

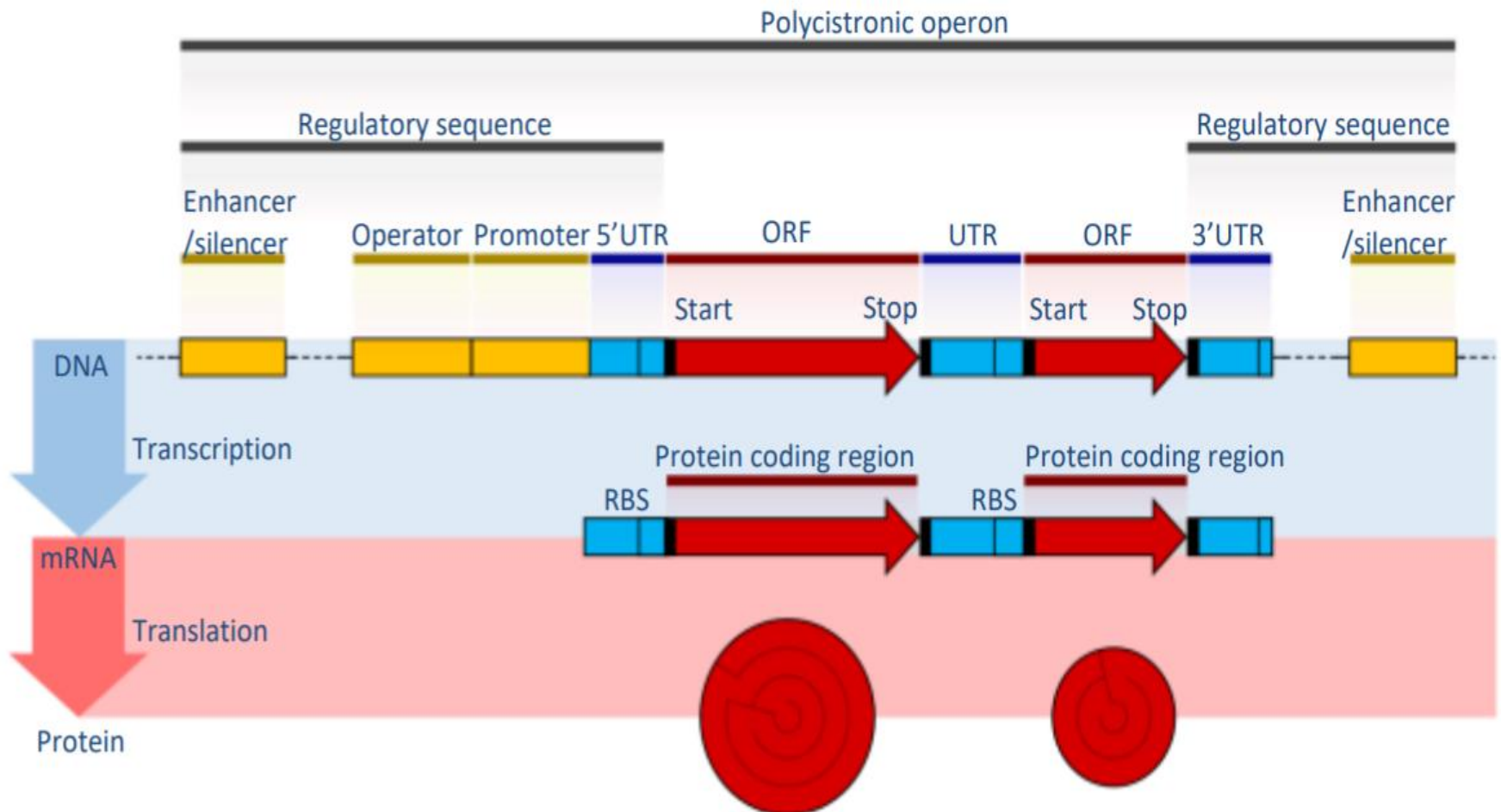
# Transcription and translation

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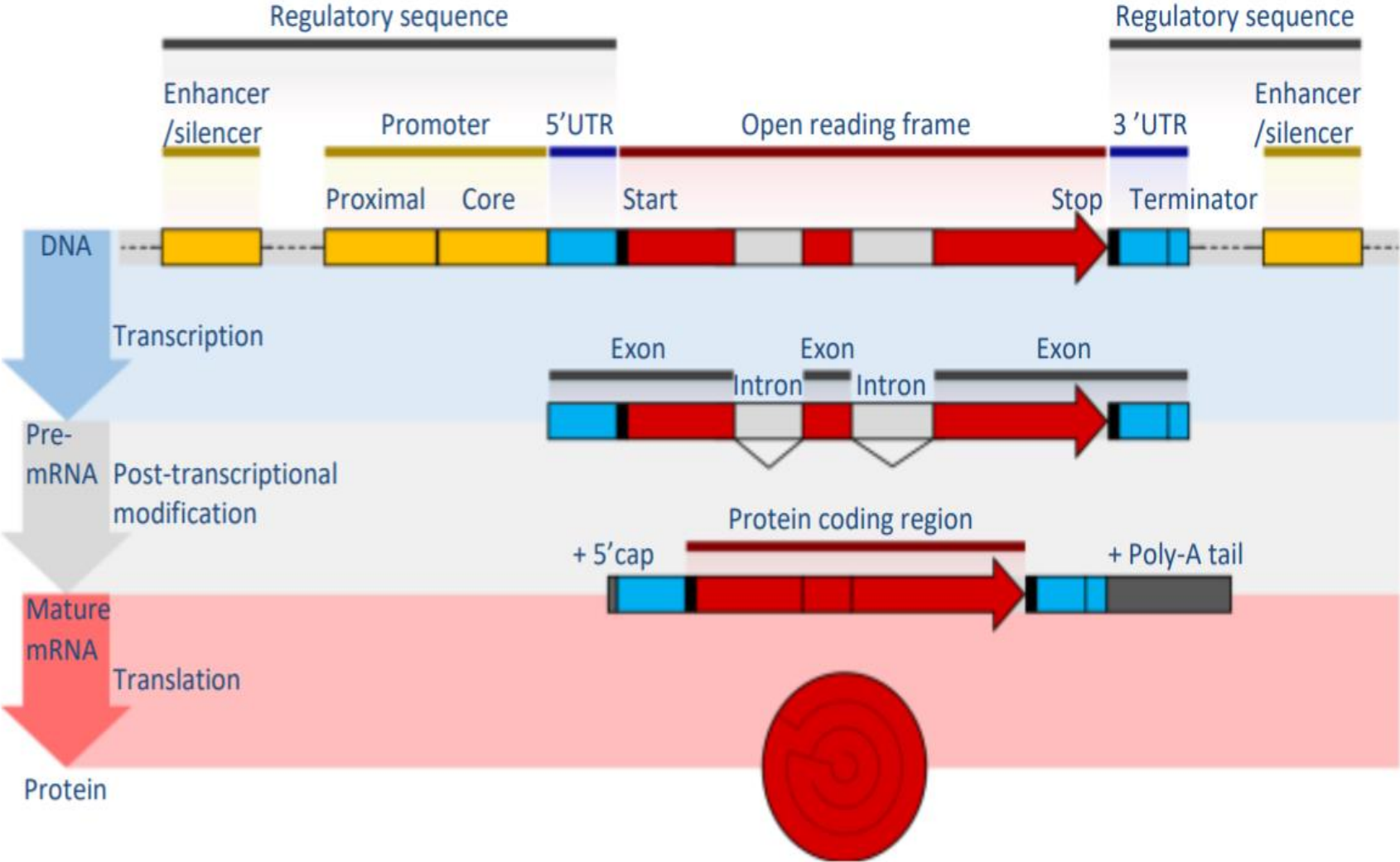
# Transcription

- Gene structure

Prokaryotic gene



# Eukaryotic gene

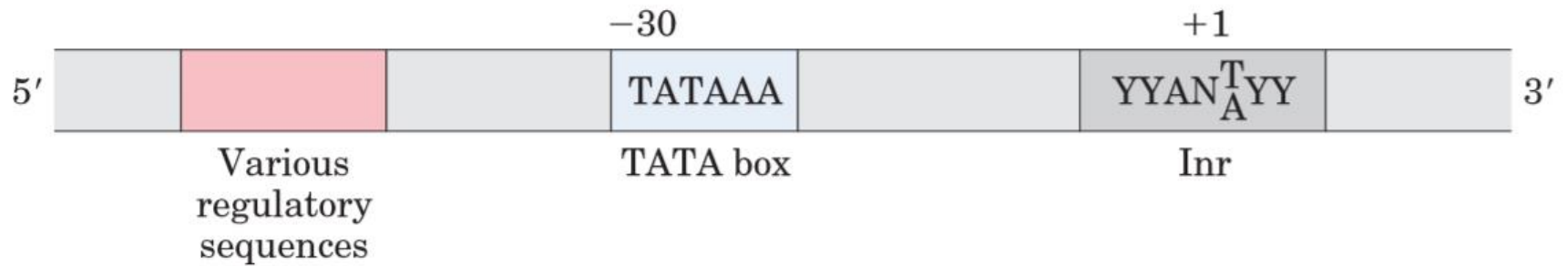


# Promoter sequences

## Prokaryotes



## Eukaryotes



## Sense and antisense strands

(5') CGCTATAGCGTTT (3')	DNA nontemplate (coding) strand
(3') GCGATATCGCAA (5')	DNA template strand
(5') CGCUAUAGCGUUU (3')	RNA transcript

The two complementary DNA strands have different roles in transcription. The strand that serves as template for RNA synthesis is called the template strand. The DNA strand complementary to the template, the nontemplate strand, or coding strand, is identical in base sequence to the RNA transcribed from the gene, with U in the RNA in place of T in the DNA.

## DNA-dependent RNA polymerase

Prokaryotes have only one RNA polymerase for all types of RNAs. Eukaryotes have three different RNA pols; for RNA pol I (rRNA), RNA pol II (mRNA) and RNA pol III (tRNA).

Prokaryotic RNA pol requires  **$\sigma$  factor which is part of RNA pol but eukaryotes require basal transcription factors. They are not the part of RNA pol.**

Other than basal TFs there accessory TFs called activators and repressors used to increase or decrease the rate of transcription.

# Mechanism of transcription

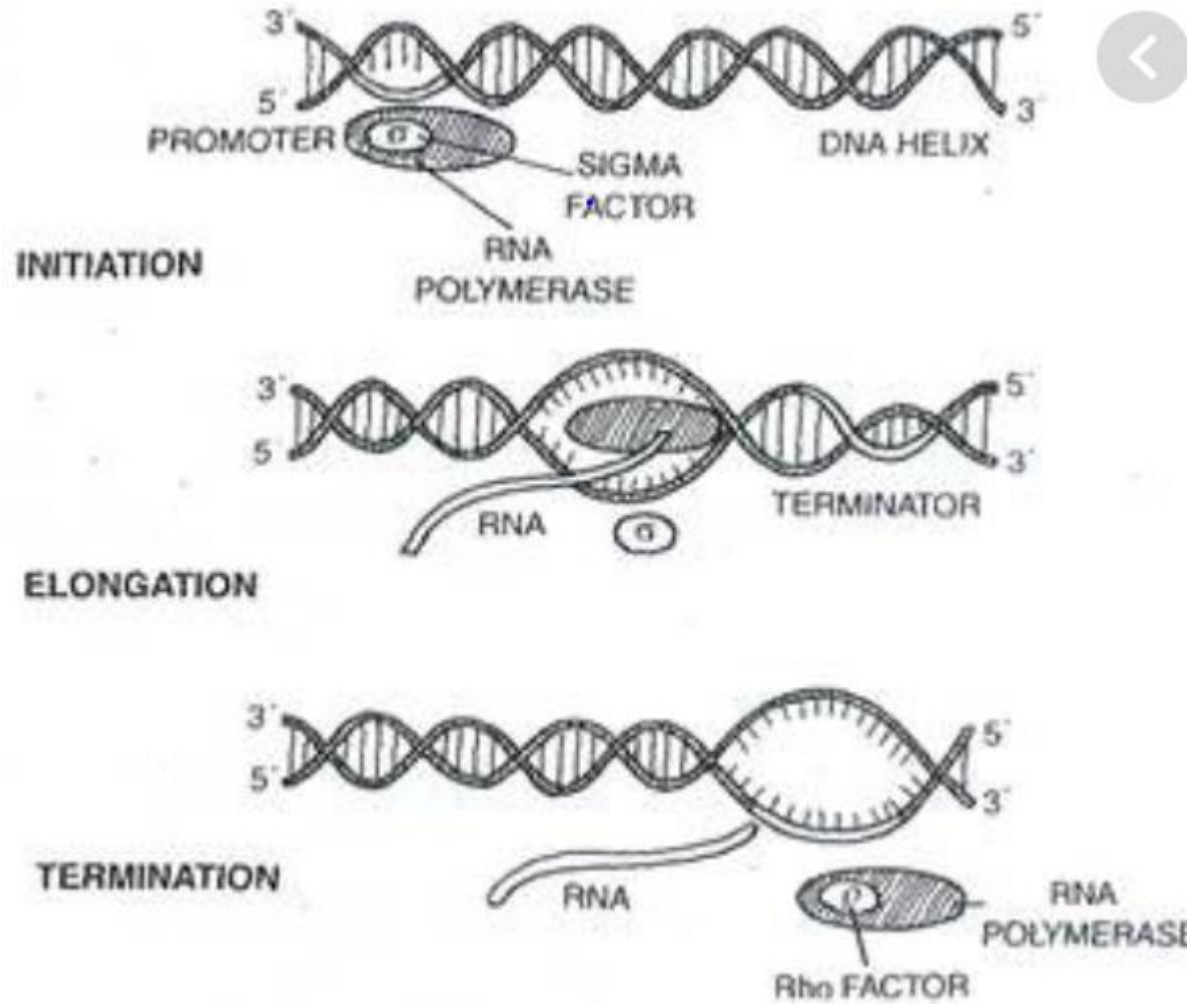
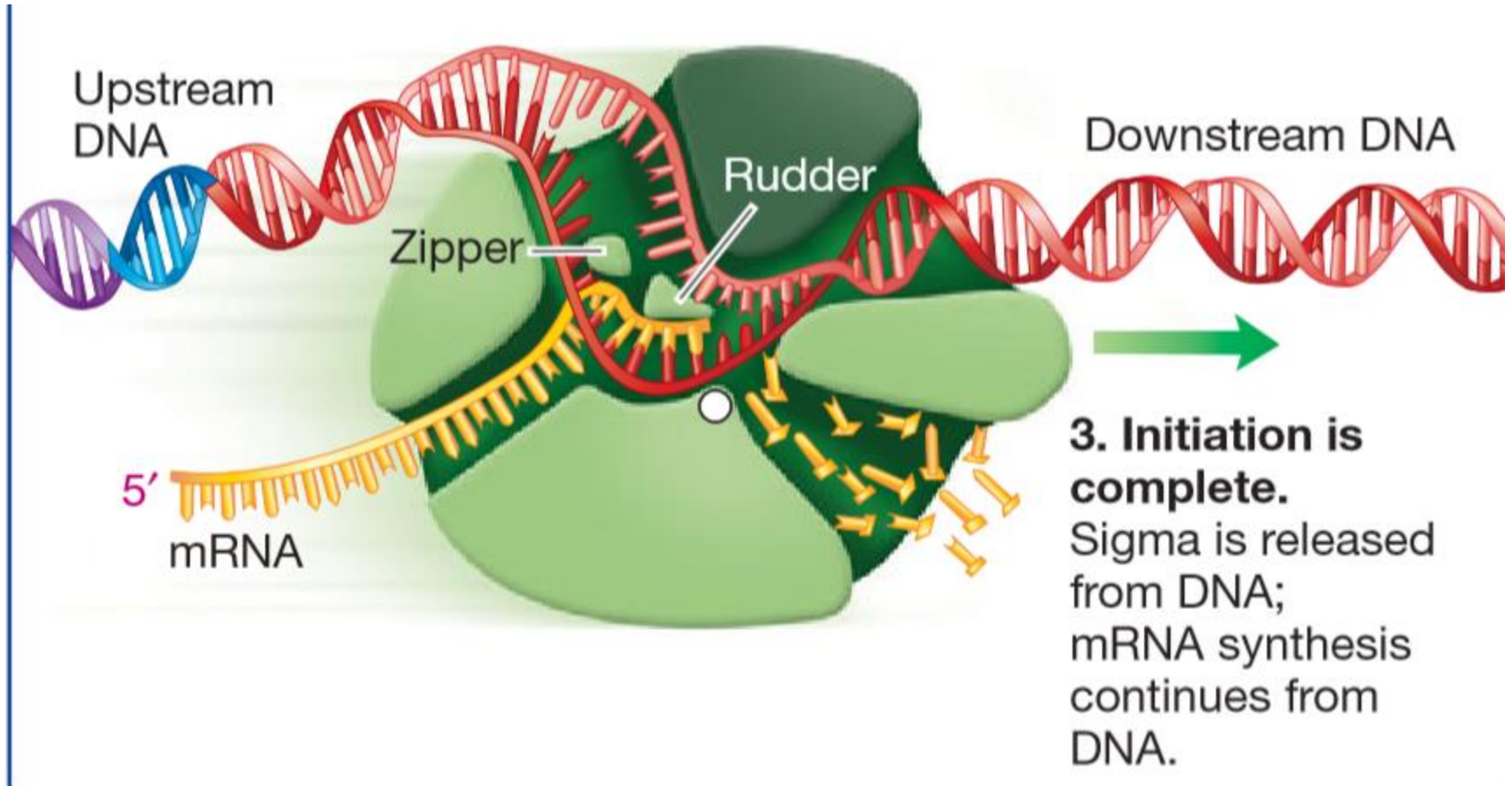


Fig. 6.17. Transcription *i.e.*, synthesis of RNA over DNA template in prokaryotes.

# RNA polymerase in action

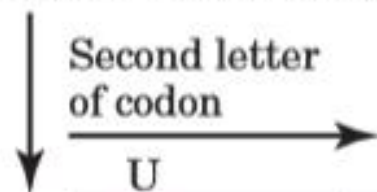




# Translation

- The particular amino acid sequence of a protein is constructed through the translation of information encoded in mRNA. This process is carried out by ribosomes.
- Amino acids are specified by mRNA codons consisting of nucleotide triplets.
- Translation requires adaptor molecules, the tRNAs, that recognize codons and insert amino acids into their appropriate sequential positions in the polypeptide.

First letter of codon (5' end)



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

# Genetic code

- Amino acids are specified by mRNA codons consisting of nucleotide triplets.
- The codon AUG signals initiation of translation. The triplets UAA, UAG, and UGA are signals for termination.
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- Codons are read in a successive, nonoverlapping fashion.
- A specific first codon in the sequence establishes the **reading frame**, in which a new codon begins every three nucleotide residues.
- There is no punctuation between codons for successive amino acid residues.
- The genetic code is degenerate: it has multiple codons for almost every amino acid.

- The standard genetic code is universal in all species, with some minor deviations in mitochondria and a few single-celled organisms.
- The third position in each codon is much less specific than the first and second and is said to wobble.

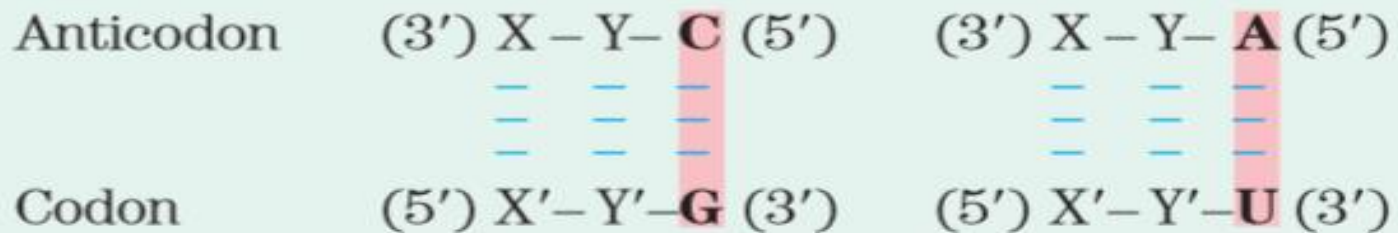
# Wobble hypothesis

- 1. The first two bases of an mRNA codon always form strong Watson-Crick base pairs with the corresponding bases of the tRNA anticodon and confer most of the coding specificity.
- 2. The first base of the anticodon (reading in the 5→3 direction; this pairs with the third base of the codon) determines the number of codons recognized by the tRNA. When the first base of the anticodon is C or A, base pairing is specific and only one codon is recognized by that tRNA. When the first base is U or G, binding is less specific and two different codons may be read. When inosine (I) is the first (wobble) nucleotide of an anticodon, three different codons can be recognized—the maximum number for any tRNA.

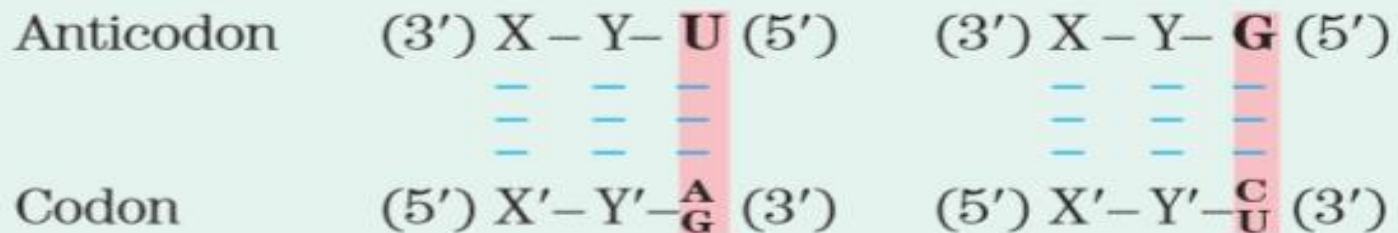
- 3. When an amino acid is specified by several different codons, the codons that differ in either of the first two bases require different tRNAs. 4. A minimum of 32 tRNAs are required to translate all 61 codons (31 to encode the amino acids and 1 for initiation).

**TABLE 27-4****How the Wobble Base of the Anticodon Determines the Number of Codons a tRNA Can Recognize**

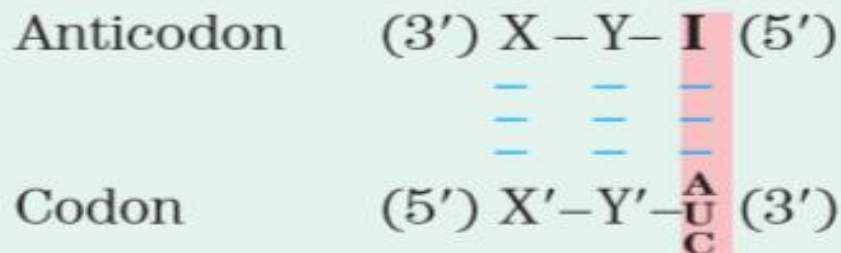
1. One codon recognized:



2. Two codons recognized:



3. Three codons recognized:

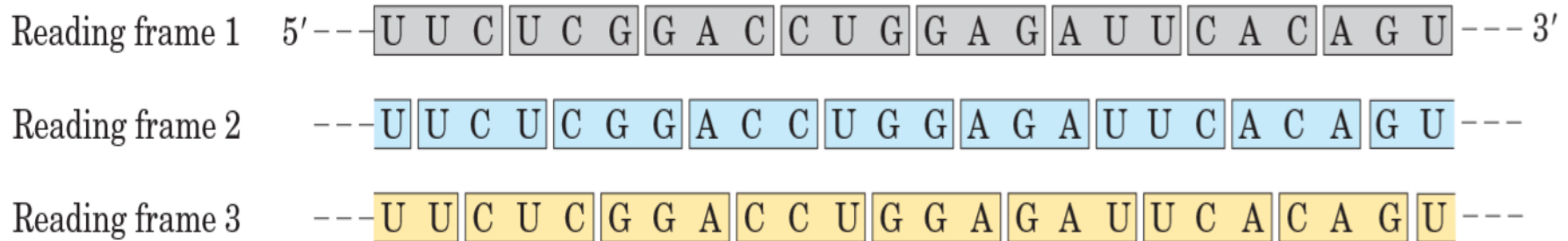


# Open reading frame

- A **reading frame** refers to one of three possible ways of reading a nucleotide sequence.
- The reading frame affects which protein is made.
- In a random sequence of nucleotides, 1 in every 20 codons in each reading frame is, on average, a termination codon.
- In general, a reading frame without a termination codon among 50 or more codons is referred to as an open reading frame (ORF).
- Long open reading frames usually correspond to genes that encode proteins.



- There are **six reading frames**: three on the positive strand, and three (which are read in the reverse direction) on the negative strand.

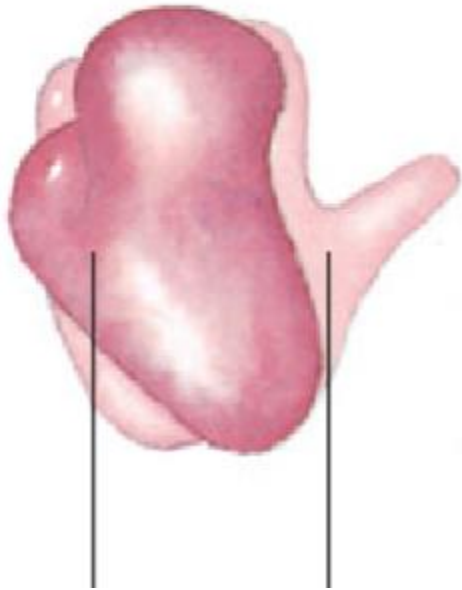


**FIGURE 27-5** Reading frames in the genetic code. In a triplet, nonoverlapping code, all mRNAs have three potential reading frames, shaded here in different colors. The triplets, and hence the amino acids specified, are different in each reading frame.

**Bacterial ribosome**

**70S**

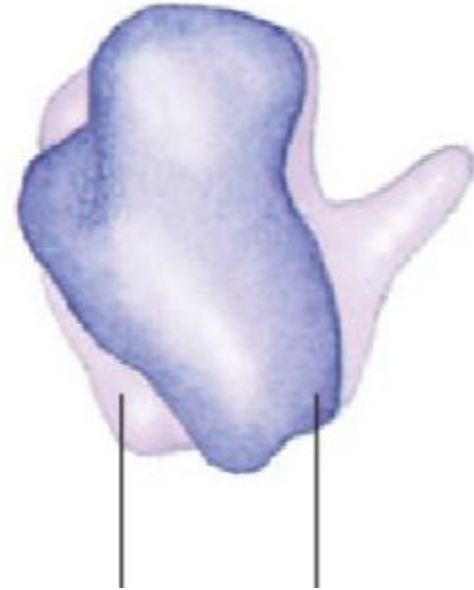
$M_r 2.7 \times 10^6$



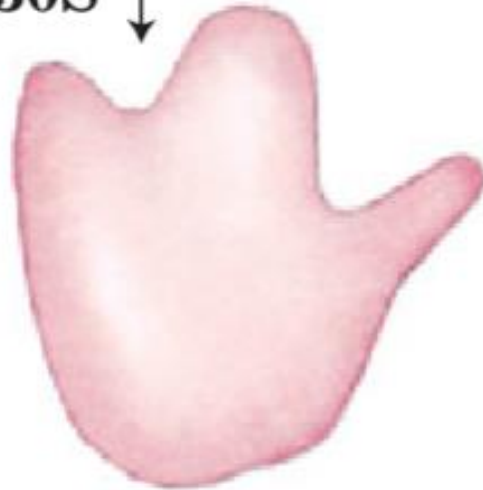
**Eukaryotic ribosome**

**80S**

$M_r 4.2 \times 10^6$



**50S**



**$M_r 1.8 \times 10^6$**

5S rRNA

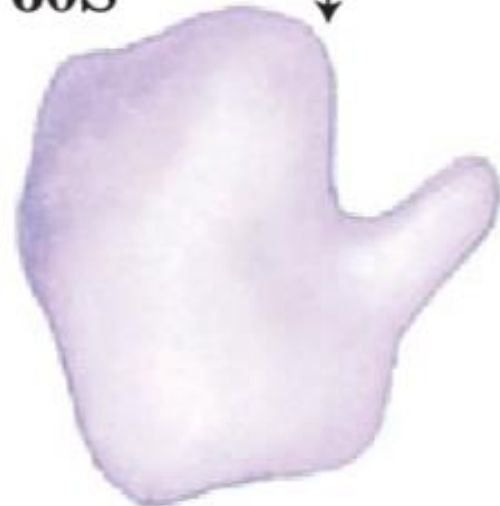
(120 nucleotides)

23S rRNA

(3,200 nucleotides)

36 proteins

**60S**



**$M_r 2.8 \times 10^6$**

5S rRNA

(120 nucleotides)

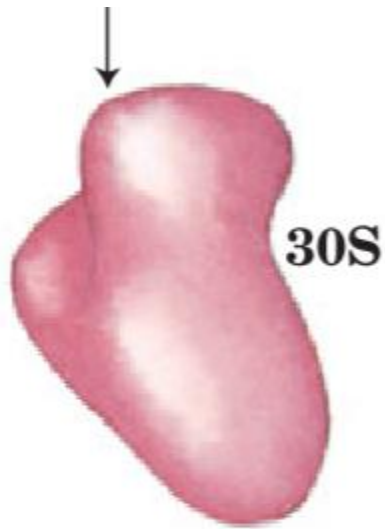
28S rRNA

(4,700 nucleotides)

5.8S rRNA

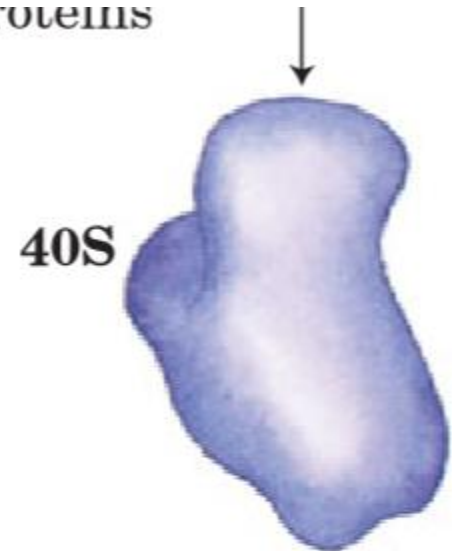
(160 nucleotides)

~ 49 proteins



$M_r 0.9 \times 10^6$   
16S rRNA  
(1,540 nucleotides)  
21 proteins

~ 49 proteins

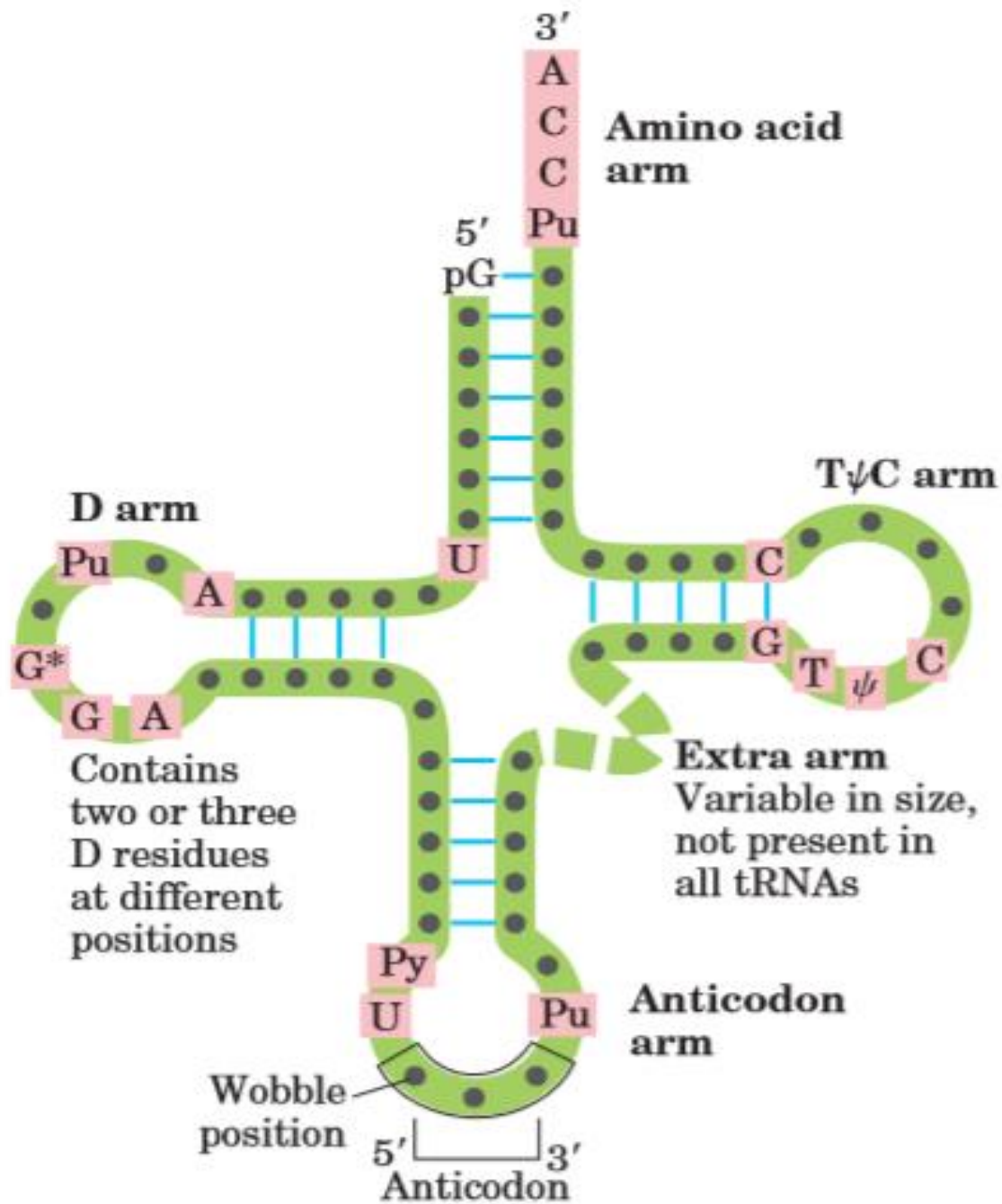


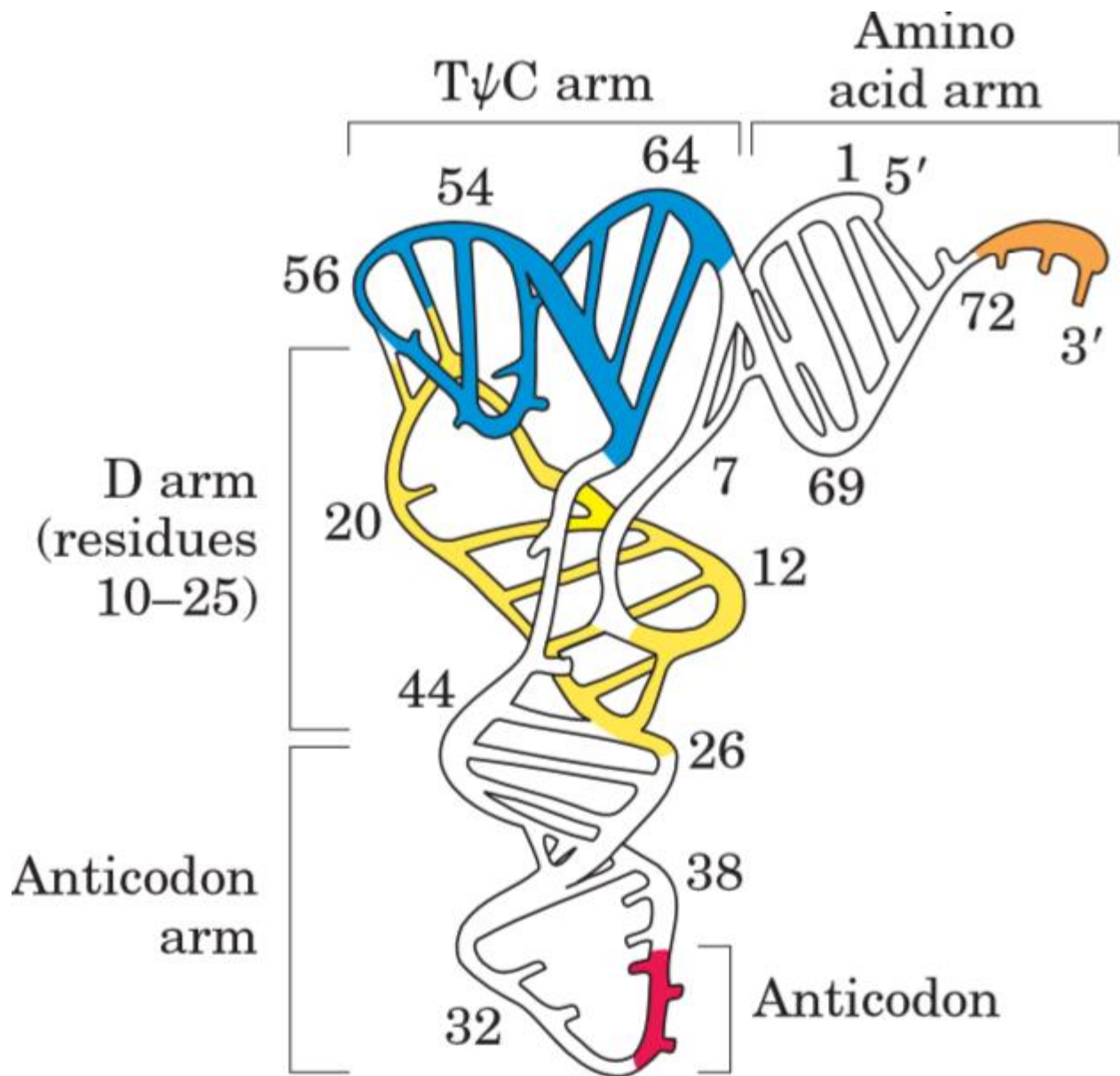
$M_r 1.4 \times 10^6$   
18S rRNA  
(1,900 nucleotides)  
~ 33 proteins

# Structure of tRNA

- Transfer RNAs are relatively small and consist of a single strand of RNA folded into a precise three-dimensional structure.
- The tRNAs in bacteria and in the cytosol of eukaryotes have between 73 and 93 nucleotide residues, corresponding to molecular weights of 24,000 to 31,000.
- When drawn in two dimensions, the hydrogenbonding pattern of all tRNAs forms a cloverleaf structure with four arms; the longer tRNAs have a short fifth arm, or extra arm (Fig. 27–17). In three dimensions, a tRNA has the form of a twisted L .

- Two of the arms of a tRNA are critical for its adaptor function. The amino acid arm can carry a specific amino acid esterified by its carboxyl group to the 2'- or 3'- hydroxyl group of the A residue at the 3' end of the tRNA.
- The anticodon arm contains the anticodon.
- The other major arms are the D arm, which contains the unusual nucleotide dihydrouridine (D), and the T $\psi$ C arm, which contains ribothymidine (T), not usually present in RNAs, and pseudouridine ( $\psi$ ).





(a)



# Stages of translation

- **Stage 1: Activation of Amino Acids**
- This reaction takes place in the cytosol, not on the ribosome. Each of the 20 amino acids is covalently attached to a specific tRNA at the expense of ATP energy, using  $Mg^{2+}$  - dependent activating enzymes known as aminoacyl-tRNA synthetases.

- **Stage 2 – Initiation**

- The mRNA bearing the code for the polypeptide to be synthesized binds to the smaller of two ribosomal subunits and to the initiating aminoacyl-tRNA. The large ribosomal subunit then binds to form an initiation complex. The initiating aminoacyl-tRNA basepairs with the mRNA codon AUG that signals the beginning of the polypeptide. This process, which requires GTP, is promoted by cytosolic proteins called initiation factors.

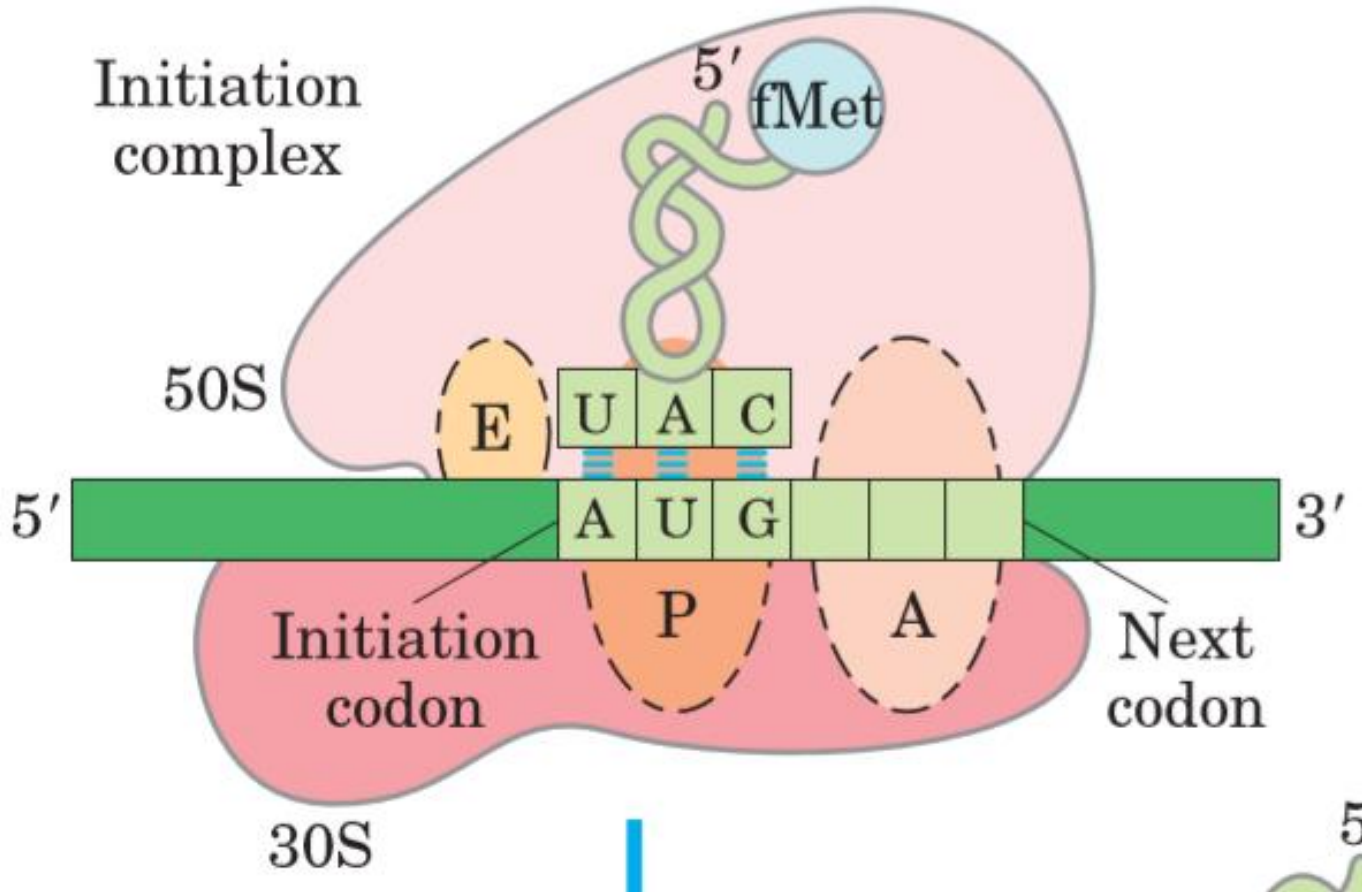
- **Stage 3: Elongation:-** The nascent polypeptide is lengthened by covalent attachment of successive amino acid units, each carried to the ribosome and correctly positioned by its tRNA, which base-pairs to its corresponding codon in the mRNA. Elongation requires cytosolic proteins known as elongation factors. The binding of each incoming aminoacyl-tRNA and the movement of the ribosome along the mRNA are facilitated by the hydrolysis of GTP as each residue is added to the growing polypeptide.
- The enzymatic activity that catalyzes peptide bond formation has been referred to as peptidyl transferase and the reaction is catalyzed by the 23S rRNA

- **Stage 4: Termination and Ribosome Recycling**

Completion of the polypeptide chain is signaled by a termination codon in the mRNA. The new polypeptide is released from the ribosome, aided by proteins called release factors, and the ribosome is recycled for another round of synthesis.

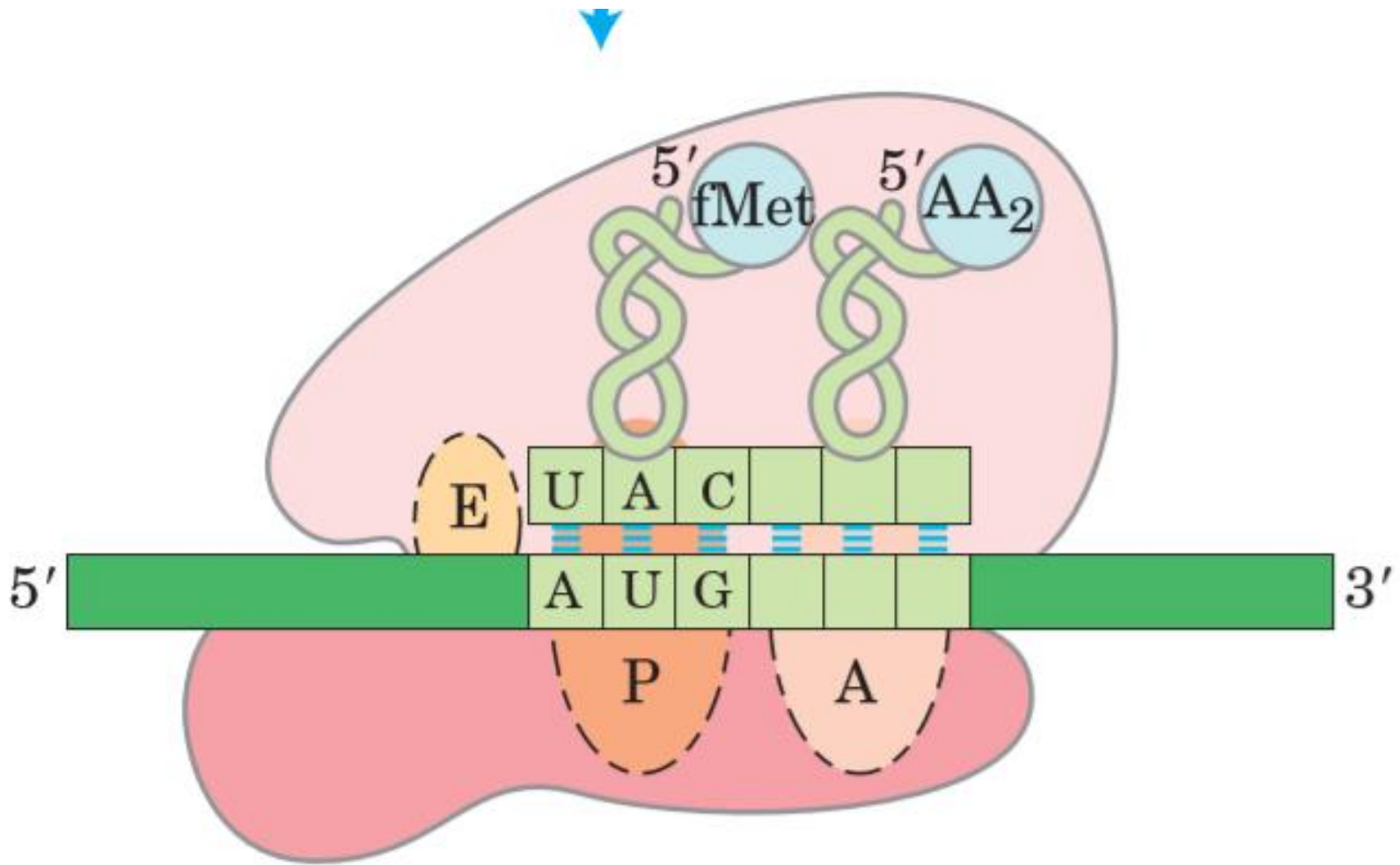
- **Stage 5: Folding and Posttranslational Processing**  
In order to achieve its biologically active form, the new polypeptide must fold into its proper three-dimensional conformation. Before or after folding, the new polypeptide may undergo enzymatic processing, including removal of one or more amino acids (usually from the amino terminus); addition of acetyl, phosphoryl, methyl, carboxyl, or other groups to certain amino acid residues; proteolytic cleavage; and/or attachment of oligosaccharides or prosthetic groups.

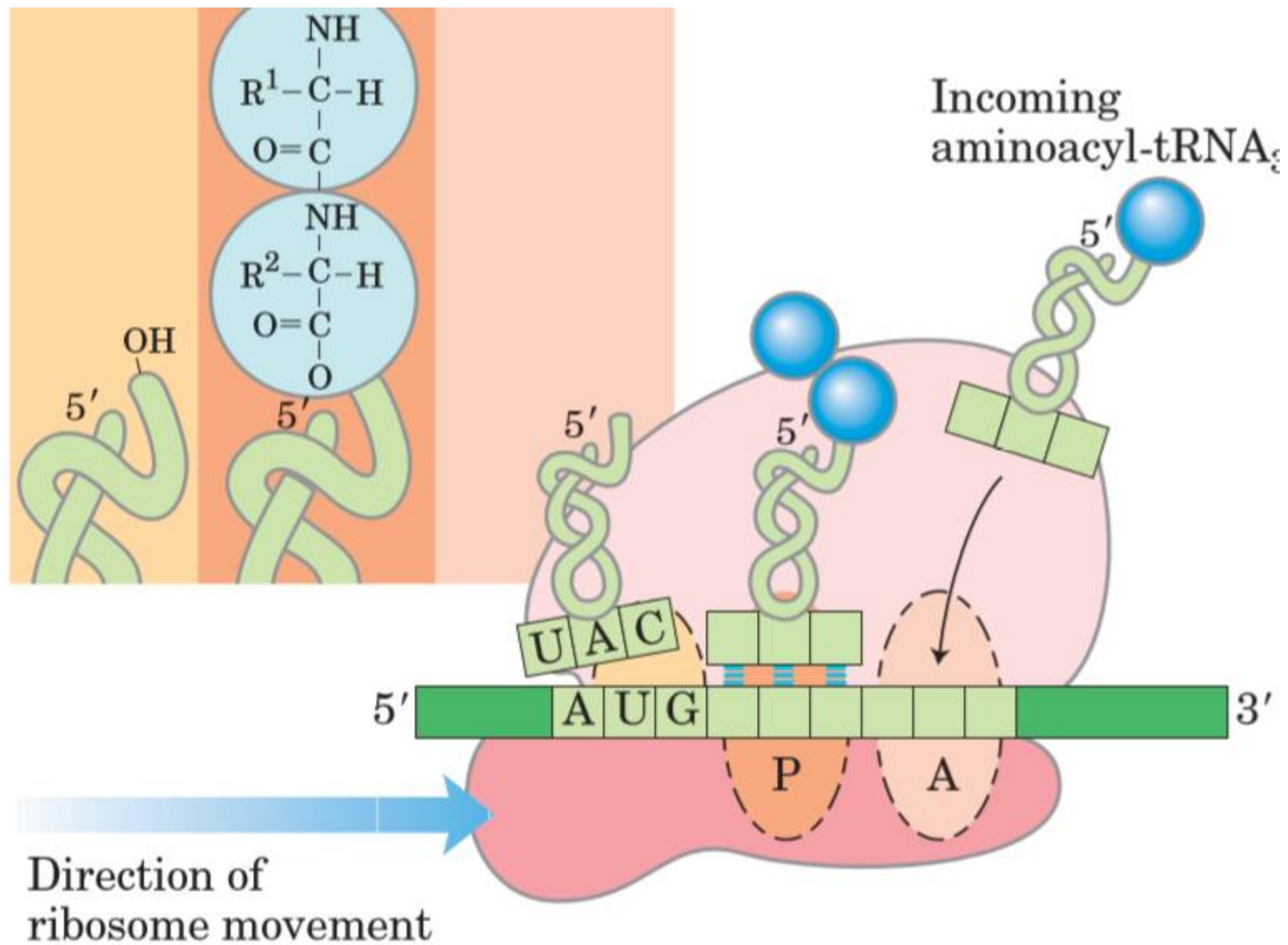
# Initiation



P designates the peptidyl site, A the aminoacyl site, and E the exit site.

# Elongation





(a)



# Termination

