



GENE EXPRESSION

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Course: Molecular Biotechnology

MA Code408

Semester 8: Week 3

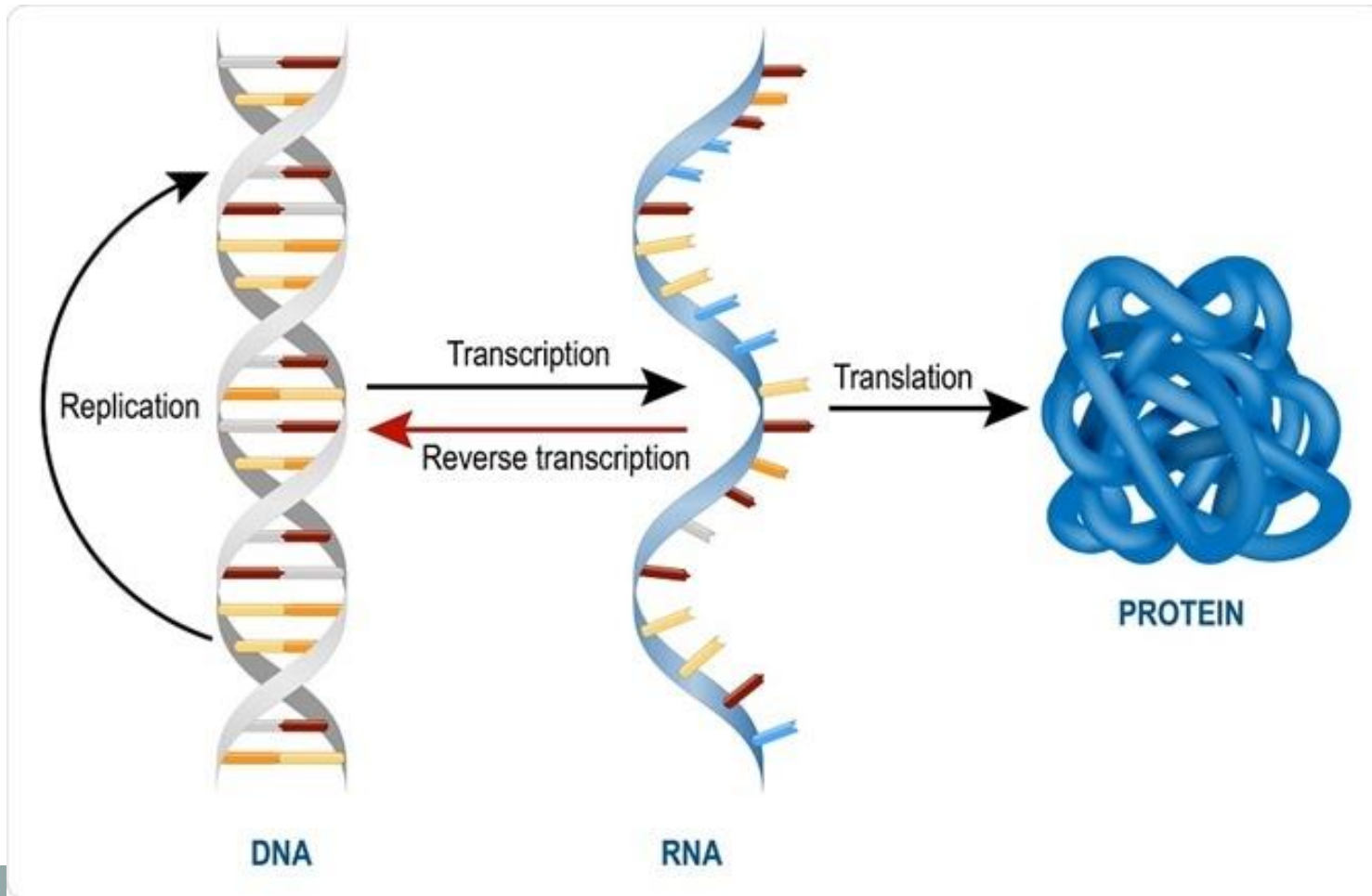
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Gene Expression

The process of gene expression is sometimes called the “**central dogma**” of molecular biology, which states that information in DNA is copied into RNA to be translated into protein. DNA carries the information for the synthesis of RNA and proteins in regions called genes.

Gene Expression





Gene Expression

DNA -----> RNA -----> Protein

Transcription & Translation

Transcription is the synthesis of complementary strand of RNA from DNA template

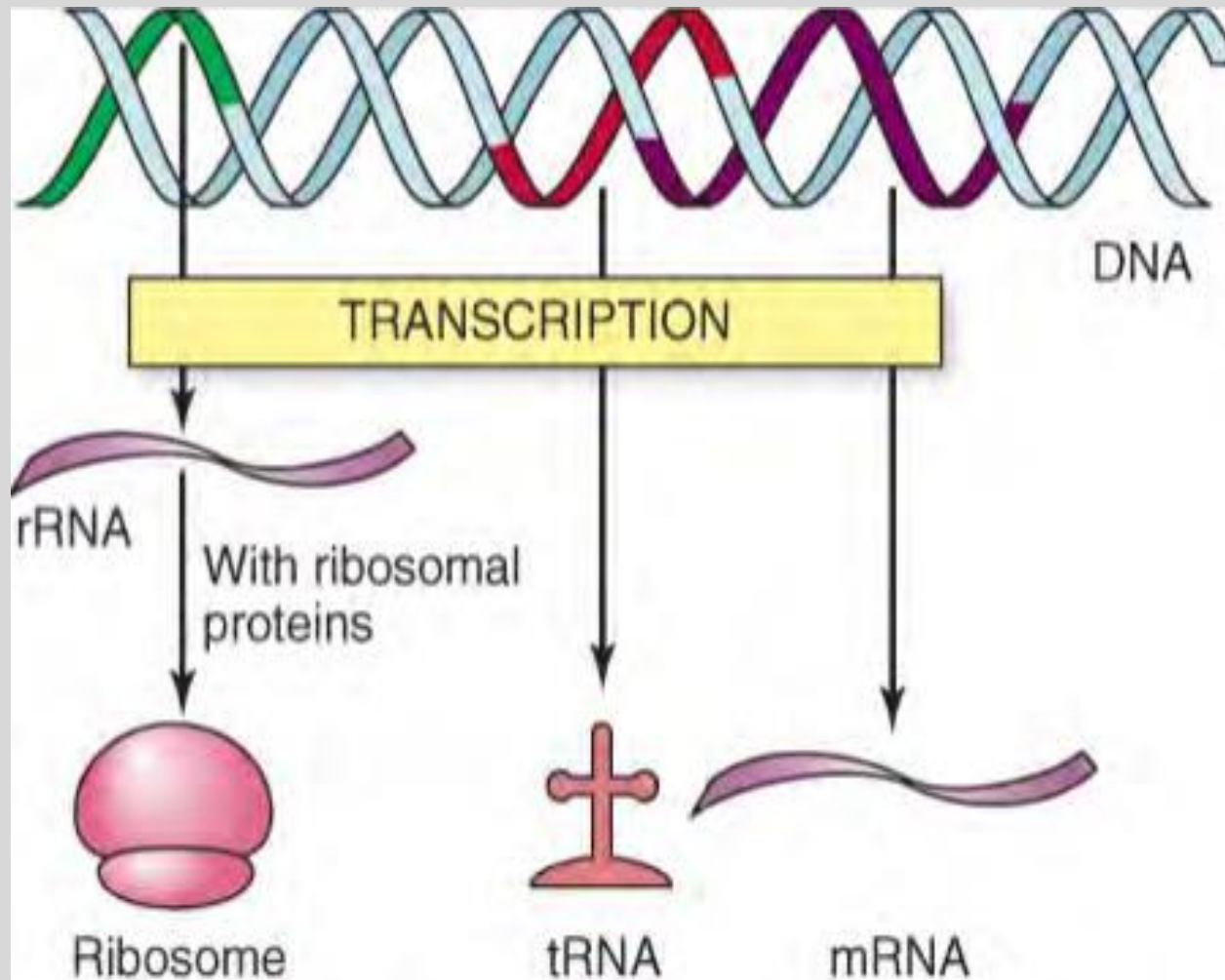
Translation is the synthesis of protein from information on mRNA template



Transcription

Transcription follows the same basepairing rules as DNA replication: T, G, C, and A in the DNA pair with A, C, G, and U, respectively, in the RNA product. The enzyme that directs transcription is called RNA polymerase.

Transcription



Transcription

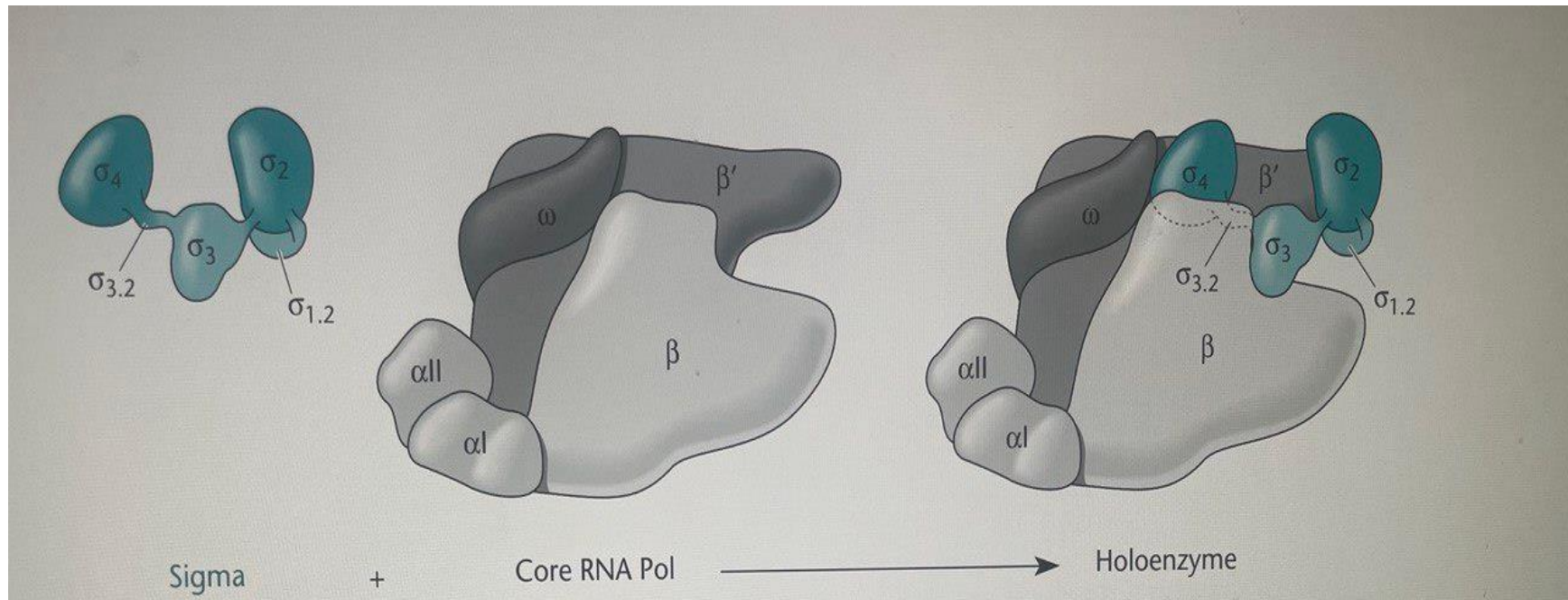
In transcription mRNA is synthesized under the direction of DNA by the enzyme RNA polymerase. An *E. coli* cell can have as many as 7,000 RNA polymerase molecules; only 2,000 to 5,000 polymerases may be active at any one time. The reaction is quite similar to that catalyzed by DNA polymerase. ATP, GTP, CTP, and UTP are used to produce an RNA copy of the DNA sequence.



RNA Polymerase

The α , β , and β' subunits are essential parts of the RNA polymerase, and the ω subunit helps in its assembly. A sixth subunit, the σ factor, is required only for initiation and cycles off the enzyme after initiation of transcription. Without the σ factor, the RNA polymerase is called the core enzyme; with σ , it is called the holoenzyme.

Schematic structure of Escherichia coli RNA polymerase



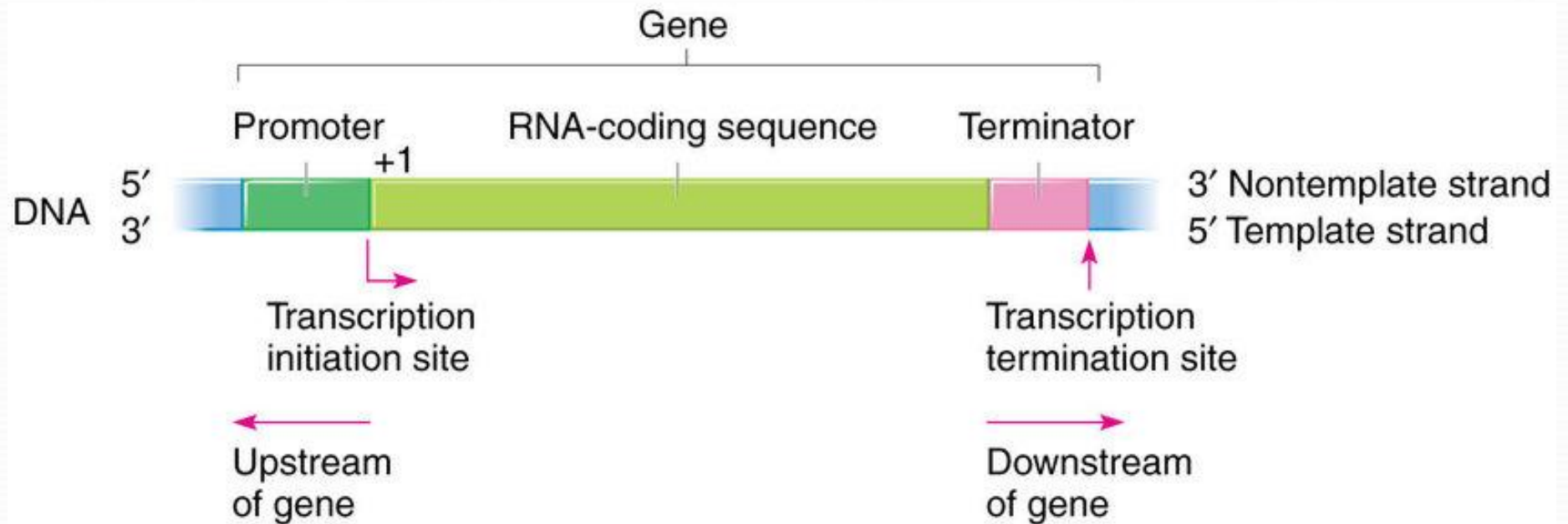
RNA Polymerase

The RNA polymerases of all bacteria are probably very similar. The eukaryotic and archaeal core RNA polymerases have more subunits and have very different sequences, but their basic overall structures are very similar to that of bacterial RNA polymerase.

Promoter

RNA polymerase holoenzyme can start making an RNA chain from a dsDNA only at certain sites. These DNA regions are called promoters, and the RNA polymerase recognizes a particular nucleotide (usually a T or C) in the promoter region of the template strand as a transcription start site. Thus, the first base in the chain is usually an A or a G laid down opposite to a T or C, respectively.

Bacterial Gene structure



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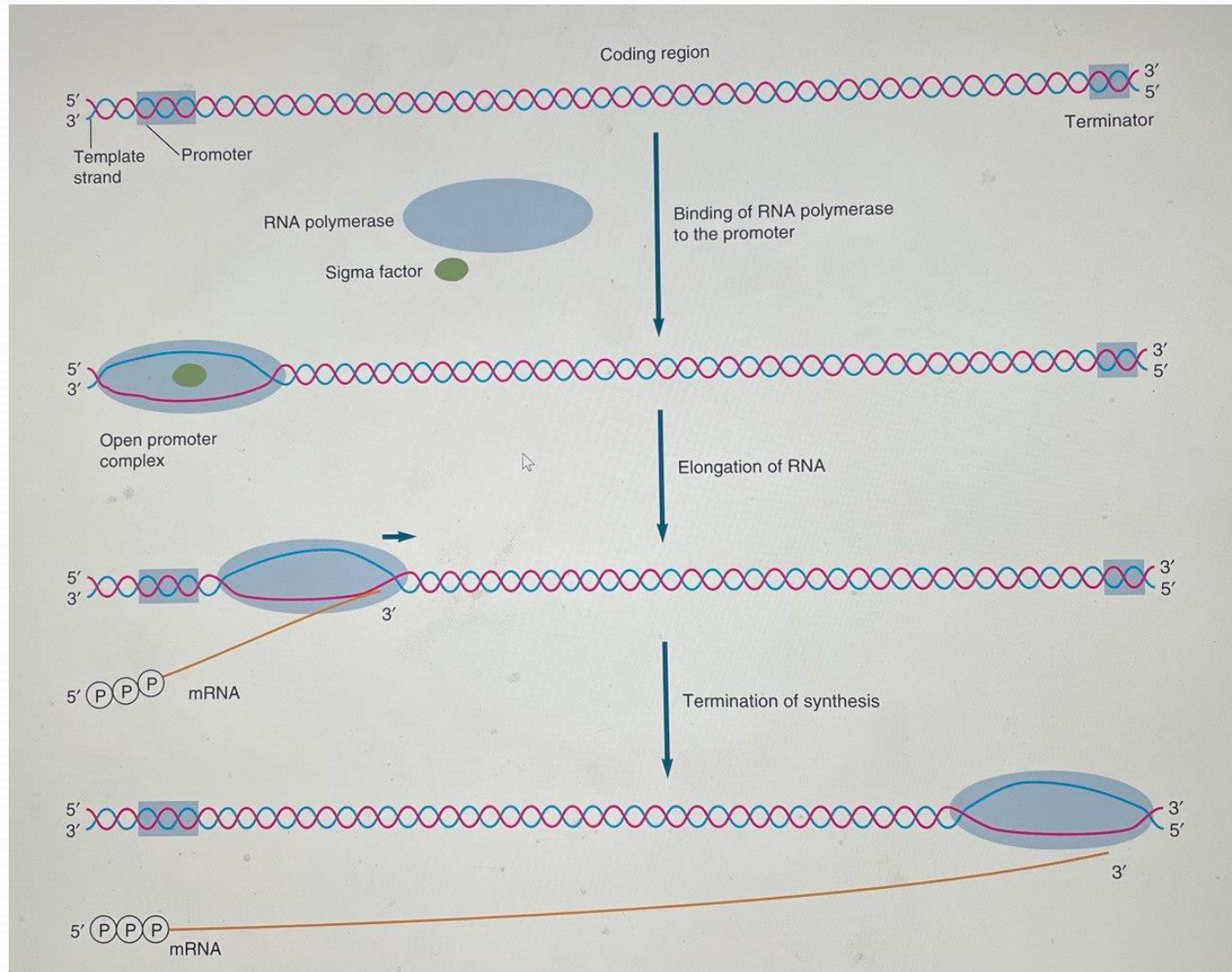
Promoter and Terminator

The Promoter and Terminator are directions for RNA polymerase to indicate the location of the gene to be transcribed.

The **ORF** is the “coding” region of the gene: it begins at the start codon and contains in order all the codons for all the amino acids in the resulting protein.

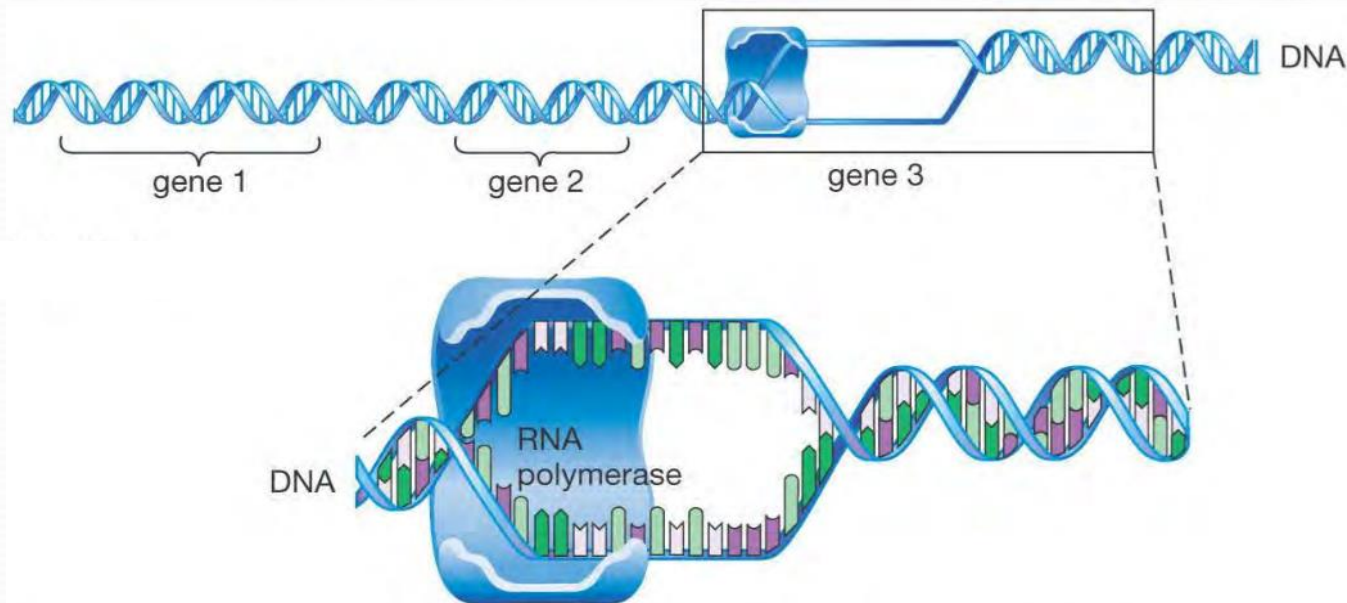
Transcription phases

Initiation, Elongation, Termination.

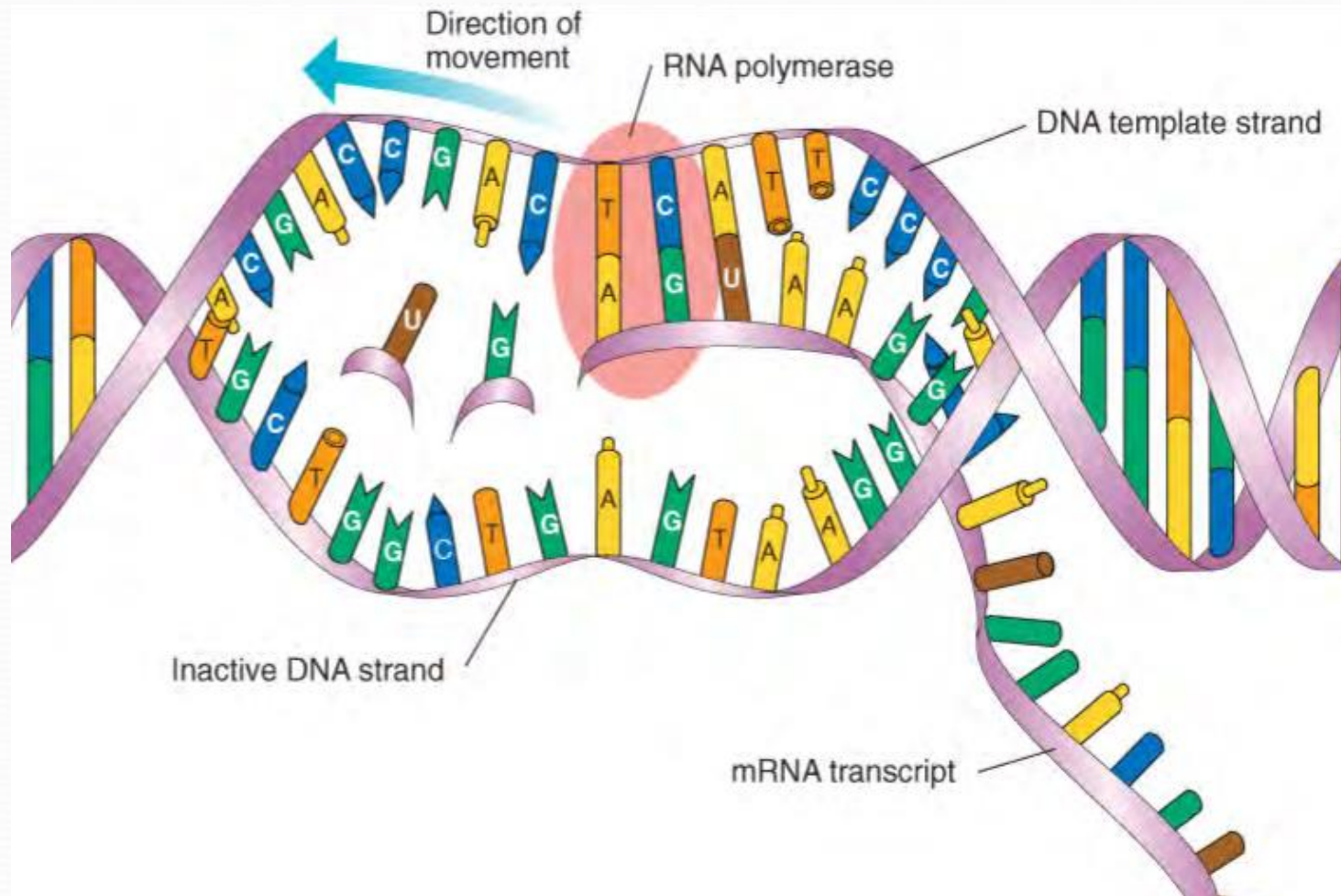


Initiation

The polymerase binds tightly to the promoter and starts building the RNA chain. The substrates, or building blocks, are the four ribonucleoside triphosphates: ATP, GTP, CTP, and UTP. The first substrate is usually a purine nucleotide. After the first nucleotide is in place, the polymerase joins a second nucleotide to the first, forming the initial phosphodiester bond in the RNA chain. Several nucleotides may be joined before the polymerase leaves the promoter and elongation begins.

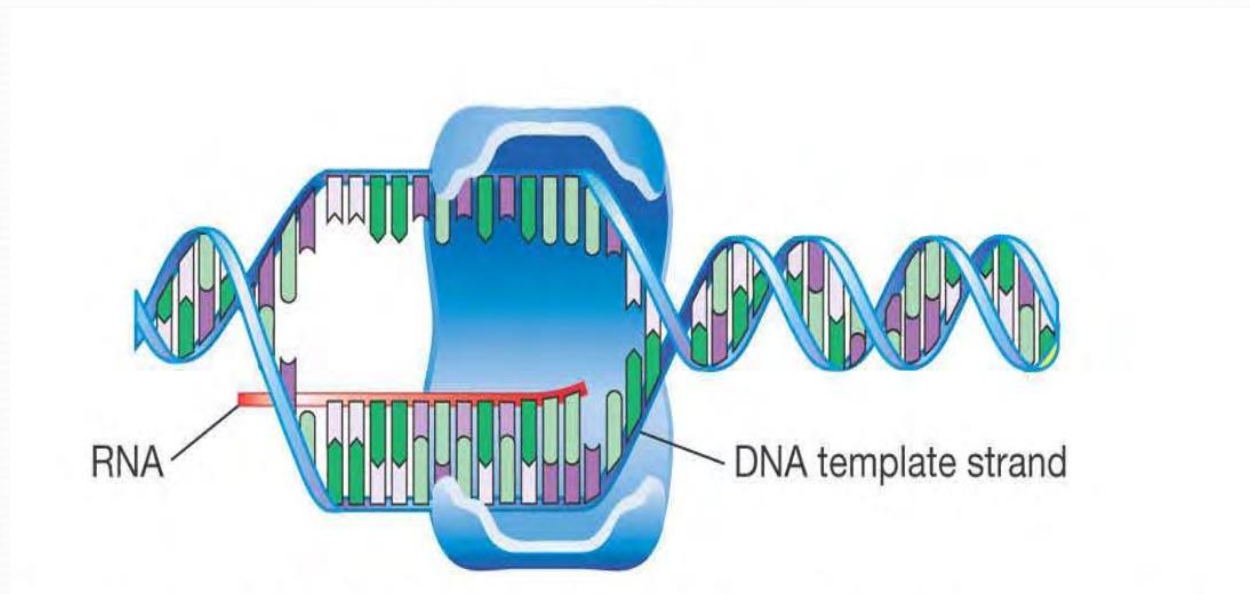


Transcription



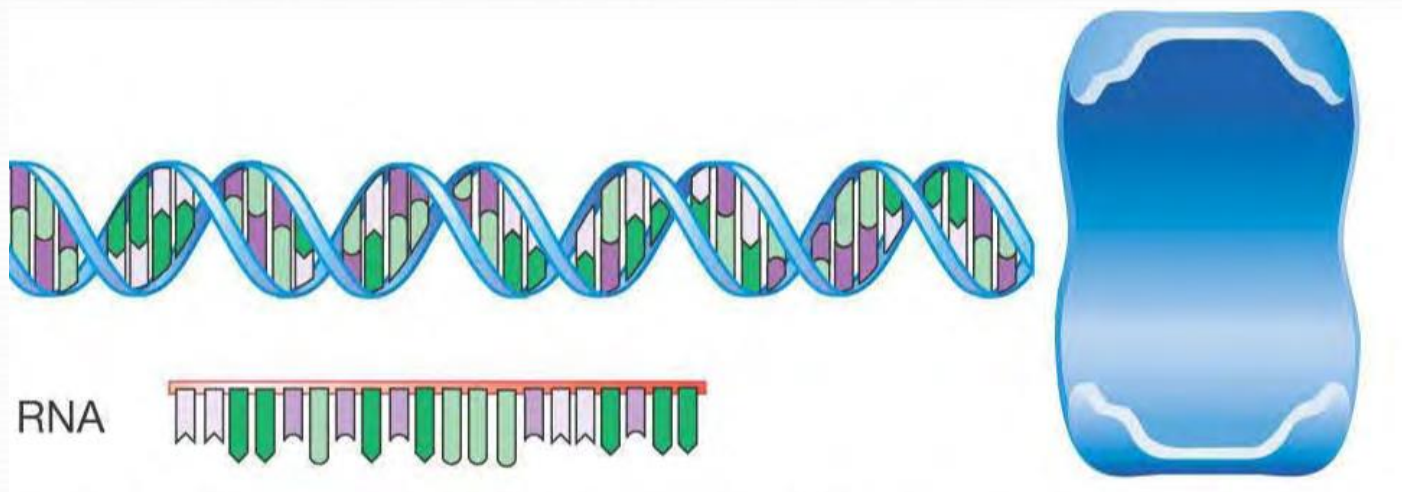
Elongation

- Only 1 DNA strand is used as a template
- RNA polymerase travels along the DNA template strand, catalyzing the addition of ribose nucleotides.
- The nucleotides in the RNA are the complementary to the template strand of the DNA.



3) Termination

Triggered by specific DNA sequences in the gene DNA completely rewinds into a double helix



Transcription in Eukaryotes

Transcription in eucaryotic microorganisms differ in several ways from procaryotic transcription. There are three major RNA polymerases, not one as in procaryotes. RNA polymerase II, associated with chromatin in the nuclear matrix, is responsible for mRNA synthesis. Polymerases I and III synthesize rRNA and tRNA, respectively.

Translation

- Protein synthesis is called translation because it translates the language of nucleic acids into the language of proteins.
- All organisms use the same codons to specify the genetic code for the particular amino acids
- 64 possible codons but only 20 amino acids
- 61 codons code for amino acids = sense codons
- 3 nonsense codons serve as the STOP signal to terminate protein synthesis.

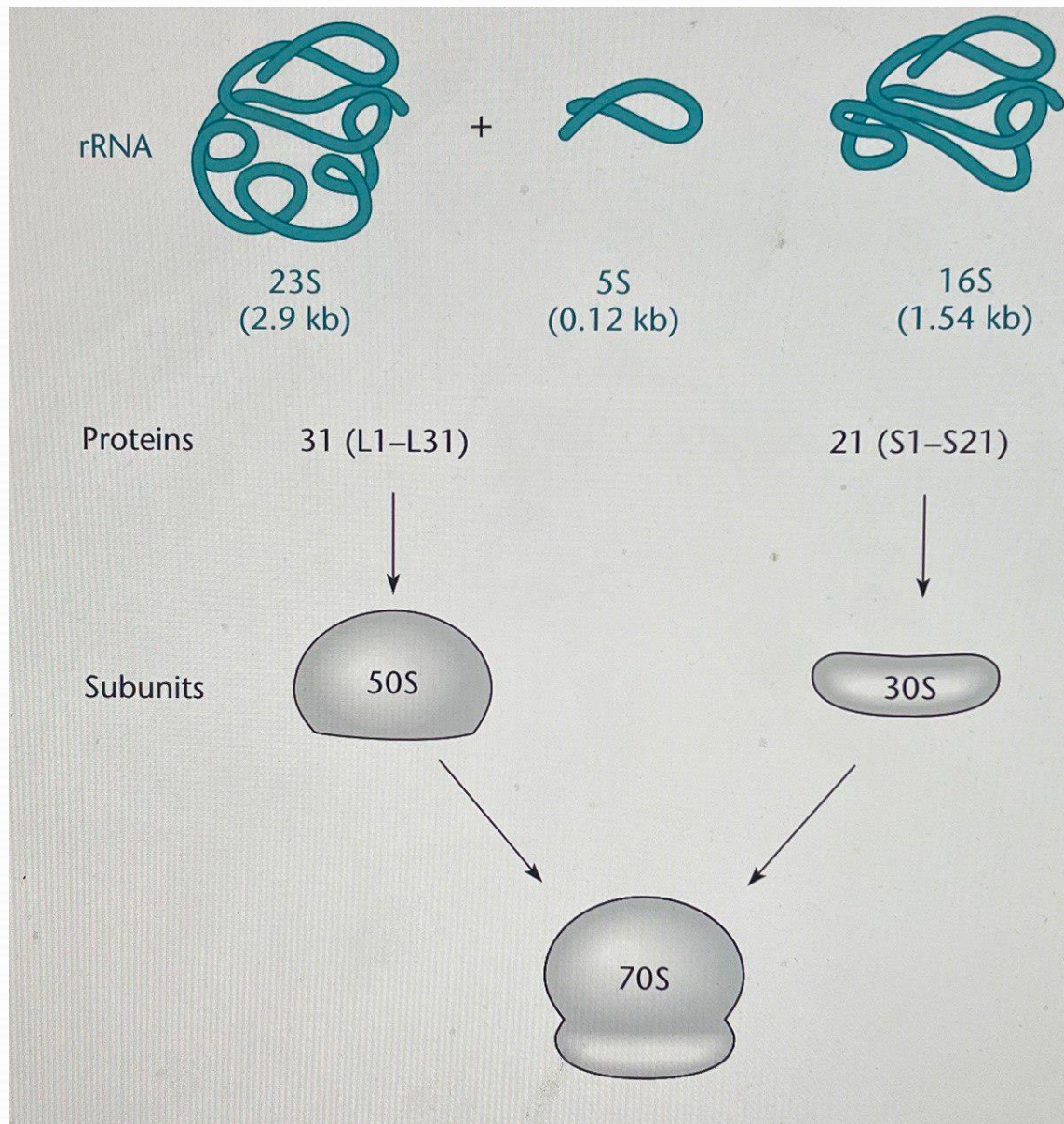
Translation

- For each sense codon there is a tRNA with a complementary antisense codon: this tRNA carries the amino acid specified by the codon.
- There are no tRNA molecules with anticodons to the 3 nonsense codons (stop codons): UAA, UAG, UGA, and thus no amino acids.
- The start codon is AUG and codes for the amino acid methionine.

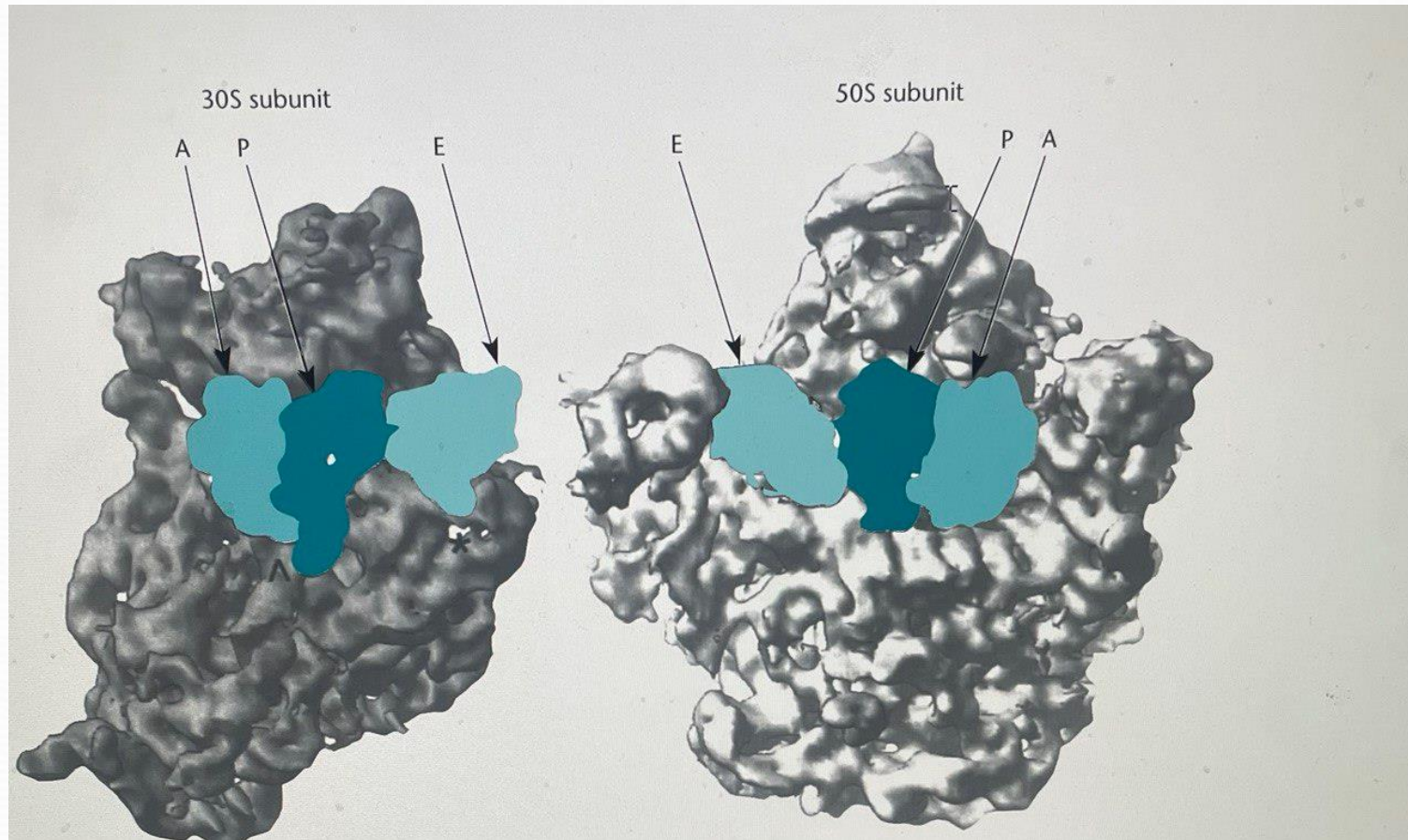
Ribosome

The ribosome is actually an enormous enzyme that performs the complicated role of polymerizing amino acids into polypeptide chains, using the information in mRNA as a guide. As such, a better name for it might have been protein polymerase, by analogy to DNA and RNA polymerases

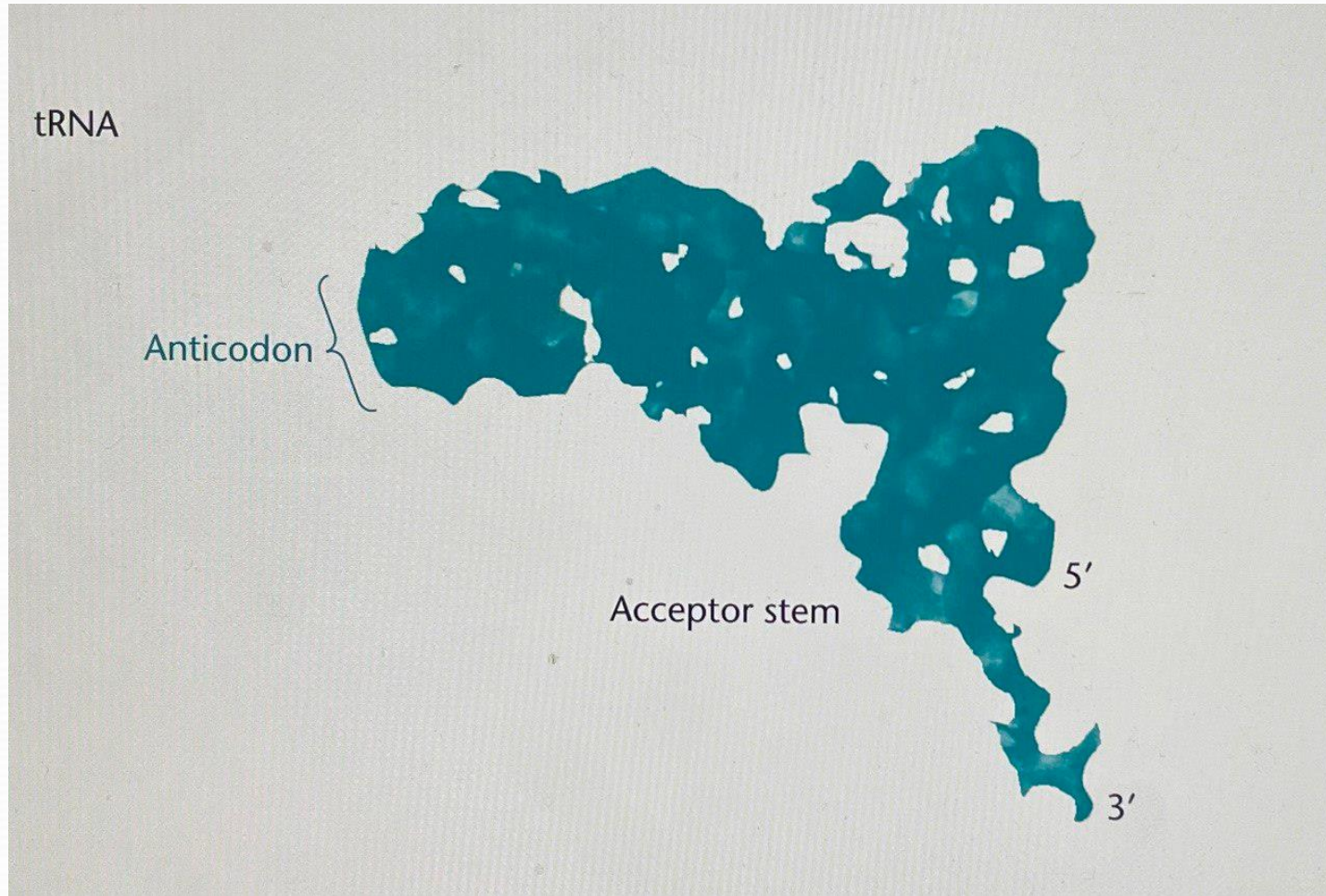
The composition of bacterial ribosome



The two subunits of the ribosome



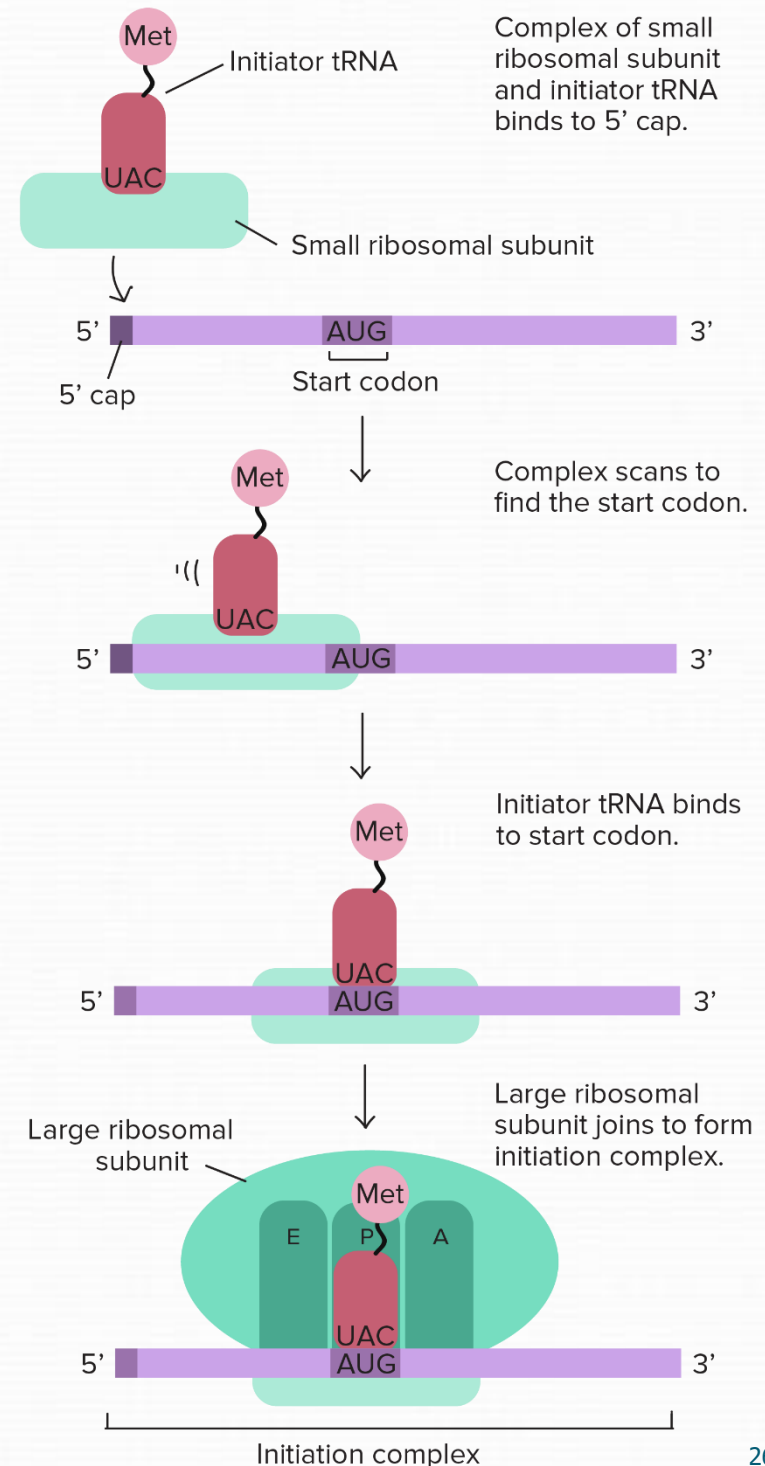
Crystal structures of a tRNA



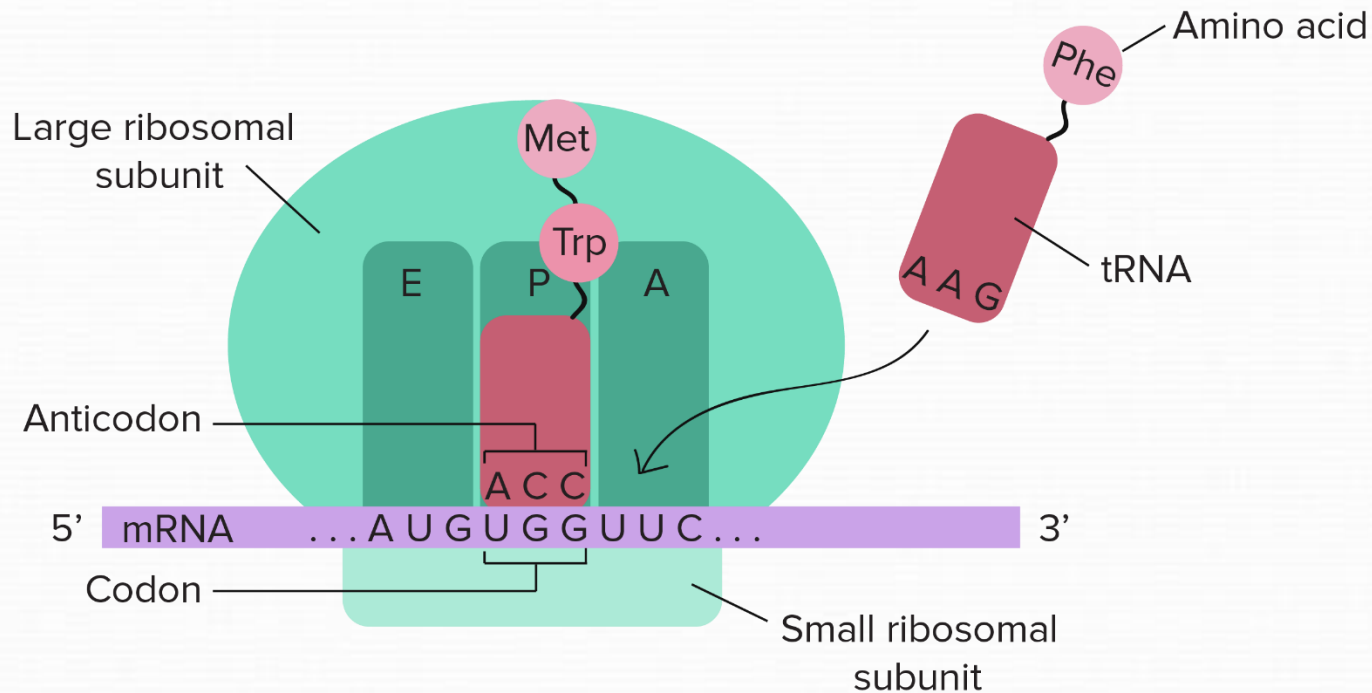
1) Initiation

Components needed to begin translation come together.

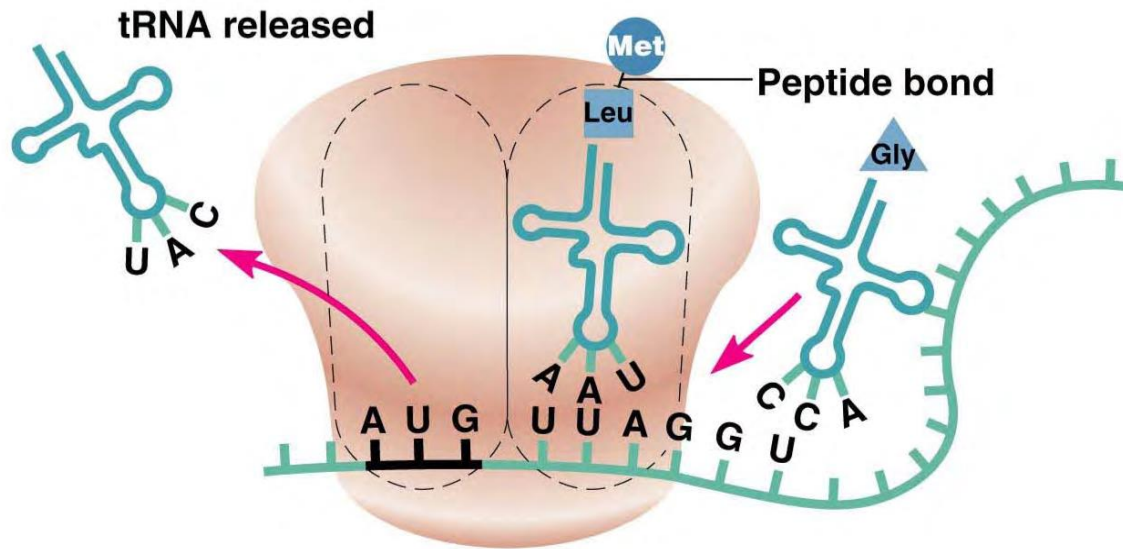
Eukaryotic translation initiation



A tRNA carrying the first amino acid is paired with the start codon on the mRNA, and the second tRNA approaches.

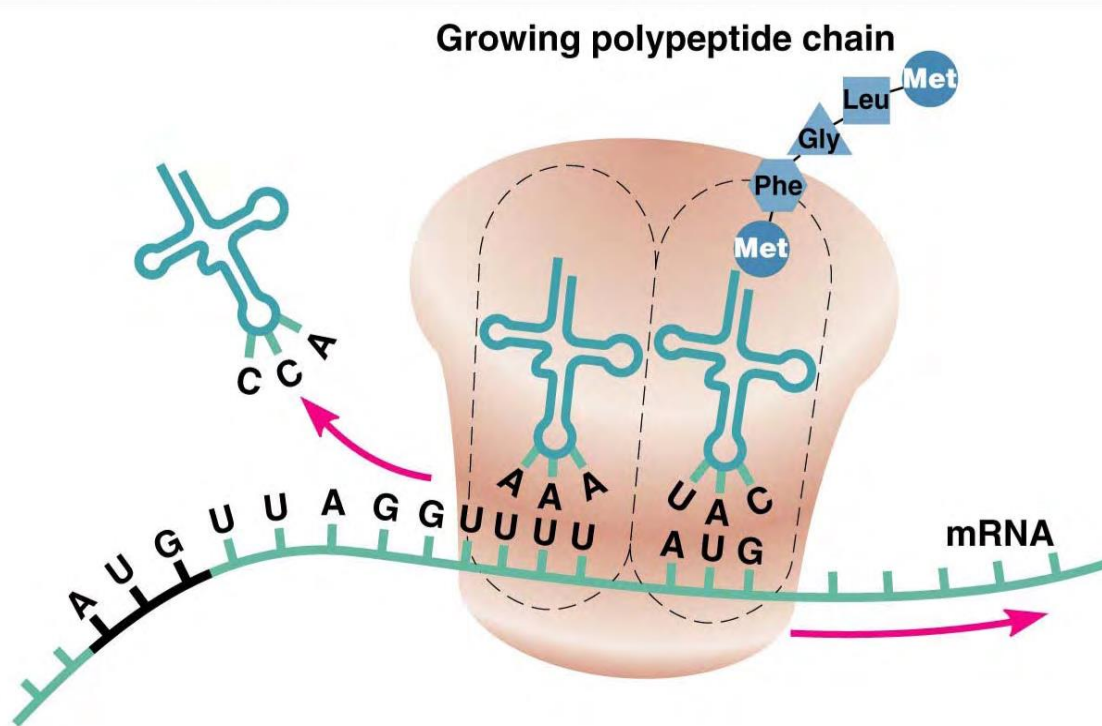


2) Elongation The second tRNA carrying second codon of mRNA and the second amino acid binds to the ribosome (A site) next to the first amino acid on ribosome (P site). The first amino acid then joins to the second by a peptide bond, and the first tRNA is released



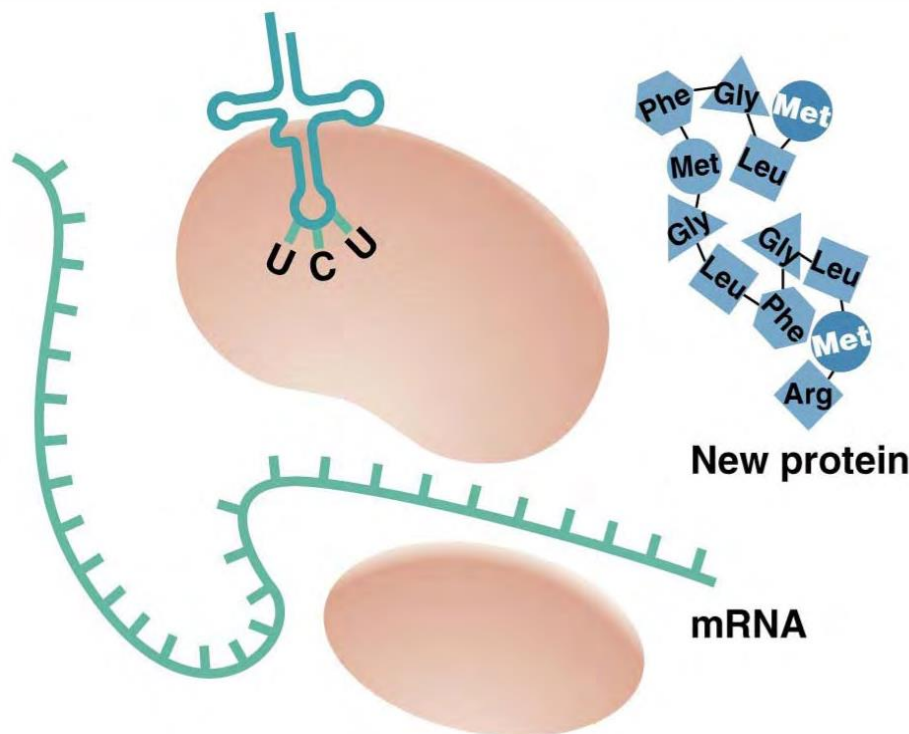
Elongation

The ribosome moves along the mRNA until the second tRNA is in the P site, and the process continues. The ribosome continues to move along the mRNA, and new amino acids are added to the polypeptide.



3) Termination

When the ribosome reaches a stop codon, the polypeptide is released. Finally, the last tRNA is released, and the ribosome comes a part. The released polypeptide forms a new protein.



Expression of the genetic code

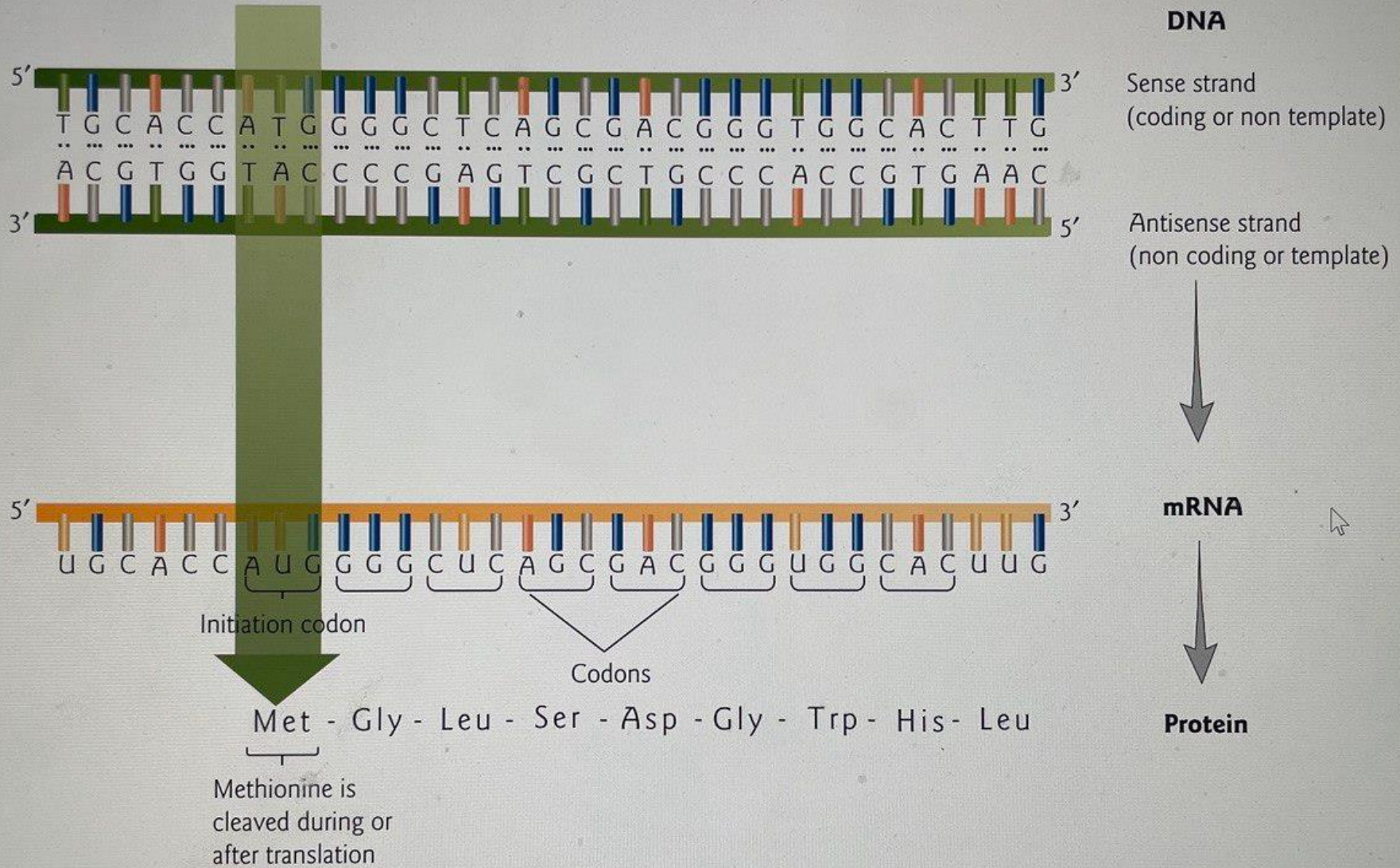


Table of the Genetic Code to amino acids

		Second position					
		U	C	A	G		
First position	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/start	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	
						Third position	

Table of 20 amino acids

Amino acid	3-letter abbreviation	1-letter abbreviation
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

Table of 20 amino acids

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	Try	W
Tyrosine	Tyr	Y
Valine	Val	V



Next Lecture

Regulation of gene Expression in bacteria



Questions and comments?