TRANSCRIPTION, TRANSLATION & THE GENETIC CODE
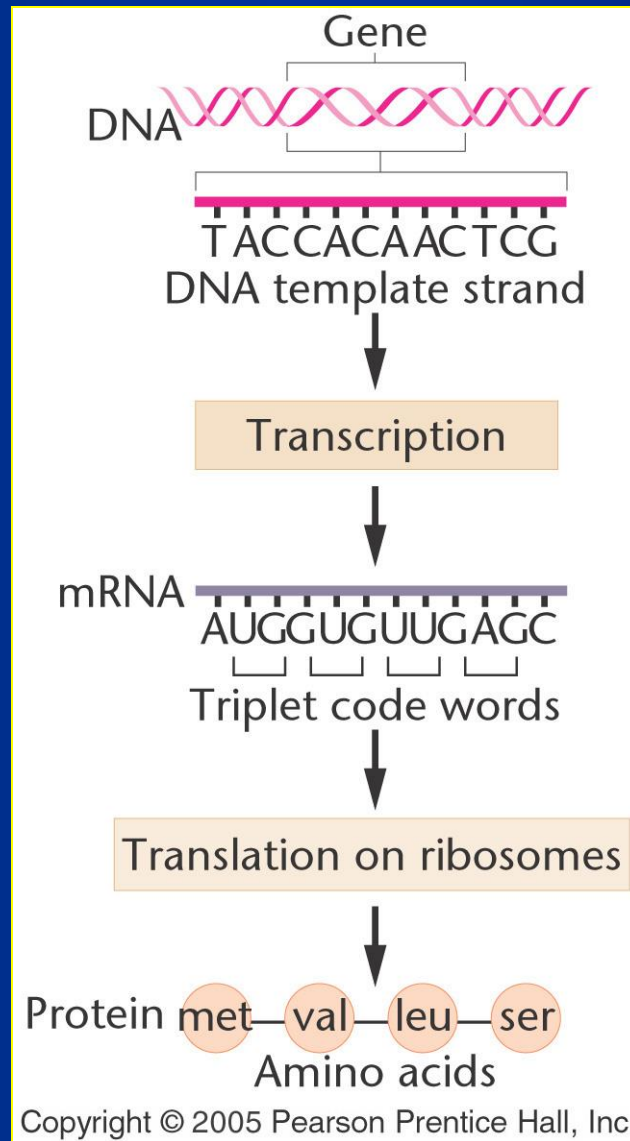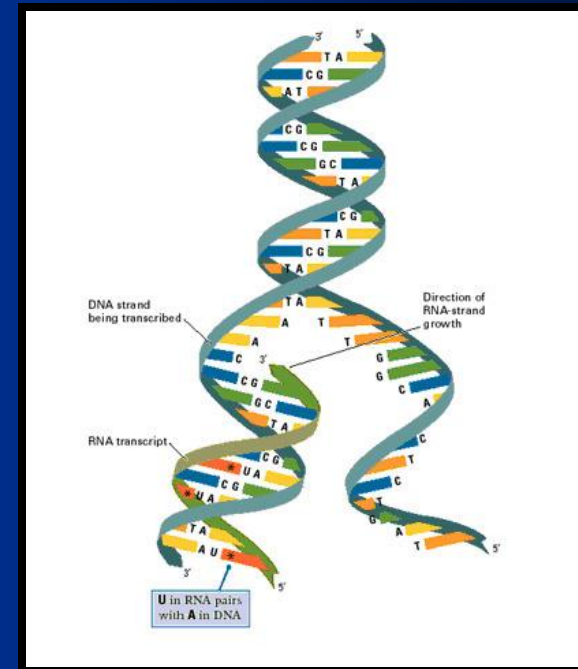
CENTRAL DOGMA Overview

Fig. 12-1

Central Dogma of Molecular Biology

- Transcription
  - DNA message is converted into mRNA format

- Translation
  - mRNA message is converted into protein

- DNA is the informational molecule which specifies the structure of proteins using RNA intermediate

Transcription

- Transcription: production of an RNA strand that is complementary in base sequence to a DNA strand
  - messenger RNA (mRNA)

- RNA is synthesized from template DNA following strand separation of the double helix

DNA strand being transcribed

Direction of RNA-strand growth

RNA transcript

U in RNA pairs with A in DNA

Base pairing between DNA and RNA

- Complementary base pairing specifies the linear sequence of bases in RNA

DNA		RNA
Adenine	pairs with	Uracil
Thymine	pairs with	Adenine
Guanine	pairs with	Cytosine
Cytosine	pairs with	Guanine

Translation

- Messenger RNA (mRNA) contains genetic code in <u>codons</u> (nucleotide triplets) that specify the sequence of amino acids in proteins

- Transfer RNA (tRNA) contain <u>anticodons</u> which are complementary to codon sequences in mRNA and position amino acids during translation

Translation

- Translation results in the synthesis of a polypeptide chain
  - Linear chain of amino acids whose sequence is specified by the sequence of codons in mRNA

- Translation occurs at the ribosomes
  - Ribosomes contain several types of ribosomal RNA (rRNA) and ribosomal proteins

The process of making mRNA from a gene is called…

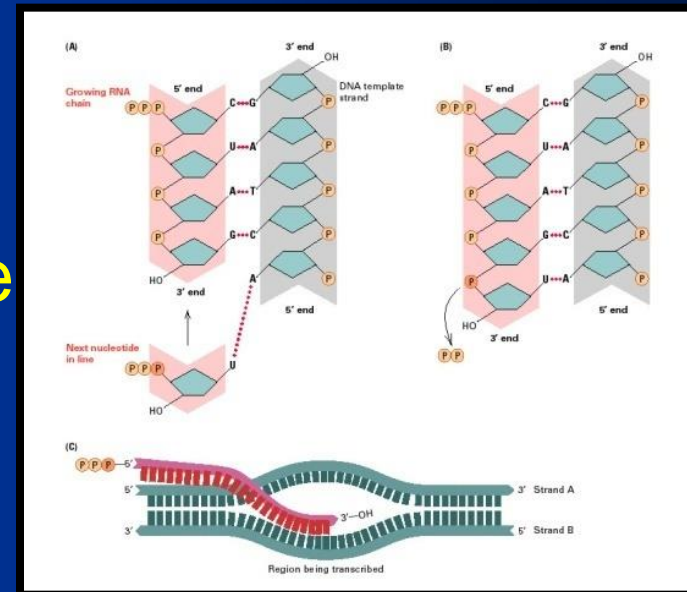- A) Transcription

- B) Translation

- C) Replication

Transcription: the Specifics

Synthesis of RNA from a DNA template

- ## Prokaryotes
    - Occurs in nucleoid/cytoplasm
    - Generate polycistronic mRNA (multiple genes in one transcript)
    - No post-transcriptional processing of mRNA required
    - Transcription & translation occurs simultaneously

- ## Eukaryotes
    - Occurs in nucleus
    - Generate monocistronic mRNA (one gene per transcript)
    - mRNA requires post-transcriptional modification
    - Transcripts transported to cytoplasm for translation

RNA Synthesis

- Antiparallel
  - RNA is copied from the template strand in the 5'-to-3' direction

- RNA synthesis does not require a primer and proceeds by the addition of nucleotides to form mRNA chain

- RNA is SS and complementary to region along one strand of DNA
  - Template vs. partner strand

The process of making mRNA from a gene occurs in the _____ direction.

- A) 3' to 5'
- B) 5' to 3'

Transcription in Prokaryotes: Template Binding

- RNA polymerase binds DNA at the promoter region
  - Holoenzyme: multiple subunits
  - σ subunit recognizes and binds promoter
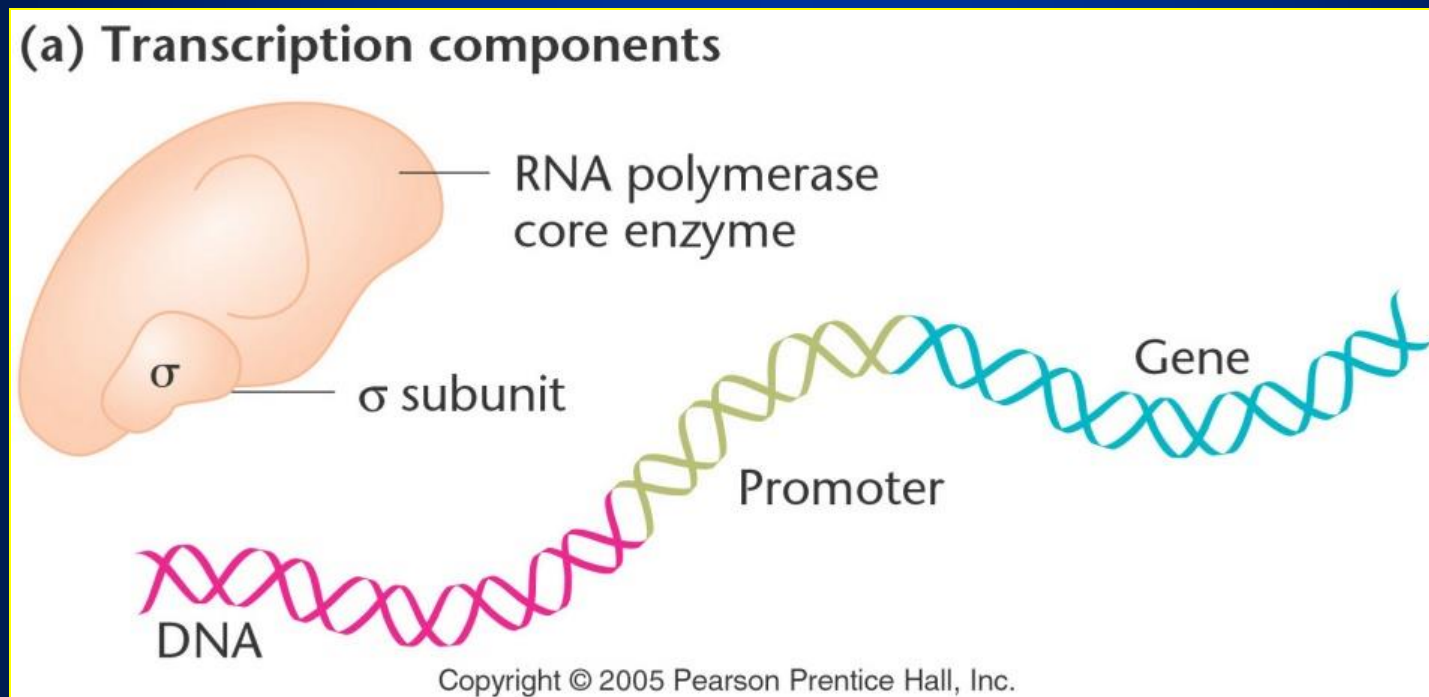 - DNA denatured downstream from promoter

(a) Transcription components

RNA polymerase core enzyme

σ subunit

σ

Gene

Promoter

DNA

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Fig. 12-8

RNA Synthesis

- Promoter: a nucleotide sequence 5' to the transcription start site; the initial binding site of RNA polymerase and transcription initiation factors

- Promoter recognition by RNA polymerase is required for initiation of transcription
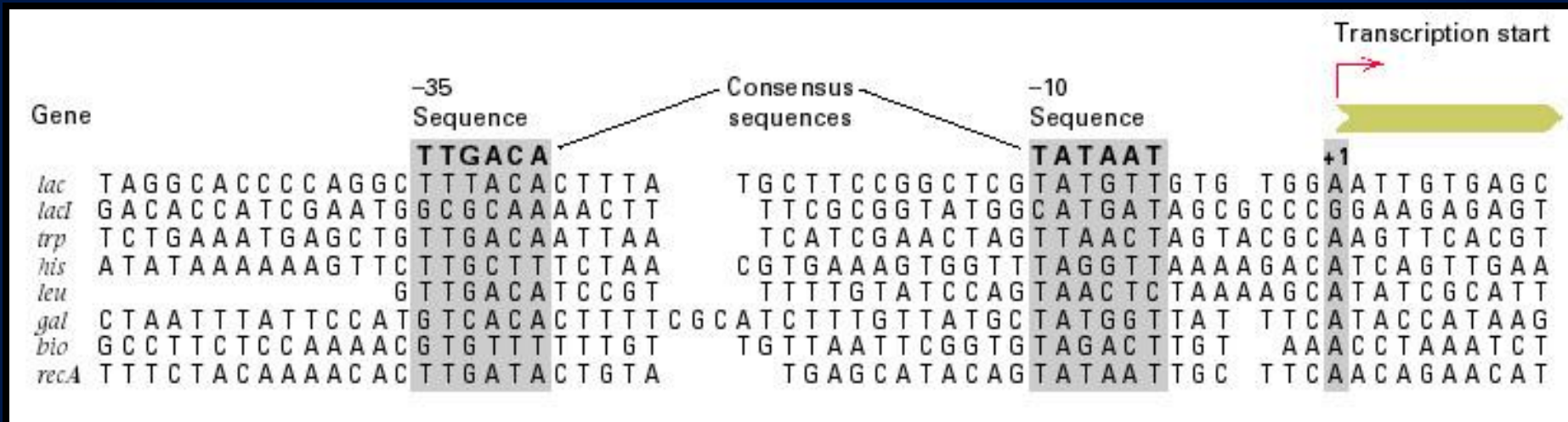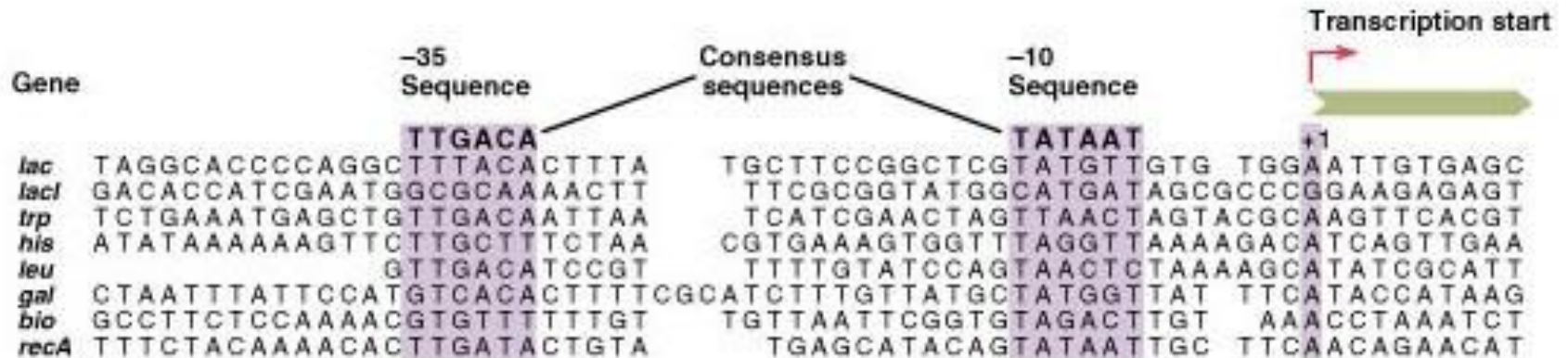
Promoters

- Contain consensus sequence
  - Similar base sequences commonly observed in promoters of different genes

RNA Synthesis

- TATA box
  - Many promoters contain consensus sequence: TATAAT (-10)
- Consensus promoter sequence at -35: TTGACA

Promoters

- Weak vs. strong promoters
  - Reflects strength and frequency of RNA Pol binding
  - Related to variation in promoter elements and consensus sequences

Which of the following is NOT true of a promoter?

- A) It is part of a DNA sequence
- B) It is located 5' to a gene
- C) It is found on mRNA
- D) It helps RNA Pol to bind to DNA

Transcription in Prokaryotes

- **Initiation & chain elongation**
  - No primer needed
  - RNA Pol adds bases 5'→3' along template strand beginning at transcription start site
  - Creates temporary RNA/DNA duplex
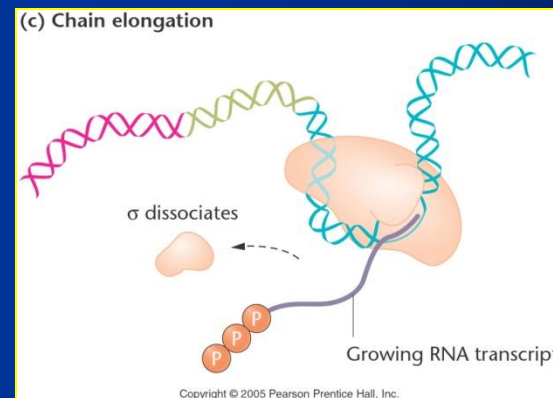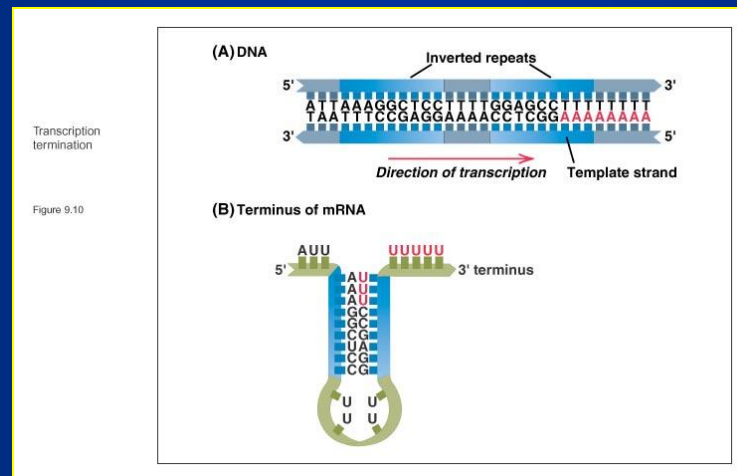 - After initiation, σ subunit dissociates & elongation continues

(c) Chain elongation

σ dissociates

Growing RNA transcript

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Fig. 12-8

Transcription in Prokaryotes

- Termination
  - Termination sequence composed of inverted repeats of nucleotides
  - Forms hairpin loop that terminates transcription
    - mRNA dissociates from polymerase
    - Polymerase dissociates from DNA

Transcription termination

Figure 9.10

(A) DNA

Inverted repeats

5' ATTAAAGGCTCCTTTTGGAGCCTTTTTTTT 3'
3' TAATTTCCGAGGAAAACCTCGGAAAAAAAA 5'

Direction of transcription   Template strand

(B) Terminus of mRNA

AUU   UUUUU
5'   AU   3' terminus
               AU
               AU
               GC
               GC
               CG
               UA
               CG
               CG
            U   U
            U   U

True or False: During prokaryotic transcription, an RNA primase must insert a primer

- A) True
- B) False

A hairpin loop is involved in which process?

- A) Transcription initiation
- B) Transcription elongation
- C) Transcription termination
- D) DNA replication initiation

Transcription in Eukaryotes

- Multiple RNA polymerases
  - I → rRNA
  - II → mRNA, nuclear RNAs, RNA processing
  - III → tRNA, 5S rRNA

Transcription in Eukaryotes

- *Cis*-acting elements
  - Part of DNA sequence
  - Aid in efficient initiation of transcription by RNA Pol II
    - TATA box (TATAAAA)
      - Facilitates denaturation of helix
      - T=A less stable than G≡C
    - CAAT box (GGCCAATCT)
    - Enhancers
      - Locations vary (upstream, downstream, within gene)

Transcription in Eukaryotes

- *Trans*-acting elements
  - Proteins that bind to DNA
  - Aid in template binding and initiation of transcription
    - Transcription factors
      - Proteins that bind to promoter and allow binding of RNA Pol II
      - TATA-binding protein

True or false: In eukaryotes a *trans*-acting element is a sequence of DNA that assists in transcription

- A) True
- B) False

Transcription in Eukaryotes

- Post-transcriptional modification of mRNA
  - Primary transcript modified into mature form that will be translated
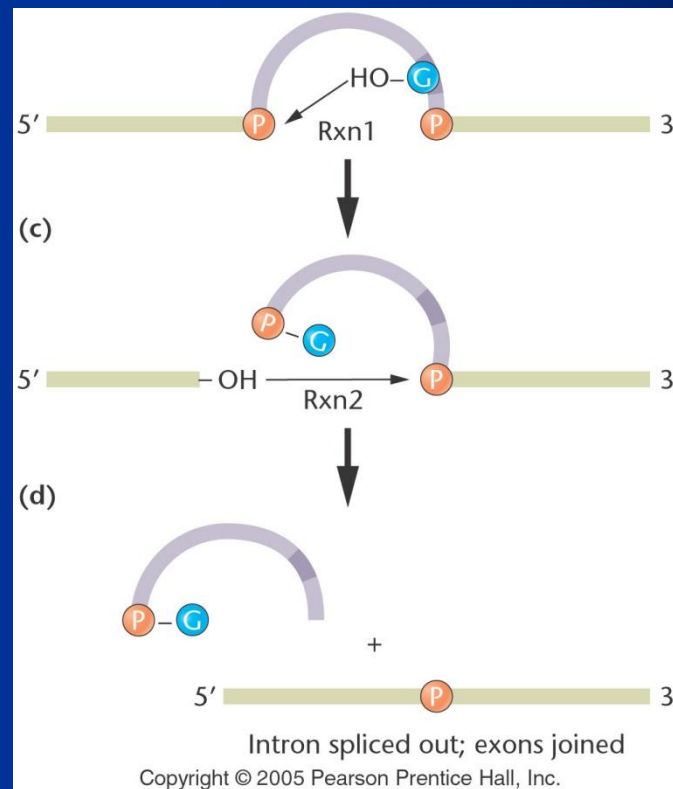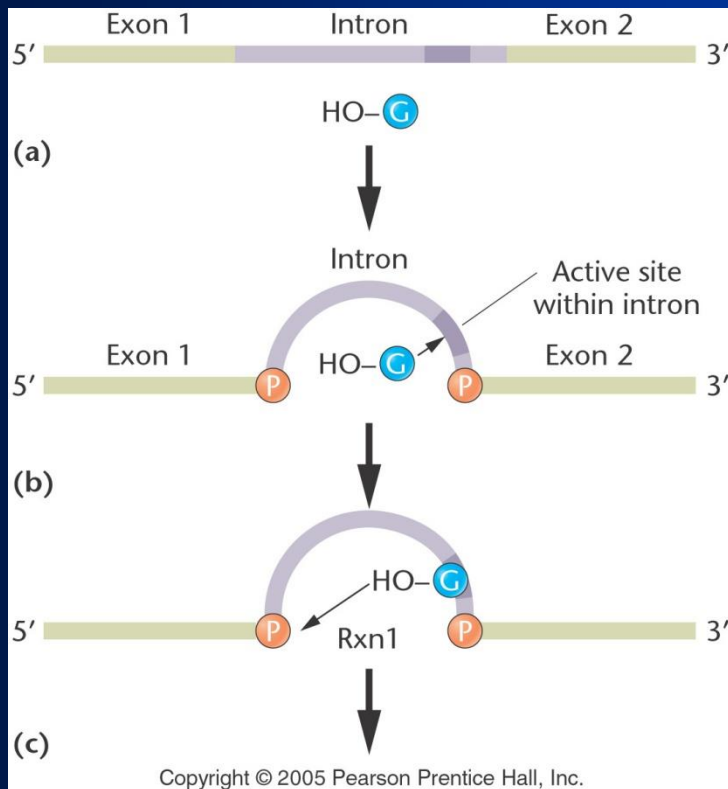 - Also called RNA processing

mRNA Processing

- Cap
  - 7-methyl guanosine (7mG) added to 5' end of mRNA
  - Protects against nucleases
  - Recognition for transport out of nucleus

- Tail
  - Poly-A sequence added to 3' end of mRNA
  - 3' terminal end cleaved at AAUAAA sequence before polyadenylation (most mRNA)
  - Protects from degradation

mRNA Processing

- Splicing
  - Splice out introns (intervening sequences) and ligate exons (coding sequences) together
    - Introns may compose 50-90% of primary transcript

Splicing Mechanisms

- Autocatalytic RNAs
  - Self-excision (ribozymes)
  - Involves guanosine cofactor

Fig. 12-11

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Intron spliced out; exons joined

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Splicing Mechanisms

- **Spliceosomes**
  - Consensus sequences at 5' (GU) and 3' (AG) of intron
  - Branch point
  - Combine with snRNAs and proteins
    - snRNPs (small nuclear ribonucleoproteins)
  - Form spliceosome complex

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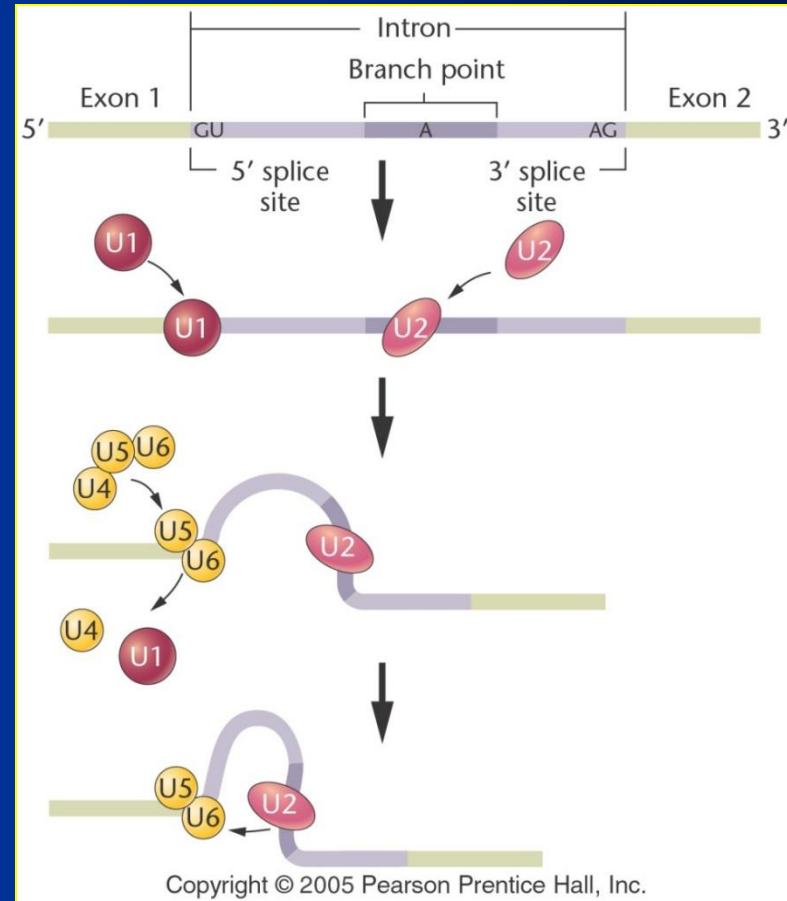
Fig. 12-13

Splicing Mechanisms

- Spliceosomes
  - Adenine within branch point attacks 5' splice site
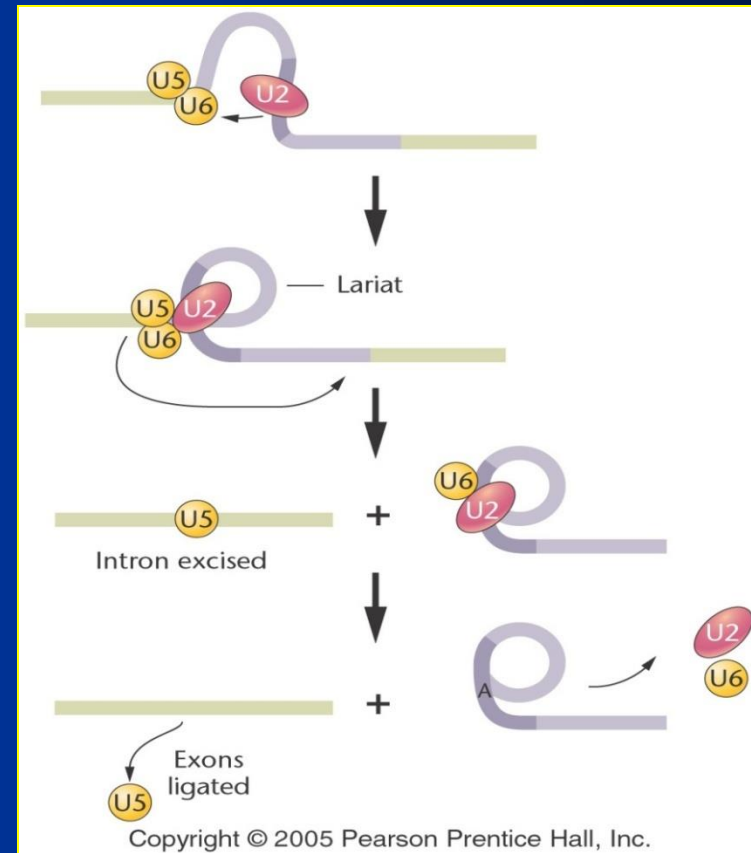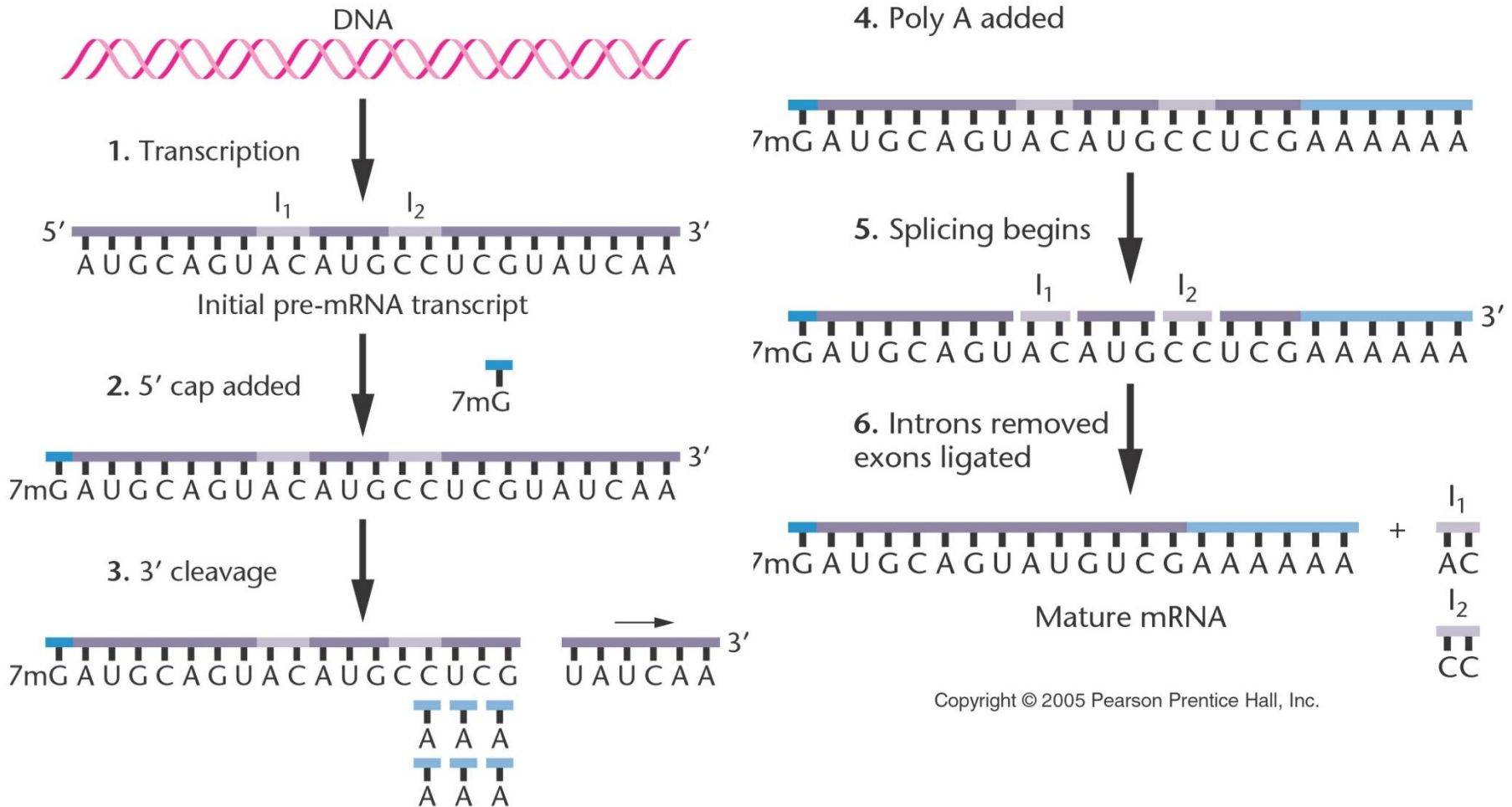 - Formation & excision of lariat
  - Exons ligated
  - Alternative splicing can lead to protein isoforms

Lariat

Intron excised

Exons ligated

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Fig. 12-13

mRNA Processing

Fig. 12-9

mRNA Processing

119

40   403

Mouse insulin

126   580

143   222   224

Rabbit β-globin

1550   246 576   398   860   370   1625

188   53   132   118   142   155

Chicken ovalbumin

Exons Introns

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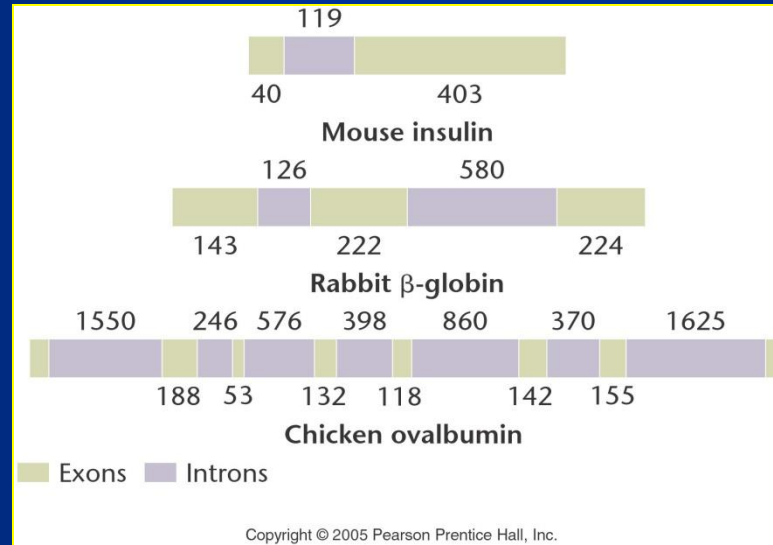
TABLE 13.7   Comparing Human Gene Size, mRNA Size, and the Number of Introns

Gene	Gene Size (kb)	mRNA Size (kb)	Number of Introns
Insulin	1.7	0.4	2
Collagen [pro-α-2(1)]	38.0	5.0	50
Albumin	25.0	2.1	14
Phenylalanine hydroxylase	90.0	2.4	12
Dystrophin	2000.0	17.0	50

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Fig. 12-11

Which of the following activities does NOT occur during mRNA processing?

- A) Addition of 7-methyl guanosine cap
- B) Addition of polyadenylyl tail
- C) Splicing out of introns
- D) Proofreading of mRNA

A snRNP consists of which of the following?

- A) small nuclear RNAs
- B) proteins
- C) spliceosome
- D) A and B only
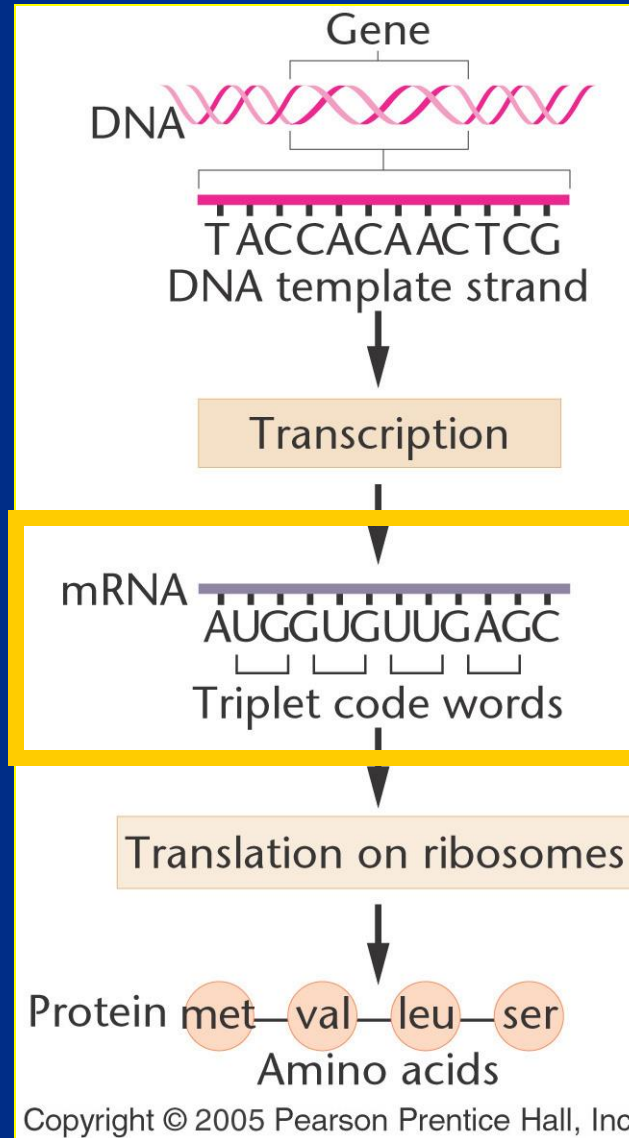- E) A, B, and C

Translation: the Specifics

GENETIC CODE

Gene

DNA

TACCACAACTCG
DNA template strand

Transcription

mRNA AUGGUGUUGAGC
Triplet code words

Translation on ribosomes

Protein met—val—leu—ser
Amino acids

Fig. 12-1

Translation

- Translation: genetic information encoded in mRNA specifies the linear sequence of amino acids in the corresponding protein

- Genetic code:

  – The sequence of bases that represent the specific amino acids (aa's) that will be assembled into a polypeptide chain and ultimately form a mature protein

Proteins

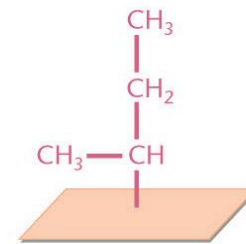- Basic building block = amino acids (20)

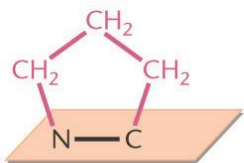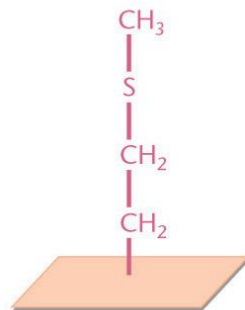1. Nonpolar: Hydrophobic
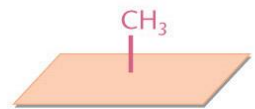
Alanine (ala, A)
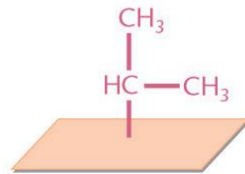
Valine (val, V)
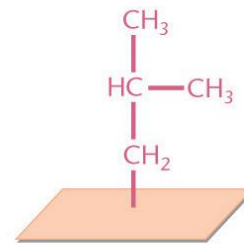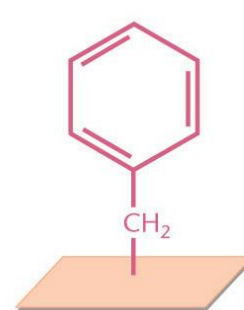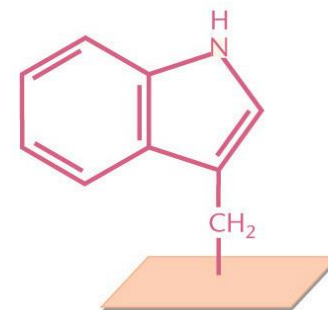
Leucine (leu, L)

Isoleucine (ile, I)

Proline (pro, P)

Methionine (met, M)

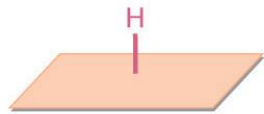Phenylalanine (phe, F)

Tryptophan (trp, W)

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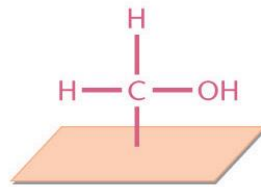
Fig. 14-15

Proteins

- Basic building block = amino acids (20)
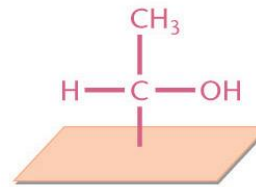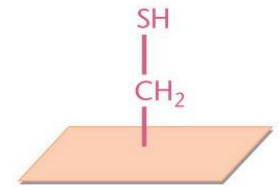

2. Polar: Hydrophilic

Glycine (gly, G)

Serine (ser, S)

Threonine (thr, T)

Cysteine (cys, C)
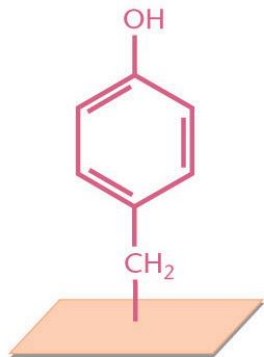
Tyrosine (tyr, Y)
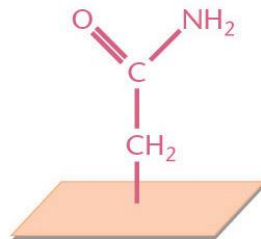
Asparagine (asn, N)
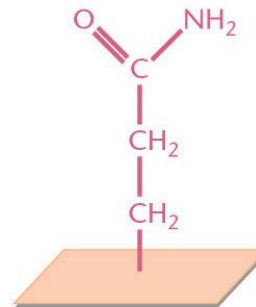
Glutamine (gln, Q)

Fig. 14-15

Proteins

- Basic building block = amino acids (20)

3. Polar: positively charged (basic)
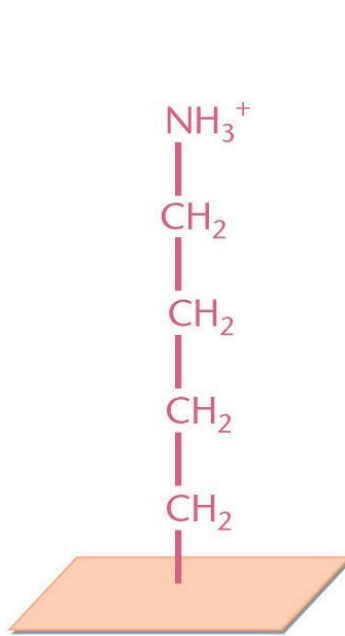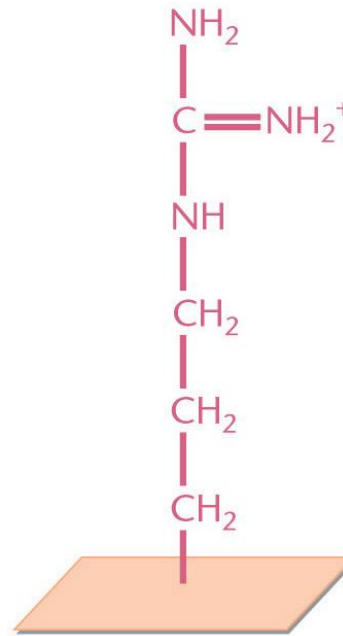
Lysine (lys, K)   Arginine (arg, R)   Histidine (his, H)
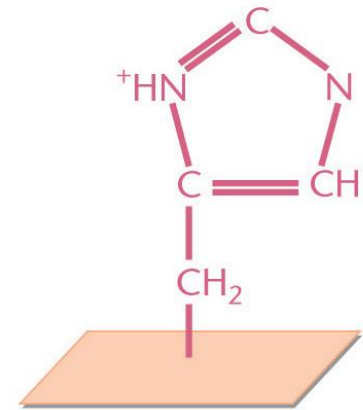
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Fig. 14-15

Proteins

- Basic building block = amino acids (20)

4. Polar: negatively charged (acidic)
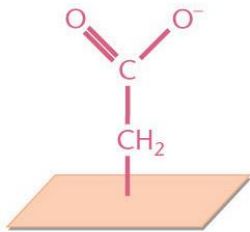
Aspartic acid (asp, D)   Glutamic acid (glu, E)

Amino group — Carboxyl group
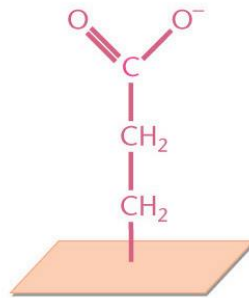
Amino acid structure
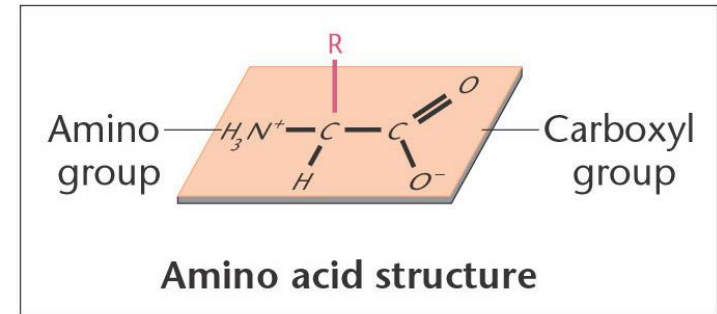
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Fig. 14-15

Proteins

- Polypeptides form by linking amino acids via peptide bonds
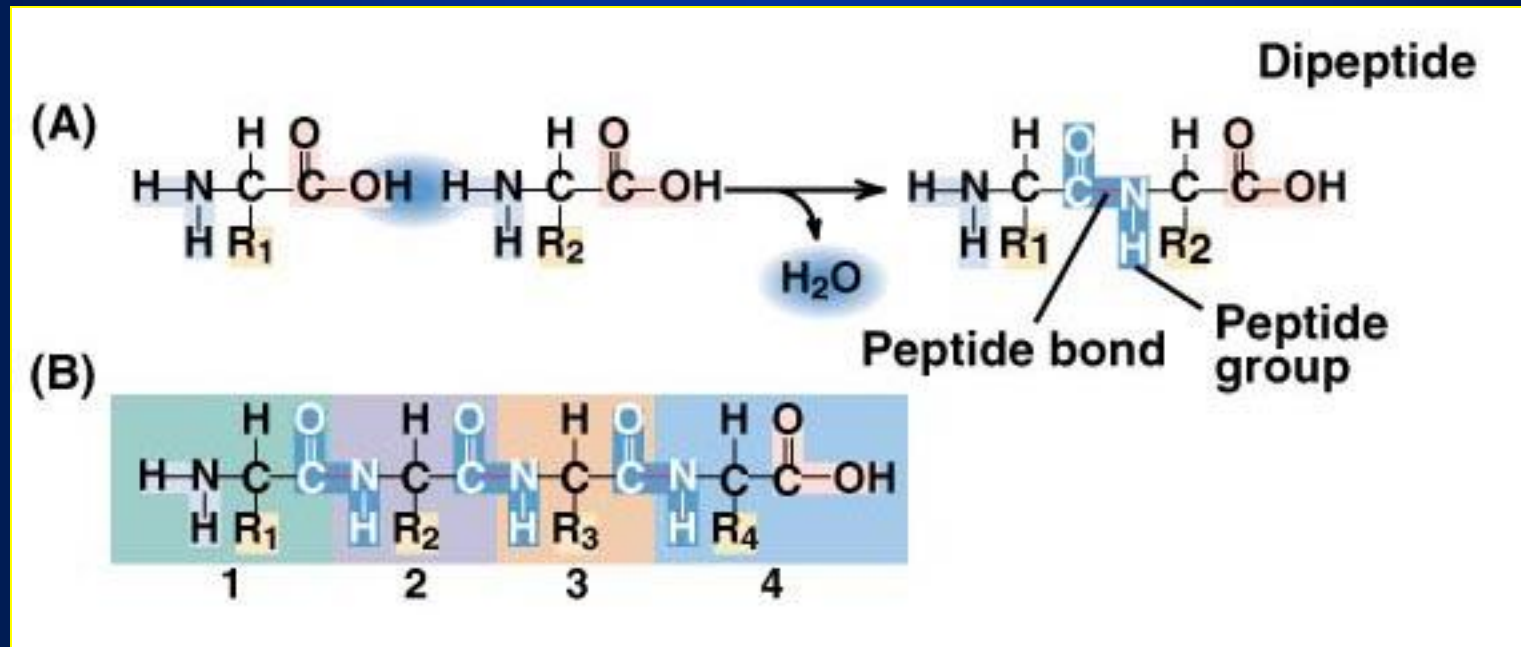
Proteins

- Folding of polypeptide chain give 3-D structure
  - Ex., myoglobin
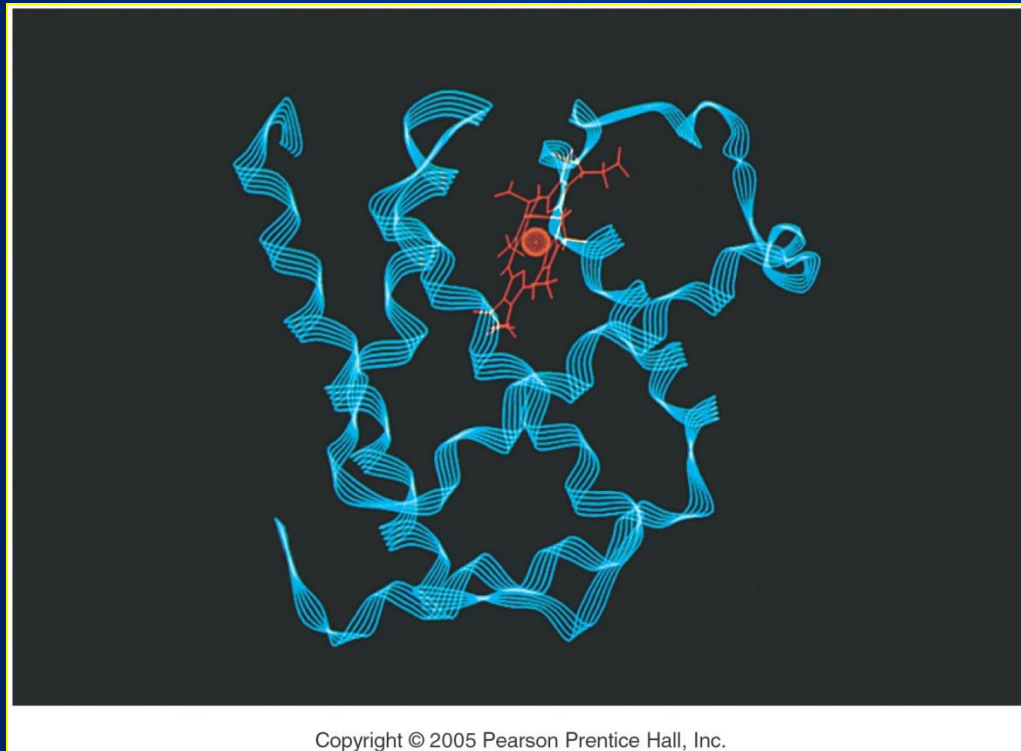
Fig. 14-18

Amino acids join together through _____ bonds to form proteins.

- A) phosphodiester
- B) peptide
- C) glycosidic
- D) ester

A protein's identity is based on its…

- A) amino acid sequence
- B) three-dimensional structure
- C) folding

Genetic Code

- How do we identify the 20 aa's used in protein synthesis from only 4 bases (AGCU)?

  – Theoretical possibilities:
    - If aa is encoded by a combination of 2 nucleotides
      – 4^2 = 16 possible outcomes

    - If aa = combination of 3 nucleotides
      – 4^3 = 64 possible outcomes

    - If aa = combination of 4 nucleotides
      – 4^4 = 256 possible outcomes

The Coding Dictionary

Fig. 12-7

The Coding Dictionary

- Sequences coding for aa's composed of 3 ribonucleotides
  - Codon

- Codons are unambiguous
  - Each codon specifies only 1 aa
  - Ex., CCC = proline

- Codons are degenerate
  - Each aa may be represented by more than 1 codon
  - Ex., proline = CCU, CCC, CCA, CCG

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The Coding Dictionary

- Codons show order
  - Degenerate codons typically grouped together

- Codons are non-overlapping
  - Each triplet read in order during translation

- Codons are nearly universal
  - Codons typically represent same aa regardless of organism

Second position

First position (5'-end)	U	C	A	G	Third position (3'-end)
U	UUU UUC *phe* / UUA UUG *leu*	UCU UCC UCA UCG *ser*	UAU UAC *tyr* / UAA *Stop* UAG *Stop*	UGU UGC *cys* / UGA *Stop* UGG *trp*	U C A G
C	CUU CUC CUA CUG *leu*	CCU CCC CCA CCG *pro*	CAU CAC *his* / CAA CAG *gln*	CGU CGC CGA CGG *arg*	U C A G
A	AUU AUC *ile* AUA / AUG *met*	ACU ACC ACA ACG *thr*	AAU AAC *asn* / AAA AAG *lys*	AGU AGC *ser* / AGA AGG *arg*	U C A G
G	GUU GUC GUA GUG *val*	GCU GCC GCA GCG *ala*	GAU GAC *asp* / GAA GAG *glu*	GGU GGC GGA GGG *gly*	U C A G

Initiation   Termination

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Exceptions to Universal Code

	Normal	Altered	
Triplet	Code Word	Code Word	Source
UGA	Termination	Tryptophan	Human and yeast mitochondria; *Mycoplasma*
CUA	Leucine	Threonine	Yeast mitochondria
AUA	Isoleucine	Methionine	Human mitochondria
AGA AGG	Arginine	Termination	Human mitochondria
UAA	Termination	Glutamine	*Paramecium; Tetrahymena; Stylonychia*
UAG	Termination	Glutamine	*Paramecium*

TABLE 13.5 Exceptions to the Universal Code

The ability of an amino acid to be coded for by more than one codon is called the _____ property of the genetic code.

- A) unambiguous
- B) degenerate
- C) univeral
- D) non-overlapping

STRUCTURAL ELEMENTS OF TRANSLATION

- mRNA
  - Transcript of genetic code
  - Template for protein manufacture
- Ribosomes
  - Site of translation
  - Free in cytoplasm or on surface of rER
  - 2 subunits
  - Composed of rRNA and protein
- tRNAs
  - Carriers of aa's to ribosomes
- Initiation & elongation factors

Ribosomes

- Ribosomes
  - Macromolecular machines that carry out protein synthesis
  - Contain ribosomal RNA (rRNA) and protein and are organized in two subunits:
    - Small subunit: 30S or 40S
    - Large subunit: 50S or 60S

  - Note: S stands for Svedberg unit, a measure of density

Ribosomes

Fig. 14-1

Prokaryotes
Monosome 70S (2.5 $\times$ 10^6 MW)

Eukaryotes
Monosome 80S (4.2 $\times$ 10^6 MW)
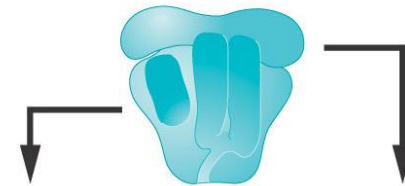
Large subunit	Small subunit	Large subunit	Small subunit
50S 1.6 $\times$ 10^6 MW	30S 0.9 $\times$ 10^6 MW	60S 2.8 $\times$ 10^6 MW	40S 1.4 $\times$ 10^6 MW
23S rRNA (2904 nucleotides) + 31 proteins + 5S rRNA (120 nucleotides)	16S rRNA (1541 nucleotides) + 21 proteins	28S rRNA (4718 nucleotides) + 49 proteins 5S rRNA (120 nucleotides) + 5.8S rRNA (160 nucleotides)	18S rRNA (1874 nucleotides) + 33 proteins

Which of the following is NOT required to build a ribosome?

- A) Protein
- B) tRNA
- C) rRNA

tRNA

- 70-90 nucleotides
- Extremely conserved structure in prokaryotes & eukaryotes
- Contain modified bases
  - Post-transcriptional modification
- Structure includes ss & ds regions

tRNA

- Cloverleaf & 3-D models

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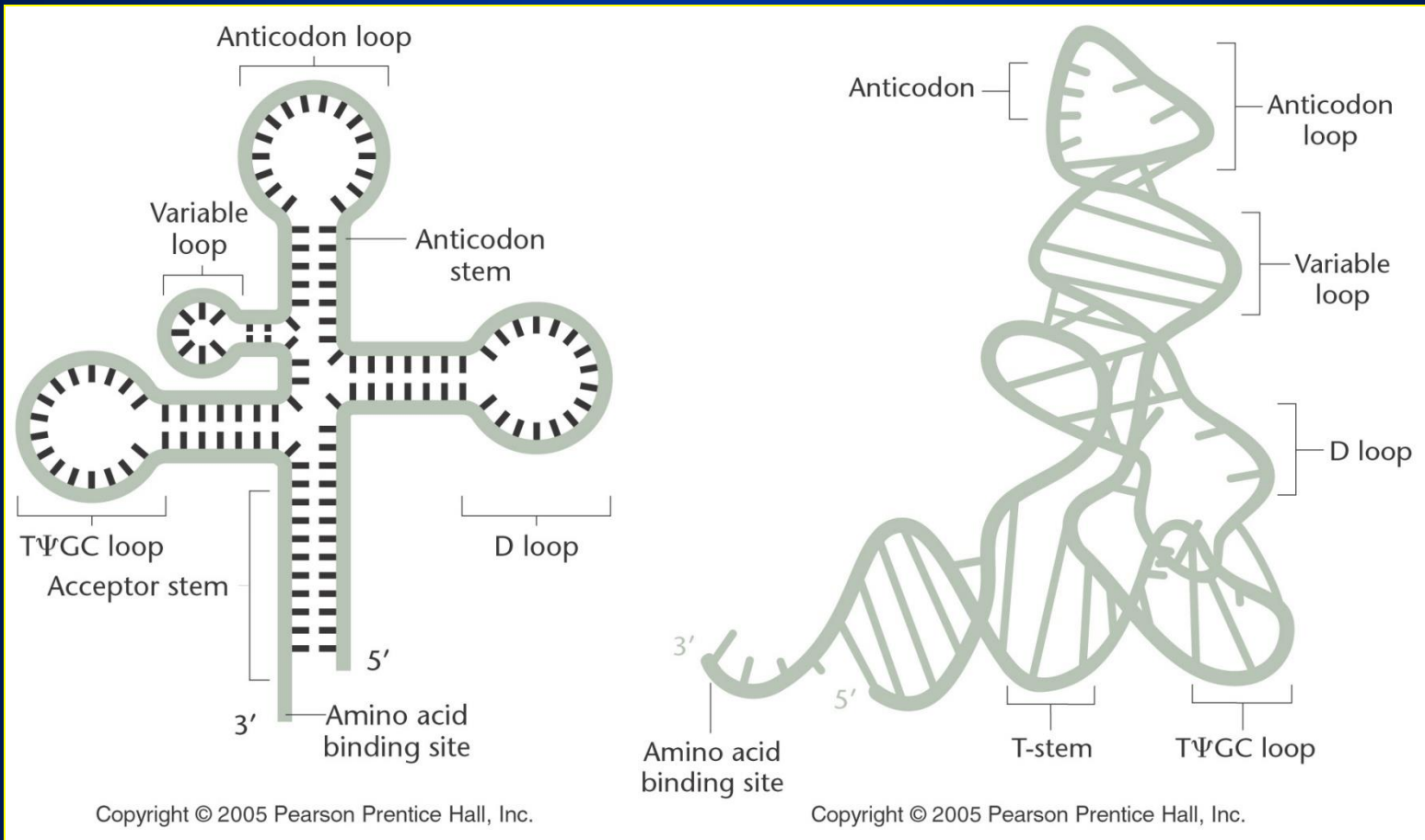
Figs. 14-3 & 4

The Wobble Hypothesis

- Theoretically need 61 tRNAs
  - Actually have:
    - 30-40 in prokaryotes
    - 50 in eukaryotes
- Fewer tRNAs than codons
- "Relaxed" base pairing allows 1 tRNA to bind multiple codons but still deliver correct amino acid

The Wobble Hypothesis

- More practical aspect of code degeneracy
  - 1st & 2nd positions typically conserved
  - 3rd position variable; "relaxed" base pairing

TABLE 13.4 Codon–Anticodon Base-Pairing Rules

Base at First Position (5′, end) of tRNA	Base at Third Position (3′, end) of mRNA
A	U
C	G
G	C or U
U	A or G
I	A, U, or C

mRNA

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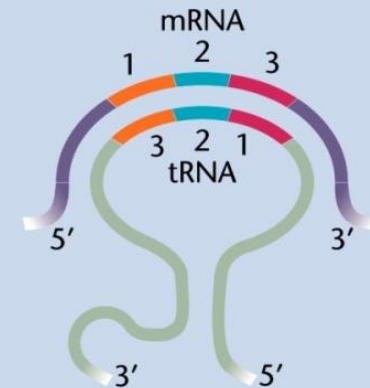
True or false: one kind of amino acid can be carried by more than one kind of tRNA

- A) True
- B) False

TRANSLATION

Translation components

Fig. 14-6

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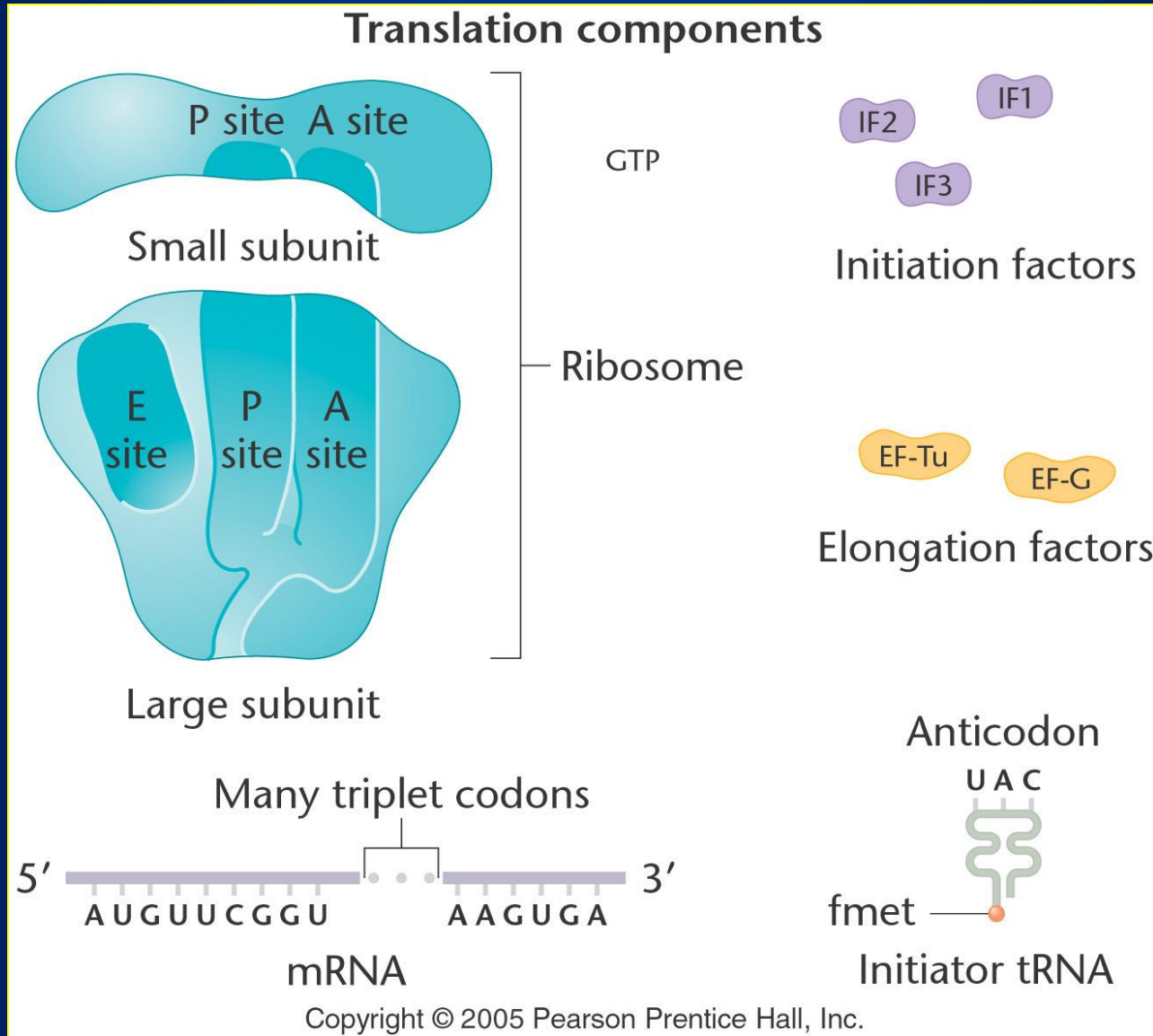
Initiation

- **Small ribosomal subunit associates with initiation factors (IFs) & GTP**
  - Stabilize small subunit

- **Binds mRNA near start codon (AUG)**
  - Prokaryotes
    - Preceeded by Shine-Dalgarno sequence (6 purines)
  - Eukaryotes
    - Kozak sequence (-ACCAUGG-)

Initiation

Step 1. mRNA binds to small subunit along with initiation factors (IF1, 2, 3)

Initiation complex

Step 2. Initiator tRNA^{fmet} binds to mRNA codon in P site; IF3 released

Step 3. Large subunit binds to complex; IF1 and IF2 released; EF-Tu binds to tRNA, facilitating entry into A site

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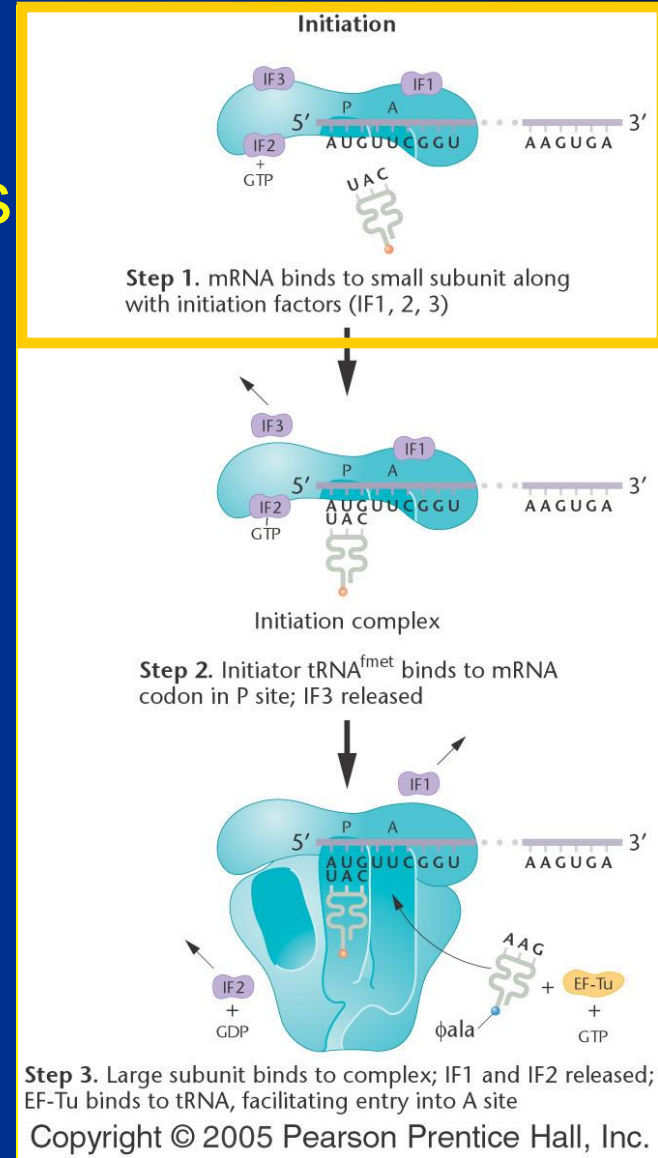
Fig. 14-6

Initiation

- Initiator tRNA binds start codon
  - Start = aa "methionine"
    - Prokaryotes (fmet)
    - Eukaryotes (met)
  - Oriented at "P" site
  - Sets reading frame

Initiation

Step 1. mRNA binds to small subunit along with initiation factors (IF1, 2, 3)

Initiation complex

Step 2. Initiator tRNA^{fmet} binds to mRNA codon in P site; IF3 released

Step 3. Large subunit binds to complex; IF1 and IF2 released; EF-Tu binds to tRNA, facilitating entry into A site
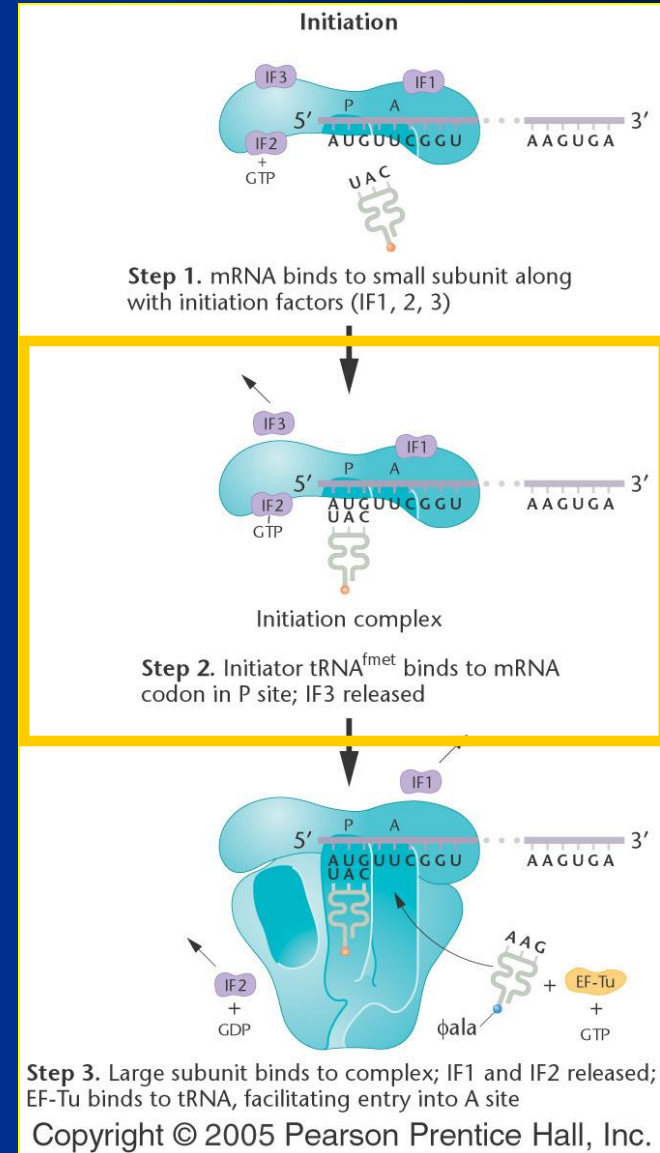
Initiation

- **Initiator tRNA binds start codon**
  - Start = aa "methionine"
    - Prokaryotes (fmet)
    - Eukaryotes (met)
  - Oriented at "P" site
  - Sets reading frame

- **Large ribosomal subunit binds & IFs released**

Initiation

Step 1. mRNA binds to small subunit along with initiation factors (IF1, 2, 3)

Initiation complex

Step 2. Initiator tRNA^{fmet} binds to mRNA codon in P site; IF3 released

Step 3. Large subunit binds to complex; IF1 and IF2 released; EF-Tu binds to tRNA, facilitating entry into A site
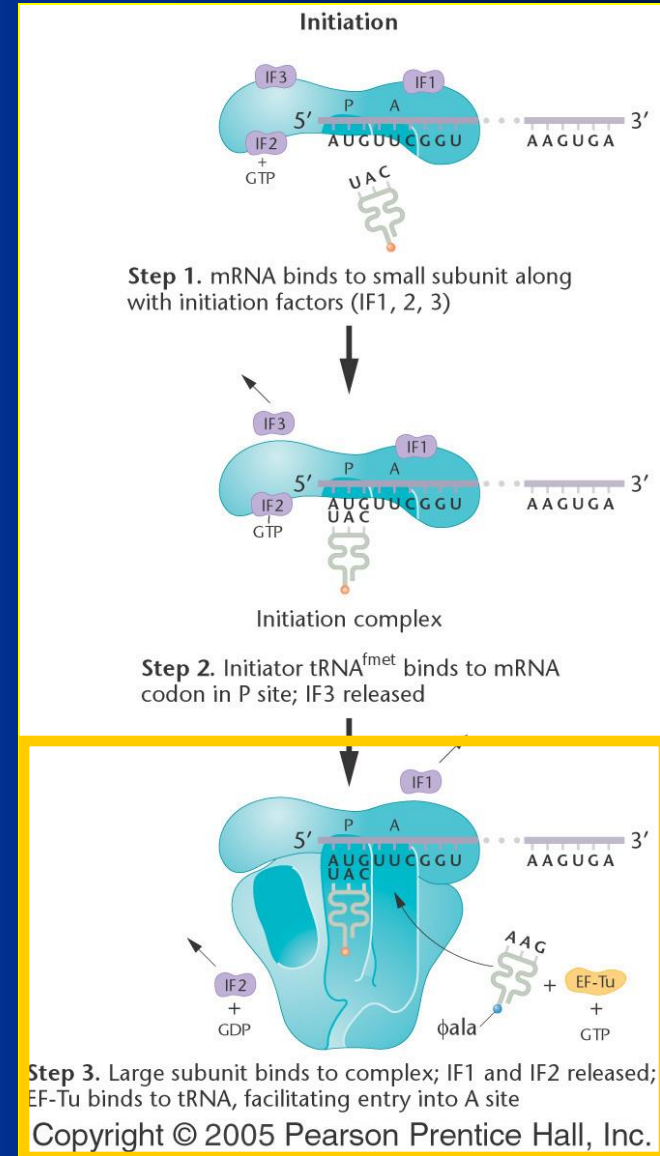
Elongation

- 2nd tRNA binds to "A" site
  - Aided by elongation factor (EF-Tu)

- Peptide bond formation
  - Peptidyl transferase
  - Part of large subunit

- Uncharged tRNA discharged from P to E site
  - Ejected

Elongation

Step 1. Second charged tRNA has entered A site, facilitated by EF-Tu; first elongation step commences

Peptide bond catalyzed by peptidyl transferase

Step 2. Dipeptide bond forms; uncharged tRNA moves to E-site and then out of ribosome

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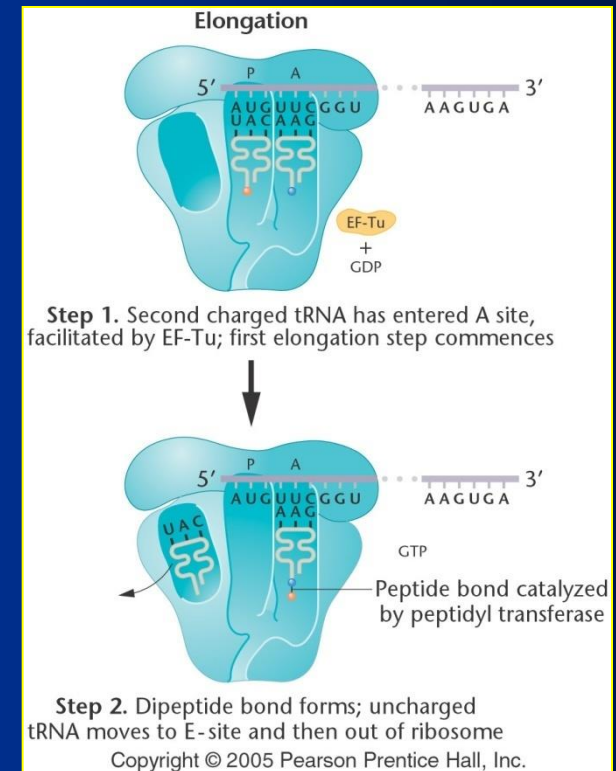
Fig. 14-7

Elongation

- **Translocation of ribosome**
  - Ribosome shifts
  - tRNA containing growing peptide now in P site
  - Involves EF-G & GTP hydrolysis

Step 3. mRNA has shifted by three bases; EF-G facilitates the translocation step; first elongation step completed

Step 4. Third charged tRNA has entered A site, facilitated by EF-Tu; second elongation step begins

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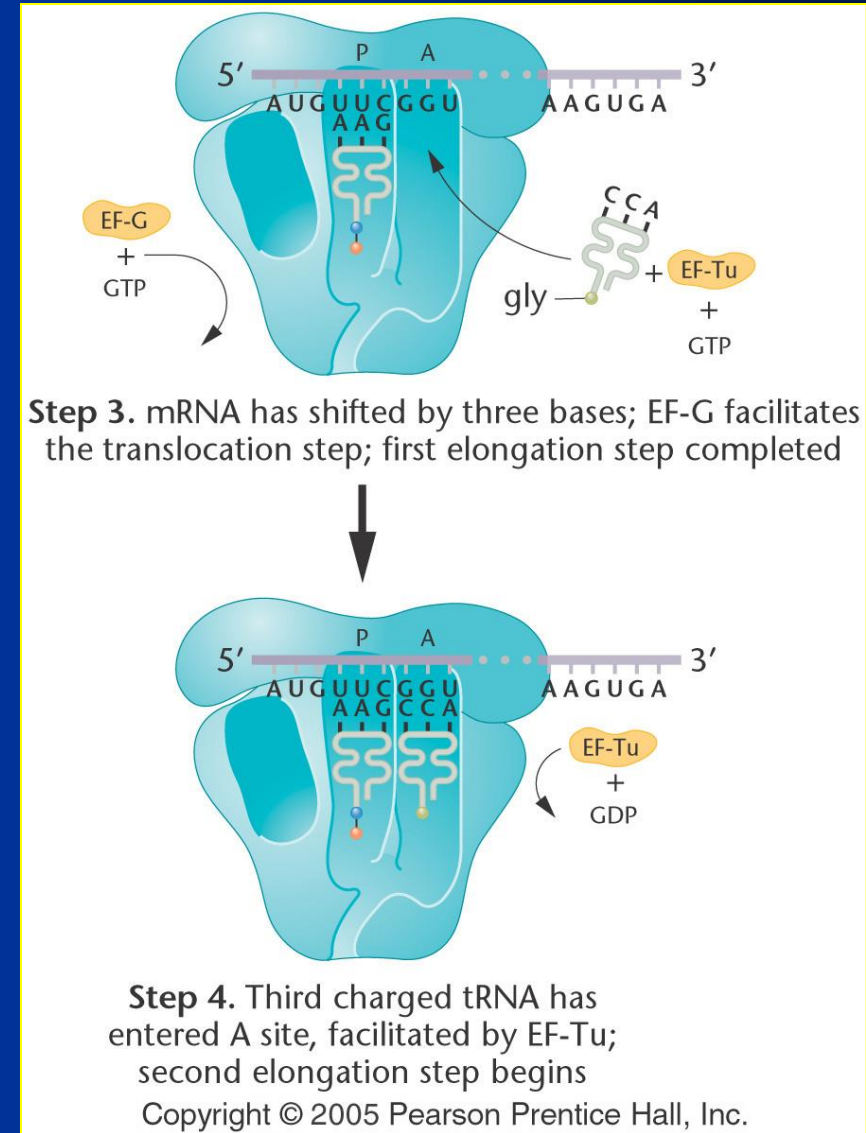
Fig. 14-7

Elongation

- Repeats until stop codon reached

Step 5. Tripeptide formed; second elongation step completed; uncharged tRNA moves to E site

Step 6. Polypeptide chain synthesized and exiting ribosome

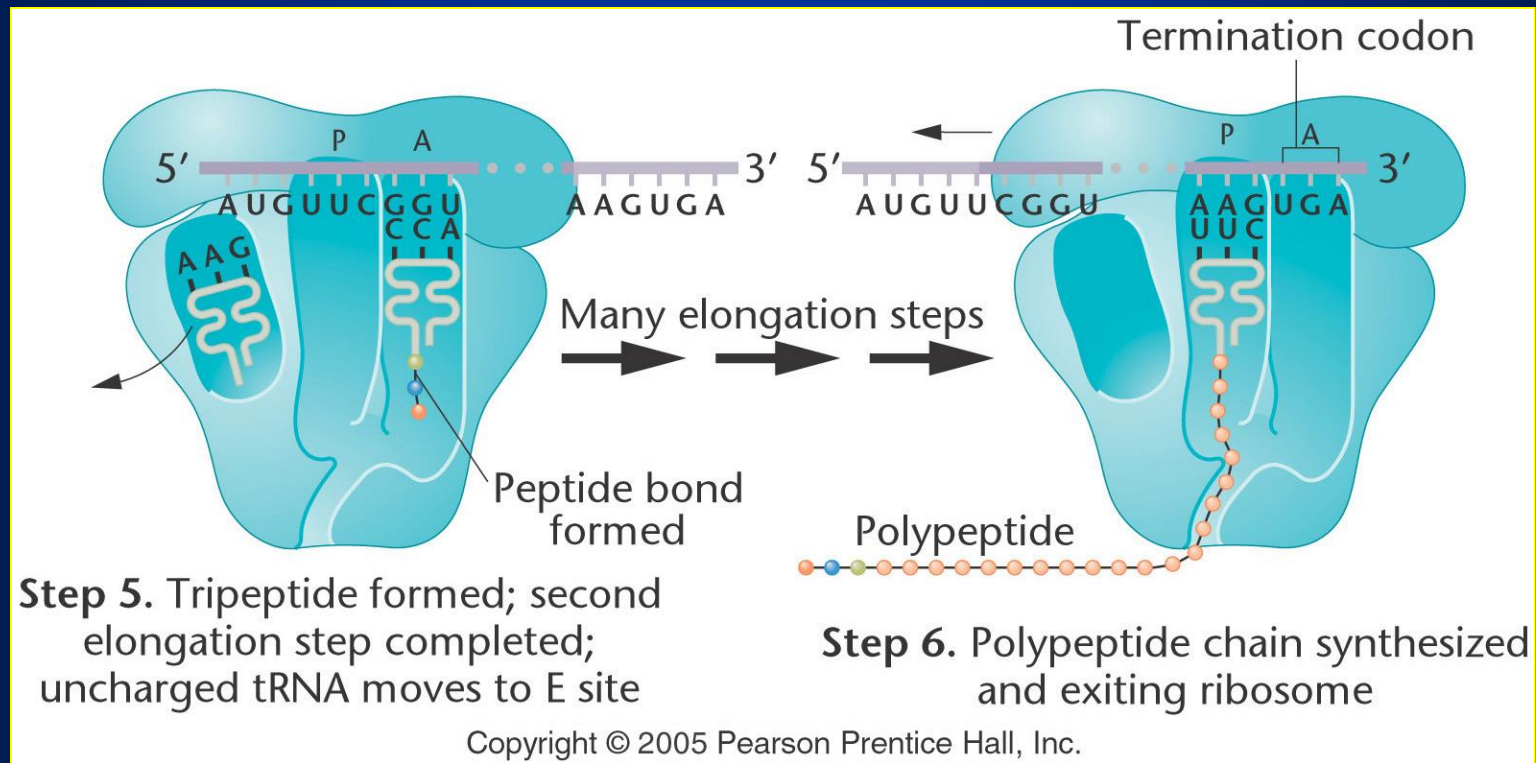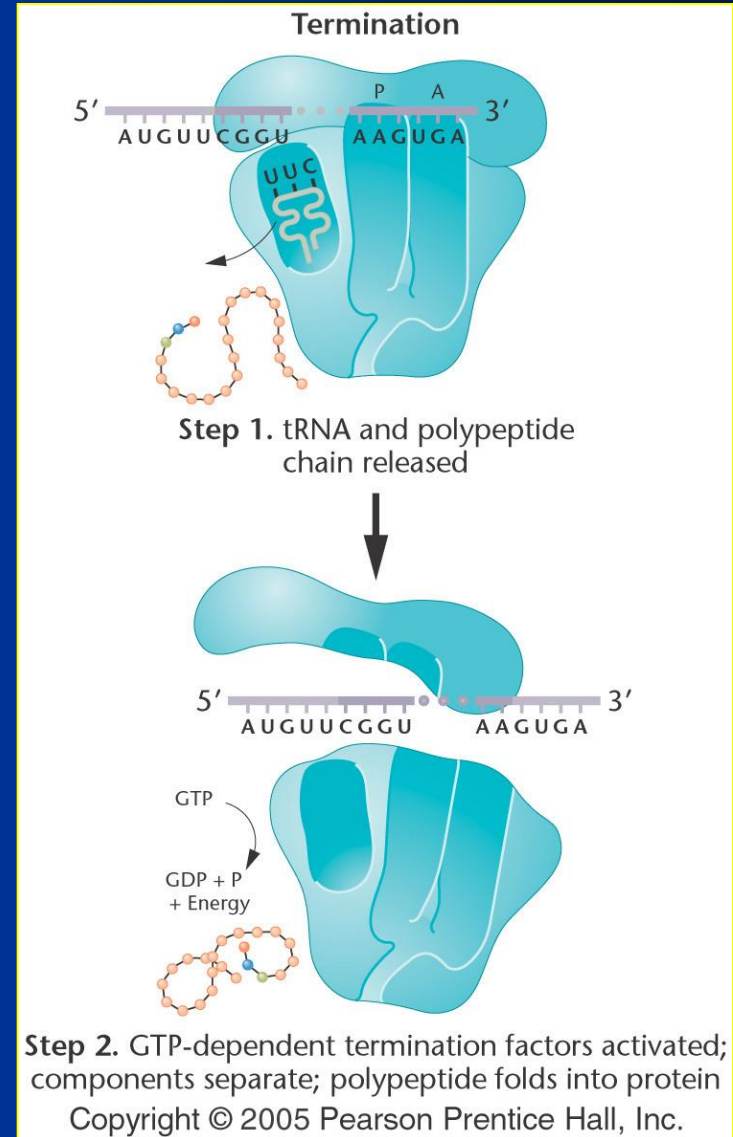Copyright © 2005 Pearson Prentice Hall, Inc.

Fig. 14-7

Termination

- ## Signaled by termination sequence
  - ### UAA, UAG, UGA
  - ### No tRNA ∴ empty A site
- ## Release factors cleave polypeptide
  - ### Requires GTP
  - ### Components dissociate

Termination

5′ A U G U U C G G U   P   A   3′
                       A A G U G A
U U C

Step 1. tRNA and polypeptide chain released

5′ A U G U U C G G U   A A G U G A   3′

GTP

GDP + P + Energy

Step 2. GTP-dependent termination factors activated; components separate; polypeptide folds into protein

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Fig. 14-7

Which of these sites in a ribosome contains the growing peptide chain?

- A) A site
- B) E site
- C) P site

Which amino acid is always the first incorporated into a peptide in eukaryotes?

- A) Cysteine
- B) Phenylalanine
- C) Proline
- D) Methionine

Post-translational Modification

- Removal of N & C terminal amino acids
- Modification of aa residues
- Addition of carbohydrate side chains
- Trimming of polypeptide
- Removal of signal sequences
- Assembly of subunits together

Translation

- Polysome: Several ribosomes can move in tandem along a messenger RNA to form a translation unit
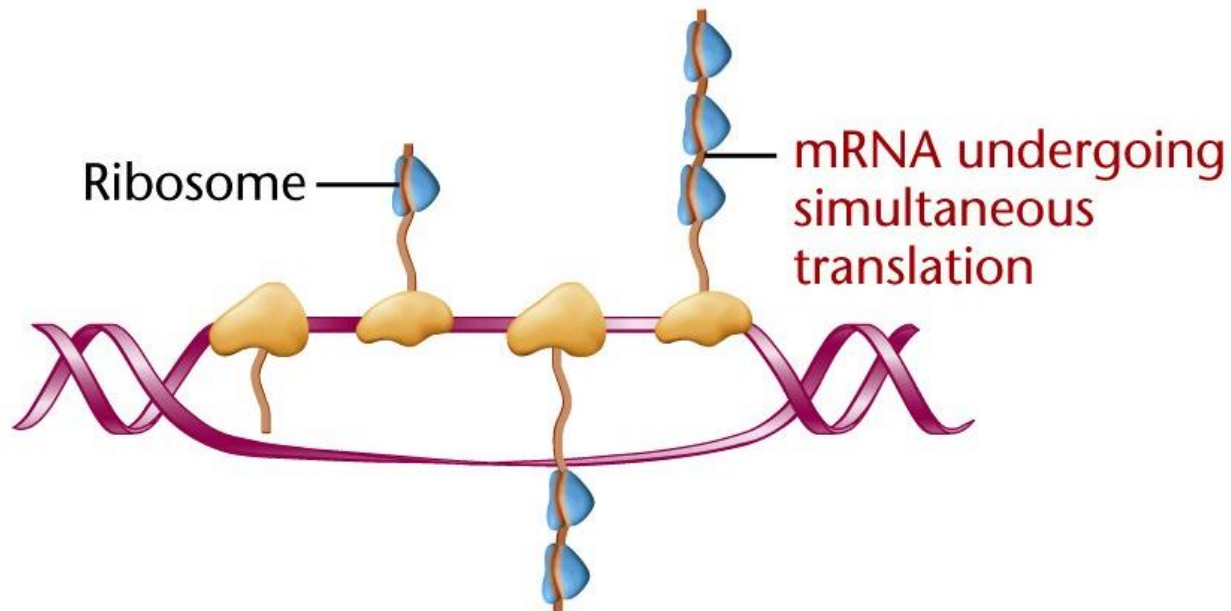

Ribosome

mRNA undergoing simultaneous translation

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True or false: a protein is complete once it is released from a ribosome.

- A) True
- B) False