Chapter 12

Genes: Expression and Regulation

DNA Transcription or RNA Synthesis

- produces three types of RNA
  - tRNA
    - carries amino acids during protein synthesis
  - rRNA
    - component of ribosomes
  - mRNA
    - directs protein synthesis

Transcription in Procaryotes

- polygenic mRNA
  - contains directions for > 1 polypeptides

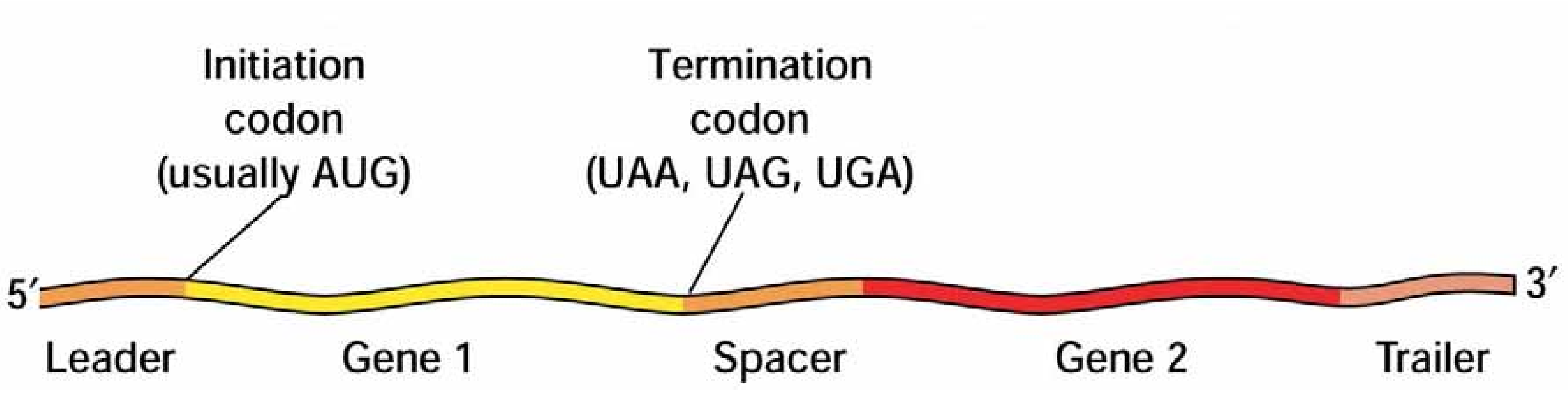


Figure 12.1

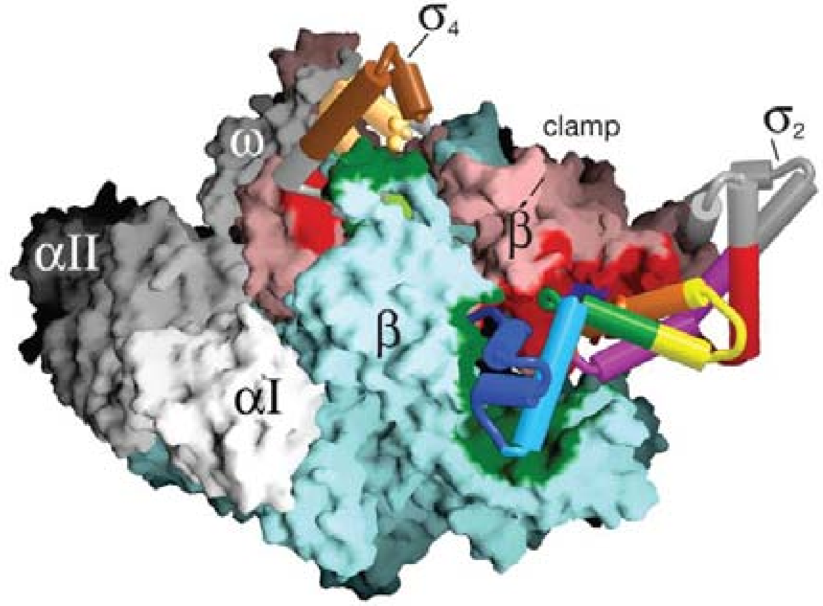
Transcription in procaryotes…

- catalyzed by a single RNA polymerase
  - large multi-subunit enzyme

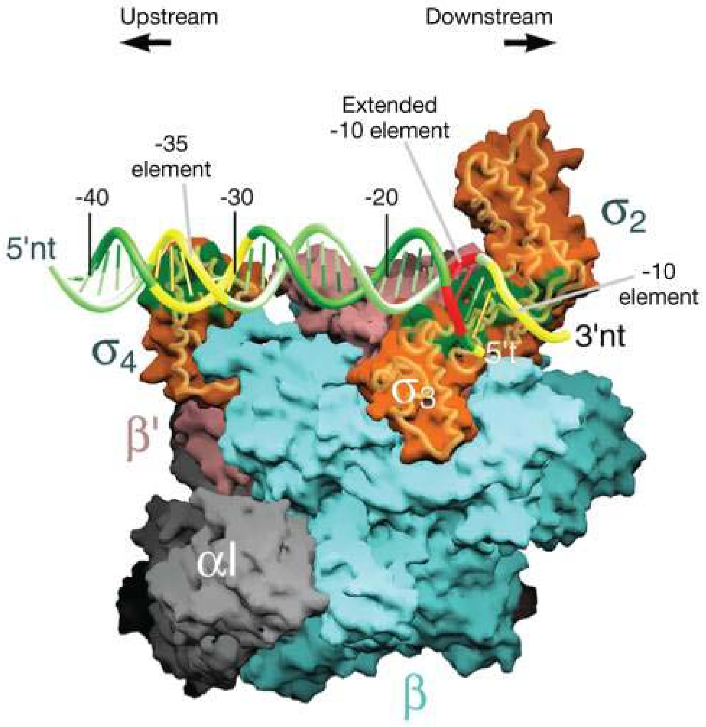
Transcription in procaryotes…

- *E. coli* RNA polymerase
  - core enzyme = $\alpha_2\beta\beta'$
  - holoenzyme = core enzyme + sigma factor (directs core enzyme to promoter)
- *Thermus aquaticus* RNA polymerase
  - core enzyme = $\alpha_2\beta\beta'\omega$
  - holoenzyme = core enzyme + sigma factor

Thermus aquaticus RNA polymerase



(a)



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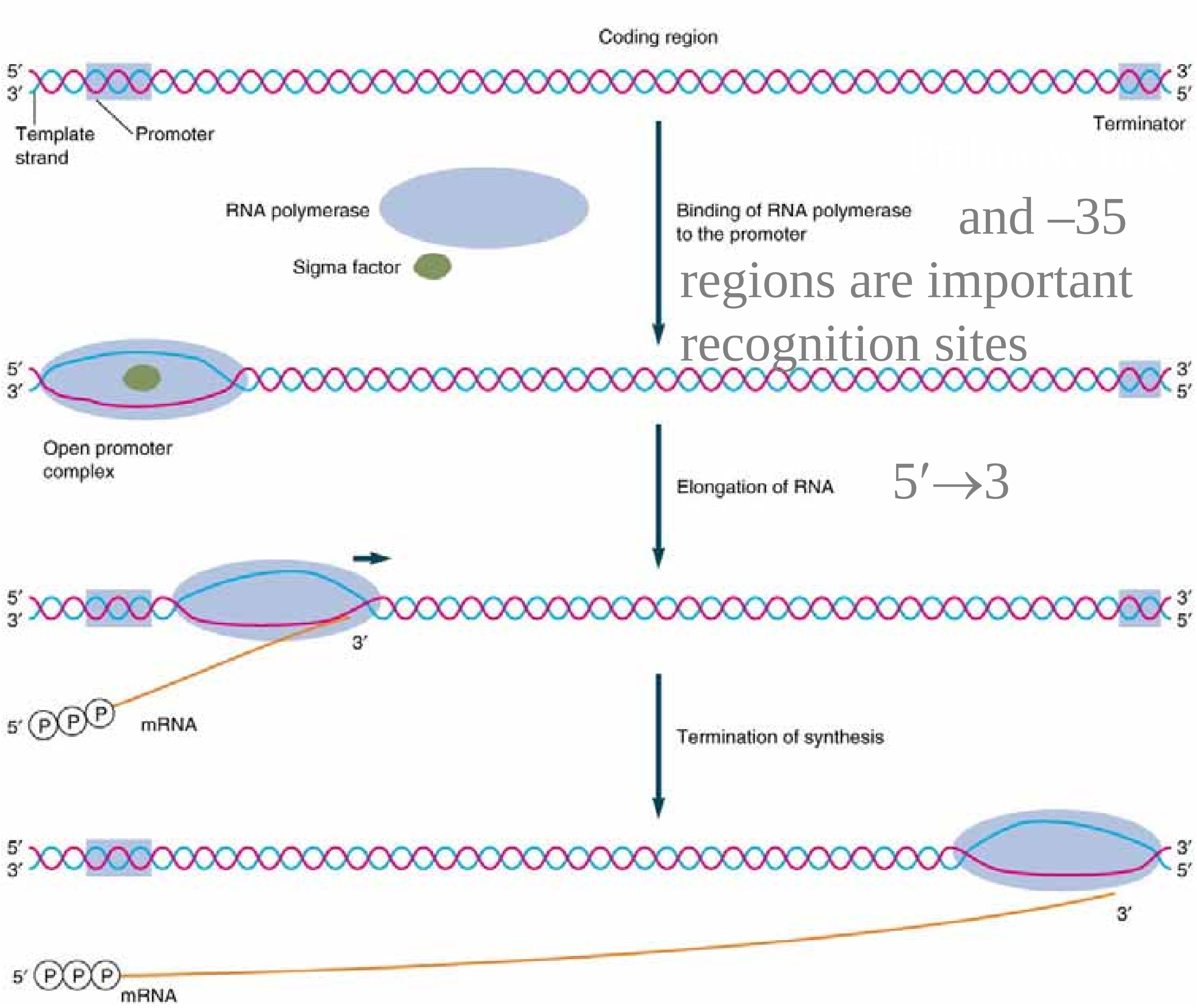


and –35 regions are important recognition sites

5′→3

Figure 12.2

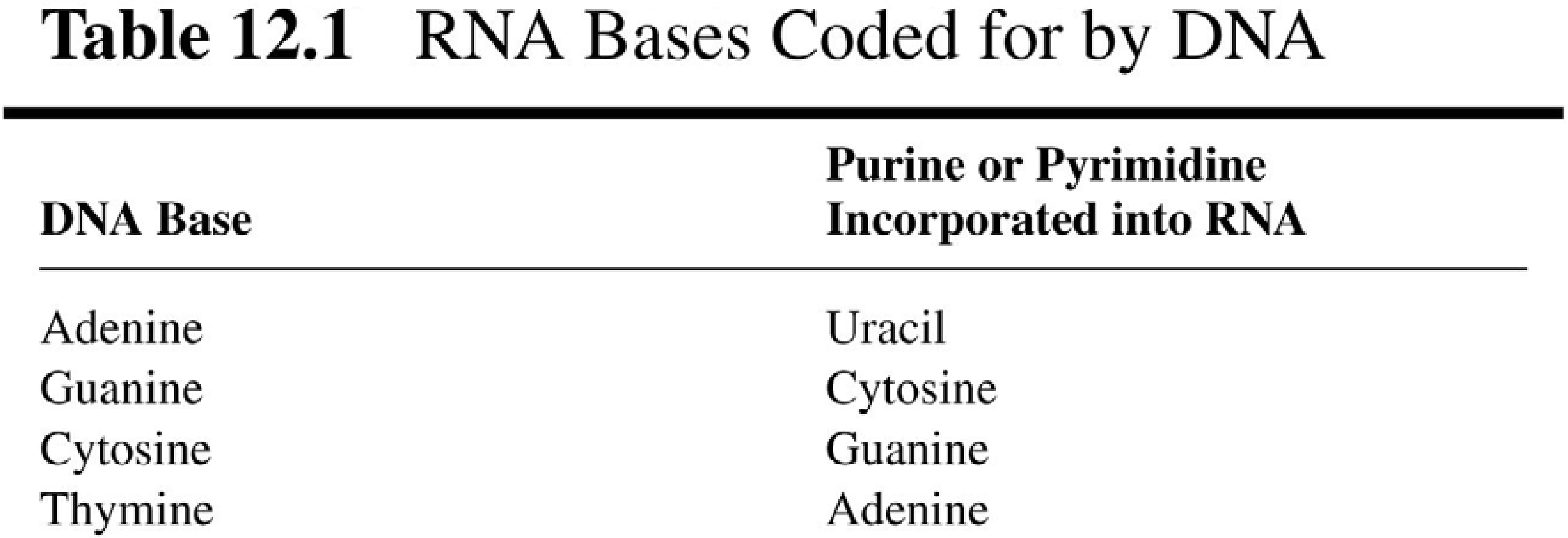
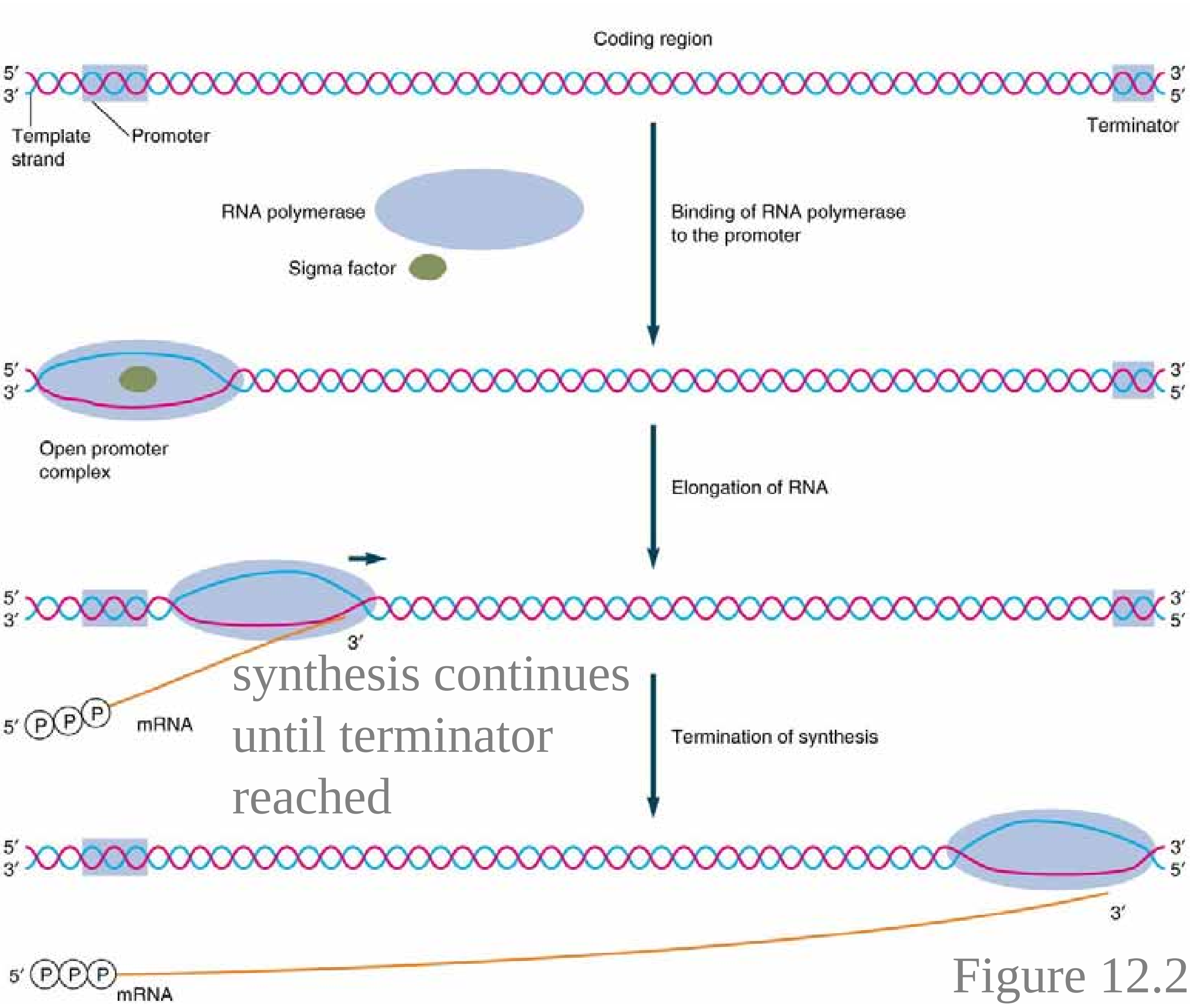


Table 12.1 RNA Bases Coded for by DNA

DNA Base	Purine or Pyrimidine Incorporated into RNA
Adenine	Uracil
Guanine	Cytosine
Cytosine	Guanine
Thymine	Adenine



synthesis continues until terminator reached

Figure 12.2

Procaryotic terminators

- two types
  - hairpin + 6 uridines
  - rho factor-dependent
    - lack polyU and often lack hairpin

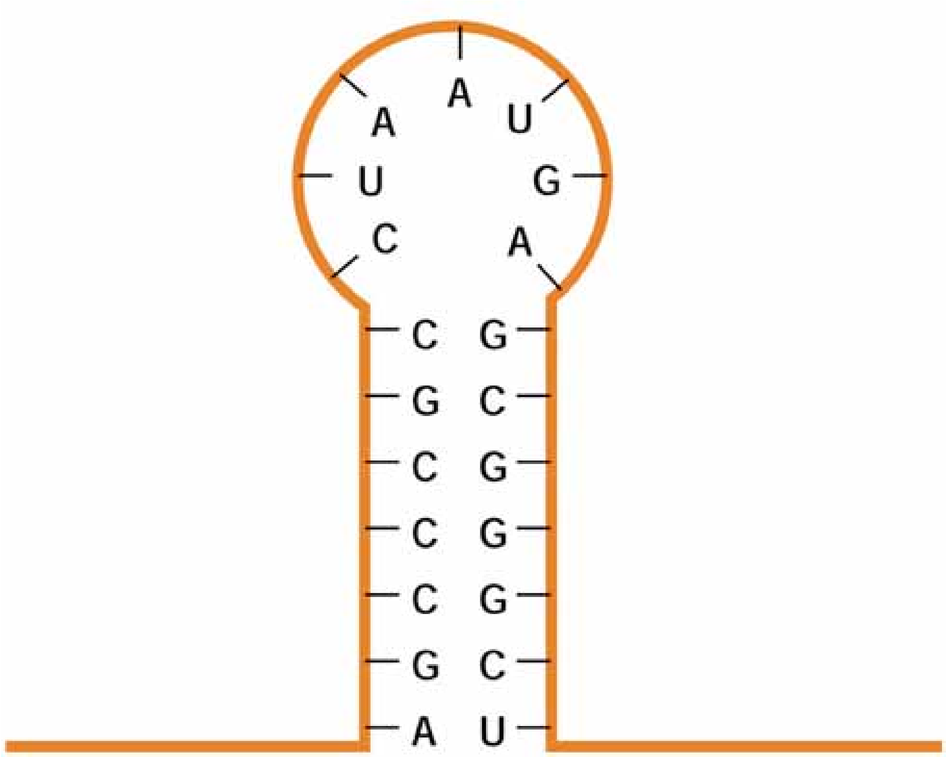


Figure 12.4

Transcription in Eucaryotes

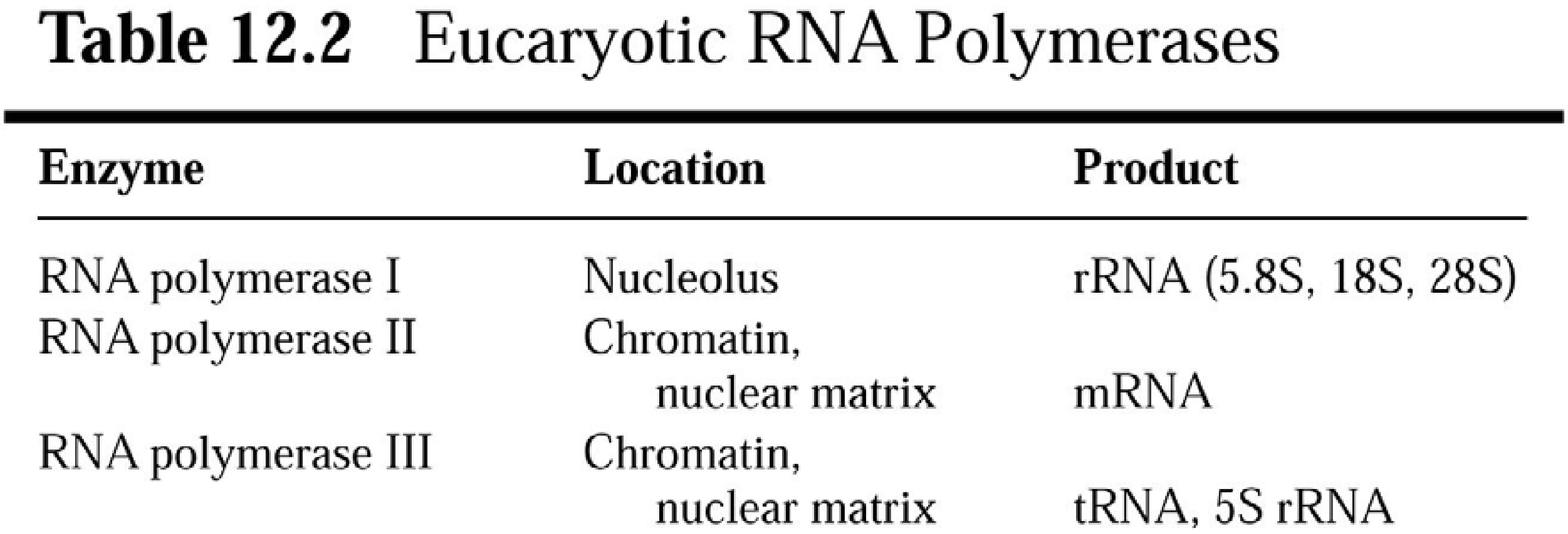
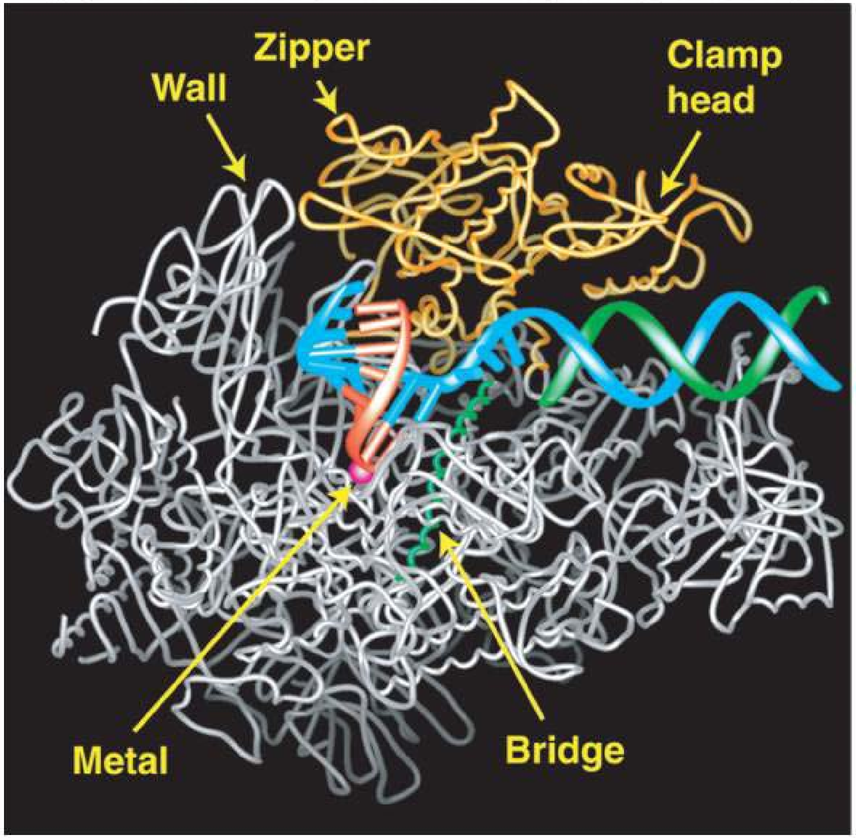


Table 12.2 Eucaryotic RNA Polymerases

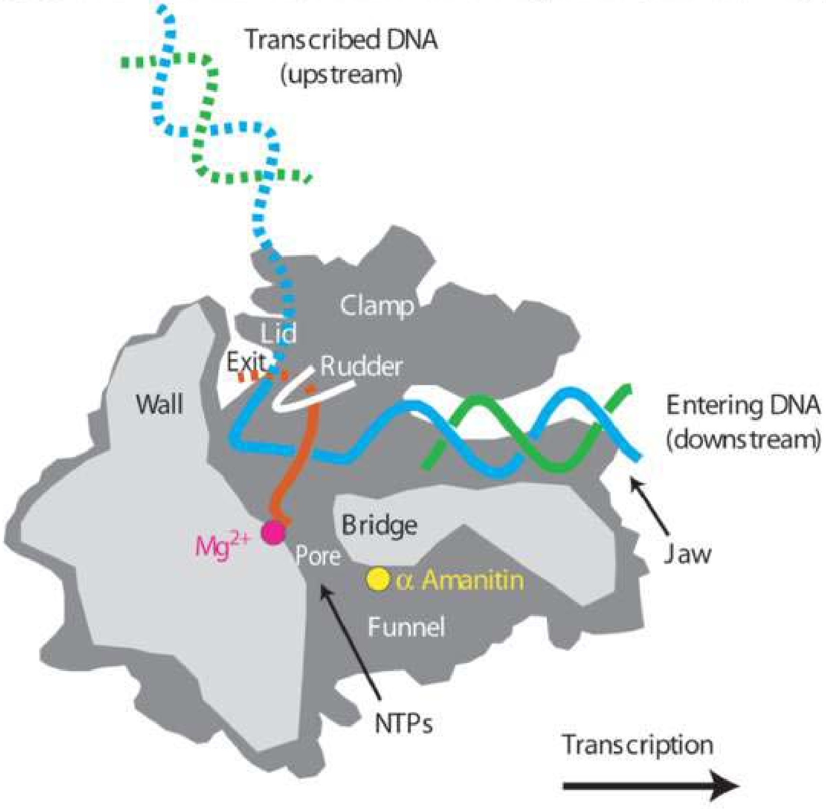
Enzyme	Location	Product
RNA polymerase I	Nucleolus	rRNA (5.8S, 18S, 28S)
RNA polymerase II	Chromatin, nuclear matrix	mRNA
RNA polymerase III	Chromatin, nuclear matrix	tRNA, 5S rRNA

- monogenic mRNA

Yeast RNA polymerase II



(c)



(d)

Transcription in eucaryotes…

- promoters contain three common elements
  - TATA box ~ 30 bases before transcription start
  - CAAT box ~ 75 bases before transcription start
  - GC box ~ 90 bases before transcription start

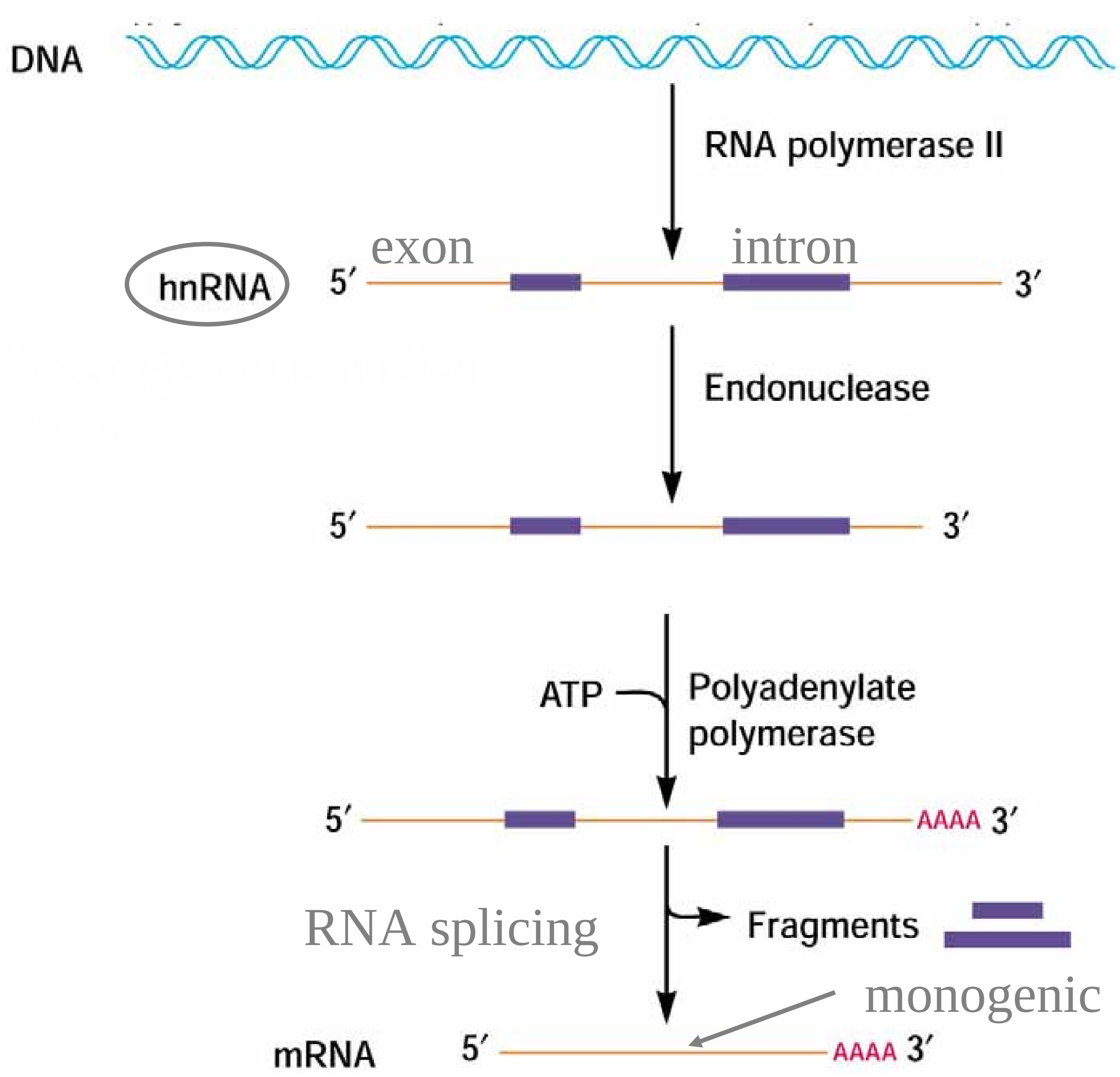
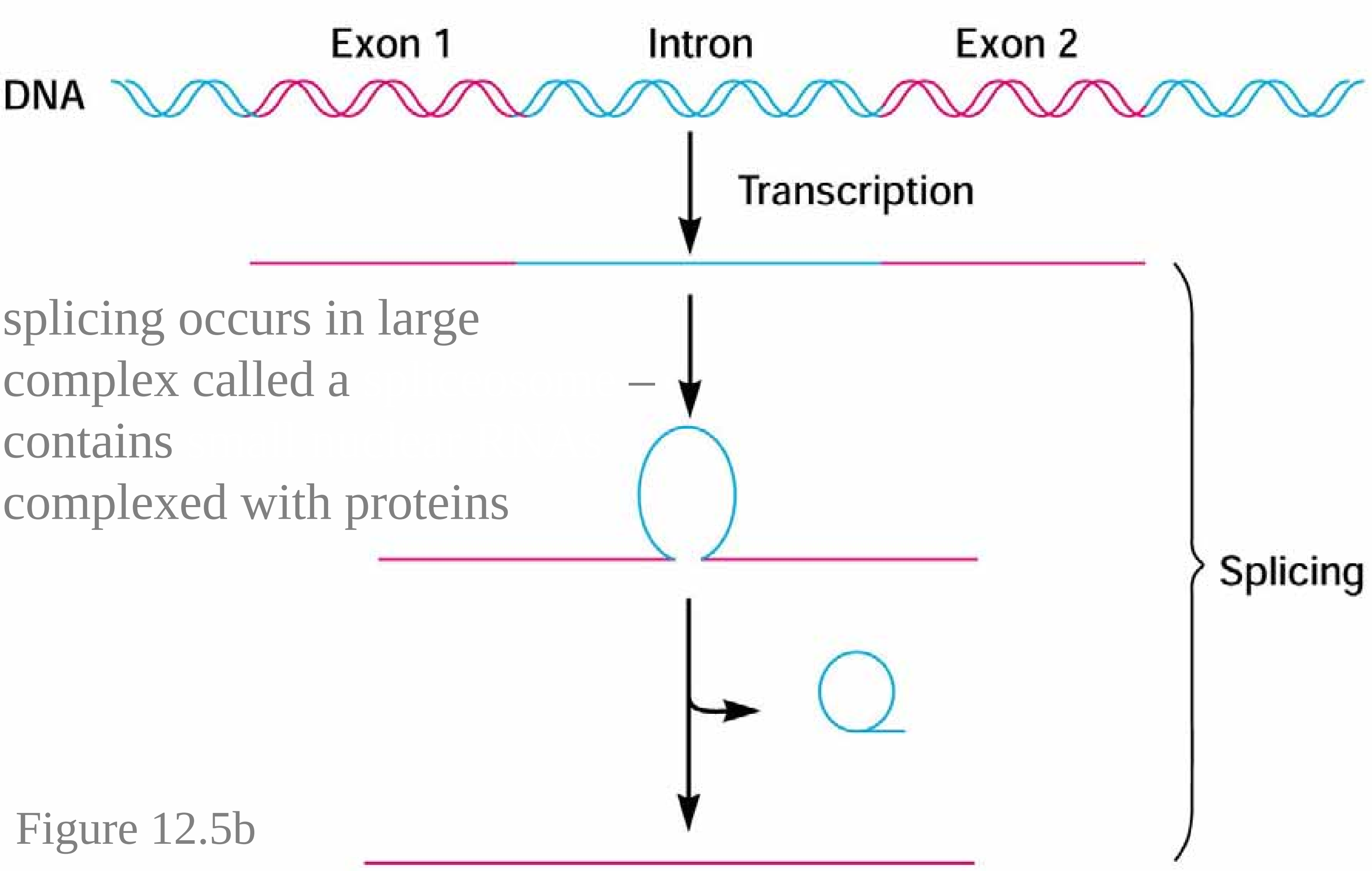

DNA
RNA polymerase II
exon
intron
hnRNA
5′
3′
heterogeneous nuclear RNA
Endonuclease
5′
3′
ATP
Polyadenylate polymerase
5′
AAAA 3′
RNA splicing
Fragments
monogenic
mRNA
5′
AAAA 3′

Figure 12.5a

RNA splicing



splicing occurs in large complex called a spliceosome – contains small nuclear RNAs complexed with proteins

Figure 12.5b

5′ cap of eucaryotic mRNA

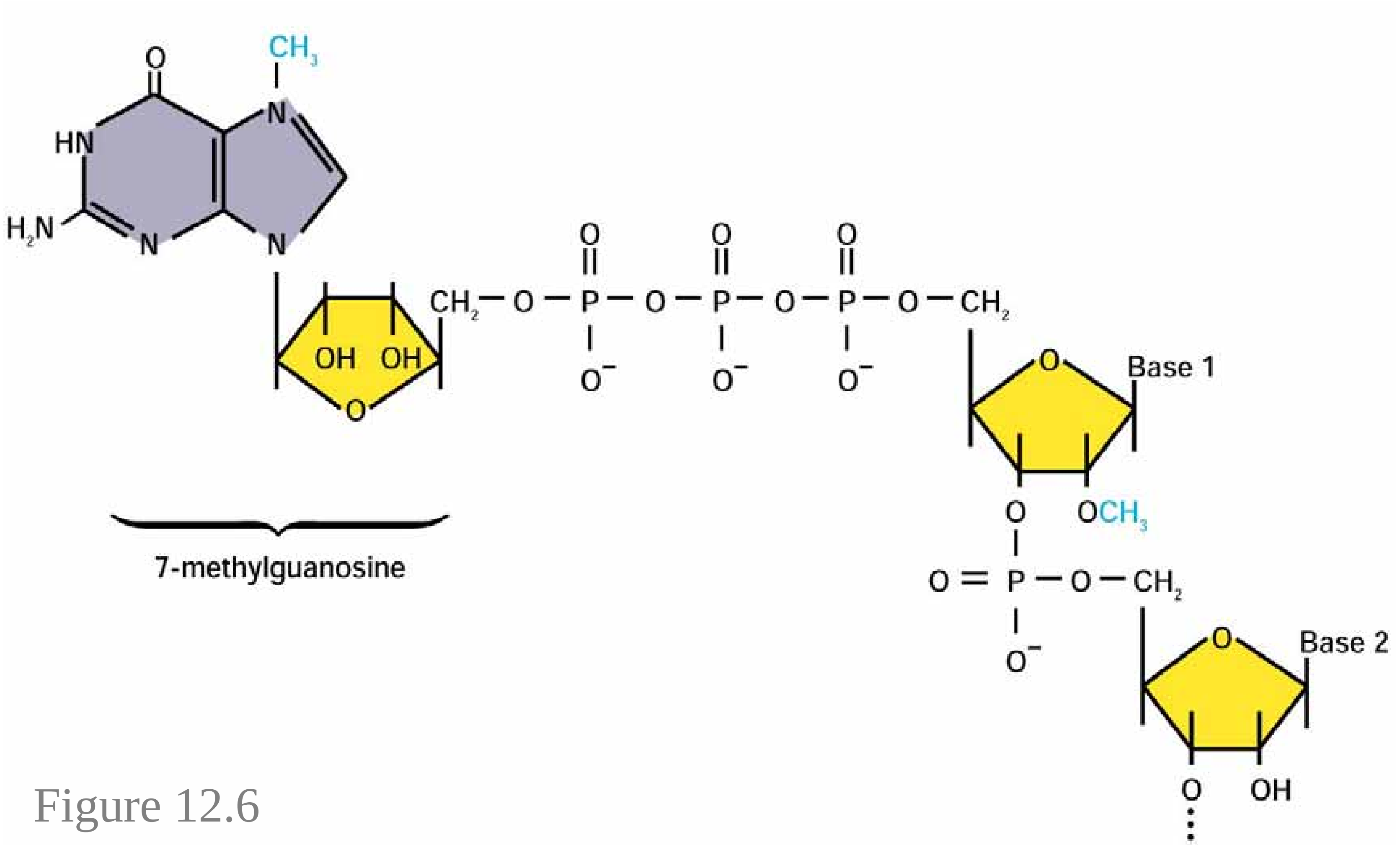
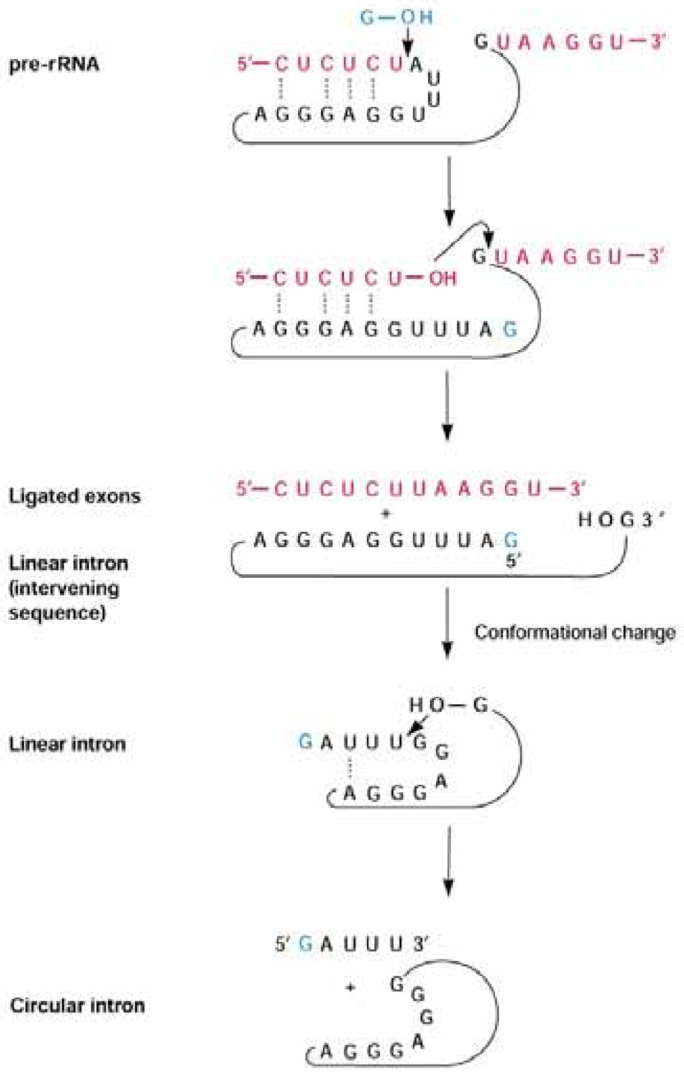


Figure 12.6

Ribozymes

- RNA molecules with catalytic activity
- e.g., self-splicing rRNA molecules



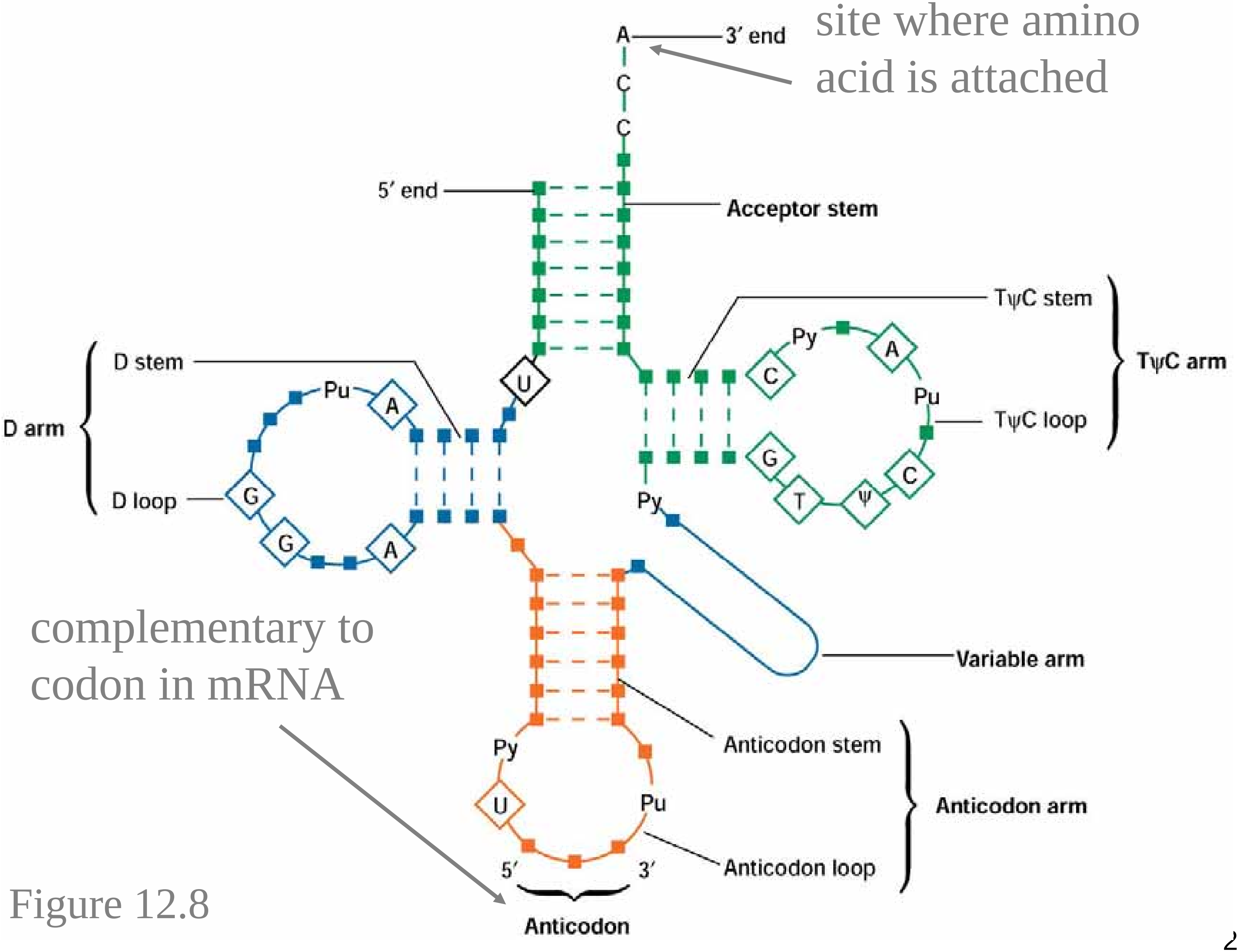
Box 12.1

Protein Synthesis

- translation
  - synthesis of polypeptide directed by sequence of nucleotides in mRNA
- direction of synthesis N terminal → C-terminal
- ribosome
  - site of translation
  - polyribosome – complex of mRNA with several ribosomes

Transfer RNA and Amino Acid Activation

- attachment of amino acid to tRNA
- catalyzed by aminoacyl-tRNA synthetases
  - – at least 20
    - each specific for single amino acid and for all the tRNAs to which each may be properly attached (cognate tRNAs)



site where amino acid is attached

complementary to codon in mRNA

Figure 12.8

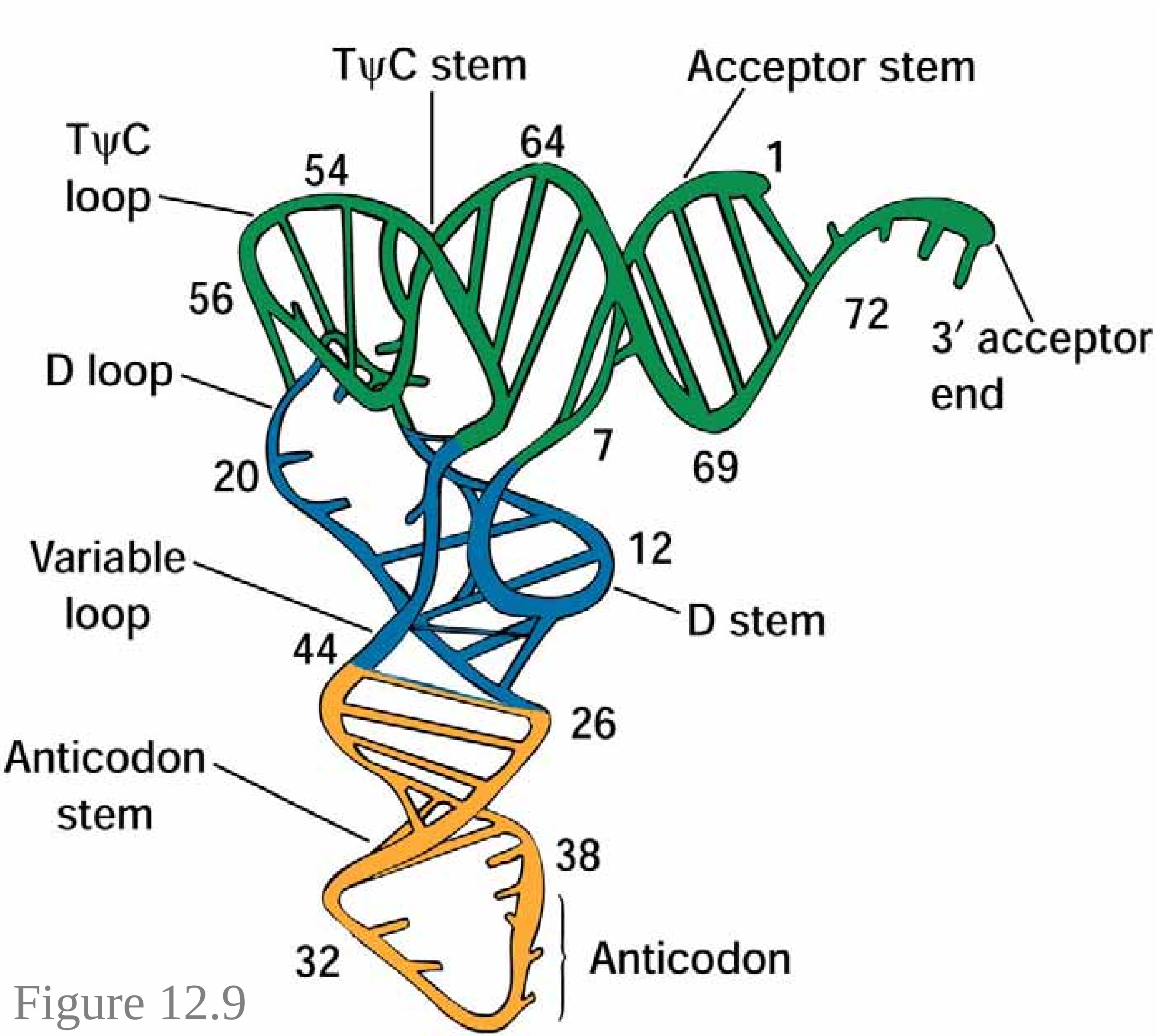

TψC stem
Acceptor stem
TψC loop
64
54
1
56
72
3′ acceptor end
D loop
7
69
20
12
Variable loop
D stem
44
26
Anticodon stem
38
32
Anticodon

Figure 12.9

Aminoacyl-tRNA

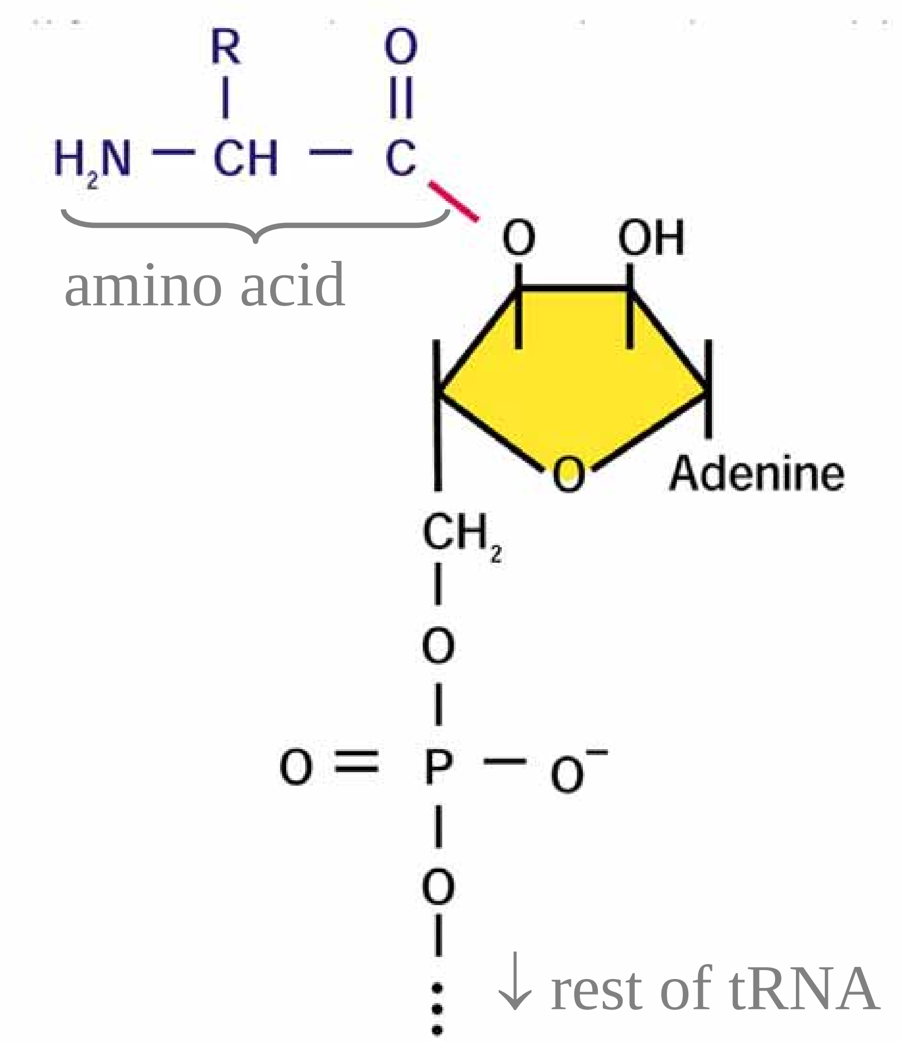
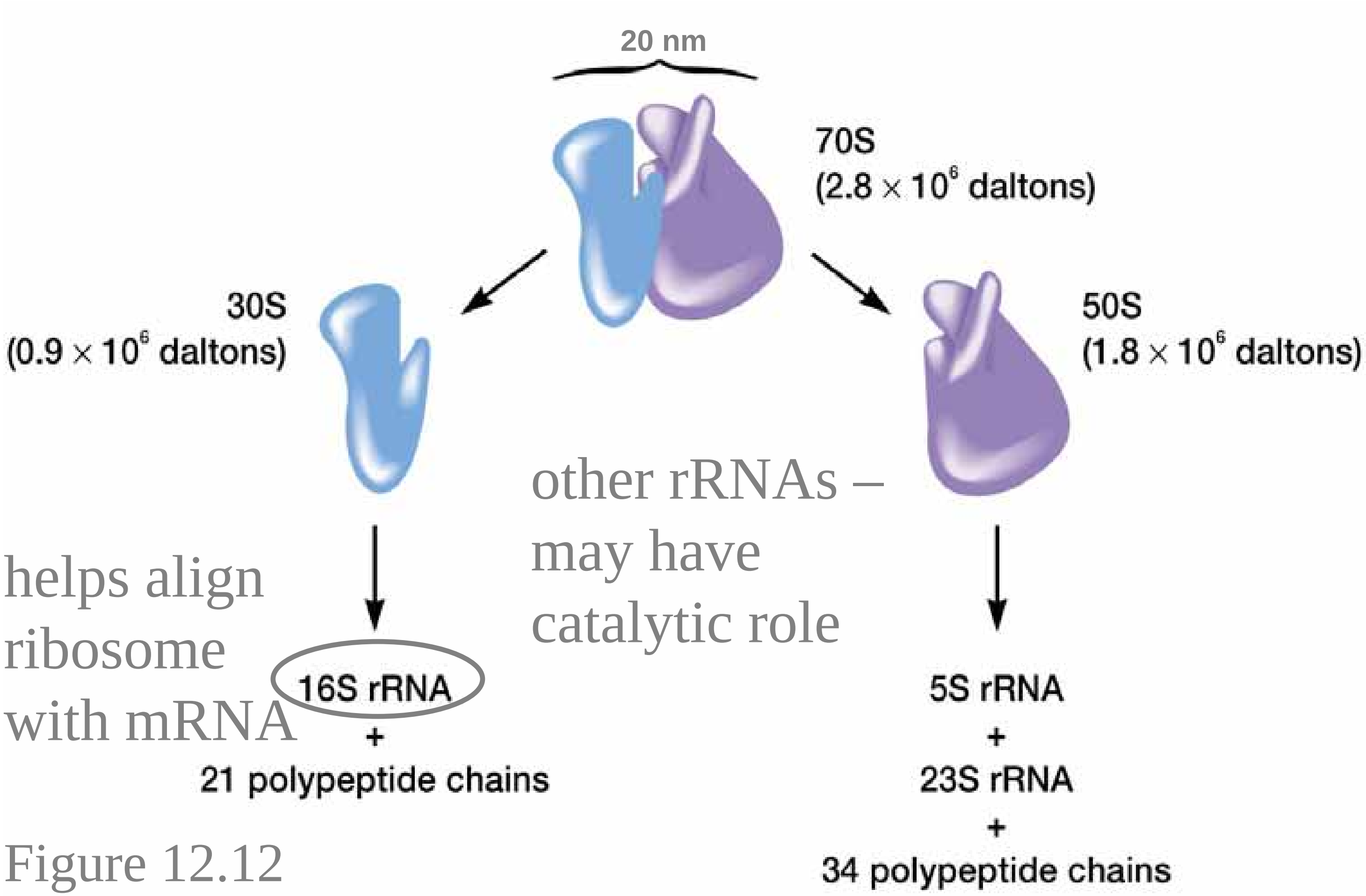


Figure 12.11

The Ribosome

- procaryotes
  - 70S ribosomes = 30S + 50S subunits
- eucaryotes
  - 80S ribosomes = 40S + 60S subunits
  - mitochondrial and chloroplast ribosomes resemble procaryotic ribosomes

The 70S ribosome



helps align ribosome with mRNA

other rRNAs – may have catalytic role

Figure 12.12

E. coli ribosome

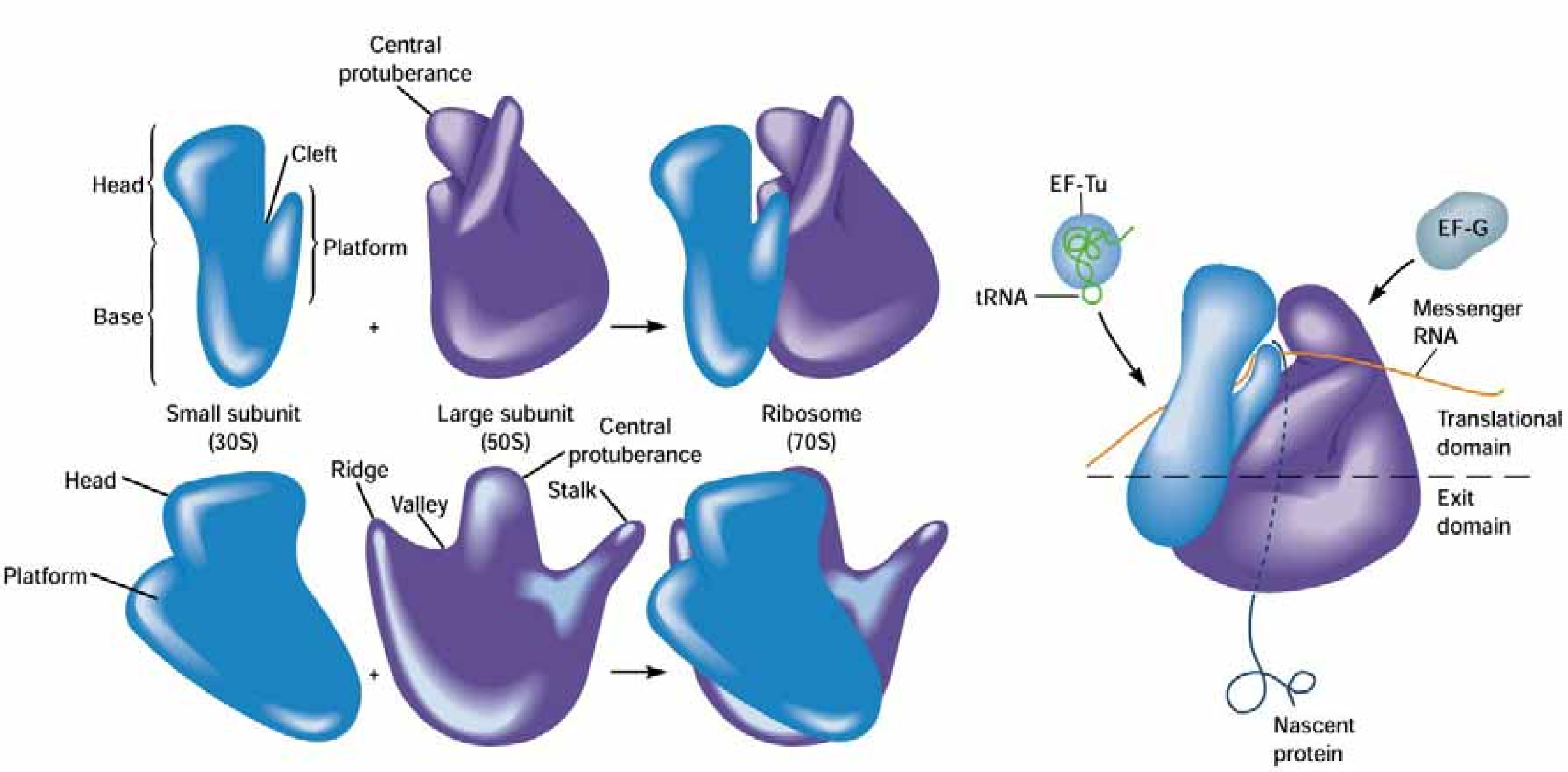
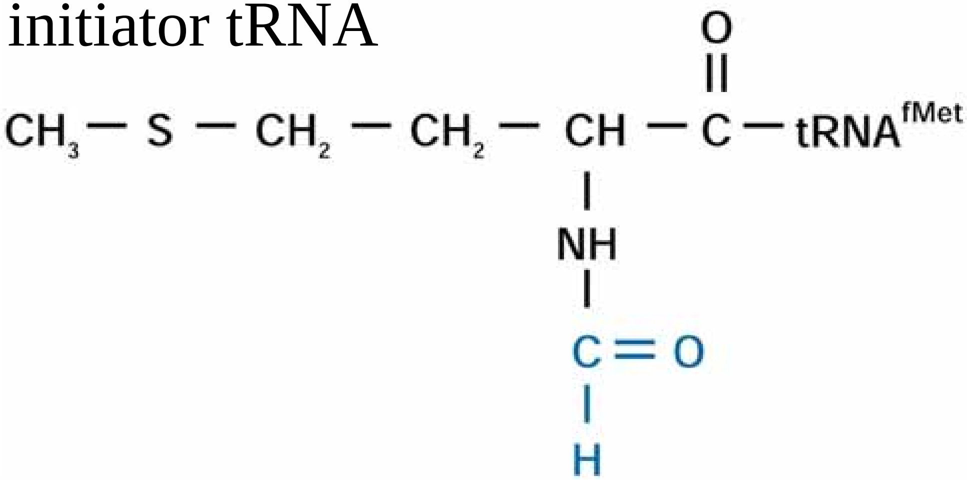


Figure 12.13

Initiation of Protein Synthesis

- involves ribosome subunits and numerous additional molecules
  - initiator tRNA
  - initiation factors (IFs)

N-formylmethionine-tRNA – bacterial initiator tRNA



$$\mathrm{CH_3-S-CH_2-CH_2-\underset{\underset{\underset{\underset{H}{|}}{C=O}}{\underset{|}{\underset{NH}{|}}}}{CH}-\overset{\overset{O}{\|}}{C}-tRNA^{fMet}}$$

Figure 12.14

archaea and eucaryotes use methionine-tRNA

- the goal – position ribosome properly at 5′ end of mRNA

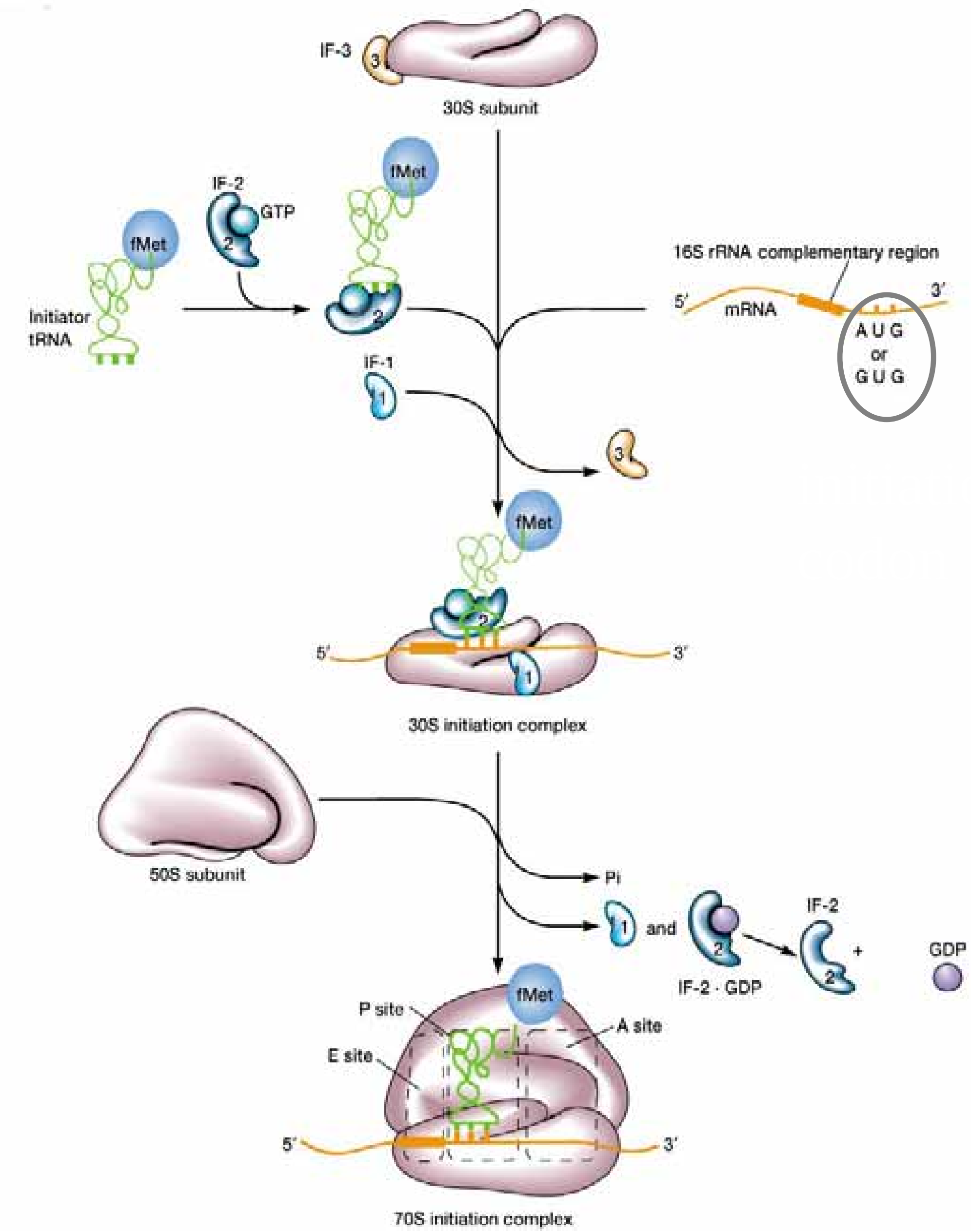


Figure 12.15

Elongation of the Polypeptide Chain

- elongation cycle
  - sequential addition of amino acids to growing polypeptide
  - consists of three phases
    - aminoacyl-tRNA binding
    - transpeptidation reaction
    - translocation
  - involves several elongation factors (EFs)

tRNA binding sites of ribosome

- peptidyl (donor; P) site
  - binds initiator tRNA or tRNA attached to growing polypeptide (peptidyl-tRNA)
- aminoacyl (acceptor; A) site
  - binds incoming aminoacyl-tRNA
- exit (E) site
  - briefly binds empty tRNA before it leaves ribosome

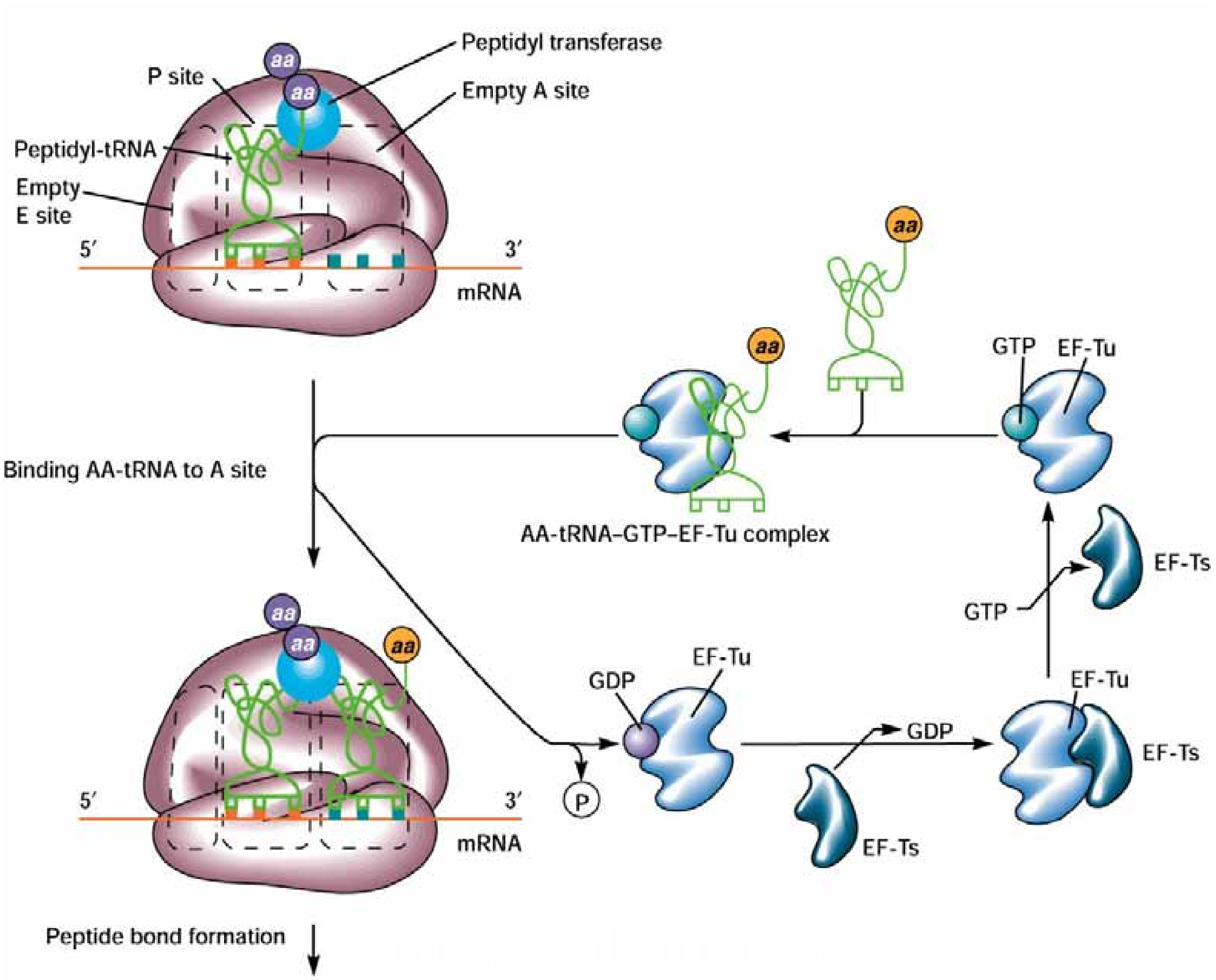
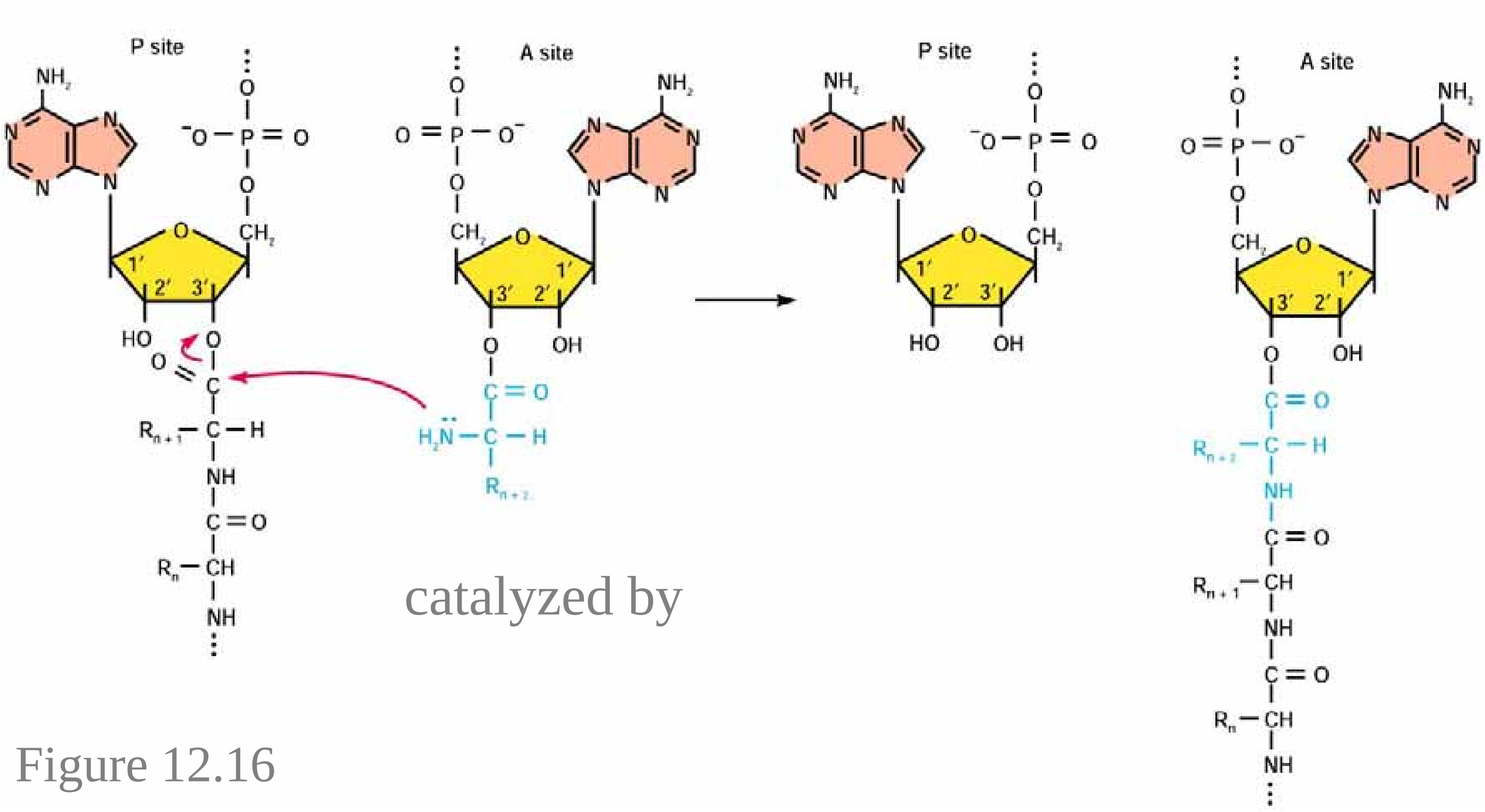

Peptidyl transferase
P site
Empty A site
Peptidyl-tRNA
Empty
E site
5′
3′
mRNA
aa
aa
aa
aa
GTP
EF-Tu
Binding AA-tRNA to A site
AA-tRNA–GTP–EF-Tu complex
EF-Ts
GTP
aa
aa
aa
GDP
EF-Tu
EF-Tu
GDP
EF-Ts
5′
3′
mRNA
P
EF-Ts
Peptide bond formation

Figure 12.16

Transpeptidation reaction



catalyzed by peptidyl transferase

Figure 12.16

translocation – three simultaneous events

1. peptidyl-tRNA moves from A site to P site

2. ribosome moves down one codon

3. empty tRNA leaves P site

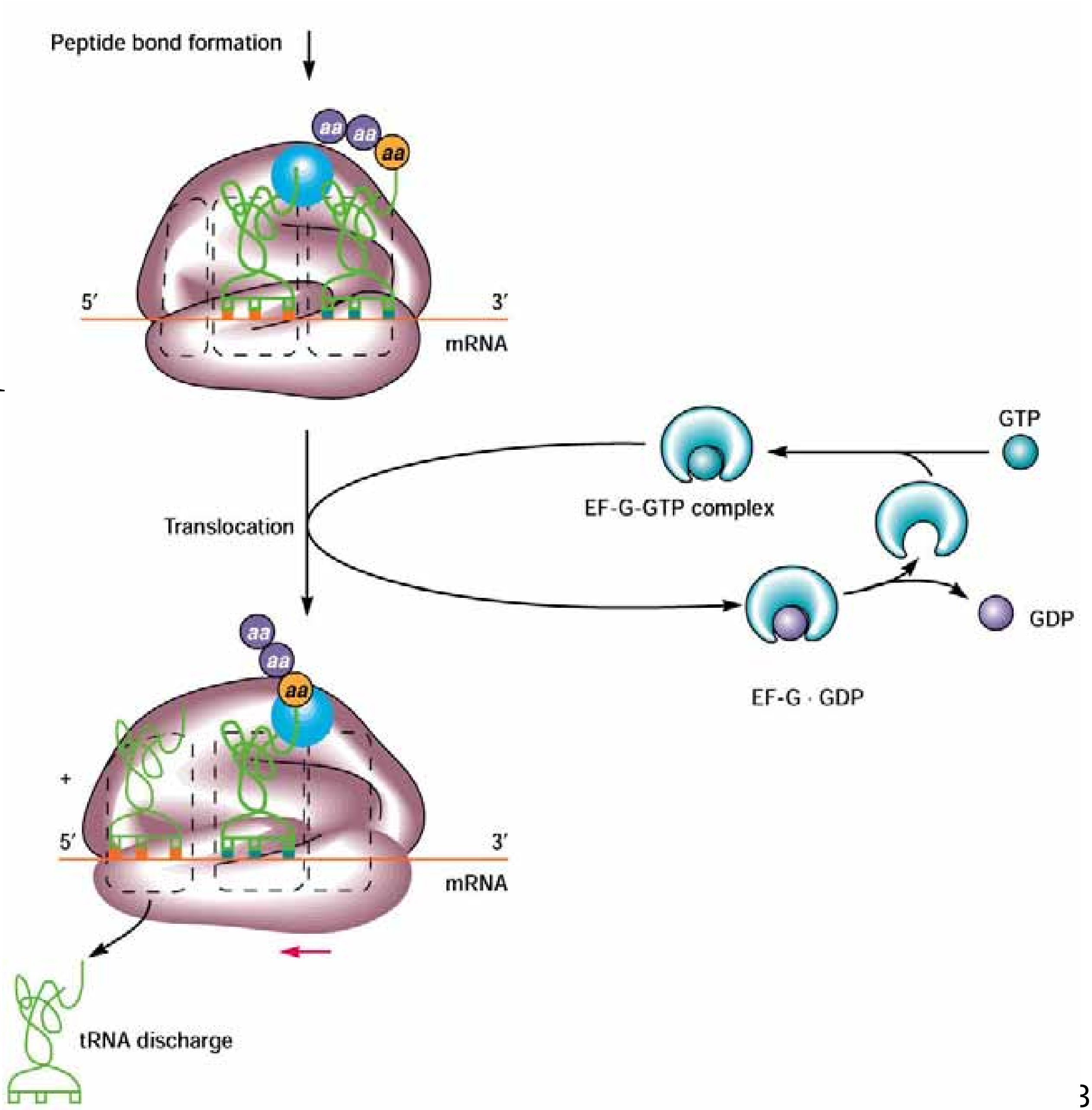


Figure 12.16

Termination of Protein Synthesis

- takes place at any one of three codons
  - nonsense (stop) codons – UAA, UAG, and UGA
- release factors (RFs)
  - aid in recognition of stop codons
  - 3 RFs function in procaryotes
  - only 1 RF active in eucaryotes

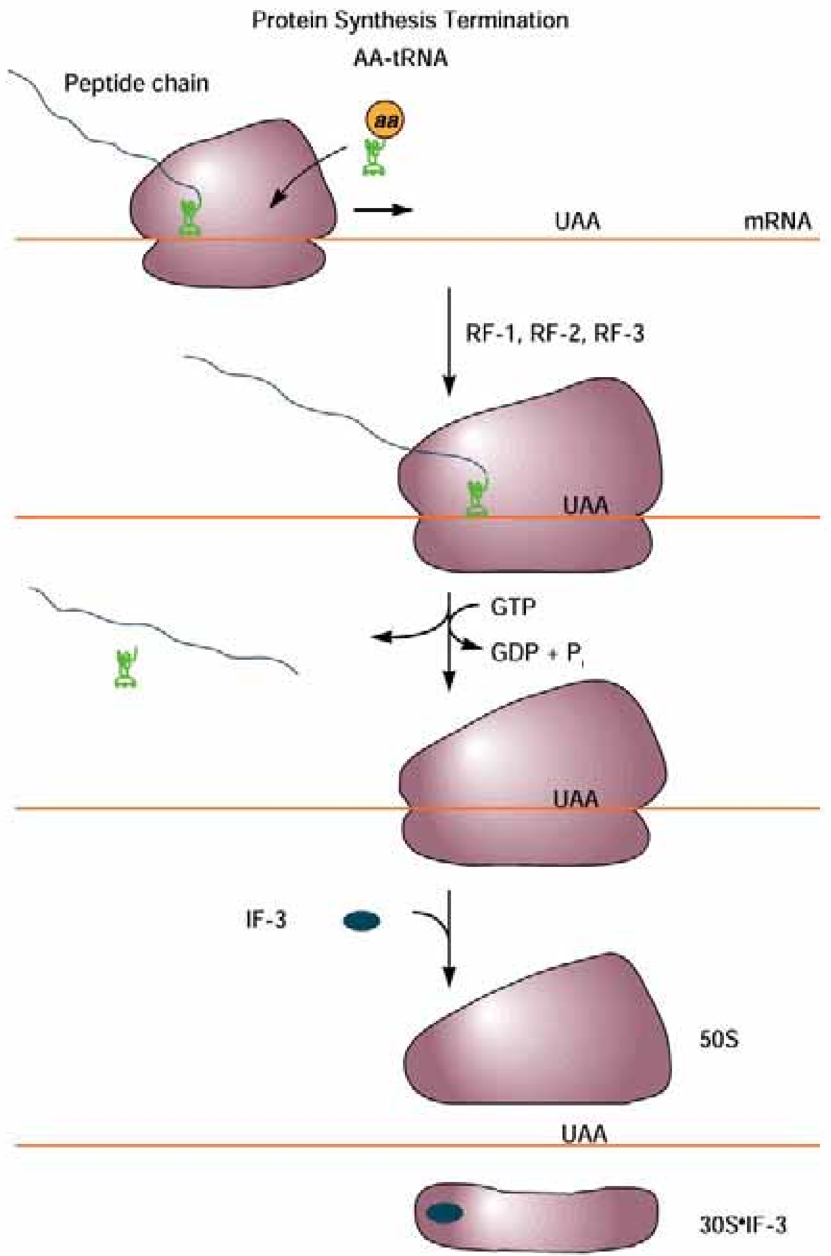

Protein Synthesis Termination
AA-tRNA
Peptide chain
aa
UAA
mRNA
RF-1, RF-2, RF-3
UAA
GTP
GDP + P_i
UAA
IF-3
50S
UAA
30S•IF-3

Figure 12.18

Protein Folding and Molecular Chaperones

- molecular chaperones
  - proteins that aid the folding of nascent polypeptides
  - protect cells from thermal damage
    - e.g., heat-shock proteins
  - aid in transport of proteins across membranes

In bacteria…

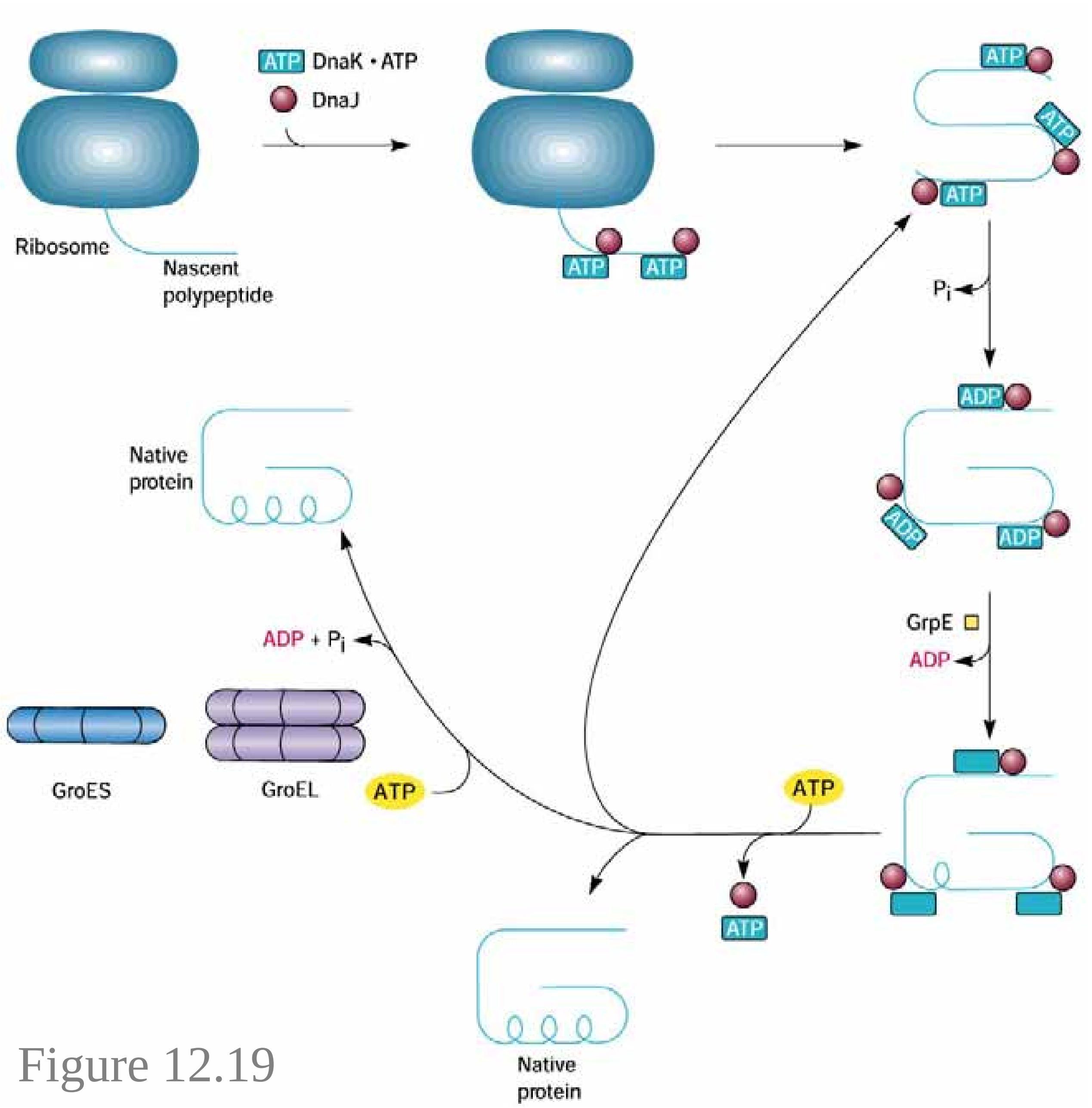


Figure 12.19

Protein folding – eucaryotes versus procaryotes

- domains
  - structurally independent regions of polypeptide
  - separated from each other by less structured portions of polypeptide
- in eucaryotes
  - domains fold independently right after being synthesized
  - molecular chaperones not as important
- in procaryotes
  - polypeptide does not fold until after synthesis of entire polypeptide
  - molecular chaperones play important role

Protein Splicing

- removal of part of polypeptide before folding
- inteins – removed portion
- exteins – portions that remain in protein

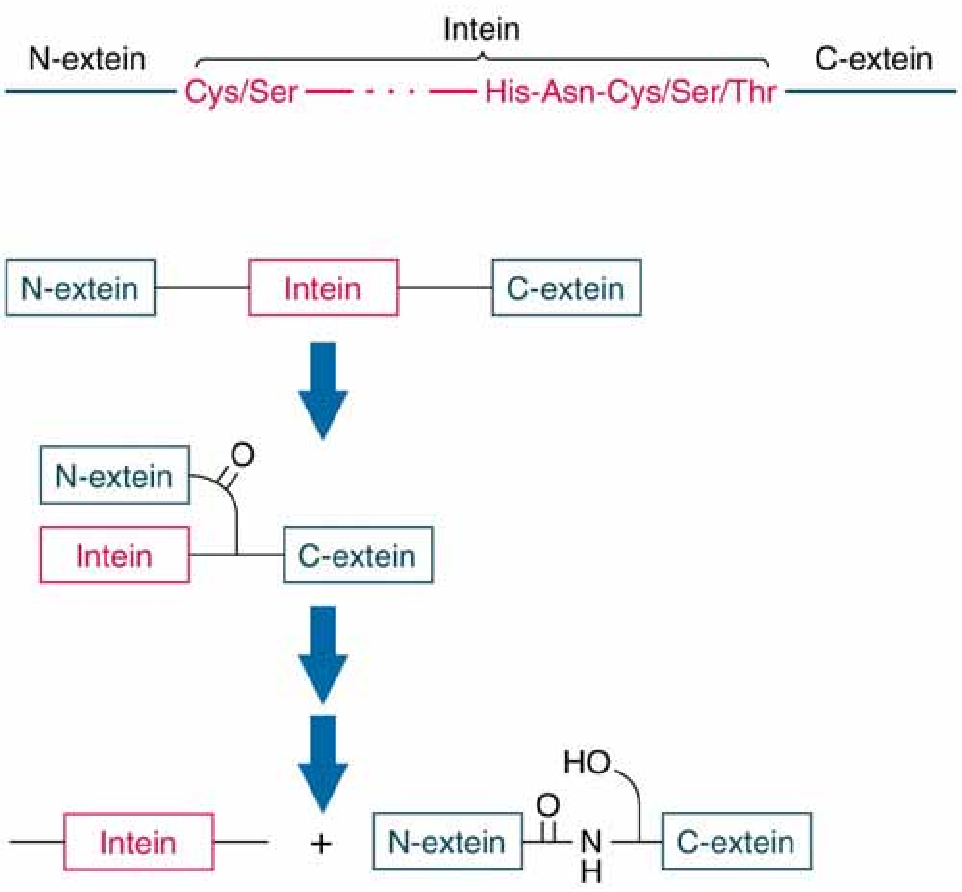


Figure 12.21

Regulation of mRNA Synthesis

- regulation of gene expression
- conserves energy and raw materials
- maintains balance between the amounts of various cell proteins
- allows organism to adapt to long-term environmental change

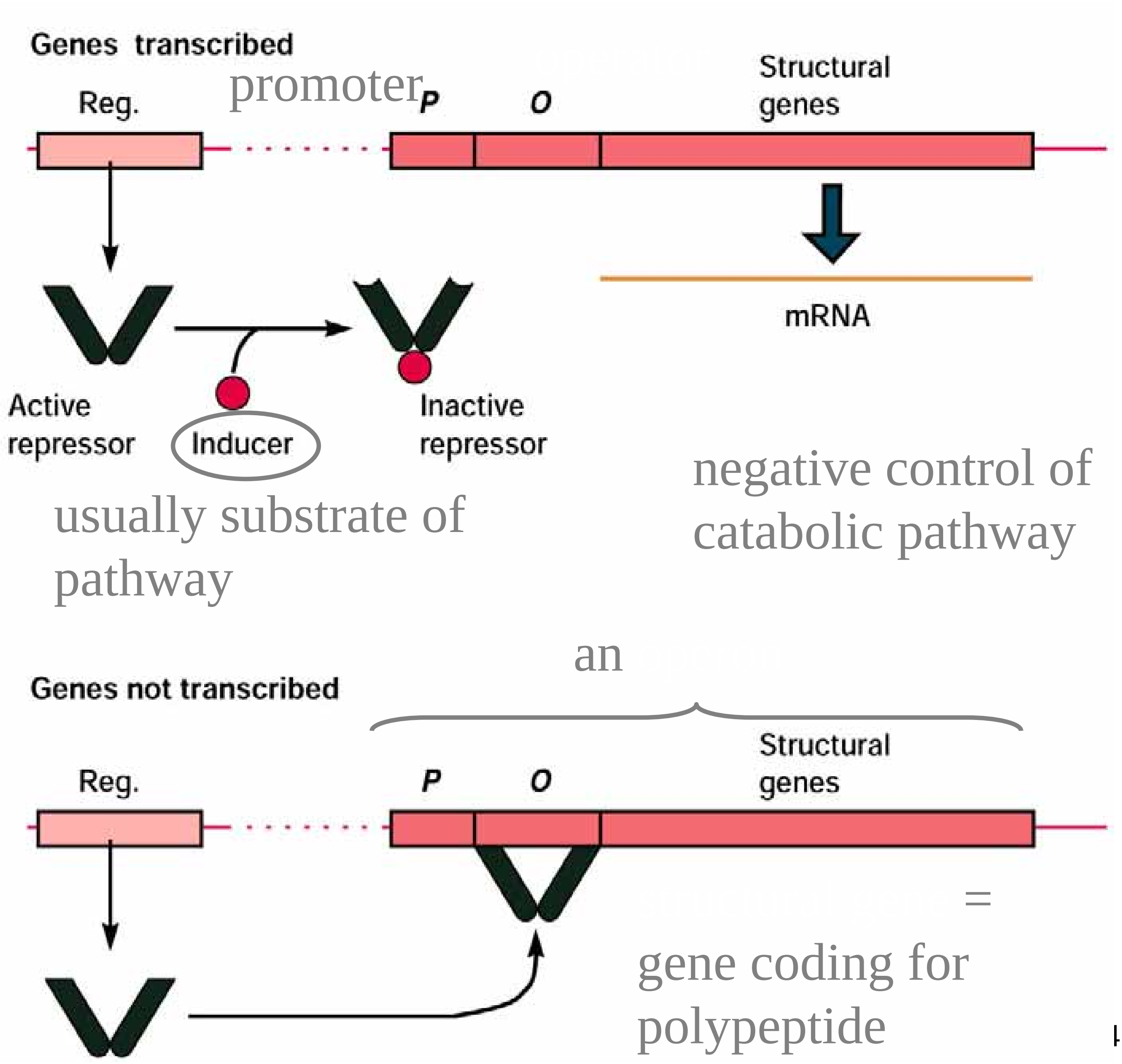
Induction and Repression

- inducible enzyme
  - level increases in presence of inducer
    - small molecule, usually substrate of catabolic pathway in which enzyme functions
- repressible enzyme
  - level decreases in presence of corepressor
    - usually end product of biosynthetic pathway in which the enzyme functions

Negative Control

- presence of regulatory protein (repressor) at regulatory site (operator) decreases mRNA synthesis
- repressor proteins
  - exist in active and inactive forms
  - inducers and corepressors alter activity of repressor

- the goal – make enzymes of pathway only when substrate of pathway is available



promoter

operator

usually substrate of pathway

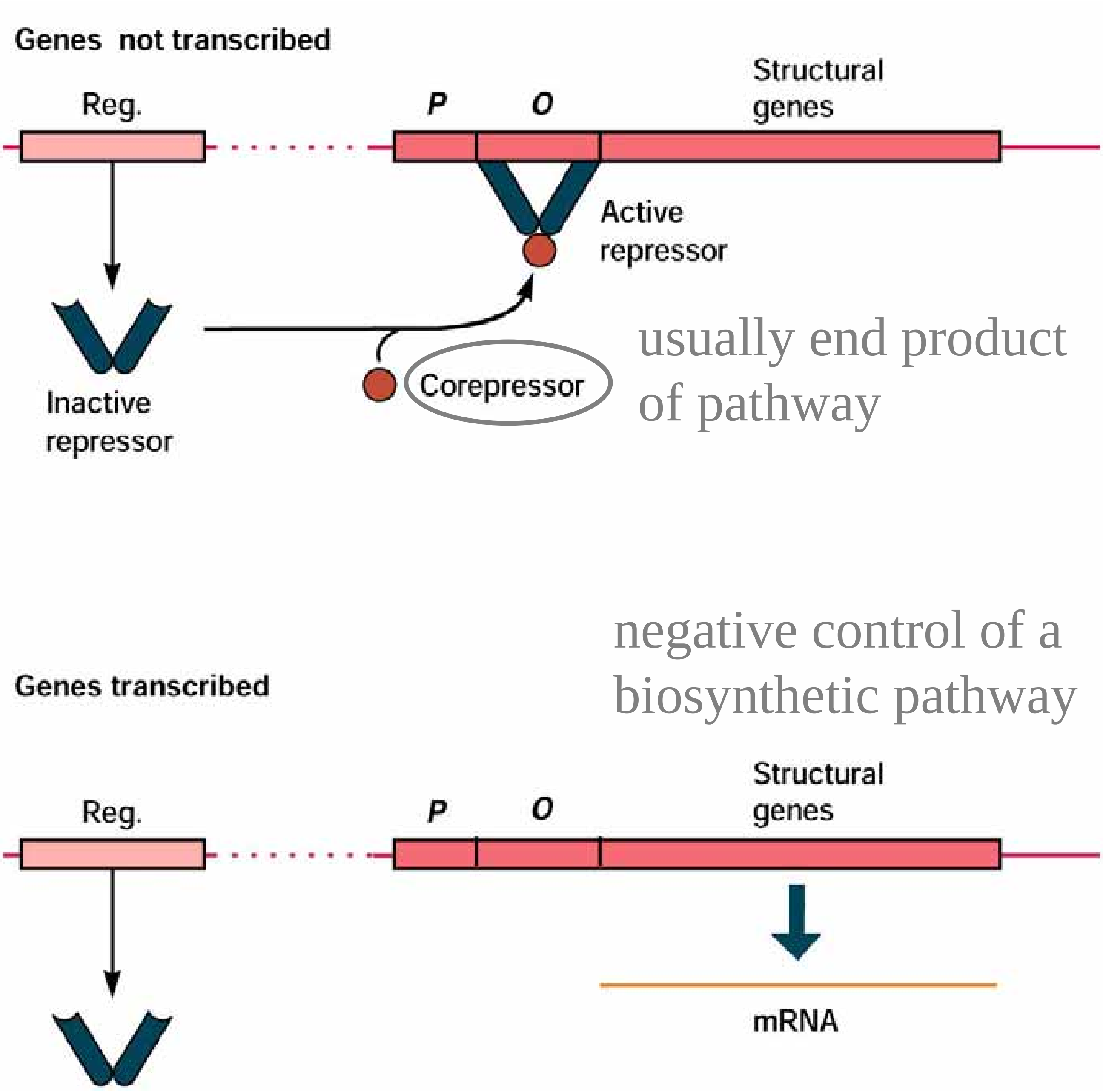
negative control of catabolic pathway

an operon

structural gene = gene coding for polypeptide

Figure12.23

- the goal – only make enzymes of pathway when end product of pathway is not available



usually end product of pathway

negative control of a biosynthetic pathway

Figure 12.24

Positive Control

- presence of a regulatory protein (activator protein) at a regulatory region promotes transcription
- e.g., lactose operon
  - regulated by catabolite activator protein (CAP) and cyclic AMP (cAMP)
    - CAP also called cyclic AMP receptor protein (CRP)

β-galactosidase reaction

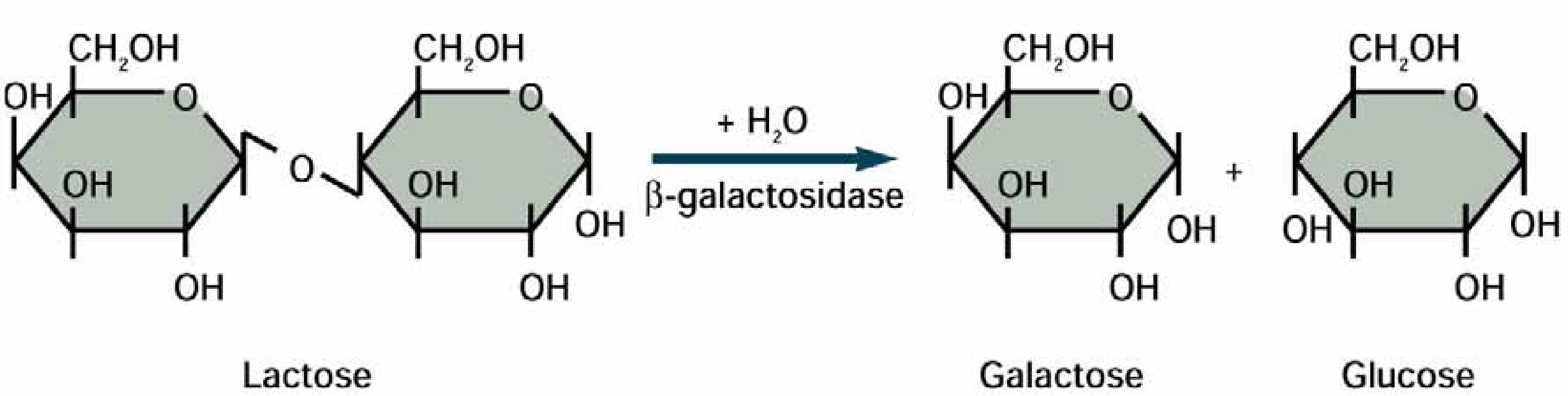


Figure 12.22

cAMP

- binds to and activates CAP

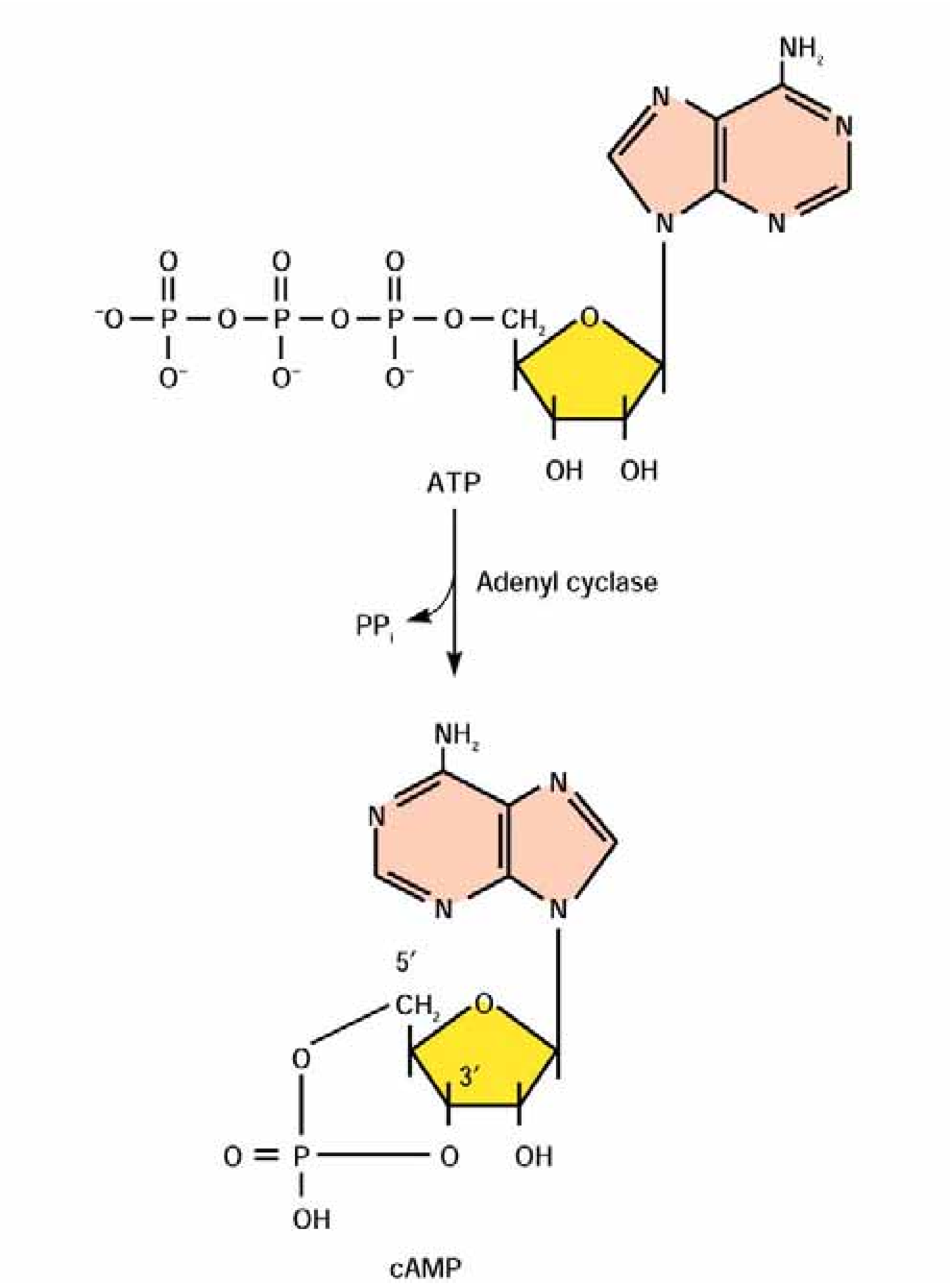
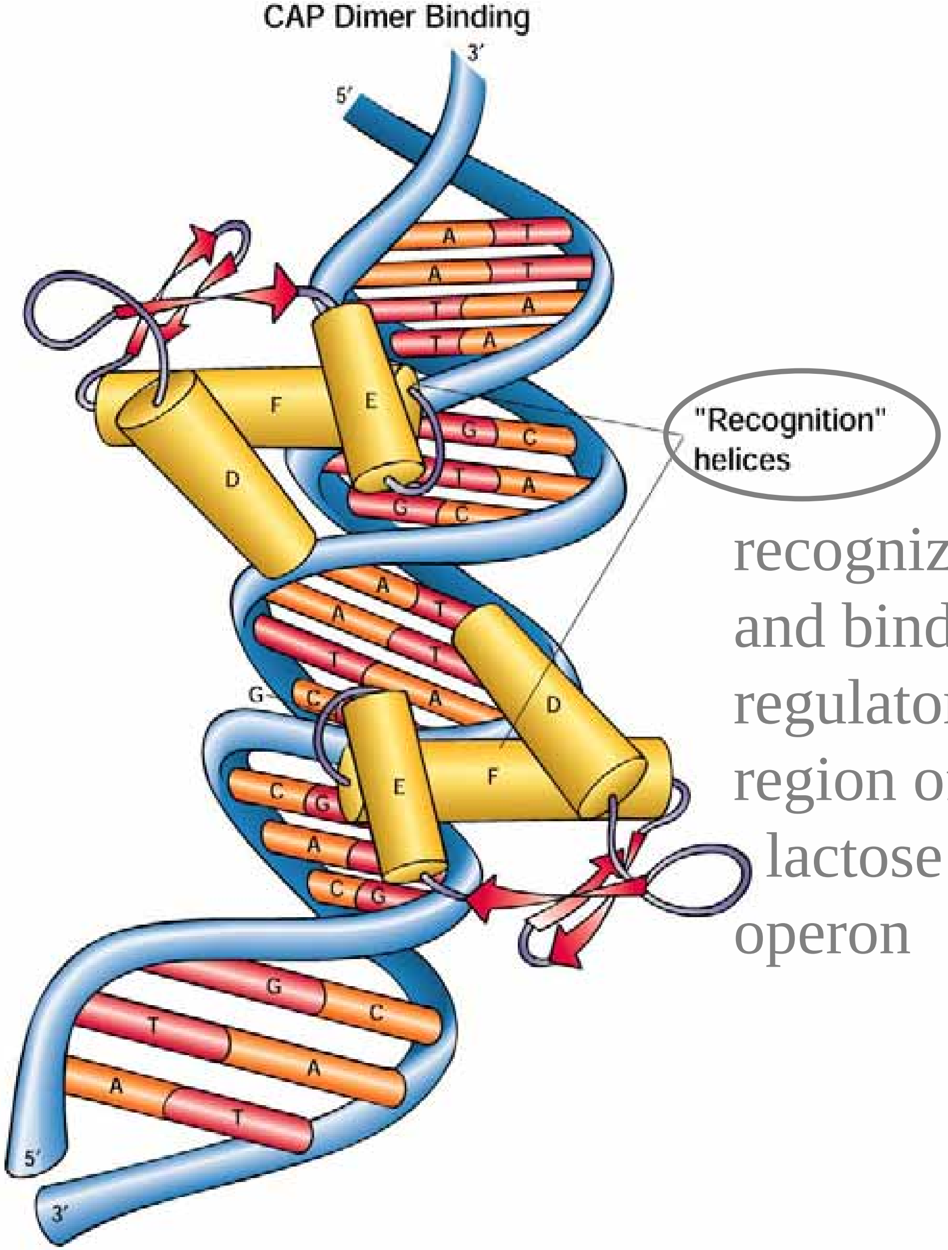


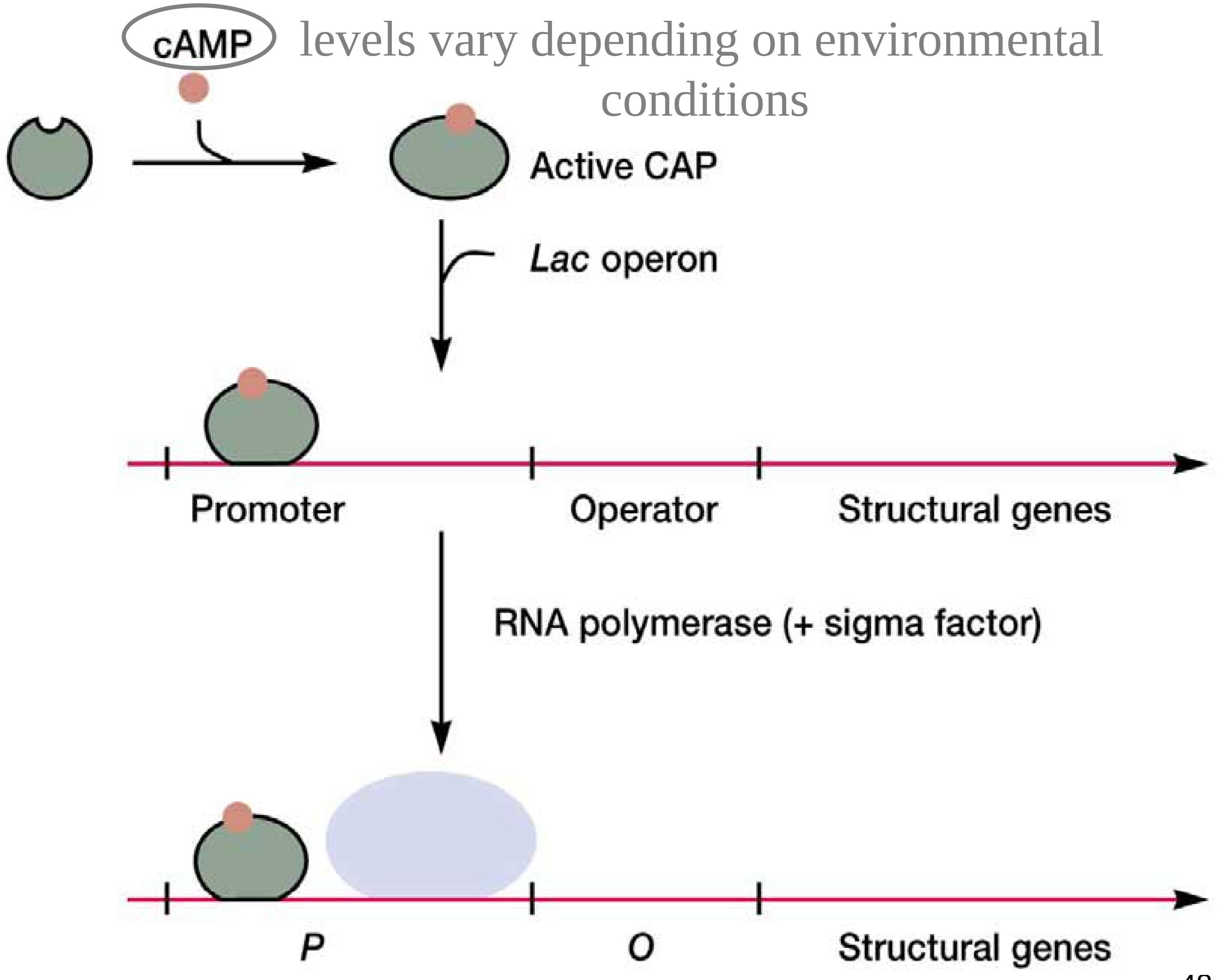
Figure 12.27

CAP



recognize and bind regulatory region of lactose operon

Figure 12.29



levels vary depending on environmental conditions

Figure 12.28

Attenuation

- regulation of transcription by the behavior of ribosomes
- observed in bacteria, where transcription and translation are tightly coupled
  - translation of a mRNA can occur as the mRNA is being synthesized

Coupled transcription and translation in procaryotes

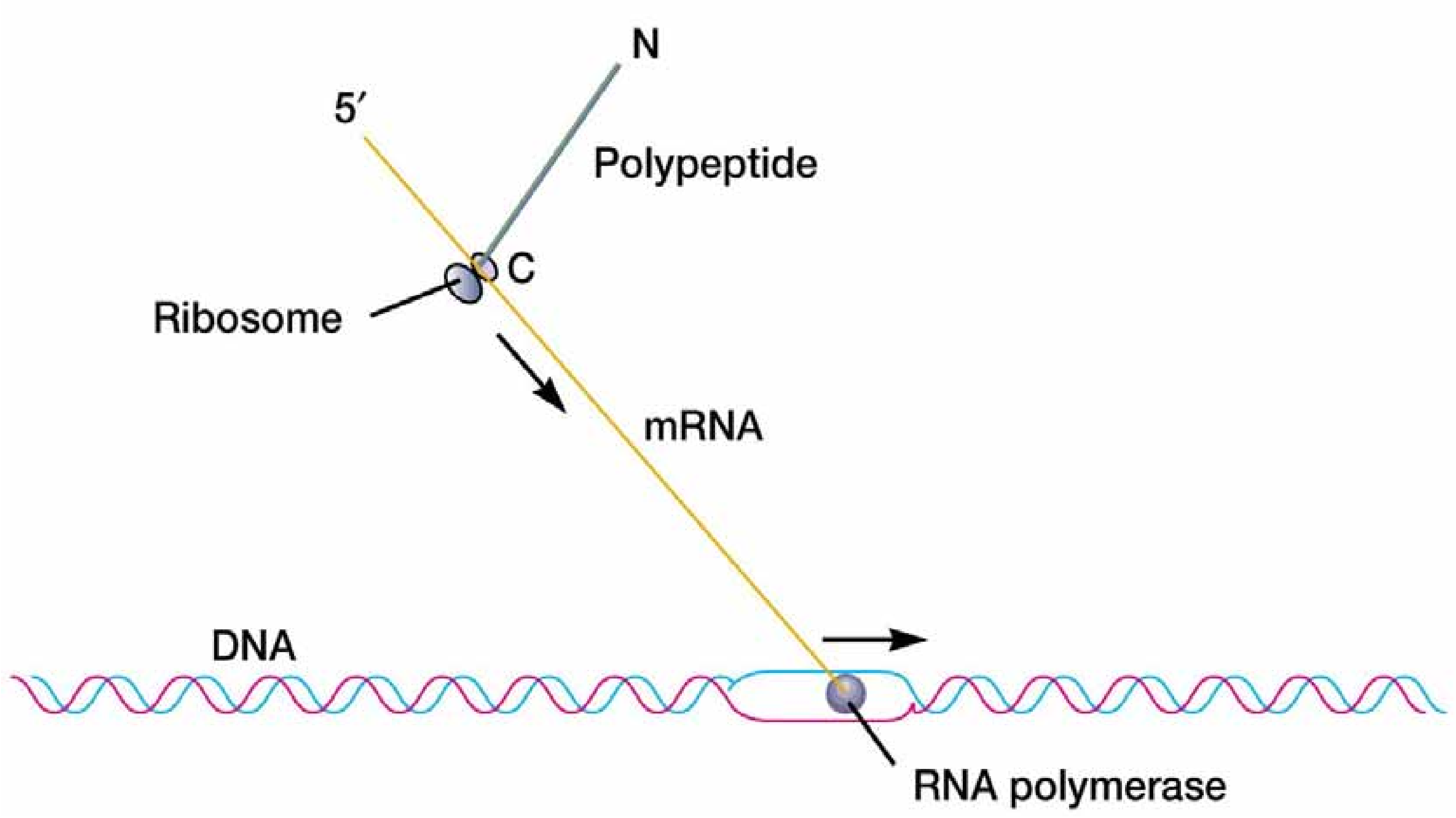
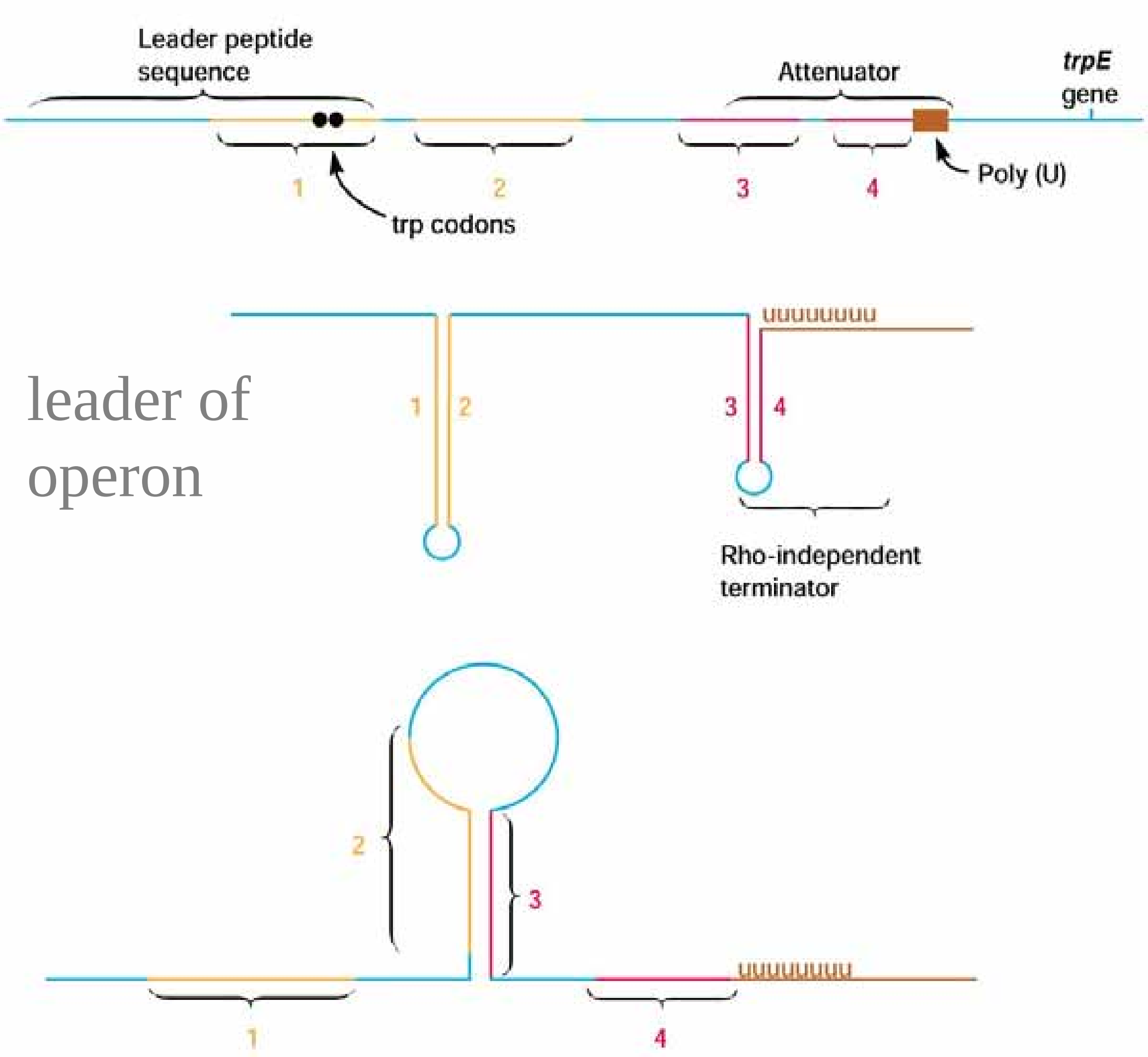


Figure 12.7

e.g., the tryptophan operon

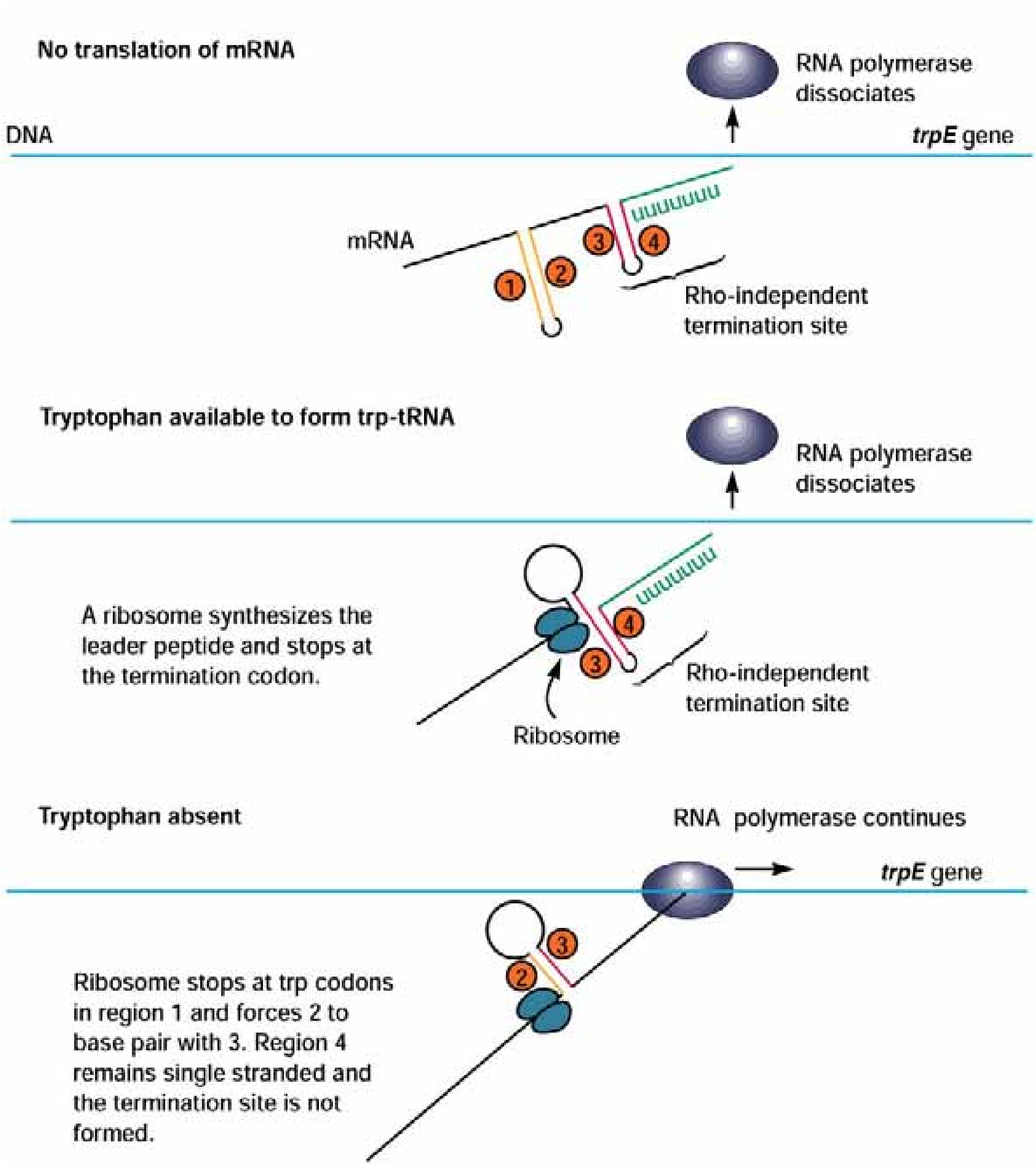
- operon that encodes enzymes for the tryptophan biosynthetic pathway

- the goal – terminate transcription if tryptophan is available



leader of operon

Figure 12.30



A ribosome synthesizes the leader peptide and stops at the termination codon.

Ribosome stops at trp codons in region 1 and forces 2 to base pair with 3. Region 4 remains single stranded and the termination site is not formed.

Figure 12.31

Global Regulatory Systems

- affect many genes and pathways simultaneously
- regulon
  - collection of genes or operons controlled by a common regulatory protein

Catabolite Repression

- occurs when operon is under control of catabolite other than initial substrate of pathway
- allows preferential use of one carbon source over another when both are available in environment
- e.g., catabolite repression of lactose and other operons by glucose
  - glucose decreases cAMP levels, thereby blocking CAP binding and decreasing mRNA synthesis

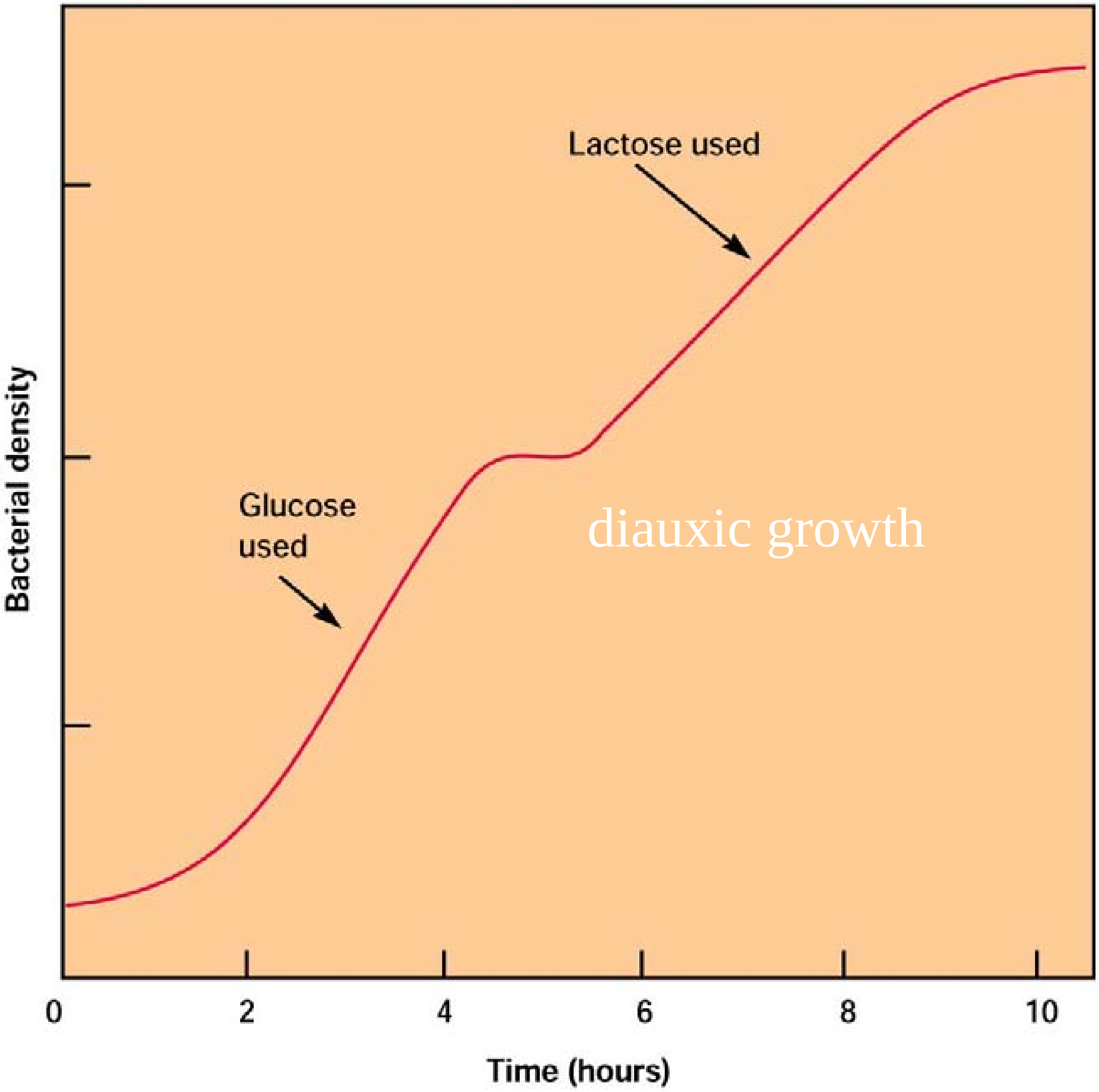

Lactose used
Glucose used
diauxic growth
Bacterial density
0
2
4
6
8
10
Time (hours)

Figure 12.32

Regulation by Sigma Factors and Control of Sporulation

- different sigma factors recognize different promoters
- substitution of sigma factors changes gene expression of many genes and operons

e.g., *Bacillus subtilis* sporulation sigma factors

- synthesized only as cell switches from vegetative growth to sporulation
- lead to transcription of sporulation-related genes

Small RNAs (sRNAs) and Regulation

- also called noncoding (nc)RNAs
- do not function as mRNA or rRNA
- appear to regulate genes by three different mechanisms
  - pair directly with other RNAs (e.g., OxyS RNA and micF RNA)
  - via RNA-protein interactions (e.g., OxyS RNA)
  - intrinsic activities (e.g., RNase P RNA and tmRNA)

e.g., OxyS RNA of *E. coli*

- made in response to hydrogen peroxide exposure
- can act as an antisense RNA
  - binds directly to mRNA and blocks translation
- can also block translation by binding a protein required for translation of a target mRNA

e.g., micF RNA of *E. coli*

- regulates synthesis of OmpF porin protein
  - porin proteins are outer membrane proteins
  - different porins produced under different conditions
    - OmpC porin made when in intestine
    - OmpF porin made when in dilute environment
- micF antisense RNA binds OmpF RNA and blocks its translation when bacterium in intestines

e.g., RNase P RNA

- the RNA component of RNase P
- has catalytic activity responsible for tRNA processing

e.g., tmRNA of *E. coli*

- helps repair problems caused by defective mRNAs that lack stop codons
- acts as both alanyl-tRNA and mRNA when ribosome stalls at end of defective mRNA
- two functions
  - releases ribosome from defective mRNA
  - adds carboxy-terminal polypeptide tag to protein, marking it for degradation

Two-Component Phosphorelay Systems

- transfer of phosphoryl groups control gene transcription and protein activity
- e.g., sporulation in *B. subtilis*
- e.g., chemotaxis in *E. coli*

Sporulation in *B. subtilis*

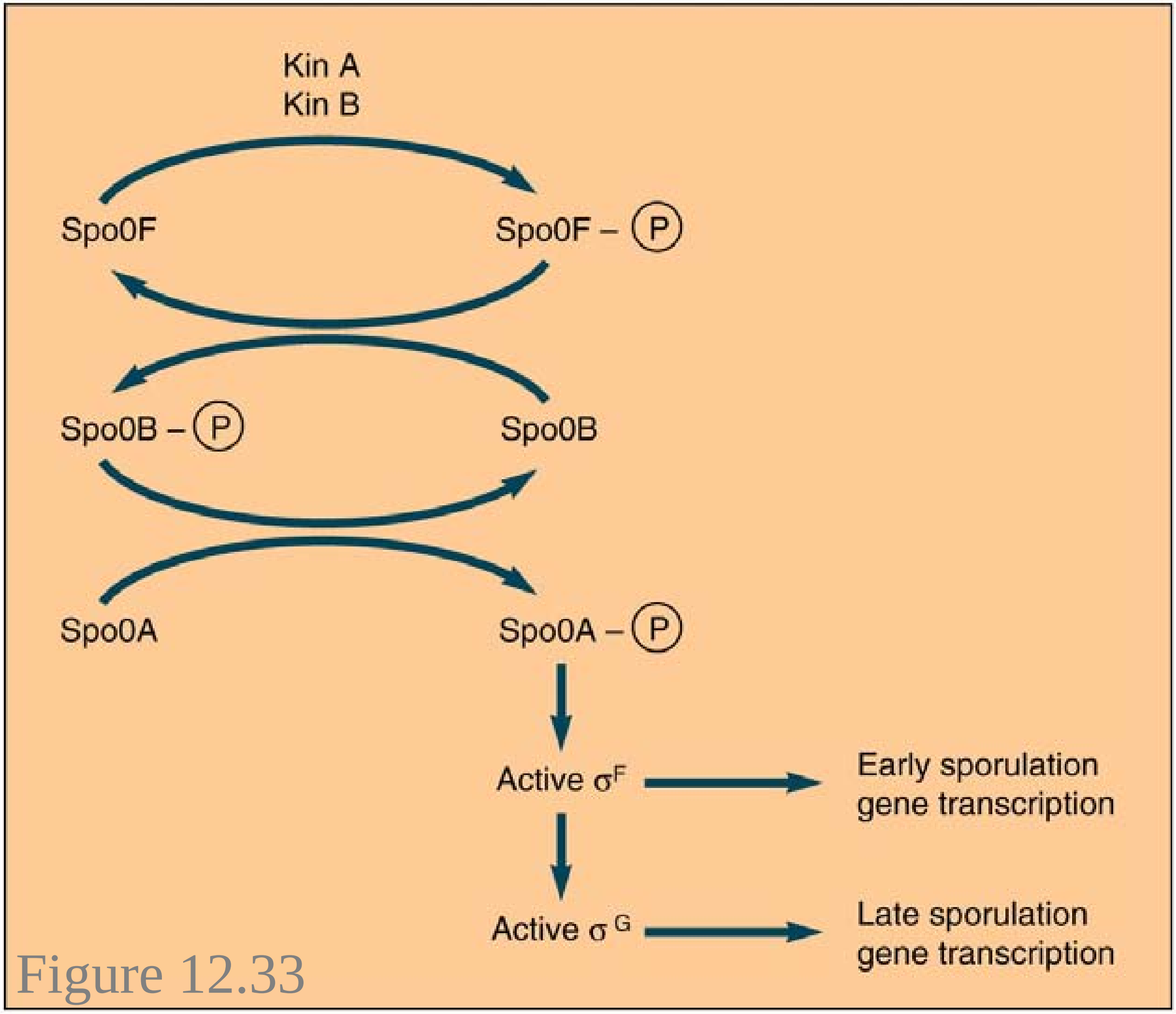


Figure 12.33

Chemotaxis in *E. coli*

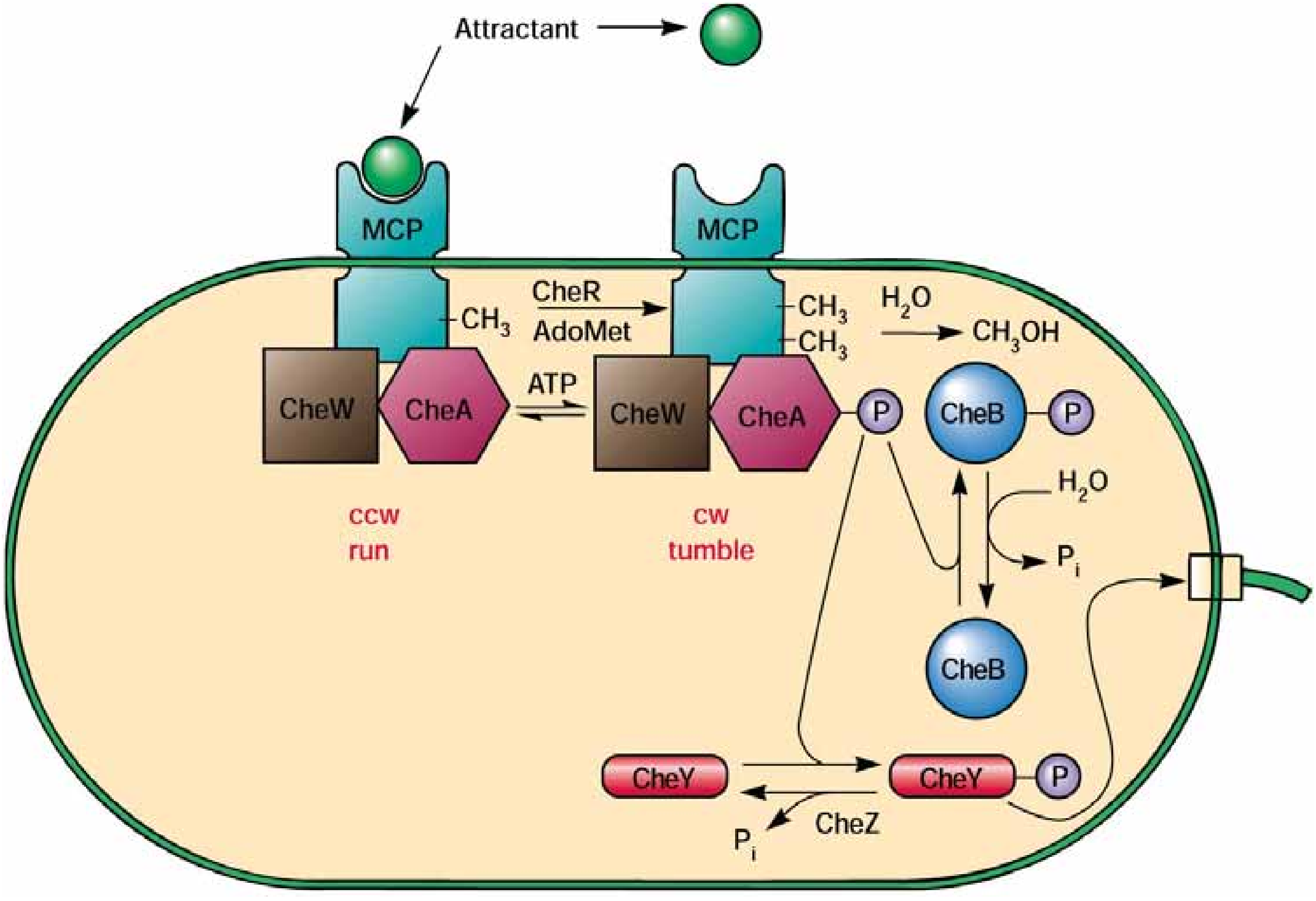


Figure 12.34

Control of the Cell Cycle

- cell cycle
  - complete sequence of events extending from formation of a new cell through next division
  - requires that DNA replication and cell division be tightly coordinated
- precise mechanisms of control are not known

Cell cycle control in *E. coli*

- two separate control pathways
  - sensitive to cell mass
  - sensitive to cell length

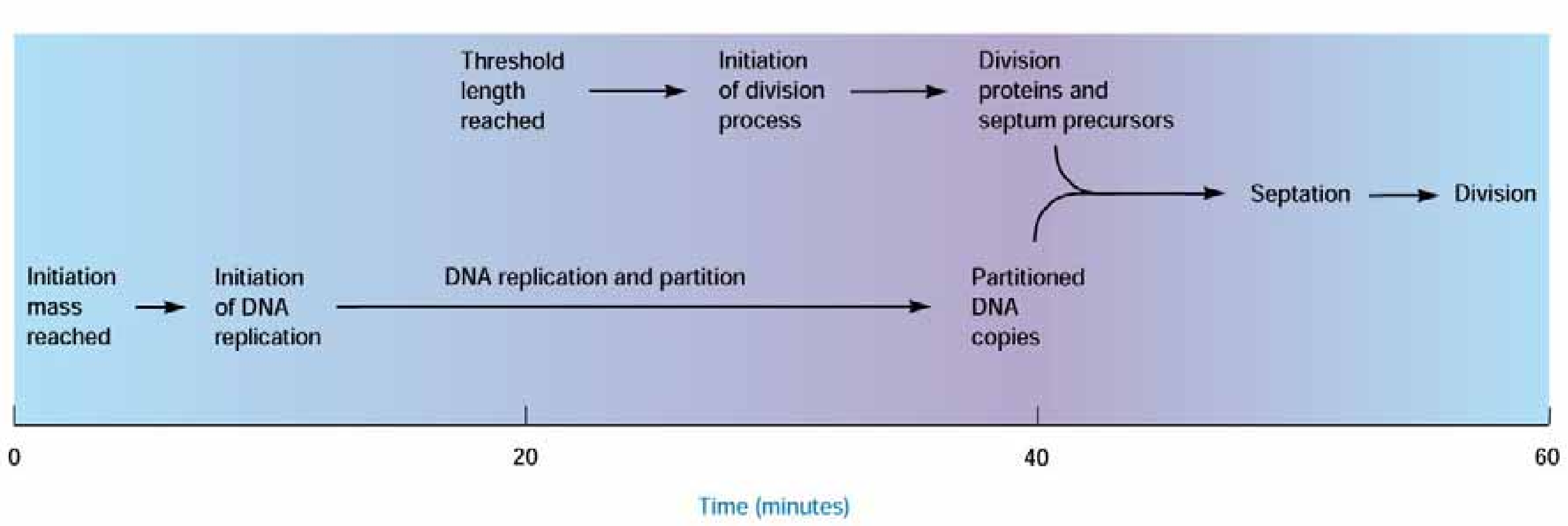


Figure 12.36

Effect of growth rate

- slow growth rate
  - DNA replicated then septation begins
- rapid growth rate
  - DNA replicated and new round of DNA replication begins before septation begins

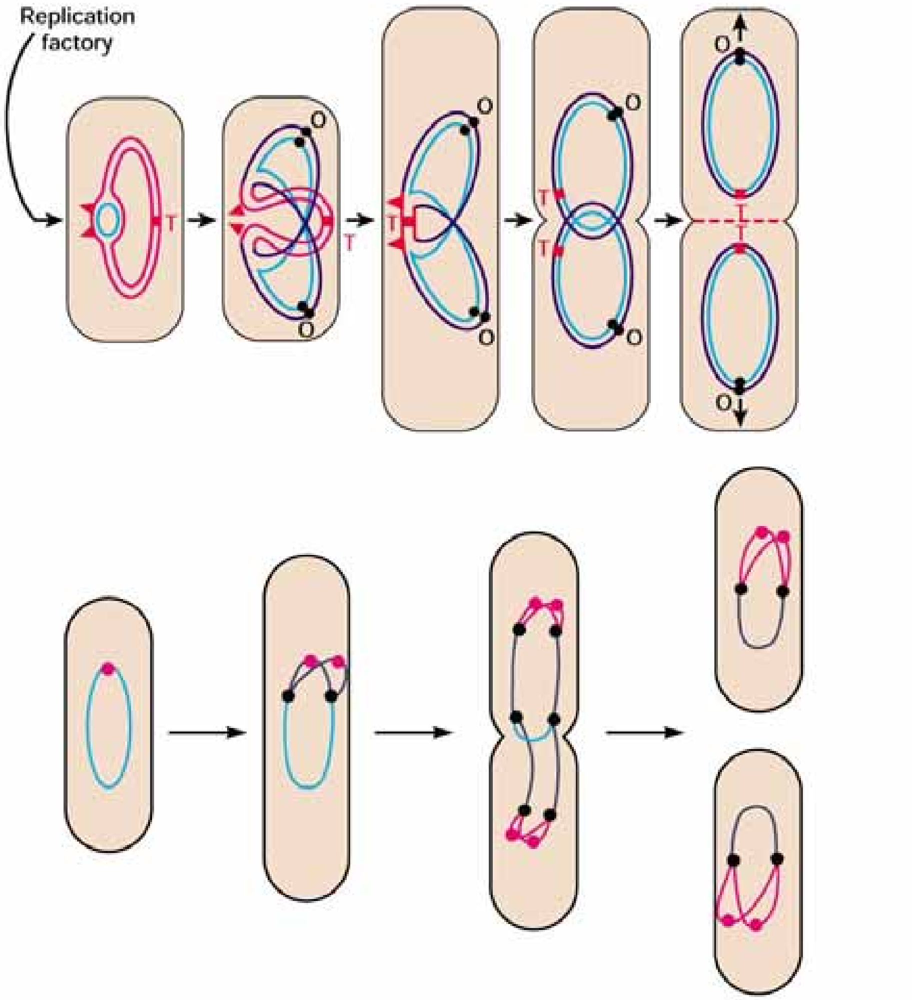


Figure 12.35