at the start codon.

Elongation: The tRNA transfers an amino acid to the tRNA corresponding to the next codon. The ribosome then moves (translocates) to the next mRNA codon to continue the process, creating an amino acid chain.

Termination: When a stop codon is reached, the ribosome releases the polypeptide.



In bacteria, translation occurs in the cytoplasm, where the large and small subunits of the ribosome bind to the mRNA. In eukaryotes, translation occurs in the cytosol or across the membrane of the endoplasmic reticulum in a process called vectorial synthesis. In many instances, the entire ribosome/mRNA complex binds to the outer membrane of the rough endoplasmic reticulum (ER); the newly created polypeptide is stored inside the ER for later vesicle transport and secretion outside of the cell.

Many types of transcribed RNA, such as transfer RNA, ribosomal RNA, and small nuclear RNA, do not undergo translation into proteins.

A number of antibiotics act by inhibiting translation. These include anisomycin, cycloheximide, chloramphenicol, tetracycline, streptomycin, erythromycin, and puromycin. Prokaryotic ribosomes have a different structure from that of eukaryotic ribosomes, and thus antibiotics can specifically target bacterial infections without any harm to a eukaryotic host's cells.

Translation initiation: Initiation factors

Prokaryotes require the use of three initiation factors: IF1, IF2, and IF3, for translation.

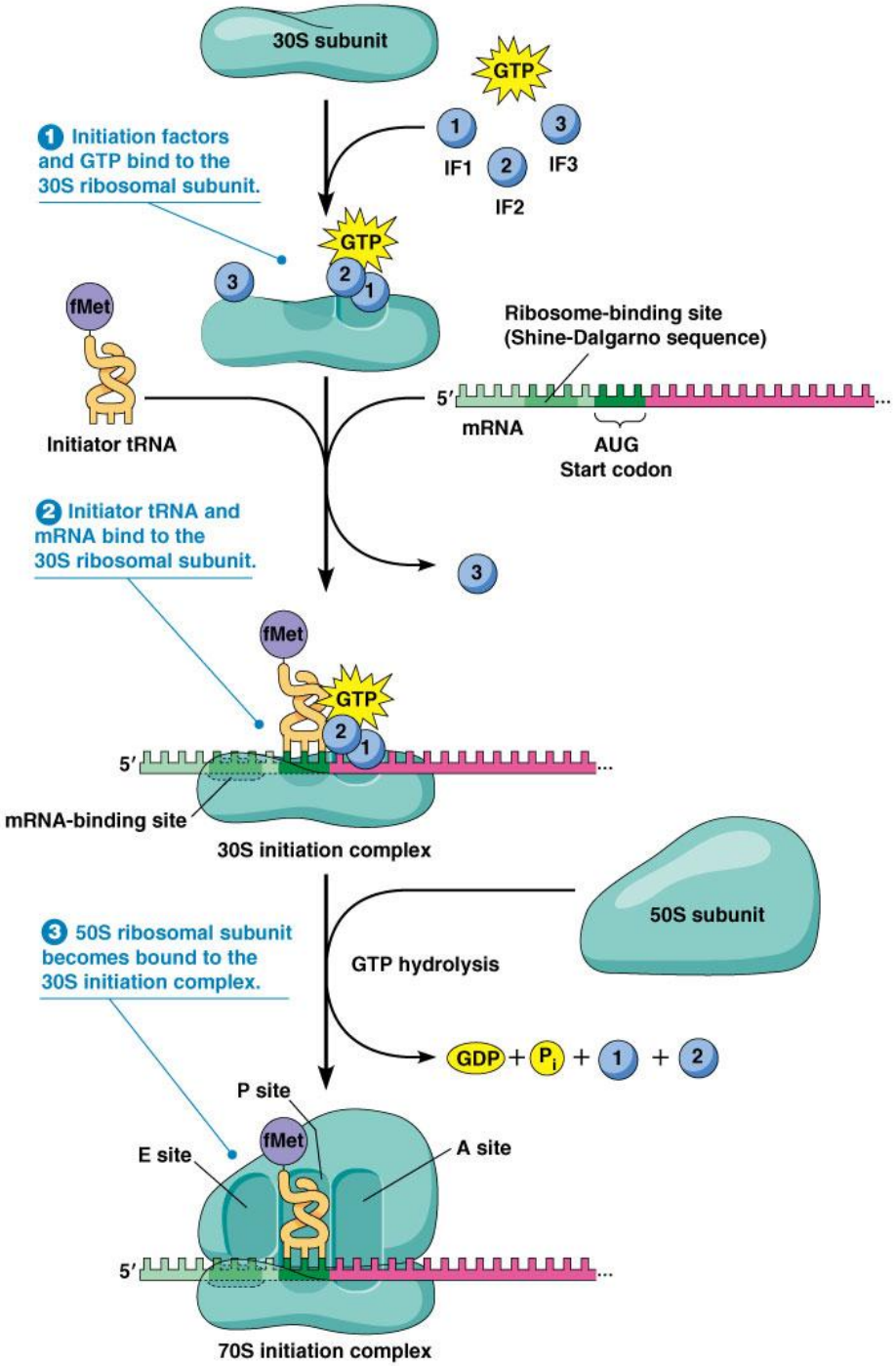
IF1 associates with the 30S ribosomal subunit in the *A site* and prevents an aminoacyl-tRNA from entering. It modulates IF2 binding to the ribosome by increasing its affinity. It may also prevent the 50S subunit from binding, stopping the formation of the 70S subunit. It also contains a β-domain fold common for nucleic acid binding proteins.

Translation initiation: Initiation factors

IF2 binds to an initiator tRNA and controls the entry of tRNA onto the ribosome. IF2, bound to GTP, binds to the 30S P site. After associating with the 30S subunit, fMet-tRNAf binds to the IF2, then IF2 transfers the tRNA into the partial P site. When the 50S subunit joins, it hydrolyzes GTP to GDP and Pi, causing a conformational change in the IF2 that causes IF2 to release and allow the 70S subunit to form.

Translation initiation: Initiation factors

IF3 is not universally found in all bacterial species but in *E. coli* it is required for the 30S subunit to bind to the initiation site in mRNA. In addition, it has several other jobs including the stabilization of free 30S subunits, enables 30S subunits to bind to mRNA and checks for accuracy against the first aminoacyl-tRNA. It also allows for rapid codon-anticodon pairing for the initiator tRNA to bind quickly to. IF3 is required by the small subunit to form initiation complexes, but has to be released to allow the 50S subunit to bind.



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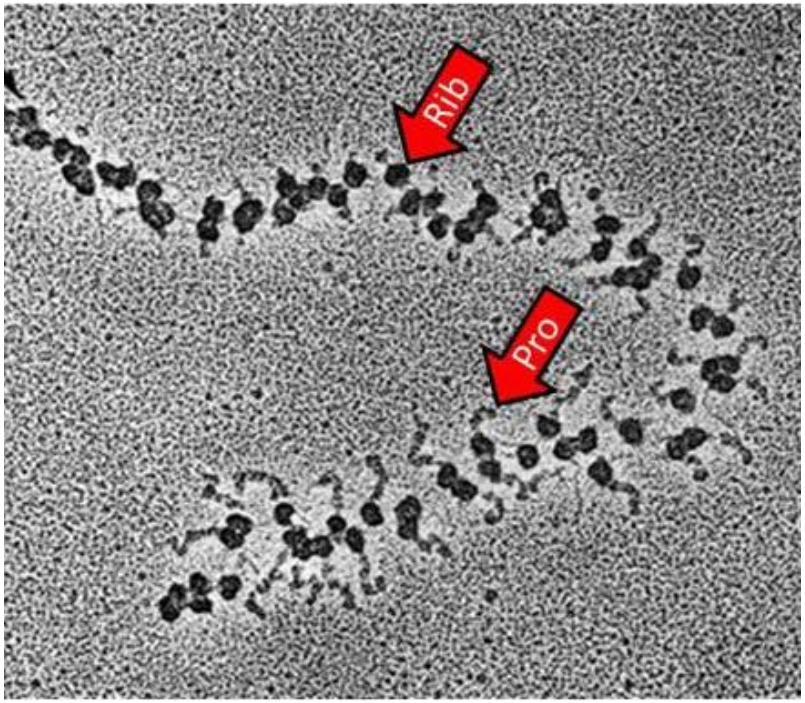
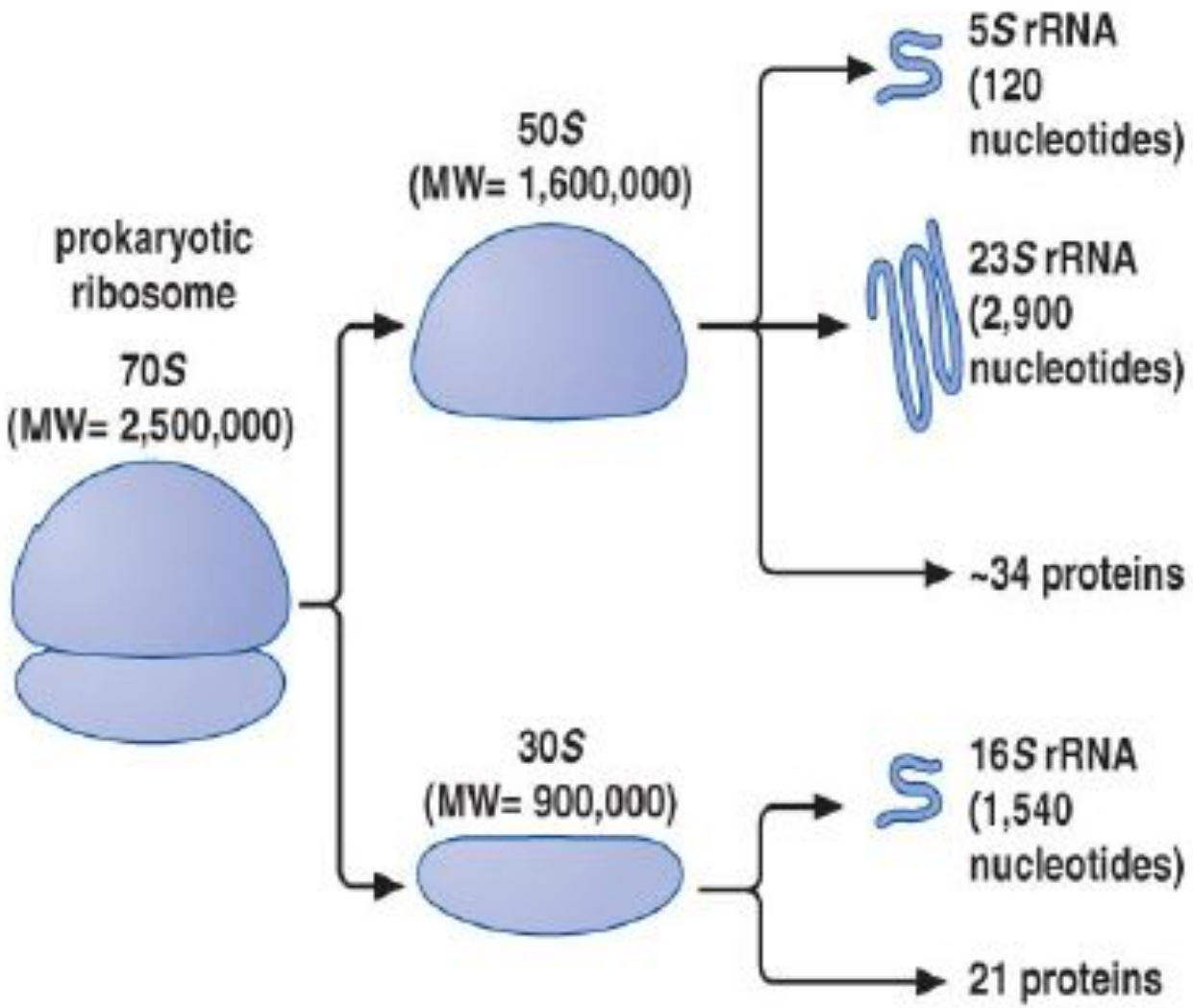
Ribosome

The fact that cells typically contain many ribosomes reflects the central importance of protein synthesis in cell metabolism. *E. coli*, for example, contain about 20,000 ribosomes, which account for approximately 25% of the dry weight of the cell, and rapidly growing mammalian cells contain about 10 million ribosomes.



Ribosome: Structure



* Each ribosome contains one copy of the rRNAs and one copy of each of the ribosomal proteins, with one exception: One protein of the 50S subunit is present in four copies.

Ribosome: rRNA

A noteworthy feature of ribosomes is that they can be formed *in vitro* by self-assembly of their RNA and protein constituents. As first described in 1968 by Masayasu Nomura, purified ribosomal proteins and rRNAs can be mixed together and, under appropriate conditions, will reform a functional ribosome.

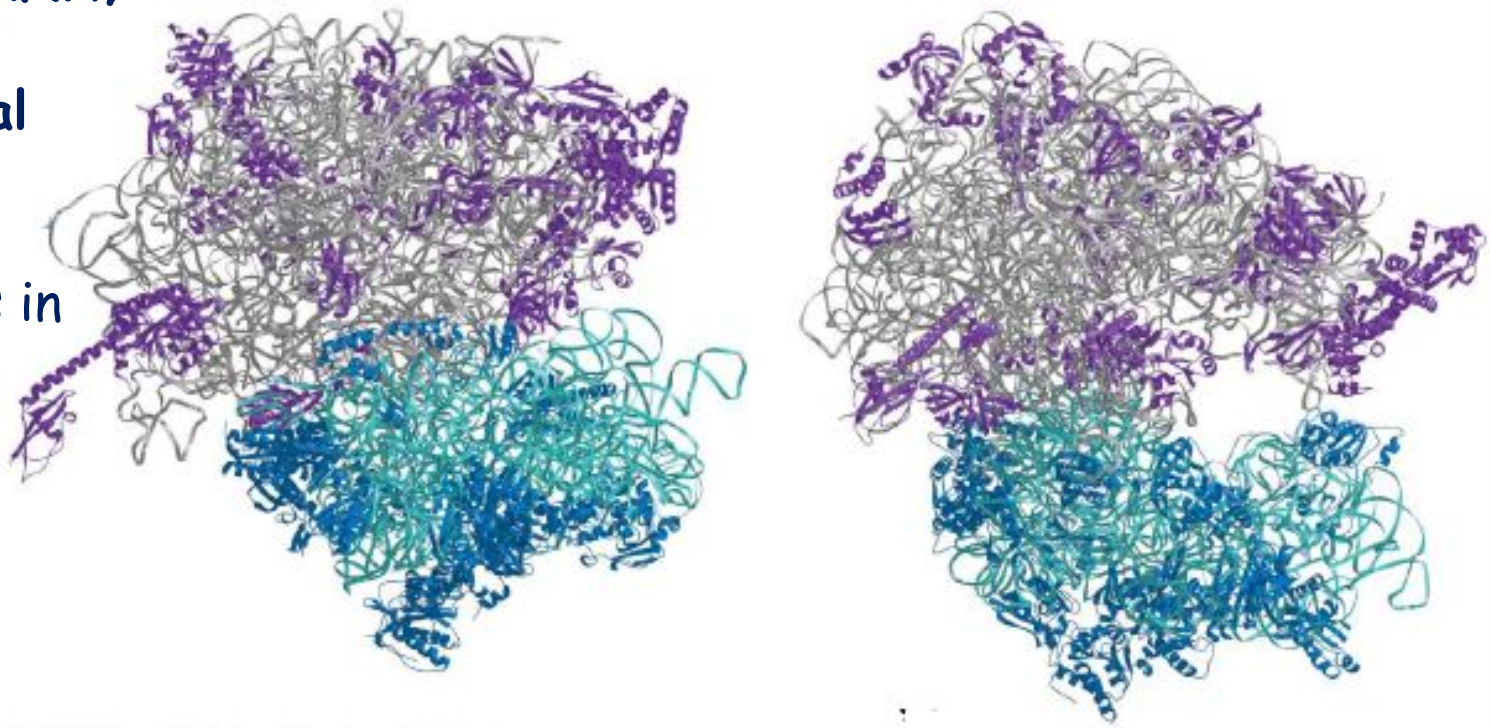
Initially, rRNAs were thought to play a structural role, providing a scaffold upon which ribosomal proteins assemble. However, with the discovery of the catalytic activity of other RNA molecules, the possible catalytic role of rRNA became widely considered. Consistent with this hypothesis, rRNAs were found to be absolutely required for the in vitro assembly of functional ribosomes.

Ribosome: rRNA

-**rRNAs** are much more than structural components of ribosome directly
responsible for the key functions of the ribosome

***peptidyl transferase center** is composed almost entirely of RNA
* 16S rRNA of small subunit is responsible for mRNA binding ;
*also function in the small subunit: **anticodon loop** and **codon** of mRNA contact **16S rRNA;**

-most **ribosomal proteins** are in periphery
*some proteins in core for stabilization reasons



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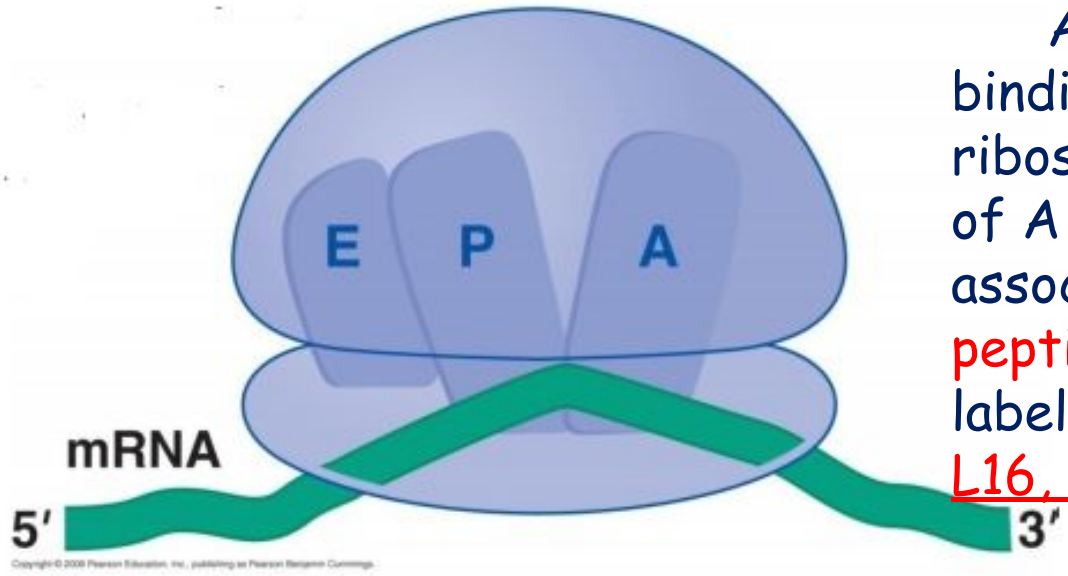
Ribosome: 16S rRNA

1)A site: binding site for aminoacyl-tRNA
2)P site: binding site for peptidyl-tRNA
3)E (denote exit) site: binding site for tRNA released after growing polypeptide chain has been transferred to the aminoacyl-tRNA (i.e., free tRNA)



Affinity label for the tRNA binding sites on the *E. coli* ribosome allowed the identification of A and P site proteins most likely associated with the peptidyl-transferase activity; labelled proteins are L27, L14, L15, L16, L2;

Additional research has demonstrated that the S1 and S21 proteins, in association with the 3'-end of 16S ribosomal RNA, are involved in the initiation of translation.

Ribosome: 16S rRNA

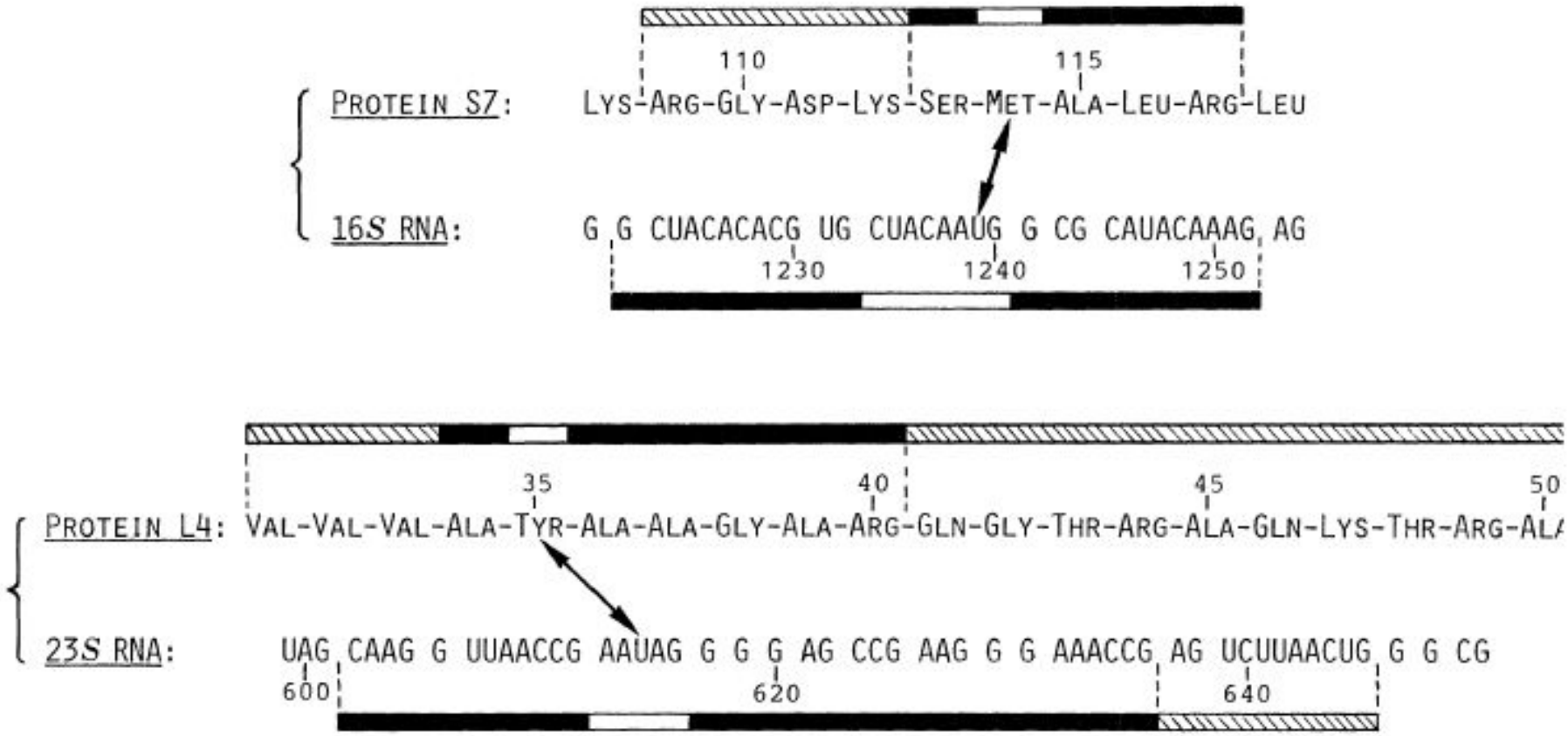
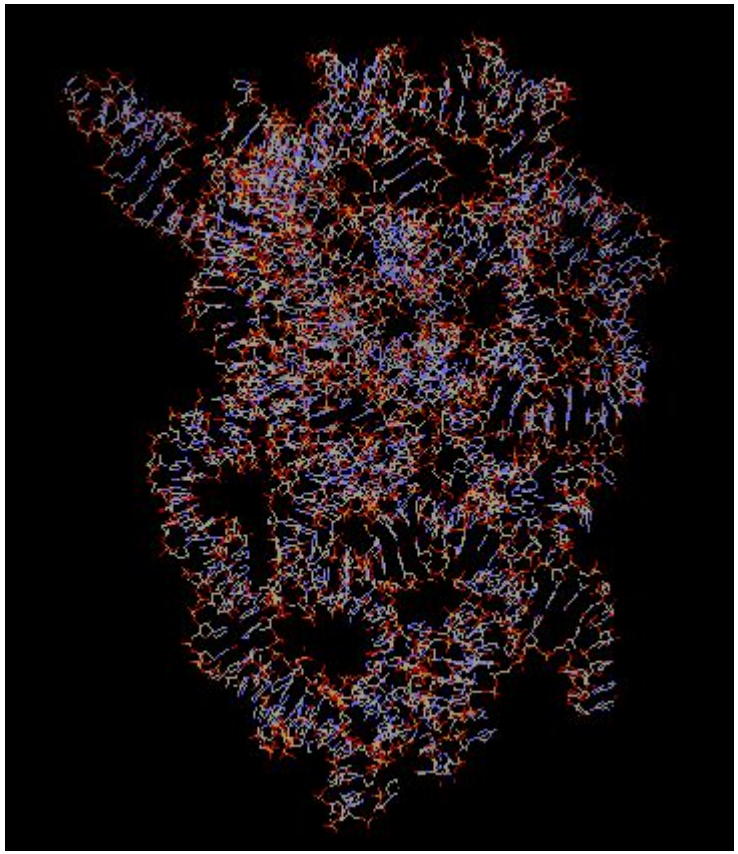


FIGURE 4. Protein–RNA crosslinking in *E. coli* ribosomal subunits by u.v. irradiation. Residue Met114 of protein S7 has been crosslinked to nucleotide U1239 of the 16*S* RNA, and Tyr35 of protein L4 to U615 of the 23*S* RNA.

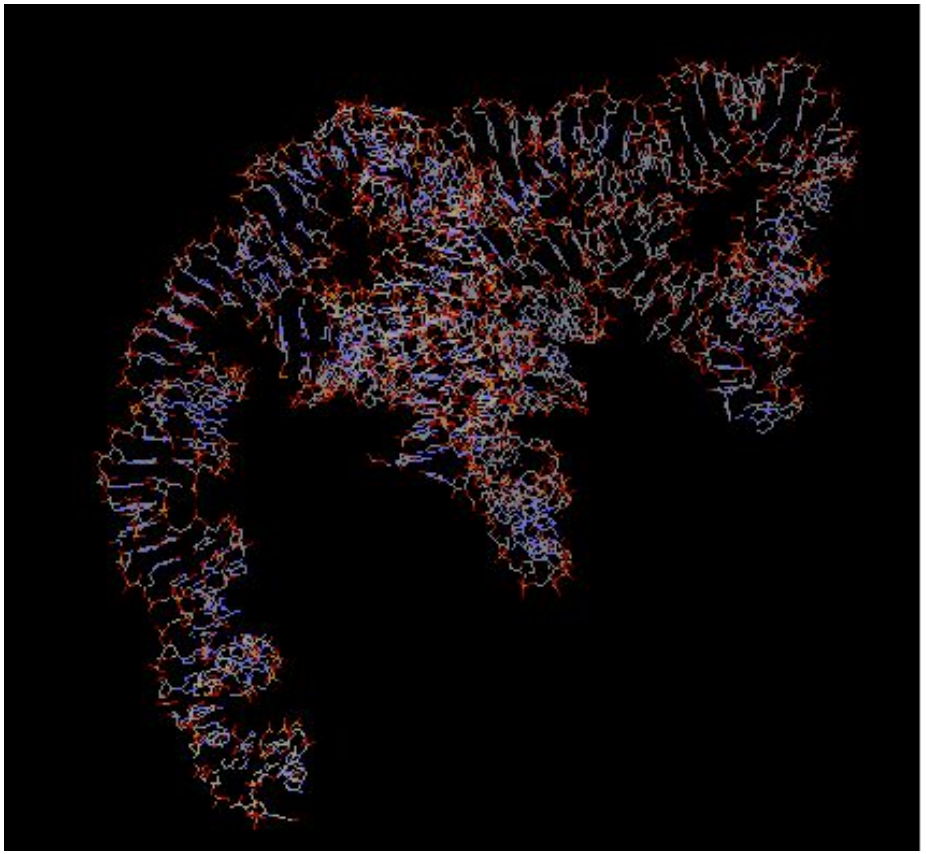
(Zwieb & Brimacombe I979)

Ribosome: 16S rRNA

The arrangement of the 16S rRNA creates a **5' domain**, **central domain**, **3' major domain**, and a **3' minor domain**.

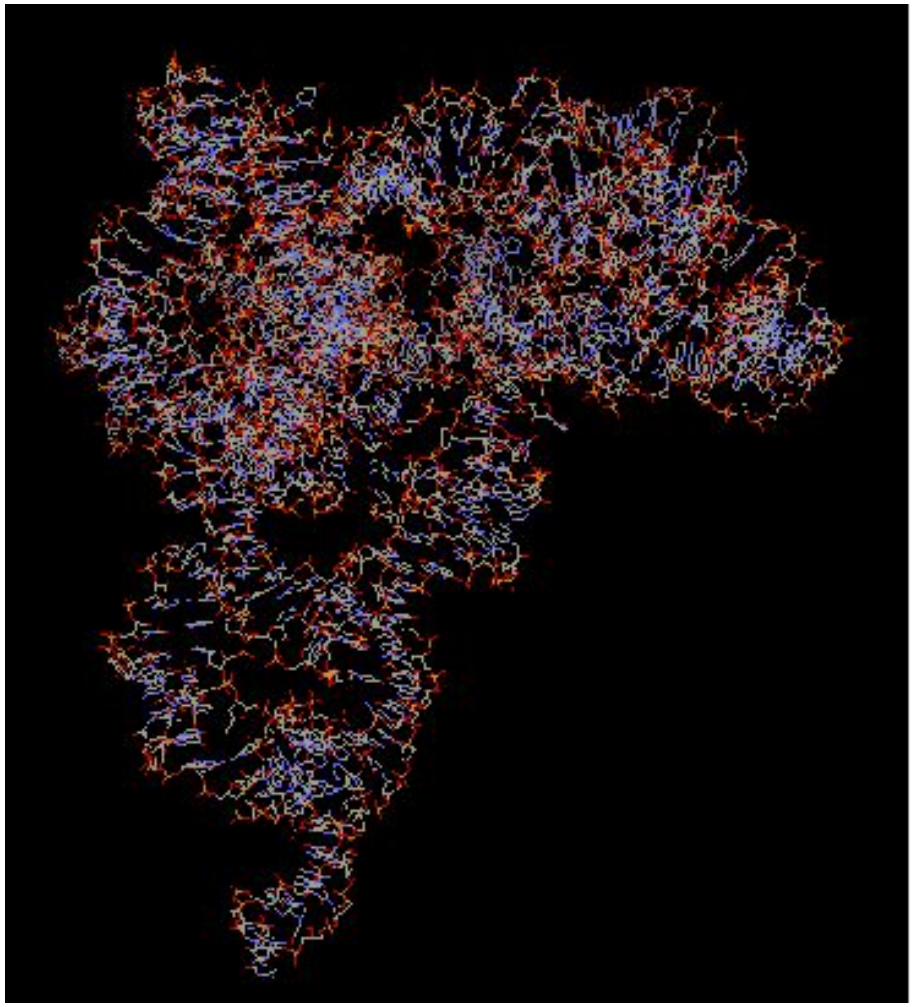


The 5' domain consists of 19 double helices that makes up the bulk of the body.

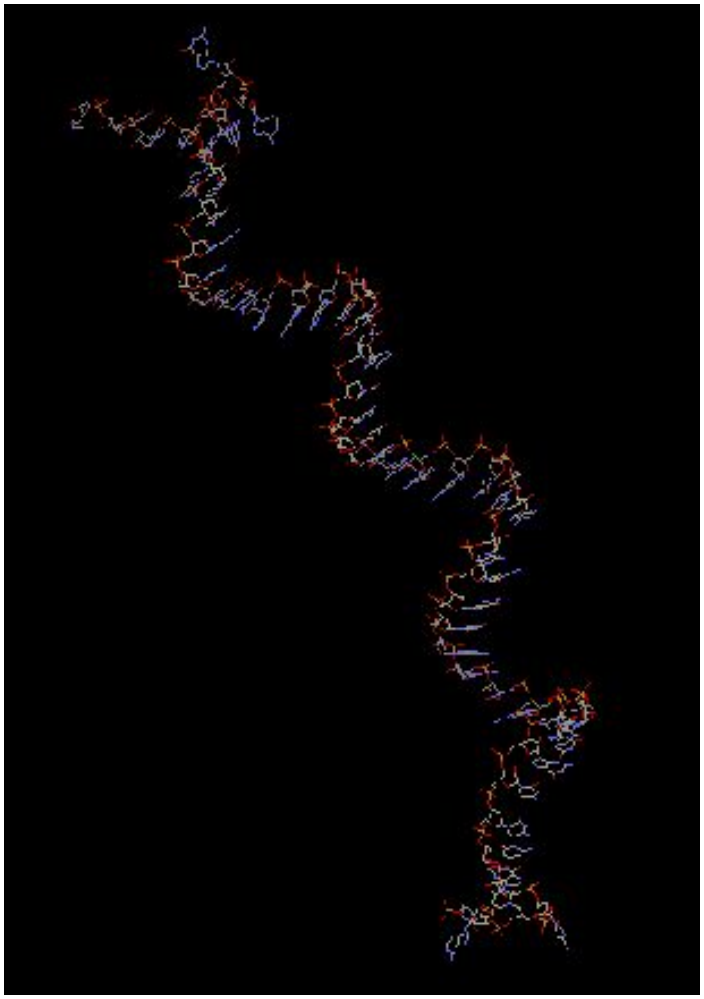


The central domain of the rRNA generates the platform and is an elongated, curved structure of nine helices, with the junction of helices 20, 21, and 22 being at the heart of it.

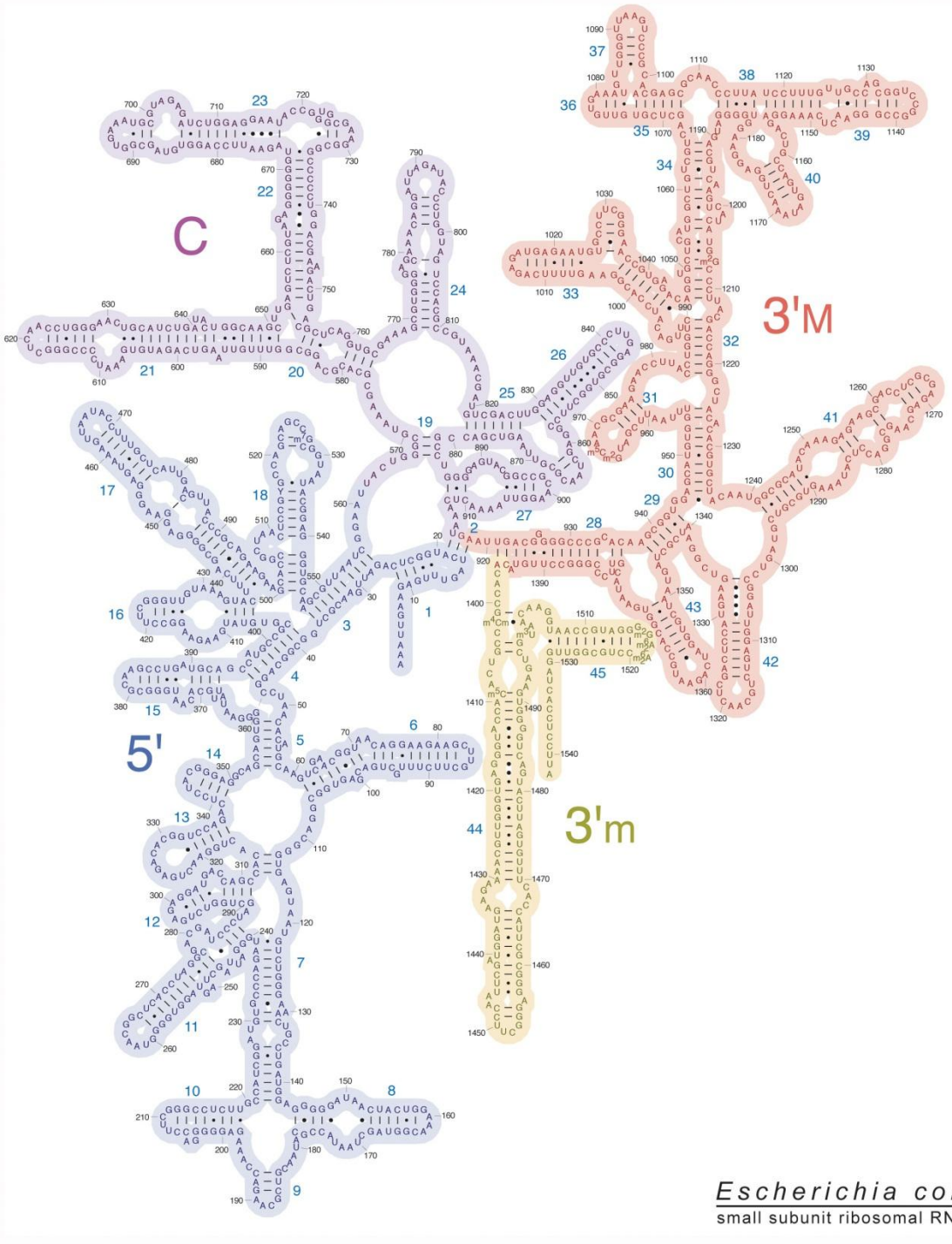
Ribosome: 16S rRNA



The 3' major domain contains 15 helical elements and composes the head.

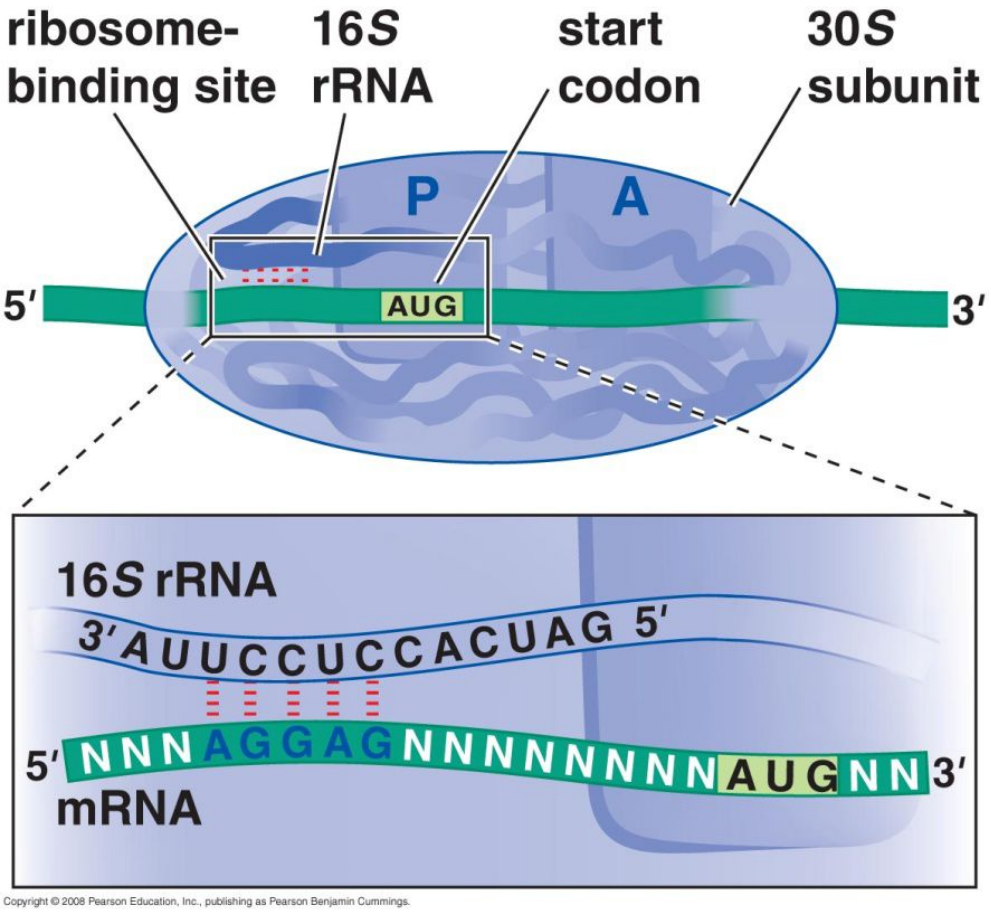


The 3' minor domain contains 2 helices and projects from the subunit to interact with the 50S subunit.


C
5'
3'M
3'm
Escherichia coli
small subunit ribosomal RNA

Ribosome: 3' end of 16S rRNA

First, the **small subunit** associates with the **mRNA**by **base-pairing** interaction between **RBS** and **16S rRNA**
-the small subunit is positioned on mRNA such that the **start codon** will be in the **P site** when the large subunit joins the complex



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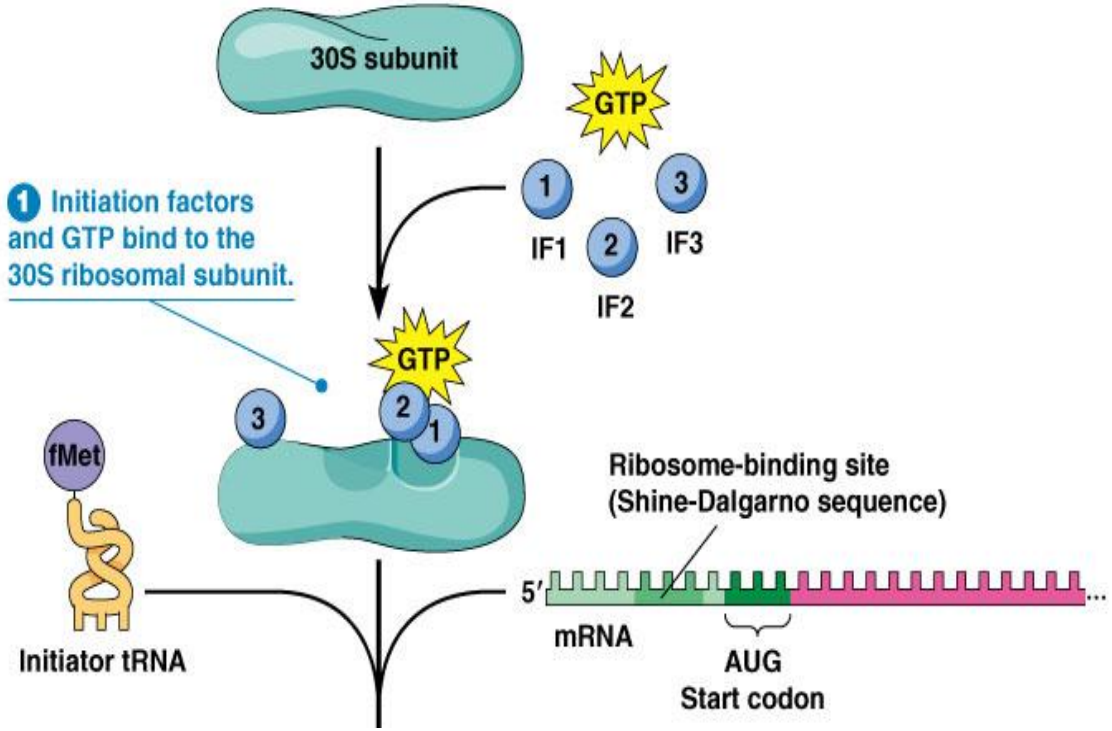
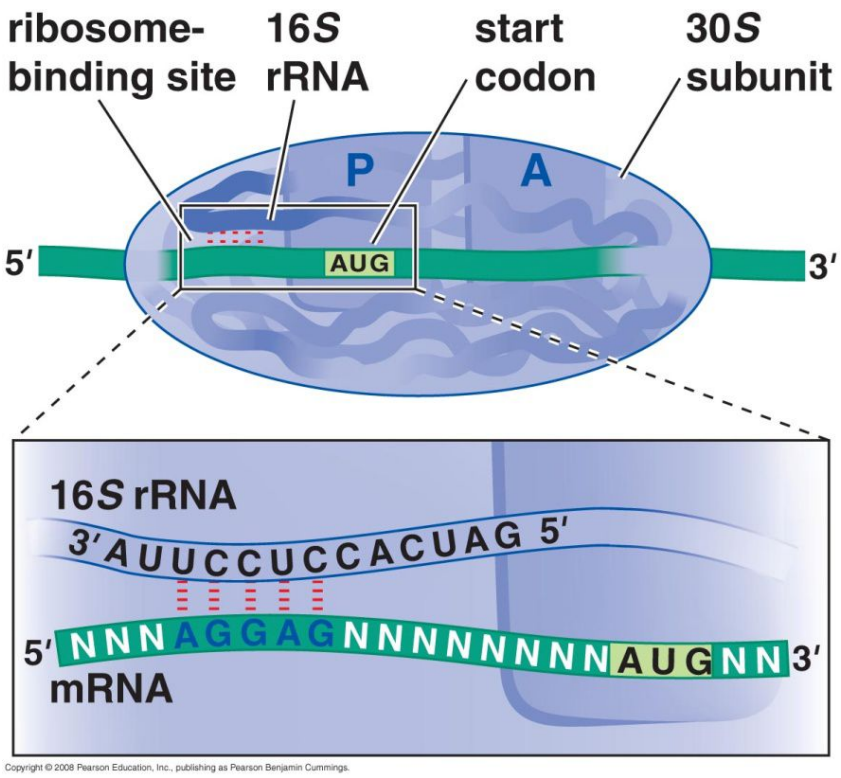
Ribosome: 3' end of 16 s rRNA

Unique localization of the 3' end of the RNA on the upper portion of the subunit platform.. Ribosomal proteins SI and S21, which have been cross-linked to the 3' end of the RNA, are localized near each other and near the end of the platform. Initiation factor IF3, to which the oxidized 3' end of the RNA has also been linked, has been itself cross-linked to ribosomal proteins S1, Sll, S12, S13, S19, and S21; antigenic determinants of each of these proteins have been localized either on the subunit platform or on nearby parts of the upper portion of the subunit



Ribosome: 3' end of 16 s rRNA



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Cross-linking studies have shown that the nucleic acid-binding domain of S1 is aligned with a region of the mRNA upstream of the SD, suggesting that S1 may interact with 5' parts of the Translation initiation region. Consistent with this observation, A/U-rich sequences in front of the SD or downstream of the initiator codon enhance protein synthesis. Disruption of the *E. coli* gene coding for S1 has been reported to be lethal.

Antibiotics affecting 16S rRNA

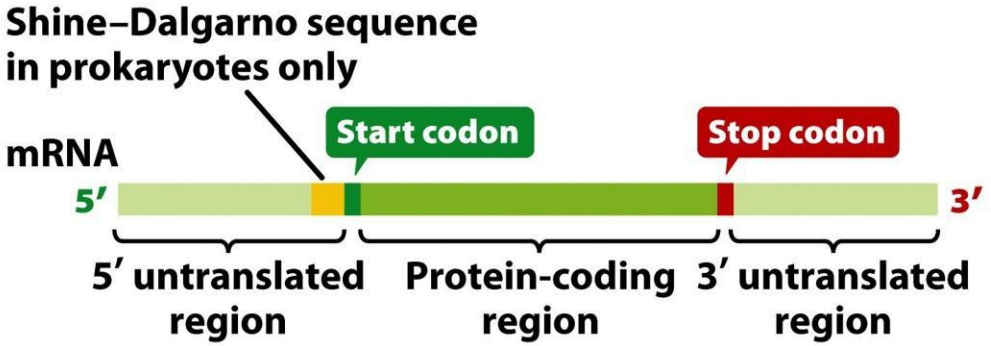
Colicin E3 (protein antibiotic from E.coli) makes a single cut in the 16S rRNA of 70S ribosomes, these include the loss of activity.

Pactamycin (Pct) was isolated from Streptomyces pactum as a potential new human antitumor drug, but in fact a potent inhibitor of translation in all three kingdoms, eukarya, bacteria, and archaea (Bhuyan et al., 1961; Mankin, 1997). For this reason, the drug is expected to interact with highly conserved regions of 16S RNA.

Streptomycin and spectinomycin are typical examples which function by binding to specific sites on prokaryotic rRNA and affecting the fidelity of protein synthesis. Binding of drug to the 16S subunit near the A-site of the 30S subunit leads to a decrease in translational accuracy and inhibition of the translocation of the ribosome.

Shine-Dalgarno sequence

The Shine-Dalgarno (SD) sequence is a ***ribosomal binding site*** in bacterial and archaeal messenger RNA, generally located around 8 bases upstream of the start codon AUG.



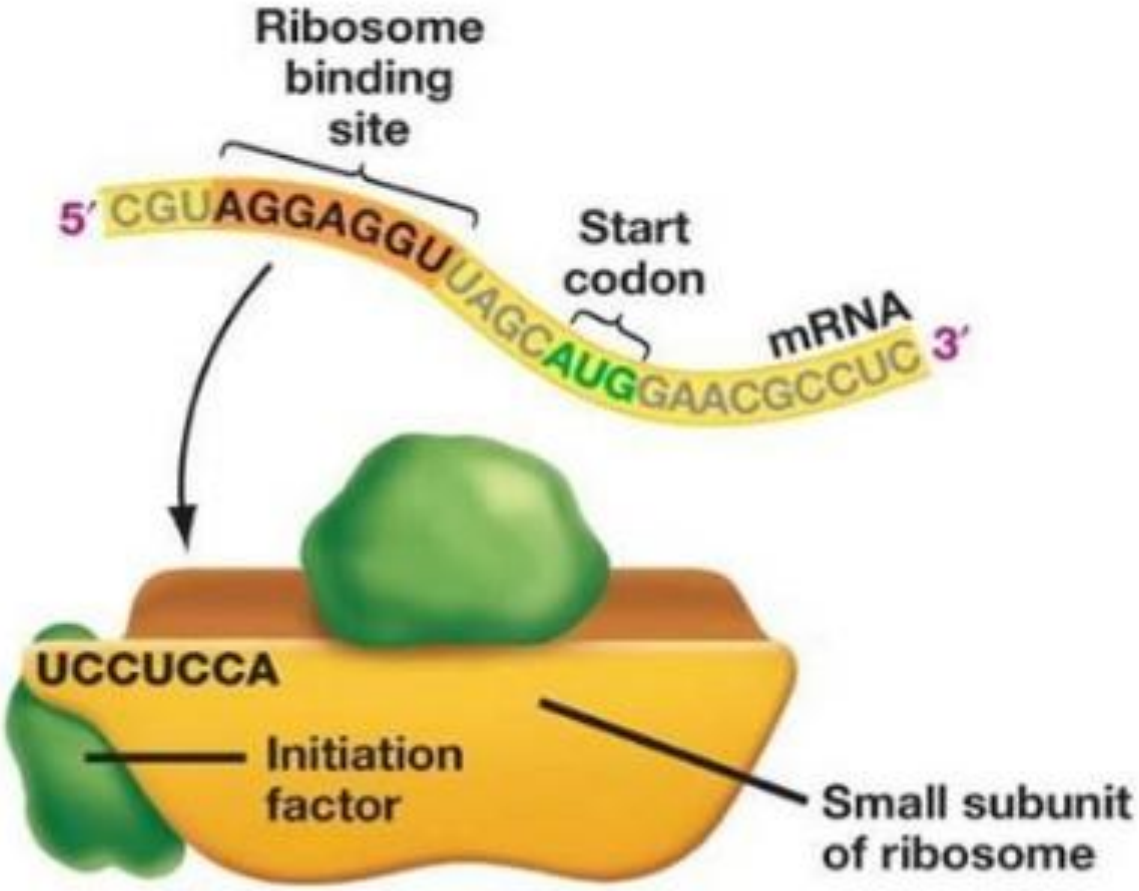
The RNA sequence helps recruit the ribosome to the messenger RNA (mRNA) to **initiate** protein synthesis by aligning the ribosome with the start codon.

The Shine-Dalgarno sequence was proposed by Australian scientists **John Shine and Lynn Dalgarno**

The Shine-Dalgarno sequence exists both in bacteria and archaea. It is also present in some chloroplast and mitochondrial transcripts.

Translation initiation in bacteria

Shine-Dalgarno sequence

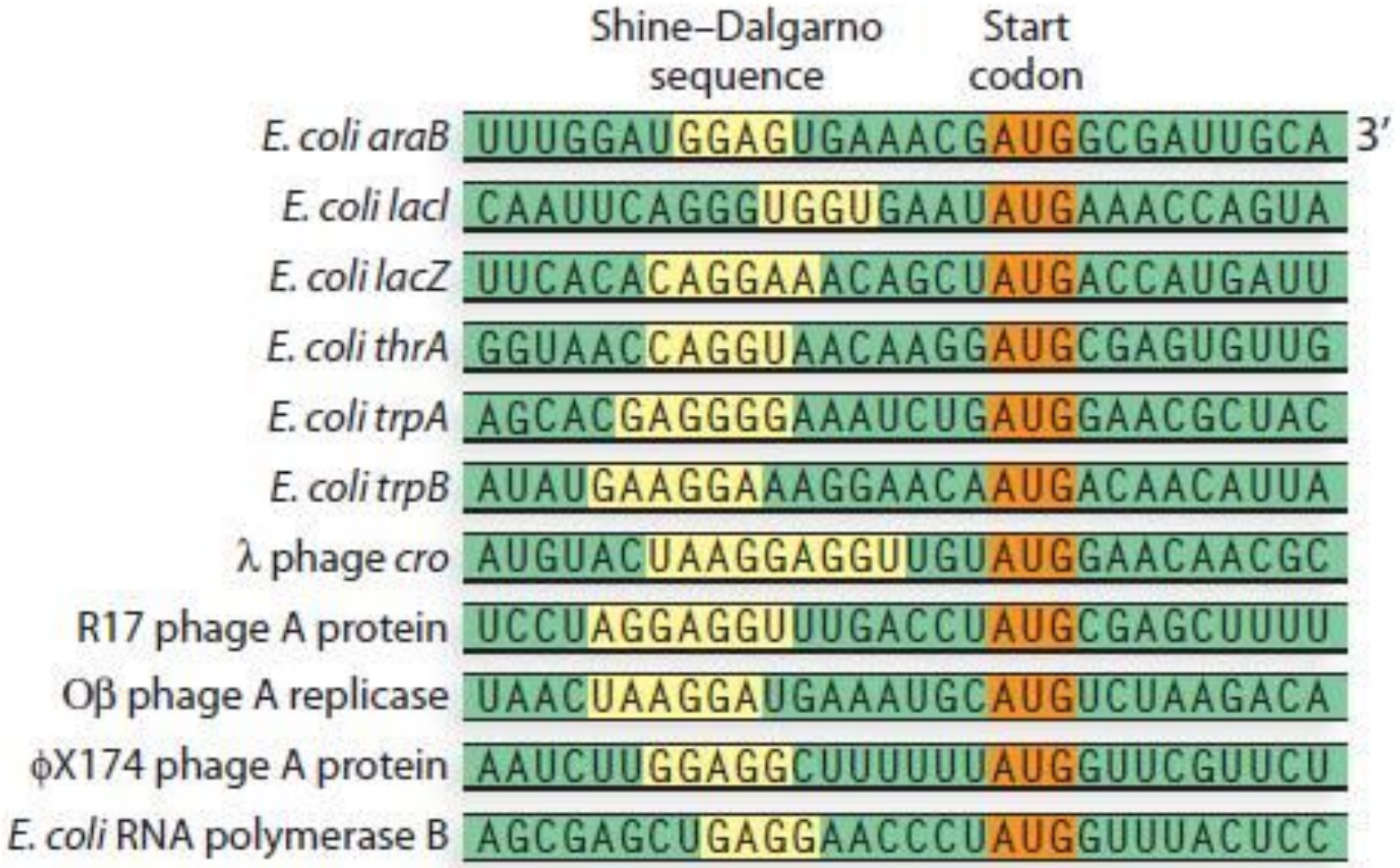


1. mRNA binds to small subunit of ribosome.

Translation initiation in bacteria

Shine-Dalgarno sequence

- 8-12 specific nucleotide sequence upstream of the start codon (of each gene/transcript).
- The sequence interacts with the complementary sequence in 16S rRNA in the small ribosomal subunit.
- Interacts specifically with the small ribosomal subunit 30S.



- several examples of the Shine-Dalgarno sequence

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Links:

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