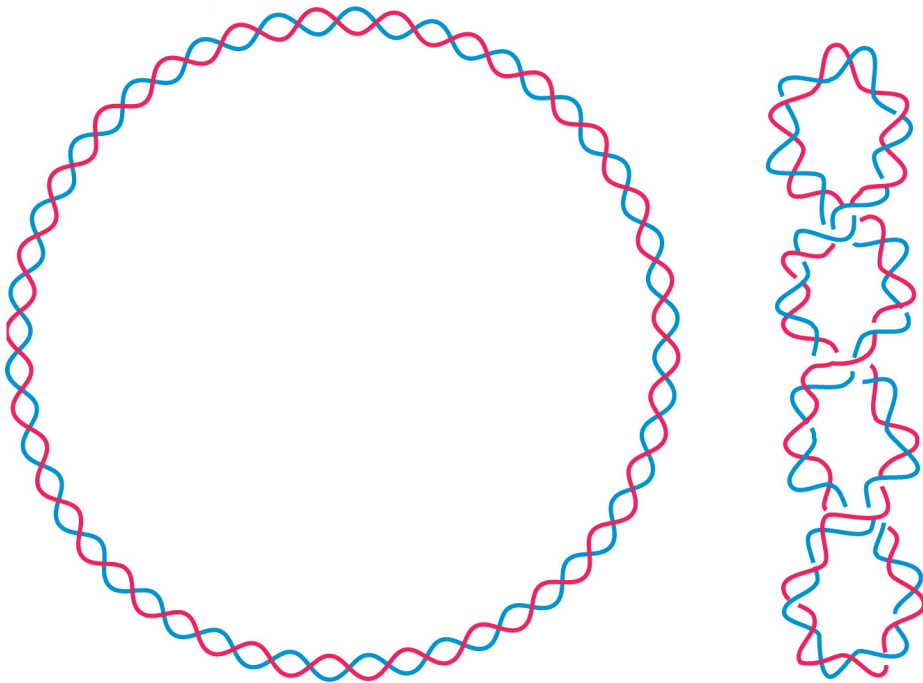


Prokaryotic DNA organization



- Circular DNA
- Condensed by packaging proteins (e.g. H-NS, IHF)
- Supercoiled

Fig. 11.8

Bidirectional replication

- Replication starts at ori (*oriC* in *E. coli*)
- Continues bidirectionally
- Chromosome attached to plasma membrane

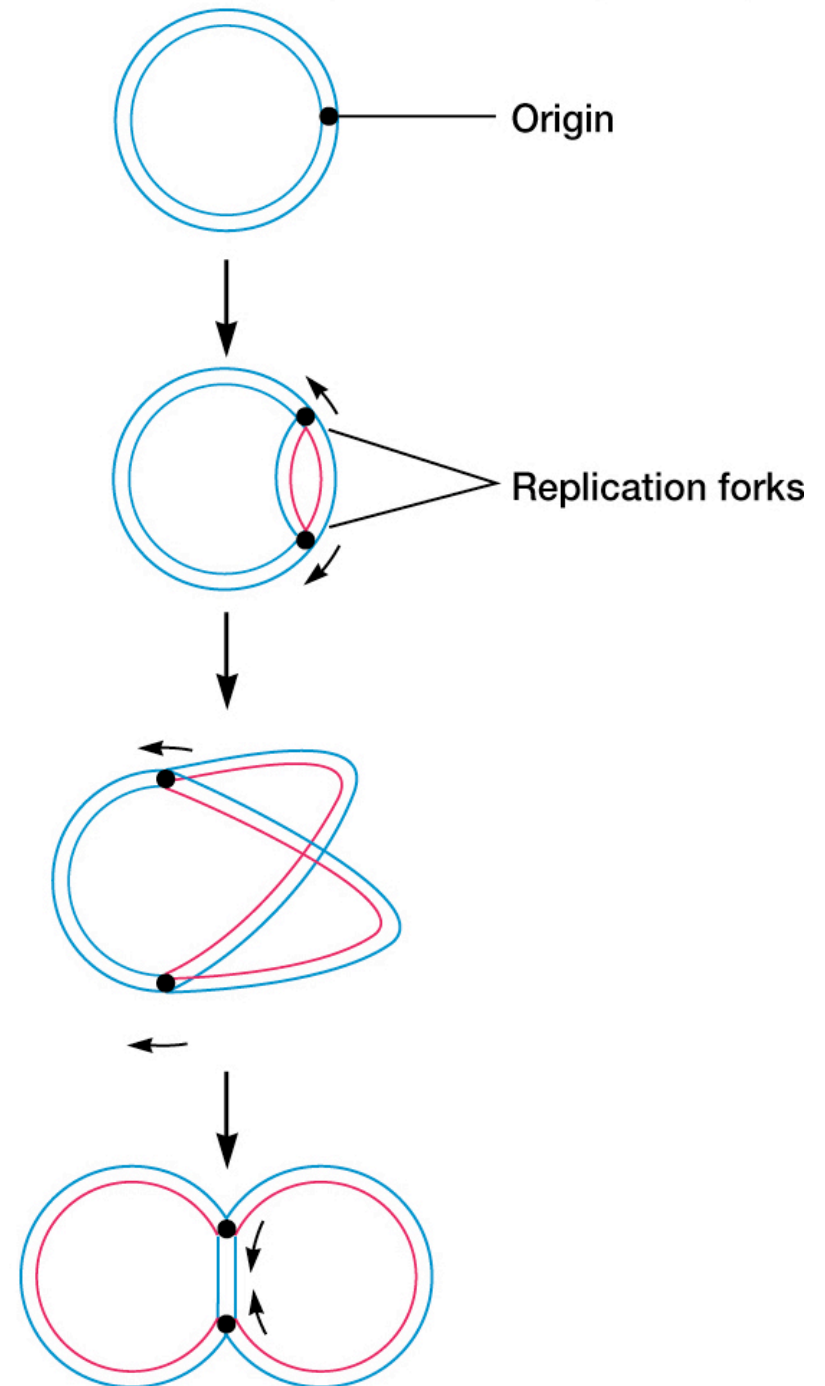


Fig. 11.11

Rolling circle replication

Used by plasmids and some bacteriophage

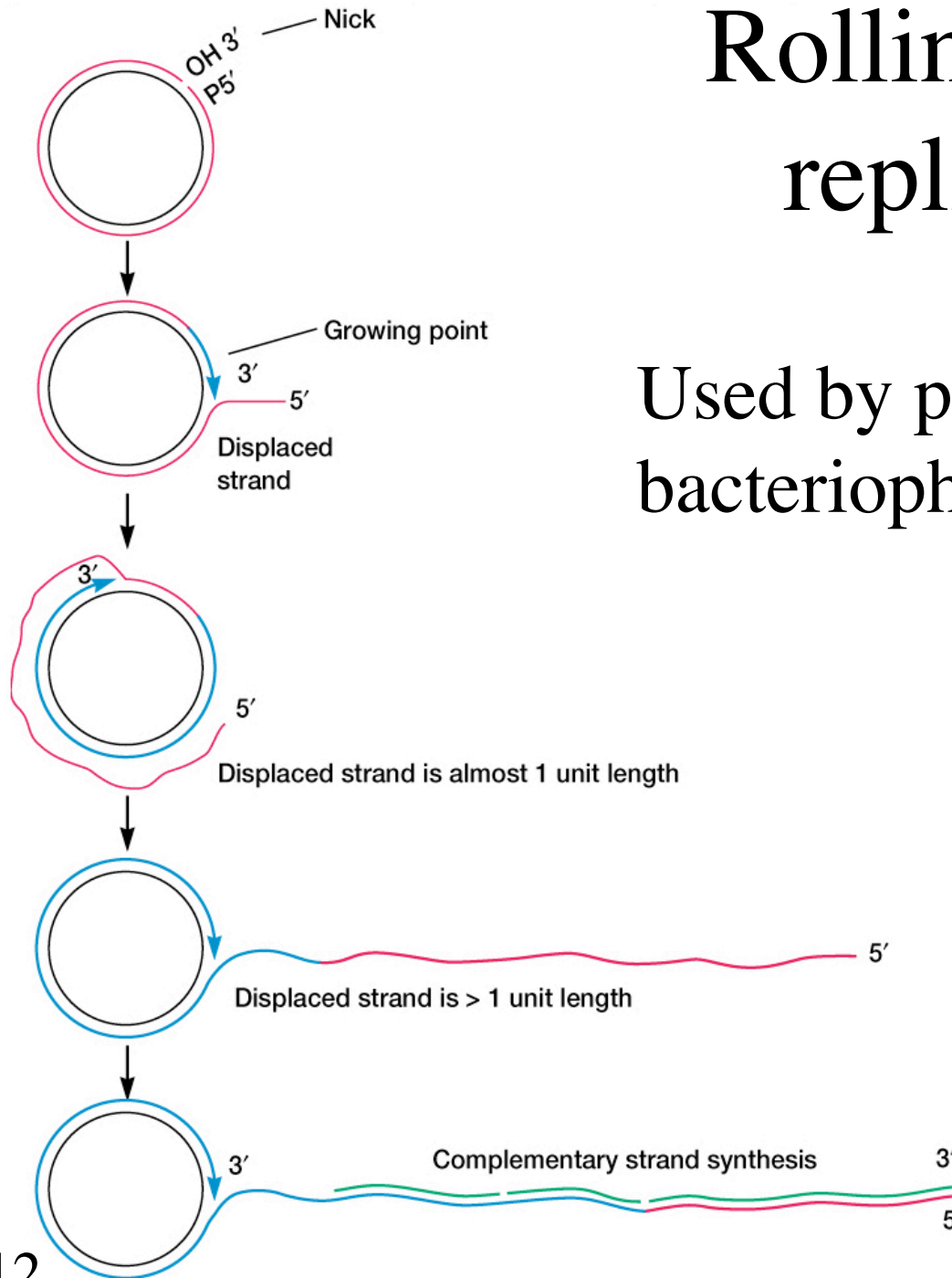
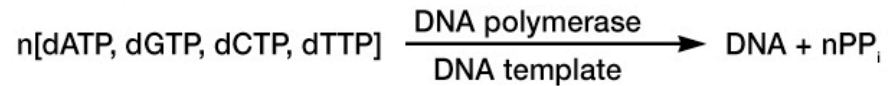


Fig. 11.12

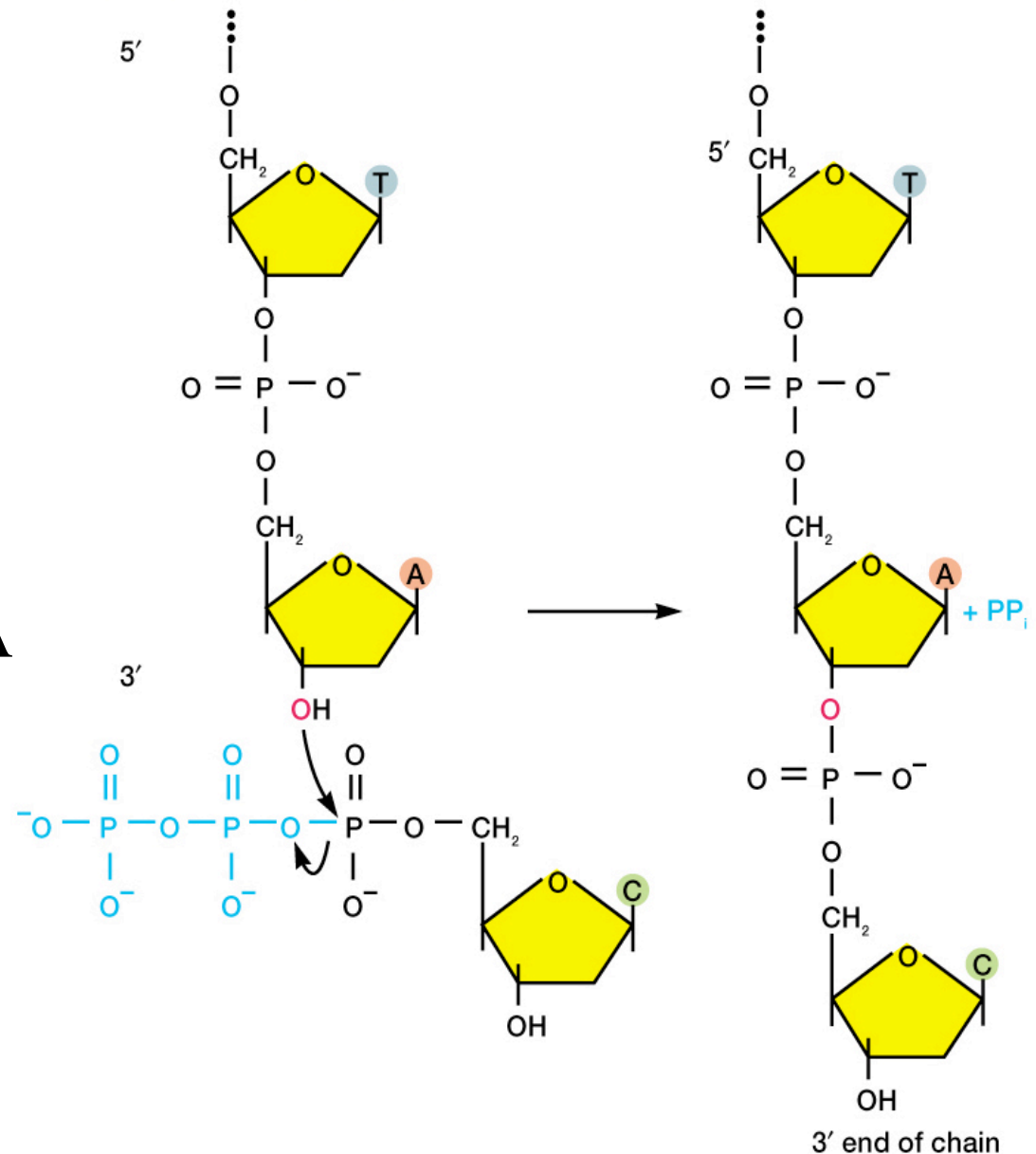
DNA polymerases

- 3 DNA polymerases
- Synthesize 5'-3'
- DNA pol III = DNA replication
- DNA pol I, II = DNA damage repair

DNA polymerase reaction



The mechanism of chain growth



Replication fork

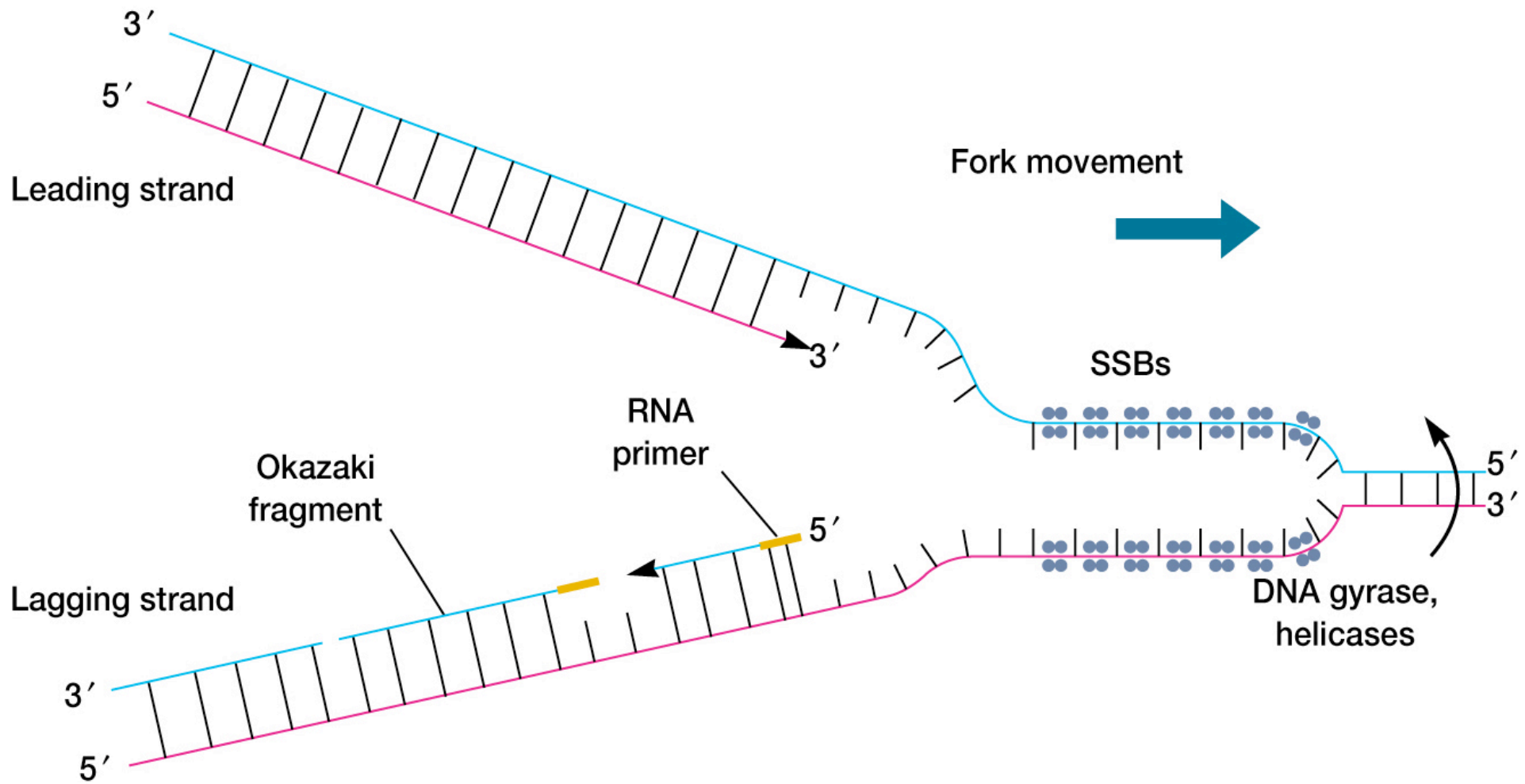
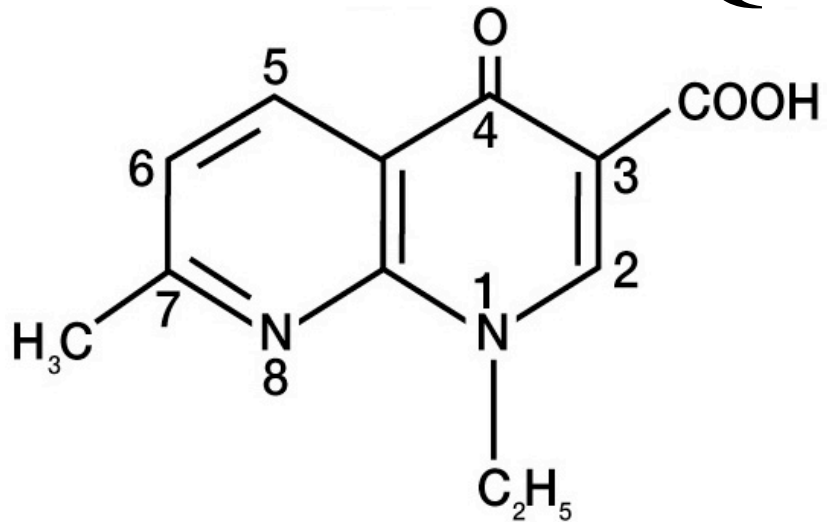
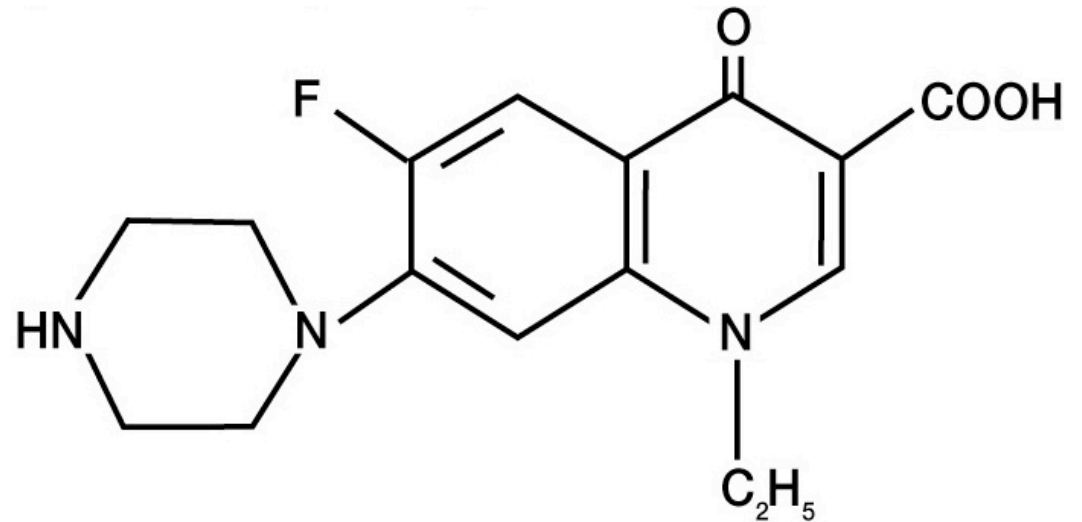


Fig. 11.15

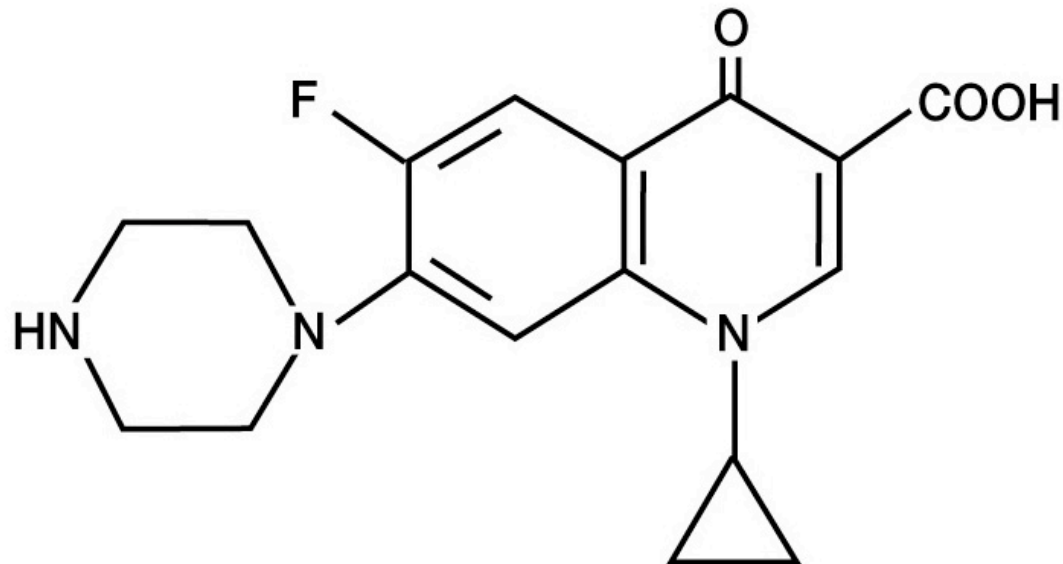
Quinolones



Nalidixic acid



Norfloxacin



Ciprofloxacin

Fig. 35.5

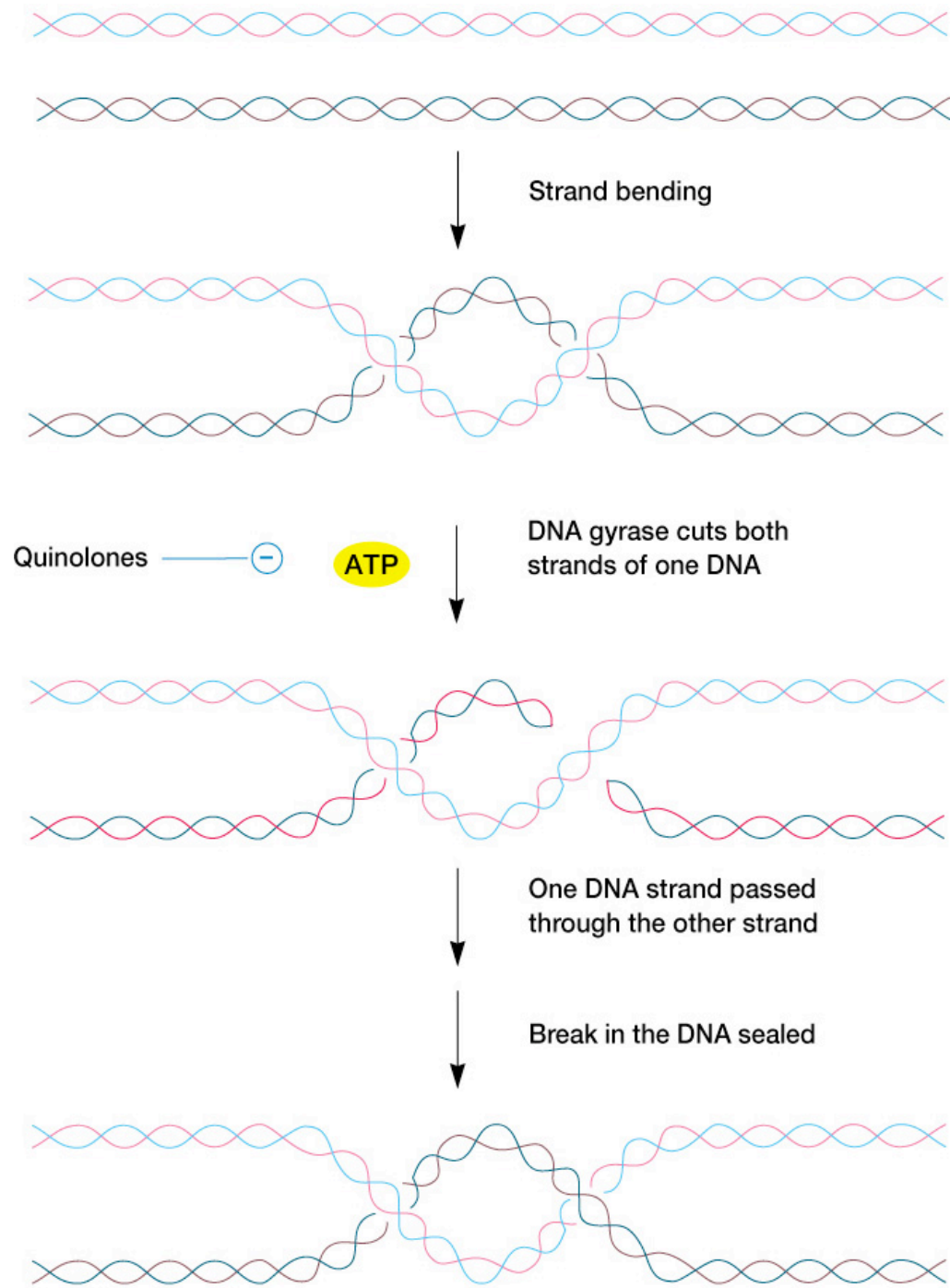


Fig. 35.6

DNA replication

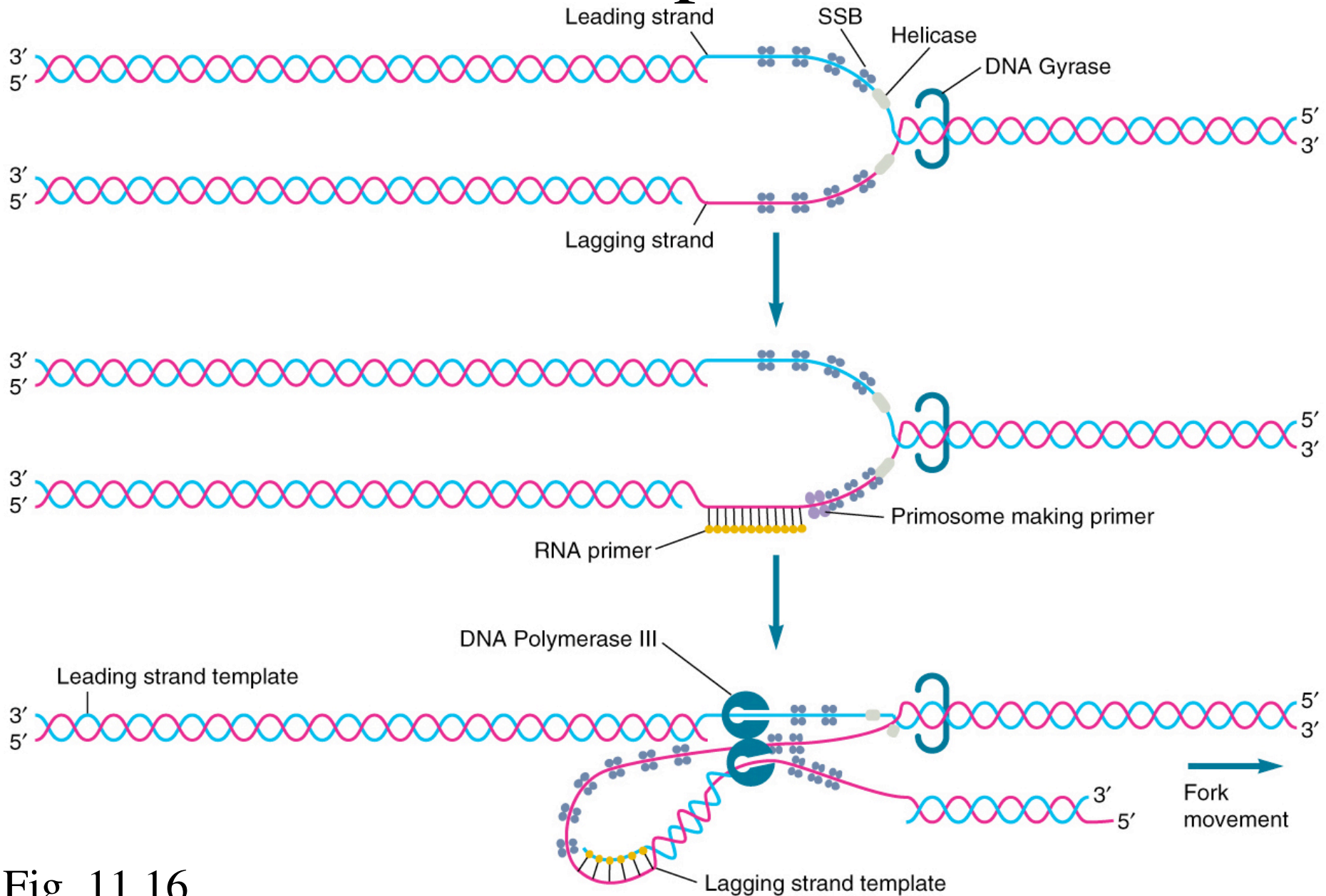


Fig. 11.16

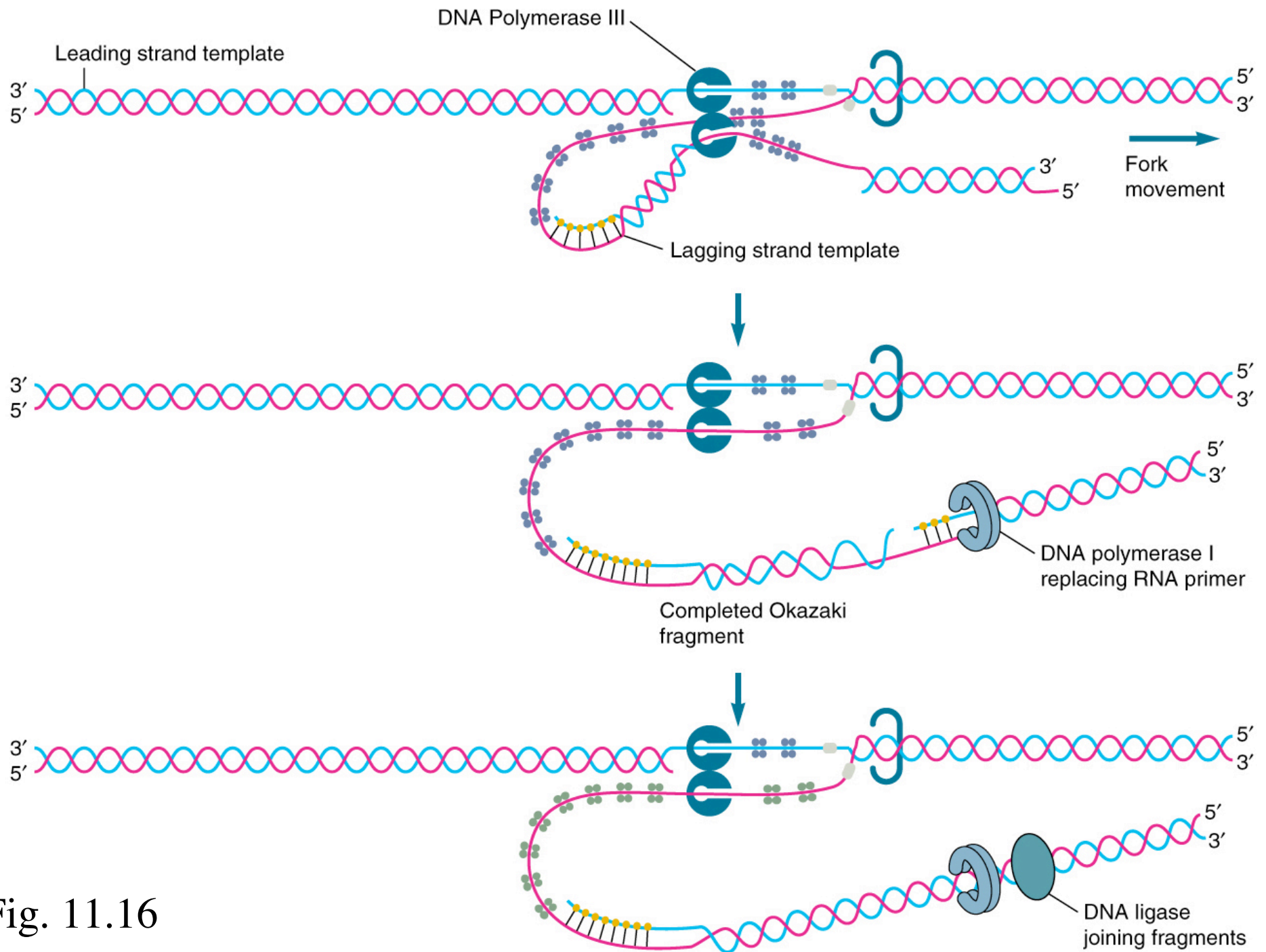


Fig. 11.16

DNA ligase

- Can ligate 5'-PO₄ to 3'-OH without insertion of nucleotide

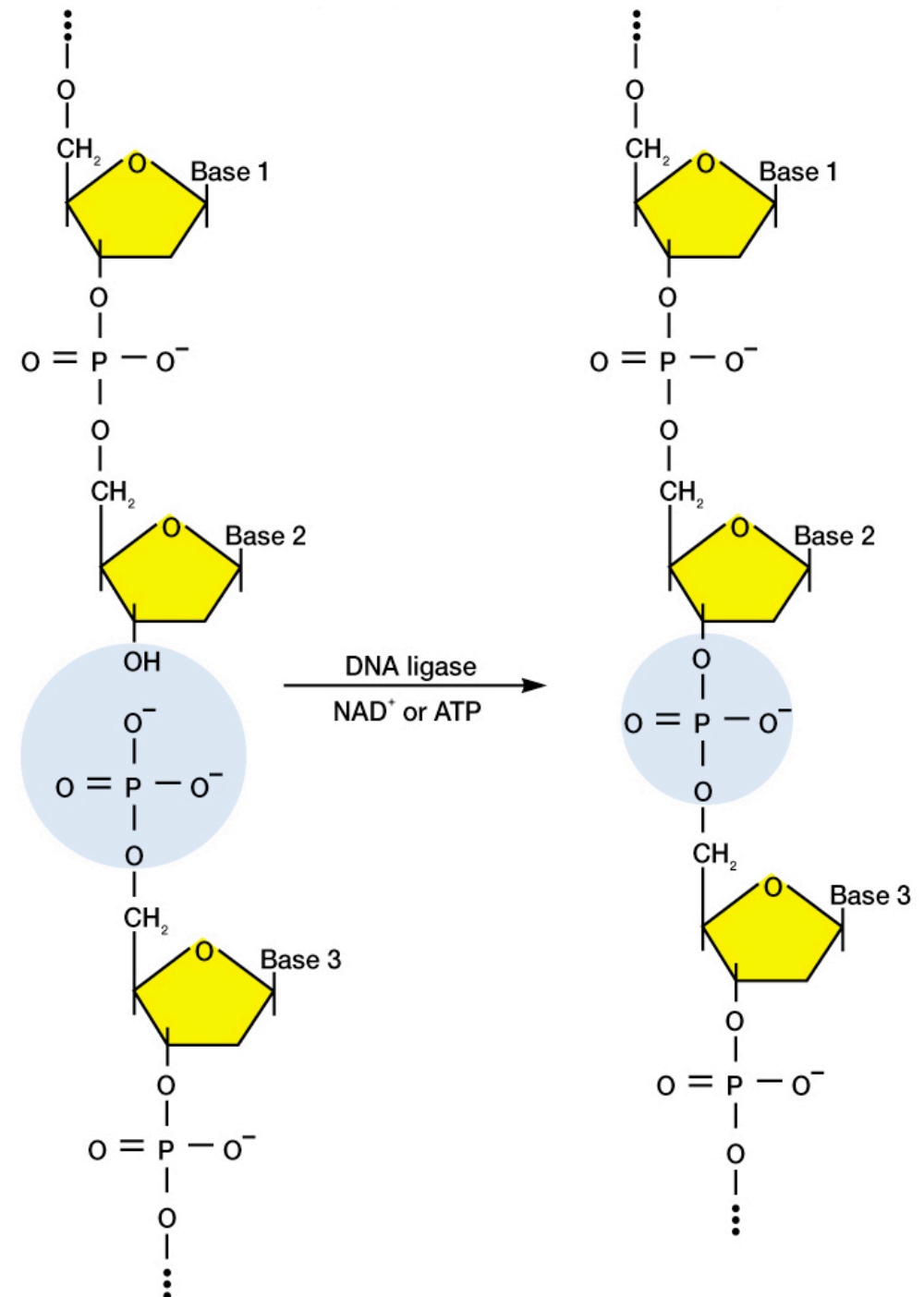


Fig. 11.17

Creating a recombinant plasmid

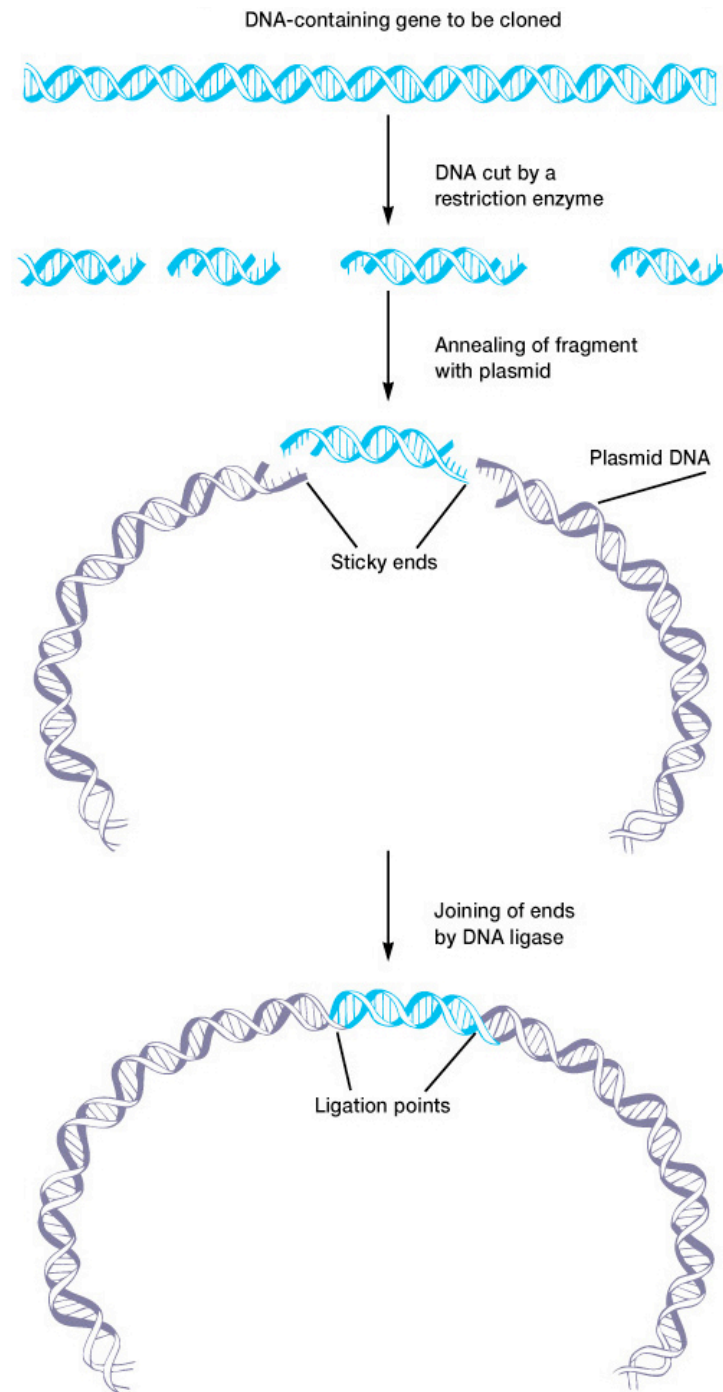


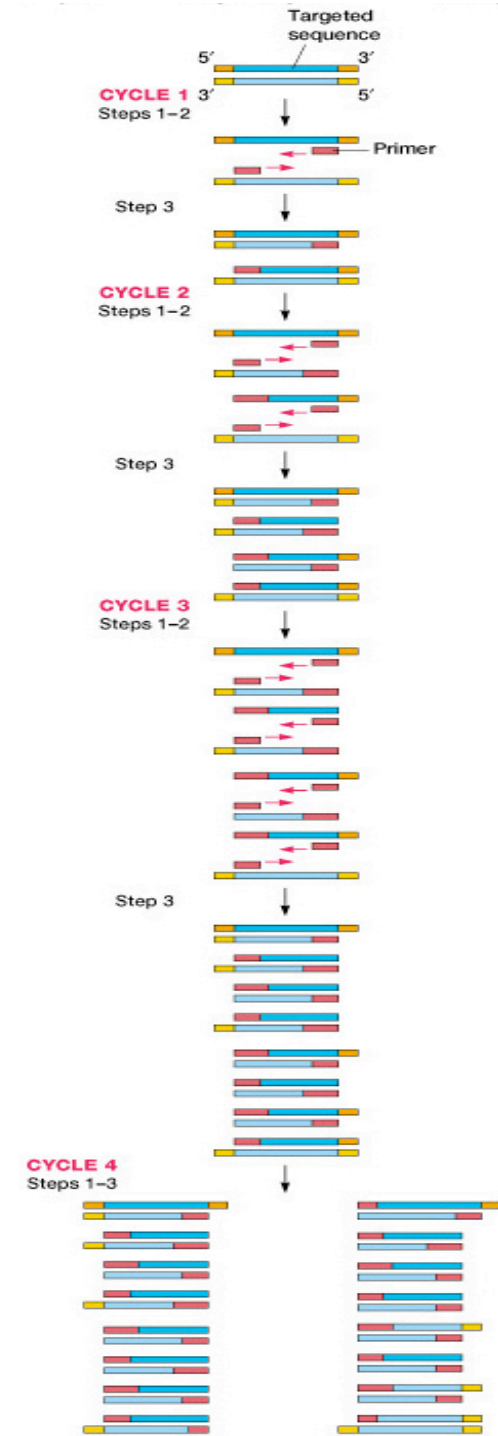
Fig. 14.4

Steps in cloning a gene

- Isolate plasmid vector
 - Plasmid DNA isolation
- Isolate gene of interest
 - Chromosomal DNA isolation
 - Polymerase chain reaction
- Cut plasmid and gene of interest with same restriction endonuclease

Polymerase chain reaction (PCR)

- Requires DNA polymerase that is not inactivated by high temperatures
- Taq, Vent polymerases isolated from thermophiles



Fig,14.8

Restriction endonucleases

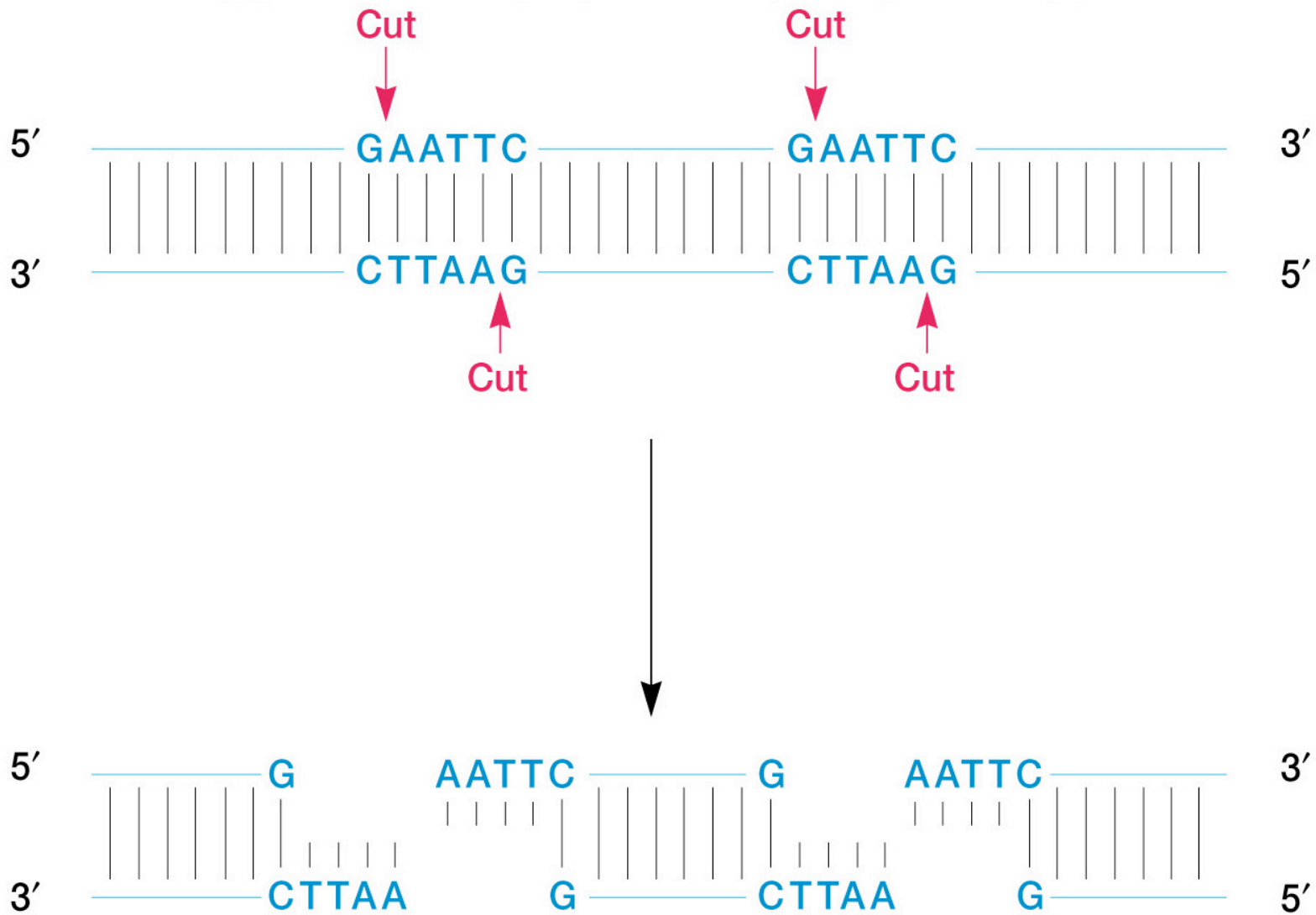


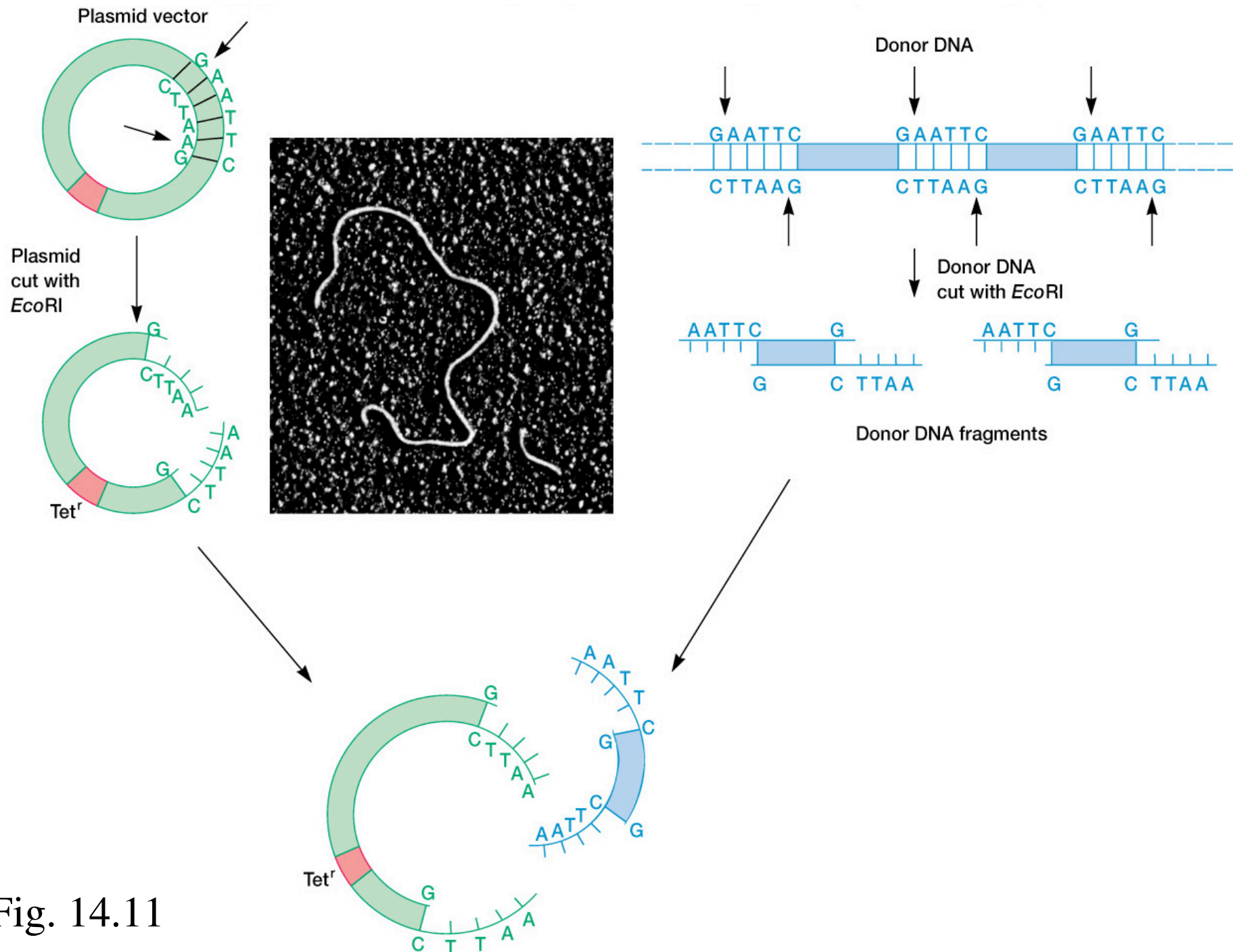
Fig. 14.2

Table 14.2 Some Type II Restriction Endonucleases and Their Recognition Sequences

Enzyme	Microbial Source	Recognition Sequence ^a	End Produced ^b
<i>AluI</i>	<i>Arthrobacter luteus</i>	$ \begin{array}{c} 5' - A - G - \downarrow C - T - 3' \\ 3' - T - C - \uparrow G - A - 5' \end{array} $	$ \begin{array}{c} C - T - 3' \\ G - A - 5' \end{array} $
<i>BamHI</i>	<i>Bacillus amyloliquefaciens H</i>	$ \begin{array}{c} 5' - G - \downarrow G - A - T - C - C - 3' \\ 3' - C - C - T - A - G - \uparrow G - 5' \end{array} $	$ \begin{array}{c} G - A - T - C - C - 3' \\ G - 5' \end{array} $
<i>EcoRI</i>	<i>Escherichia coli</i>	$ \begin{array}{c} 5' - G - \downarrow A - A - T - T - C - 3' \\ 3' - C - T - T - A - A - \uparrow G - 5' \end{array} $	$ \begin{array}{c} A - A - T - T - C - 3' \\ G - 5' \end{array} $
<i>HaeIII</i>	<i>Haemophilus aegyptius</i>	$ \begin{array}{c} 5' - G - G - \downarrow C - C - 3' \\ 3' - C - C - \uparrow G - G - 5' \end{array} $	$ \begin{array}{c} C - C - 3' \\ G - G - 5' \end{array} $
<i>HindIII</i>	<i>Haemophilus influenzae b</i>	$ \begin{array}{c} 5' - A - \downarrow A - G - C - T - T - 3' \\ 3' - T - T - C - G - A - \uparrow A - 5' \end{array} $	$ \begin{array}{c} A - G - C - T - T - 3' \\ A - 5' \end{array} $
<i>NotI</i>	<i>Nocardia otitidis-caviarum</i>	$ \begin{array}{c} 5' - G - C - \downarrow G - G - C - C - G - C - 3' \\ 3' - C - G - C - C - G - G - \uparrow C - G - 5' \end{array} $	$ \begin{array}{c} G - G - C - C - G - C - 3' \\ C - G - 5' \end{array} $
<i>PstI</i>	<i>Providencia stuartii</i>	$ \begin{array}{c} 5' - C - T - G - C - A - \downarrow G - 3' \\ 3' - G - \uparrow A - C - G - T - C - 5' \end{array} $	$ \begin{array}{c} G - 3' \\ A - C - G - T - C - 5' \end{array} $
<i>SalI</i>	<i>Streptomyces albus</i>	$ \begin{array}{c} \text{ilter to} \\ 5' - G - \downarrow T - C - G - A - C - 3' \\ 3' - C - A - G - C - T - \uparrow G - 5' \end{array} $	$ \begin{array}{c} T - C - G - A - C - 3' \\ G - 5' \end{array} $

^aThe arrows indicate the sites of cleavage on each strand.

^bOnly the end of the right-hand fragment is shown.



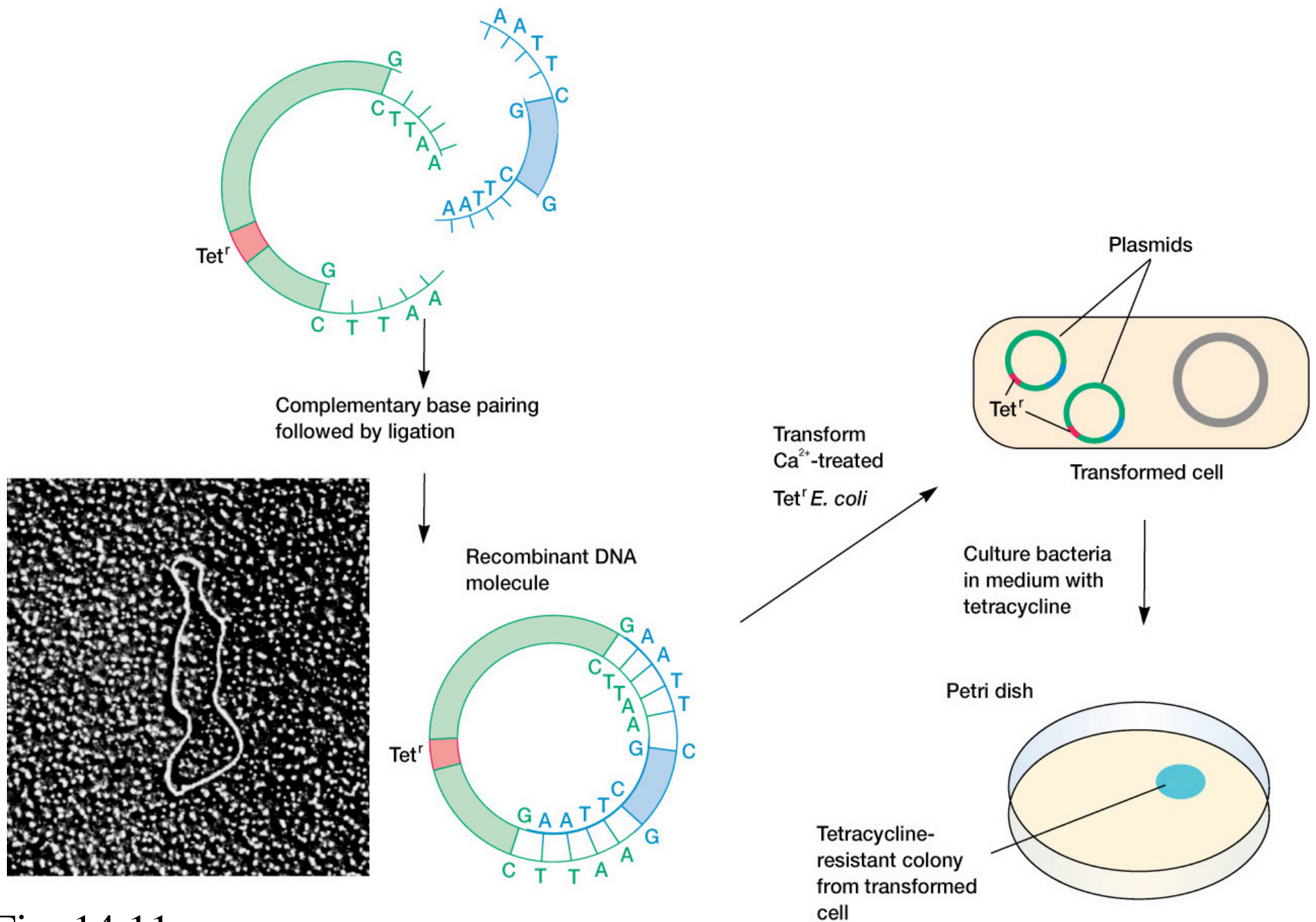


Fig. 14.11

Table 14.4 Some Human Peptides and Proteins
Synthesized by Genetic Engineering

Peptide or Protein	Potential Use
α_1 -antitrypsin	Treatment of emphysema
α -, β -, and γ -interferons	As antiviral, antitumor, and anti-inflammatory agents
Blood-clotting factor VIII	Treatment of hemophilia
Calcitonin	Treatment of osteomalacia
Epidermal growth factor	Treatment of wounds
Erythropoietin	Treatment of anemia
Growth hormone	Growth promotion
Insulin	Treatment of diabetes
Interleukins-1, 2, and 3	Treatment of immune disorders and tumors
Macrophage colony stimulating factor	Cancer treatment
Relaxin	Aid to childbirth
Serum albumin	Plasma supplement
Somatostatin	Treatment of acromegaly
Streptokinase	Anticoagulant
Tissue plasminogen activator	Anticoagulant
Tumor necrosis factor	Cancer treatment

Transcription of RNA

mRNA

rRNA

tRNA

sRNA

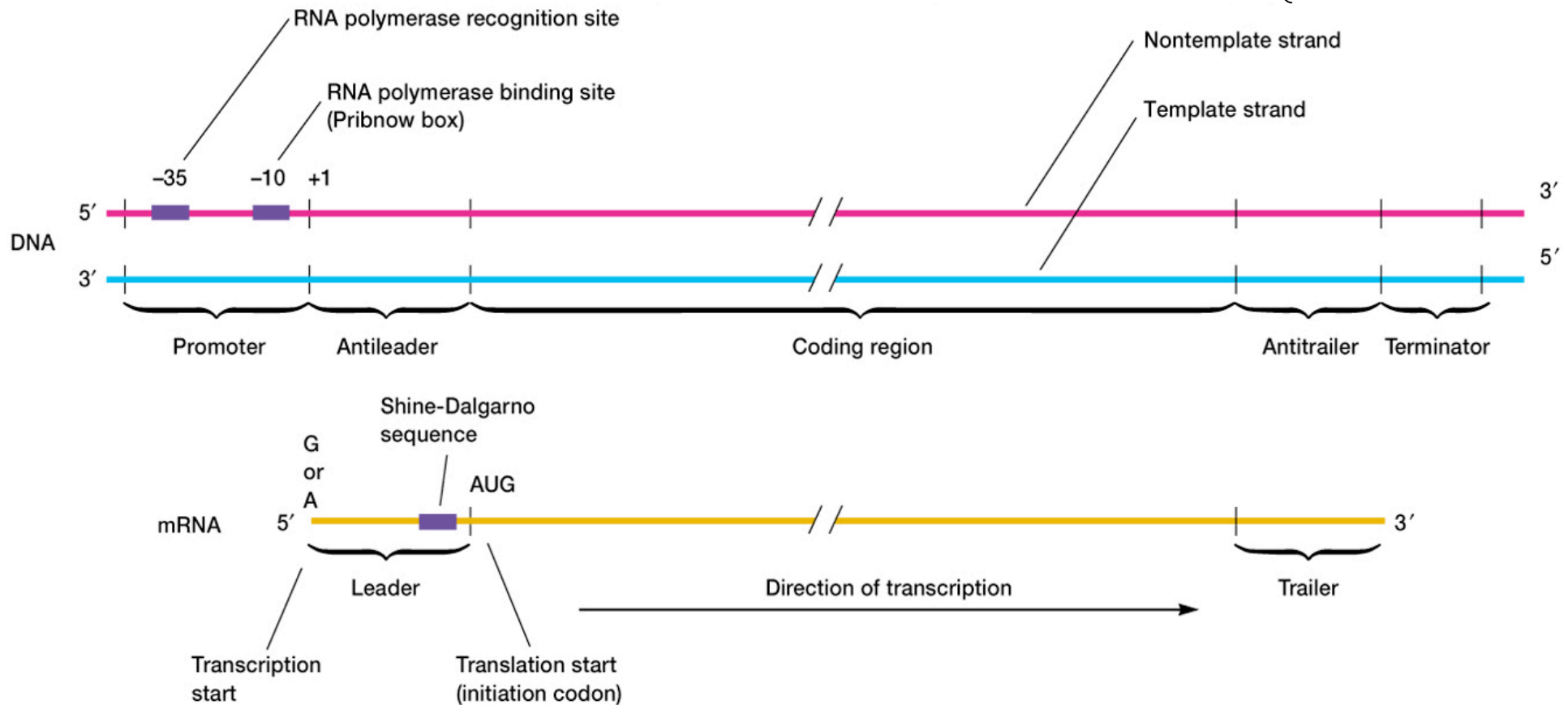


Fig. 11.21

Prokaryotic Promoter

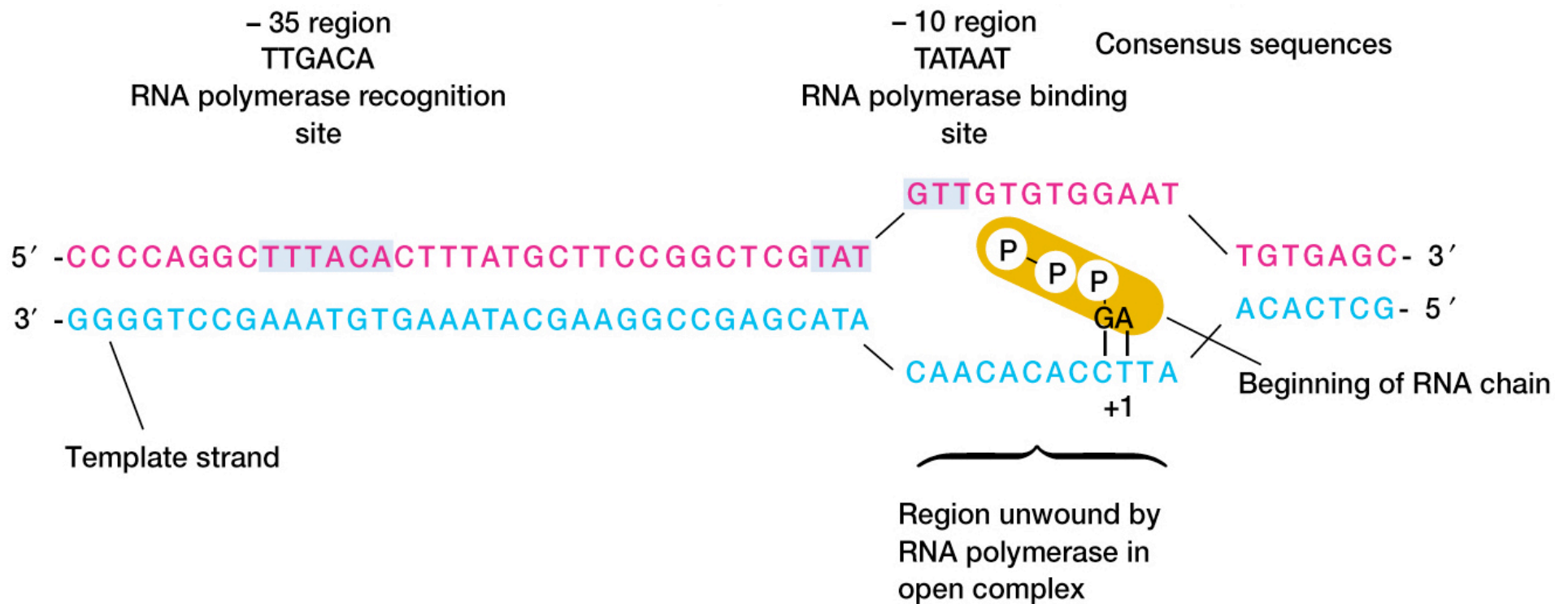


Fig. 11.22

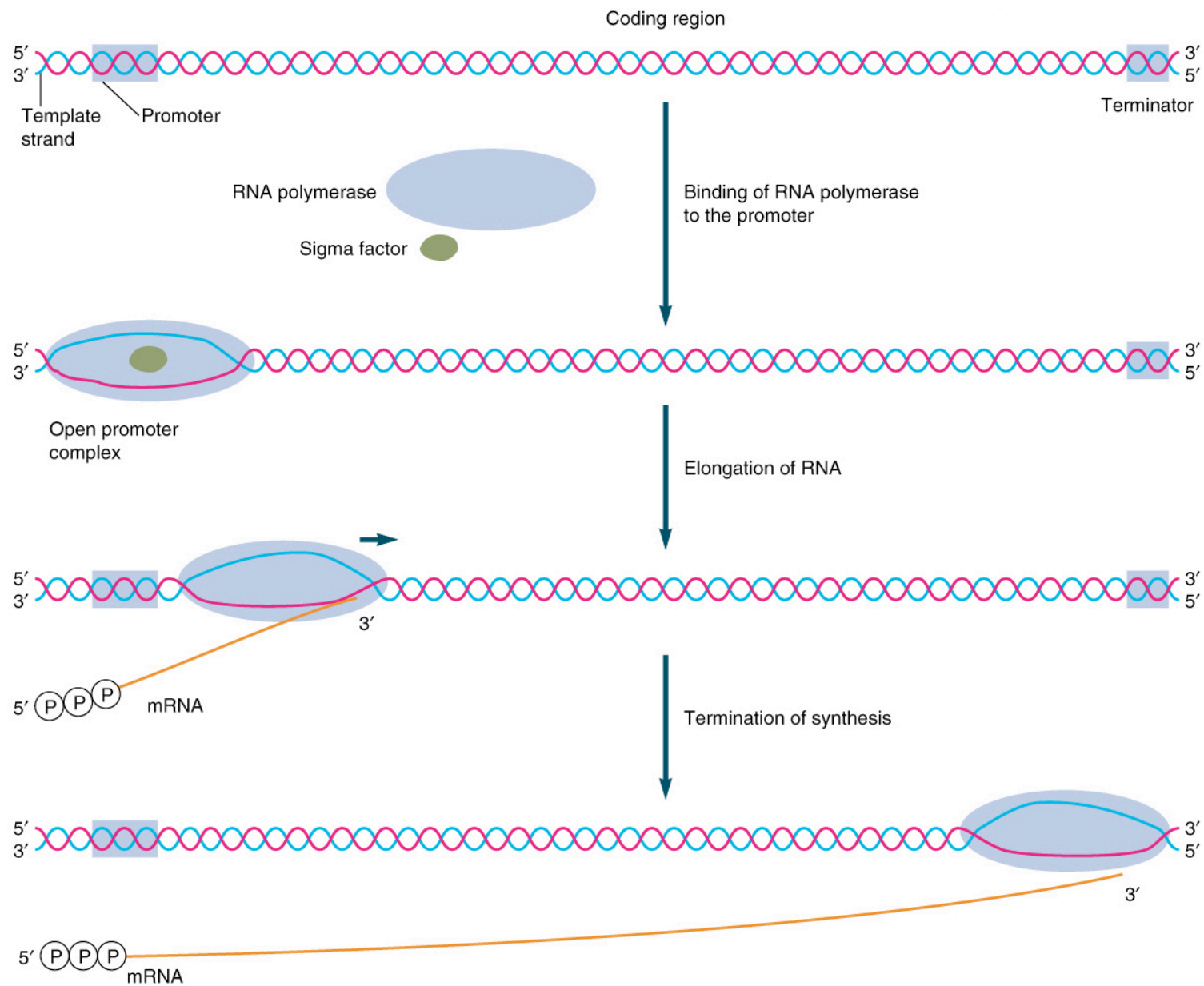
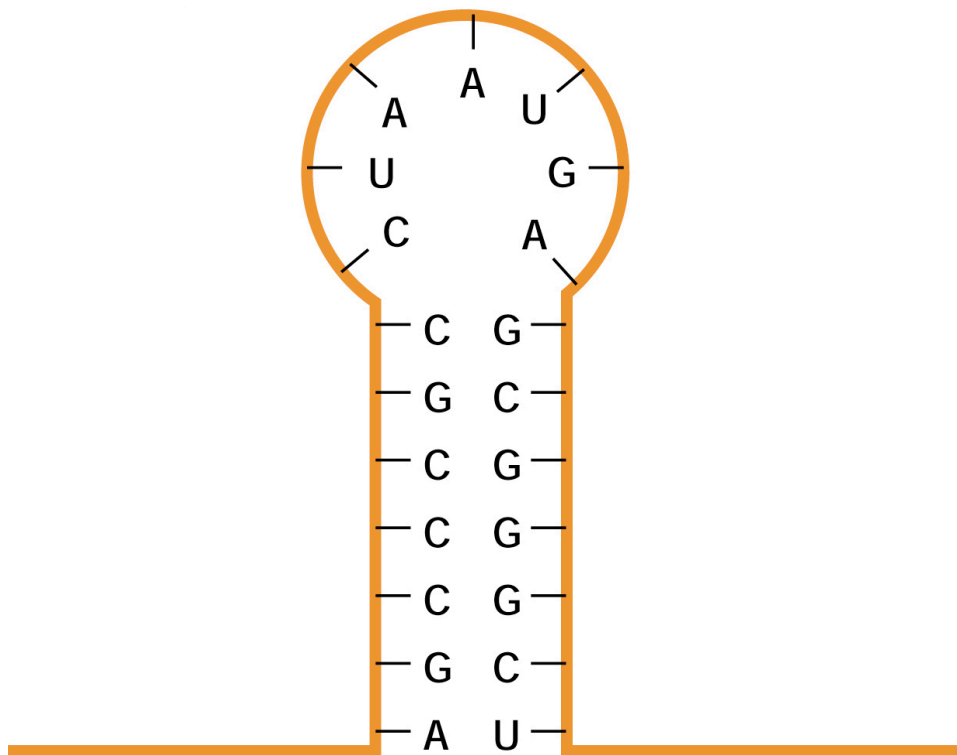


Fig. 12.2

Prokaryotic Terminator



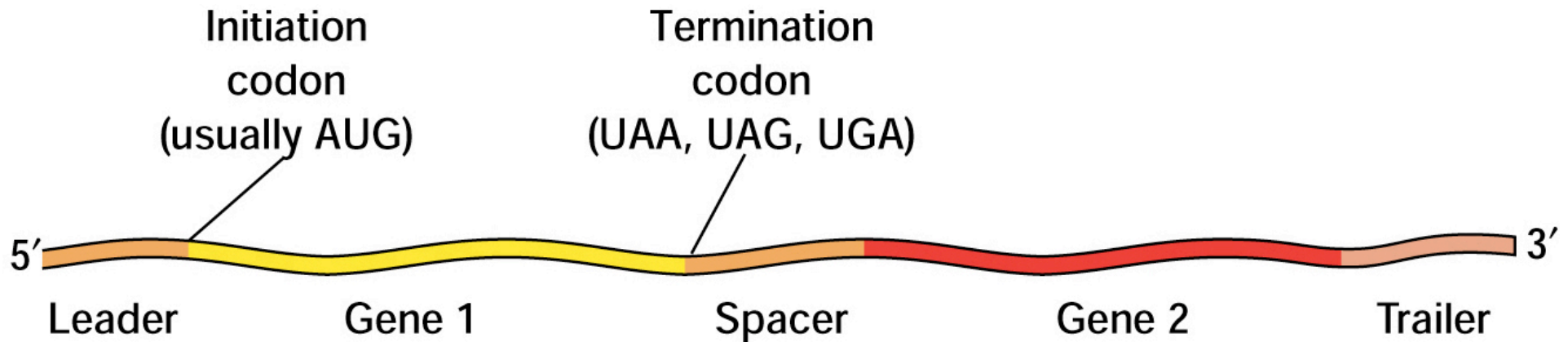
- **Rho-independent=**
 - 6 uridines follow hairpin
 - Requires no accessory proteins
- **Rho-dependent=**
 - No uridines after hairpin
 - Requires Rho to displace RNA polymerase

Fig. 12.3

Rifampin

- Binds to RNA polymerase
- Inhibits transcription, killing cell

Polycistronic mRNA



- Multiple genes on single transcript
- Transcription from a single promoter
- Usually genes on a polycistronic mRNA are related to a specific function or structure
- No introns in within genes

Fig. 12.1

Coupled transcription-translation

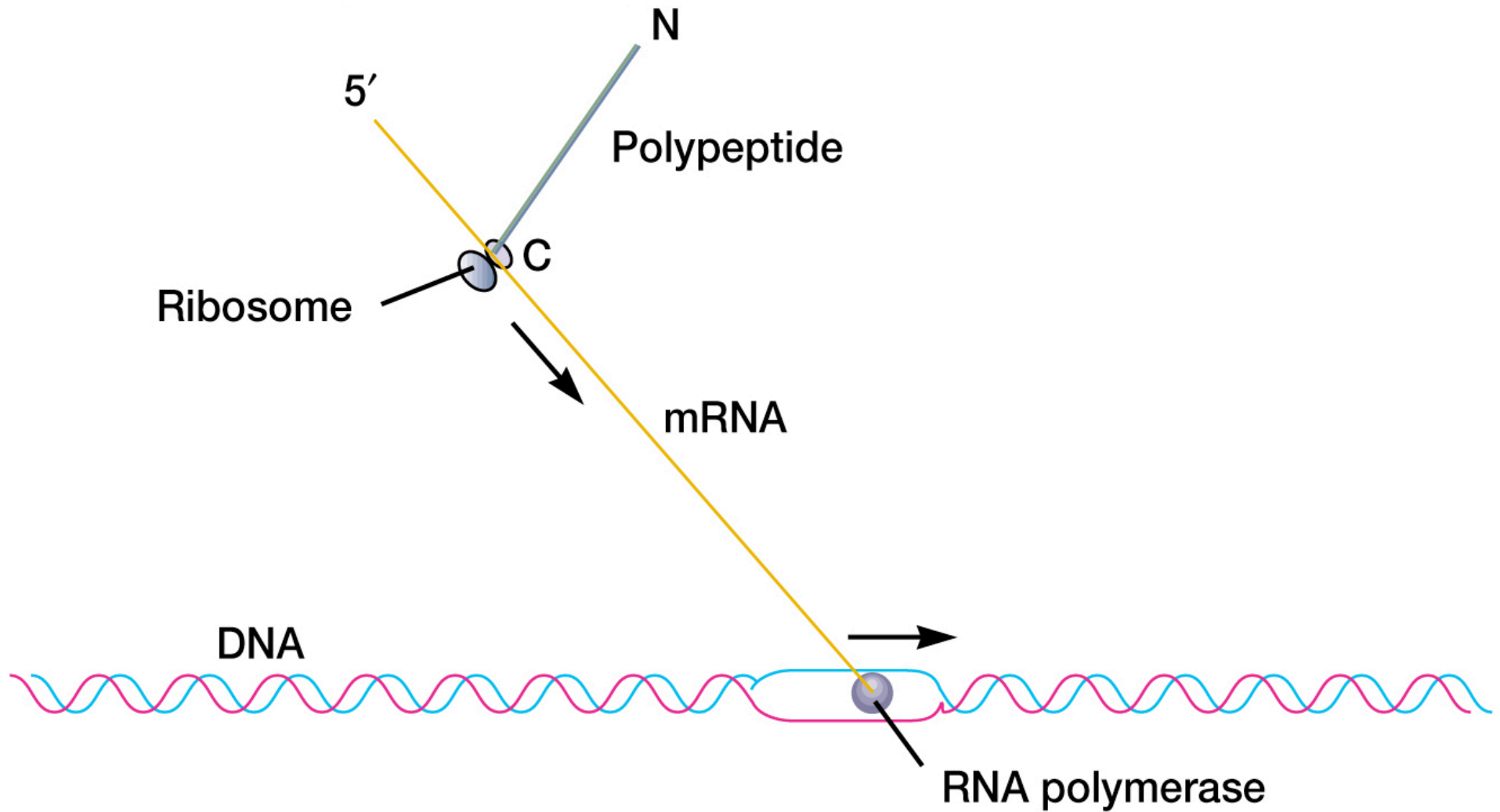


Fig. 12.6

Prokaryotic Ribosome

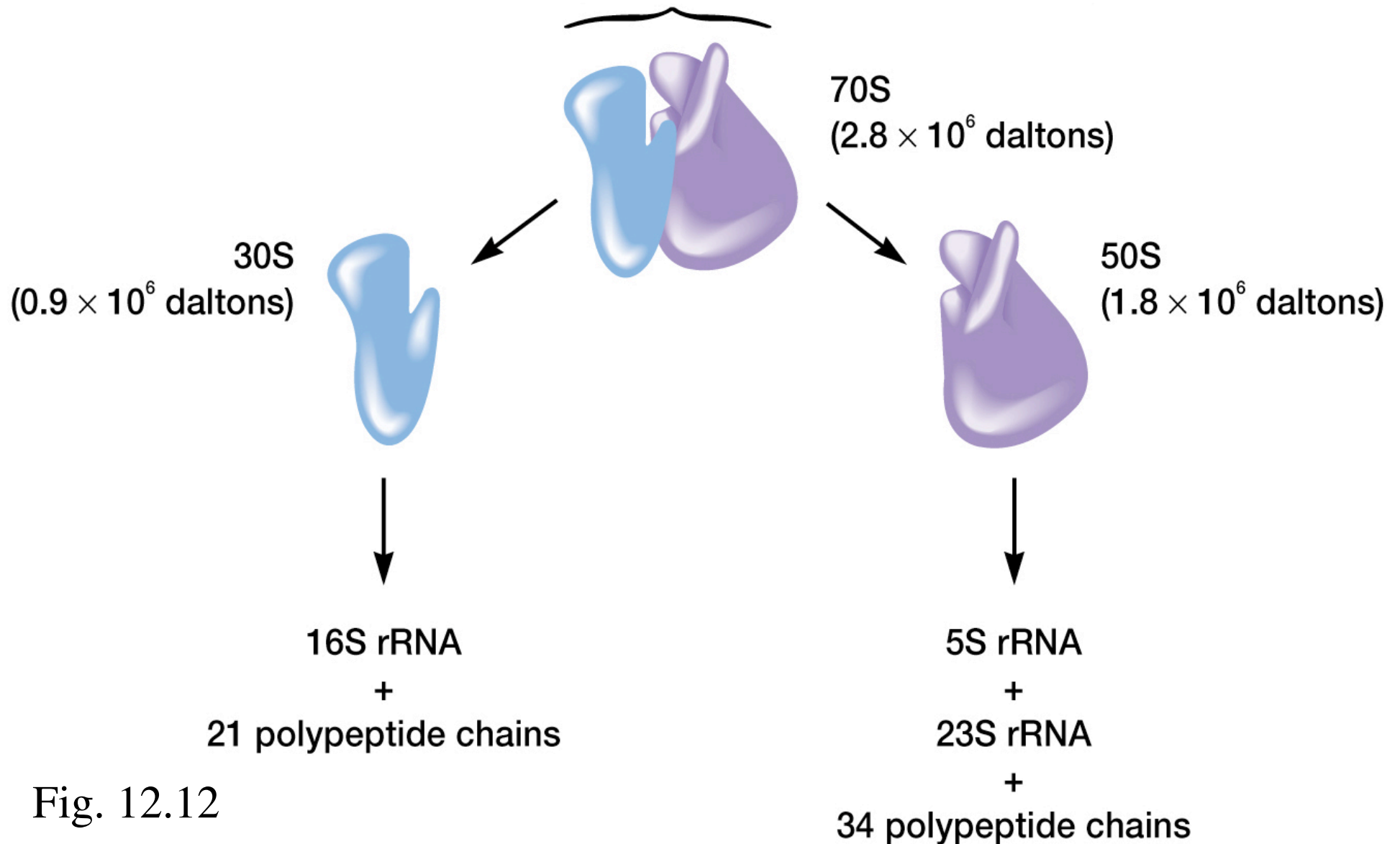


Fig. 12.12

Translational Domain

- Formed by association of 30S and 50S subunits
- 16S rRNA binds to and aligns mRNA

16S rRNA

3'-UUCCUU-5'

mRNA

5'-AAGGAA-3'

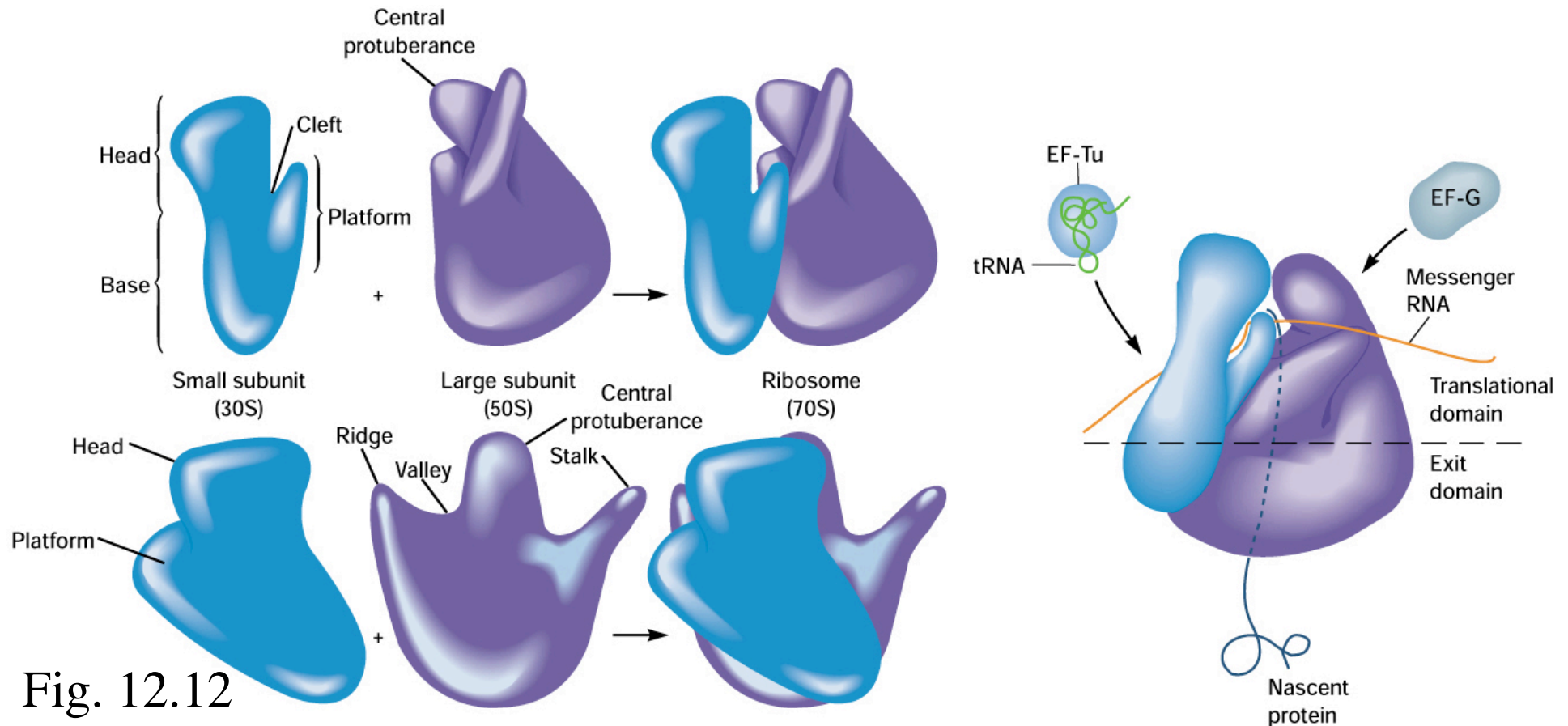


Fig. 12.12

tRNA structure

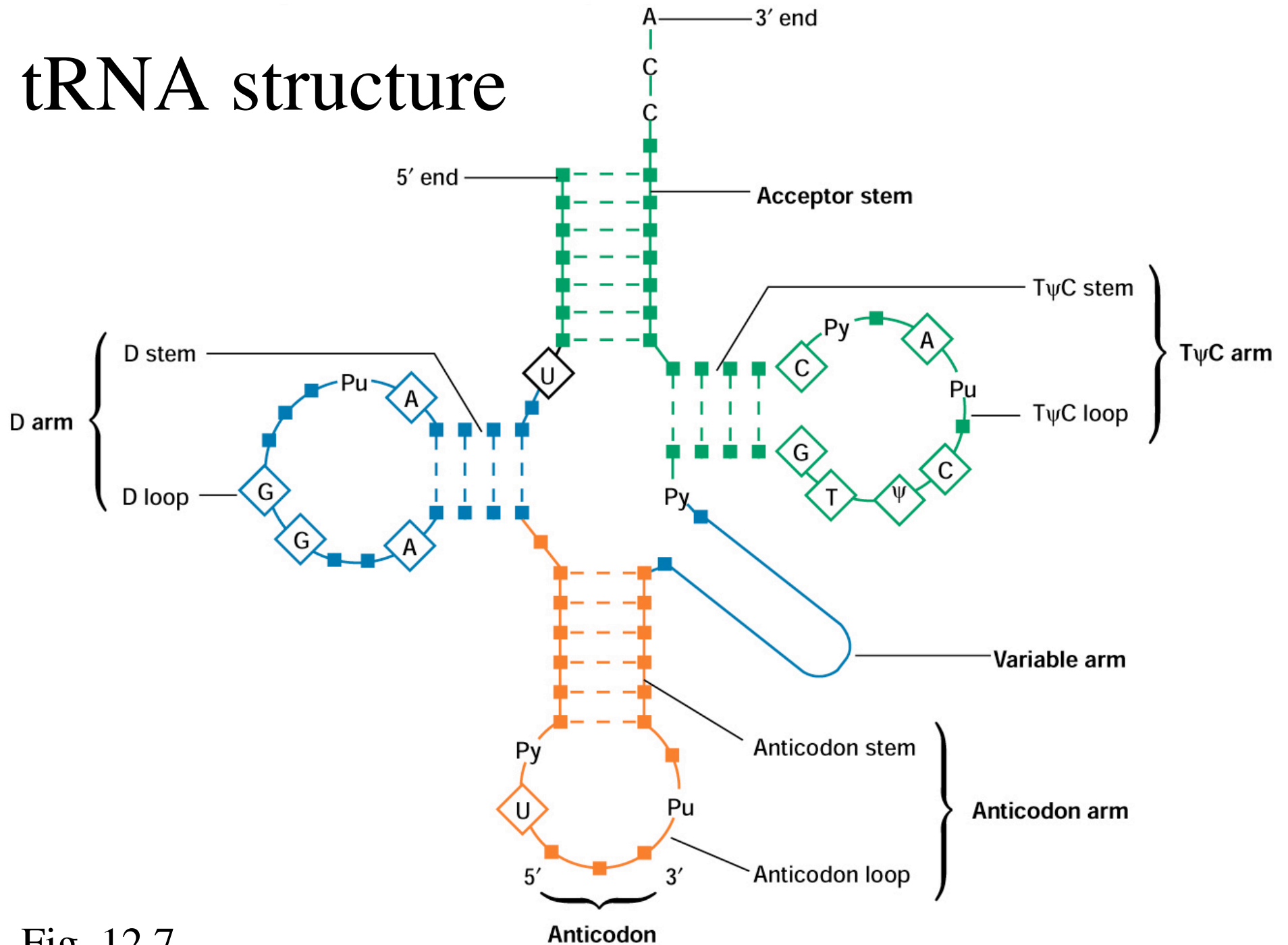
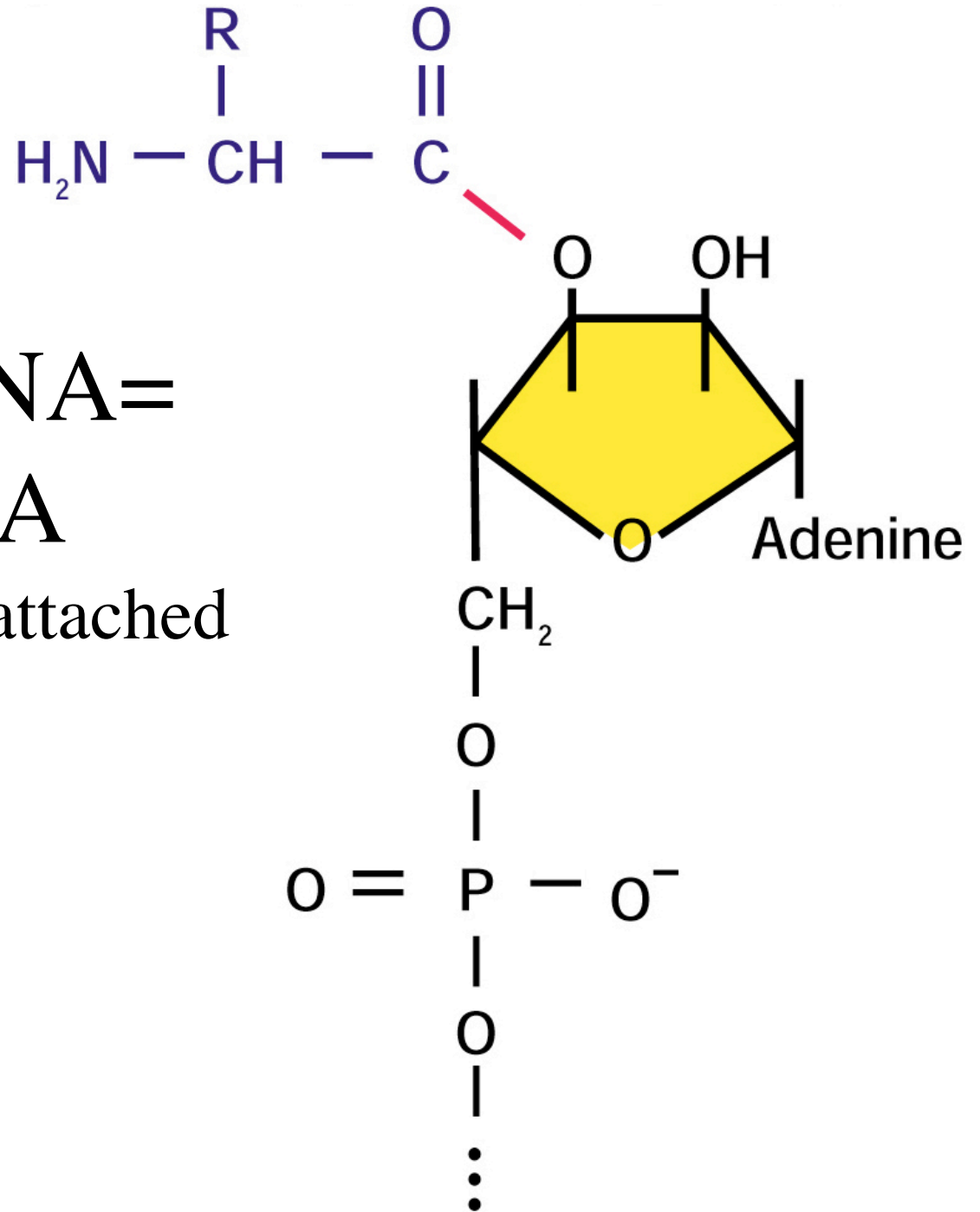


Fig. 12.7



Amino-acyl tRNA=

“Charged” tRNA

-cognate amino acid attached

to 3' end

Fig. 12.10

Table 11.1 The Genetic Code

		<i>Second Position</i>				
		U	C	A	G	
<i>First Position (5' End)^a</i>	U	UUU } Phe	UCU } Ser	UAU } Tyr	UGU } Cys	U C A G
		UUC }	UCC }	UAC }	UGC }	
		UUA } Leu	UCA }	UAA } STOP	UGA } STOP	
		UUG }	UCG }	UAG }	UGG } Trp	
	C	CUU } Leu	CCU } Pro	CAU } His	CGU } Arg	U C A G
		CUC }	CCC }	CAC }	CGC }	
		CUA }	CCA }	CAA } Gln	CGA }	
		CUG }	CCG }	CAG }	CGG }	
	A	AUU } Ile	ACU } Thr	AAU } Asn	AGU } Ser	U C A G
		AUC }	ACC }	AAC }	AGC }	
		AUA }	ACA }	AAA } Lys	AGA } Arg	
		AUG } Met	ACG }	AAG }	AGG }	
	G	GUU } Val	GCU } Ala	GAU } Asp	GGU } Gly	U C A G
		GUC }	GCC }	GAC }	GGC }	
		GUA }	GCA }	GAA } Glu	GGA }	
		GUG }	GCG }	GAG }	GGG }	

^aThe code is presented in the RNA form. Codons run in the 5' to 3' direction. See text for details.

Initiation of Protein Synthesis

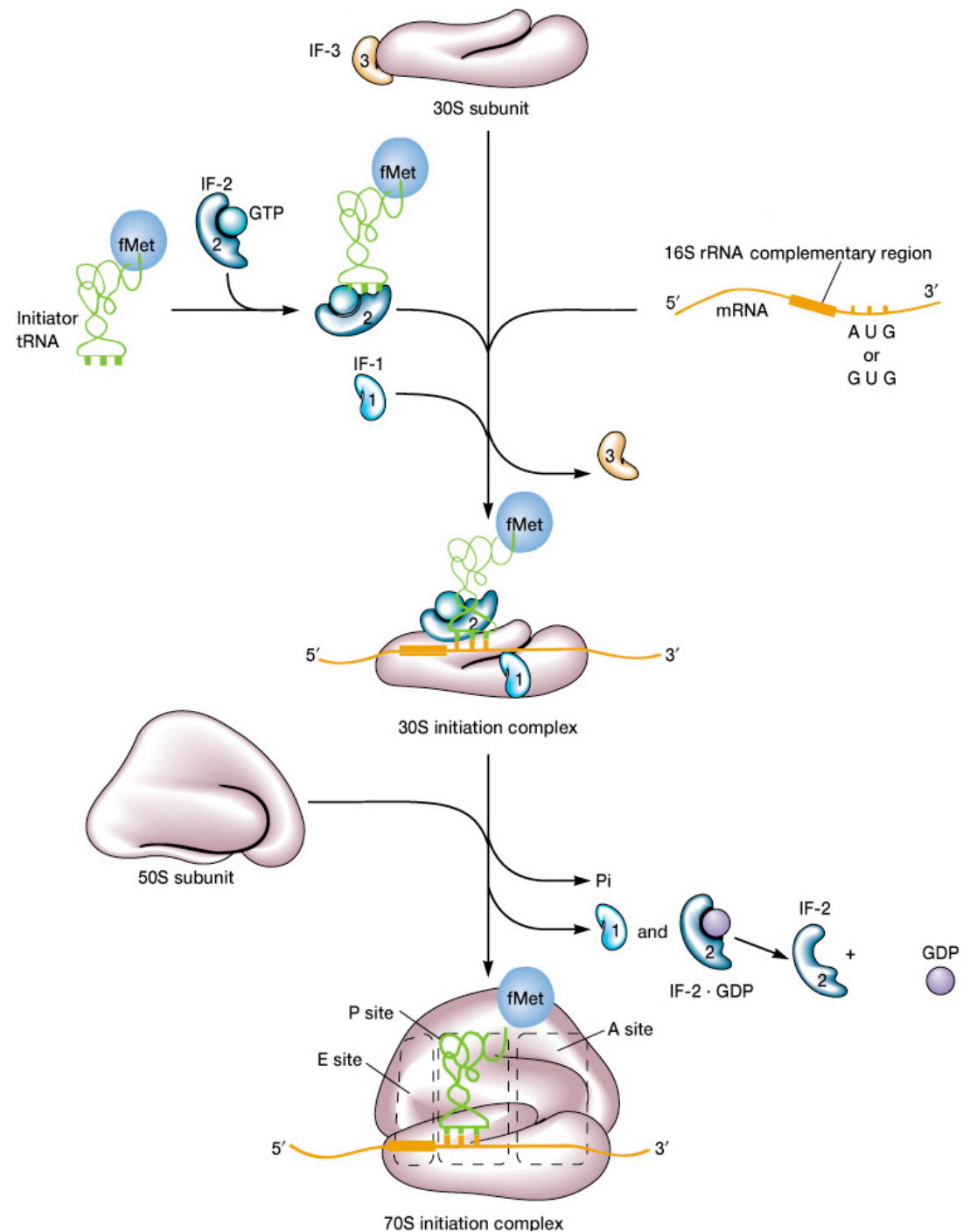


Fig. 12.14

Alignment to allow in-frame translation

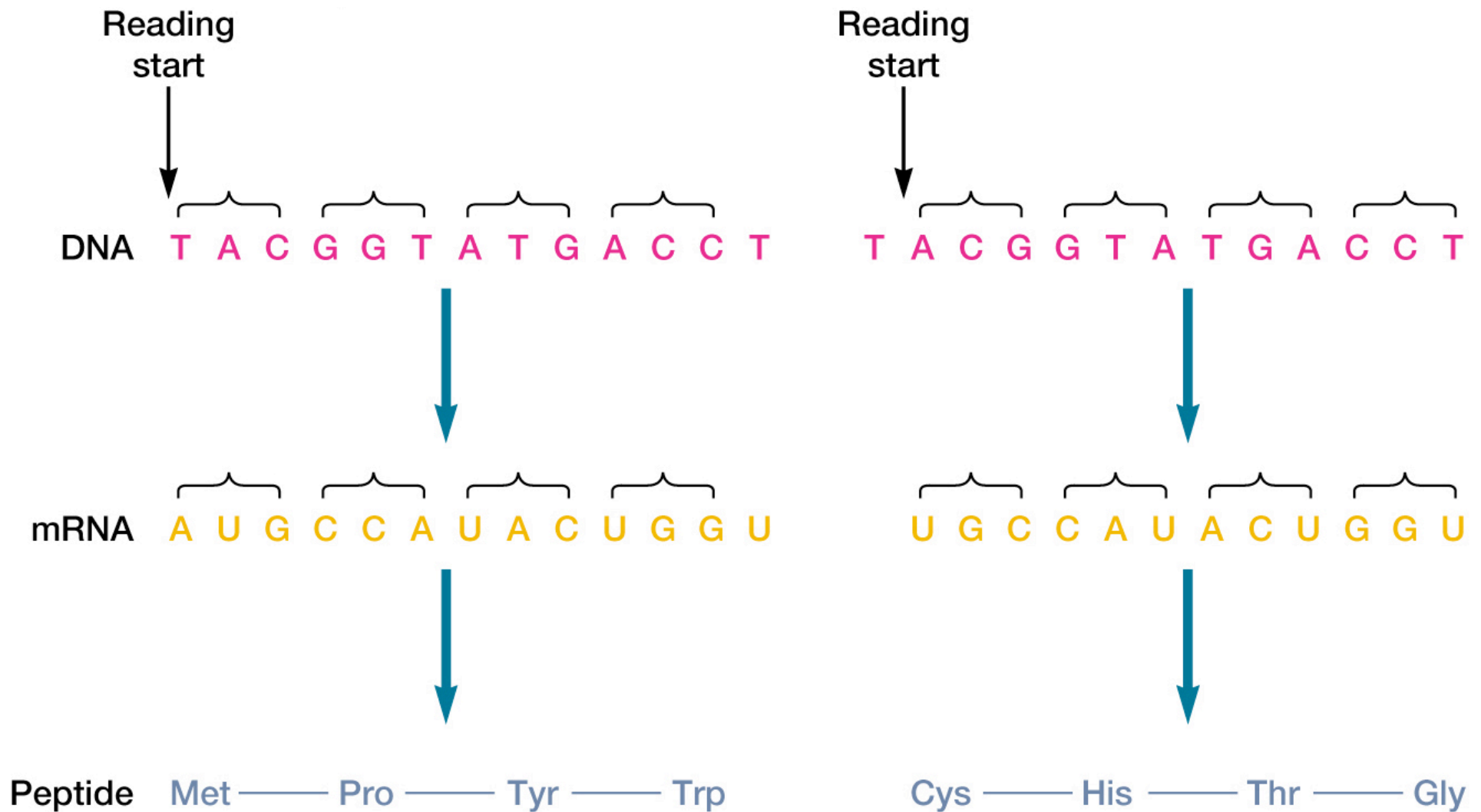


Fig. 11.19

Initiation of Protein Synthesis

tRNA
mRNA

3'-UAC-5'
5'-AUG-3'

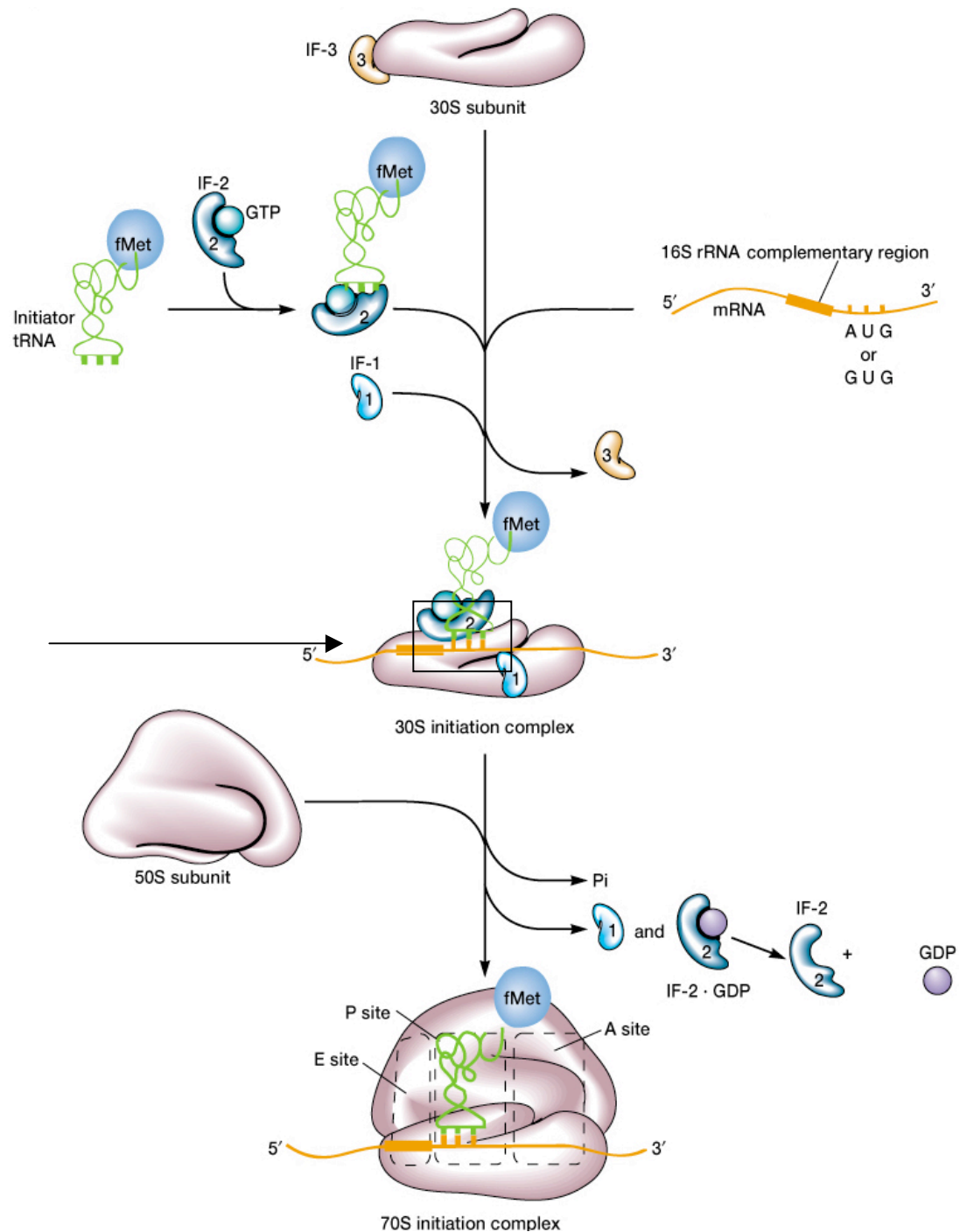


Fig. 12.14

Prokaryotic Initiator tRNA

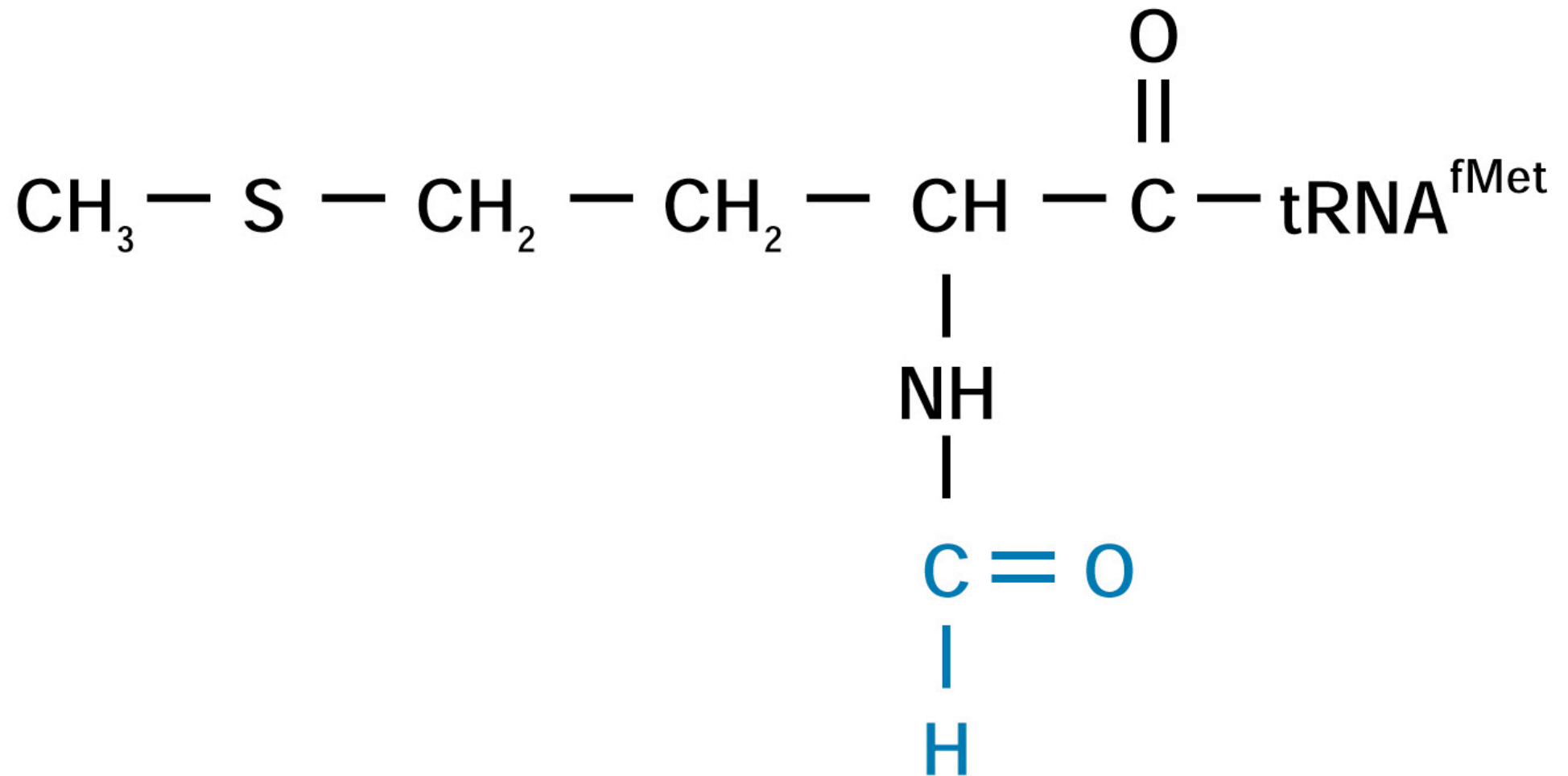


Fig. 12.13

Elongation

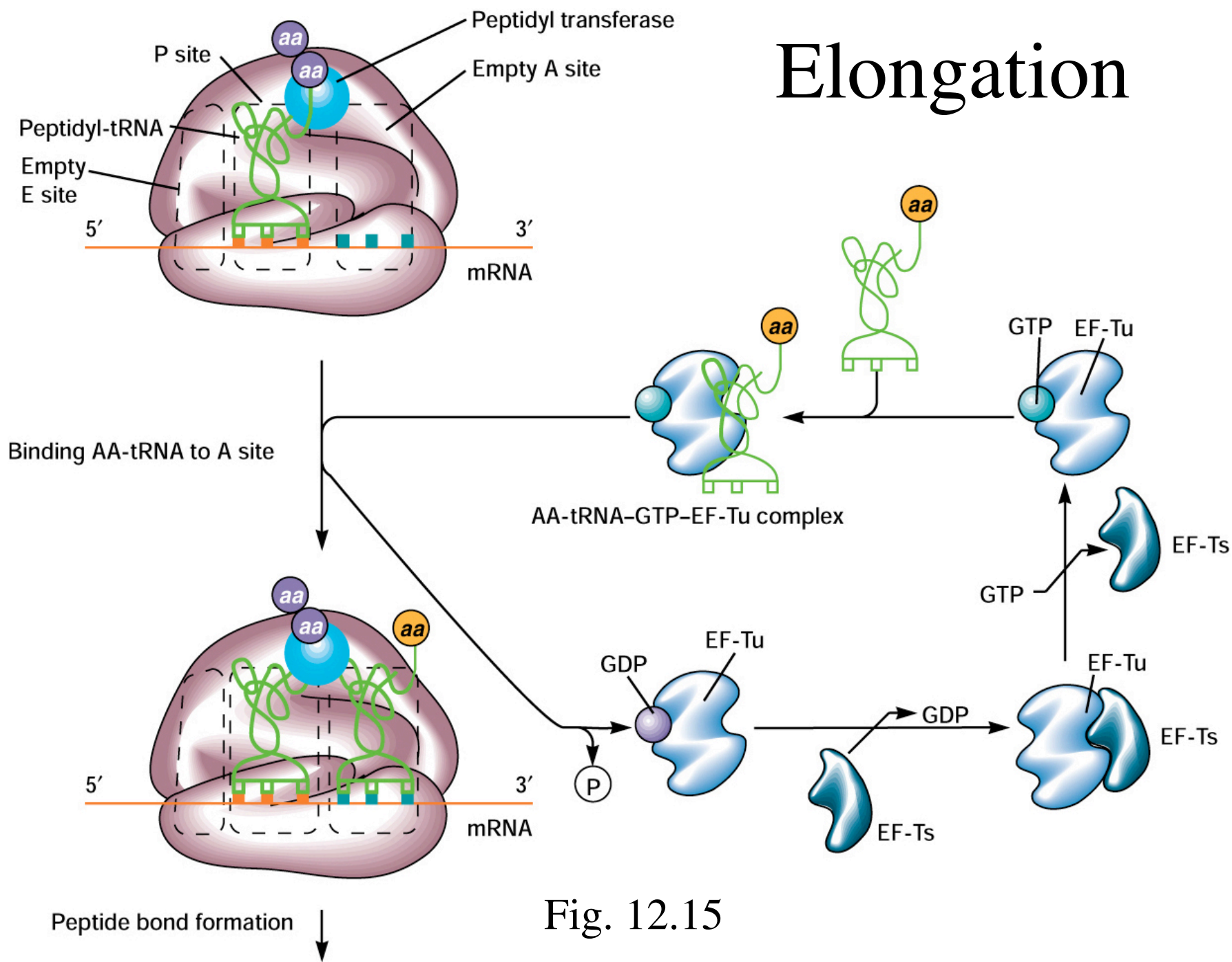


Fig. 12.15

Peptide Bond Formation

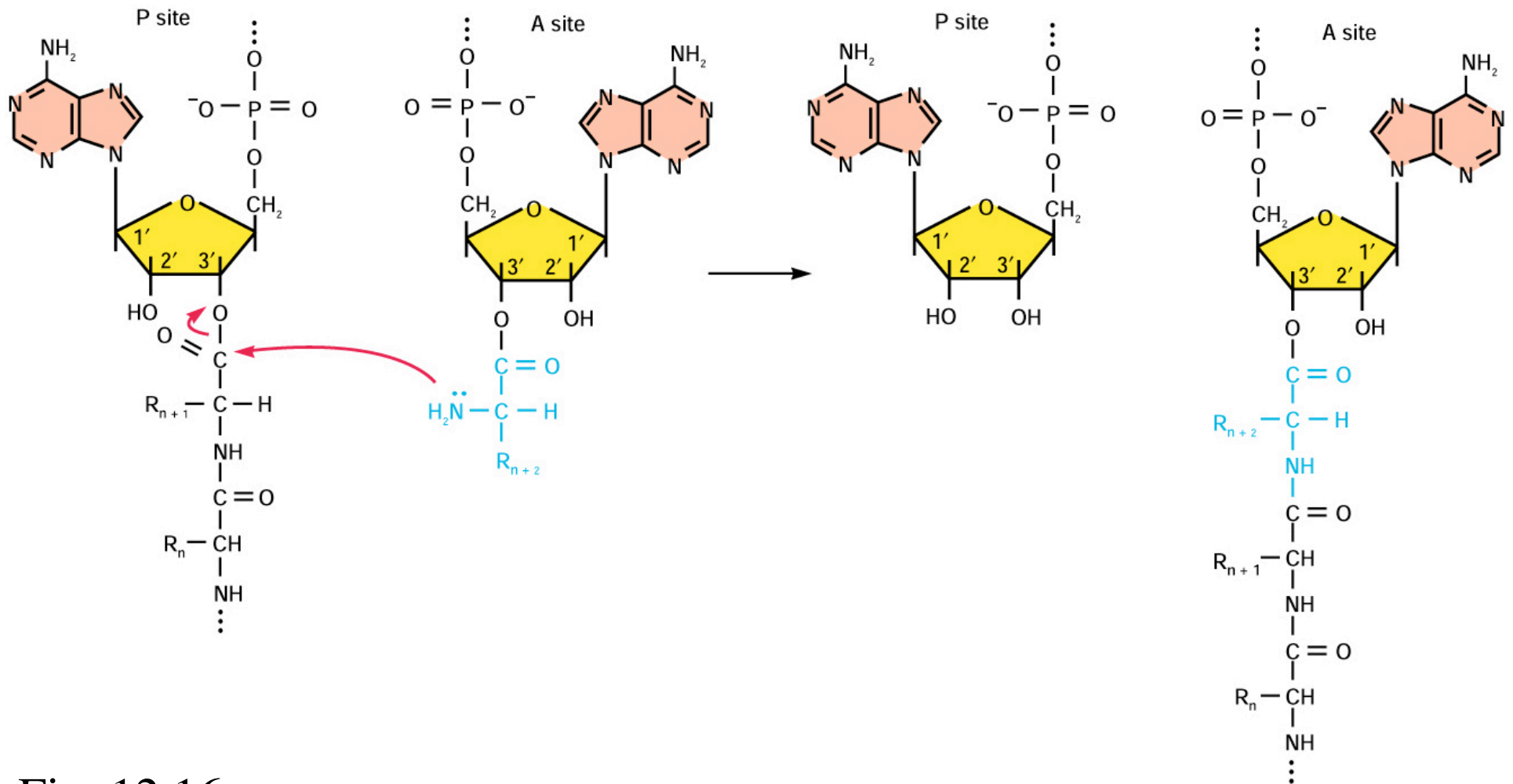
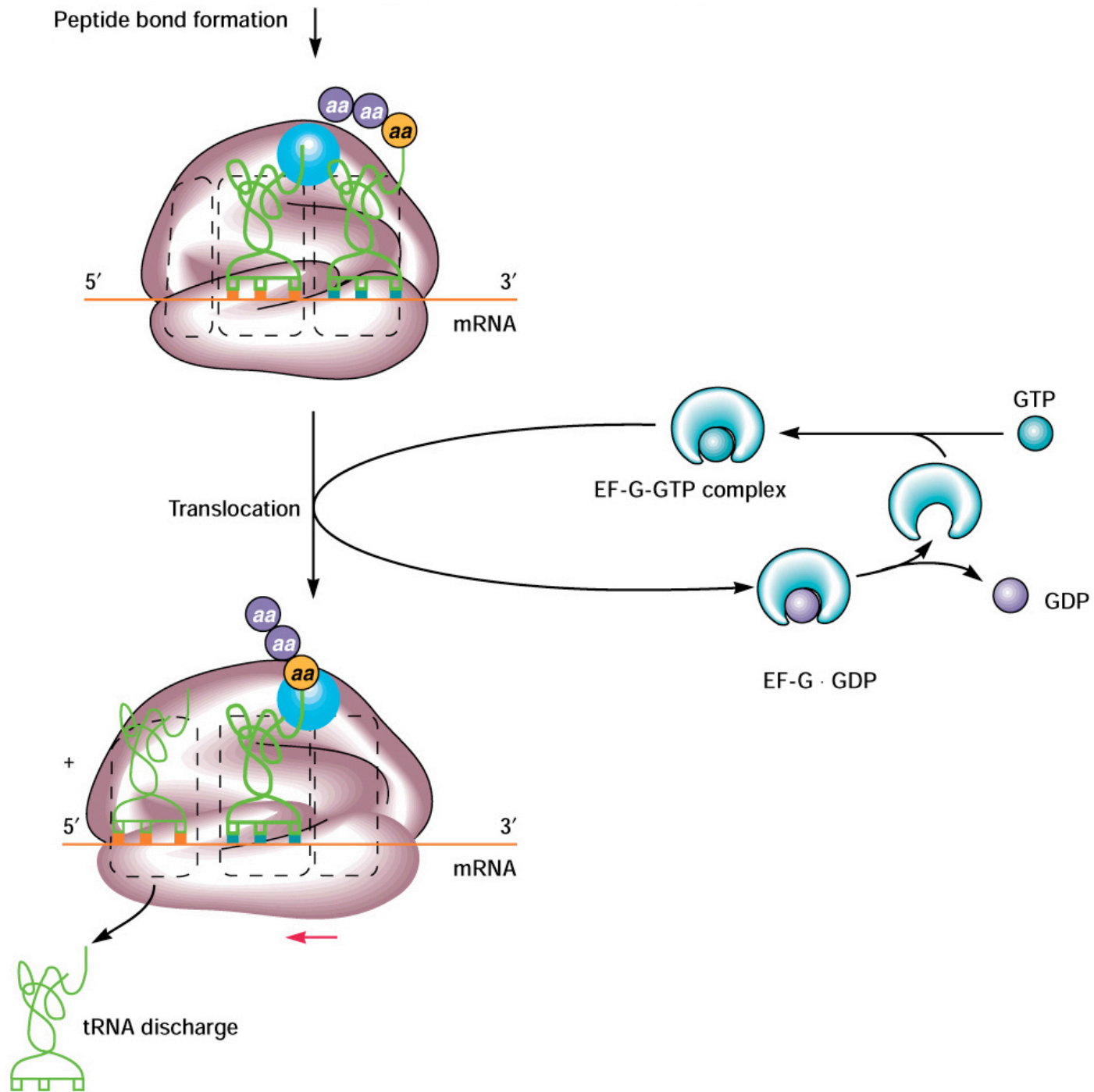


Fig. 12.16



Termination

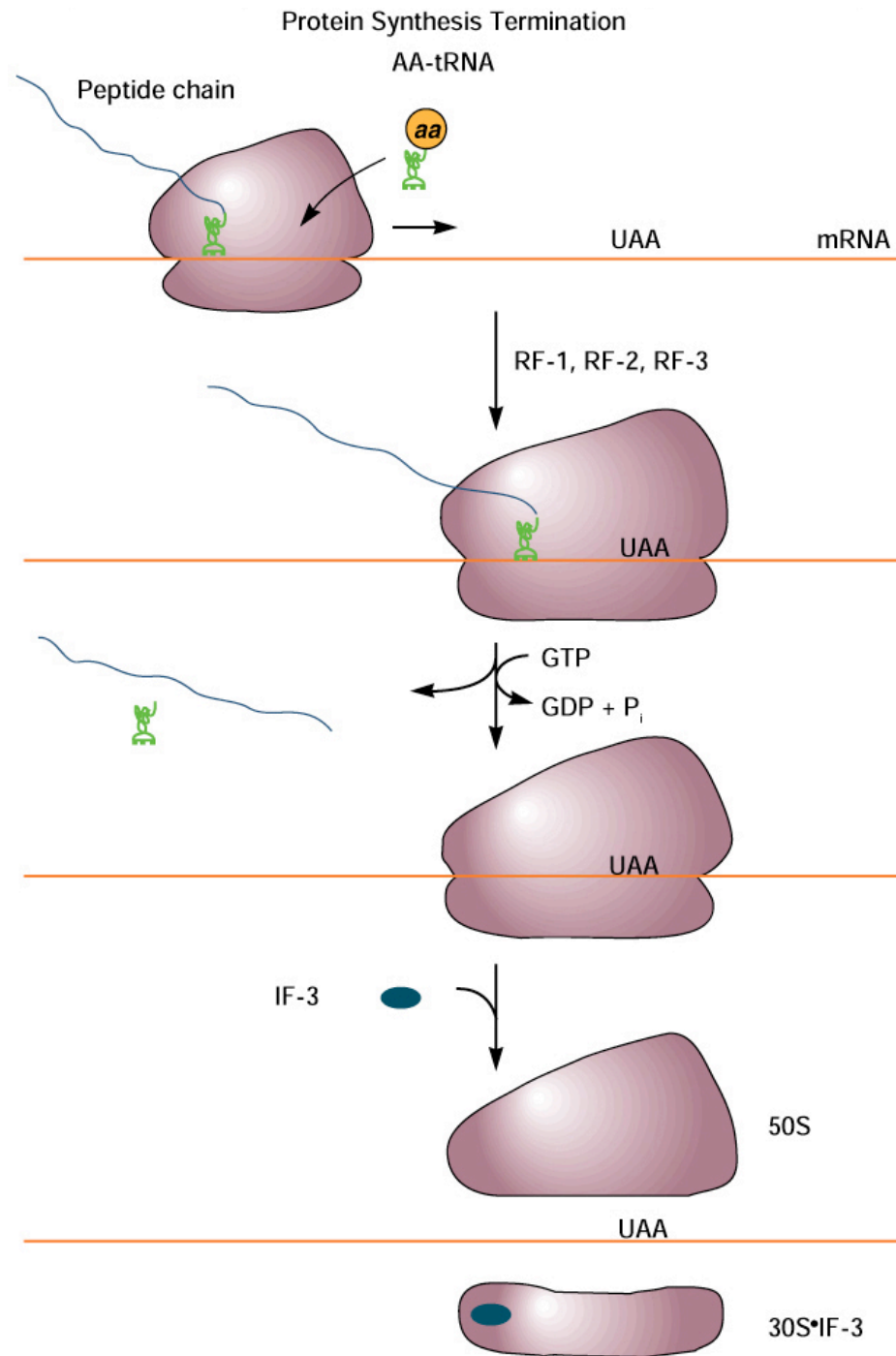


Fig. 12.17

Tetracyclines

- Includes doxycycline, chlortetracycline
- Binds to 30S subunit
- Interferes with aminoacyl-tRNA binding to A site

Tetracycline (chlortetracycline, doxycycline)

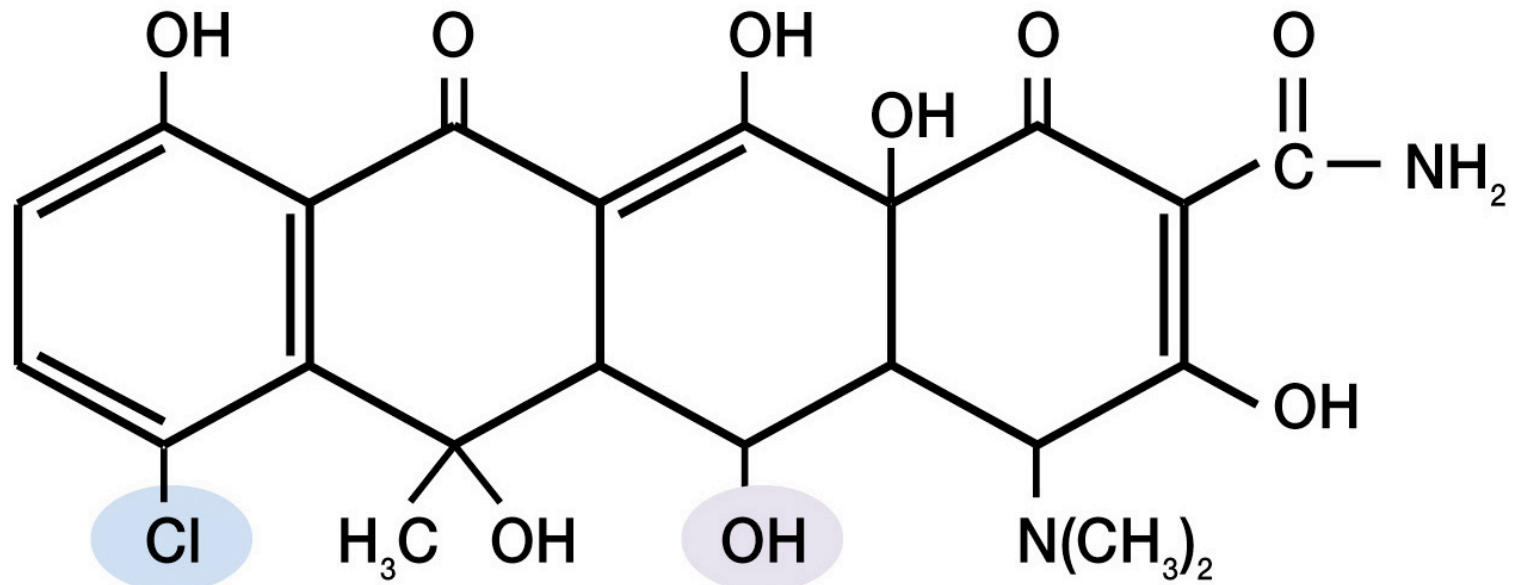


Fig. 35.9

Macrolide antibiotics

- Includes clindamycin, azithromycin
- Binds 23S rRNA of 50S subunit
- Inhibits peptide chain elongation

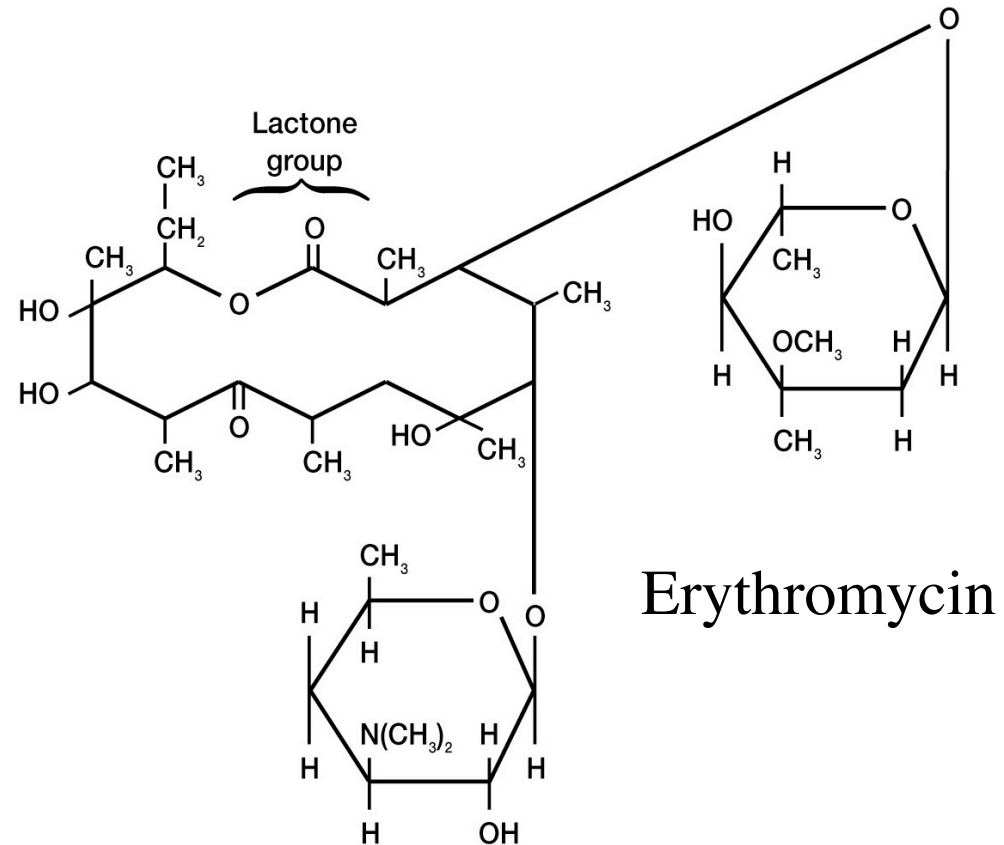
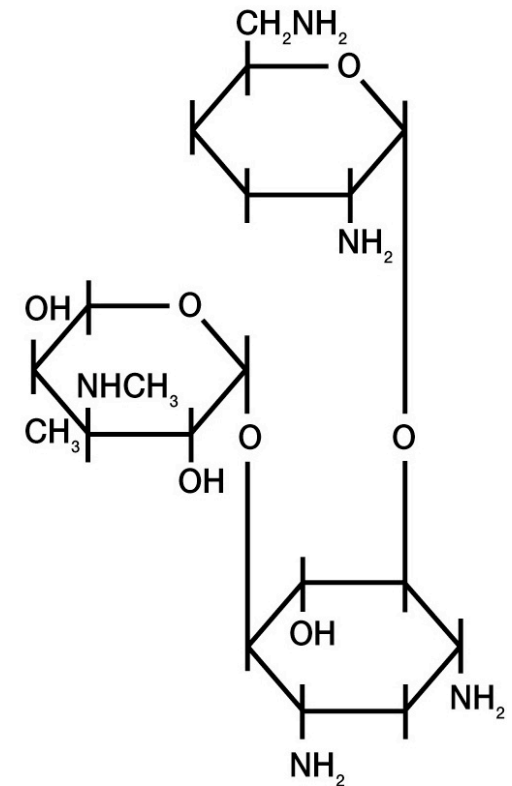
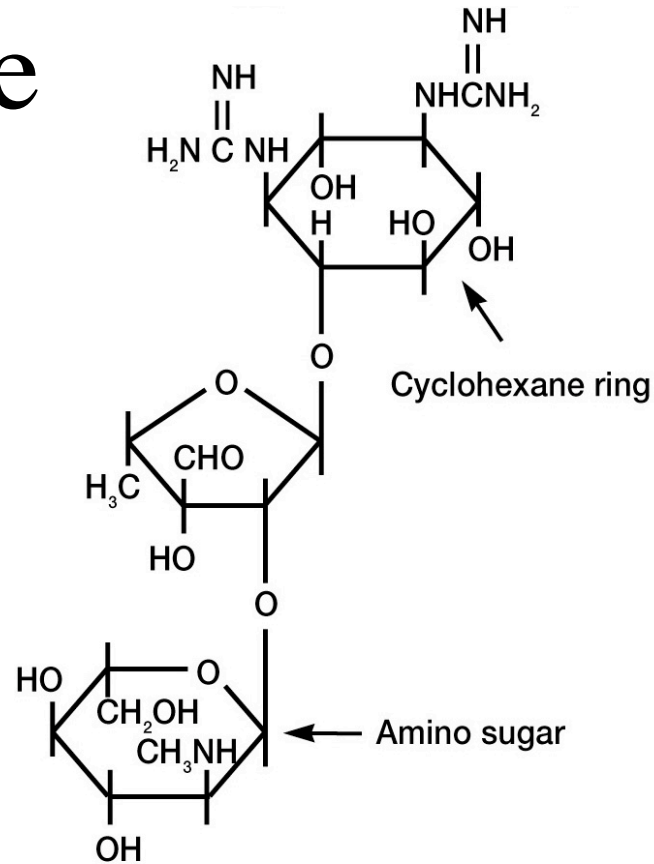


Fig. 35.11

Aminoglycoside antibiotics



Streptomycin

Gentamicin C_{1a}

- Includes streptomycin, gentamicin, neomycin, tobramycin, kanamycin
- Binds to 30S subunit
- Inhibits protein synthesis
- Causes misreading of mRNA

Fig. 35.10