Lecture Series 9

From DNA to Protein: Genotype to Phenotype

From DNA to Protein: Genotype to Phenotype

A. Genes and the Synthesis of Polypeptides

B. DNA, RNA, and the Flow of Information aka The Central Dogma

C. Transcription: DNA-Directed RNA Synthesis

D. RNA Processing

E. The Genetic Code

From DNA to Protein: Genotype to Phenotype

F. The Key Players in Translation

G. Translation: RNA-Directed Polypeptide Synthesis

H. Regulation of Translation

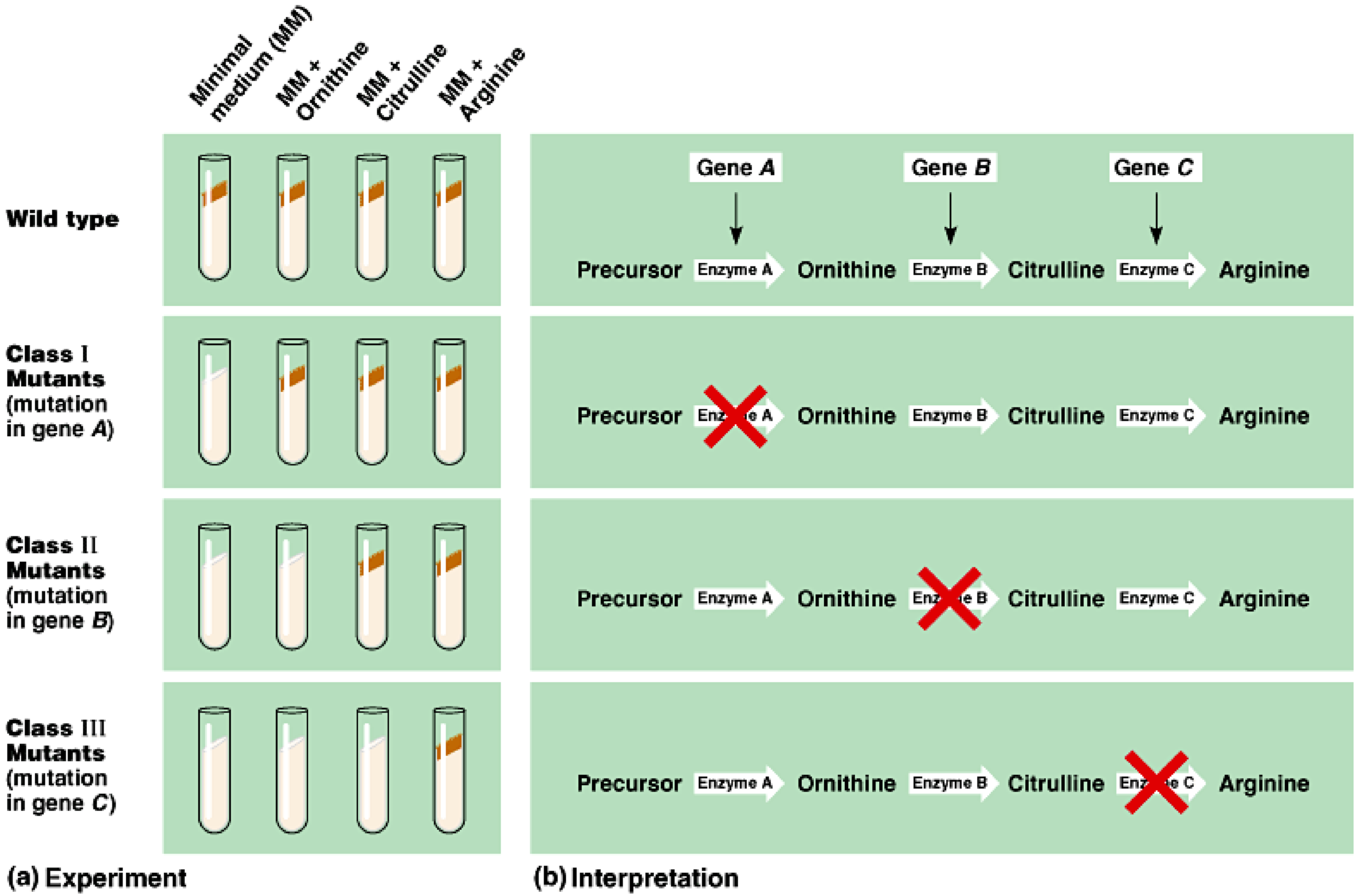
I. Posttranslational Events

J. Mutations: Heritable Changes in Genes

A. Genes and the Synthesis of Polypeptides

- Genes are made up of DNA and are expressed in the phenotype as polypeptides.
- Beadle and Tatum's experiments with the bread mold *Neurospora* resulted in mutant strains lacking a specific enzyme in a biochemical pathway. These results led to the one-gene, one-polypeptide hypothesis.

Beadle and Tatum's evidence for the one gene-one enzyme hypothesis

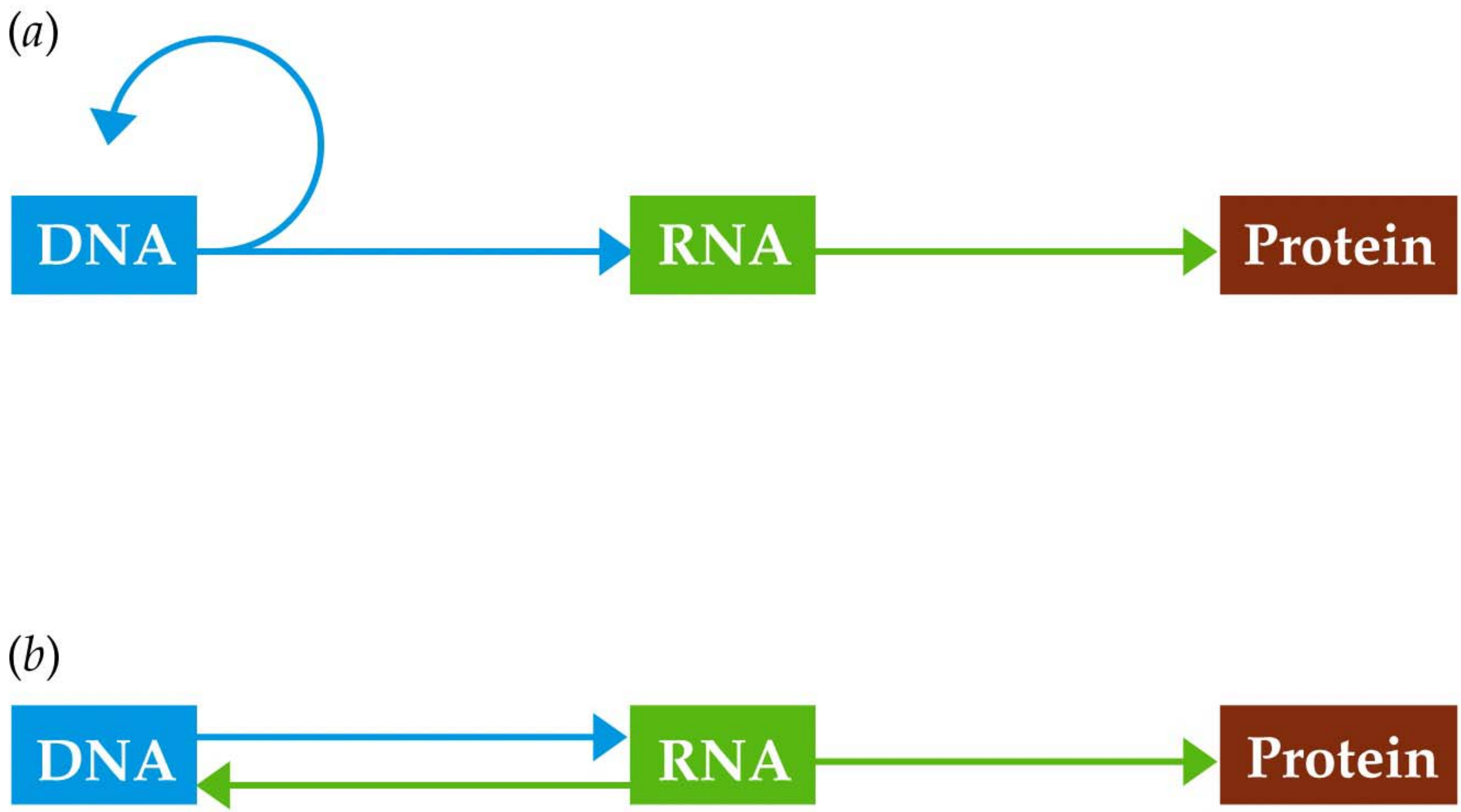


A. Genes and the Synthesis of Polypeptides

- Certain hereditary diseases in humans have been found to be caused by a defective enzyme.
- These observations supported the one-gene, one-polypeptide hypothesis.

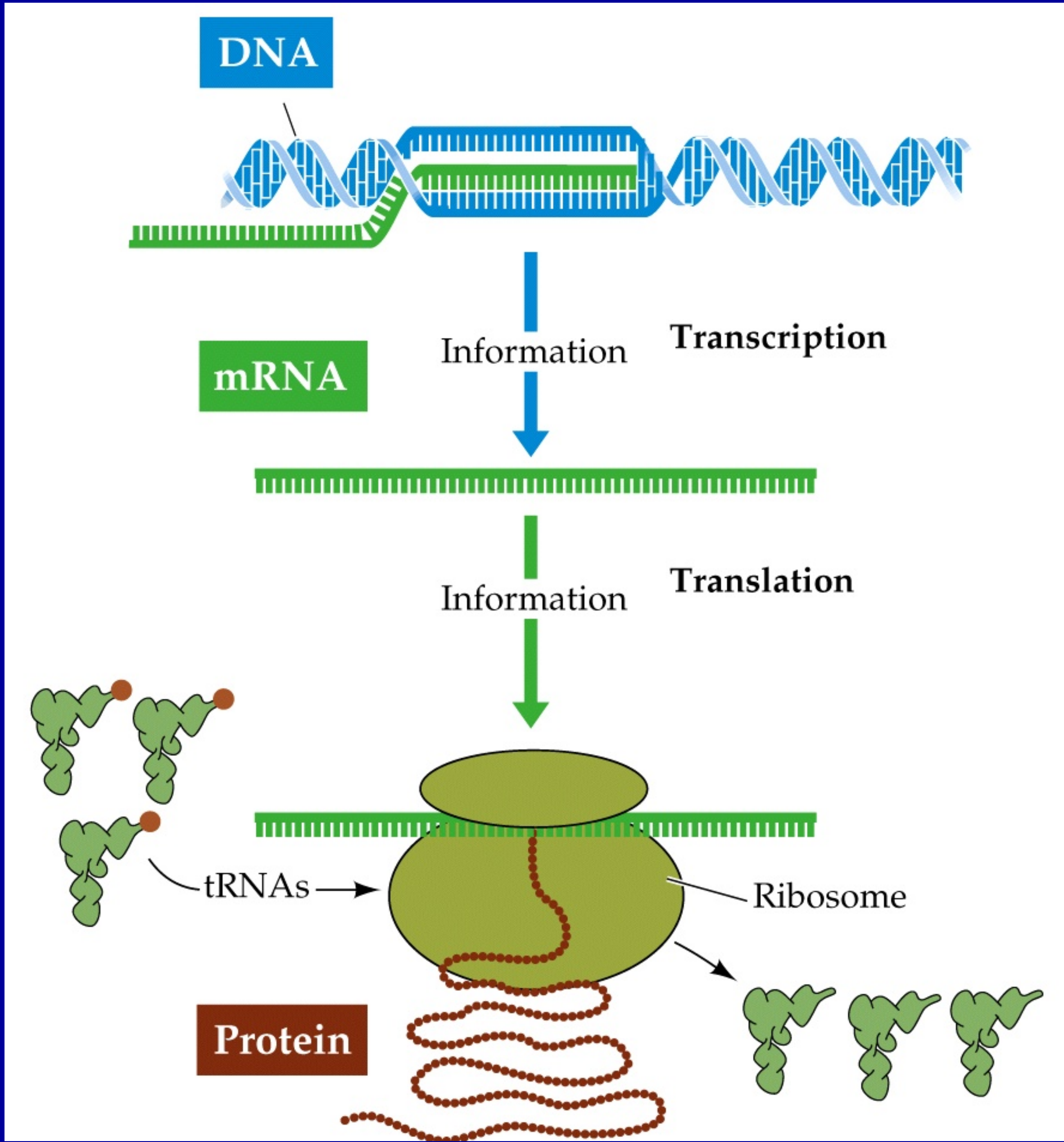
B. DNA, RNA, and the Flow of Information

- RNA differs from DNA in three ways: It is single-stranded, its sugar molecule is ribose rather than deoxyribose, and its fourth base is uracil rather than thymine.
- The **central dogma** of molecular biology is DNA $\rightarrow$ RNA $\rightarrow$ protein. Unidirectional when genes are expressed.

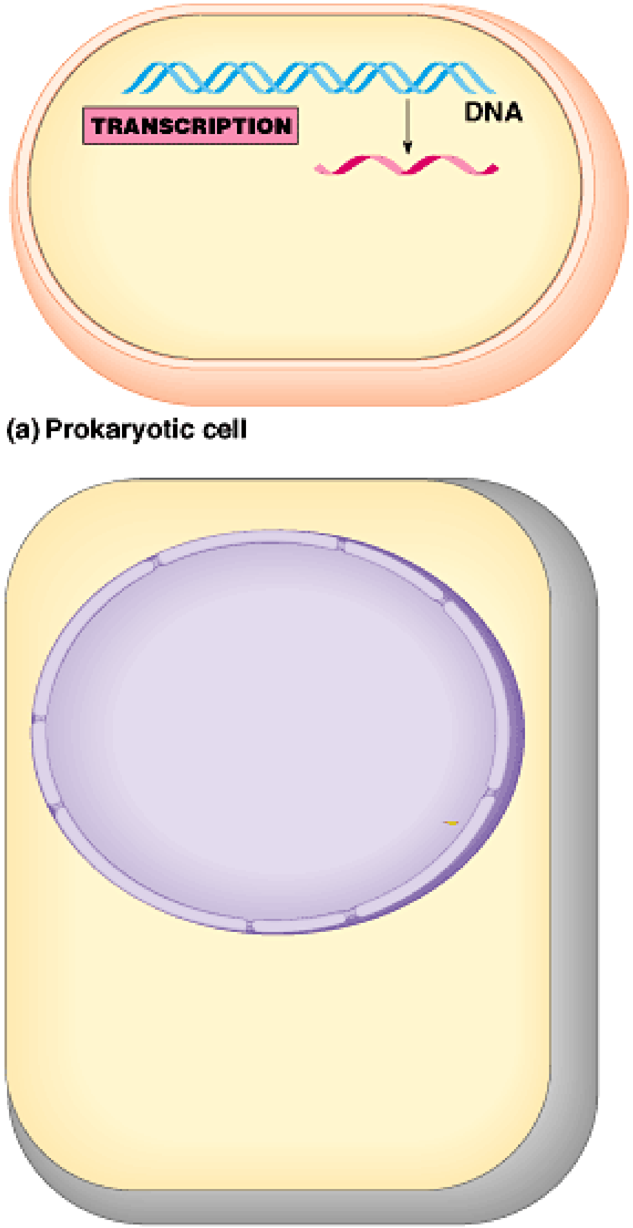

(a)
DNA
RNA
Protein
(b)
DNA
RNA
Protein

B. DNA, RNA, and the Flow of Information

- A gene is expressed in two steps: First, DNA is transcribed to RNA; then RNA is translated into protein.
- In retroviruses, the rule for transcription is reversed: RNA $\rightarrow$ DNA. Other RNA viruses exclude DNA altogether, going directly from RNA to protein.


DNA
mRNA
Information
Transcription
Information
Translation
tRNAs
Ribosome
Protein

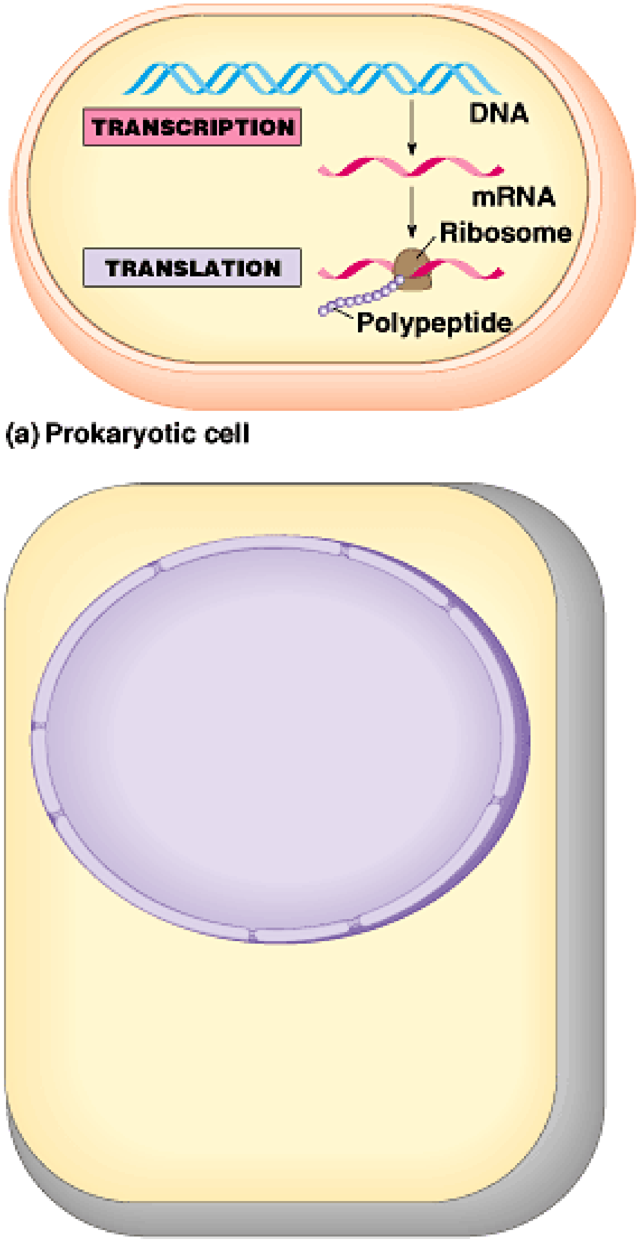
Overview: the roles of transcription and translation in the flow of genetic information (Layer 1)



(a) Prokaryotic cell

(b) Eukaryotic cell

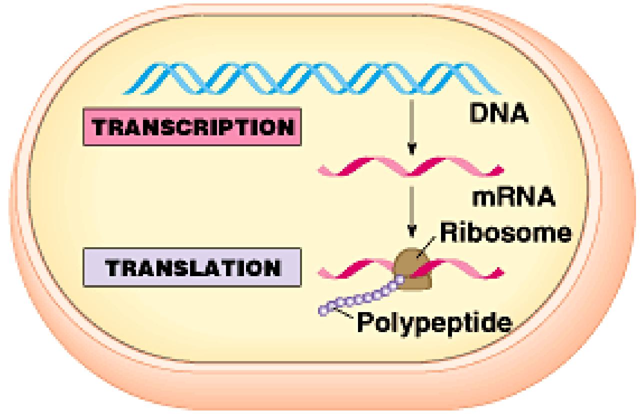
Overview: the roles of transcription and translation in the flow of genetic information (Layer 2)



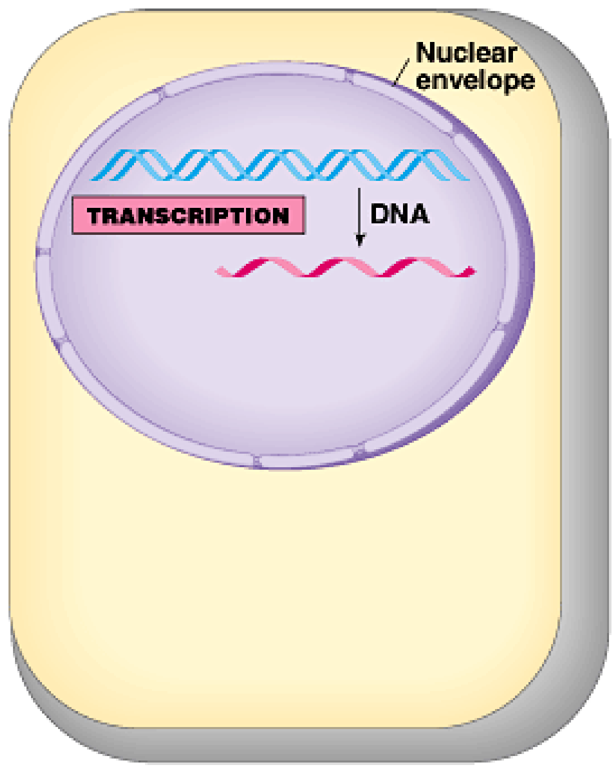
(a) Prokaryotic cell

(b) Eukaryotic cell

Overview: the roles of transcription and translation in the flow of genetic information (Layer 3)

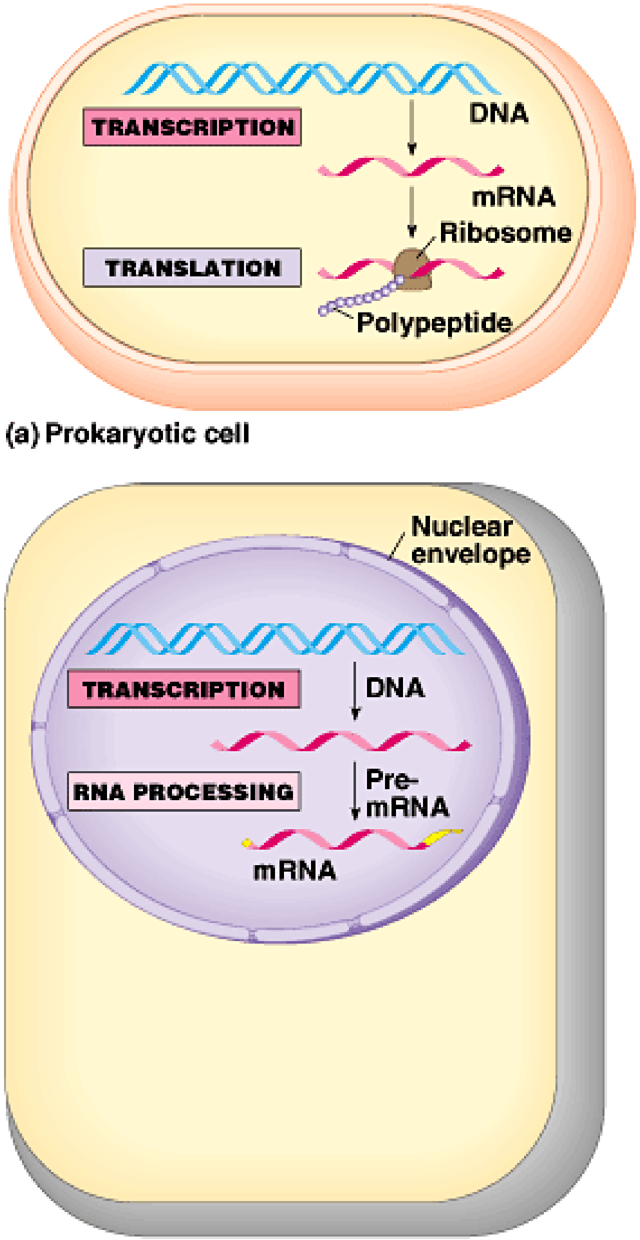


(a) Prokaryotic cell



(b) Eukaryotic cell

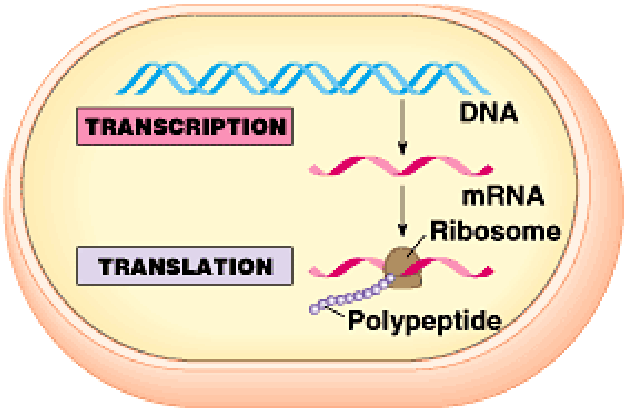
Overview: the roles of transcription and translation in the flow of genetic information (Layer 4)



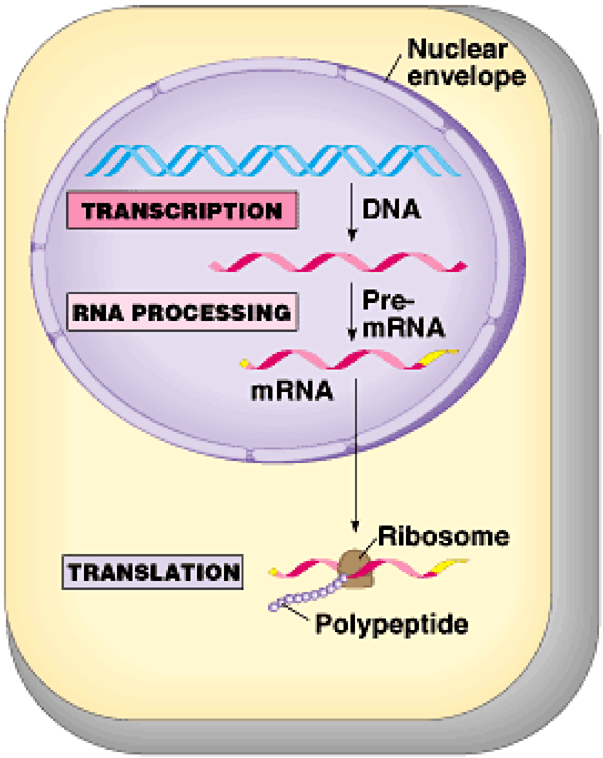
(a) Prokaryotic cell

(b) Eukaryotic cell

Overview: the roles of transcription and translation in the flow of genetic information (Layer 5)



(a) Prokaryotic cell



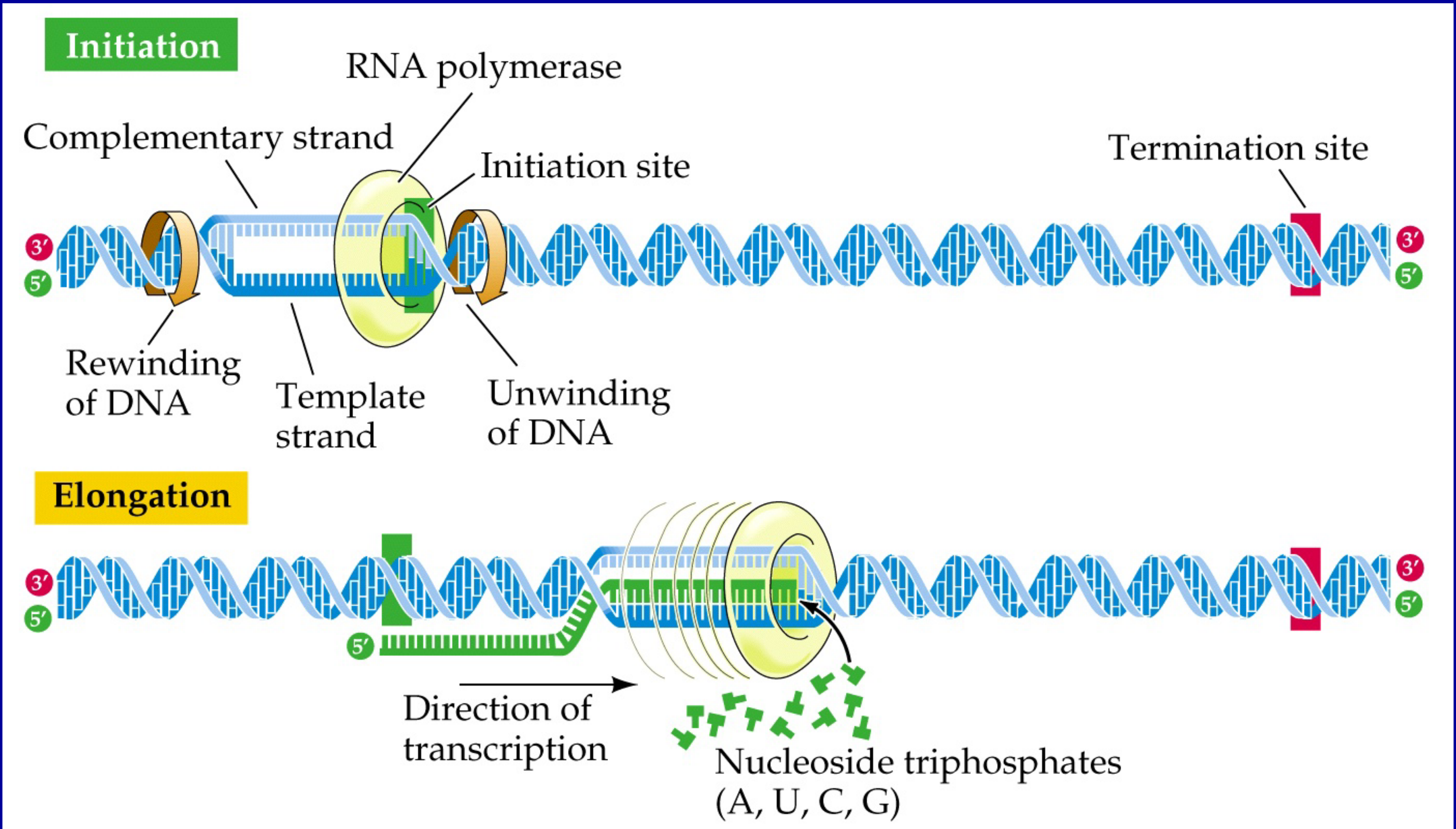
(b) Eukaryotic cell

C. Transcription: DNA-Directed RNA Synthesis

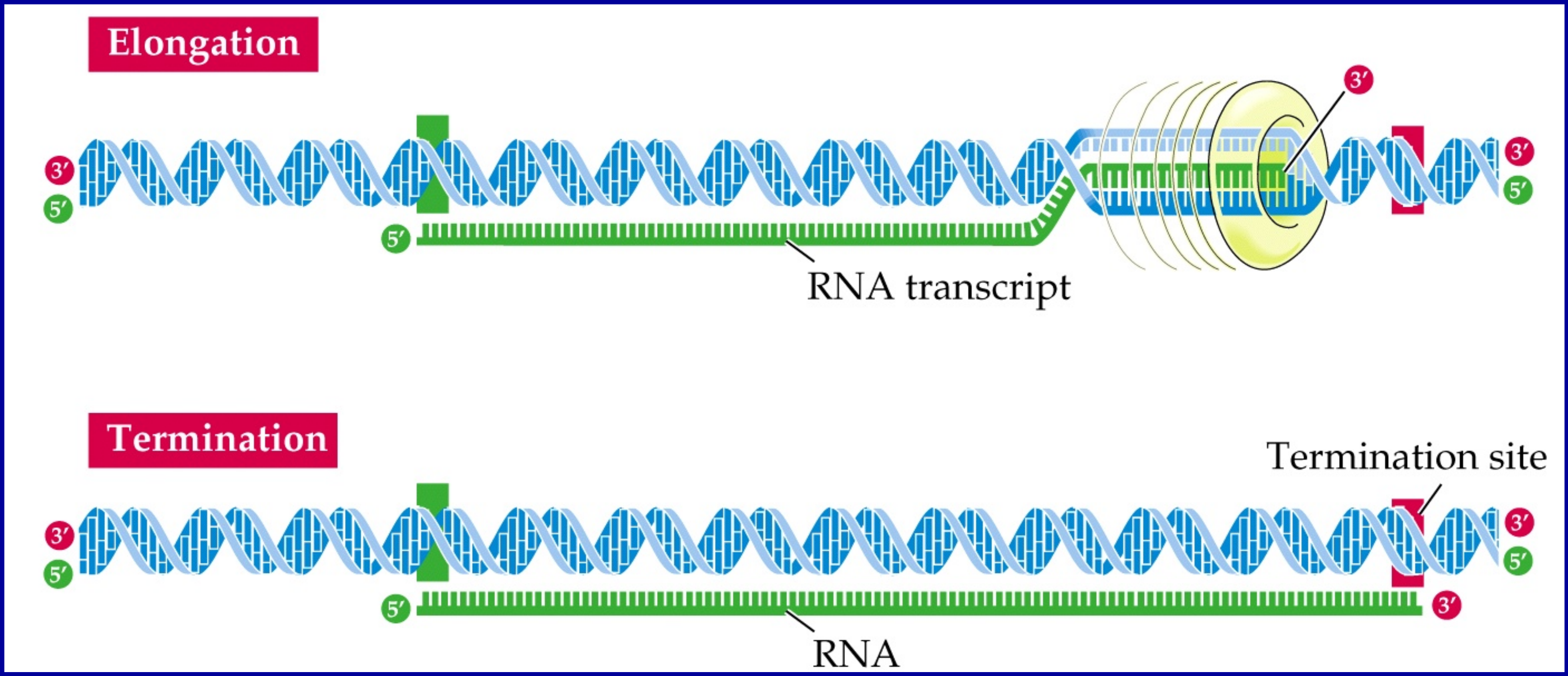
- RNA is transcribed from a DNA template after the bases of DNA are exposed by unwinding of the double helix.
- In a given region of DNA, only one of the two strands can act as a template for transcription.
- RNA polymerase catalyzes transcription from the template strand of DNA.

C. Transcription: DNA-Directed RNA Synthesis

- Three step process: Initiation, Elongation and Termination.
- The initiation of transcription requires that RNA polymerase recognize and bind tightly to a promoter sequence on DNA.
- RNA elongates in a 5'-to-3' direction, antiparallel to the template DNA.
- Special sequences and protein helpers terminate transcription.

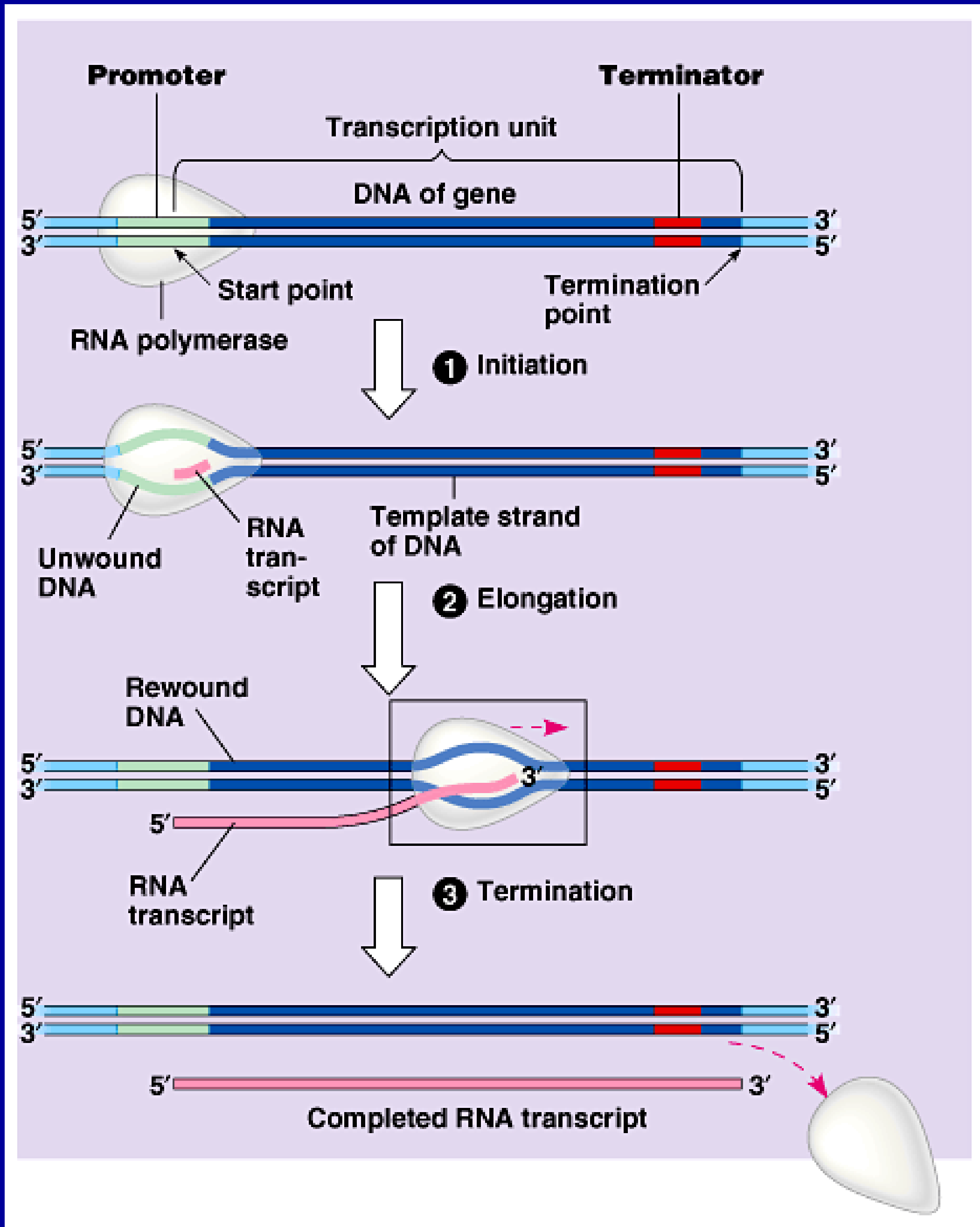


Initiation
RNA polymerase
Complementary strand
Initiation site
Termination site
3′
5′
3′
5′
Rewinding of DNA
Template strand
Unwinding of DNA
Elongation
3′
5′
3′
5′
5′
Direction of transcription
Nucleoside triphosphates (A, U, C, G)

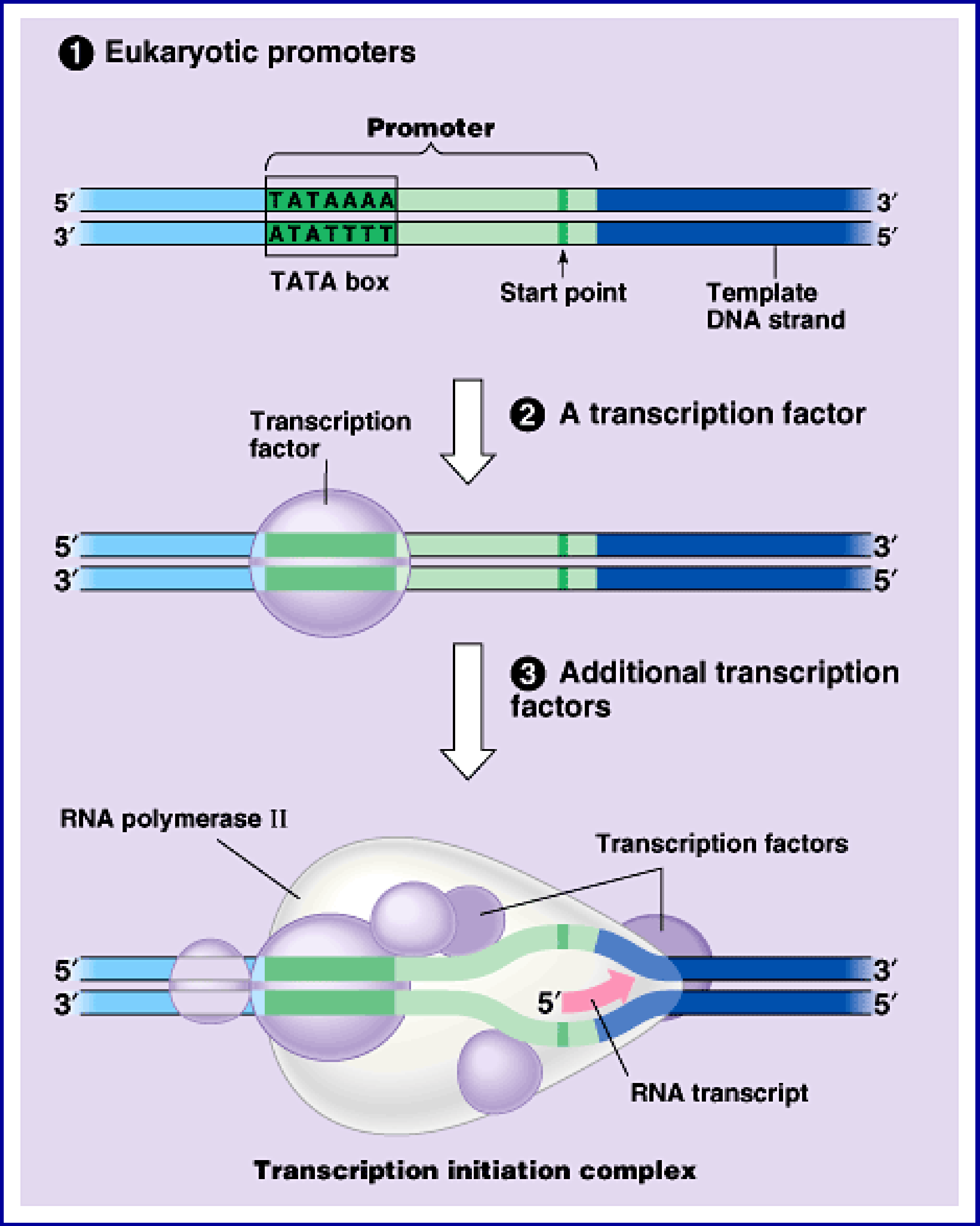


Elongation
3′
3′
5′
3′
5′
5′
RNA transcript
Termination
Termination site
3′
5′
3′
5′
5′
3′
RNA

The stages of transcription



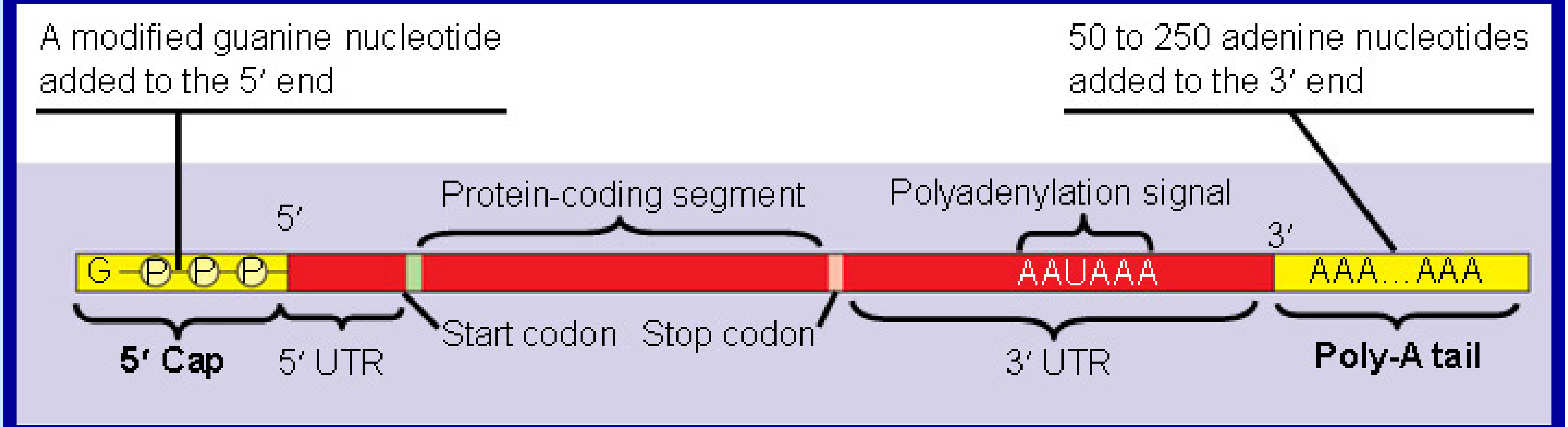
The initiation of transcription at a eukaryotic promoter



D. RNA Processing

- After transcription, the pre-mRNA is altered by the addition of a G cap at the 5' end and a poly A tail at the 3' end.
- UTR is untranslated region even though they are transcribed on the mRNA.

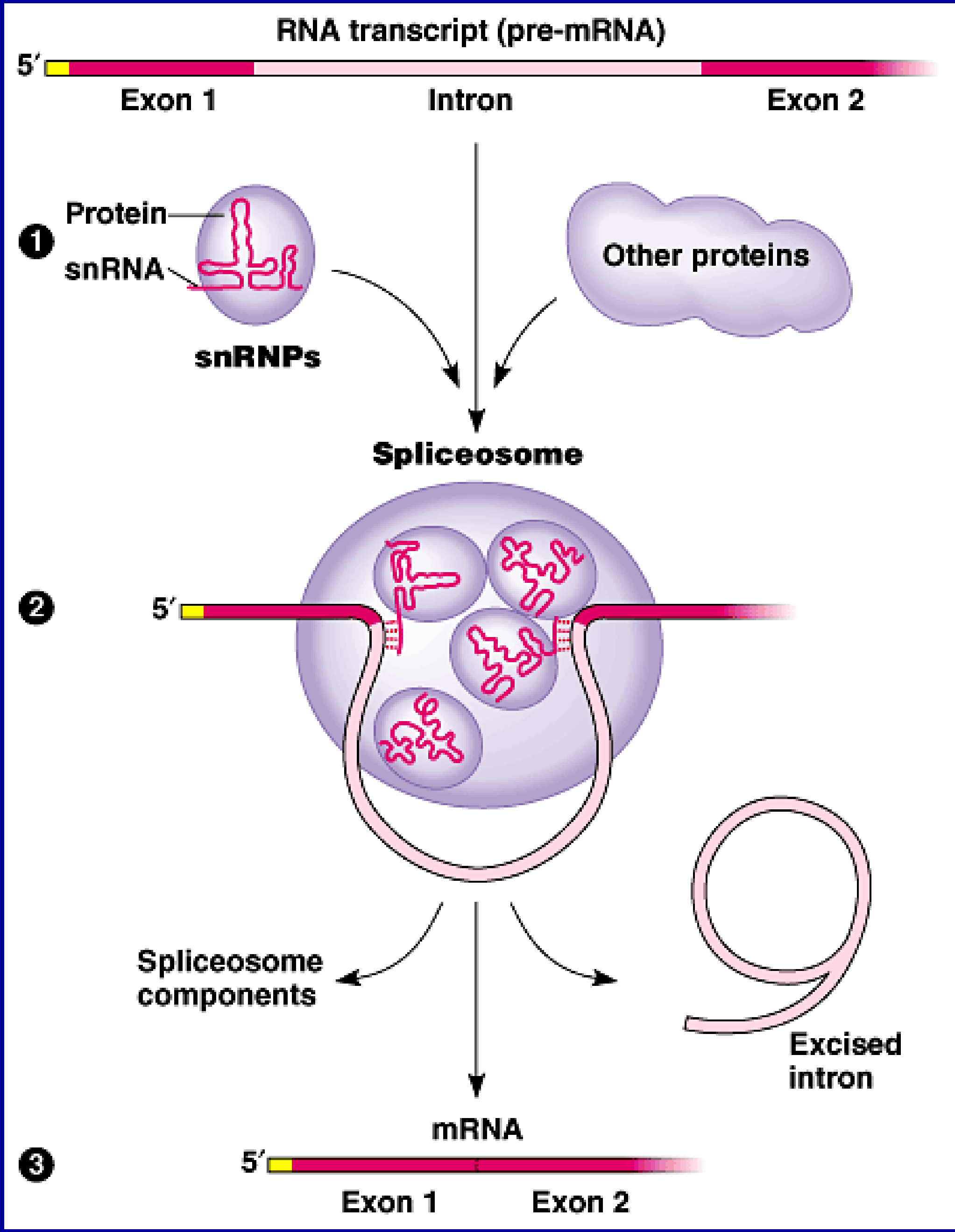
RNA processing; addition of the 5′ cap and poly(A) tail



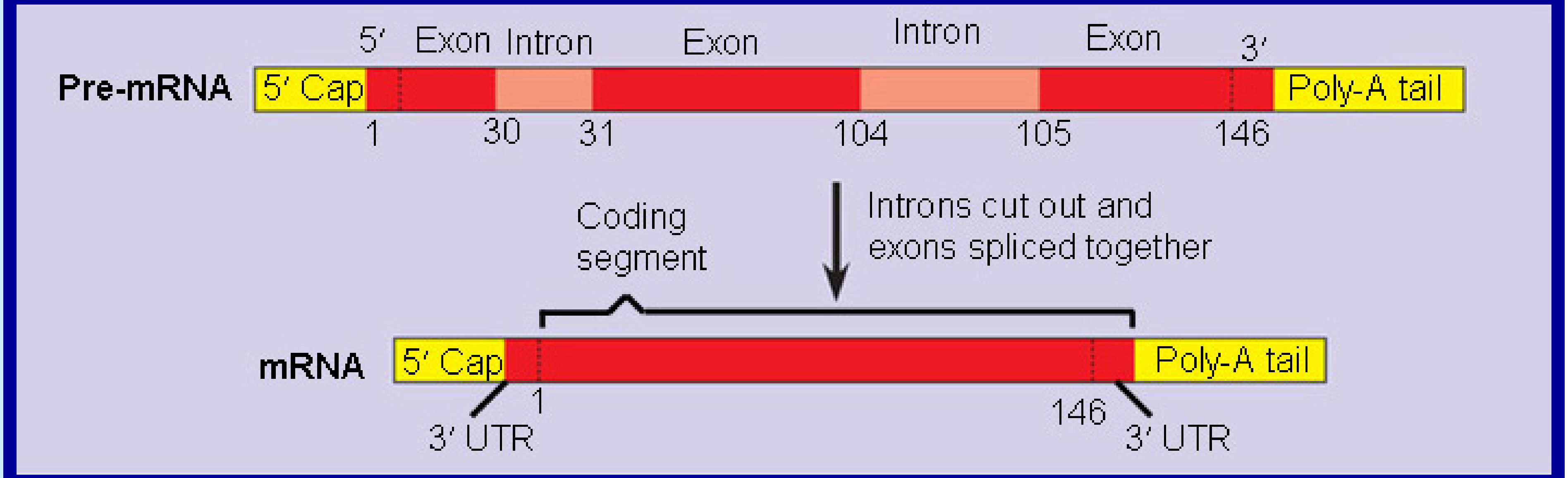
D. RNA Processing

- The introns are removed from the mRNA precursor by the spliceosome, a complex of RNA’s and proteins.
- These RNA’s are snRNAs which are ~300 bases long.
- Yet another ribozyme.

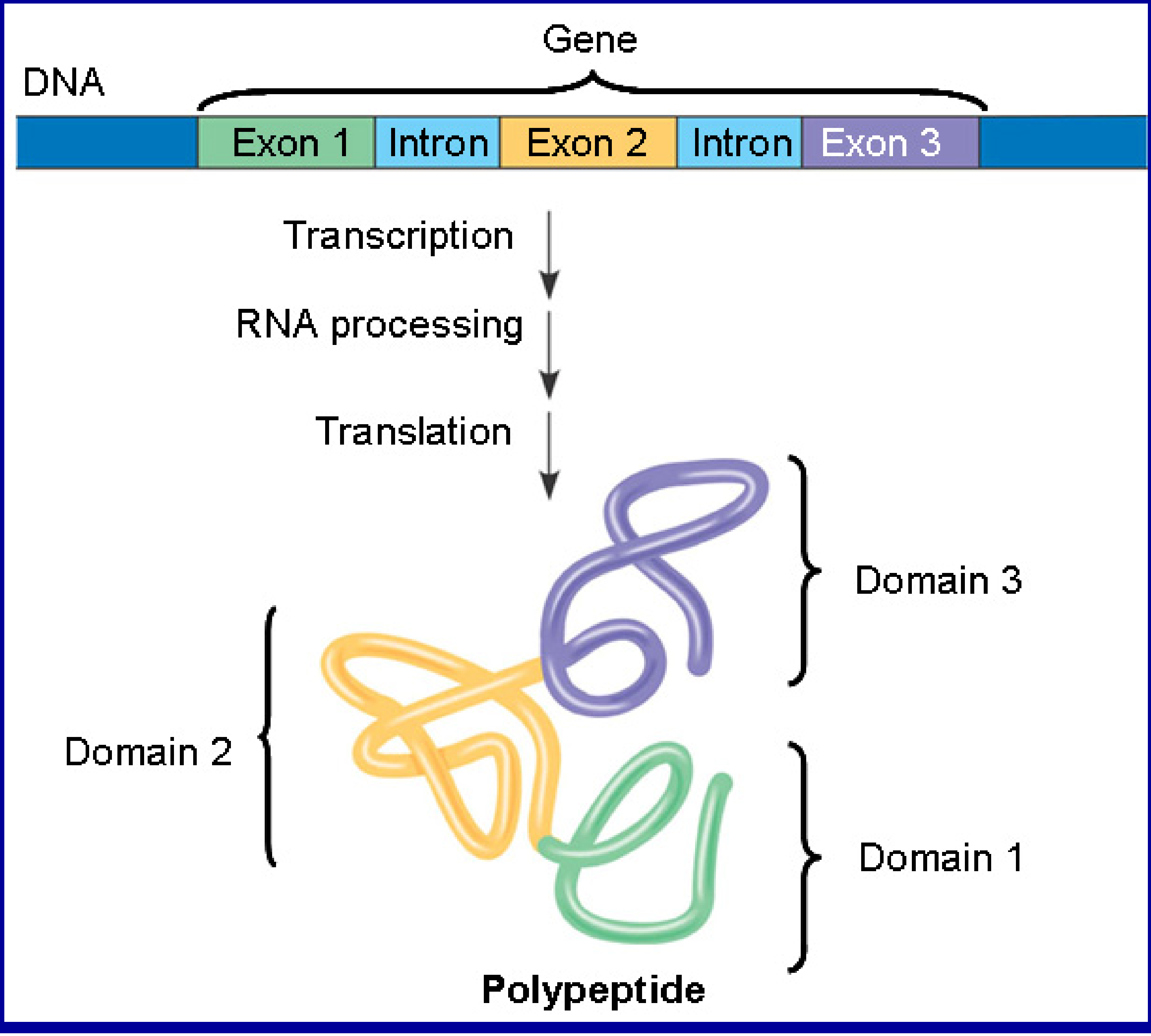
The roles of snRNPs and spliceosomes in mRNA splicing



RNA processing: RNA splicing



Correspondence between exons and protein domains



E. The Genetic Code

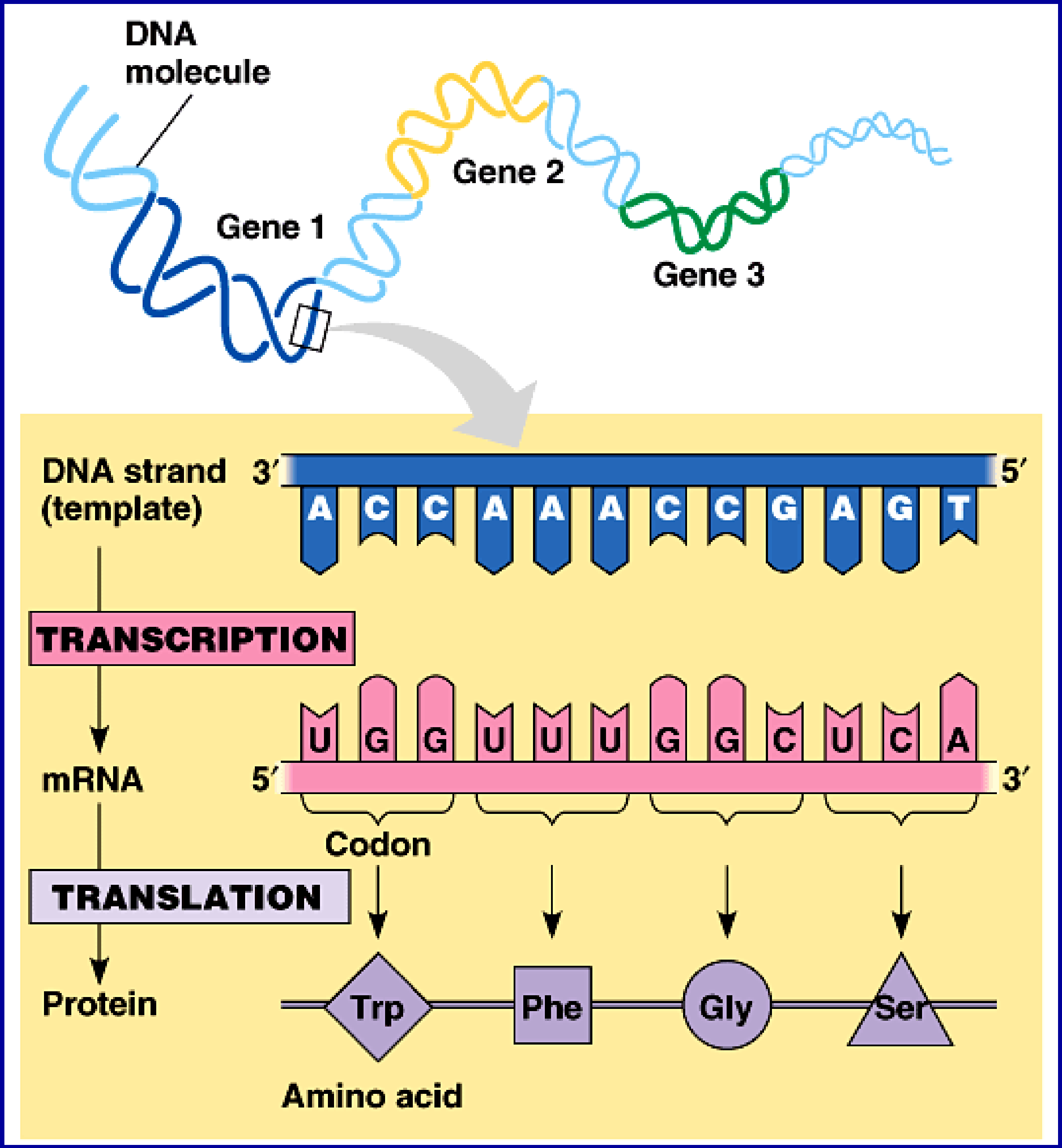
- The genetic code consists of triplets of nucleotides (codons). Since there are four bases, there are 64 possible codons.
- One mRNA codon indicates the starting point of translation and codes for methionine. Three stop codons indicate the end of translation. The other 60 codons code only for particular amino acids.

E. The Genetic Code

- Since there are only 20 different amino acids, the genetic code is redundant; that is, there is more than one codon for certain amino acids. However, a single codon does not specify more than one amino acid.
- The genetic code is degenerate but not ambiguous!

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU, UUC Phenyl-alanine UUA, UUG Leucine	UCU, UCC, UCA, UCG Serine	UAU, UAC Tyrosine UAA Stop codon UAG Stop codon	UGU, UGC Cysteine UGA Stop codon UGG Tryptophan	U C A G
C	CUU, CUC, CUA, CUG Leucine	CCU, CCC, CCA, CCG Proline	CAU, CAC Histidine CAA, CAG Glutamine	CGU, CGC, CGA, CGG Arginine	U C A G
A	AUU, AUC, AUA Isoleucine AUG Methionine; start codon	ACU, ACC, ACA, ACG Threonine	AAU, AAC Asparagine AAA, AAG Lysine	AGU, AGC Serine AGA, AGG Arginine	U C A G
G	GUU, GUC, GUA, GUG Valine	GCU, GCC, GCA, GCG Alanine	GAU, GAC Aspartic acid GAA, GAG Glutamic acid	GGU, GGC, GGA, GGG Glycine	U C A G

The triplet code



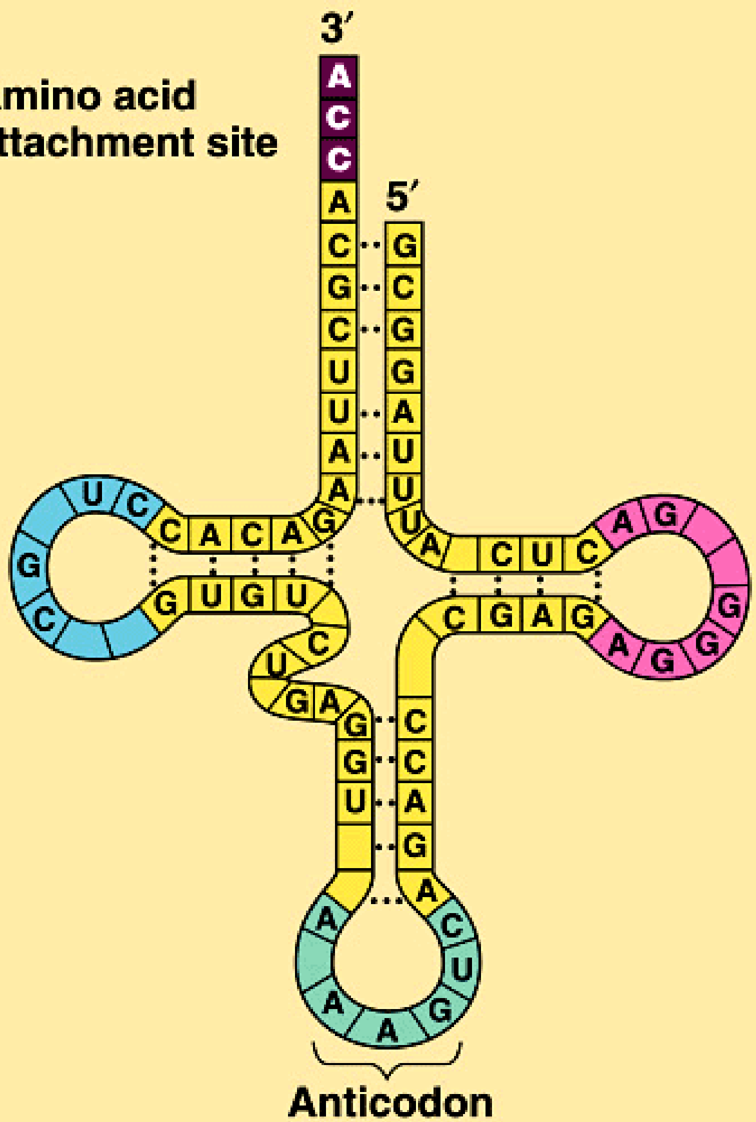
F. The Key Players in Translation

- In prokaryotes, translation begins before the mRNA is completed.
- In eukaryotes, transcription occurs in the nucleus and translation occurs in the cytoplasm.
- Translation requires three components: tRNA’s, activating enzymes, and ribosomes.

F. The Key Players in Translation

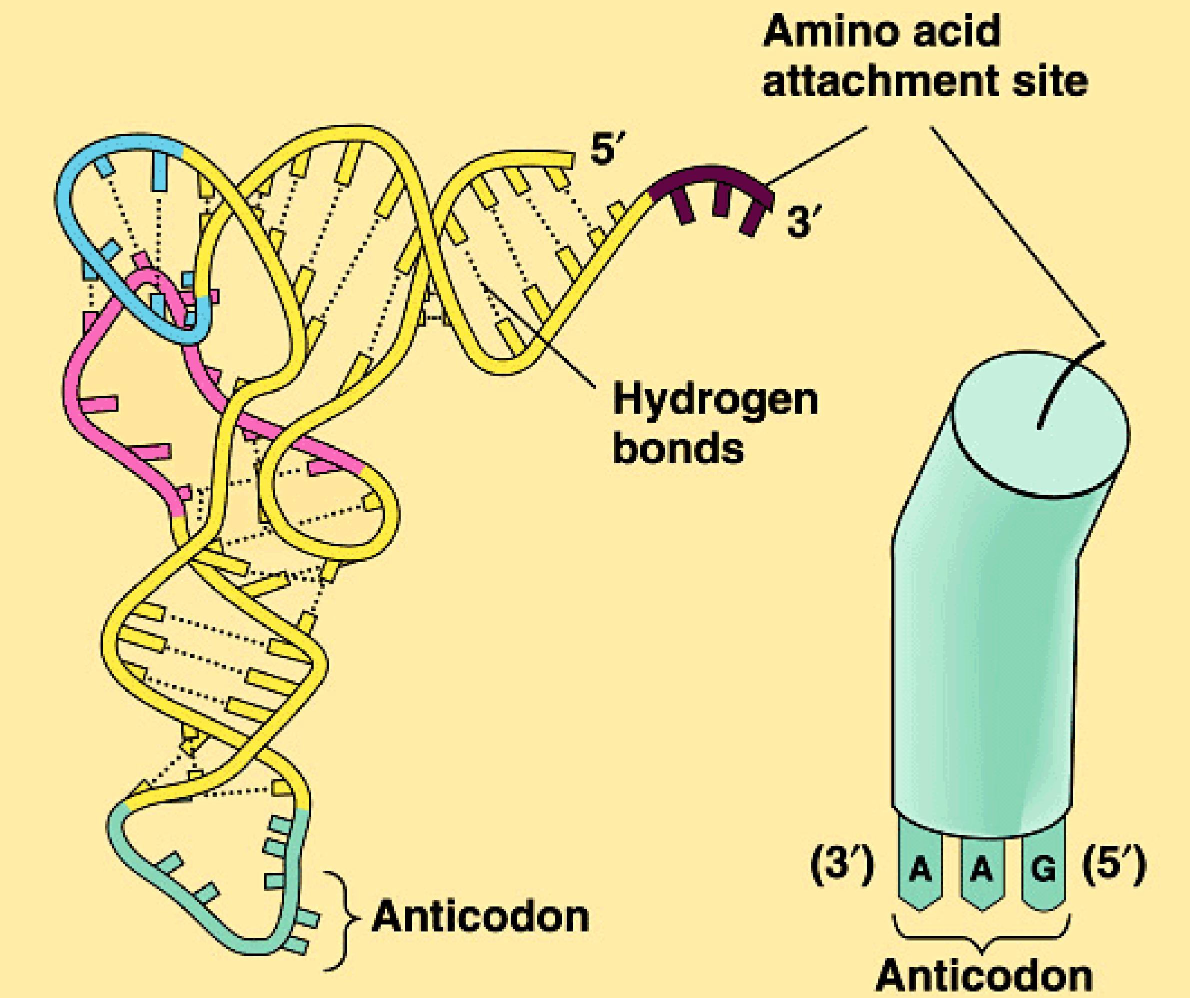
- In translation, amino acids are linked in codon-specified order in mRNA.
- This is achieved by an adapter, transfer RNA (tRNA), which binds the correct amino acid and has an anticodon complementary to the mRNA codon.

The structure of transfer RNA (tRNA)



(a) Two-dimensional structure

The structure of transfer RNA (tRNA)

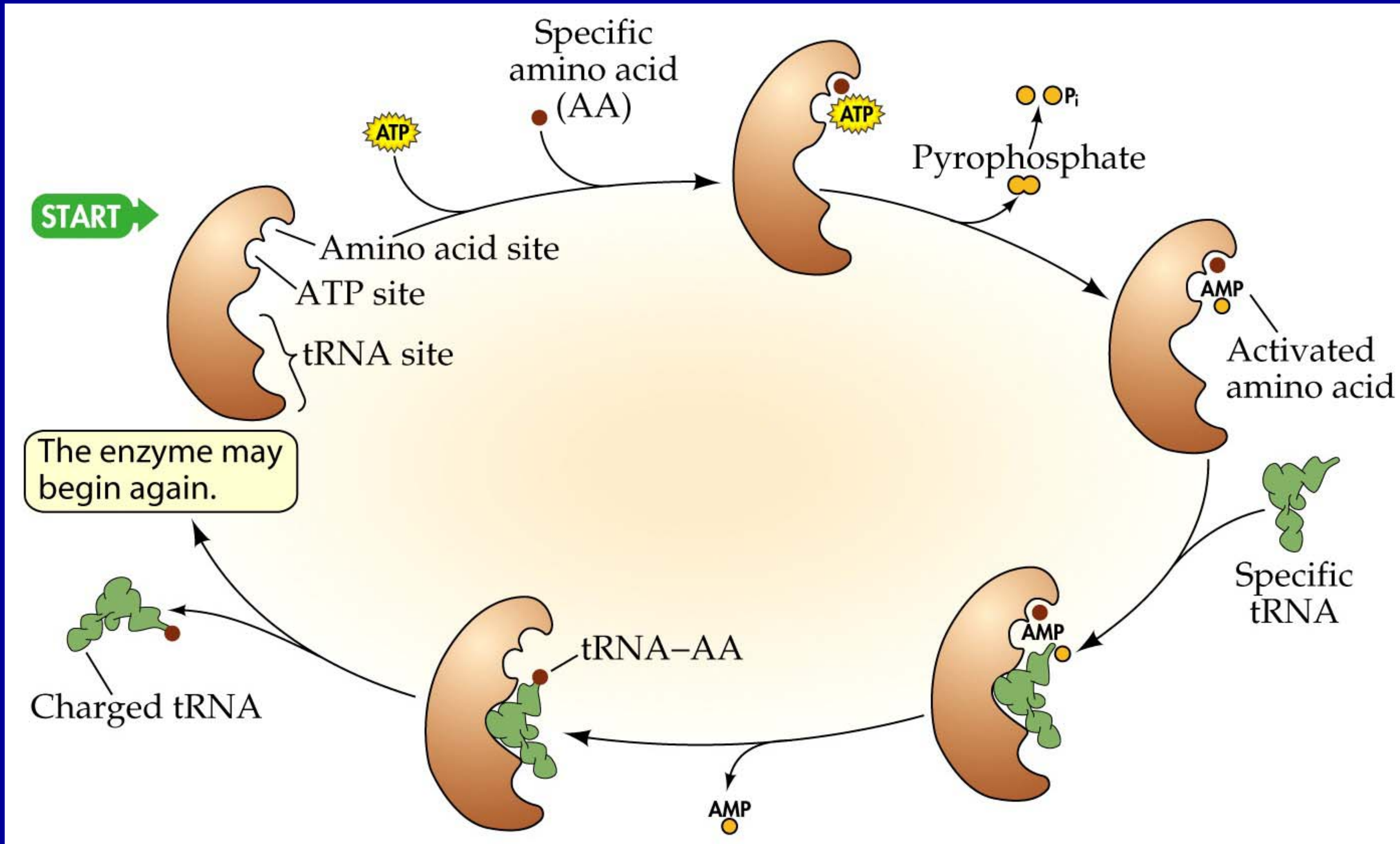


(b) Three-dimensional structure

(c) Symbol

F. The Key Players in Translation

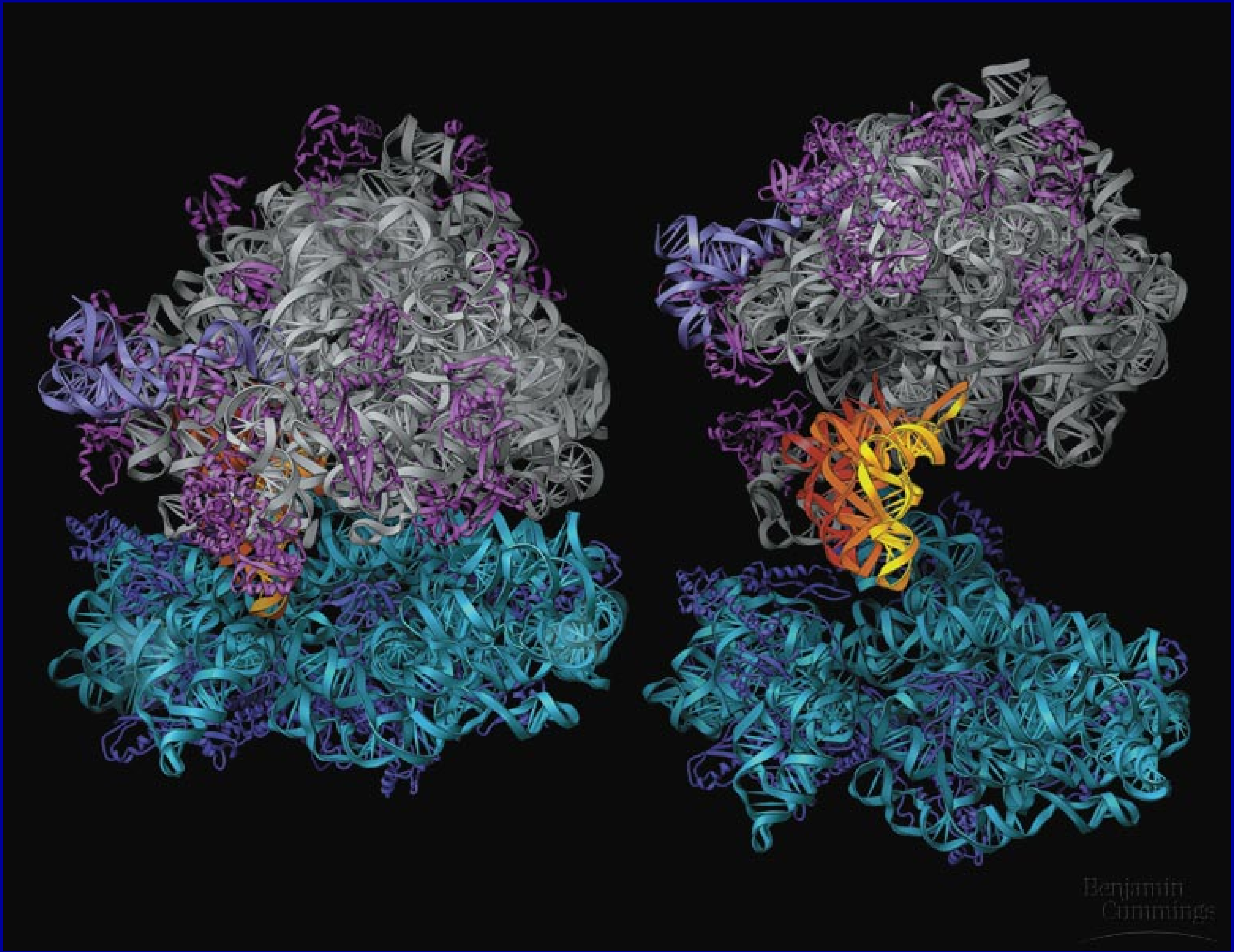
- The aminoacyl-tRNA synthetases, a family of activating enzymes, attach specific amino acids to their appropriate tRNA’s, forming charged tRNA’s.
- These are the ultimate translators in the cell.


START
Specific
amino acid
(AA)
ATP
Amino acid site
ATP site
tRNA site
ATP
Pyrophosphate
P i
AMP
Activated
amino acid
Specific
tRNA
AMP
tRNA–AA
AMP
Charged tRNA
The enzyme may
begin again.

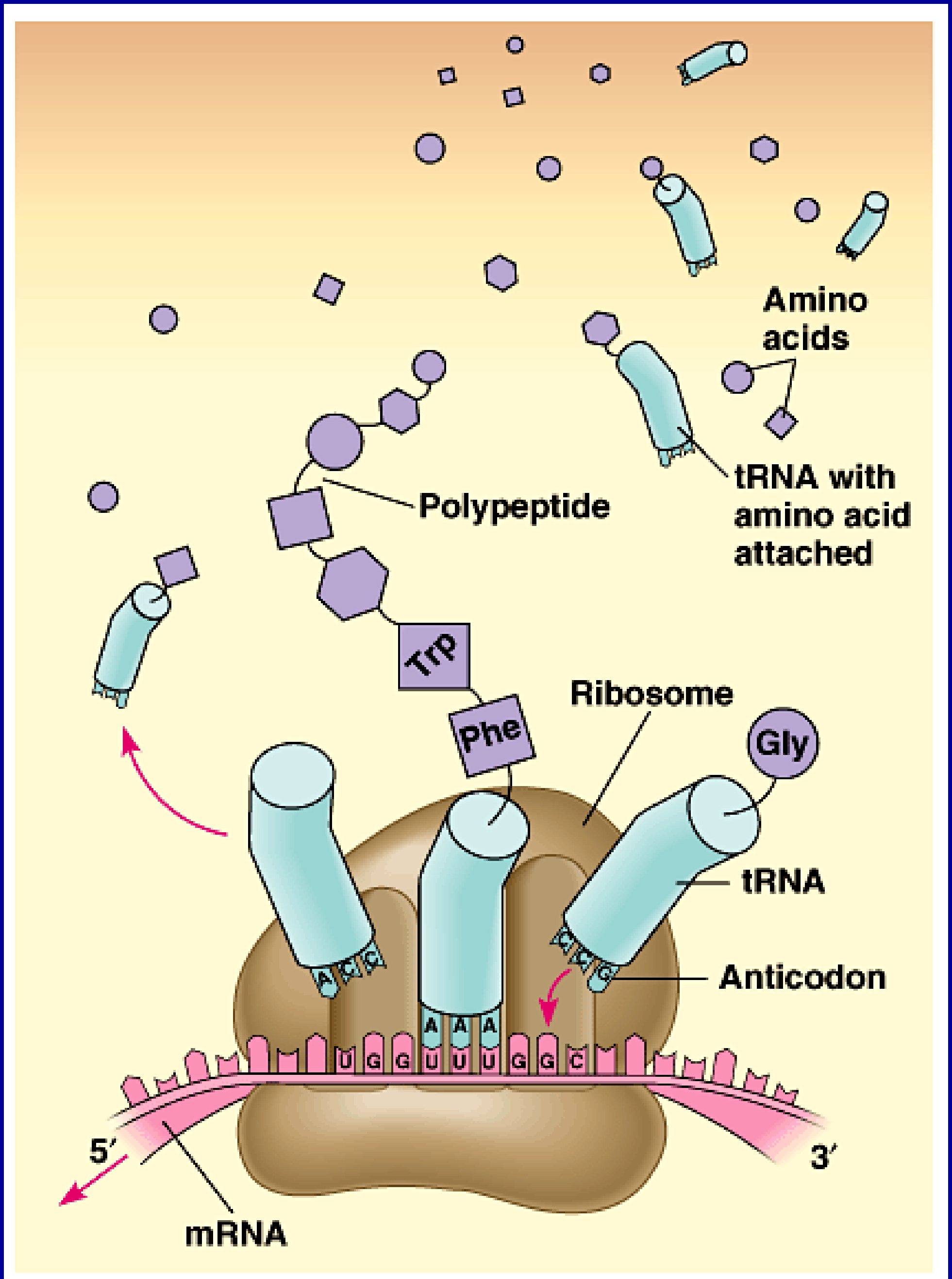
F. The Key Players in Translation

- The mRNA meets the charged tRNA’s at a ribosome.
- The ribosome is the staging area for protein synthesis or translation.
- Ribosomes are roughly 60% RNA and 40% proteins.

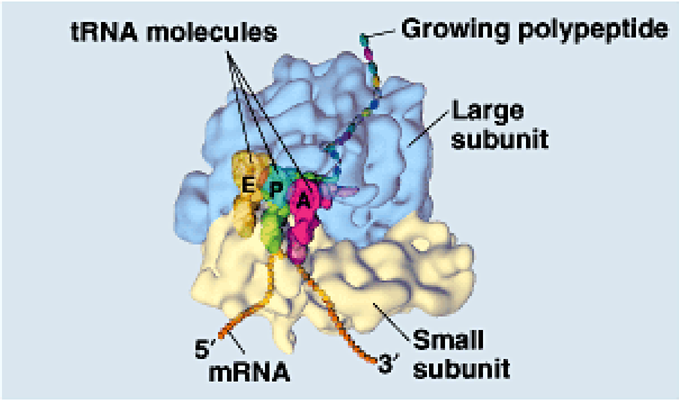
The Ribosome (another ribozyme)



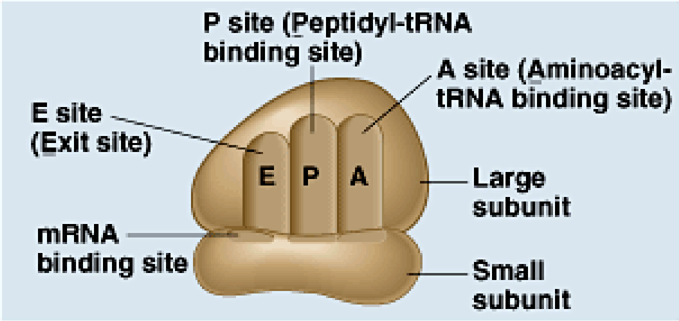
Translation: the basic concept



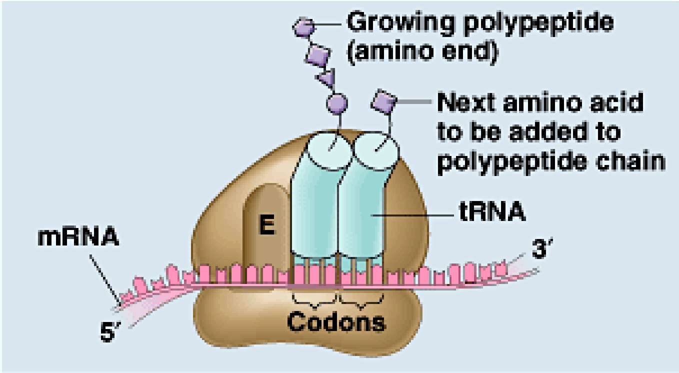
The anatomy of a functioning ribosome



(a) Computer model of functioning ribosome



(b) Schematic model showing binding sites

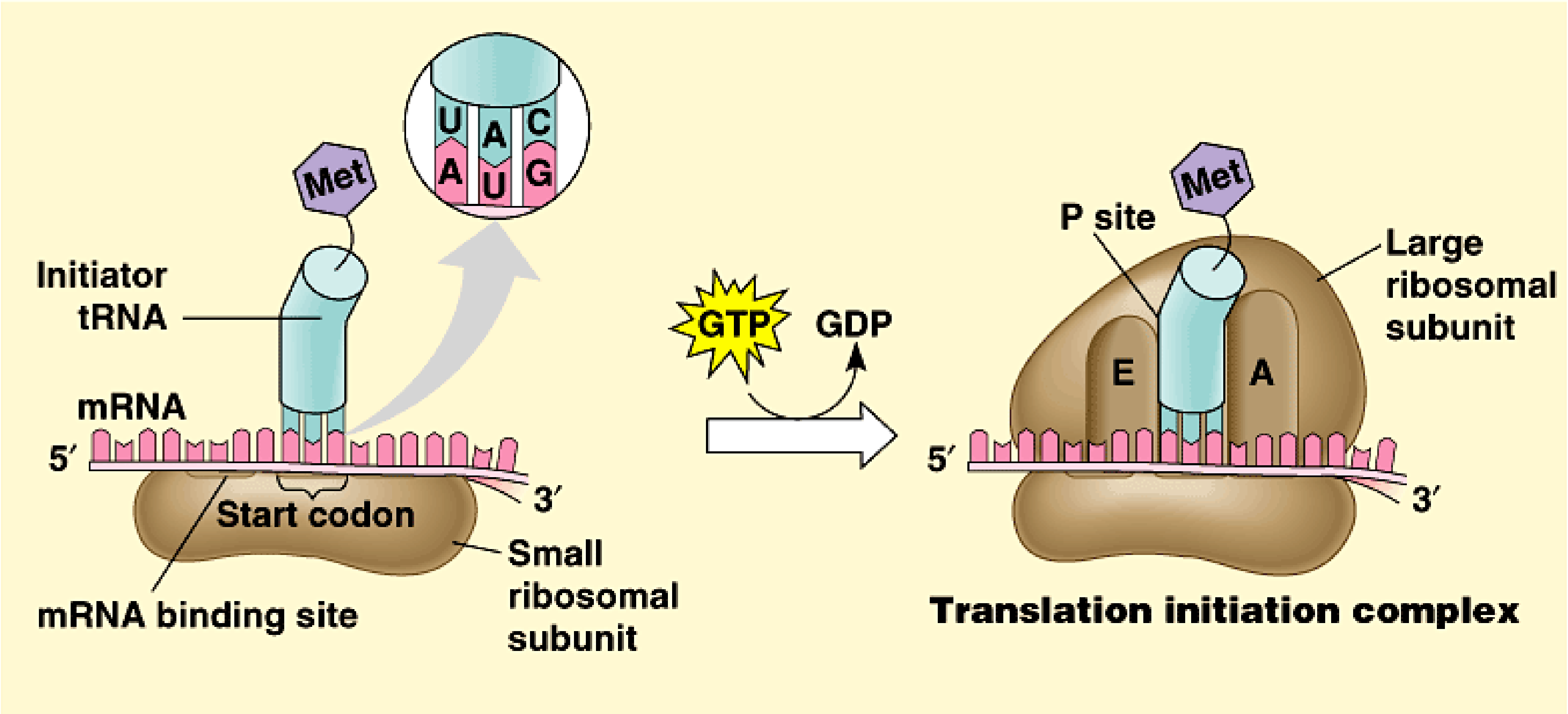


(c) Schematic model with mRNA and tRNA

G. Translation: RNA-Directed Polypeptide Synthesis

- Three step process: Initiation, Elongation and Termination.
- An initiation complex consisting of an amino acid-charged tRNA and a small ribosomal subunit bound to mRNA triggers the beginning of translation.
- Initiation complex includes the use of various initiations factors and of 1 GTP.

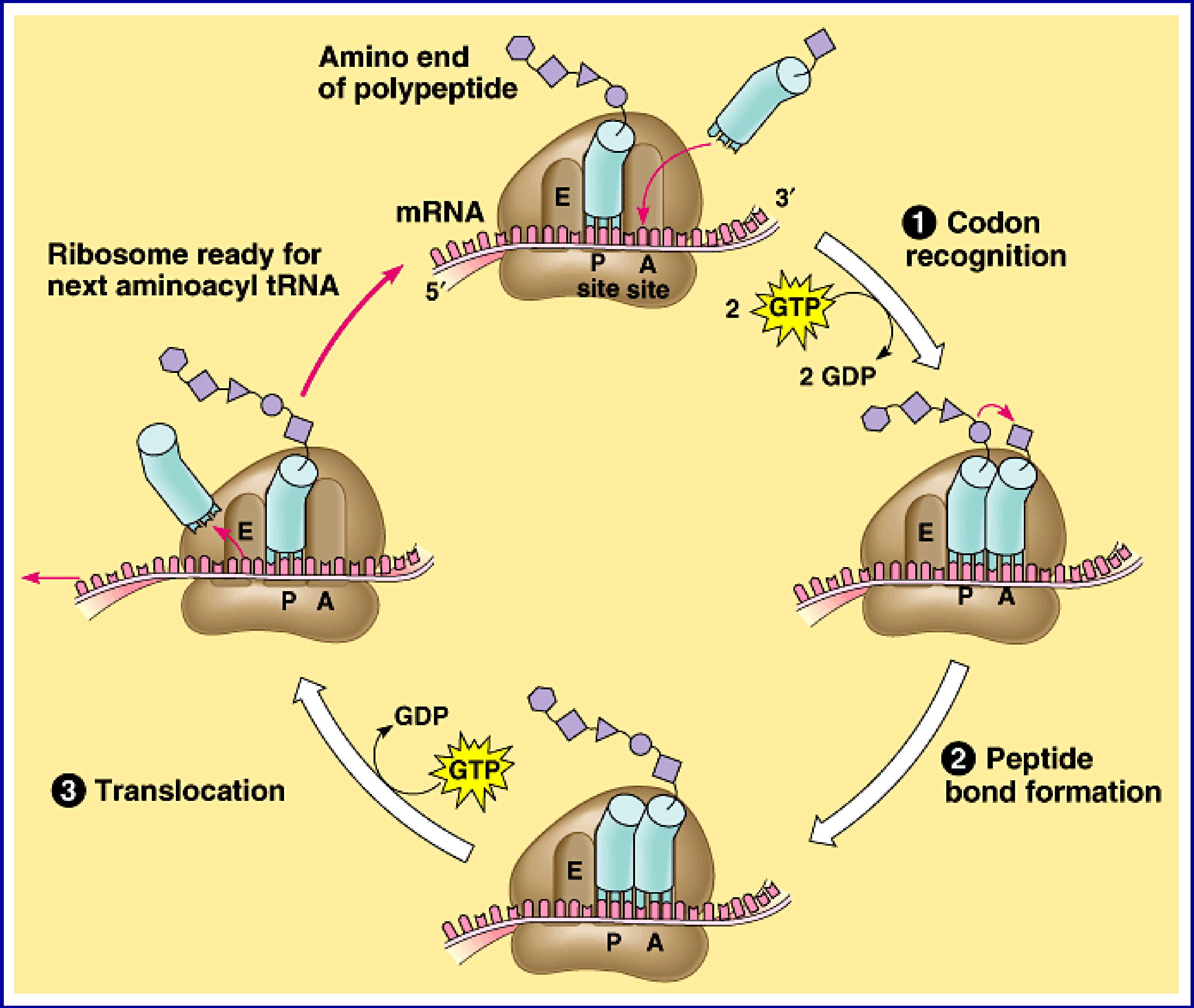
The initiation of translation



G. Translation: RNA-Directed Polypeptide Synthesis

- Polypeptides grow from the N terminus toward the C terminus. The ribosome moves along the mRNA one codon at a time.
- Elongation has three steps: Codon Recognition, Peptide Bond Formation and Translocation.
- Elongation also requires elongation factors and 3 GTPs per amino acid added.

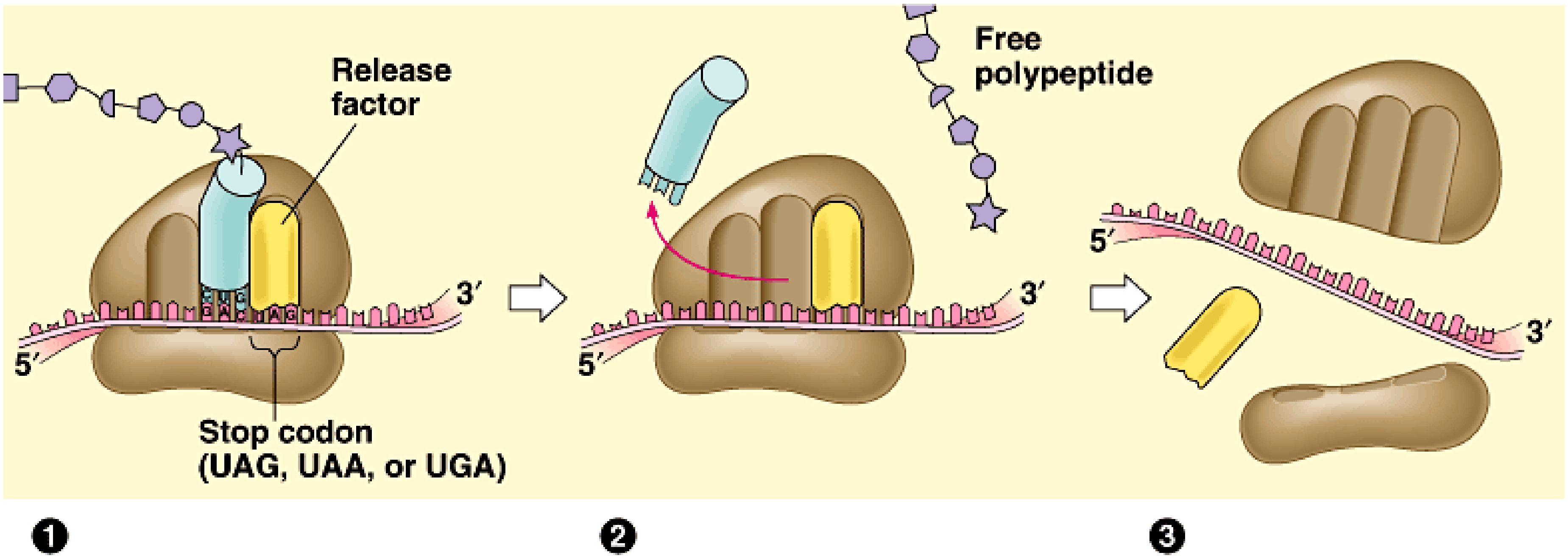
The elongation cycle of translation



G. Translation: RNA-Directed Polypeptide Synthesis

- The presence of a stop codon in the A site of the ribosome causes translation to terminate.

The termination of translation



H. Regulation of Translation

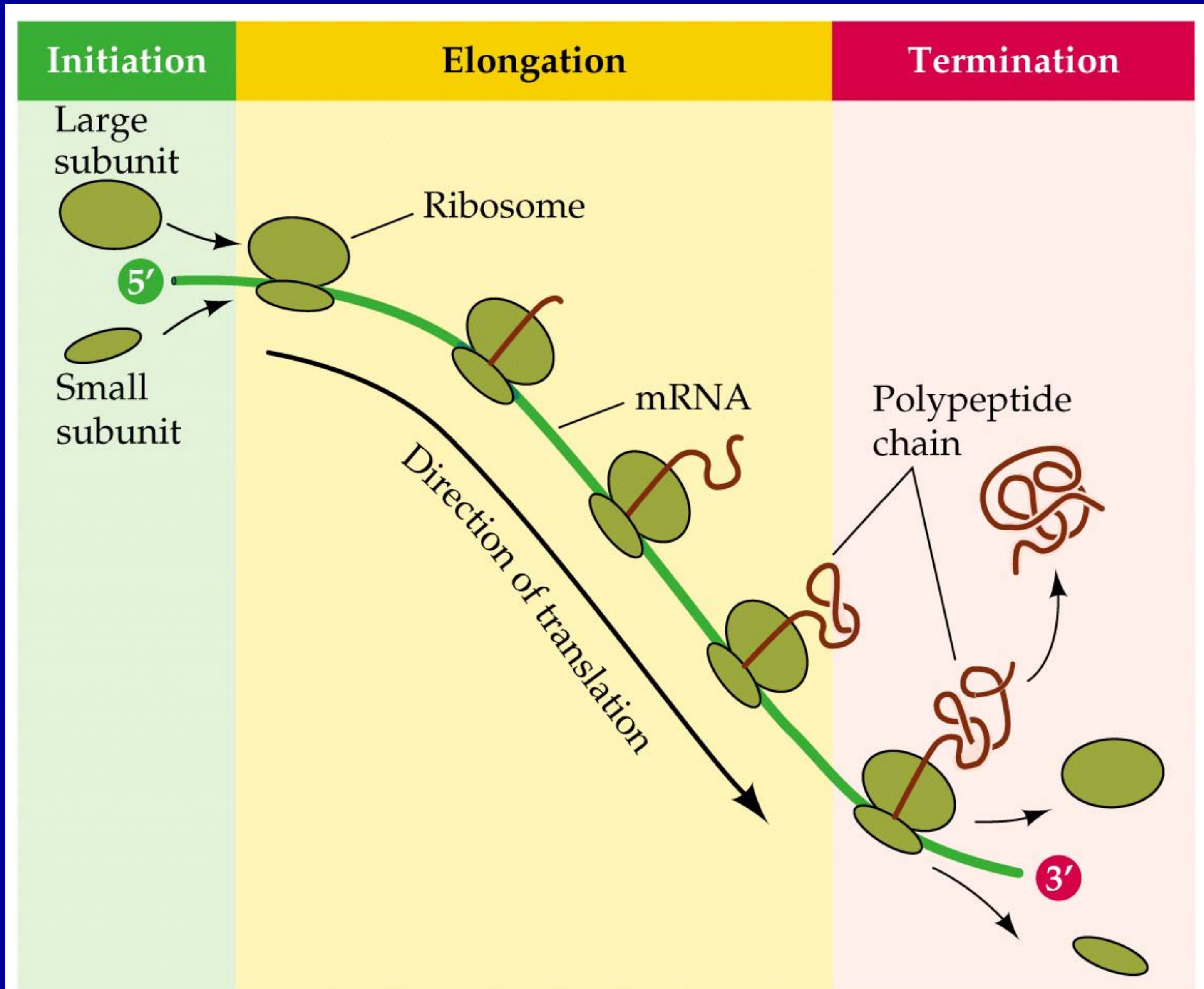
- Some antibiotics work by blocking events in translation.

12.2 Antibiotics that Inhibit Bacterial Protein Synthesis

ANTIBIOTIC	STEP INHIBITED
Chloromycetin	Formation of peptide bonds
Erythromycin	Translocation of mRNA along ribosome
Neomycin	Interactions between tRNA and mRNA
Streptomycin	Initiation of translation
Tetracycline	Binding of tRNA to ribosome

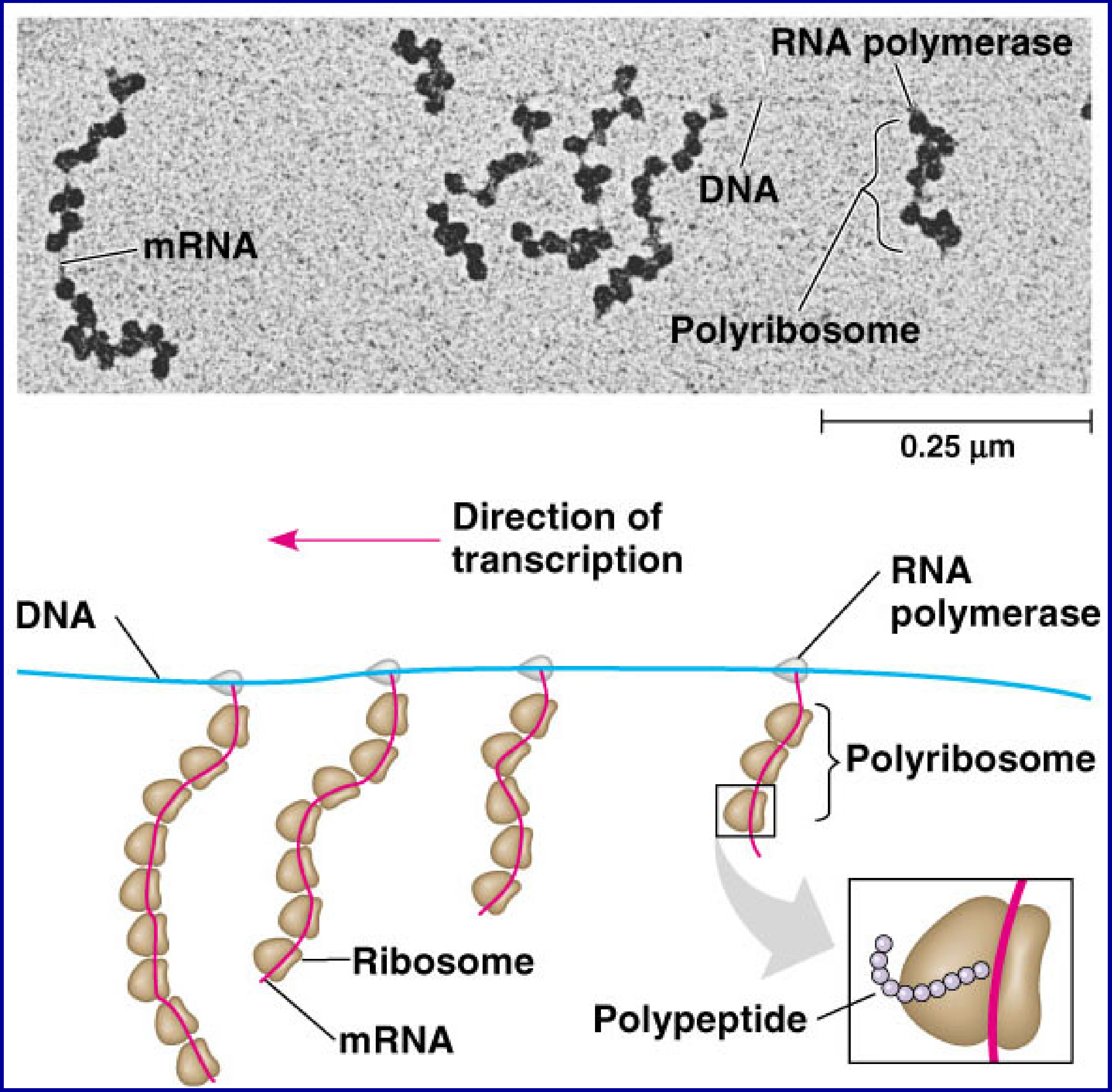
H. Regulation of Translation

- In a polysome, more than one ribosome moves along the mRNA at one time.

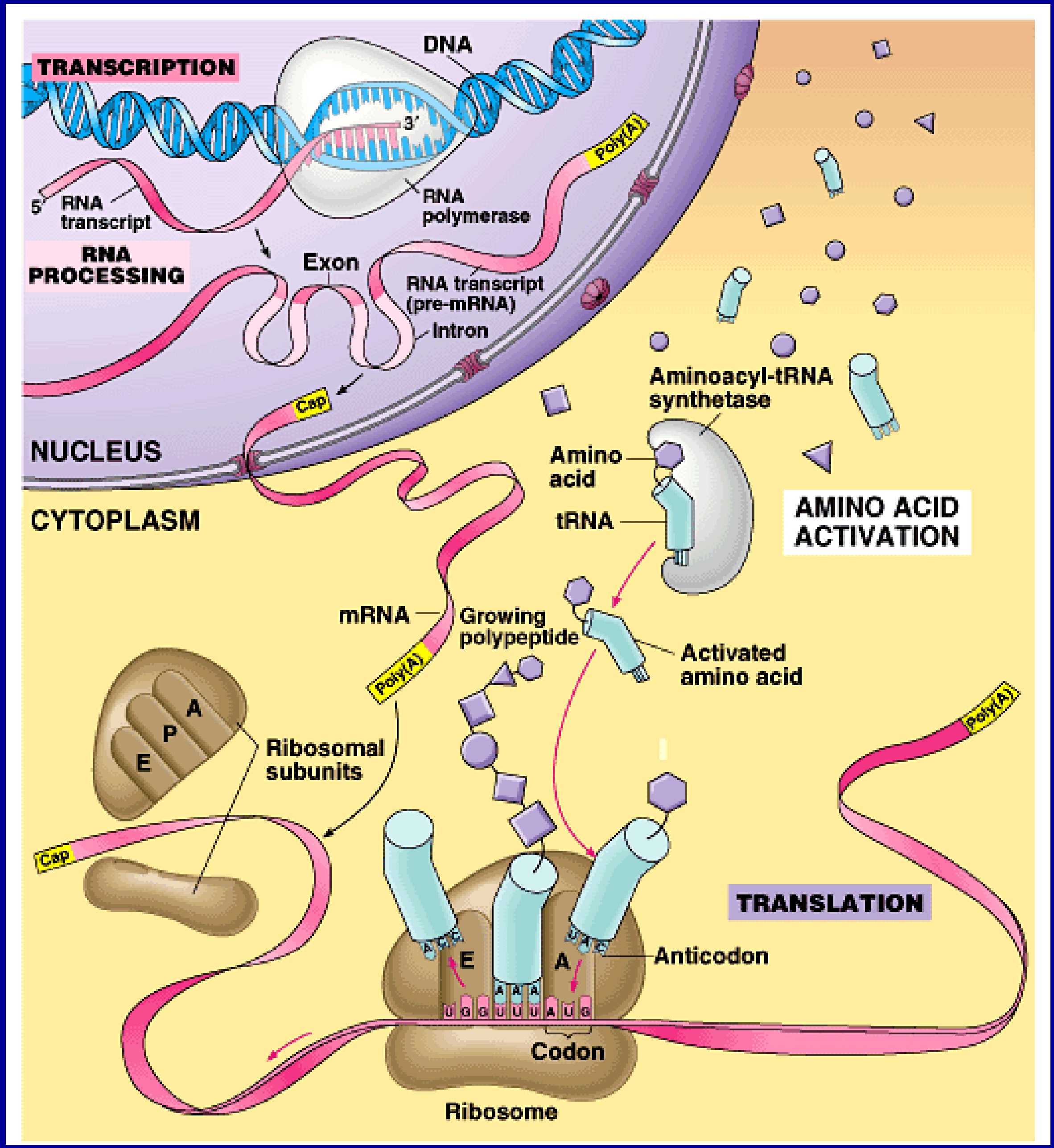


Initiation
Elongation
Termination
Large subunit
Ribosome
5′
Small subunit
mRNA
Direction of translation
Polypeptide chain
3′

Coupled transcription and translation in bacteria

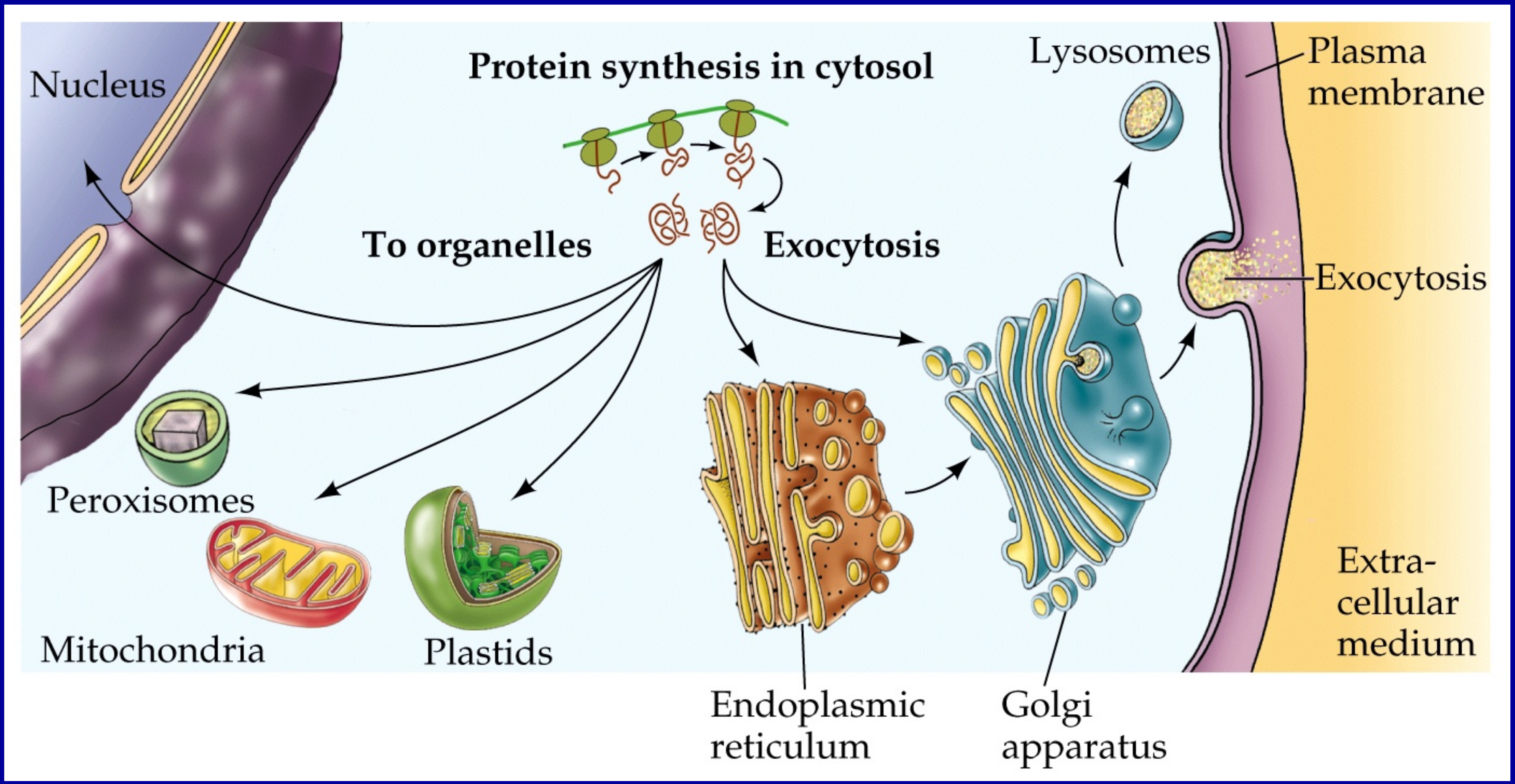


A summary of transcription and translation in a eukaryotic cell



I. Posttranslational Events

- Signals contained in the amino acid sequences of proteins direct them to cellular destinations.


Protein synthesis in cytosol
Nucleus
To organelles
Exocytosis
Lysosomes
Plasma membrane
Exocytosis
Peroxisomes
Mitochondria
Plastids
Extra-cellular medium
Endoplasmic reticulum
Golgi apparatus

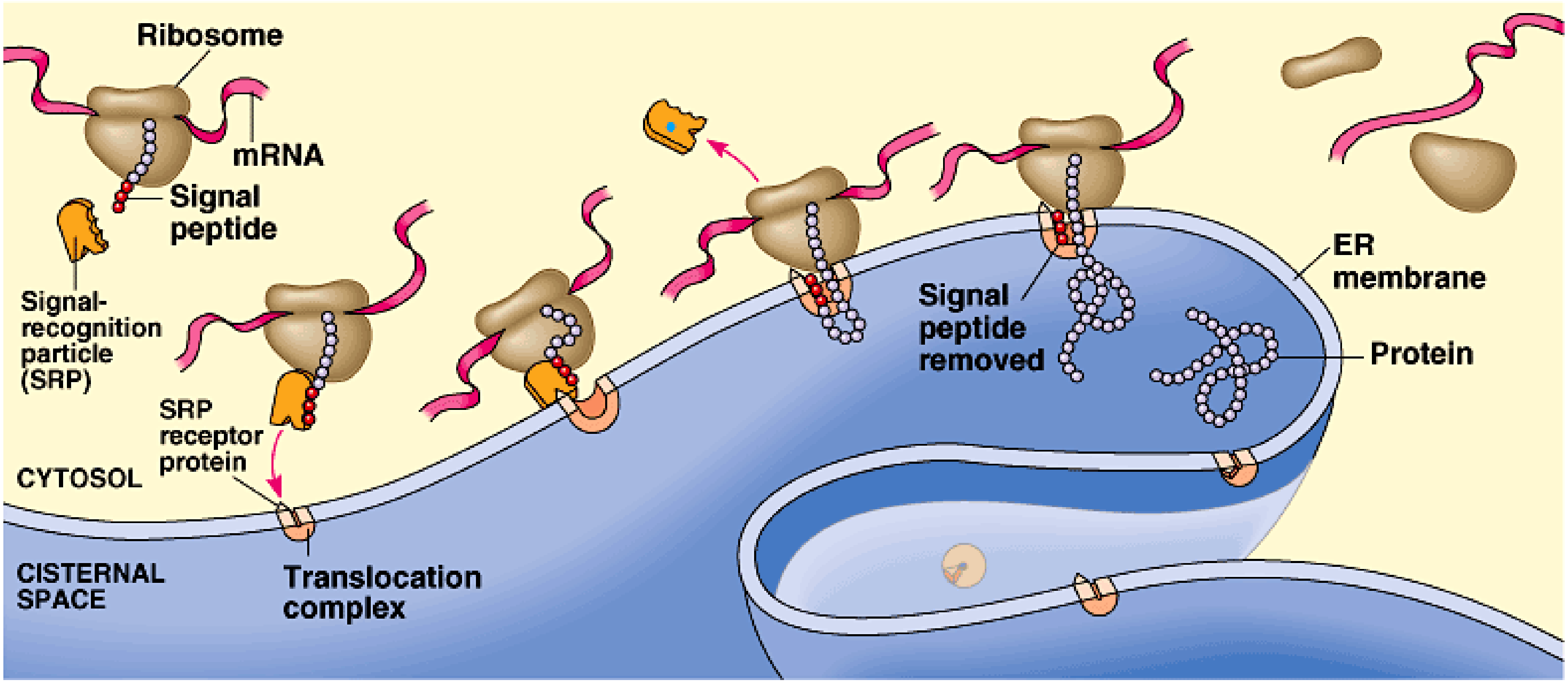
I. Posttranslational Events

- Protein synthesis begins on free ribosomes in the cytoplasm. Those proteins destined for the nucleus, mitochondria, and plastids are completed there and have signals that allow them to bind to and enter destined organelles.

I. Posttranslational Events

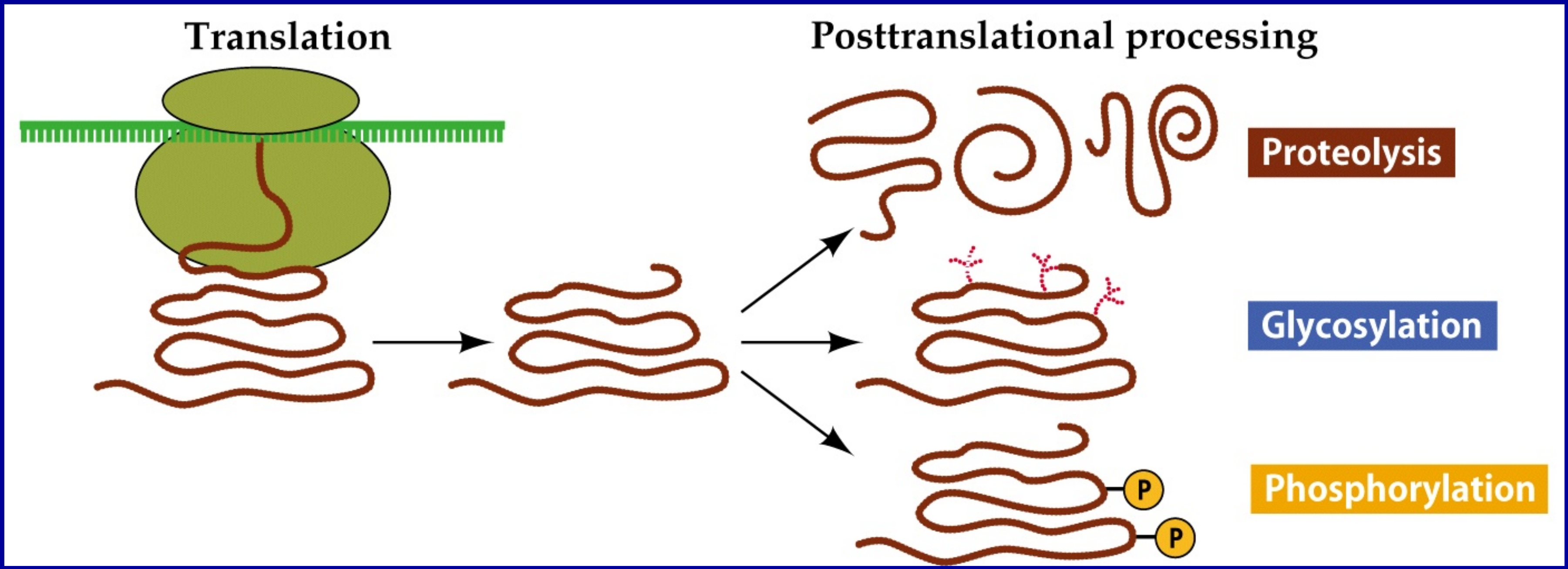
- Proteins destined for the ER, Golgi apparatus, lysosomes, and outside the cell complete their synthesis on the ER surface. They enter the ER by the interaction of a hydrophobic signal sequence with a channel in the membrane.

The signal mechanism for targeting proteins to the ER



I. Posttranslational Events

- Covalent modifications of proteins after translation include proteolysis, glycosylation, and phosphorylation.


Translation
Posttranslational processing
Proteolysis
Glycosylation
P
P
Phosphorylation

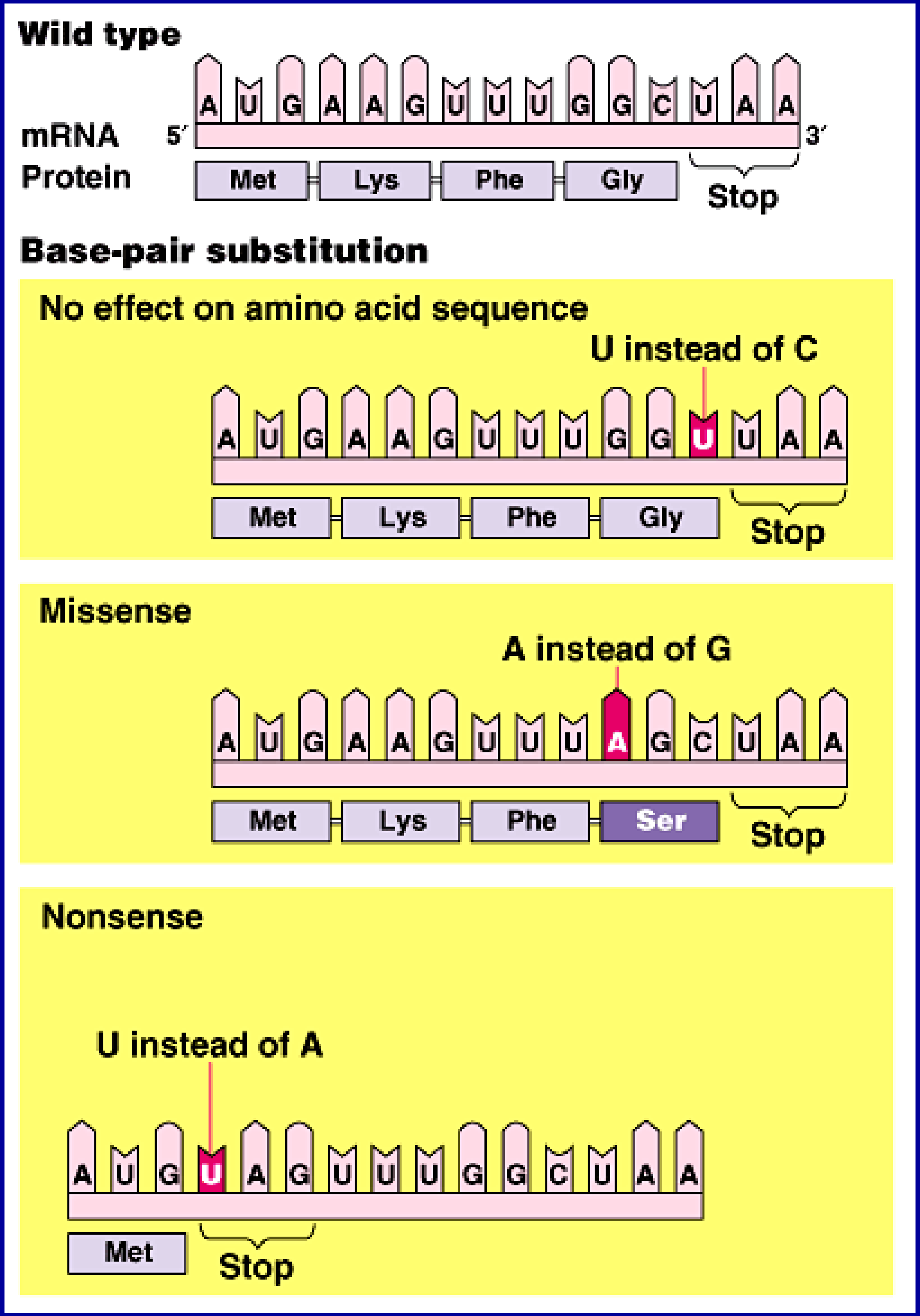
J. Mutations: Heritable Changes in Genes

- Mutations in DNA are often expressed as abnormal proteins. However, the result may not be easily observable phenotypic changes.
- Raw materials for evolution to operate.
- Some mutations appear only under certain conditions, such as exposure to a certain environmental agent or condition.

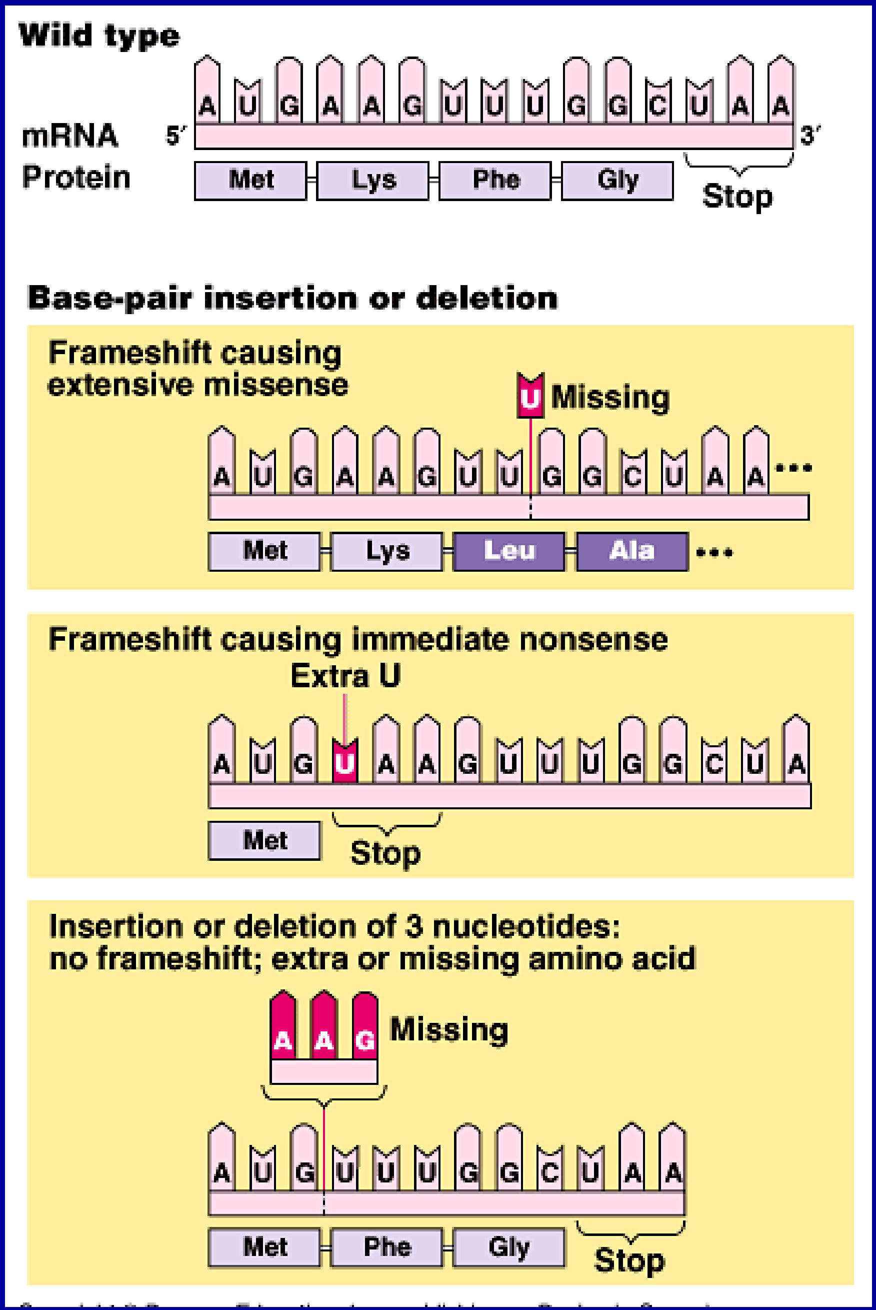
J. Mutations: Heritable Changes in Genes

- Point mutations (silent, missense, nonsense, or frame-shift) result from alterations in single base pairs of DNA.

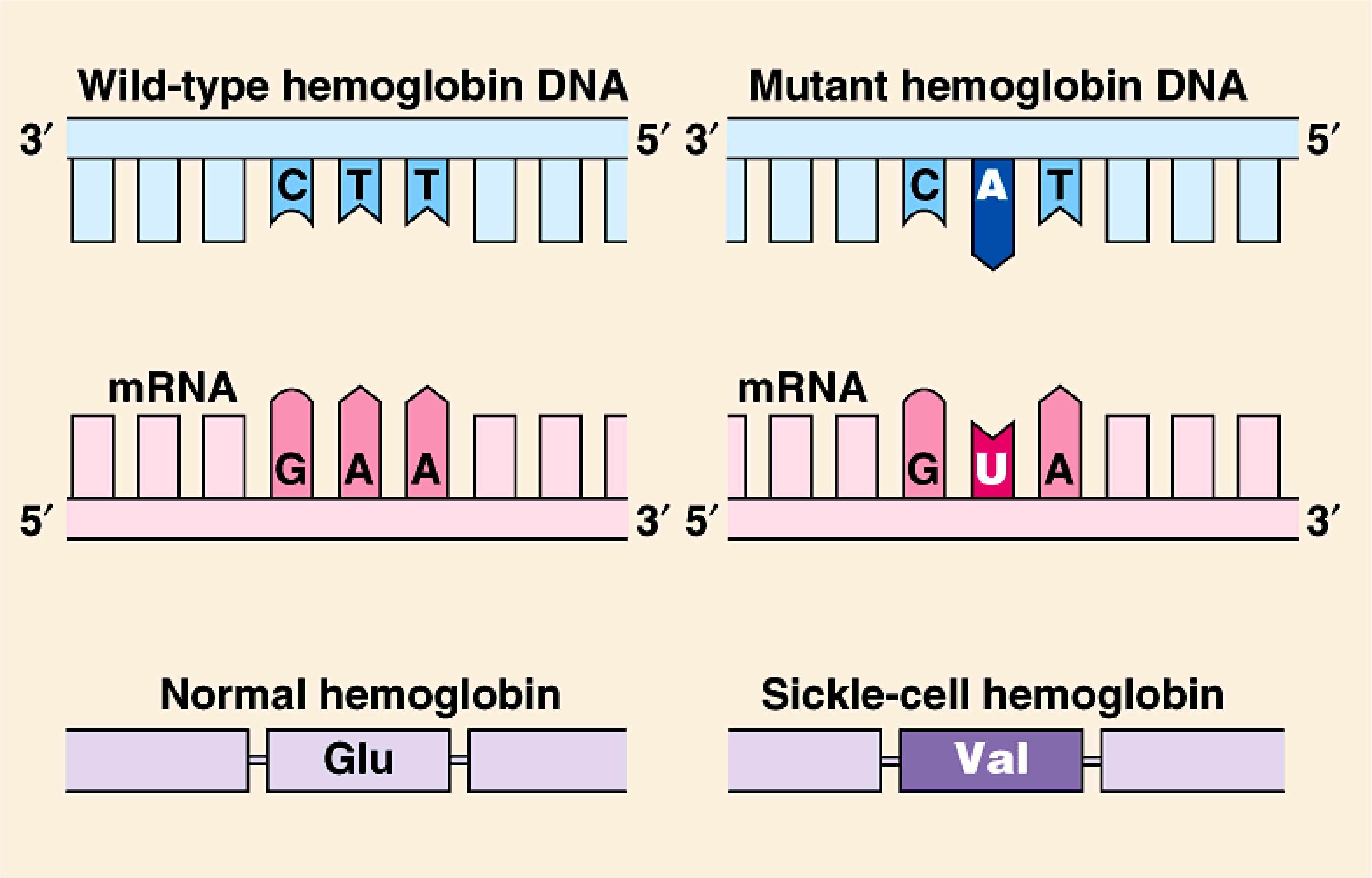
Categories and consequences of point mutations: Base-pair substitution



Categories and consequences of point mutations: Base-pair insertion or deletion



The molecular basis of sickle-cell disease: a point mutation



J. Mutations: Heritable Changes in Genes

- Chromosomal mutations (deletions, duplications, inversions, or translocations) involve large regions of a chromosome.

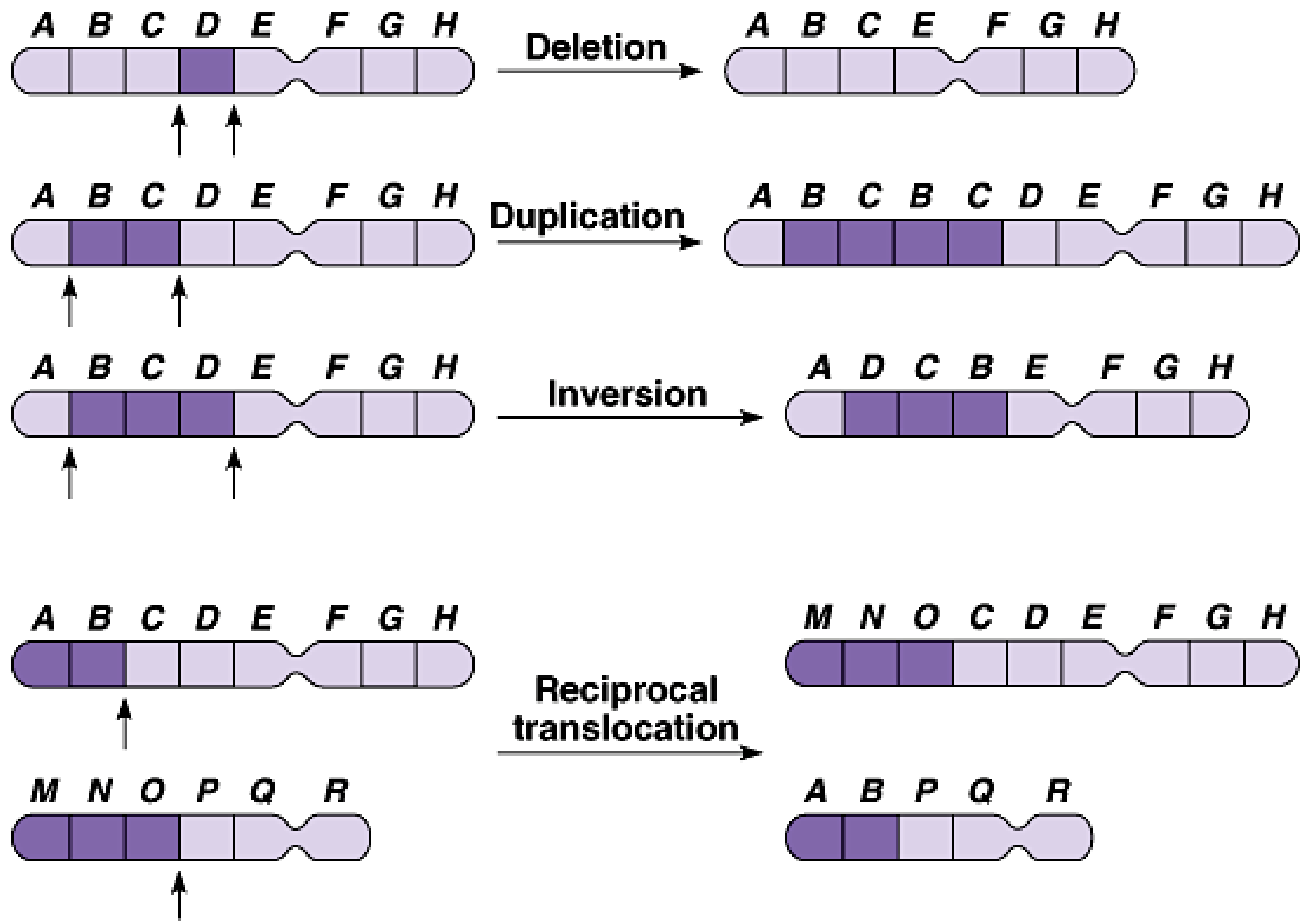
Alterations of chromosome structure

(a) A **deletion** removes a chromosomal segment.

(b) A **duplication** repeats a segment.

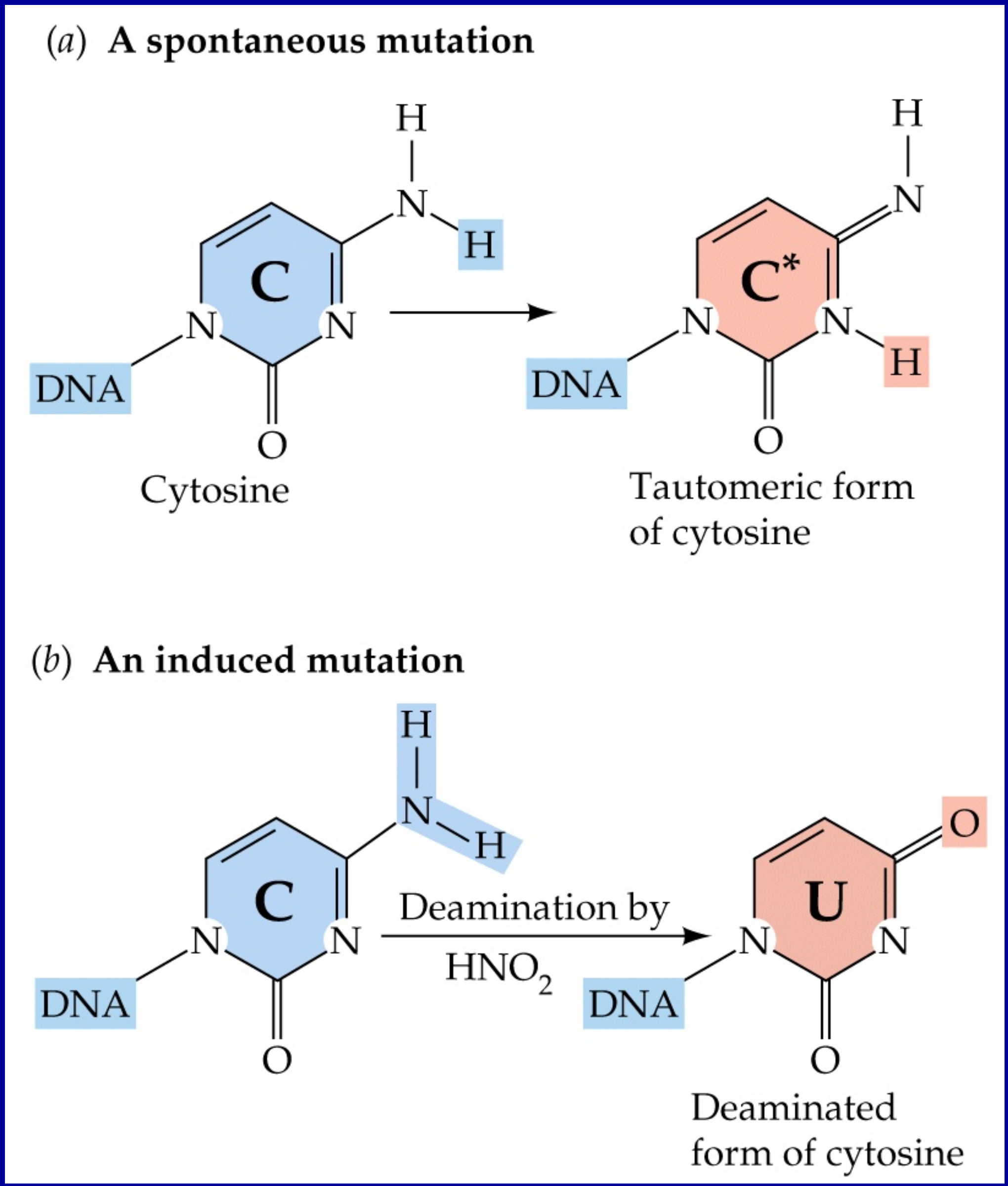
(c) An **inversion** reverses a segment within a chromosome.

(d) A **translocation** moves a segment from one chromosome to another, non-homologous one.



J. Mutations: Heritable Changes in Genes

- Mutations can be spontaneous or induced.
- Spontaneous mutations occur because of instabilities in DNA or chromosomes.
- Induced mutations occur when an outside agent damages DNA.


(a) A spontaneous mutation
H
N
H
C
N
N
DNA
O
Cytosine
H
N
C*
N
N
H
DNA
O
Tautomeric form of cytosine
(b) An induced mutation
H
N
H
C
N
N
DNA
O
Deamination by
HNO2
O
U
N
N
DNA
O
Deaminated form of cytosine

(*c*) **The consequences of either mutation**