

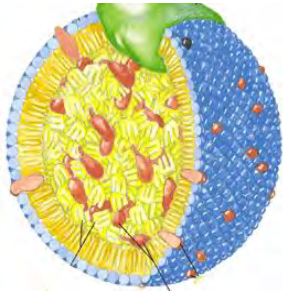


BIOCHEMISTRY REVIEW

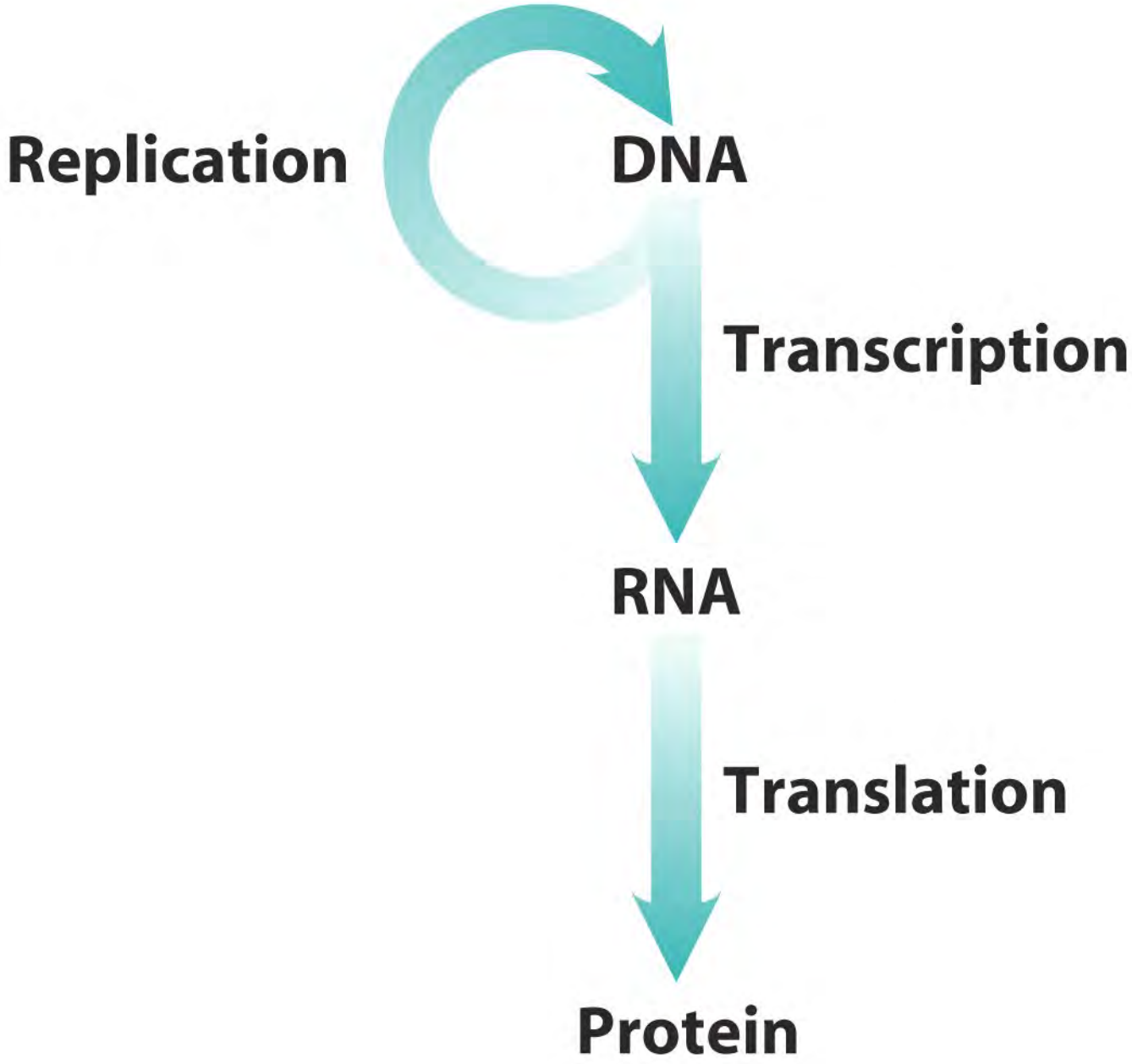
Overview of Biomolecules

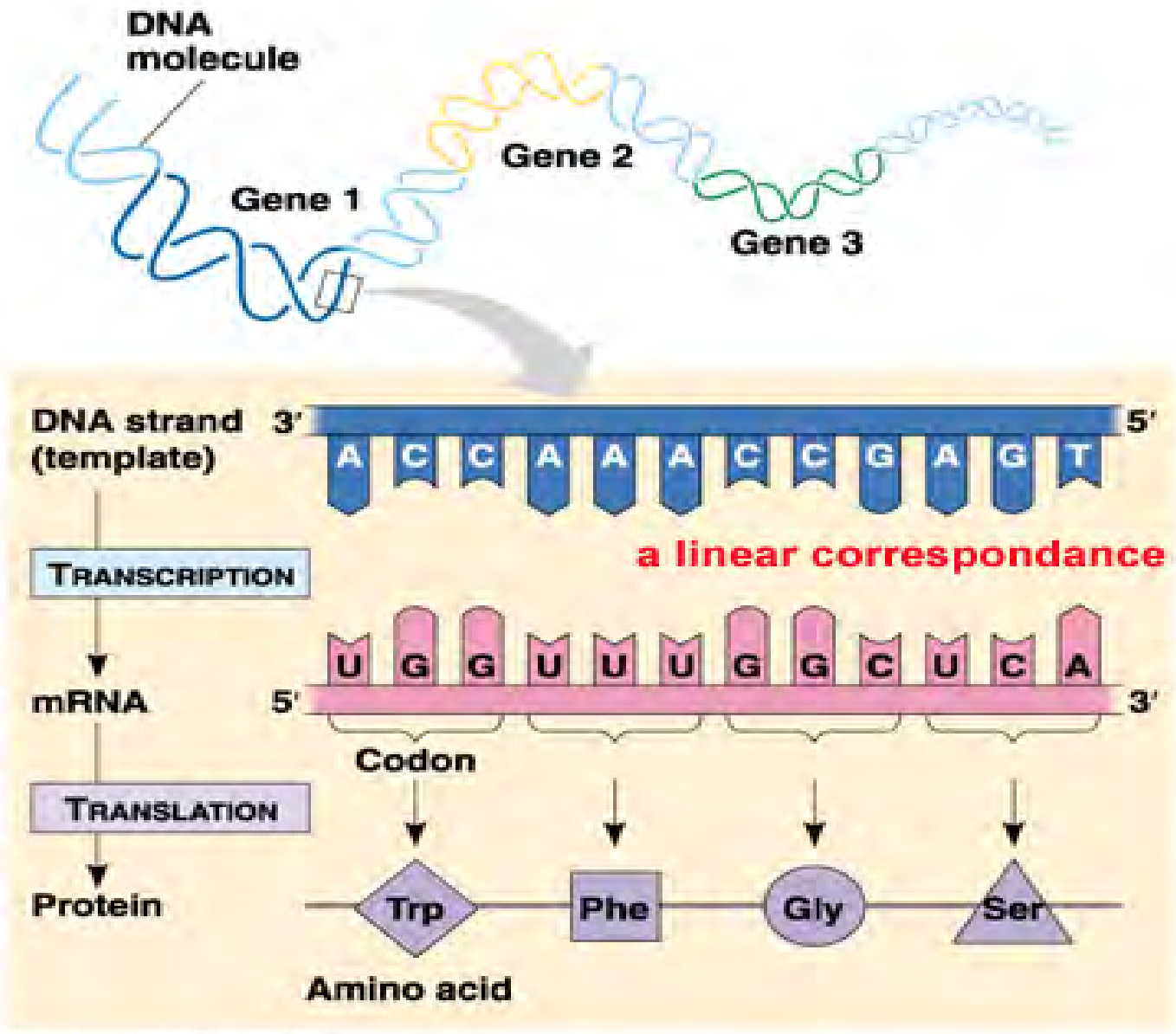
Chapter 12

Transcription

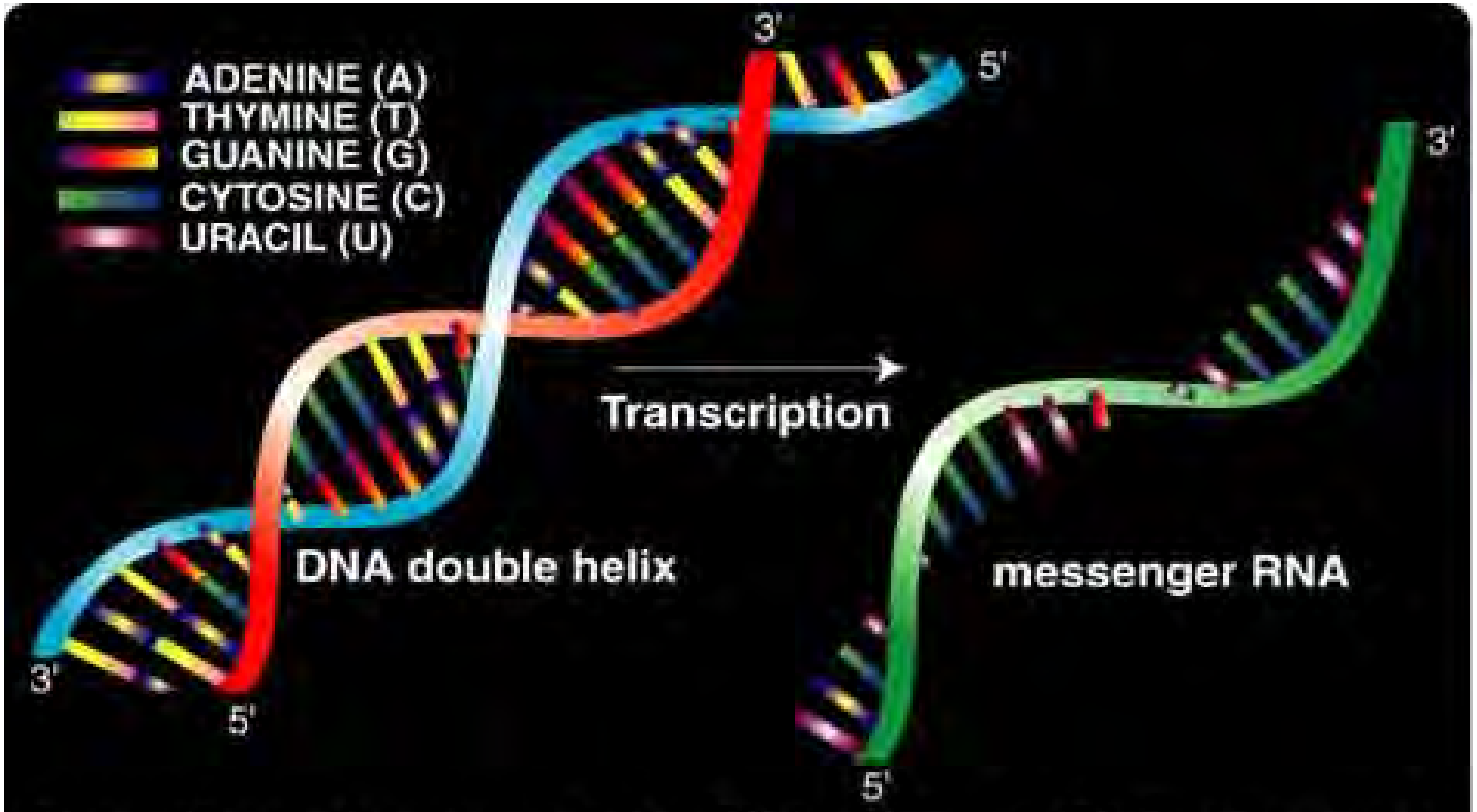





Replication
DNA
Transcription
RNA
Translation
Protein



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Formation of messenger RNA from a DNA template strand. The strand in blue, the 3'-5' strand is the template strand. The RNA polymerase transcribes the information in this strand . During this transcription, A becomes U, T becomes A, G becomes C and C becomes G.



(a) Monocistronic



(b) Polycistronic



Are You Getting It??



Which are general characteristics of transcription?
(multiple answers)

a) An entire DNA molecule is transcribed at one time.
b) A DNA double-helix is denatured to create a template.
c) Base-pairs that can form include G≡C and A=U.
d) The RNA strand made is complementary to a DNA strand.
e) Monocistronic mRNA consists entirely of coding regions.
f) Polycistronic mRNA contains non-coding spacers.



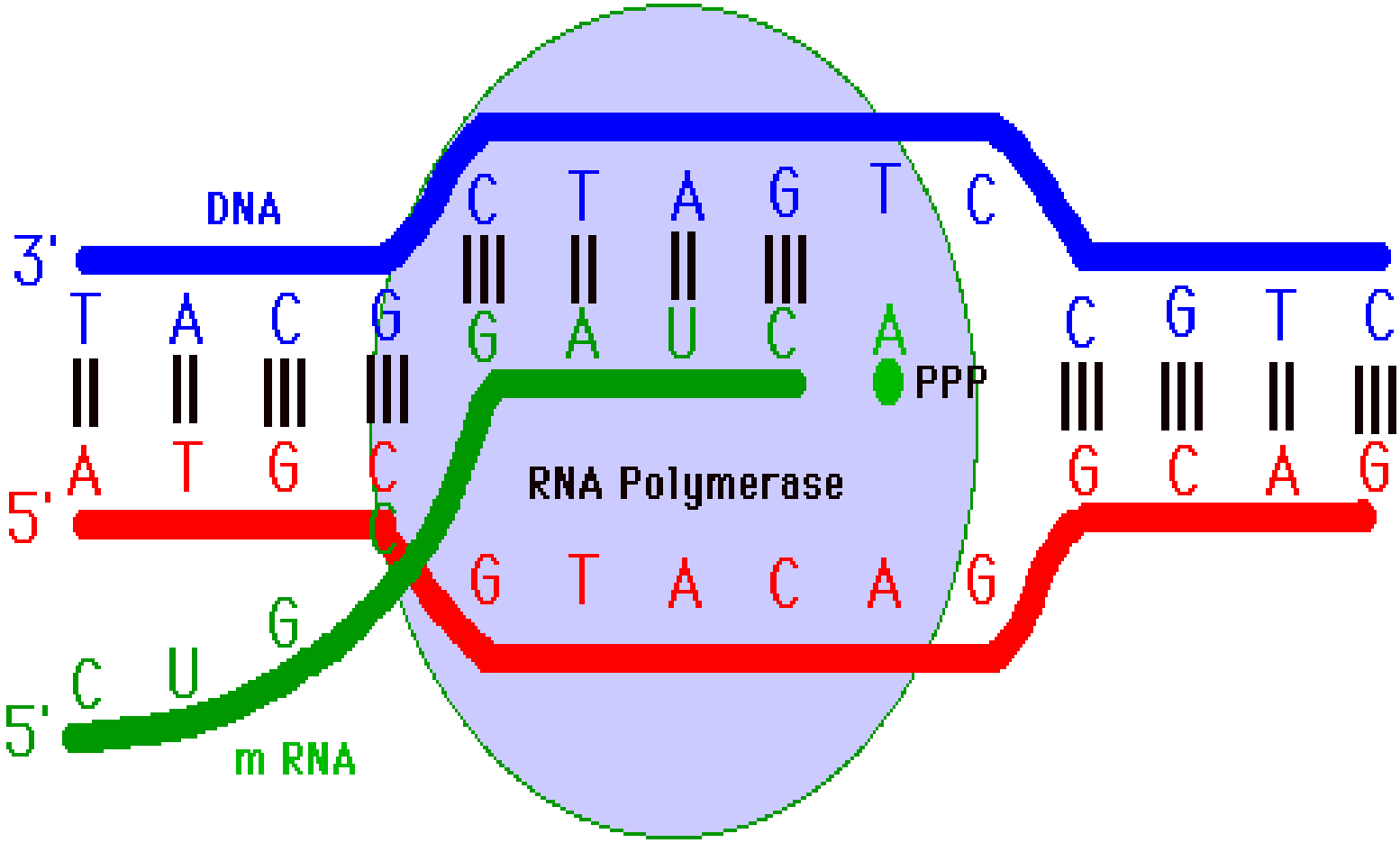
Are You Getting It??

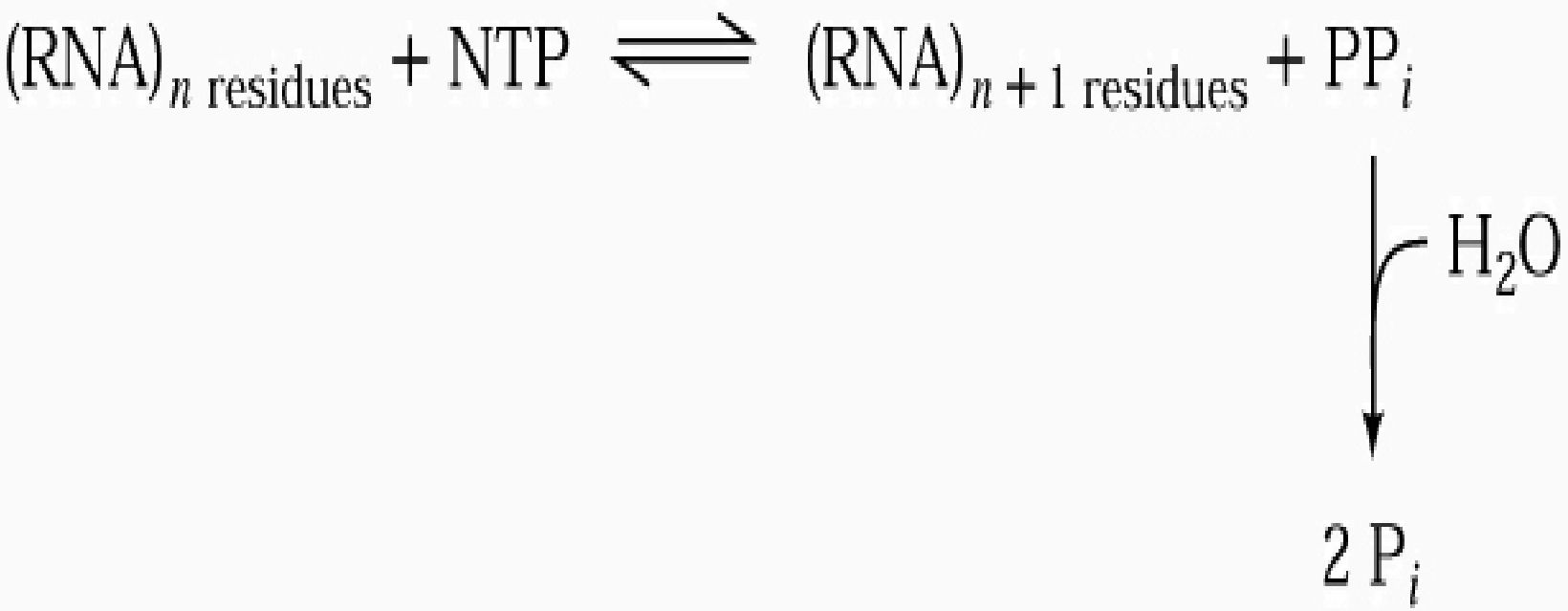


Answer

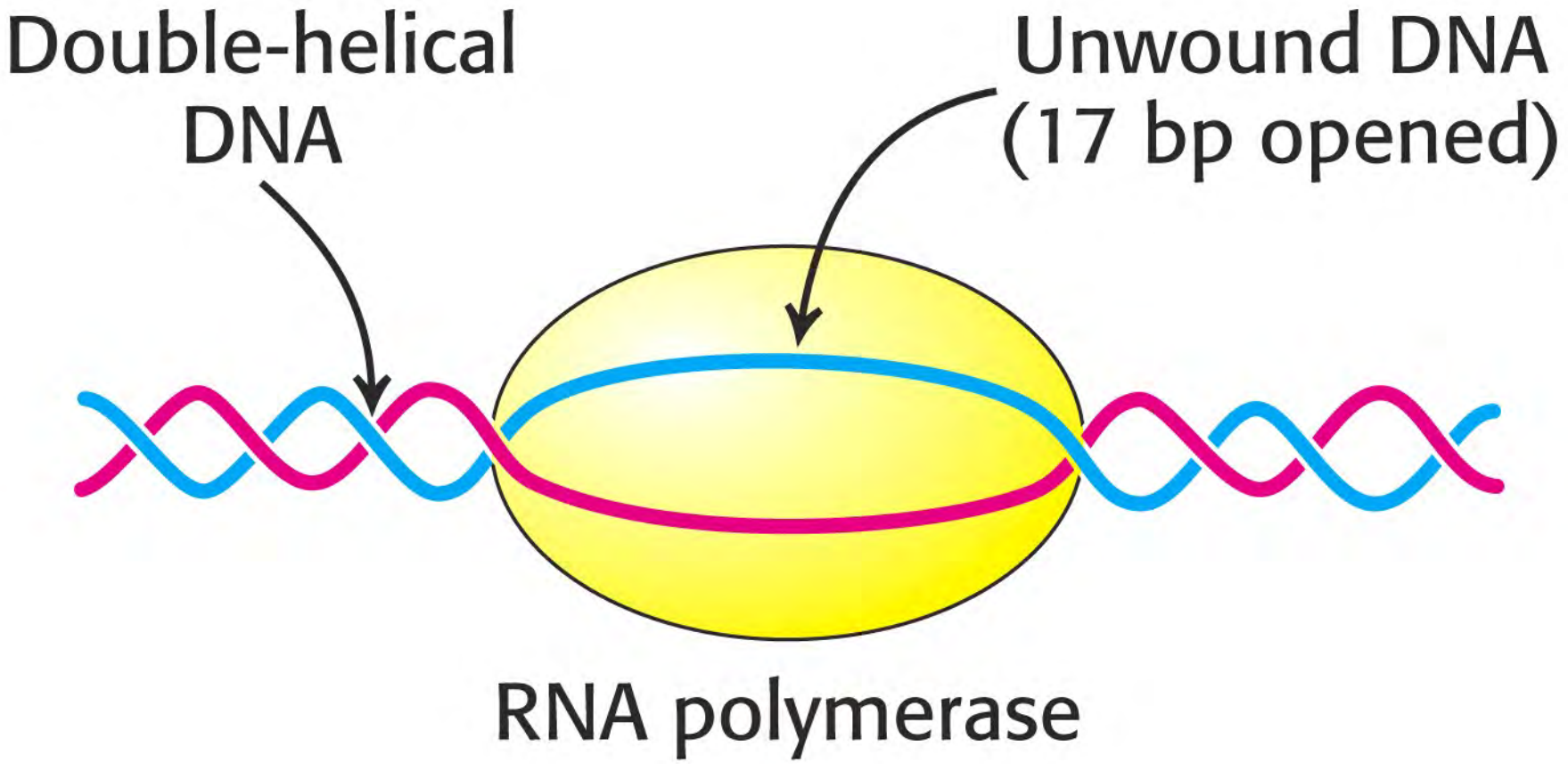
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e) **Monocistronic mRNA consists entirely of coding regions.**
f) ***Polycistronic mRNA contains non-coding spacers.***


DNA
3'
5'
5'
m RNA
RNA Polymerase
PPP
C T A G T C
T A C G
G A U C A
C G T C
A T G C
G C A G
G T A C A G
G
C U



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Double-helical
DNA
Unwound DNA
(17 bp opened)
RNA polymerase

6.1 TRANSCRIPTION: THE PRINCIPLE

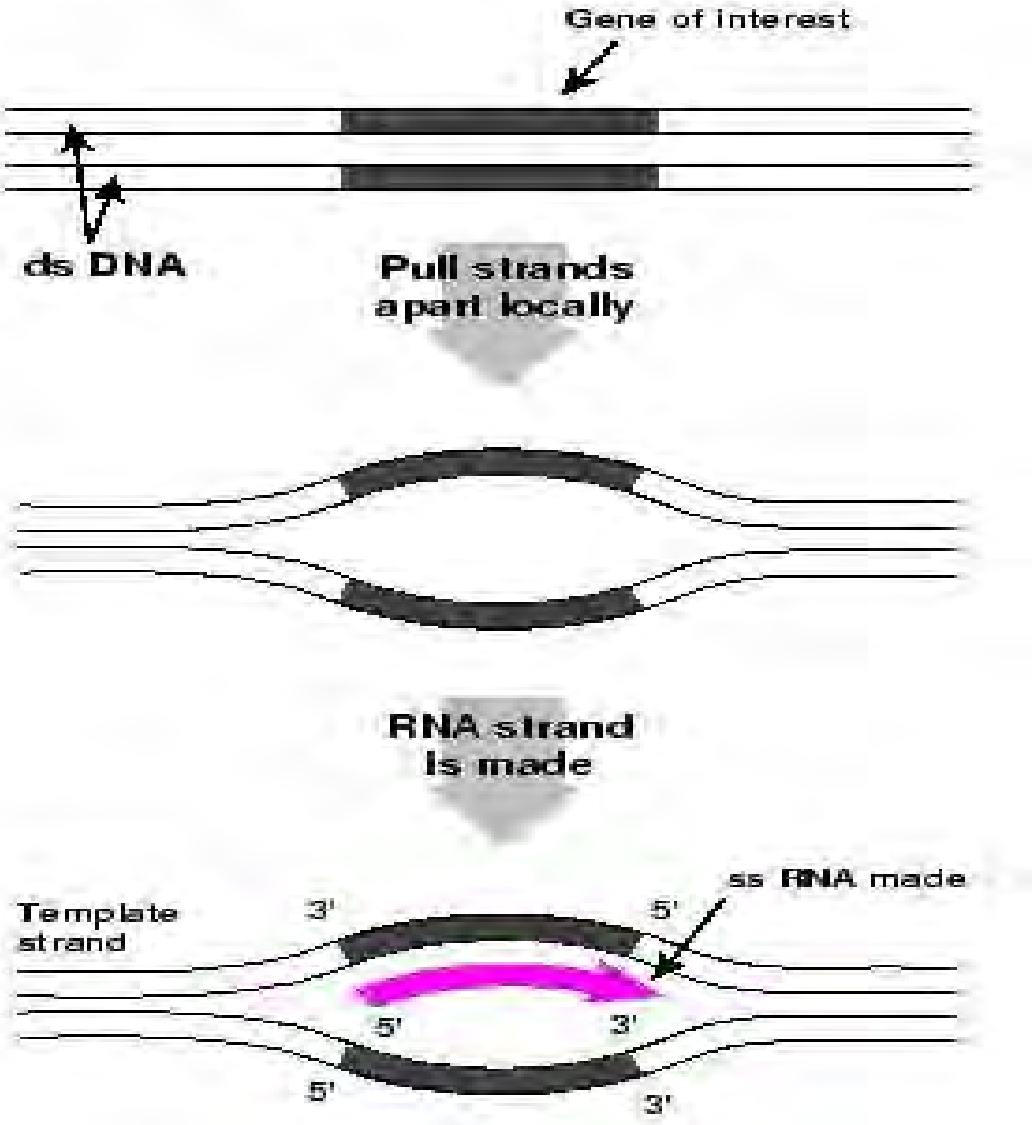
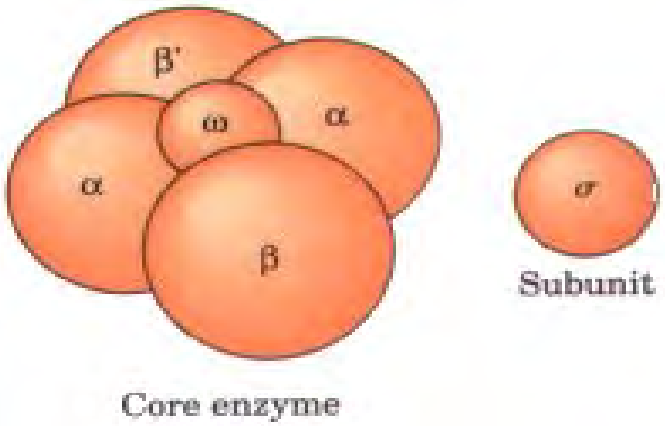
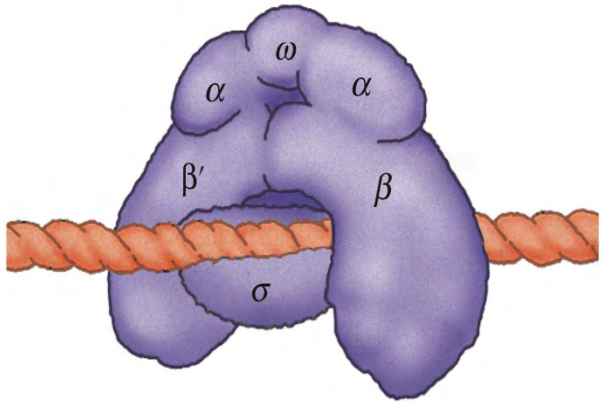


TABLE 28.1 Subunits of RNA polymerase from *E. coli*

Subunit	Gene	Number	Mass (kd)
α	*rpoA*	2	37
β	*rpoB*	1	151
β′	*rpoC*	1	155
σ^{70}	*rpoD*	1	70





THE CELL, Fourth Edition, Figure 7.1 © 2006 ASM Press and Sinauer Associates, Inc.



Are You Getting It??



Which are characteristics of E. coli RNA polymerase?
(multiple answers)

a) It is a multimeric protein that forms a holoenzyme.
b) It uses ribonucleoside triphosphates as substrates.
c) It can copy either DNA strand in a gene.
d) It polymerizes in the 3'→ 5' direction.
e) It has helicase activity.
f) It has proof-reading activity.



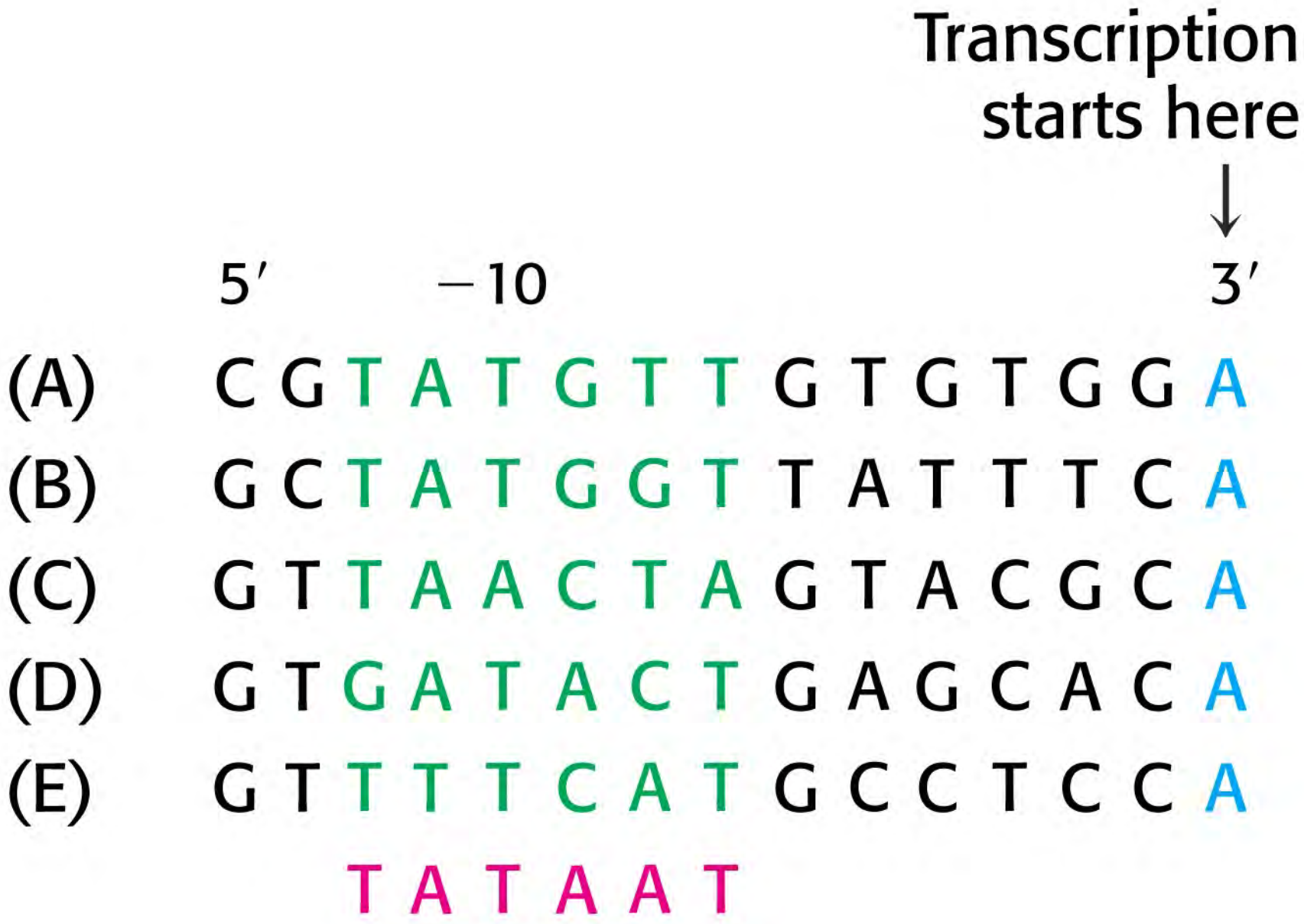
Are You Getting It??



Answer

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d) **It polymerizes in the 3'→ 5' direction.**
e) ***It has helicase activity.***
f) **It has proof-reading activity.**


Transcription starts here
5′
−10
3′
(A) C G T A T G T T G T G T G G A
(B) G C T A T G G T T A T T T C A
(C) G T T A A C T A G T A C G C A
(D) G T G A T A C T G A G C A C A
(E) G T T T T C A T G C C T C C A
T A T A A T

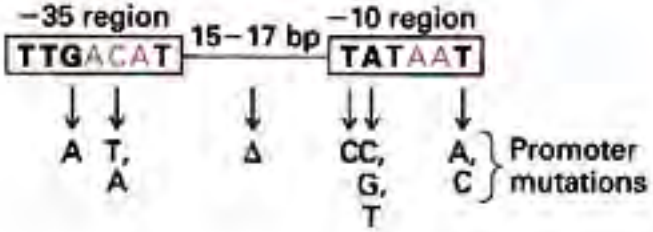
(a) Strong *E.coli* promoters

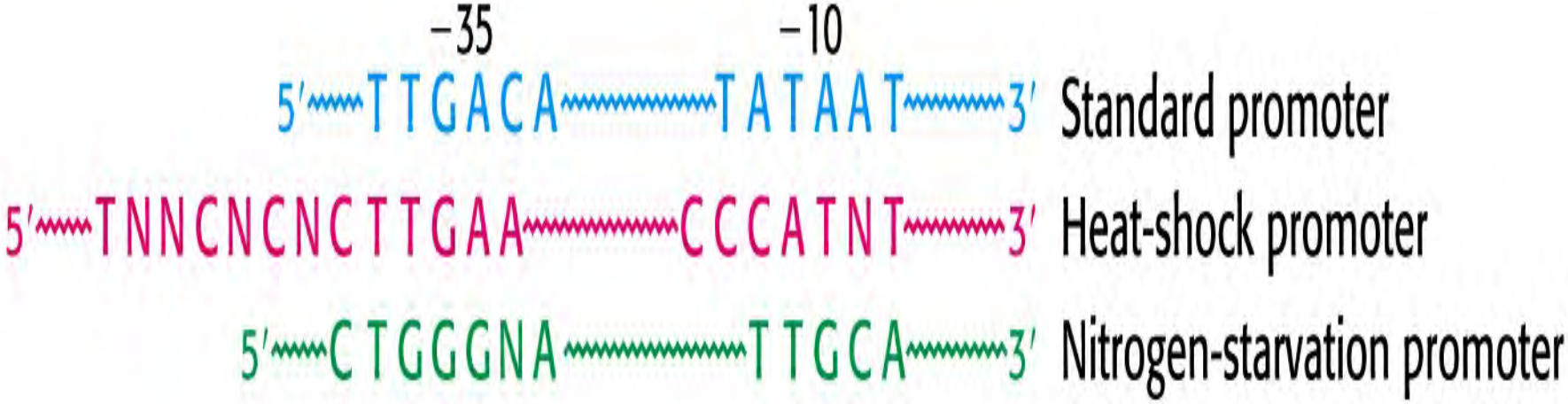
tyr tRNA	TCTCAACGTAACAC	TTTACA	GCGGCG··CGTCATTTGA
rrn D1	GATCAAAAAAATAC	TTGTGC	AAAAAA··TTGGGATCCC
rrn X1	ATGCATTTTTCCGC	TTGTCT	TCCTGA··GCCGACTCCC
rrn $(DXE)_2$	CCTGAAATTCAGGG	TTGACT	CTGAAA··GAGGAAAGCG
rrn E1	CTGCAATTTTTCTA	TTGCGG	CCTGCG··GAGAACTCCC
rrn A1	TTTTAAATTTCCTC	TTGTCA	GGCCGG··AATAACTCCC
rrn A2	GCAAAAATAAATGC	TTGACT	CTGTAG··CGGGAAGGCG
λ P_R	TAACACCGTGCGTG	TTGACT	ATTTTA·CCTCTGGCGG
λ P_L	TATCTCTGGCGGTG	TTGACA	TAAATA·CCACTGGCGG
T7 A3	GTGAAACAAAACGG	TTGACA	ACATGA·AGTAAACACG
T7 A1	TATCAAAAAGAGTA	TTGACT	TAAAGT·CTAACCTATA
T7 A2	ACGAAAAACAGGTA	TTGACA	ACATGAAGTAACATGCAG
fd VIII	GATACAAATCTCCG	TTGTAC	TTTGTT··TCGCGCTTGG

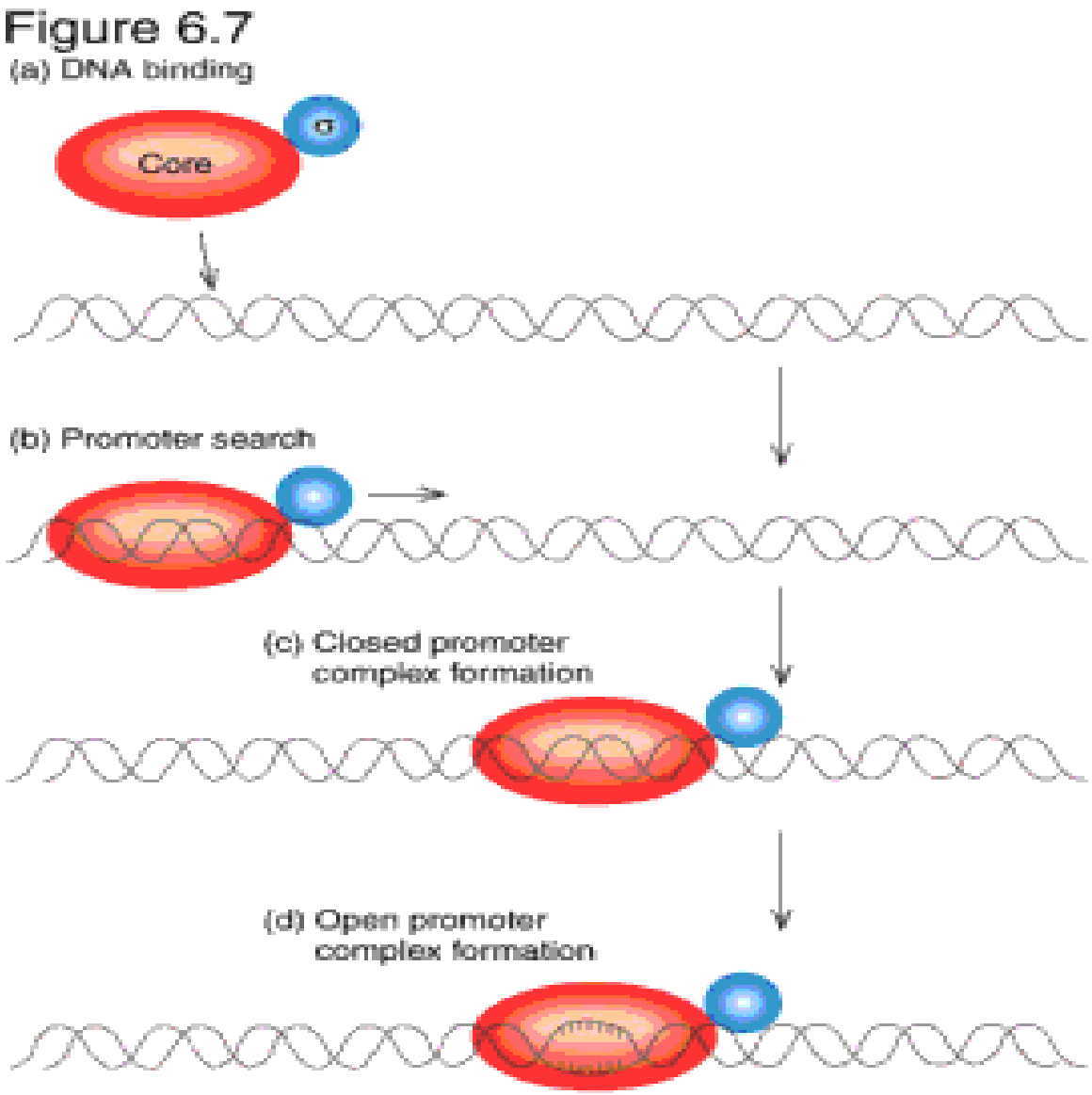
	GTCATTTGA	TATGATG	C·GCCCCGCTTCCCGATAAGGG
	TTGGGATCCC	TATAATG	CGCCTCCGTTGAGACGACAACG
	GCCGACTCCC	TATAATG	CGCCTCCATCGACACGGCGGAT
	GAGGAAAGCG	TAATATA	C·GCCACCTCGCGACAGTGAGC
	GAGAACTCCC	TATAATG	CGCCTCCATCGACACGGCGGAT
	AATAACTCCC	TATAATG	CGCCACCACTGACACGGAACAA
	CGGGAAGGCG	TATTATG	C·ACACCCGCGCCGCTGAGAA
	CCTCTGGCGG	TGATAATG	G··TTGCATGTACTAAGGAGGT
	CCACTGGCGG	TGATACTG	A··GCACATCAGCAGGACGCAC
	AGTAAACACG	GTACGATG	T·ACCACATGAAACGACAGTGA
	CTAACCTATA	GGATACTT	A·CAGCCATCGAGAGGGACACG
	AAGTAACATG	CAGTAAGAT	AC·AAATCGCTAGGTAACACTAG
	TCGCGCTTGG	TATAATC	G·CTGGGGGTCAAAGATGAGTG

−35 −10 +1

(b) Consensus sequences of σ^{70} promoters




−35
−10
5′ TTGACA TATAAT 3′
Standard promoter
5′ TNNCNCNCTTGAA CCCATNT 3′
Heat-shock promoter
5′ CTGGGNA TTGCA 3′
Nitrogen-starvation promoter


Figure 6.7
(a) DNA binding
Core
σ
(b) Promoter search
(c) Closed promoter complex formation
(d) Open promoter complex formation



Are You Getting It??



Which are properties of E. coli promoters?

(multiple answers)

a) All promoters have the same base sequence.
b) The -10 sequence is A=T rich.
c) The -35 sequence is where transcription starts.
d) Promoters are recognized by the σ subunit.
e) Promoters are located downstream of the gene.



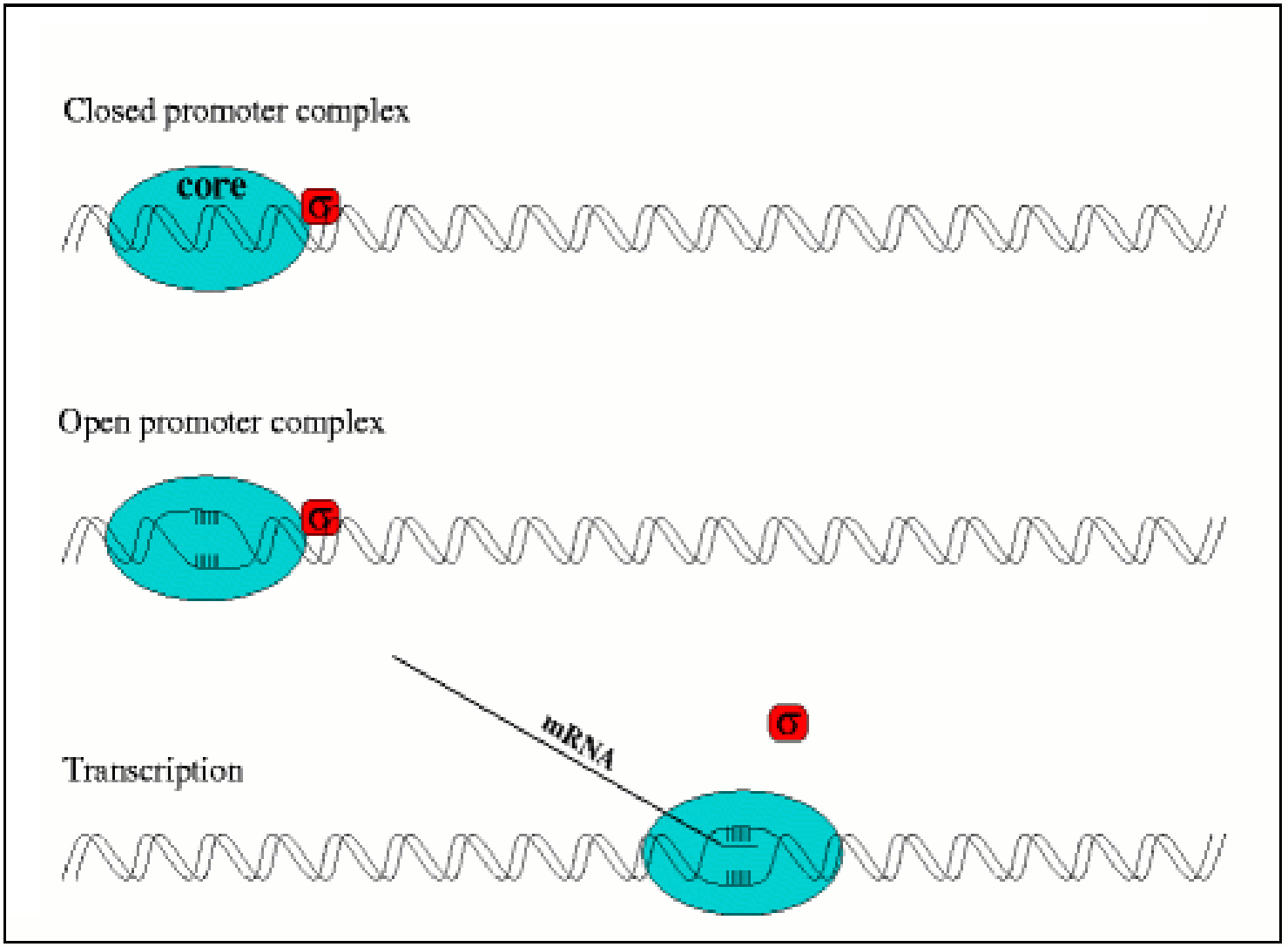
Are You Getting It??

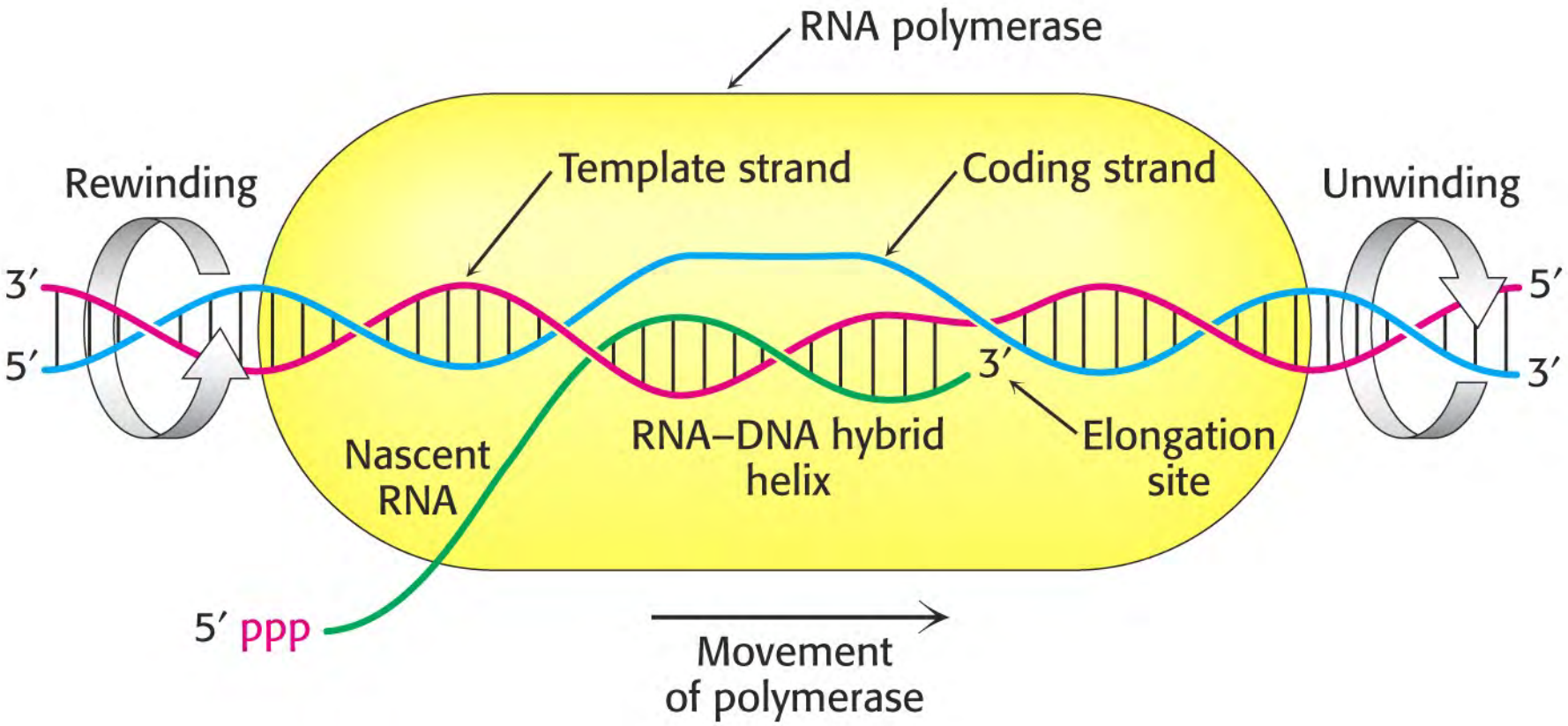


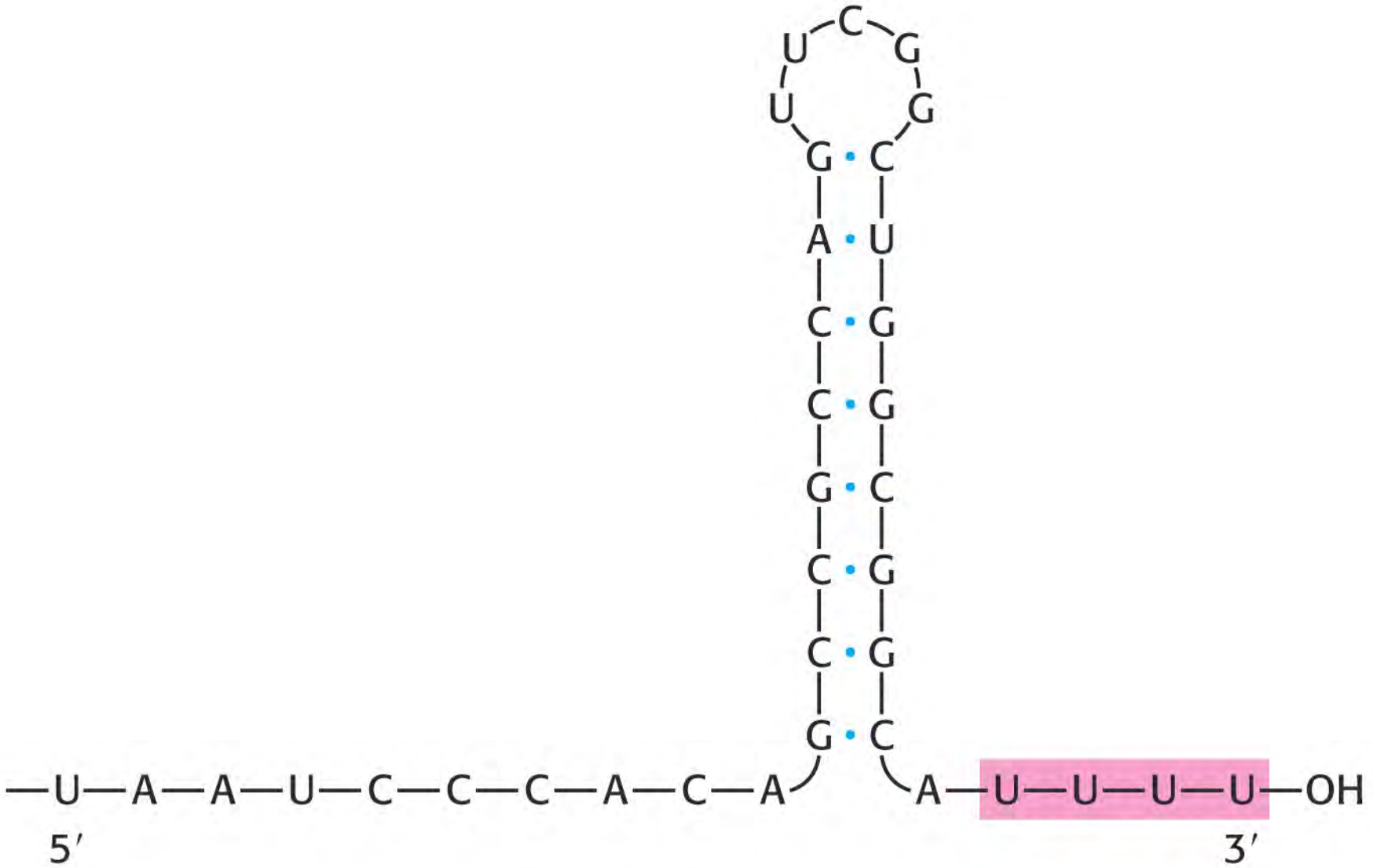
Answer

Which are properties of E. coli promoters?

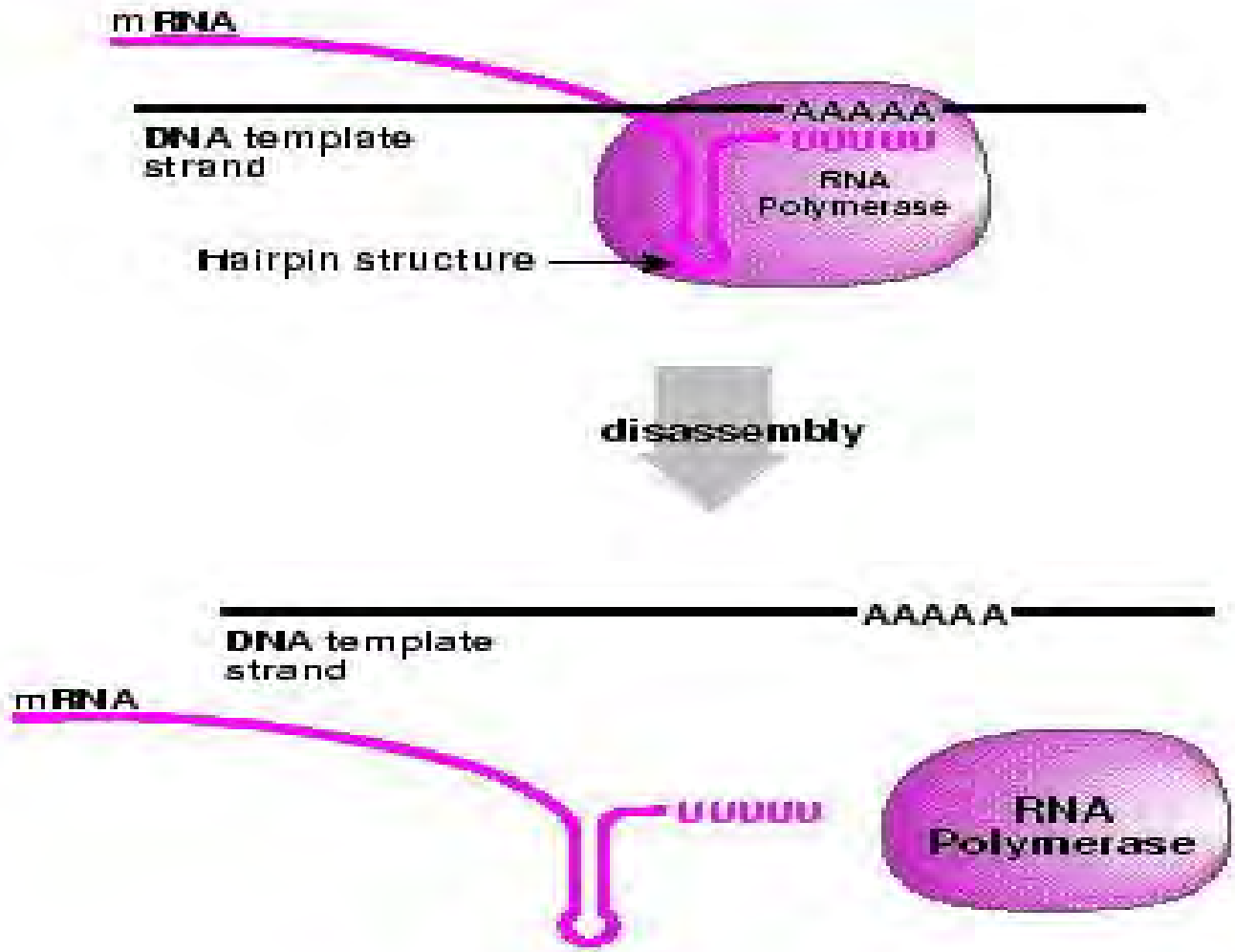
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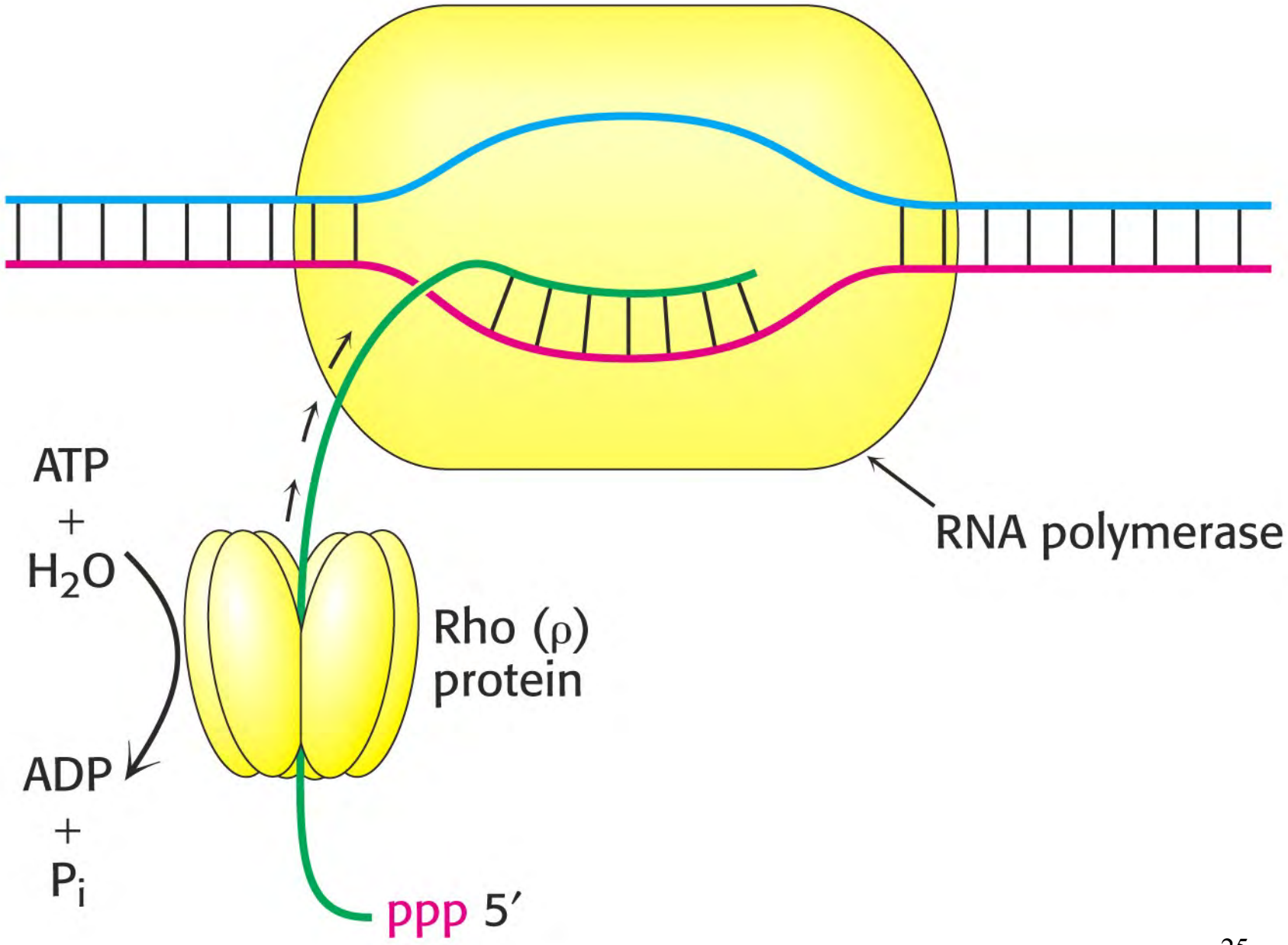

Closed promoter complex
core
σ
Open promoter complex
σ
mRNA
σ
Transcription


RNA polymerase
Rewinding
Template strand
Coding strand
Unwinding
3′
5′
5′
3′
3′
Nascent RNA
RNA–DNA hybrid helix
Elongation site
5′ ppp
Movement of polymerase

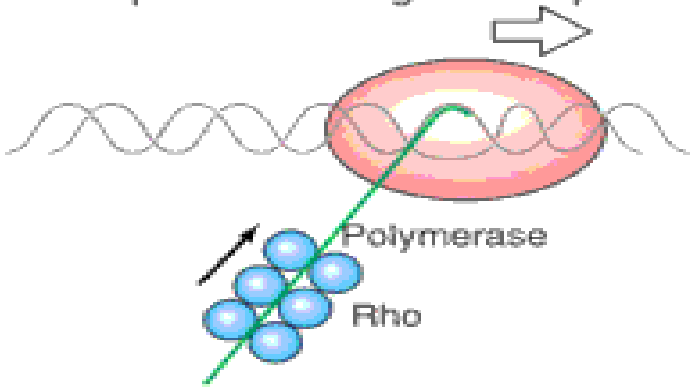

C
U
G
U
G
G
C
A
U
C
G
C
G
G
C
C
G
C
G
G
C
—U—A—A—U—C—C—C—A—C—A
A—U—U—U—U—OH
5′
3′

6.10 TERMINATION OF TRANSCRIPTION

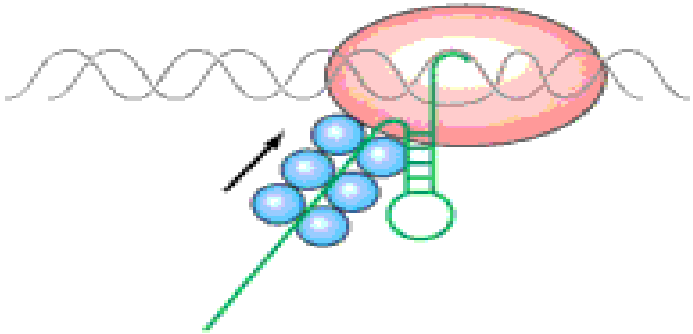



ATP
+
H_2O
ADP
+
P_i
Rho (ρ)
protein
RNA polymerase
ppp 5′

(a) Rho binds to transcript at rho loading site and pursues polymerase.



(b) Hairpin forms; polymerase pauses; rho catches up.



(c) Rho helicase releases transcript and causes termination.

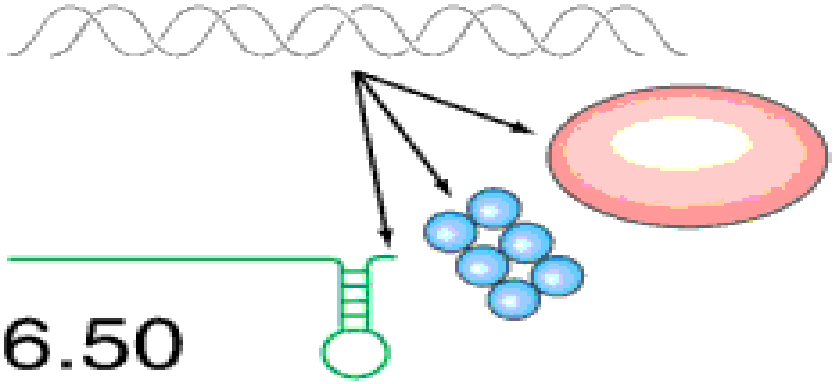


Figure 6.50



Are You Getting It??



Which events occur during transcription in E. coli?
(multiple answers)

a) **RNA polymerase denatures a small area of DNA.**
b) **RNA polymerase makes phosphodiester bonds.**
c) **The mRNA forms hydrogen bonds with the DNA.**
d) **Termination of transcription uses a hairpin structure.**
e) **The rho protein is a type of RNA polymerase.**



Are You Getting It??



Answer

Which events occur during transcription in E. coli?

a) *RNA polymerase denatures a small area of DNA.*
b) *RNA polymerase makes phosphodiester bonds.*
c) *The mRNA forms hydrogen bonds with the DNA.*
d) *Termination of transcription uses a hairpin structure.*
e) **The rho protein is a type of RNA polymerase.**

TABLE 28.2 Eukaryotic RNA polymerases

Type	Location	Cellular transcripts	Effects of α-amanitin
I	Nucleolus	18S, 5.8S, and 28S rRNA	Insensitive
II	Nucleoplasm	mRNA precursors and snRNA	Strongly inhibited
III	Nucleoplasm	tRNA and 5S rRNA	Inhibited by high concentrations

5′ T_{82} A_{97} T_{93} A_{85} A_{63} A_{88} A_{50} 3′

TATA box

5′ G G N C A A T C T 3′

CAAT box

5′ G G G C G G 3′

GC box

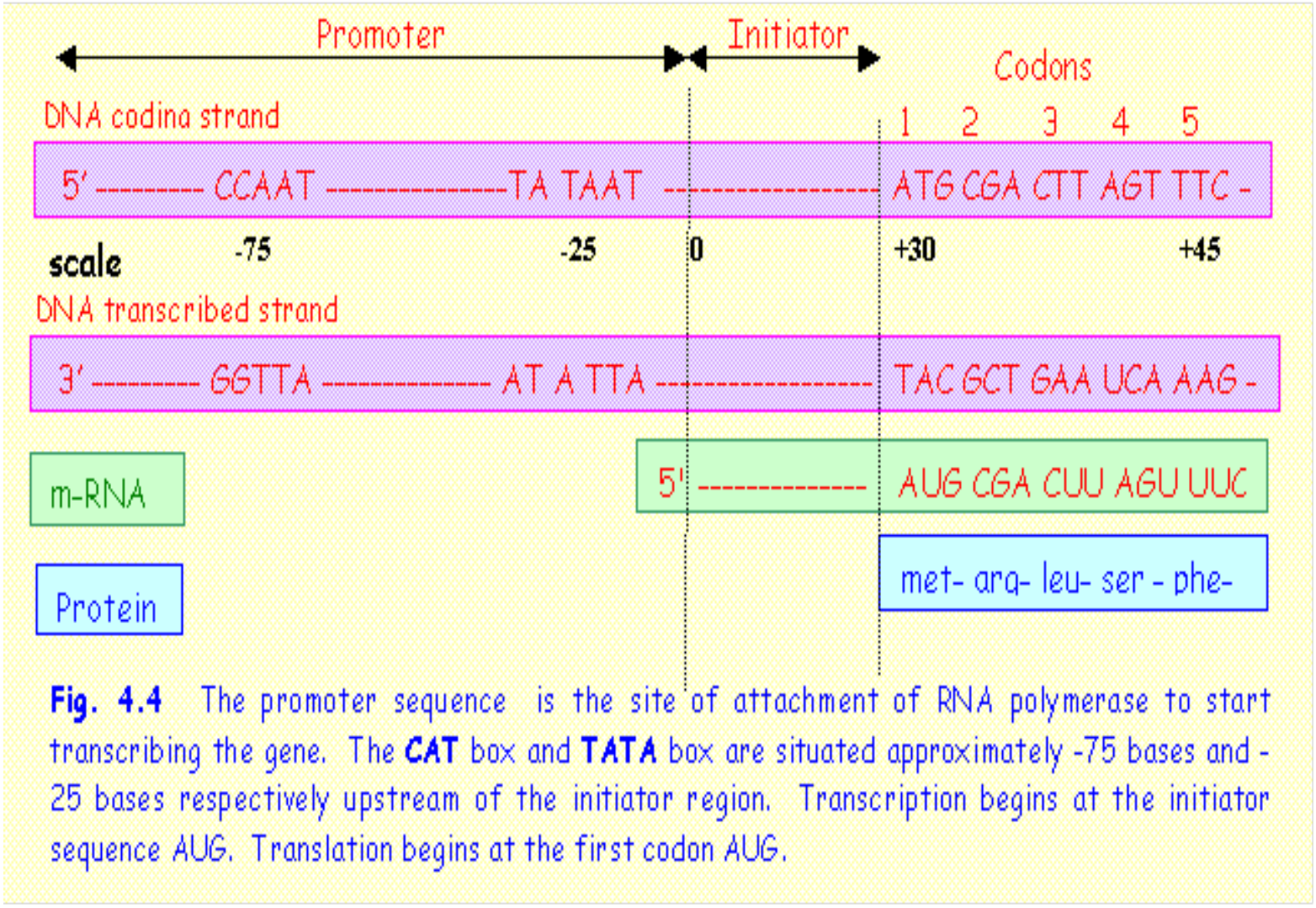
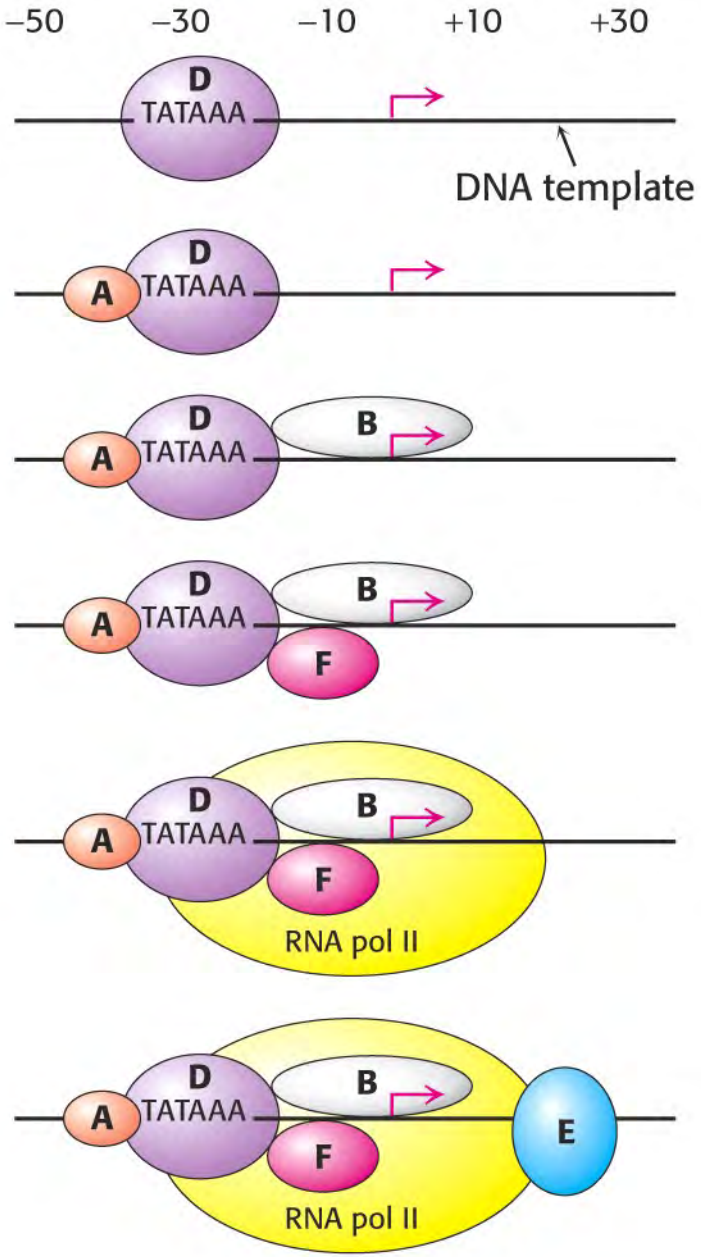
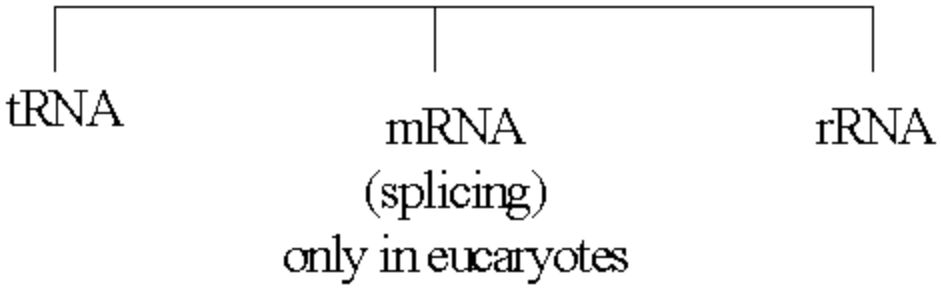


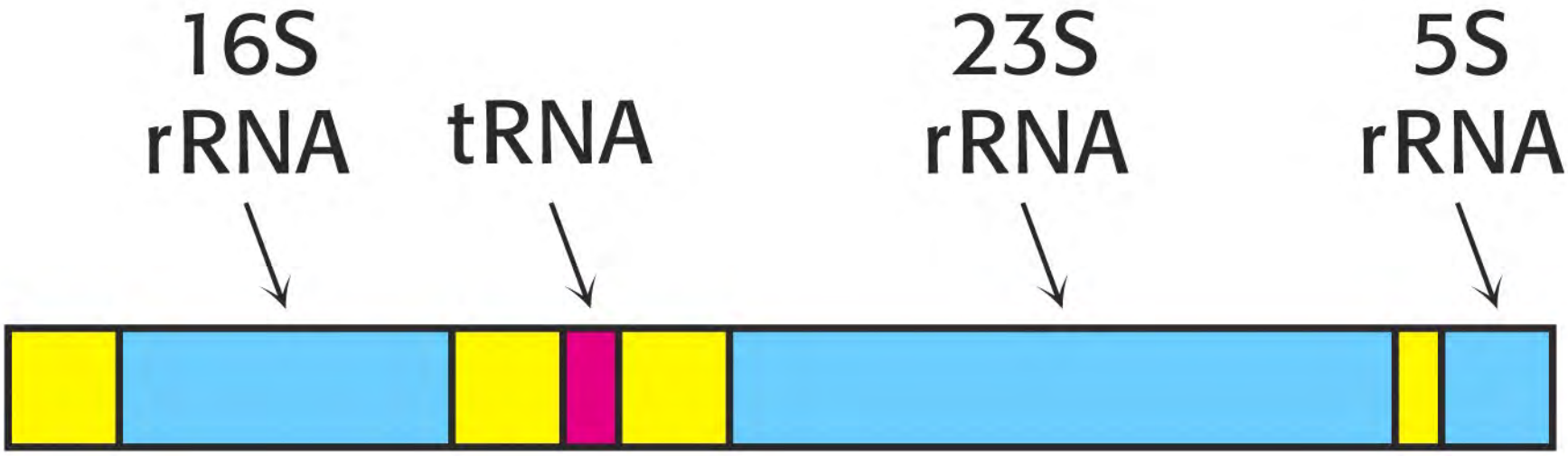
Fig. 4.4 The promoter sequence is the site of attachment of RNA polymerase to start transcribing the gene. The **CAT** box and **TATA** box are situated approximately -75 bases and -25 bases respectively upstream of the initiator region. Transcription begins at the initiator sequence AUG. Translation begins at the first codon AUG.

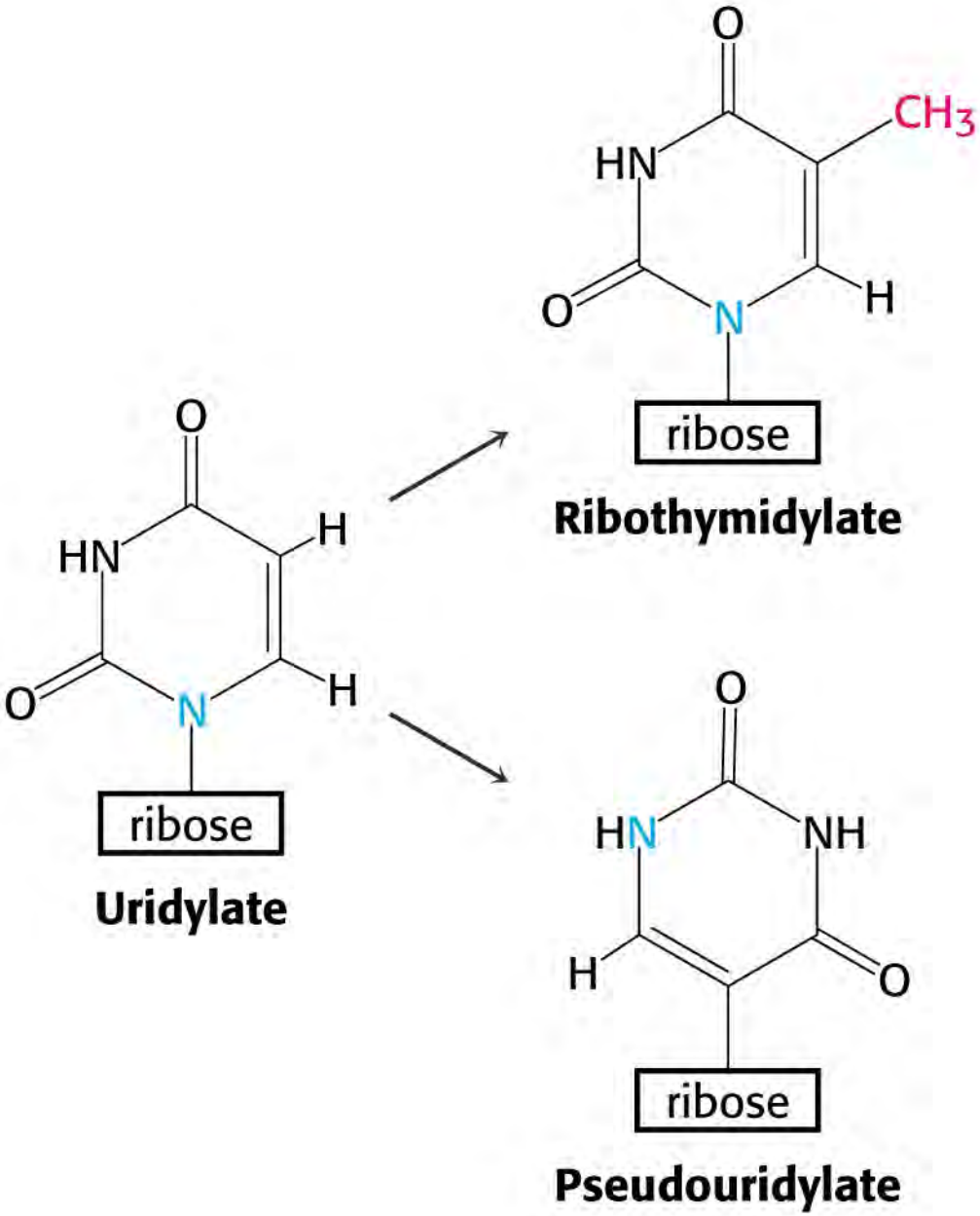

–50
–30
–10
+10
+30
D
TATAAA
DNA template
A
B
F
RNA pol II
E

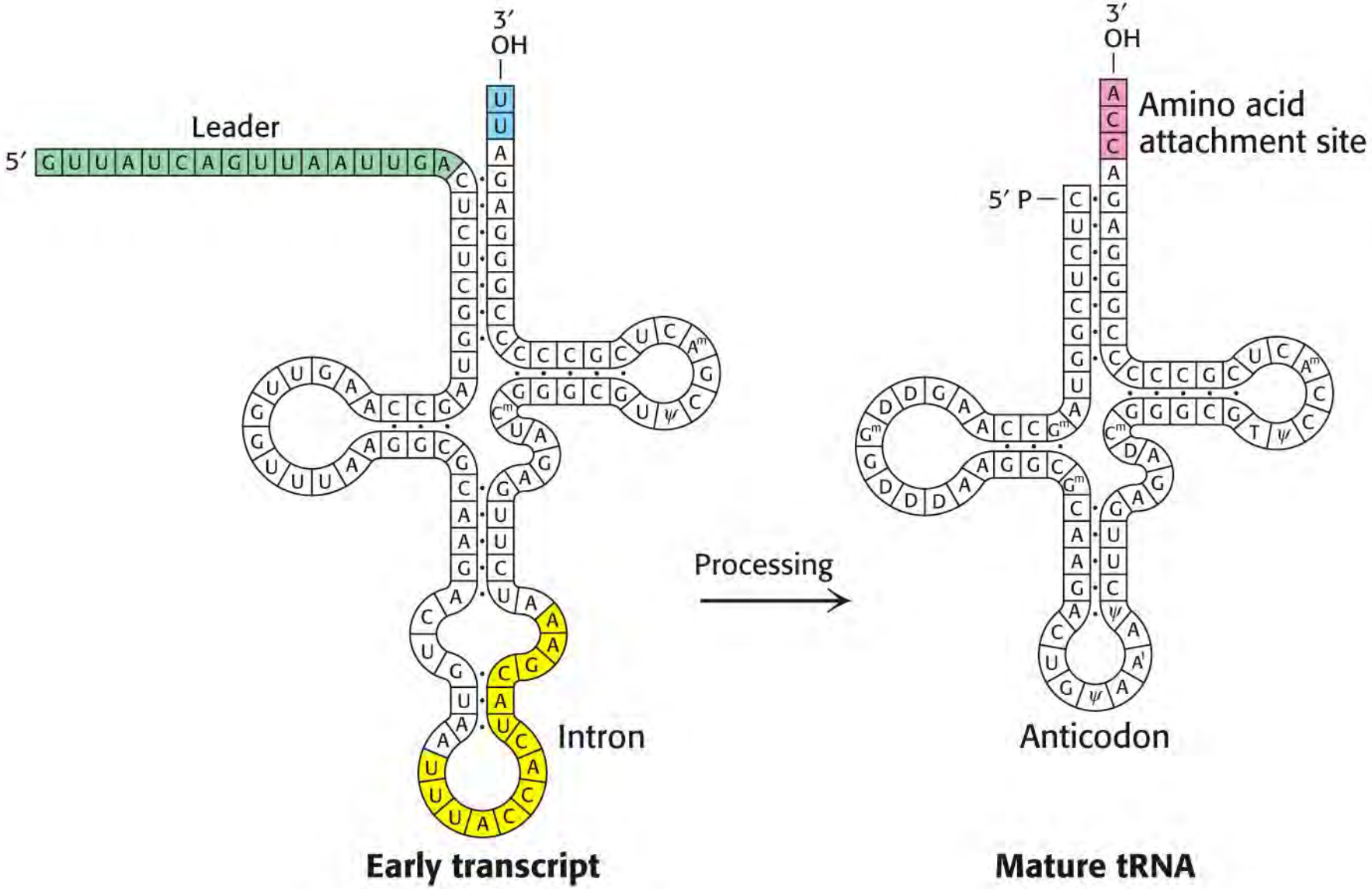
Processing of rRNA and tRNA



In eucaryotes and procaryotes, rRNAs are synthesized as a long precursor, and further cleaved to generate mature transcripts.


16S
rRNA
tRNA
23S
rRNA
5S
rRNA


O
HN
CH3
O
N
H
ribose
Ribothymidylate
O
HN
H
O
N
H
ribose
Uridylate
O
HN
NH
H
O
ribose
Pseudouridylate


3′
OH
Leader
5′
Intron
Early transcript
Processing
3′
OH
Amino acid
attachment site
5′ P
Anticodon
Mature tRNA



Are You Getting It??



Which are characteristics of RNA processing in E. coli?
(multiple answers)

a) mRNAs are extensively modified.
b) rRNAs do not require processing.
c) tRNAs undergo modification of bases.
d) RNA transcripts are cut by specific nucleases.
e) RNA processing is carried out by transcription factors.



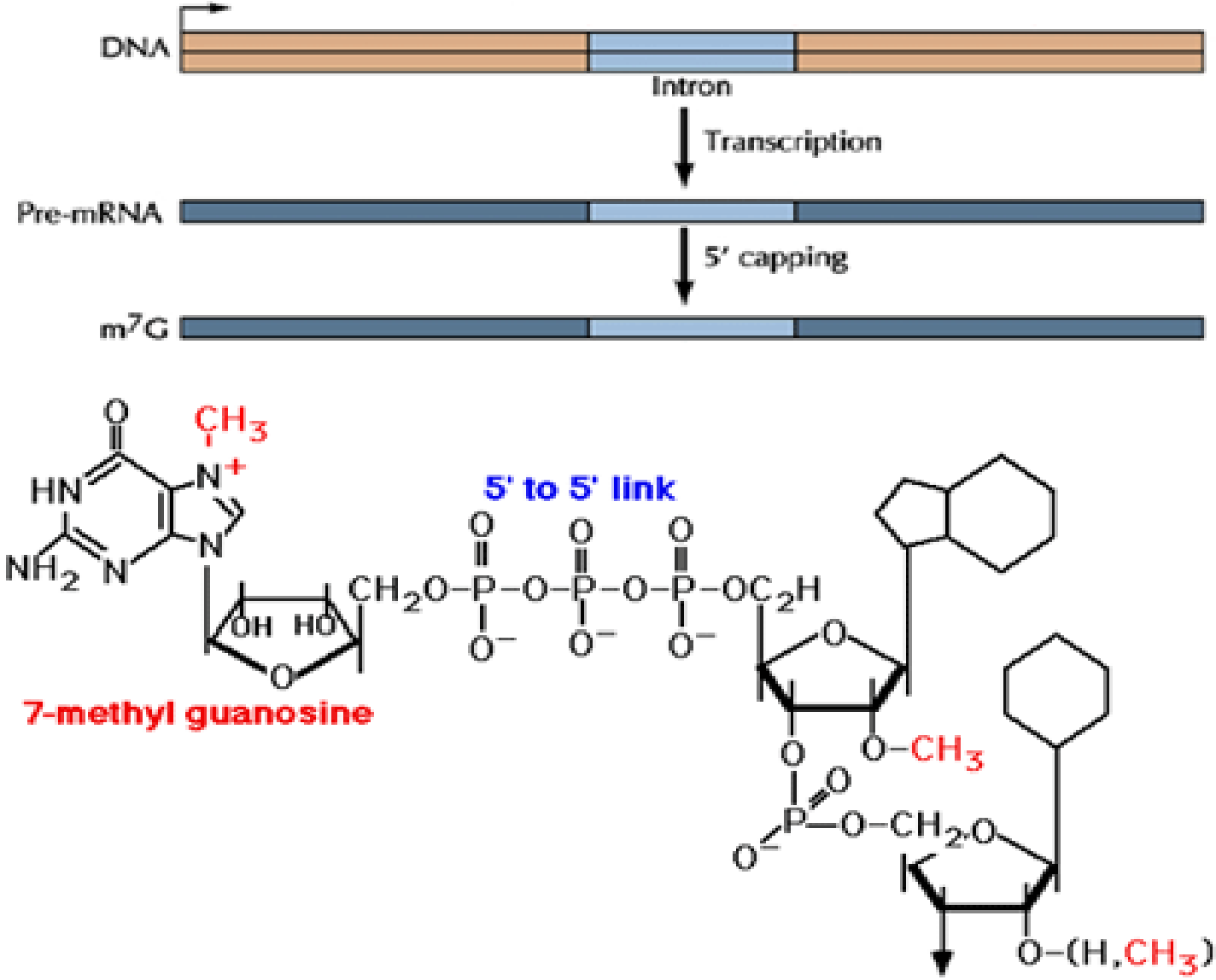
Are You Getting It??

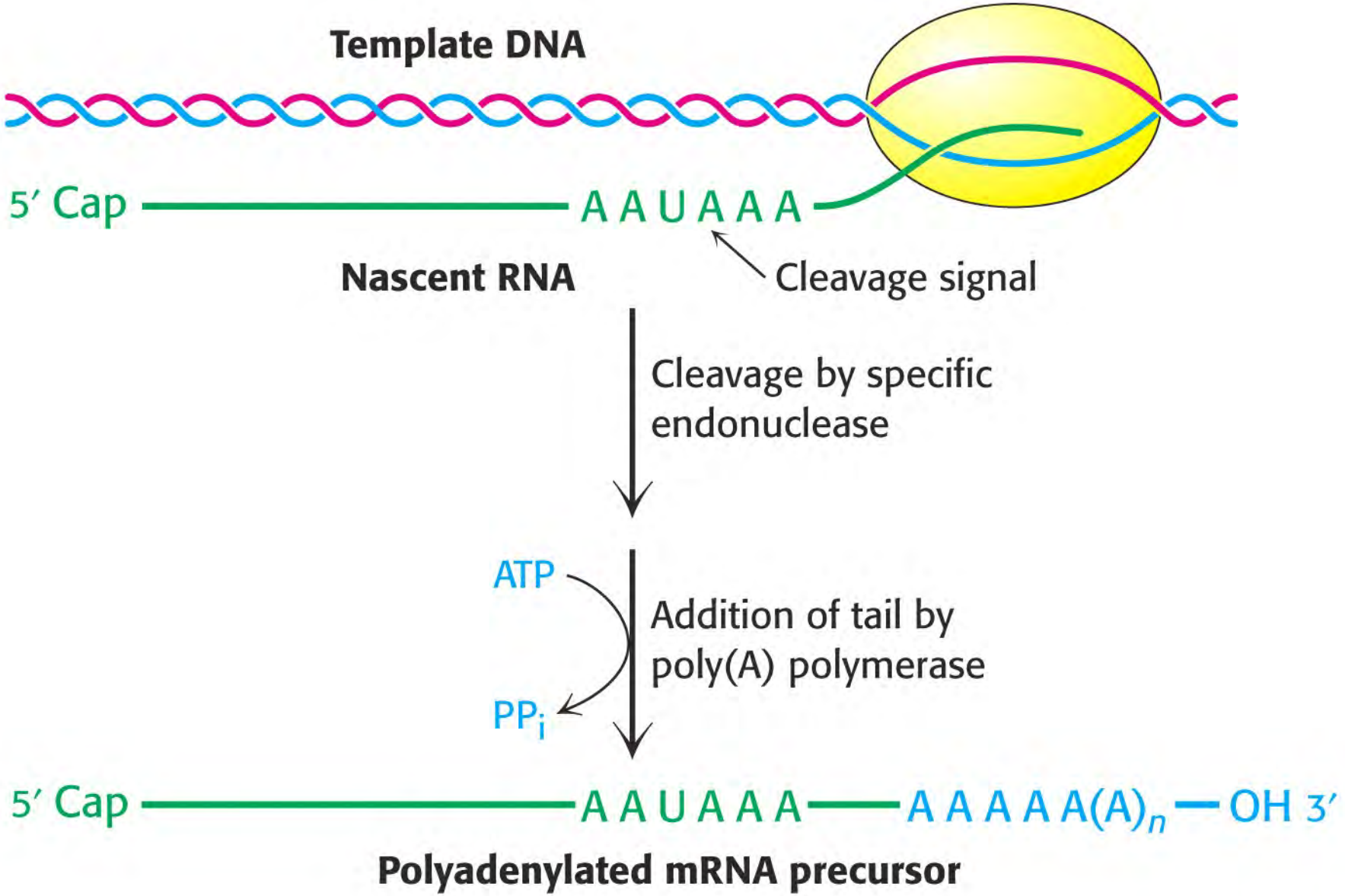


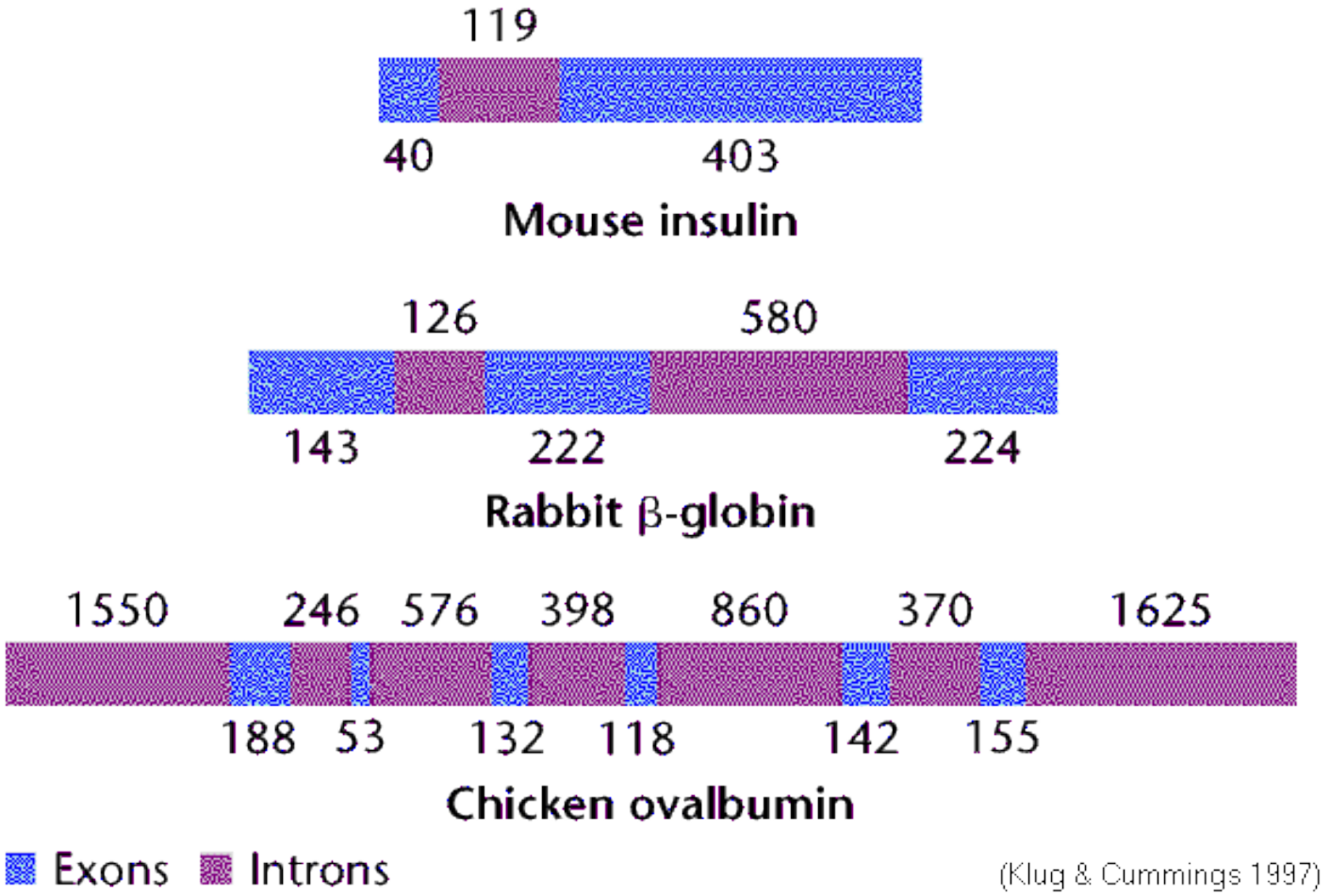
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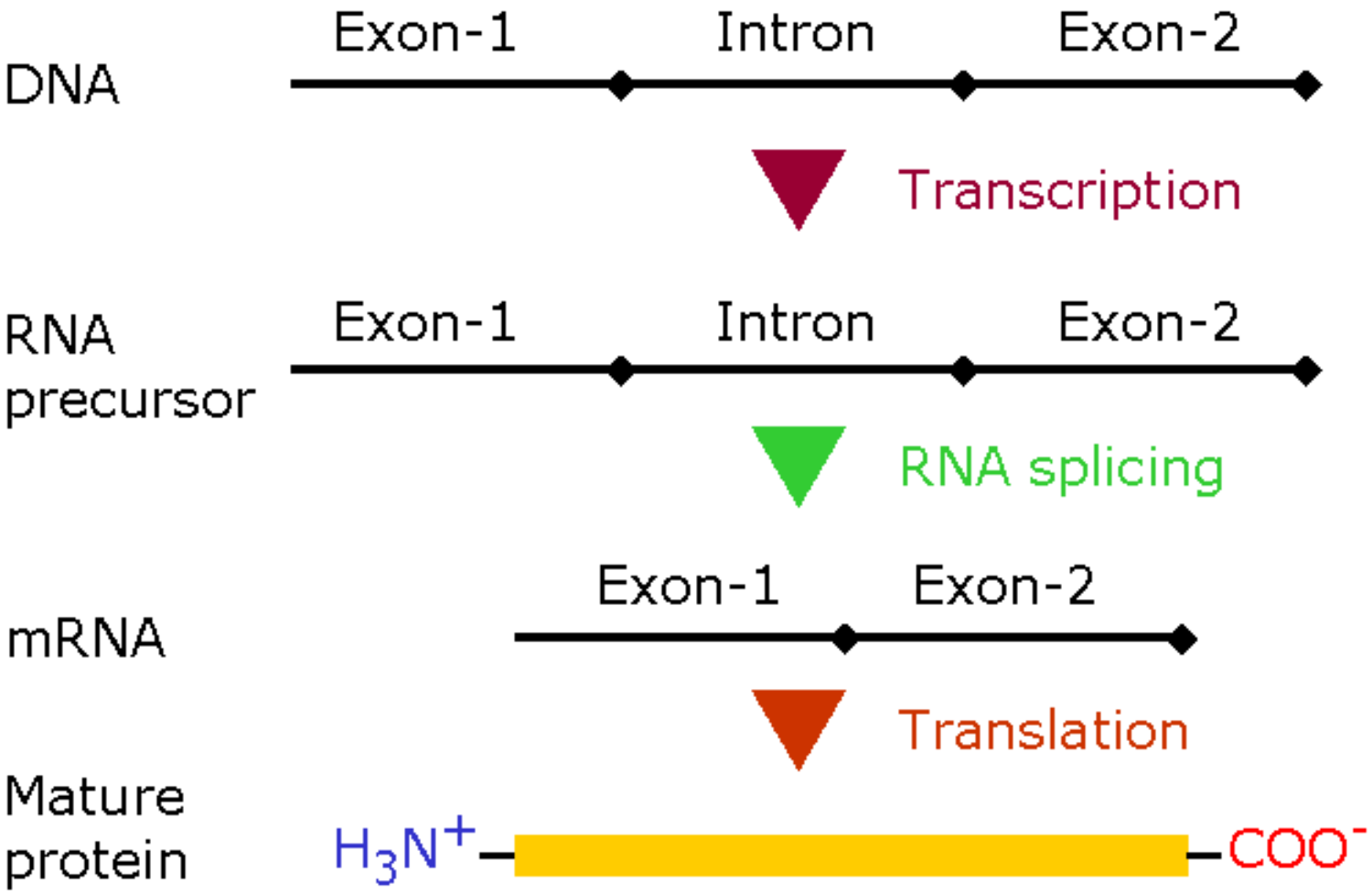

DNA
Intron
Transcription
Pre-mRNA
5′ capping
m7G
5' to 5' link
7-methyl guanosine


Template DNA
5′ Cap
A A U A A A
Nascent RNA
Cleavage signal
Cleavage by specific endonuclease
ATP
PPi
Addition of tail by poly(A) polymerase
5′ Cap
A A U A A A
A A A A A(A)n
OH 3′
Polyadenylated mRNA precursor



(Klug & Cummings 1997)

RNA splicing





Are You Getting It??



Which are characteristics of mRNA processing in eukaryotes? ***(multiple answers)***

a) **mRNA is processed to produce hnRNA.**
b) **The 5'- end is modified with a methylguanosine nucleotide.**
c) **The 3'- end is modified with a sequence of adenosine nucleotides.**
d) **Introns are cut out of the gene and joined together.**
e) **Exons from adjacent genes are spliced together.**



Are You Getting It??



Answer

Which are characteristics of mRNA processing in eukaryotes?

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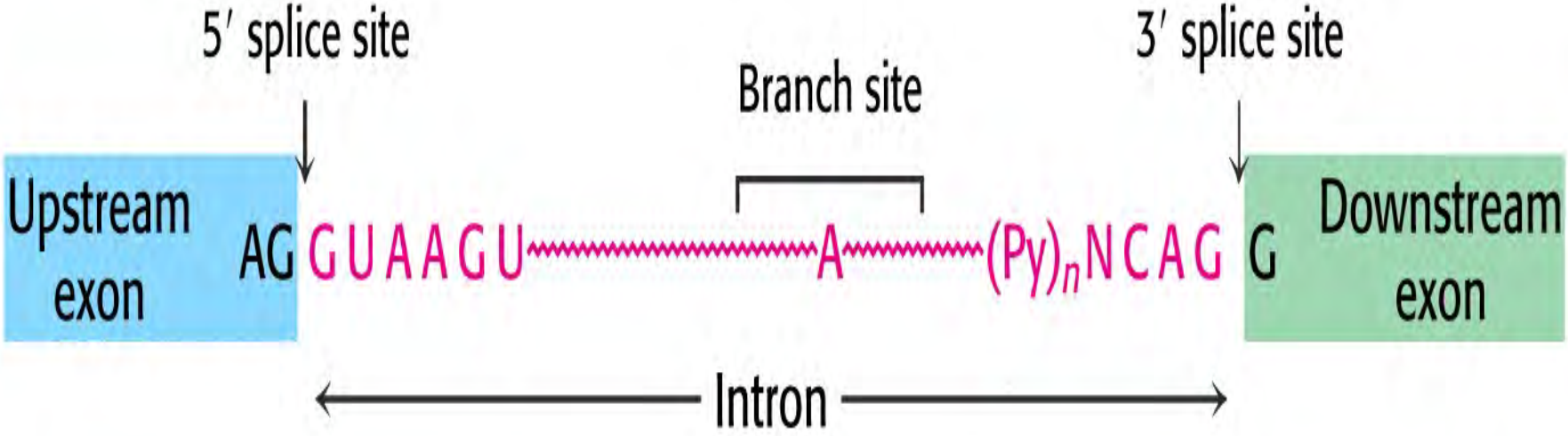
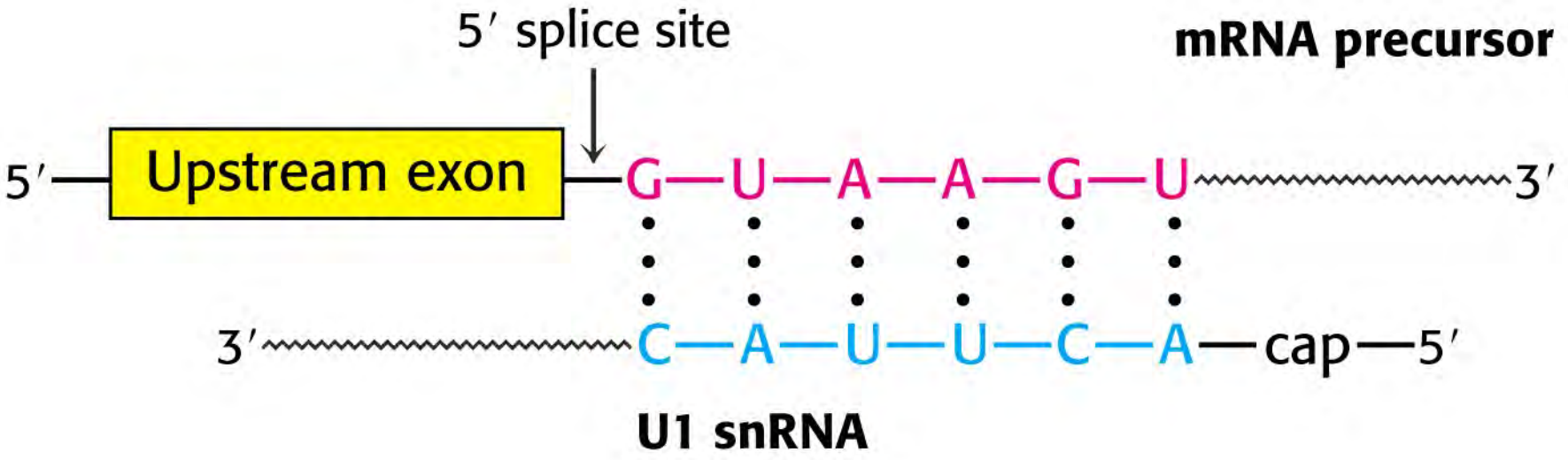
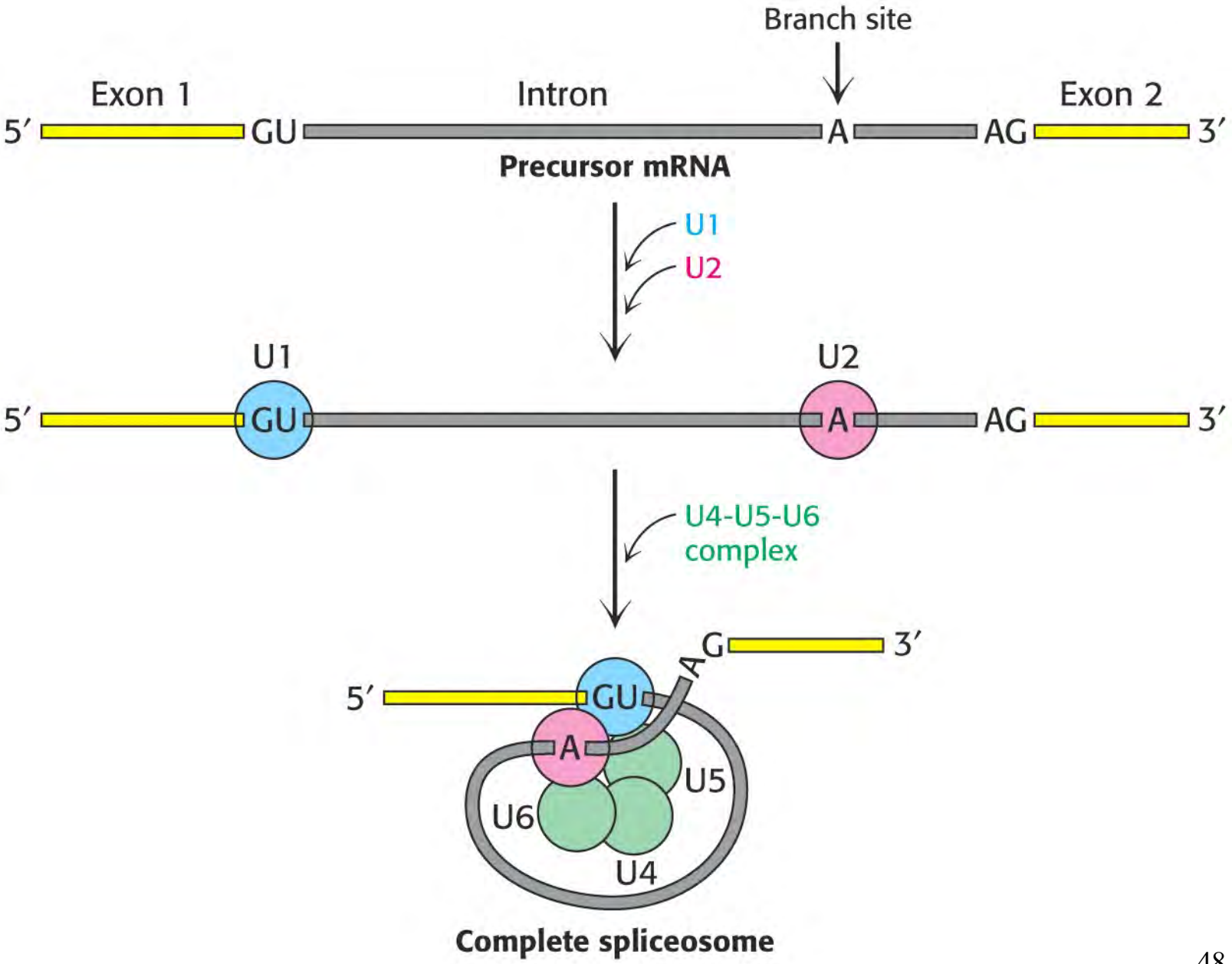
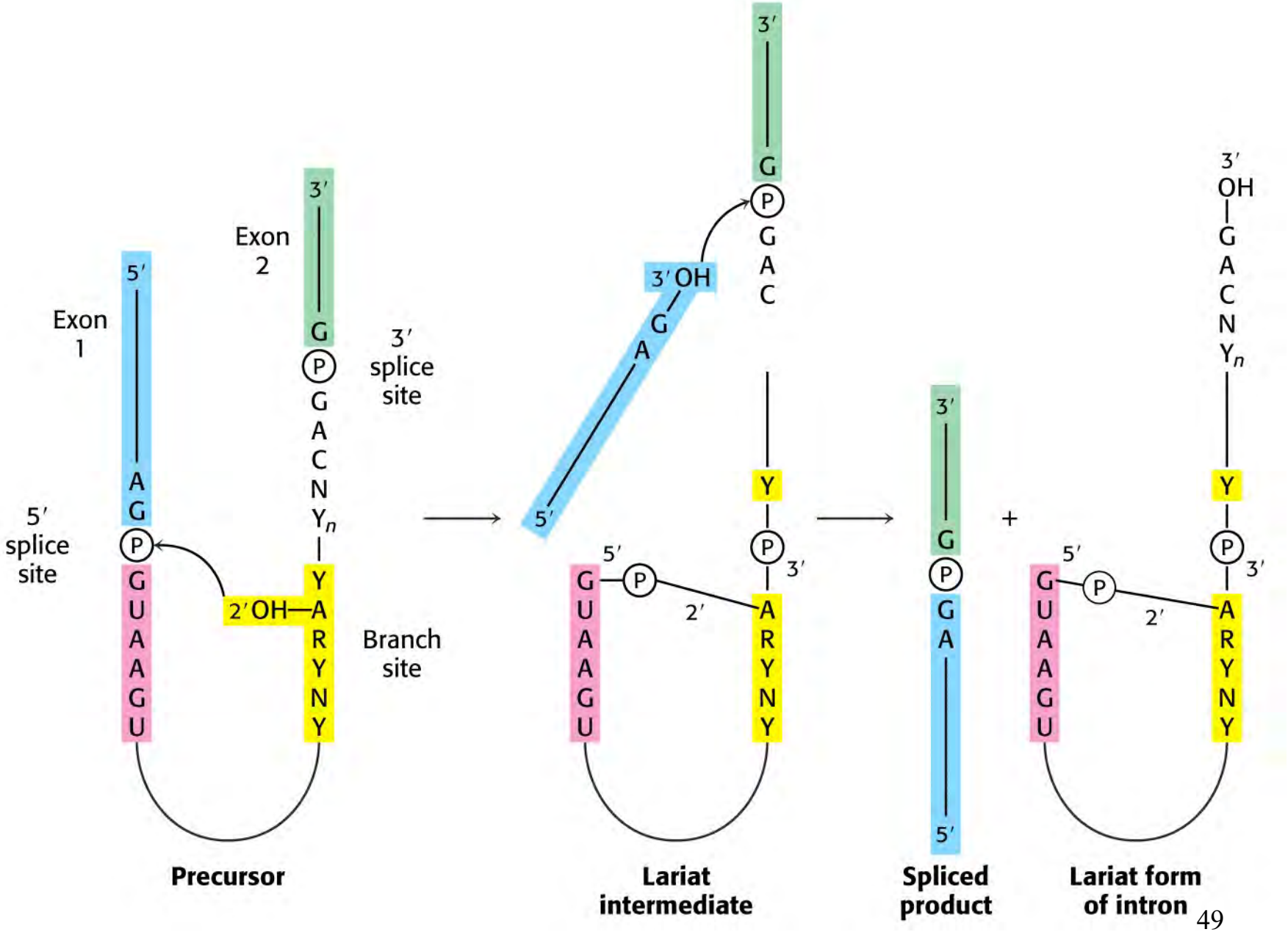

5′ splice site
Branch site
3′ splice site
Upstream exon
AG
GUAAGU
A
(Py)nNCAG
G
Downstream exon
Intron

TABLE 28.3 Small nuclear ribonucleoprotein particles (snRNPs) in the splicing of mRNA precursors

snRNP	Size of snRNA (nucleotides)	Role
U1	165	Binds the 5′ splice site and then the 3′ splice site
U2	185	Binds the branch site and forms part of the catalytic center
U5	116	Binds the 5′ splice site
U4	145	Masks the catalytic activity of U6
U6	106	Catalyzes splicing

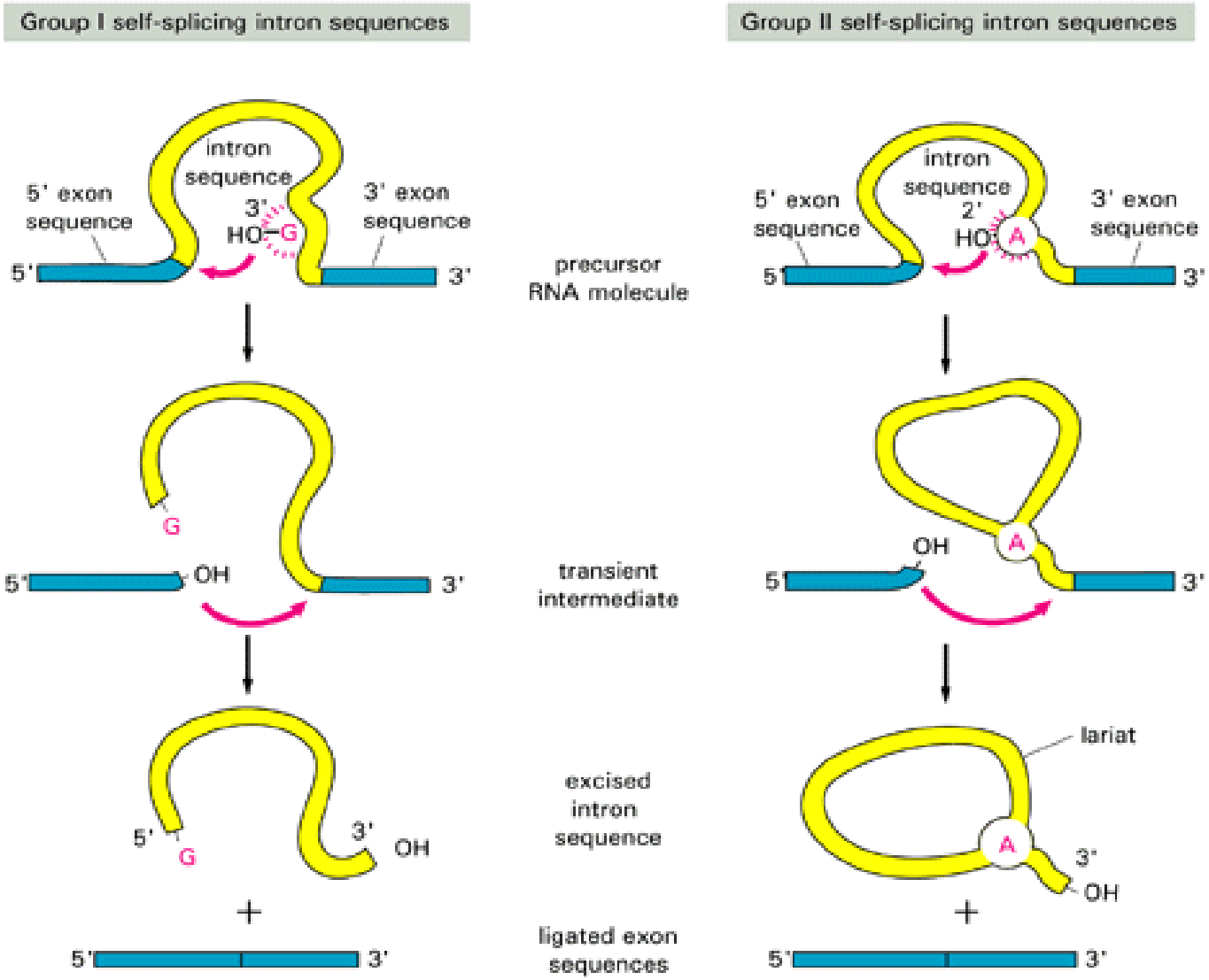

5′ splice site
mRNA precursor
5′
Upstream exon
G—U—A—A—G—U
3′
3′
C—A—U—U—C—A—cap—5′
U1 snRNA

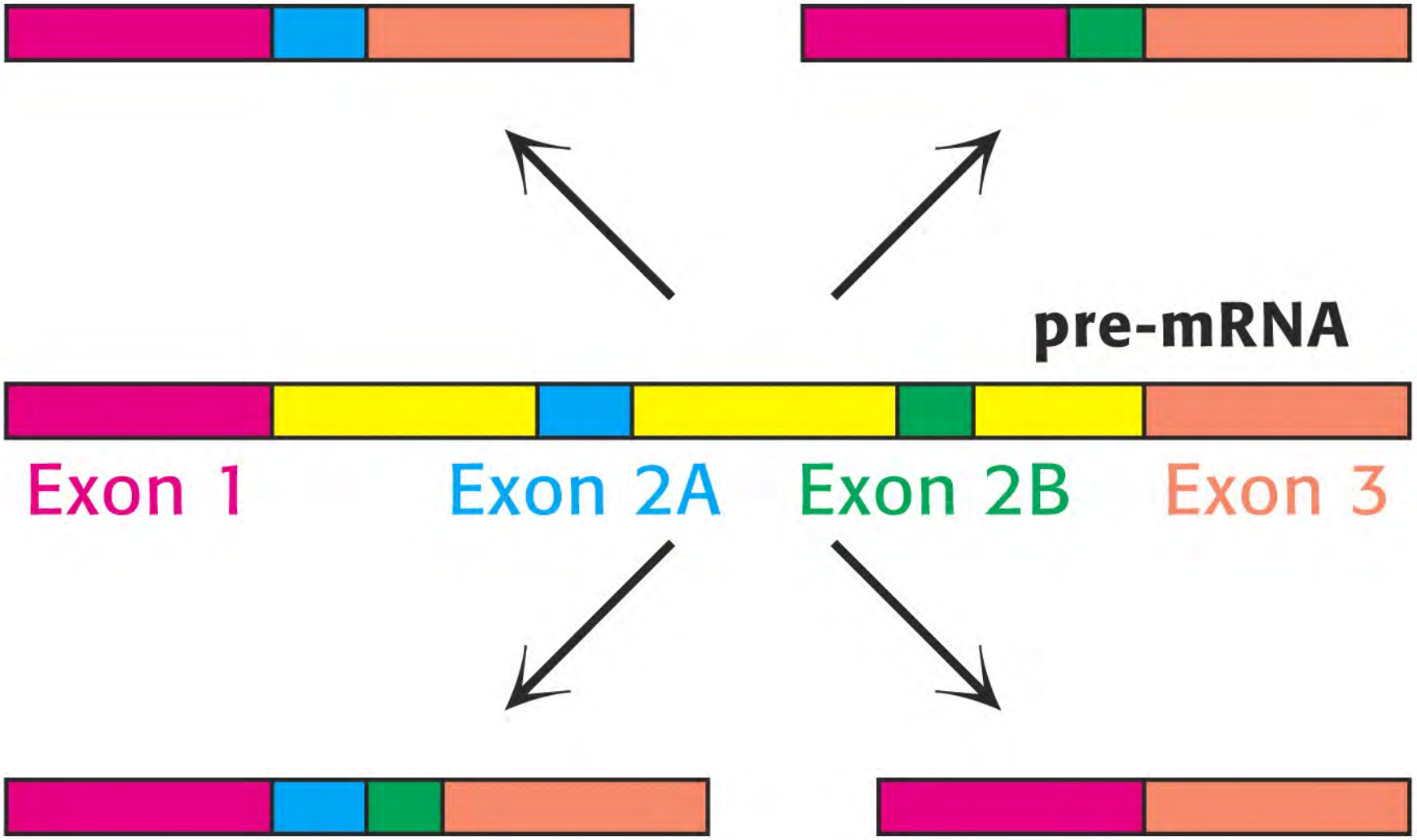

Branch site
Exon 1
Intron
Exon 2
5′
GU
A
AG
3′
Precursor mRNA
U1
U2
U1
U2
5′
GU
A
AG
3′
U4-U5-U6
complex
G
3′
5′
GU
A
U5
U6
U4
Complete spliceosome


Exon 1
5′
A G
5′ splice site
P
G U A A G U
Exon 2
3′
G
P
G A C N Y$_n$
2′ OH
Y A R Y N Y
Branch site
3′ splice site
Precursor
3′ OH
G
A
5′
3′
G
P
G A C
Y
P
3′
5′
P
2′
G U A A G U
A R Y N Y
Lariat intermediate
3′
G
P
G A
5′
Spliced product
+
3′
OH
G A C N Y$_n$
Y
P
3′
5′
P
2′
G U A A G U
A R Y N Y
Lariat form of intron

Introns

Type	Mechanism	Occurence
Pre-mRNA	**spliceosome**	**eukaryotic nucleus-mRNA**
Group I	**Self-splicing G-cofactor**	**lower eukaryotic rRNA, a few rRNA and tRNAs in fungi and plant mitochondria and chloroplasts**
Group II	**Self-splicing**	**a few structral, tRNA and rRNA in fungi and plant mitochondria and chloroplasts**


Group I self-splicing intron sequences
Group II self-splicing intron sequences
intron sequence
5' exon sequence
3' exon sequence
3'
HO
G
5'
3'
precursor RNA molecule
G
5'
OH
3'
transient intermediate
5'
G
3'
OH
excised intron sequence
5'
3'
ligated exon sequences
intron sequence
2'
A
lariat


pre-mRNA
Exon 1
Exon 2A
Exon 2B
Exon 3



Are You Getting It??



Which are characteristics of RNA splicing?

(multiple answers)

a) All introns are removed by the same mechanism.
b) All genes have the same number of introns.
c) Each pre-mRNA can be processed in only one way.
d) snRNAs can bind to splice sites and branch sites.
e) Lariat structures can form during splicing.
f) RNA can act as an enzyme during splicing.



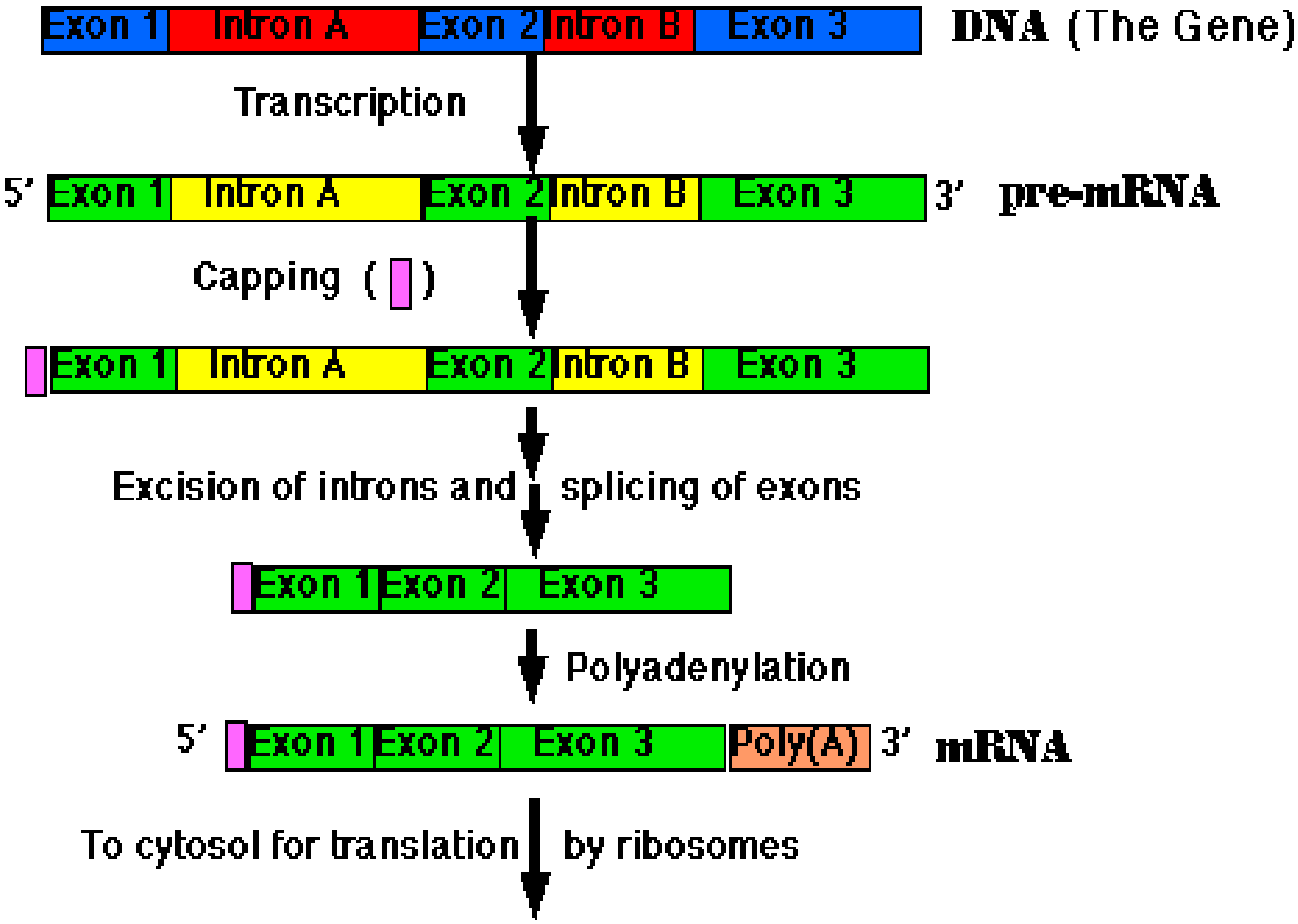
Are You Getting It??



Answer

Which are characteristics of RNA splicing?

a) **All introns are removed by the same mechanism.**
b) **All genes have the same number of introns.**
c) **Each pre-mRNA can be processed in only one way.**
d) ***snRNAs can bind to splice sites and branch sites.***
e) ***Lariat structures can form during splicing.***
f) ***RNA can act as an enzyme during splicing.***


Exon 1
Intron A
Exon 2
Intron B
Exon 3
DNA (The Gene)
Transcription
5'
Exon 1
Intron A
Exon 2
Intron B
Exon 3
3'
pre-mRNA
Capping ( )
Exon 1
Intron A
Exon 2
Intron B
Exon 3
Excision of introns and splicing of exons
Exon 1
Exon 2
Exon 3
Polyadenylation
5'
Exon 1
Exon 2
Exon 3
Poly(A)
3'
mRNA
To cytosol for translation by ribosomes