Last Class

- DNA replication
- Chromosome replication
- DNA repair
- General Recombination

Site-specific recombination

- Moves specialized nucleotide sequence (mobile genetic elements) between non-homologous sites within a genome.
- Transpositional site-specific recombination
- Conservative site-specific recombinatinon

Transpositional site-specific recombination

- Modest target site selectivity and insert mobile genetic elements into many sites
- Transposase enzyme cuts out mobile genetic elements and insert them into specific sites.

Three of the many types of mobile genetic elements found in bacteria

Transposase gene: encoding enzymes for DNA breakage and joining
Red segments: DNA sequences as recognition sites for enzymes
Yellow segments: antibiotic genes

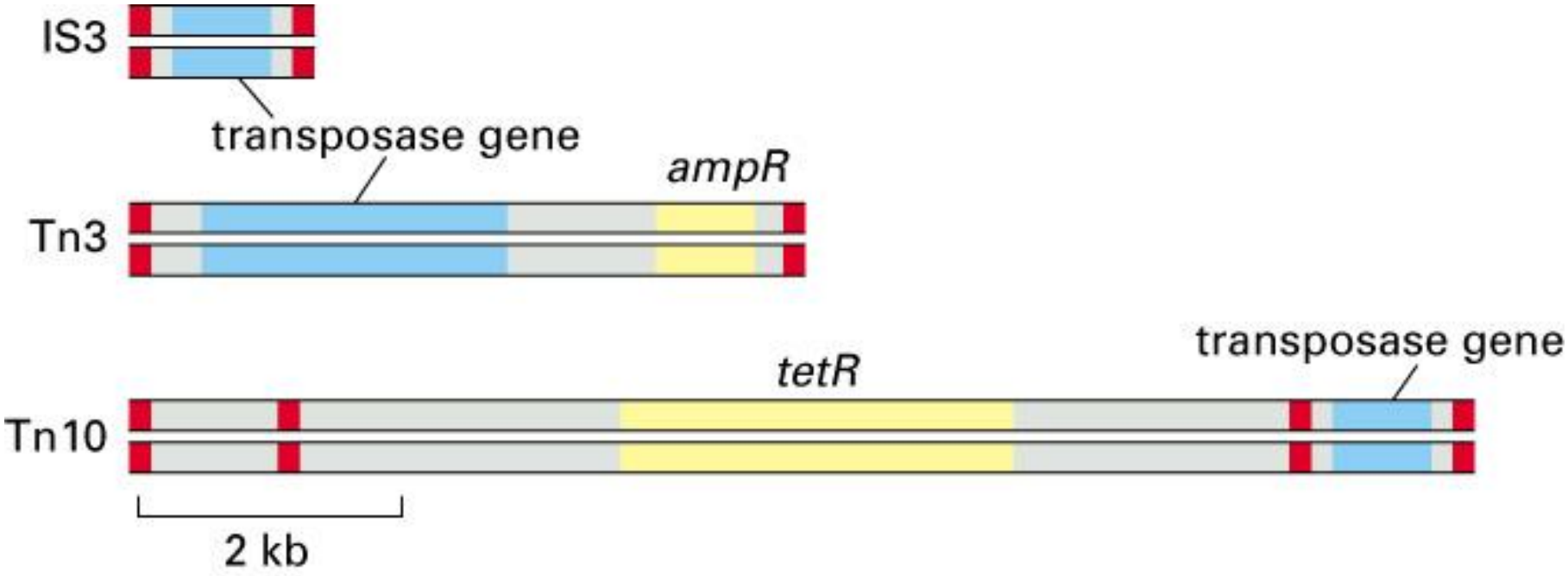


Figure 5–69. Molecular Biology of the Cell, 4th Edition.

TABLE 5–3 Three Major Classes of Transposable Elements

CLASS DESCRIPTION AND STRUCTURE	GENES IN COMPLETE ELEMENT	MODE OF MOVEMENT	EXAMPLES
DNA-only transposons			
			
short inverted repeats at each end	encodes transposase	moves as DNA, either excising or following a replicative pathway	P element (*Drosophila*) Ac-Ds (maize) Tn3 and IS1 (*E.coli*) Tam3 (snapdragon)
Retroviral-like retrotransposons			
			
directly repeated long terminal repeats (LTRs) at ends	encodes reverse transcriptase and resembles retrovirus	moves via an RNA intermediate produced by promoter in LTR	Copia (*Drosophila*) Ty1 (yeast) THE-1 (human) Bs1 (maize)
Nonretroviral retrotransposons			
			
Poly A at 3′ end of RNA transcript; 5′ end is often truncated	encodes reverse transcriptase	moves via an RNA intermediate that is often produced from a neighboring promotor	F element (*Drosophila*) L1 (human) Cin4 (maize)

These elements range in length from 1000 to about 12,000 nucleotide pairs; each family contains many members, only a few of which are listed here. In addition to transposable elements, there are selected viruses that can move in and out of host cell chromosomes; these viruses are related to the first two classes of transposons.

Cut and Paste Transposition
DNA-only

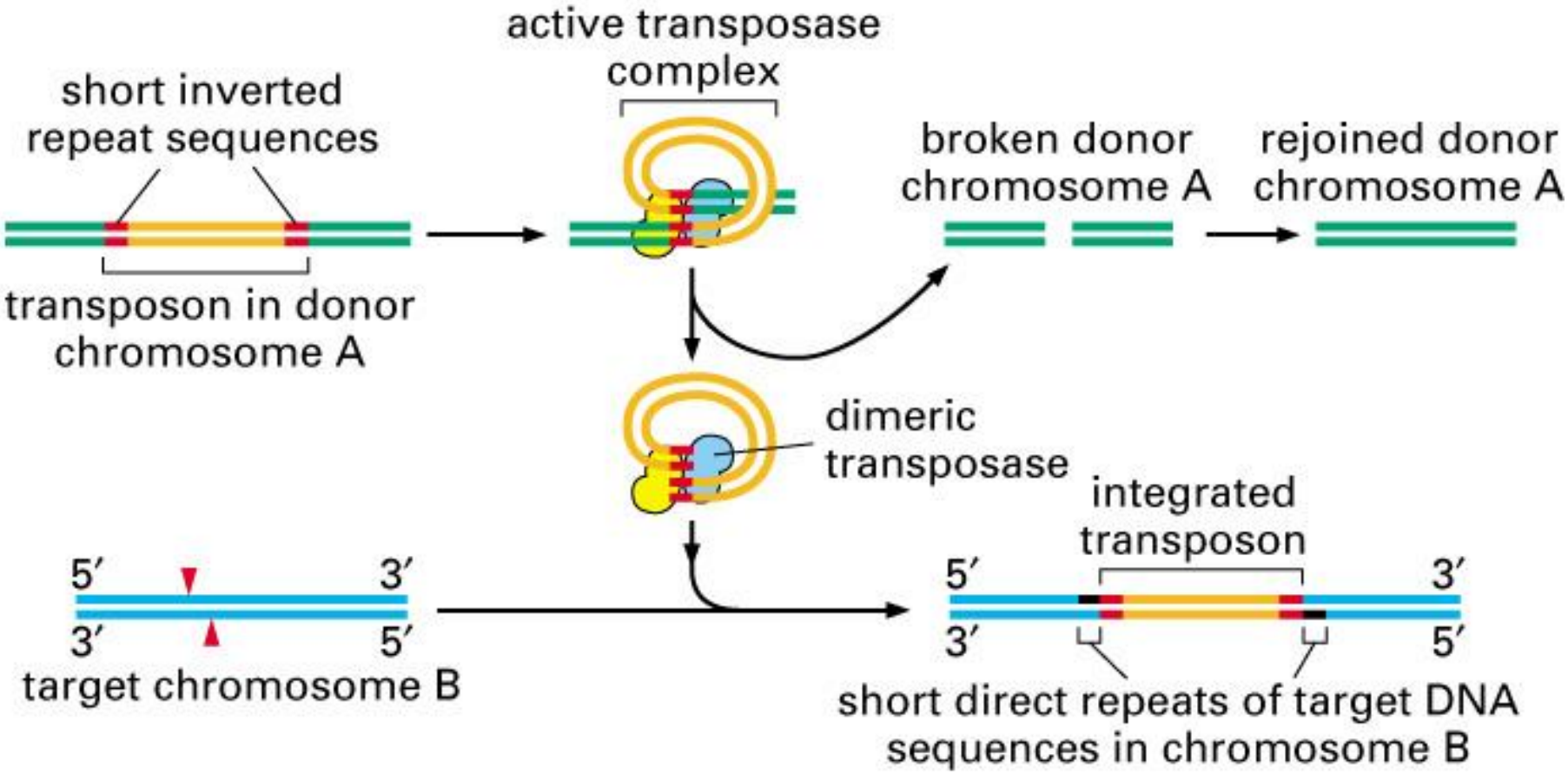
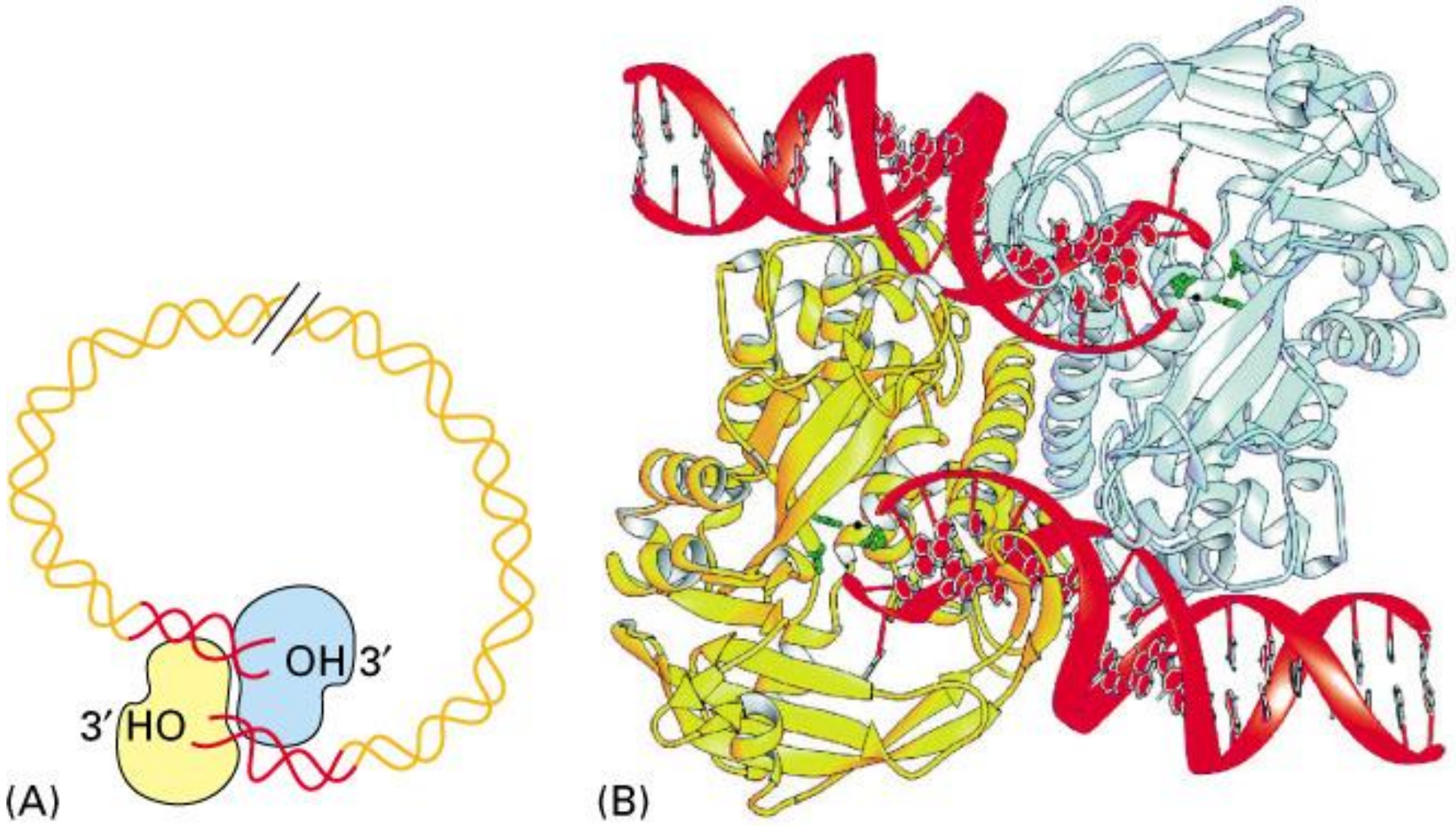


Figure 5–70. Molecular Biology of the Cell, 4th Edition.

The structure of the central intermediate formed by transposase (integrase)



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Replicative Transposition

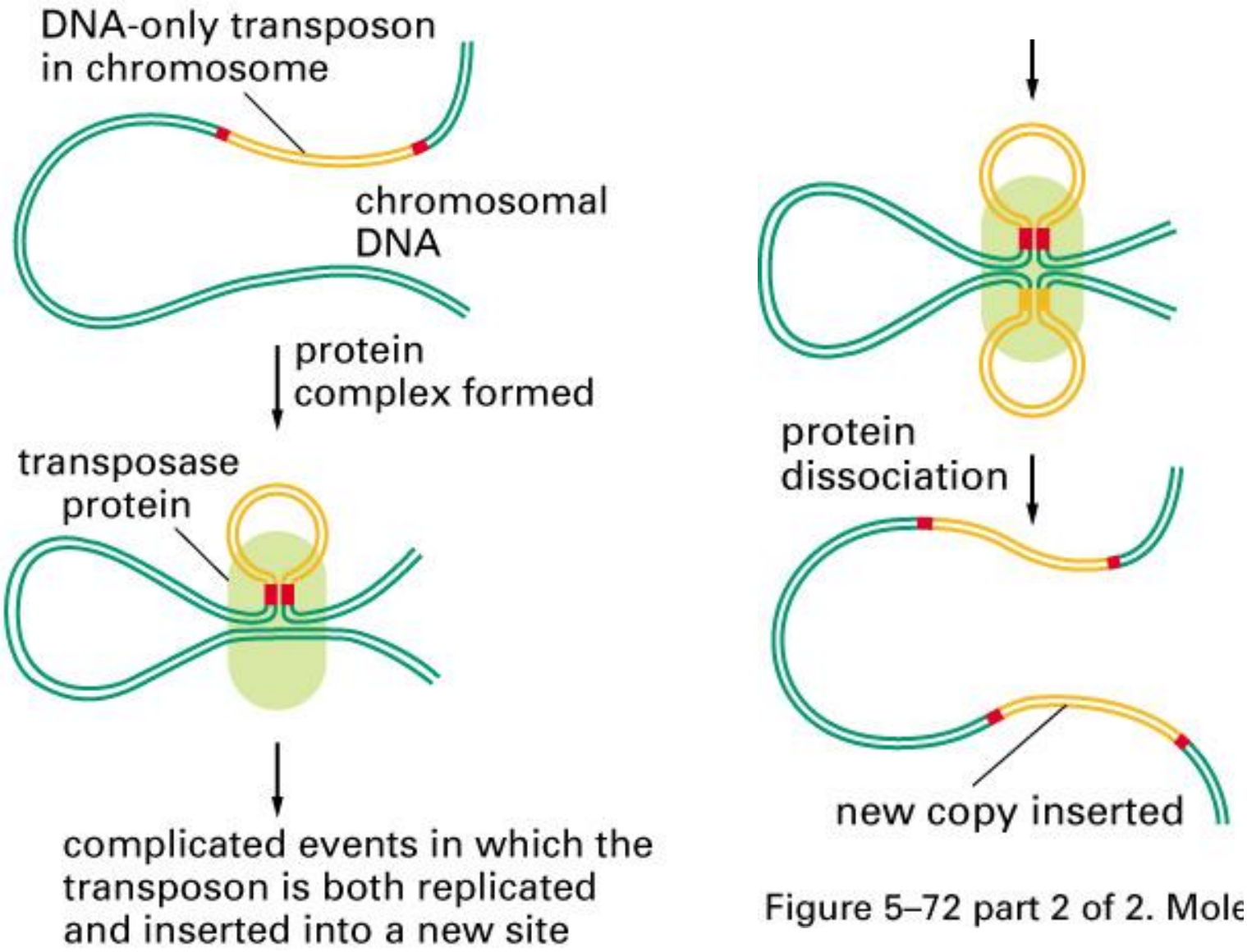


Figure 5–72 part 2 of 2. Mole

Retrovirus-based Transposition
Retroviral-like retrotransposition

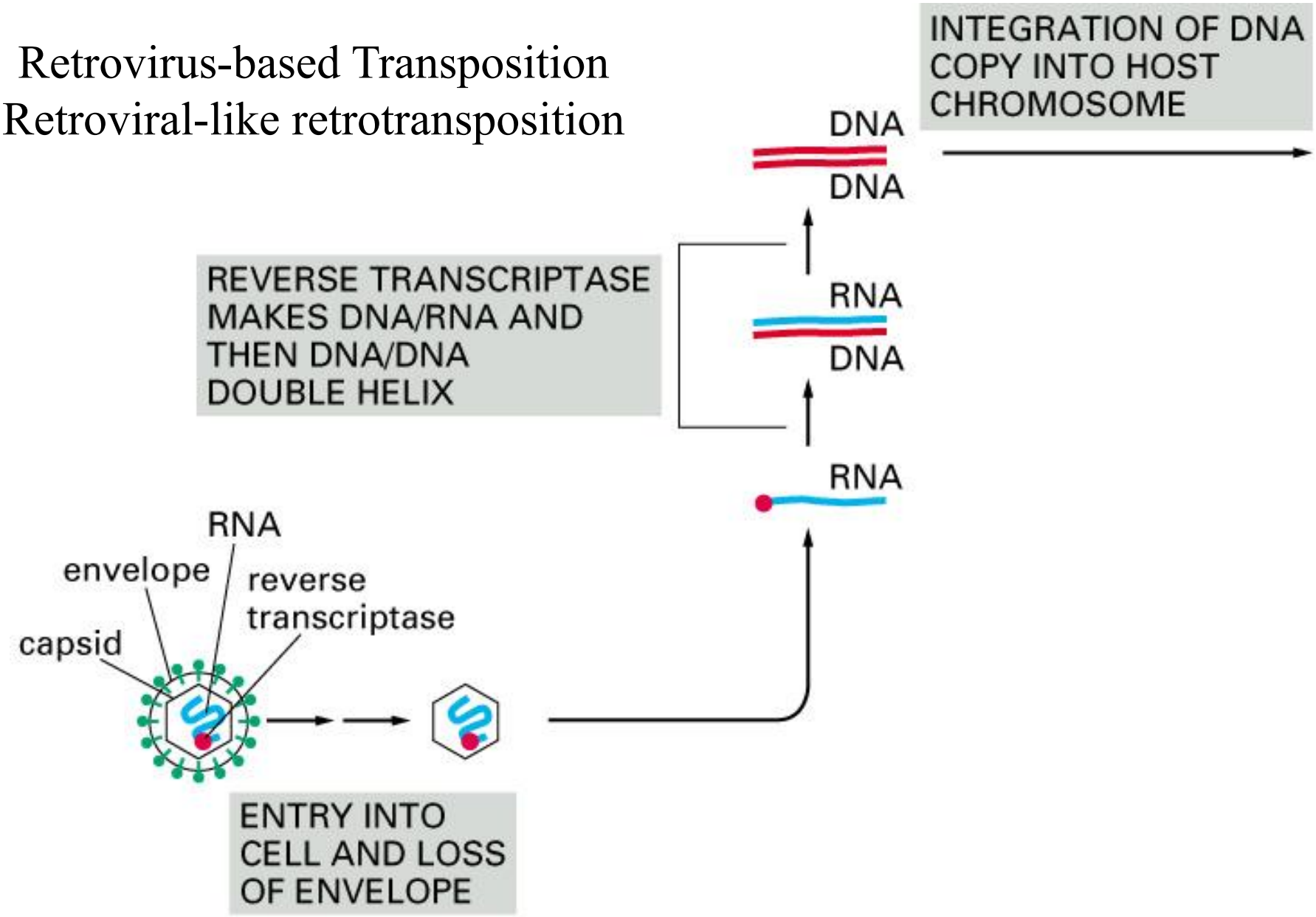


Figure 5–73 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

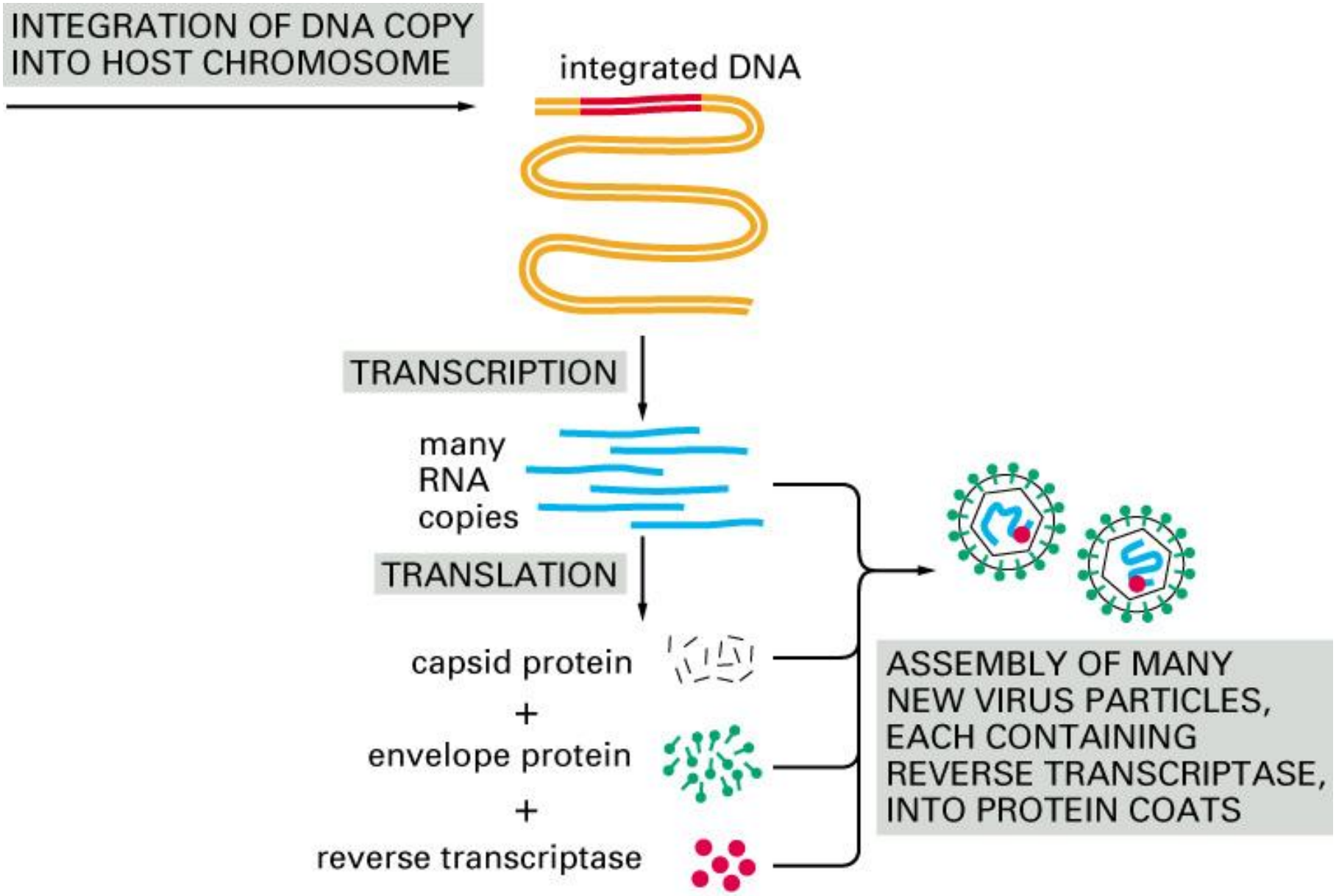
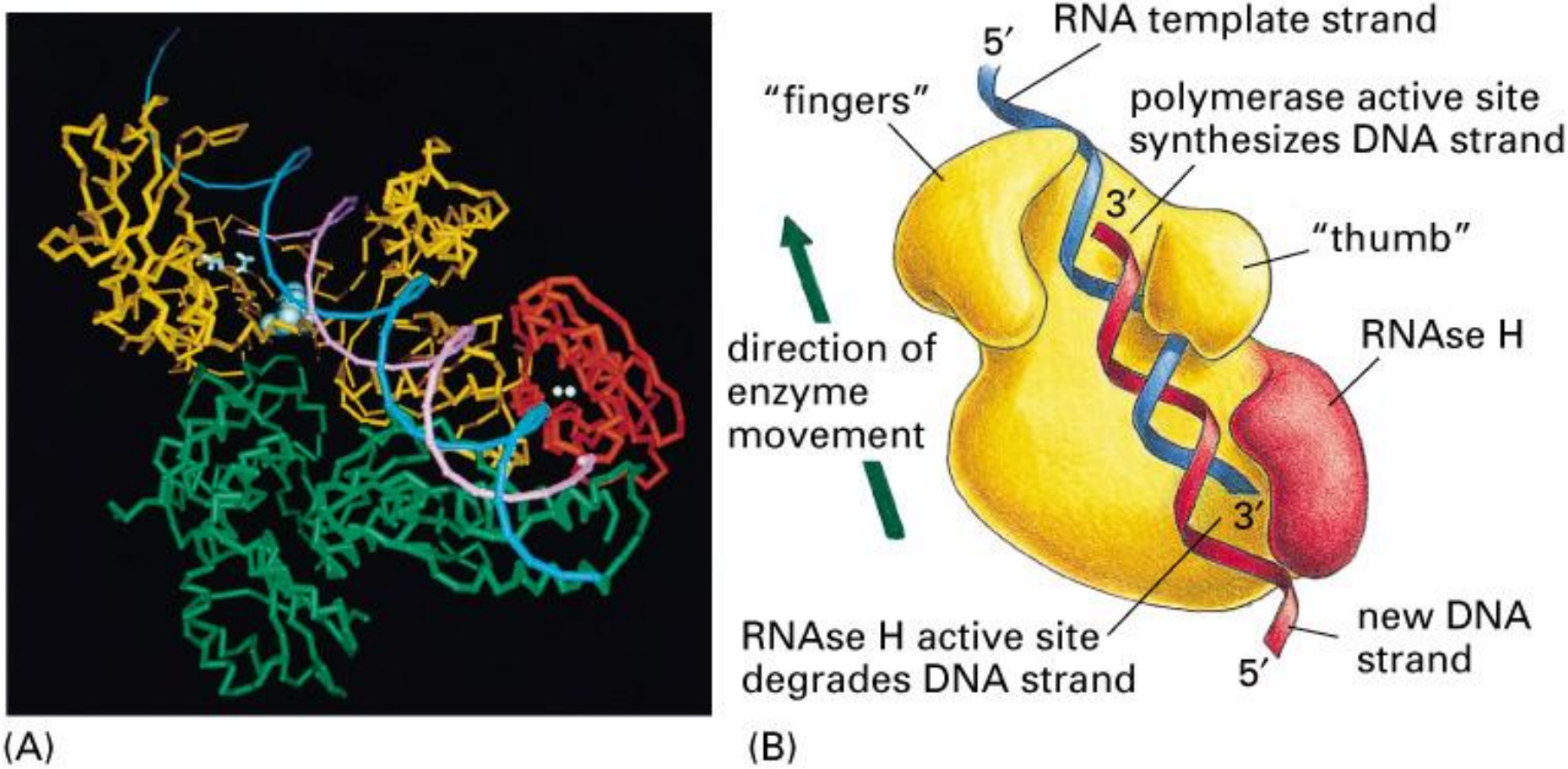


Figure 5–73 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Reverse Transcriptase
From RNA to DNA



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Non-retroviral retrotransposition
L1 Element

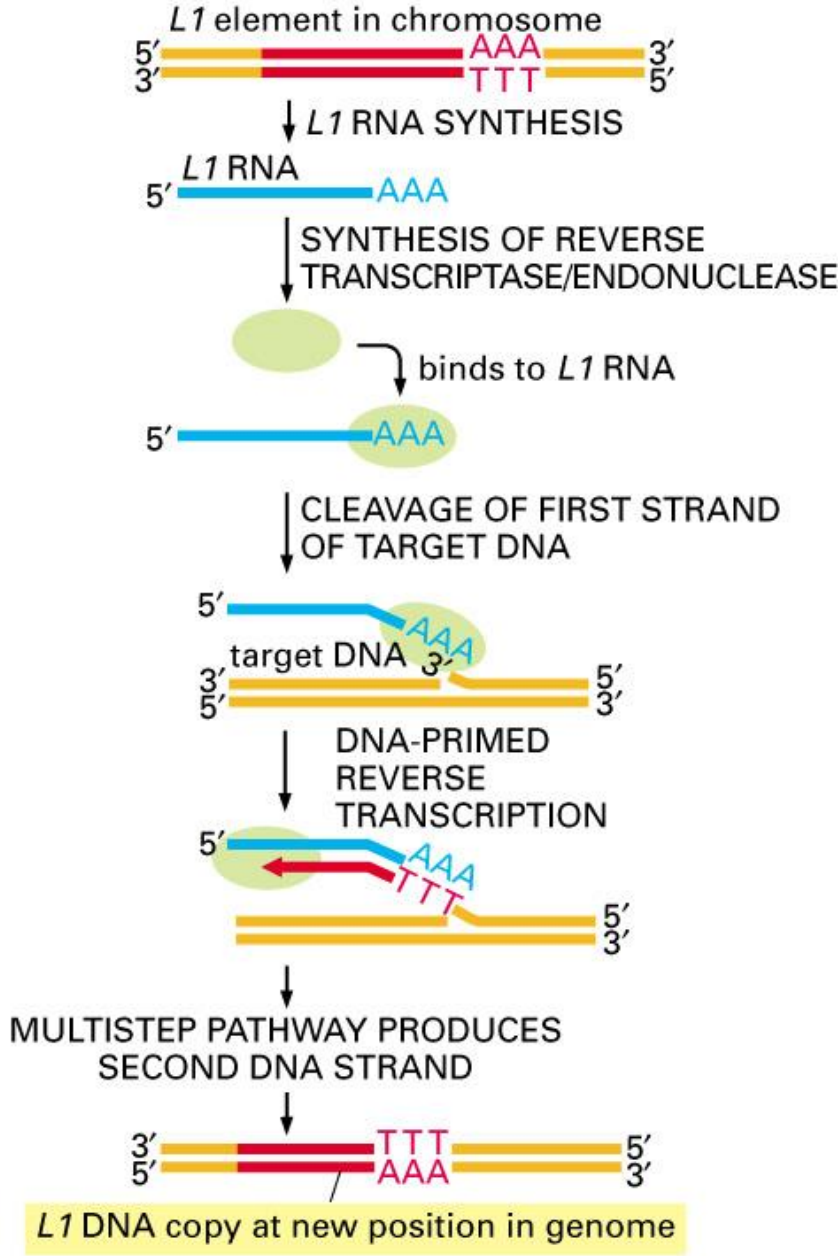


Figure 5–76. Molecular Biology of the Cell, 4th Edition.

Conservative Site Specific Recombination
Integration vs. inversion
Notice the arrows of directions

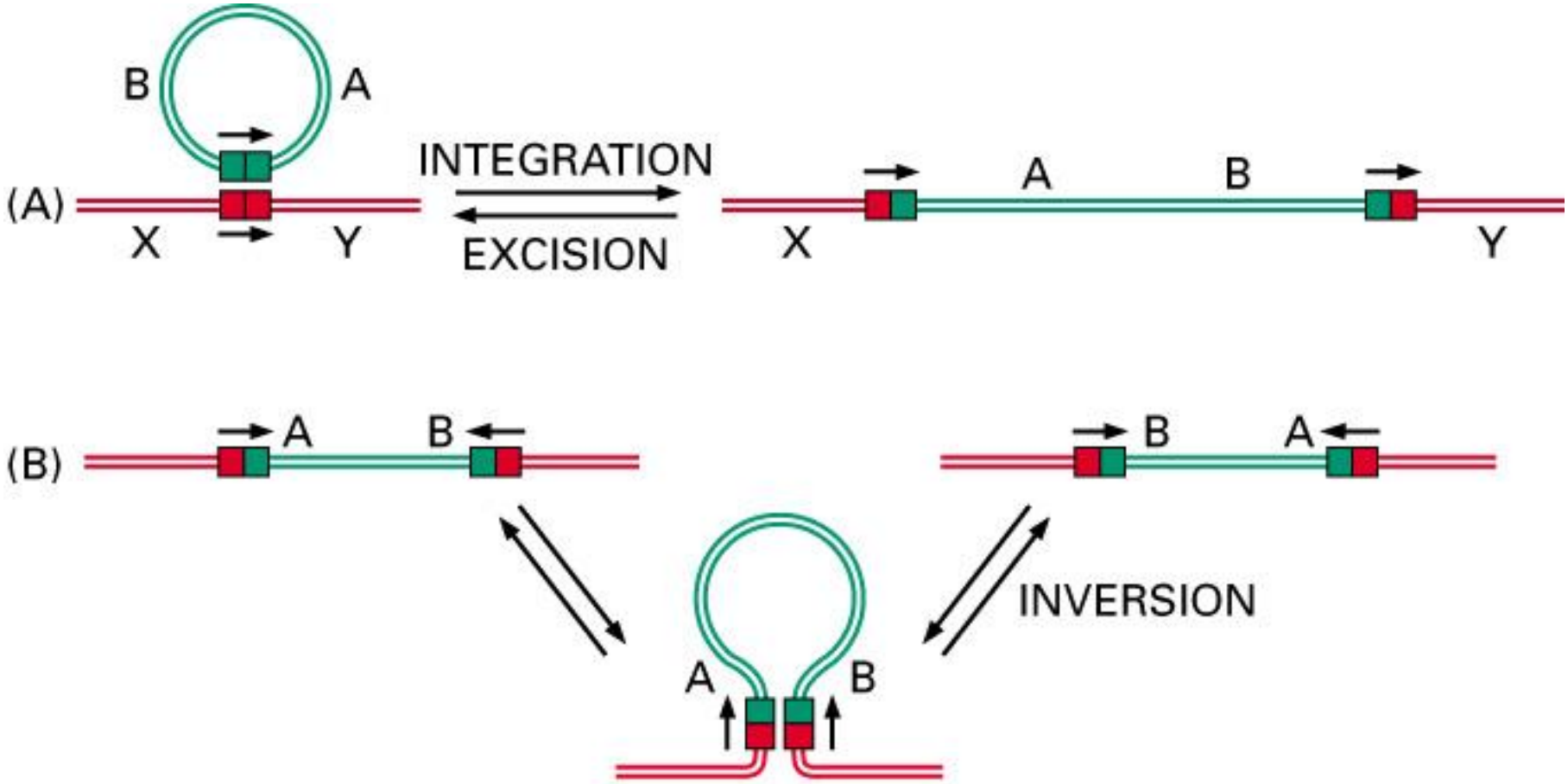


Figure 5–79. Molecular Biology of the Cell, 4th Edition.

Bacteriophase Lambda

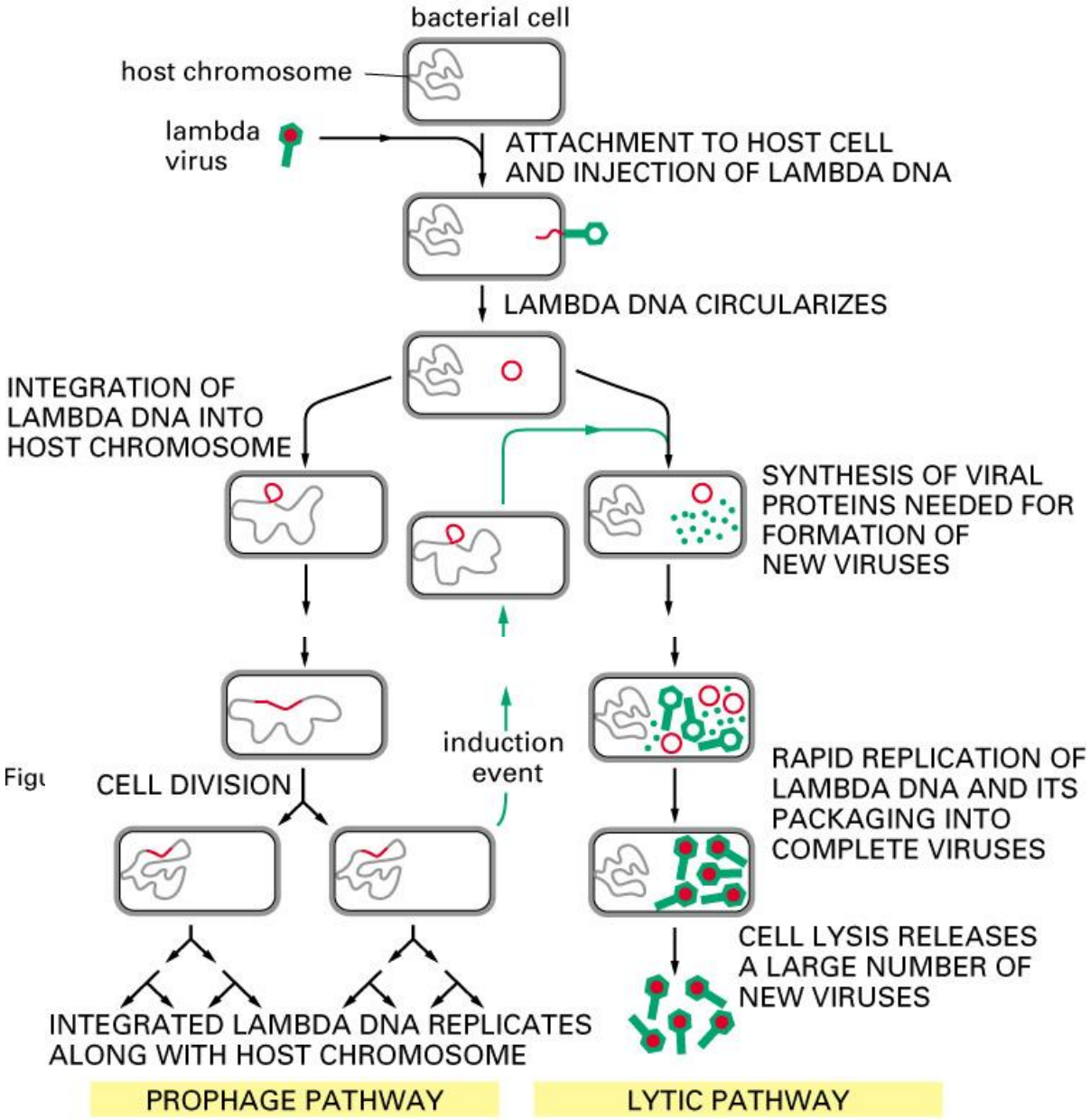


Figure 5–81 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Genetic Engineering to control Gene expression

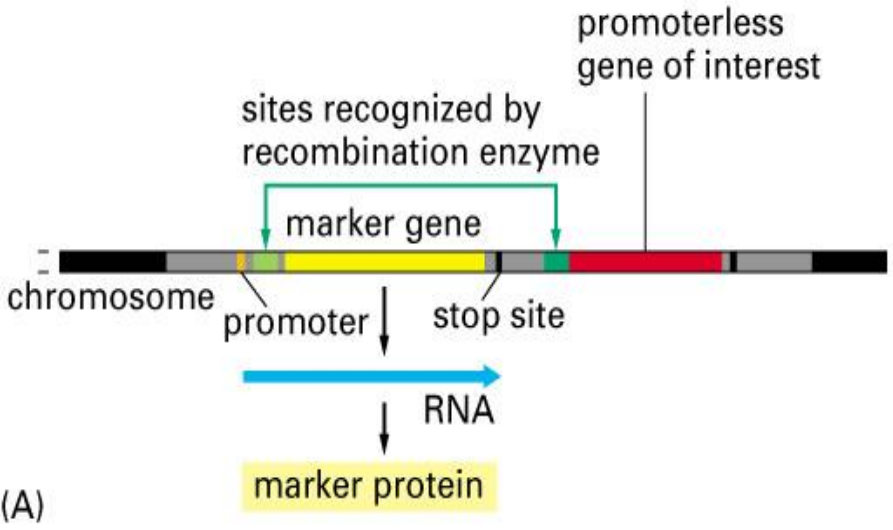


Figure 5–82 part 1 of 3. Molecular Biology of the Cell, 4th Edition

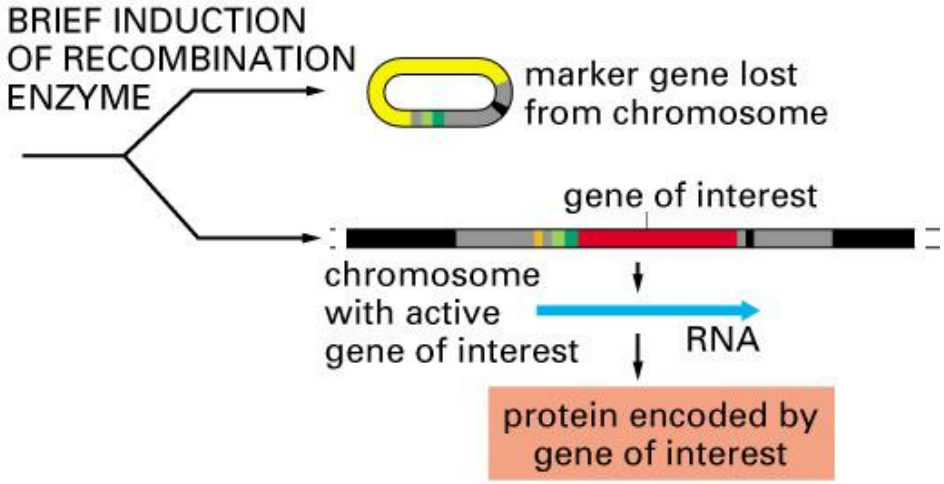


Figure 5–82 part 2 of 3. Molecular Biology of the Cell, 4th Edition.

Summary

- DNA site-specific recombination
- transpositional; conservative
- Transposons: mobile genetic elements
- Transpositional: DNA only transposons, retroviral-like retrotransposons, nonretroviral retrotransposons

How Cells Read the Genome: From DNA to Protein

1. Transcription
2. RNA Modification and Splicing
3. RNA transportation
4. Translation
5. Protein Modification and Folding

DNA->RNA-> Proteins

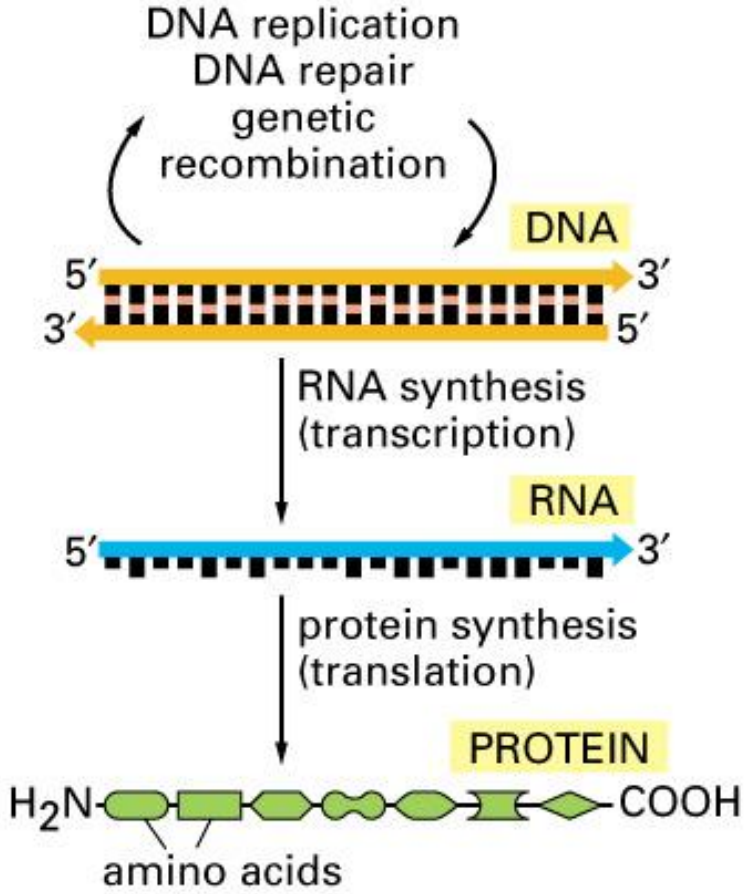


Figure 6–2. Molecular Biology of the Cell, 4th Edition.

Genes expressed with different efficiency

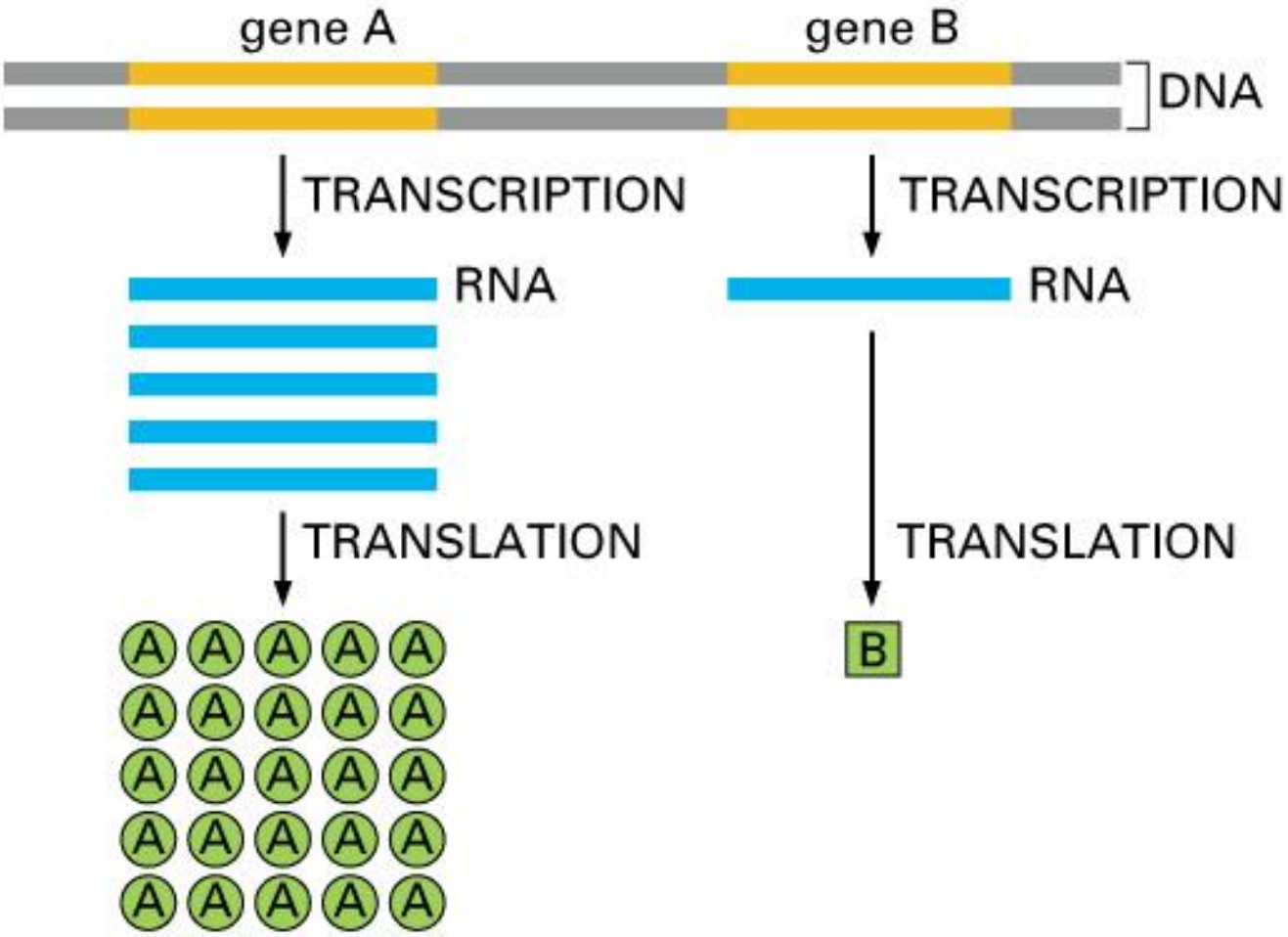


Figure 6–3. Molecular Biology of the Cell, 4th Edition.

The chemical structure differences between DNAs and RNAs

1. ribose, deoxyribose
2. Uracil and thymine

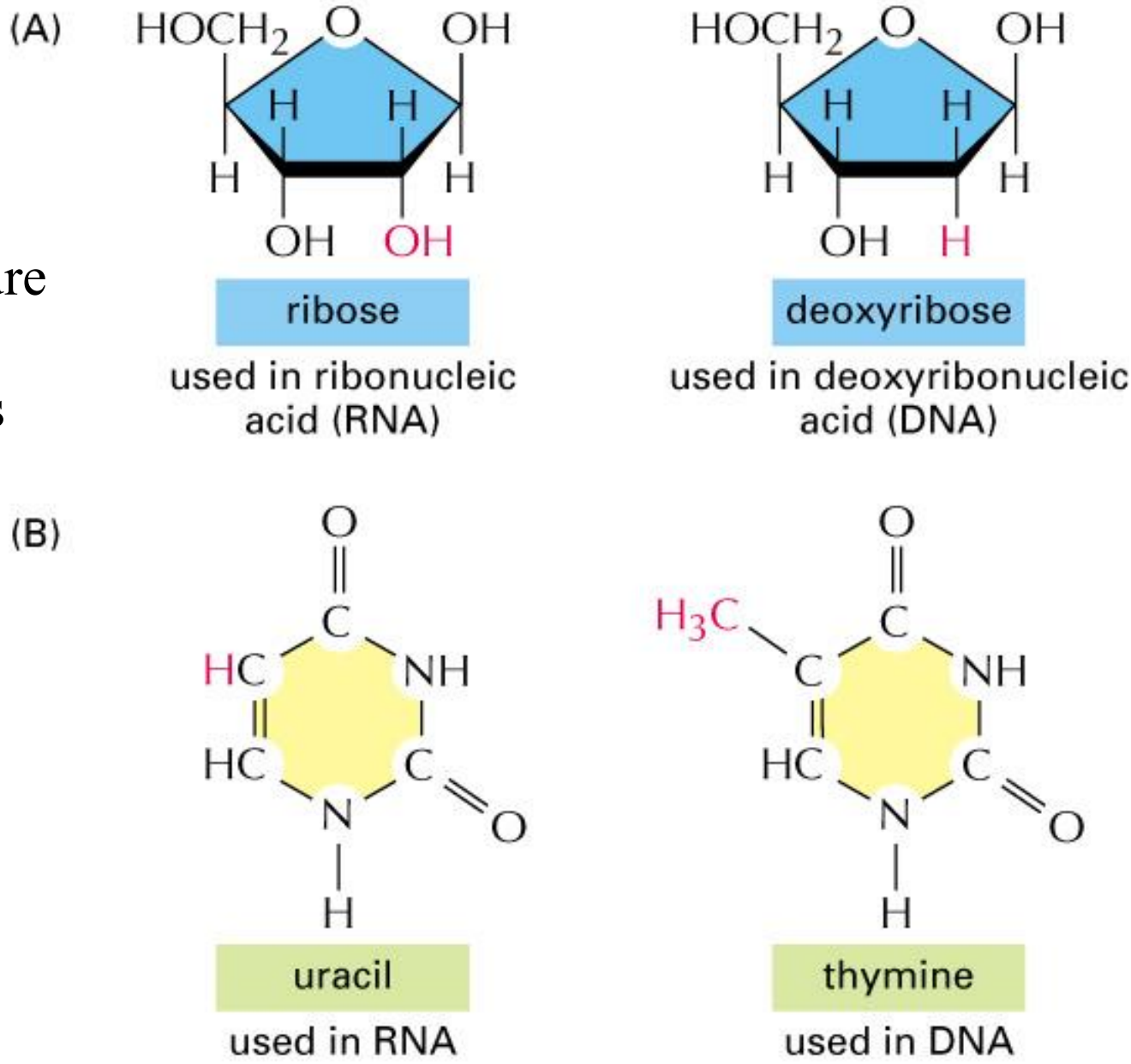


Figure 6–4 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

RNAs

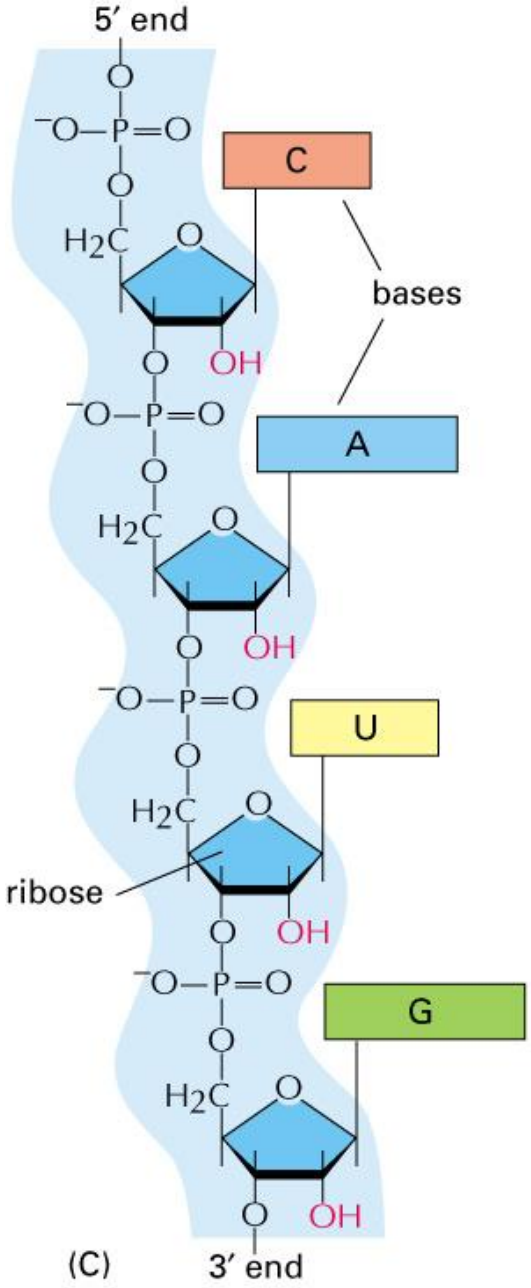


Figure 6–4 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

RNA base pairs
A-U; G-C

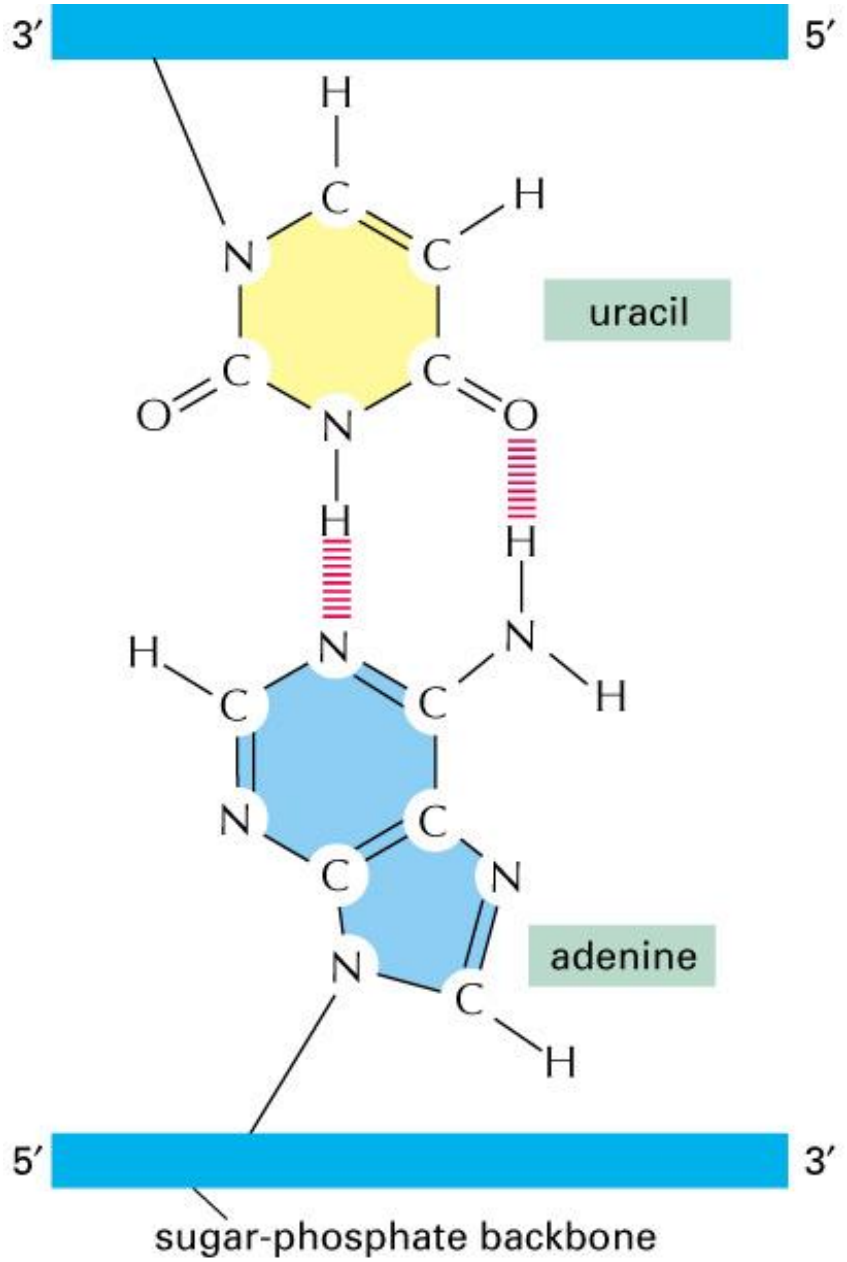


Figure 6–5. Molecular Biology of the Cell, 4th Edition.

RNA Structures

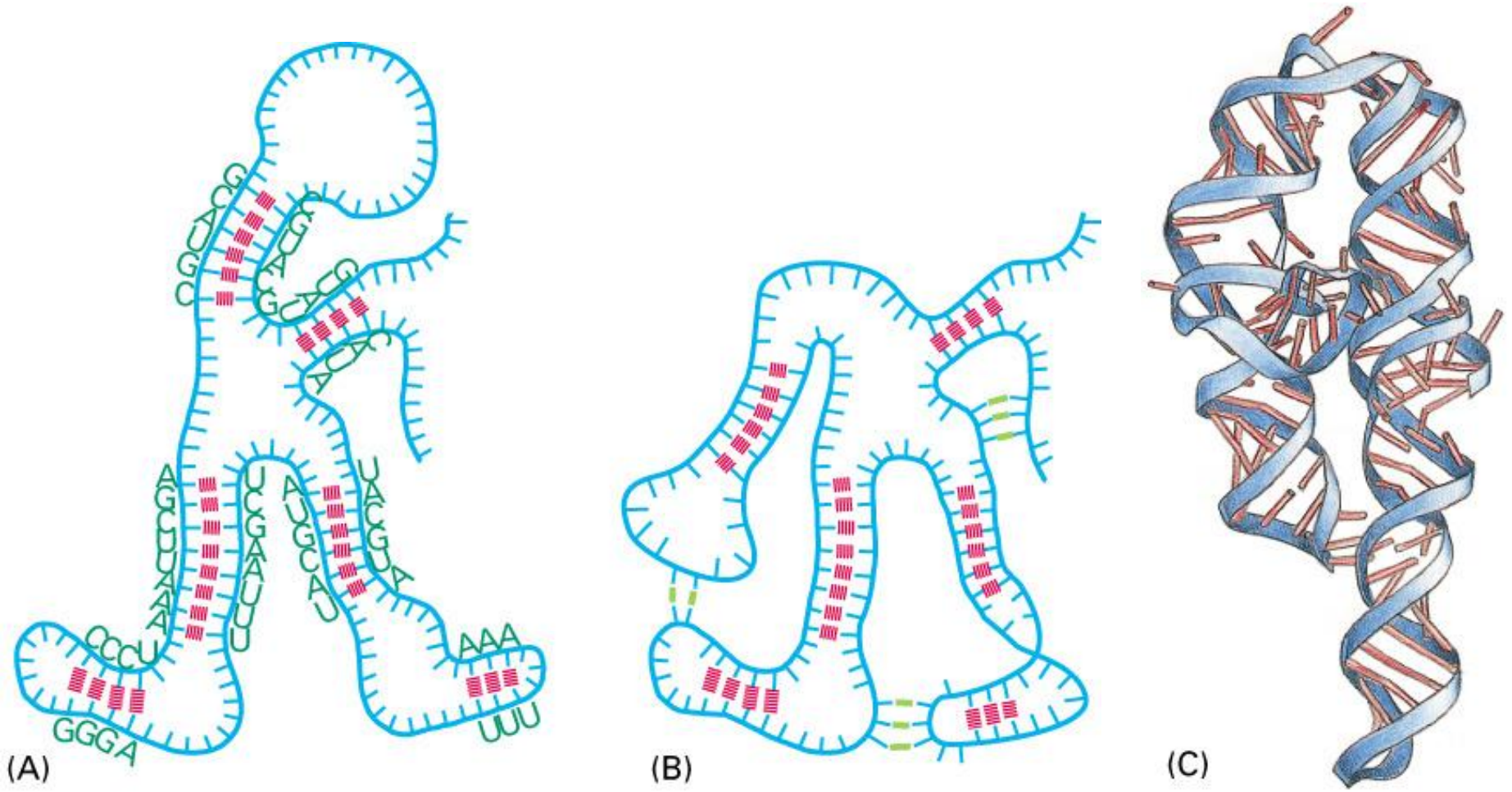


Figure 6–6 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Figure 6–6 part 2 of 2. Mol

DNA transcription to RNA

No need of primers, 10^4 error rate
Why called transcription?

mRNA: messenger RNA, 3-5%
rRNA: Ribosomal RNA, major amount
tRNA: transfer RNA
snRNA: small nuclear RNA

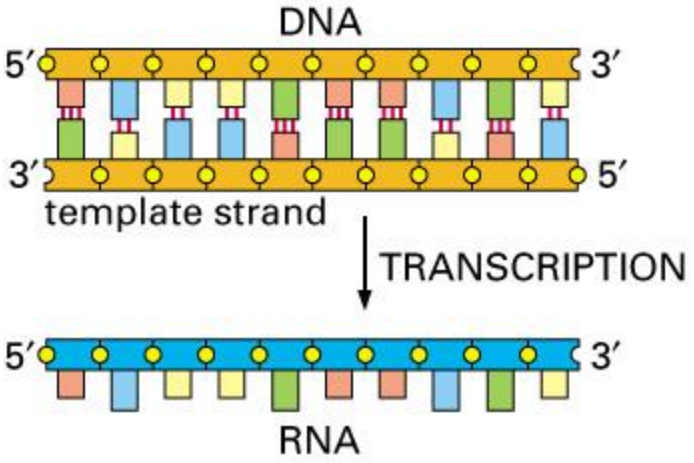


Figure 6–7. Molecular Biology of the Cell, 4th Edition.

RNA Polymerases
RNA polymerase I: rRNA
RNA polymerase II: mRNA
RNA polymerase III: tRNA

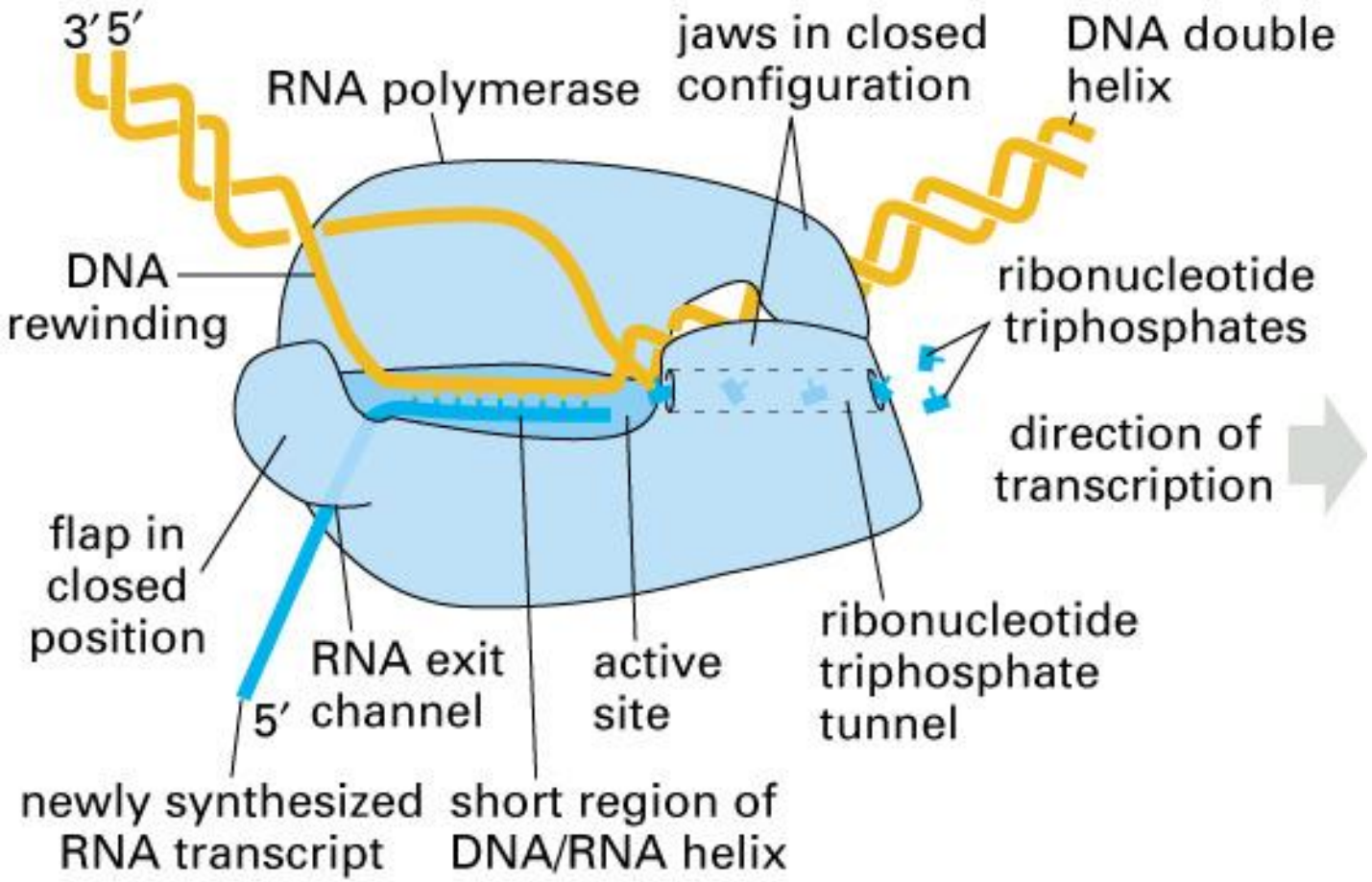


Figure 6–8. Molecular Biology of the Cell, 4th Edition.

EM images of 2 genes under transcription

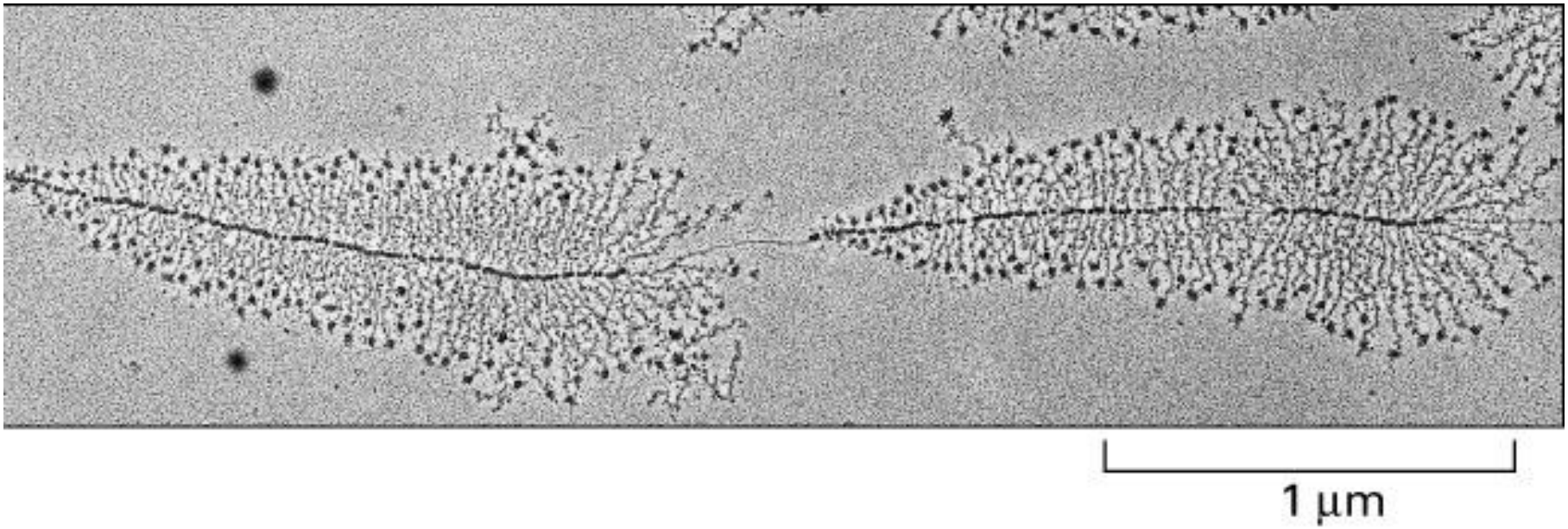


Figure 6–9. Molecular Biology of the Cell, 4th Edition.

Transcription
Cycle
Promoter
Terminator
sigma factor

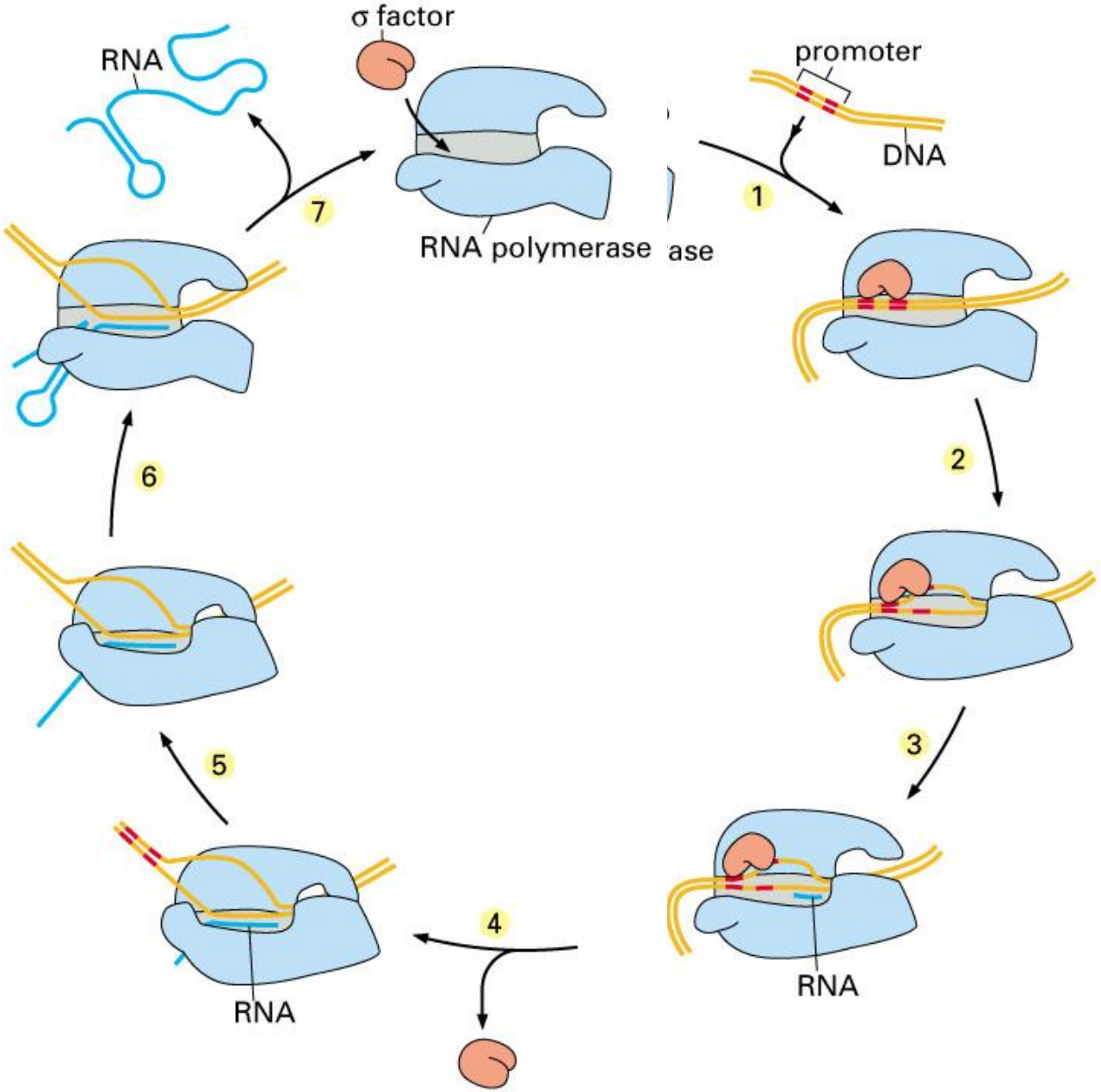


Figure 6–10 part 2 of 2. Molecular Biology of the Cell, 4. Molecular Biology of the Cell, 4th Edition.

RNA polymerase orientation

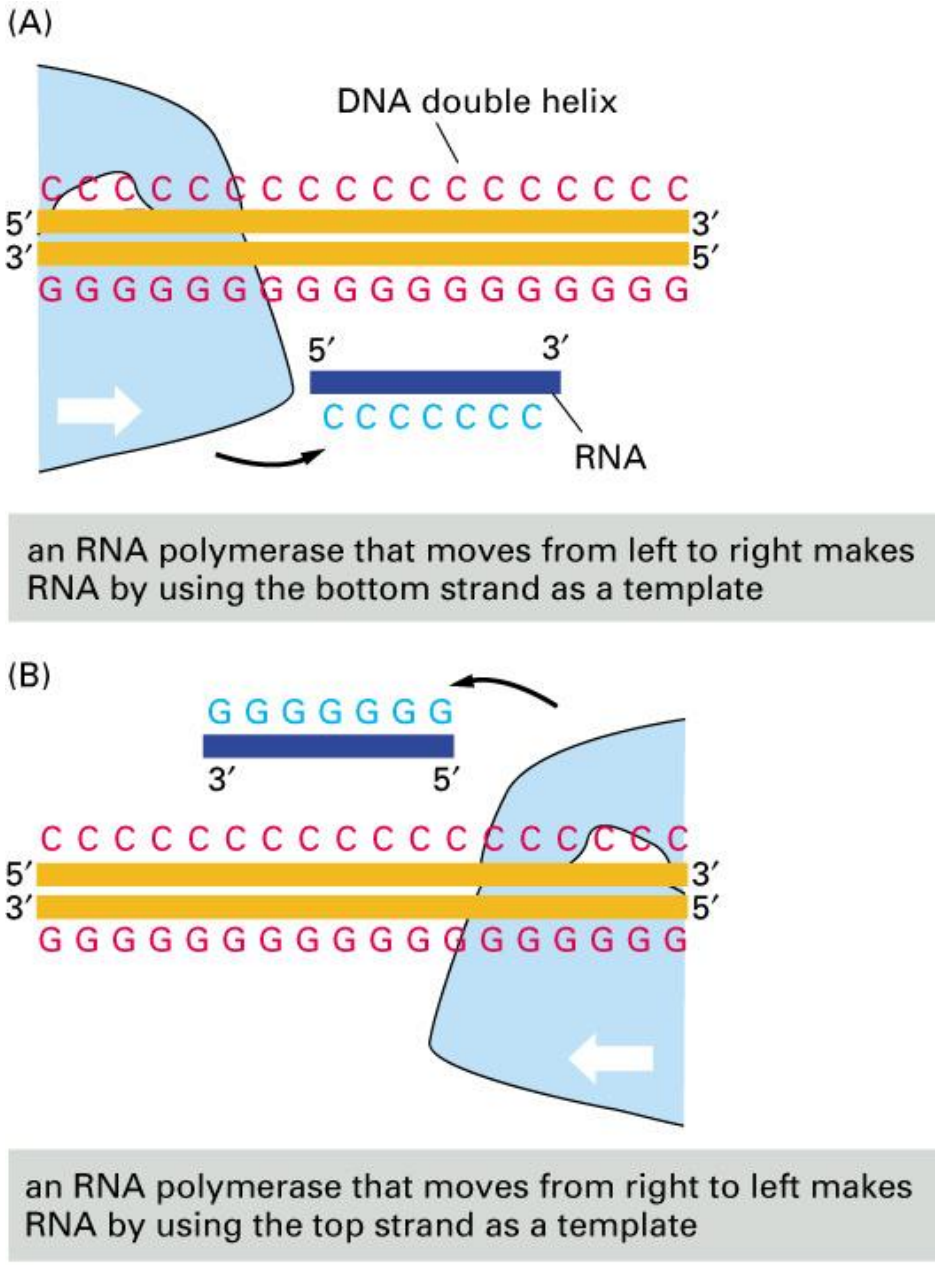


Figure 6–13. Molecular Biology of the Cell, 4th Edition.

RNA polymerase orientation and Gene products

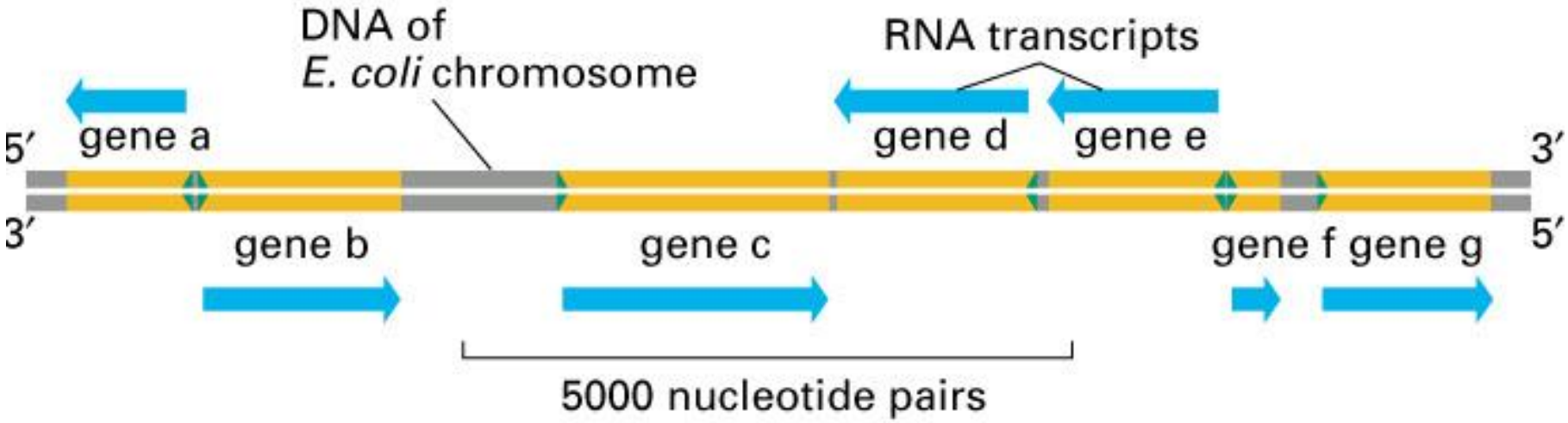


Figure 6–14. Molecular Biology of the Cell, 4th Edition.

Initiation of transcription with RNA polymerase II in eucaryotes
TF: transcription factor
TBP: TATA box binding protein
Promoter upstream of real starting sequence of transcription

TFIIH open DNA double helix and phosphorylate C-tail of polymerase and allow the release and transcription

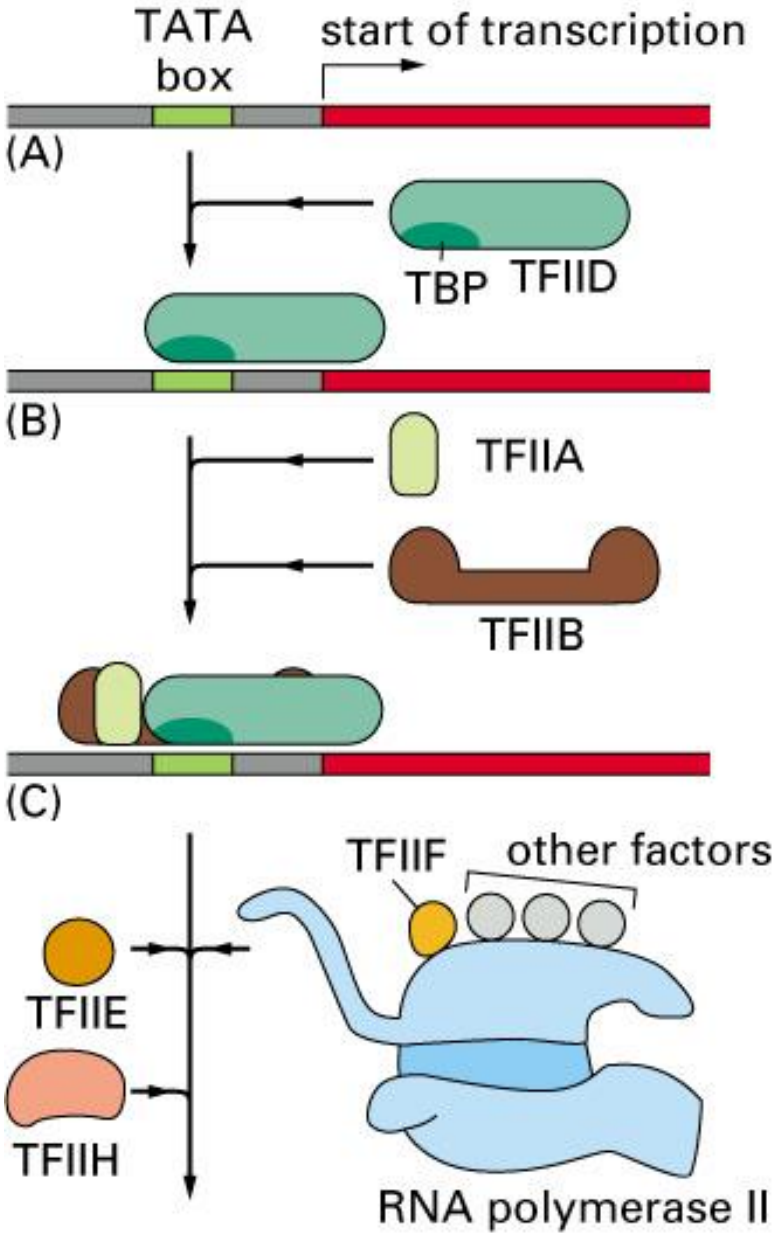


Figure 6–16 part 1 of 2. Molecular Biology

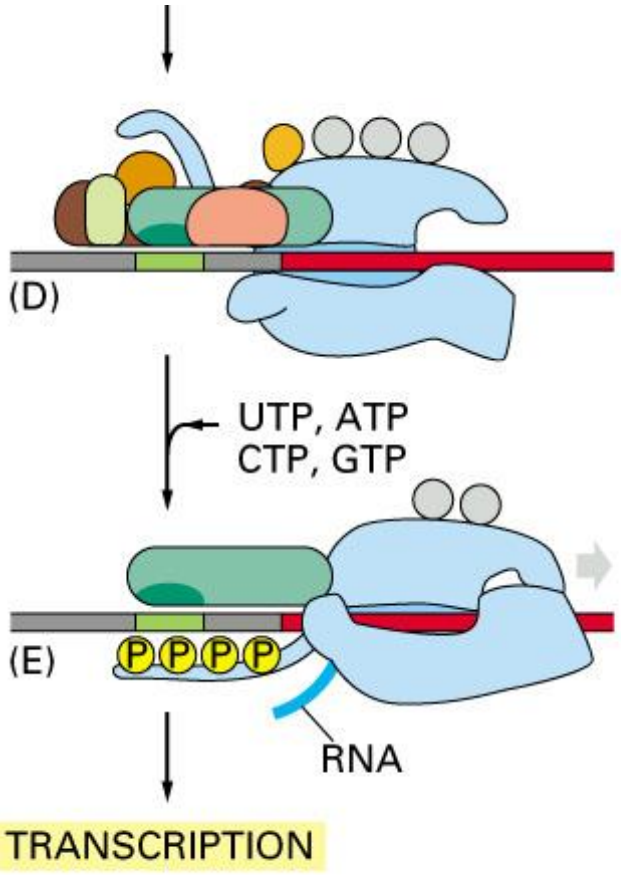


Figure 6–16 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

The importance of RNA polymerase II tail

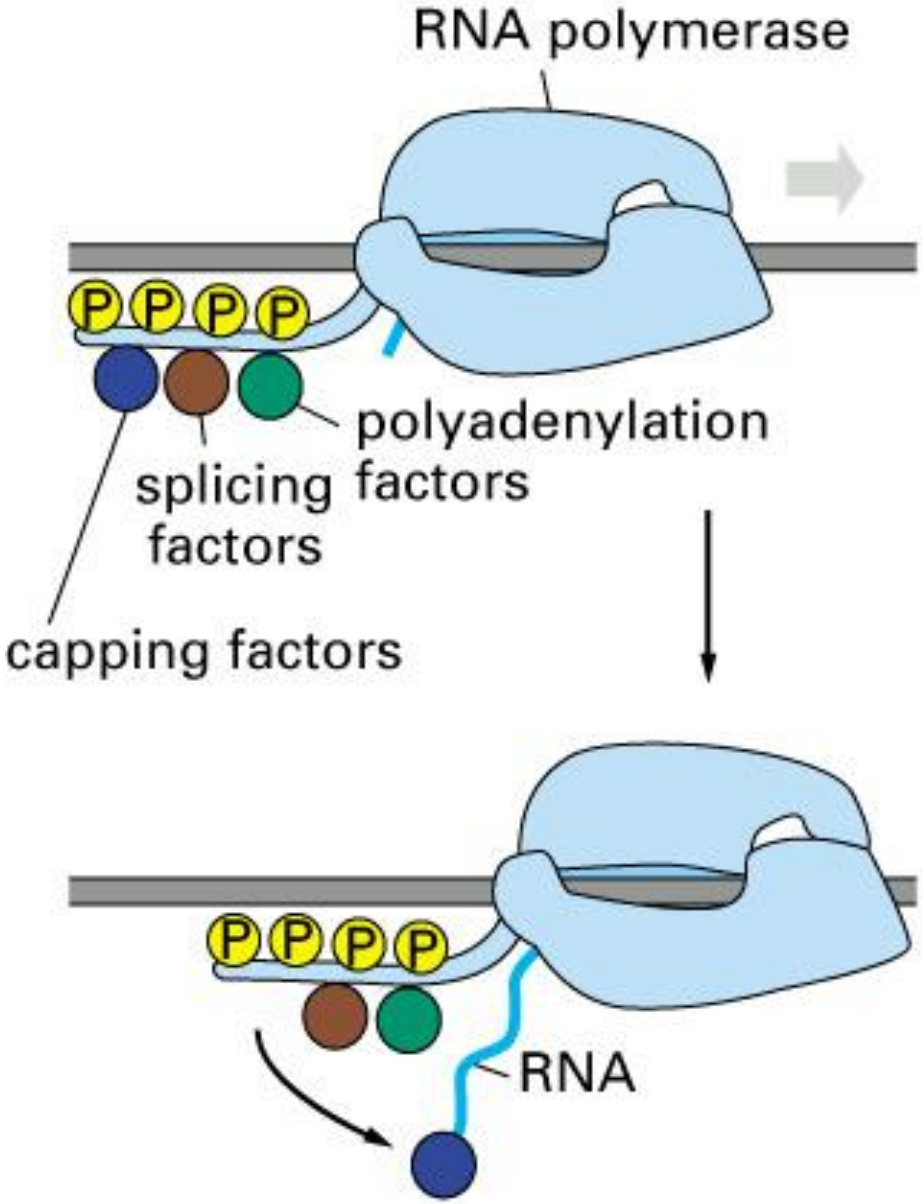
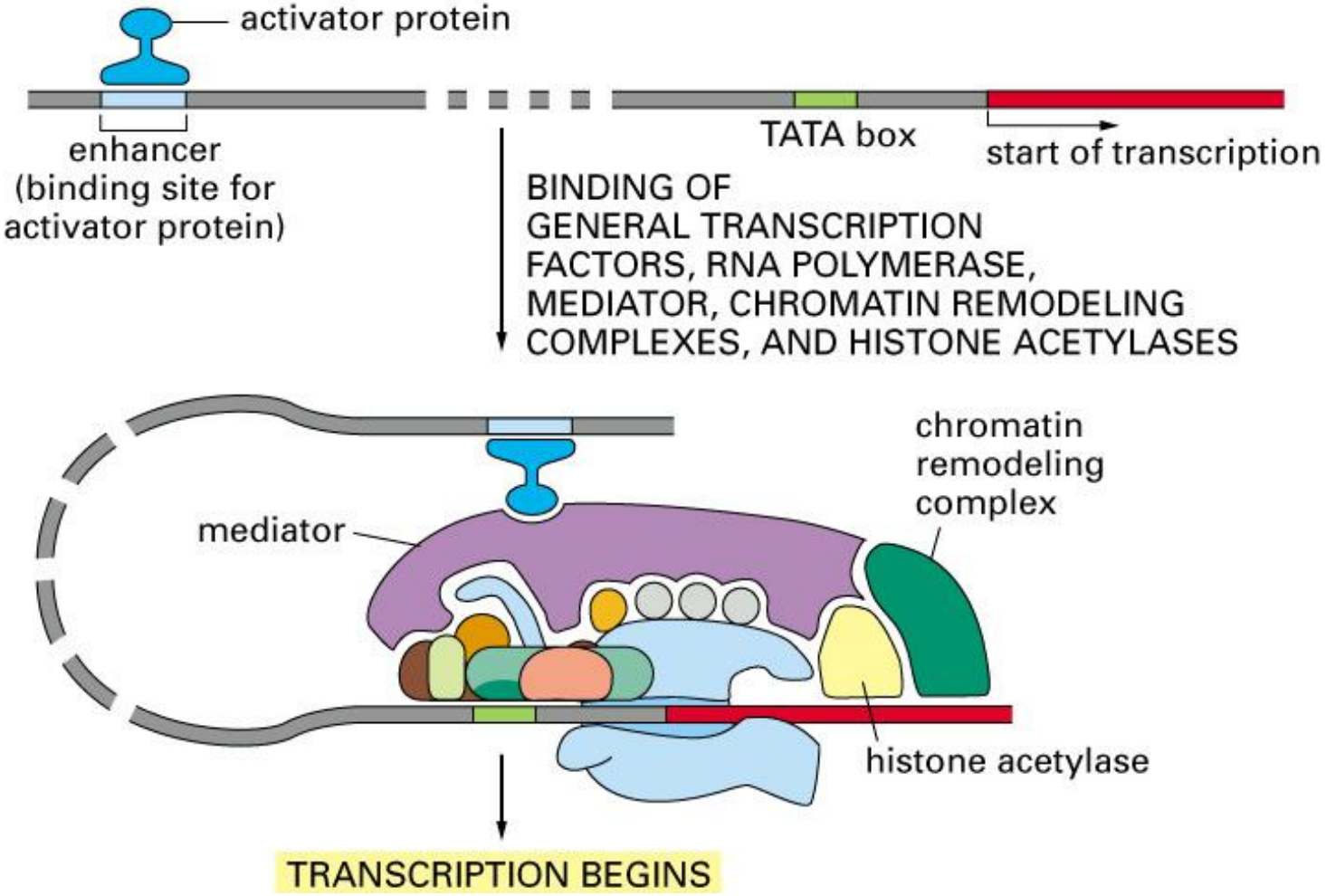


Figure 6–23. Molecular Biology of the Ce

Initiation of transcription with RNA polymerase II in eucaryotic cells

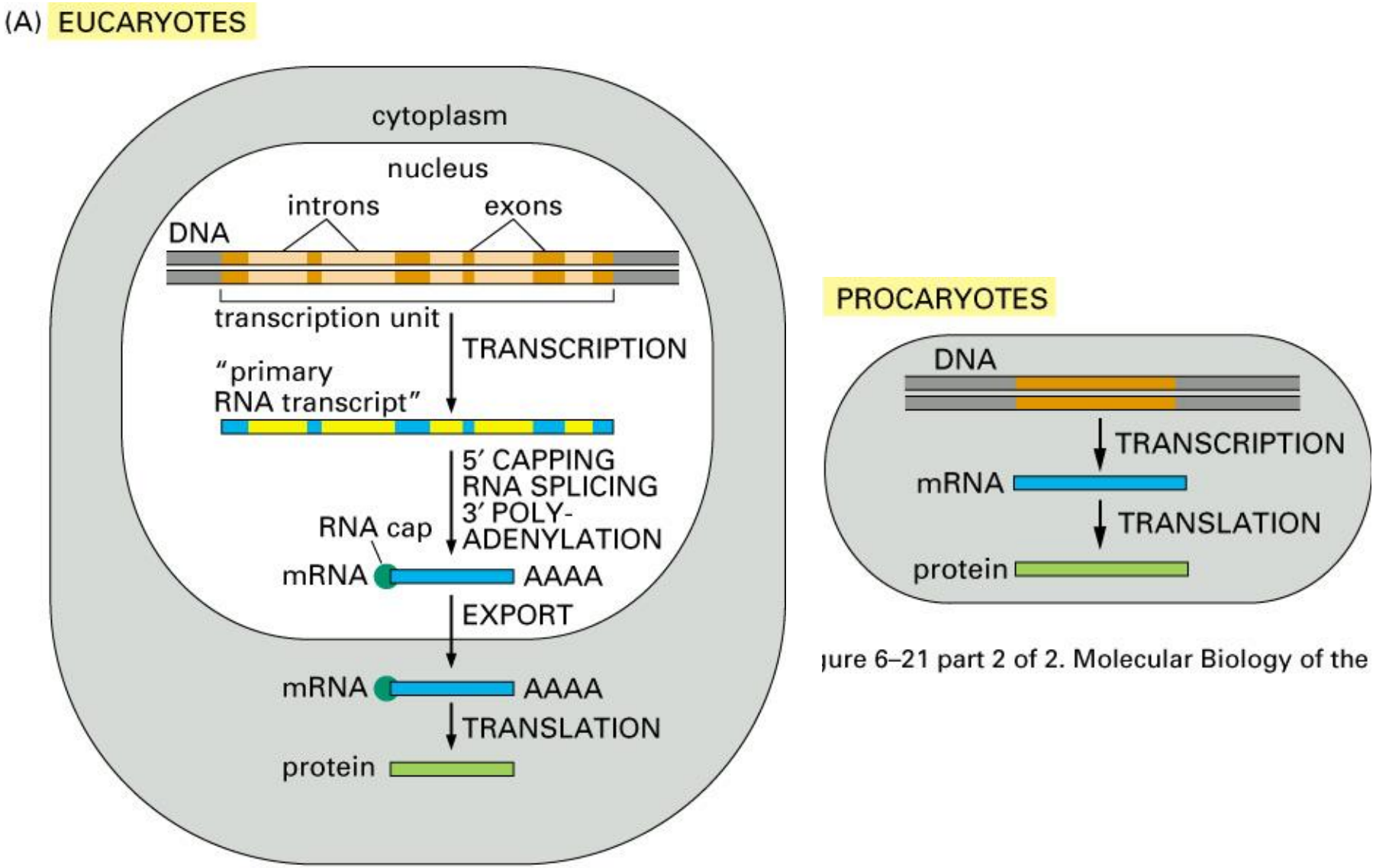
Remember Nucleasomes

Enhancer, mediator, chromatin remodeling complex, histone acetylase



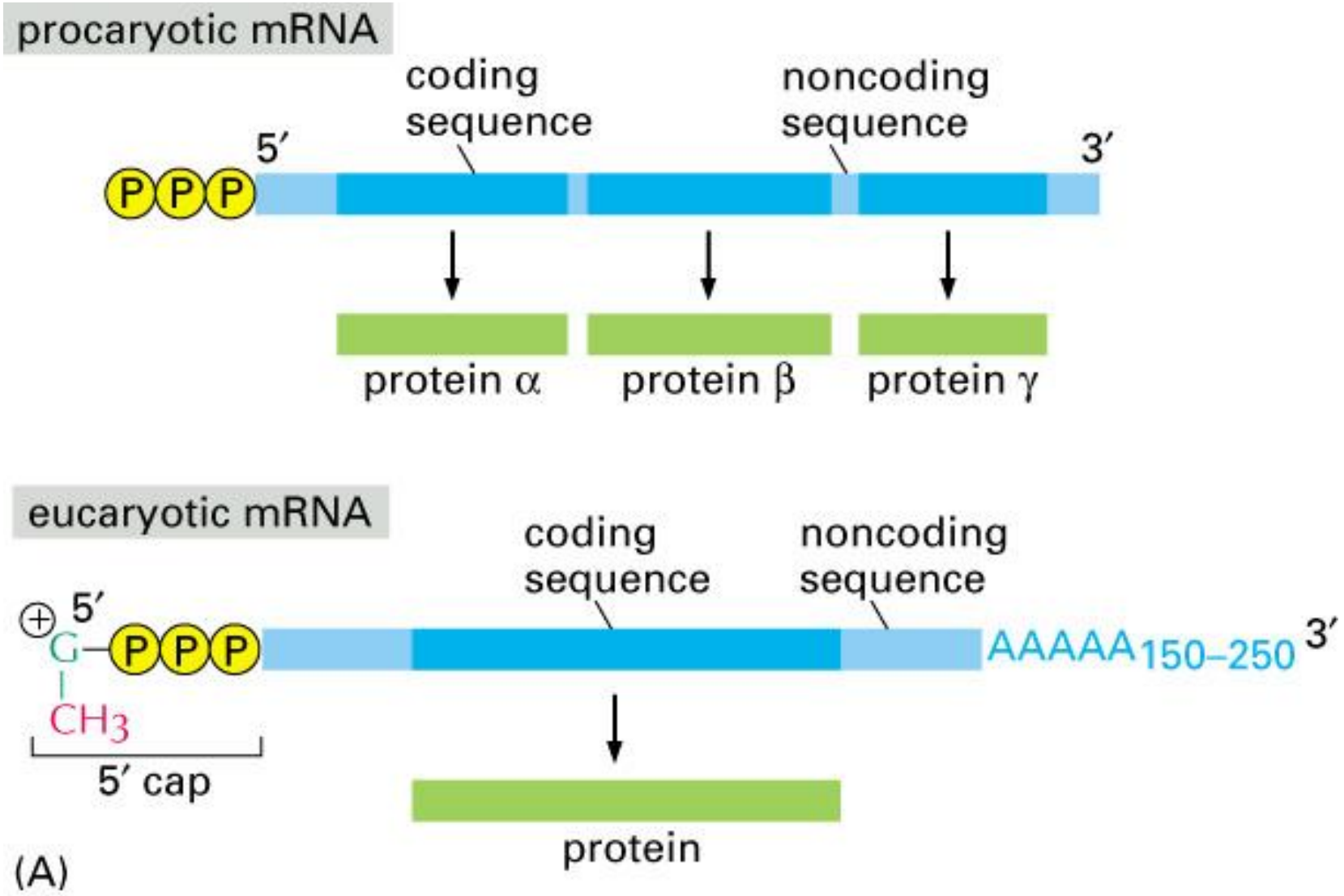
Genes to proteins

The comparison between eucaryotes (substantially complex) and procaryotes (simple)

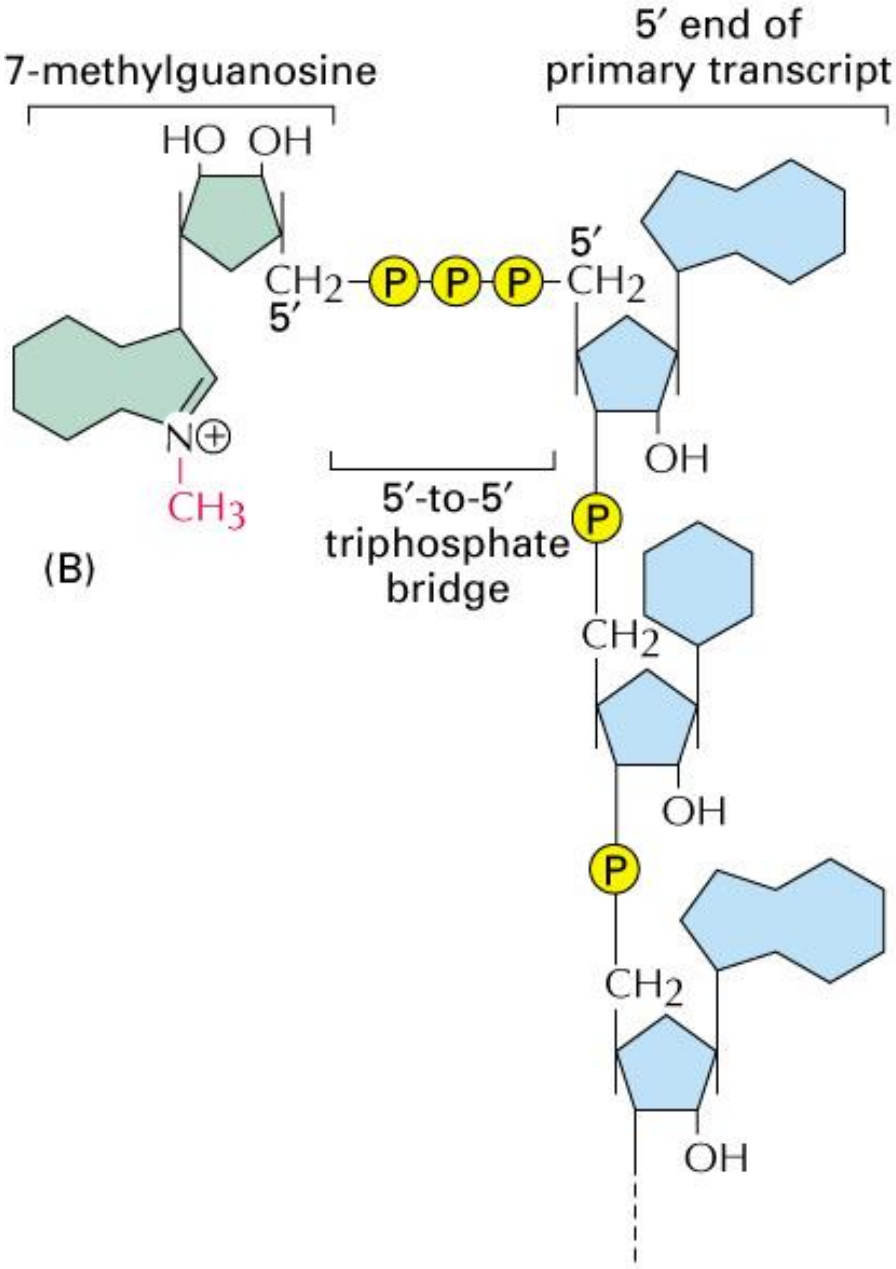


ure 6–21 part 2 of 2. Molecular Biology of the

mRNA between procaryotic and eucaryotic cells
5’ capping and 3’ polyadenylation



5’ capping



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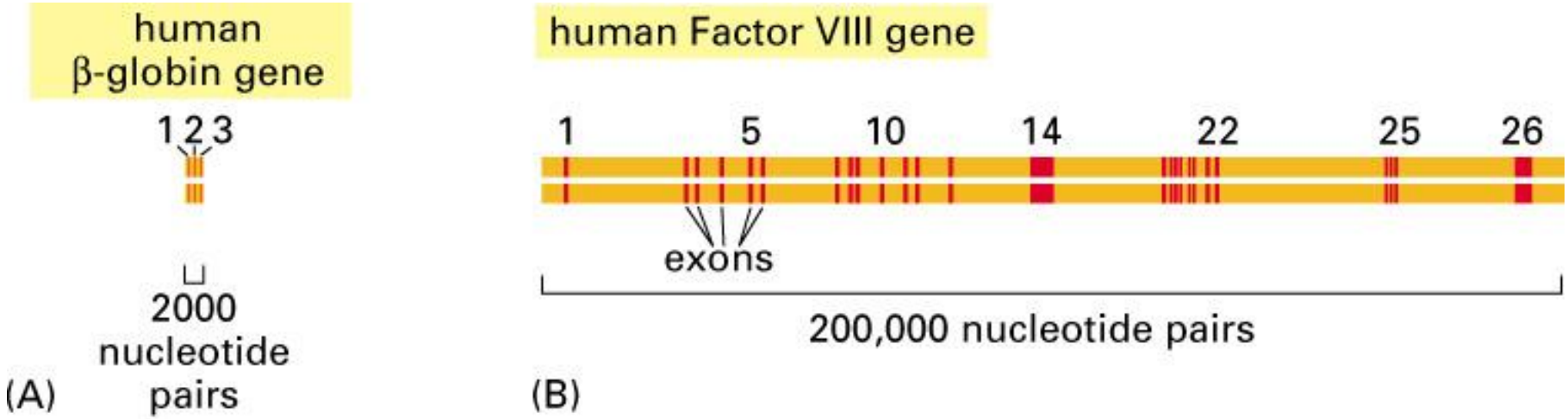
Splicing effects on gene products
RNA splicing
Exons: expressed sequences
Introns: intervening sequences



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RNA splicing reactions

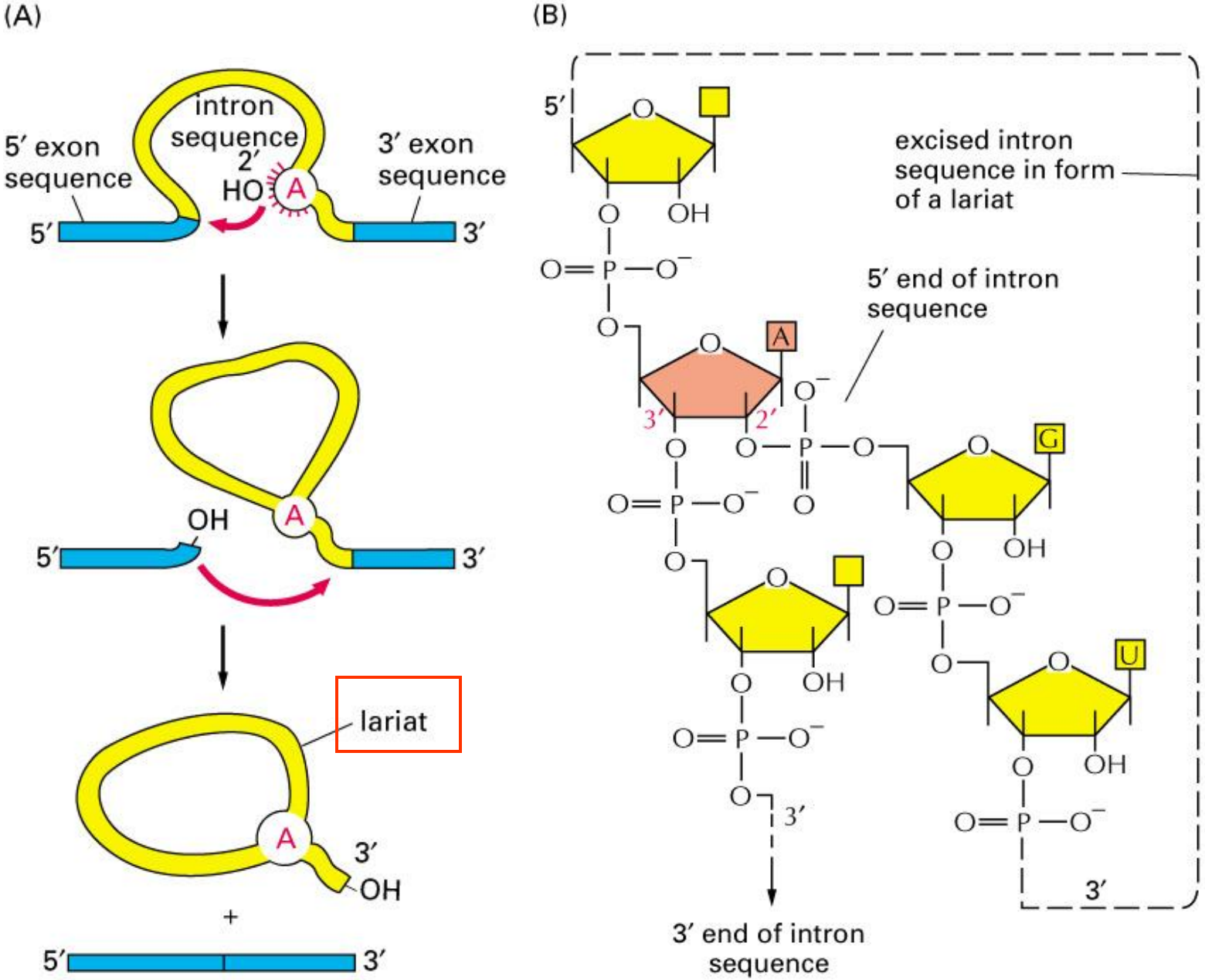


Figure 6–26 part 1 of 2. Molecular Biology c Figure 6–26 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

3 Important sequences for Splicing to occur
R: A or G; Y: C or U

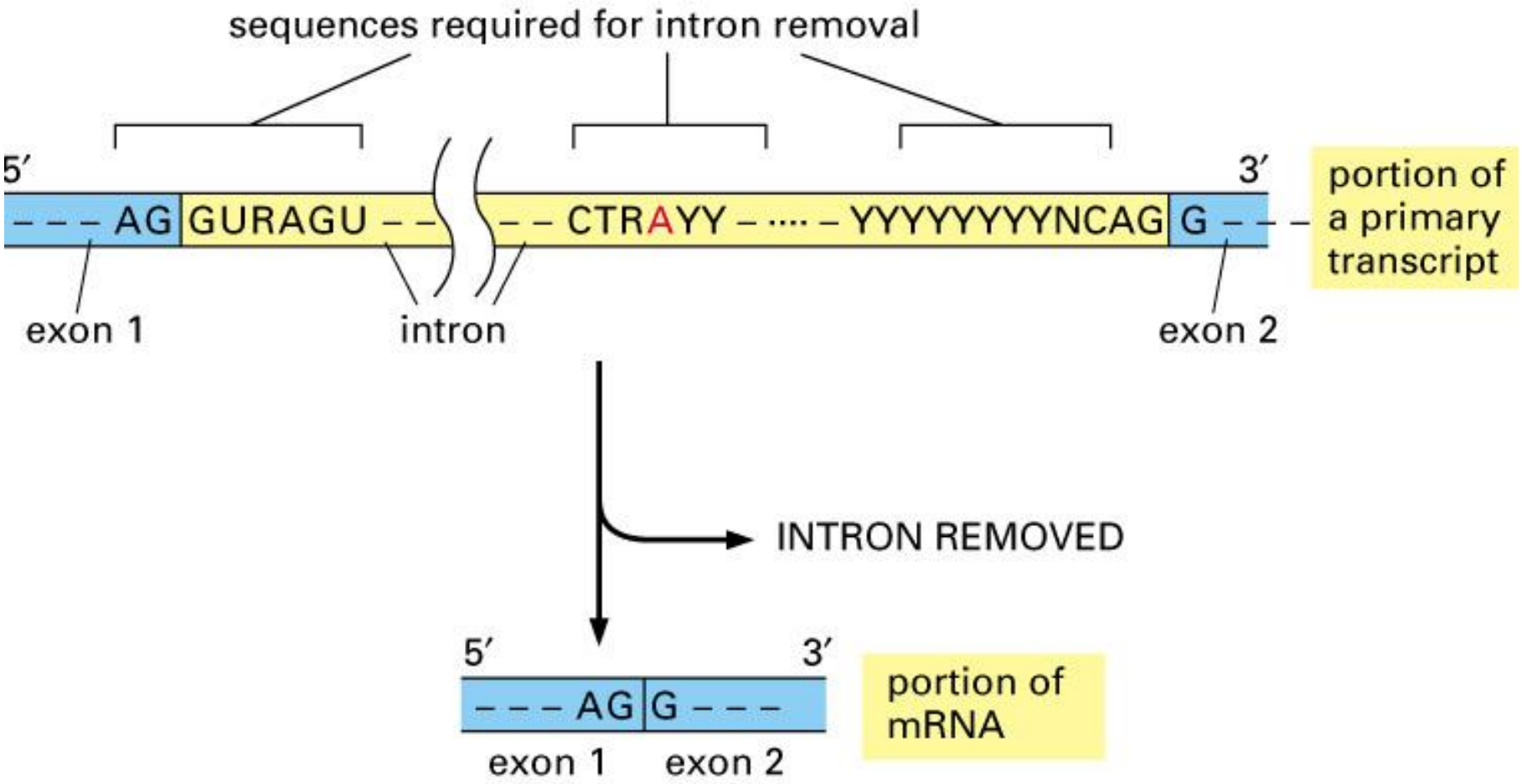


Figure 6–28. Molecular Biology of the Cell, 4th Edition.

RNA Splicing mechanism
BBP: branch-point binding protein
U2AF: a helper protein

snRNA: small nuclear RNA
snRNP: small nuclear ribonucleoprotein
Components for splicesome

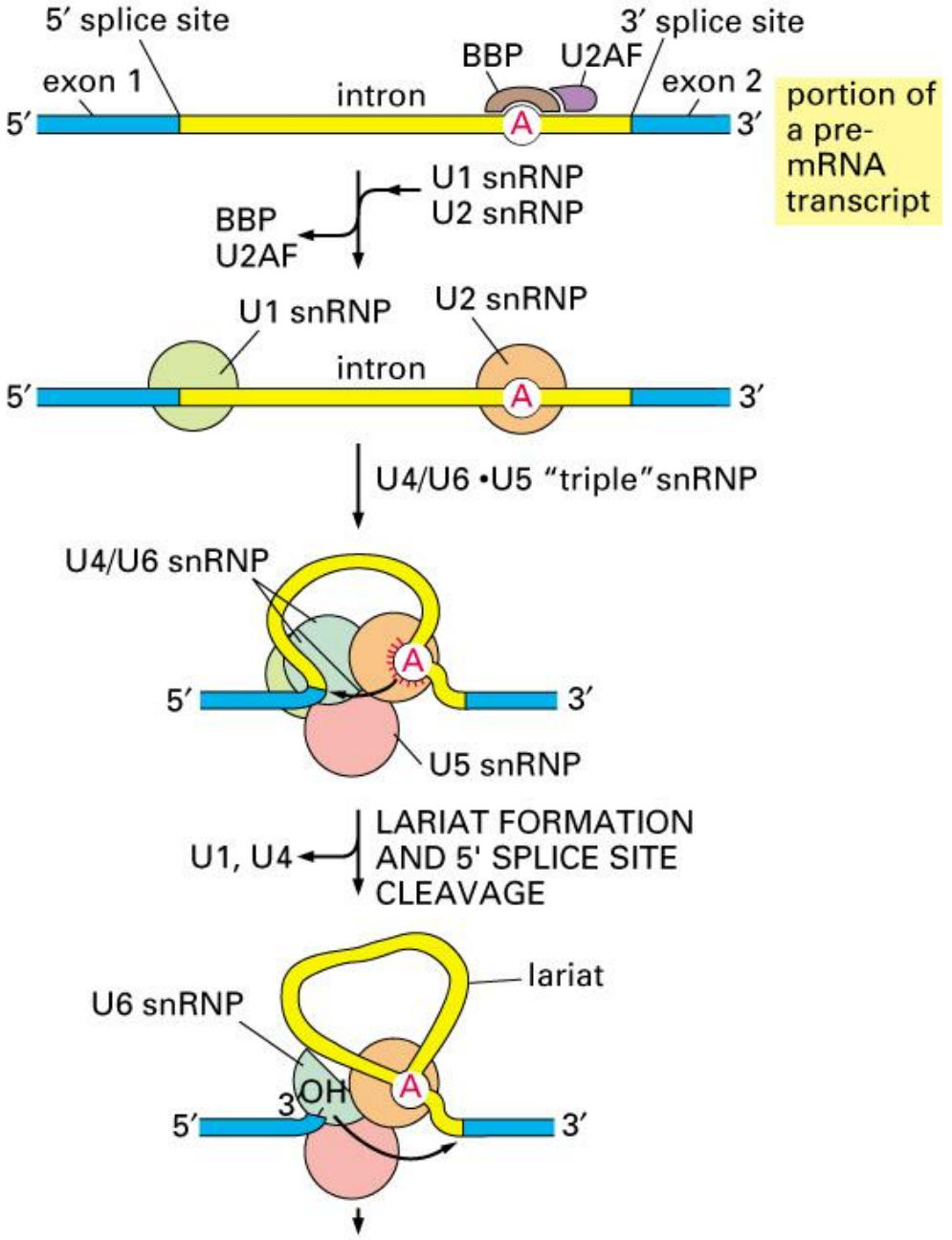
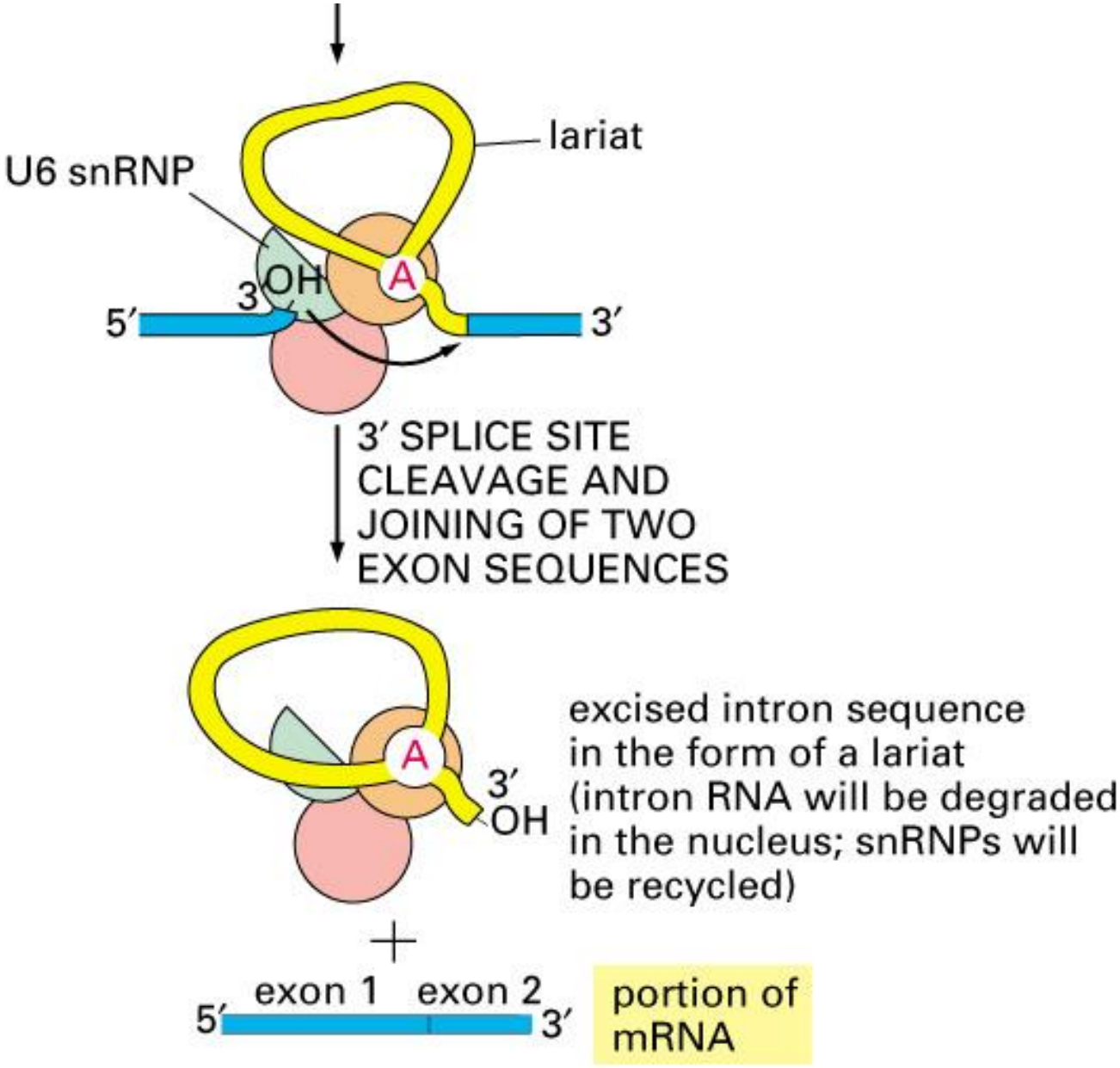


Figure 6–29 part 1 of 2. Molecular Biology of the Cell, 4th Edition.



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Further mechanism to mark Exon and Intron difference

CBC: capping binding complex

hnRNP: heterogeneous nuclear ribonucleoprotein, binding to introns

SR: rich in serine and arginines, binding to exons

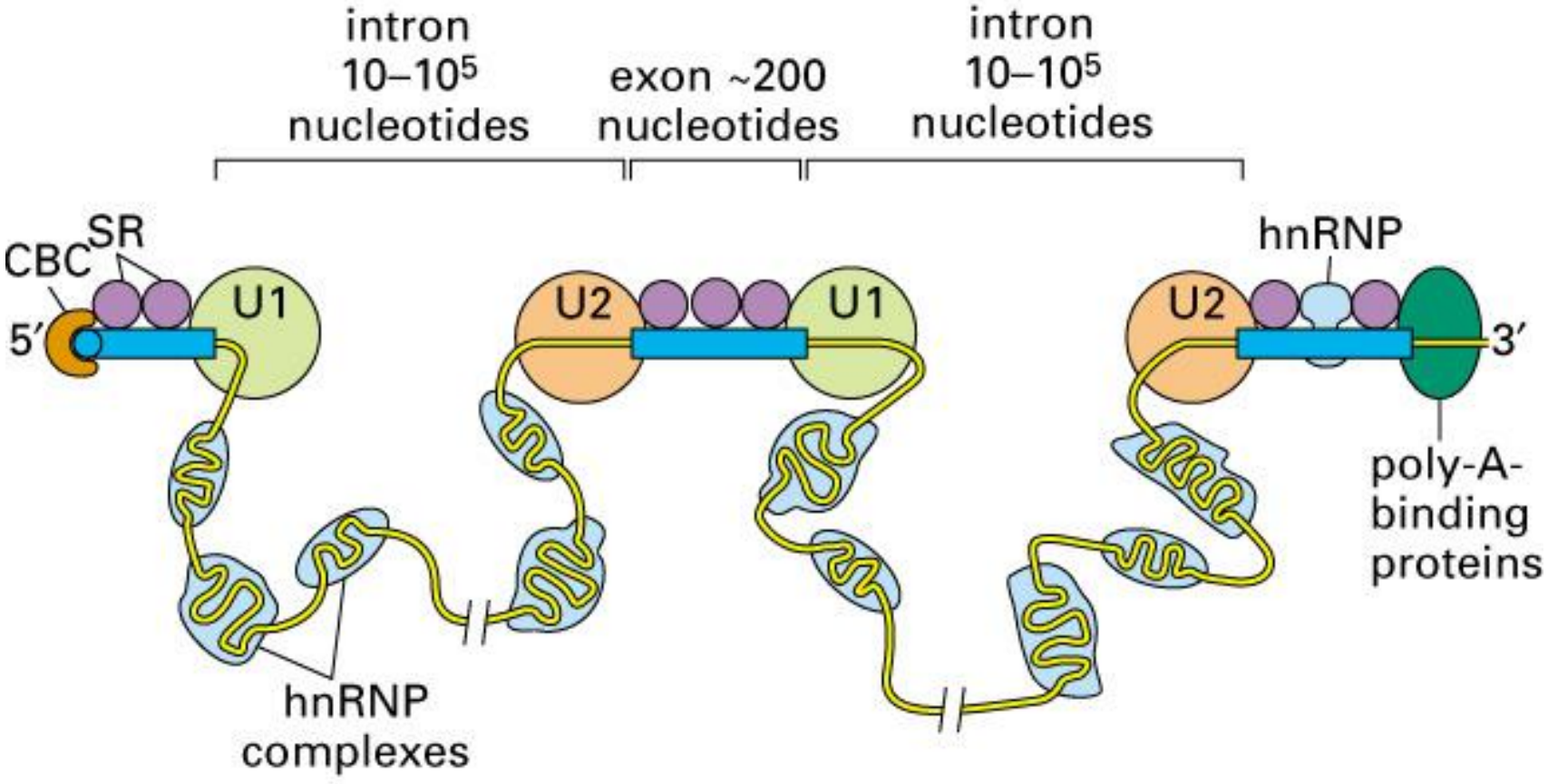


Figure 6–33. Molecular Biology of the Cell, 4th Edition.

Consensus sequence for 3' process

AAUAAA: CstF (cleavage stimulation factor F)

GU-rich sequence: CPSF (cleavage and polyadenylation specificity factor)

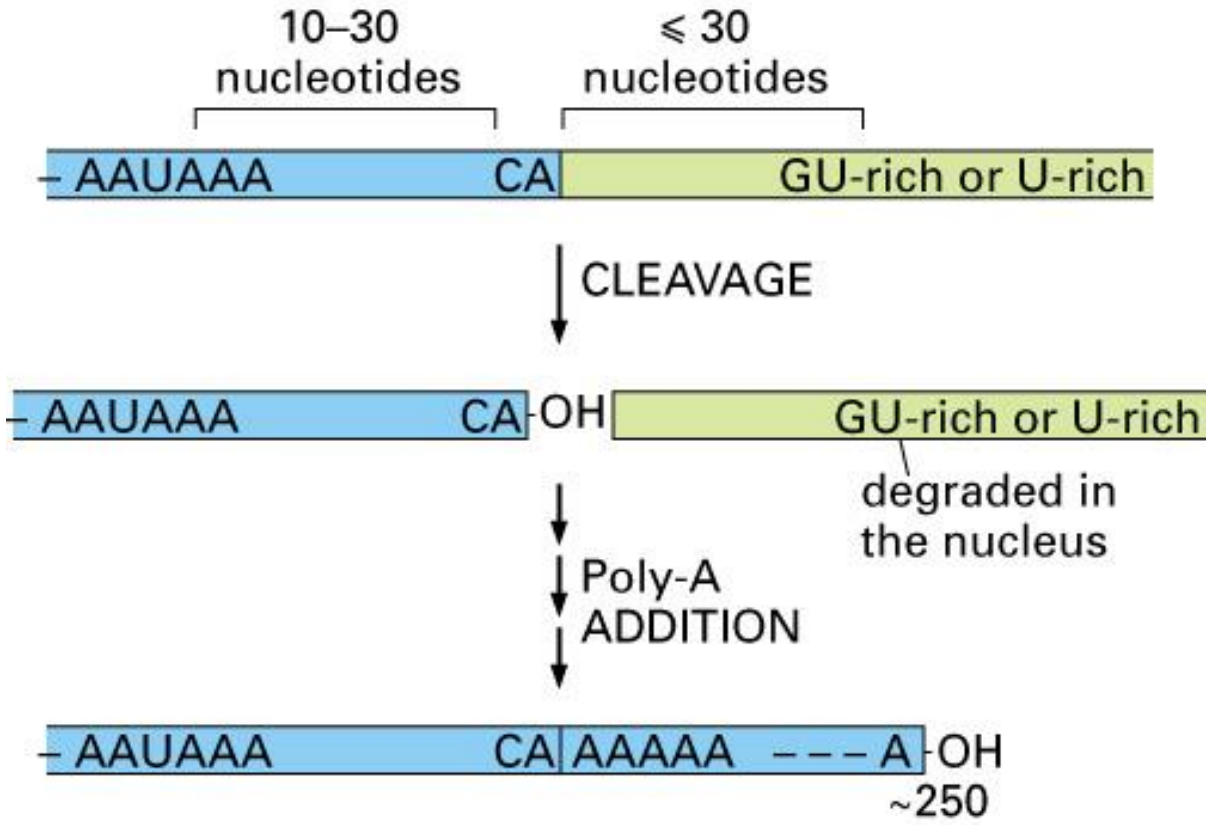


Figure 6–37. Molecular Biology of the Cell, 4th Edition.

Major steps for 3' end of eucaryotic mRNA

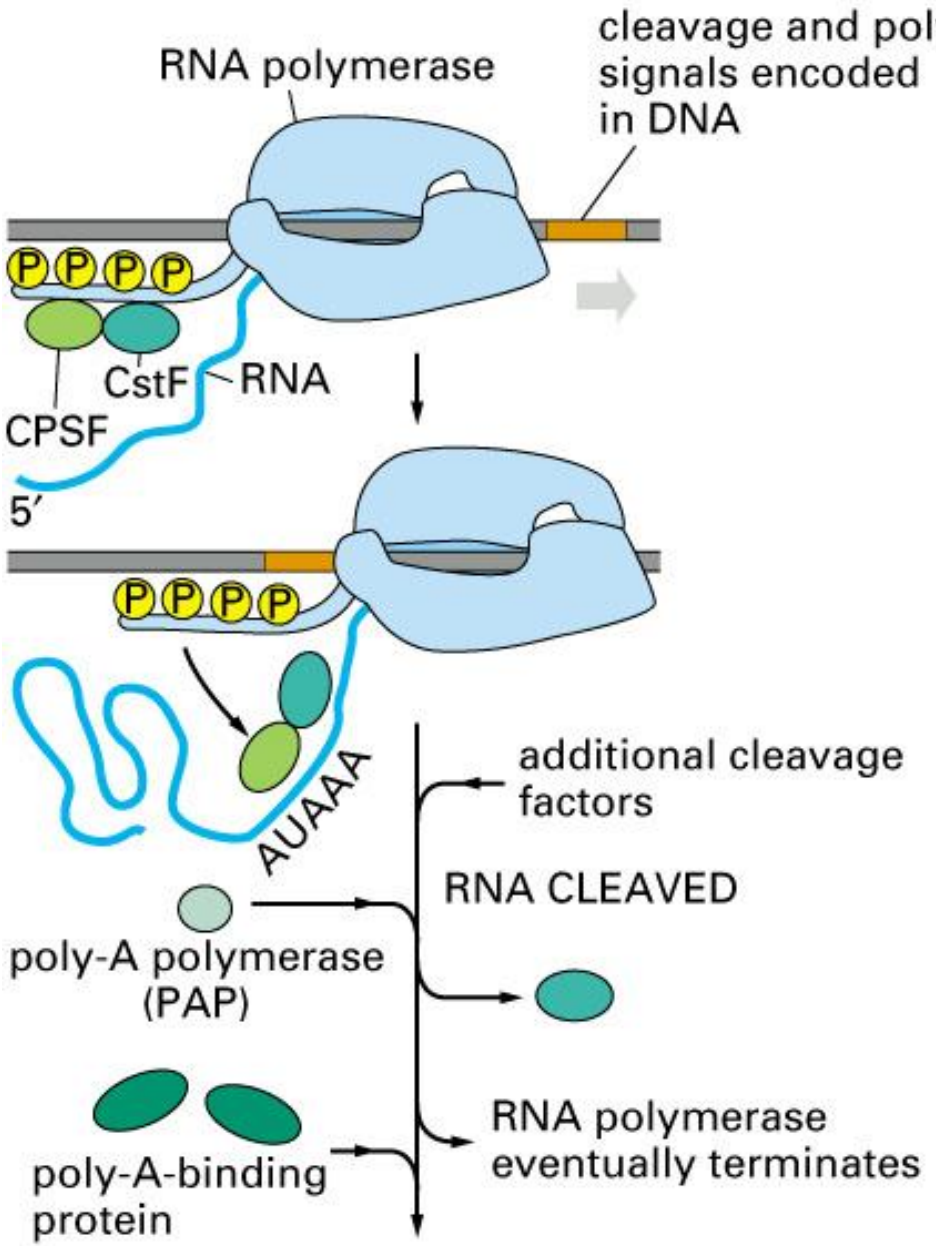


Figure 6–38 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

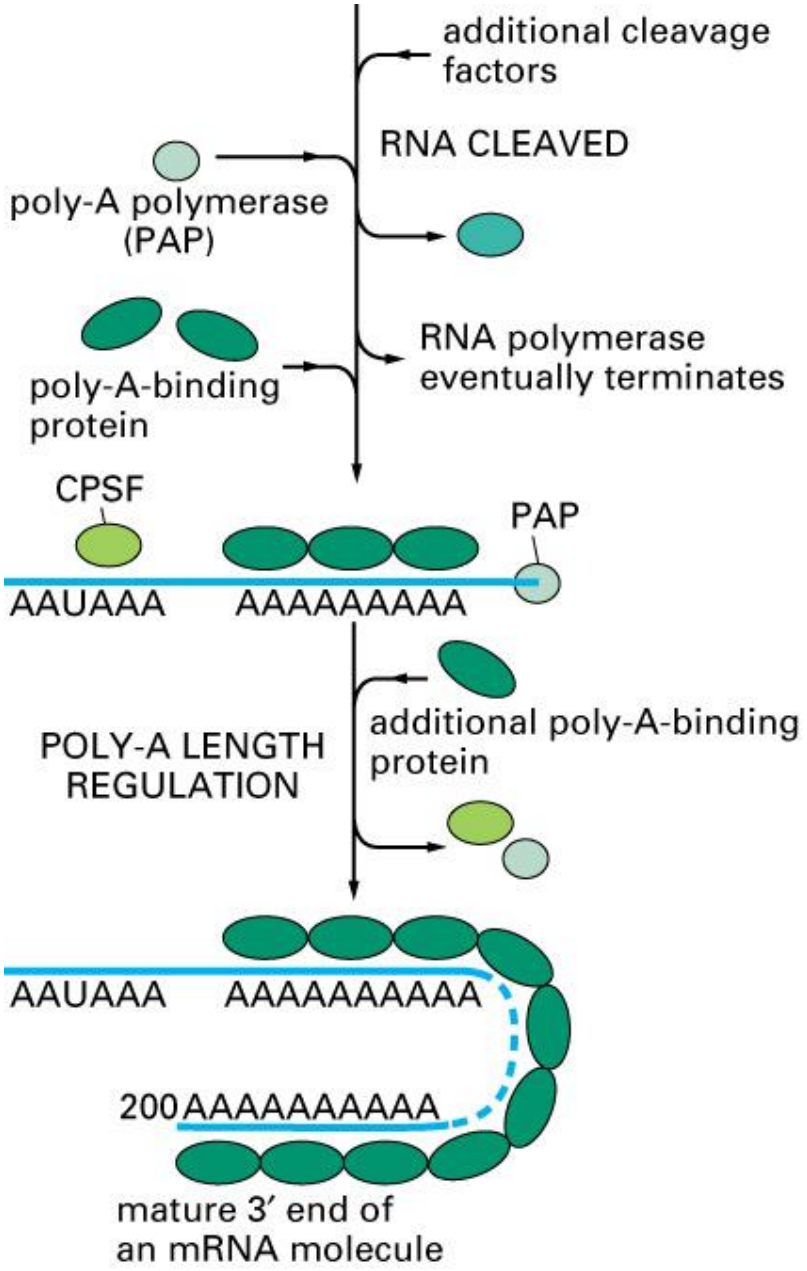


Figure 6–38 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Transportation through nuclear pore complex

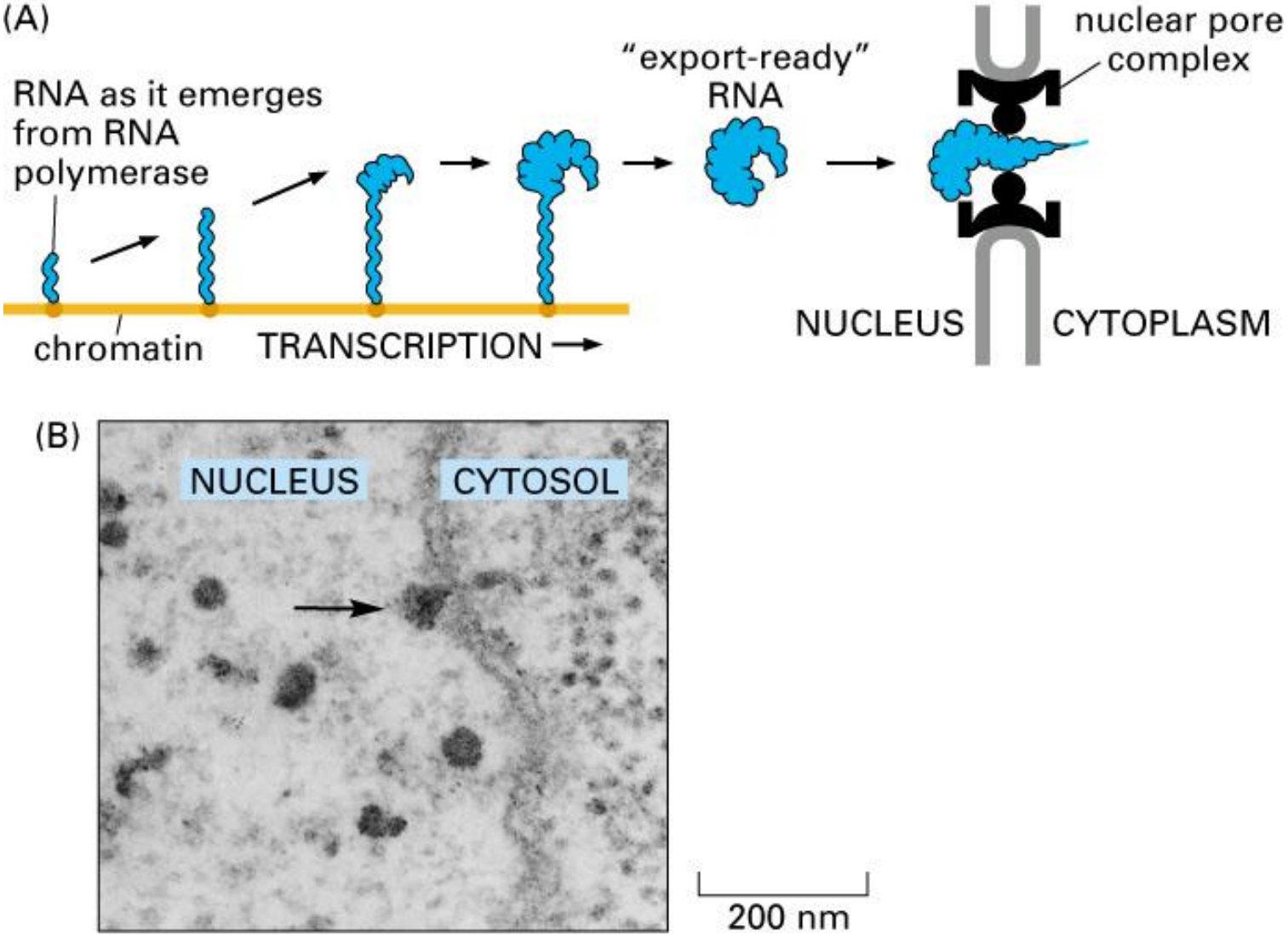


Figure 6–39. Molecular Biology of the Cell, 4th Edition.

Exporting mechanism
hnRNP binds to intron and help the recognition to destroy RNA introns

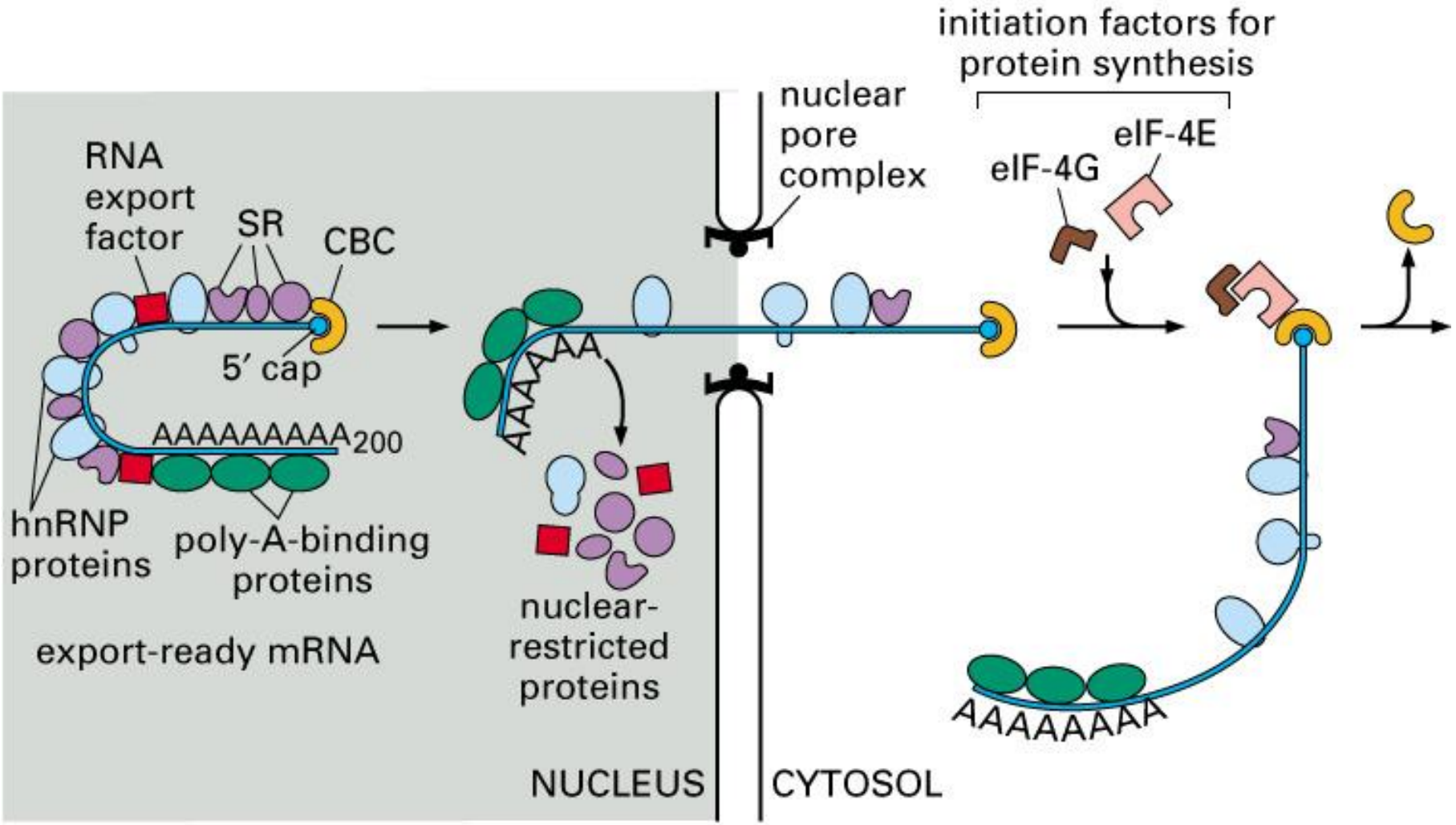
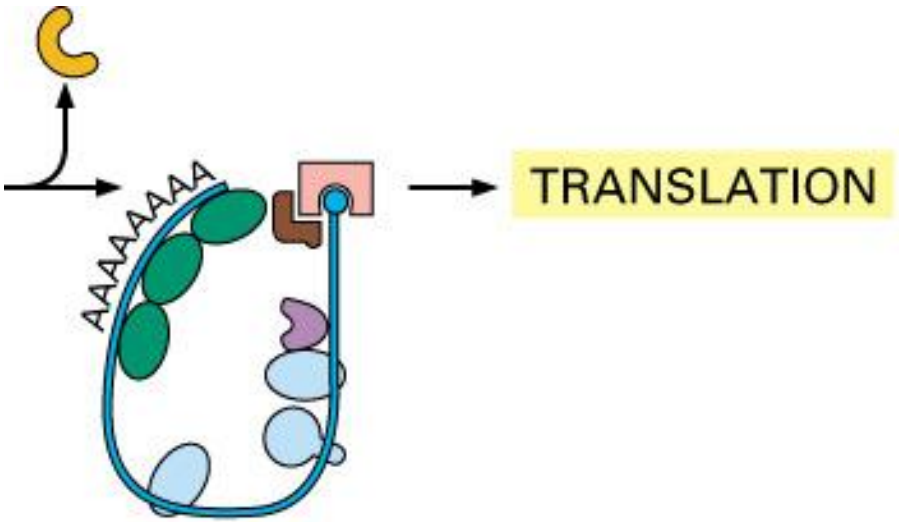


Figure 6–40 part 1 of 2. Molecular Biology of the Cell, 4th Edition.



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RNA modifications

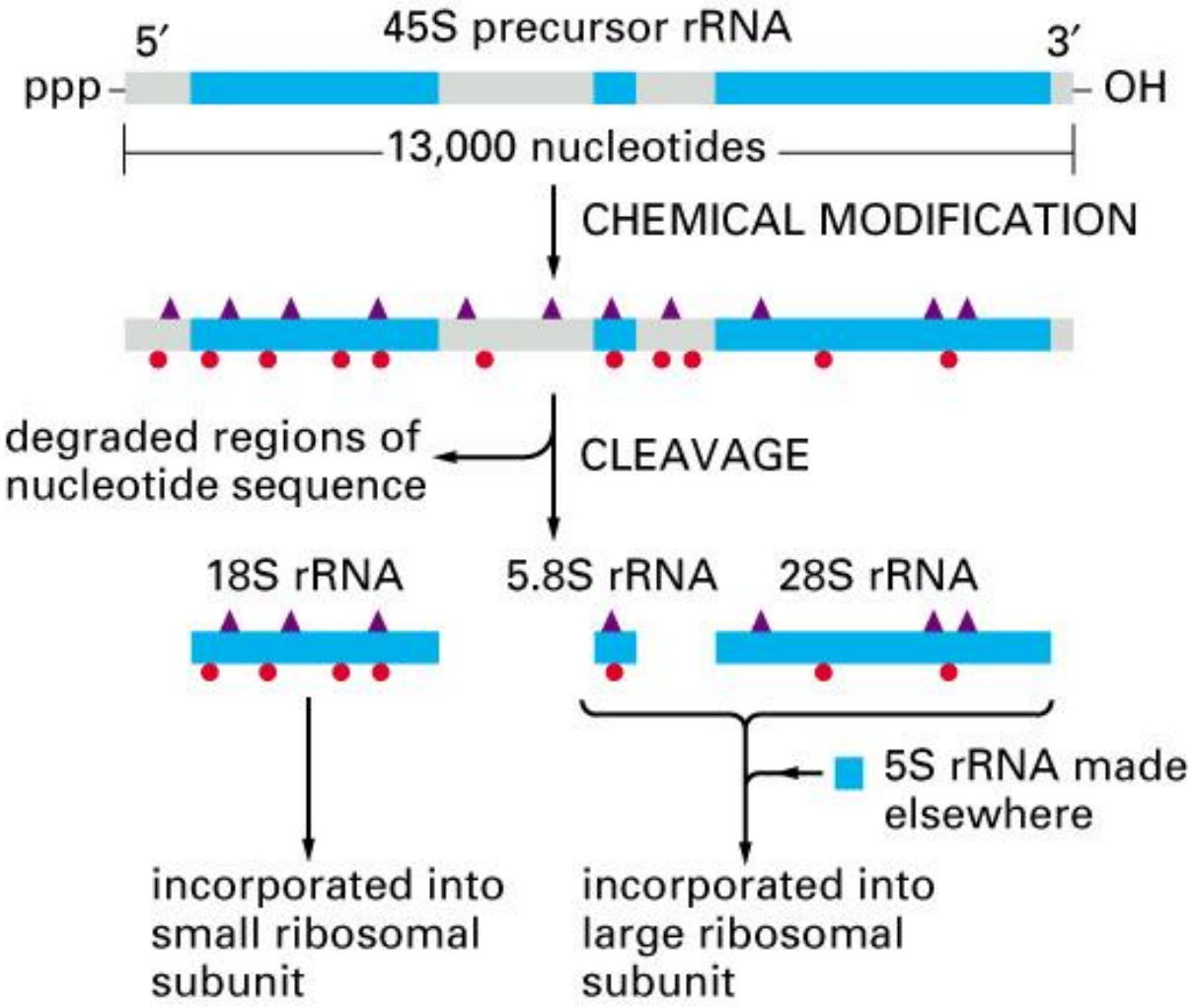


Figure 6–42. Molecular Biology of the Cell, 4th Edition.

Nucleolus
For rRNA processing

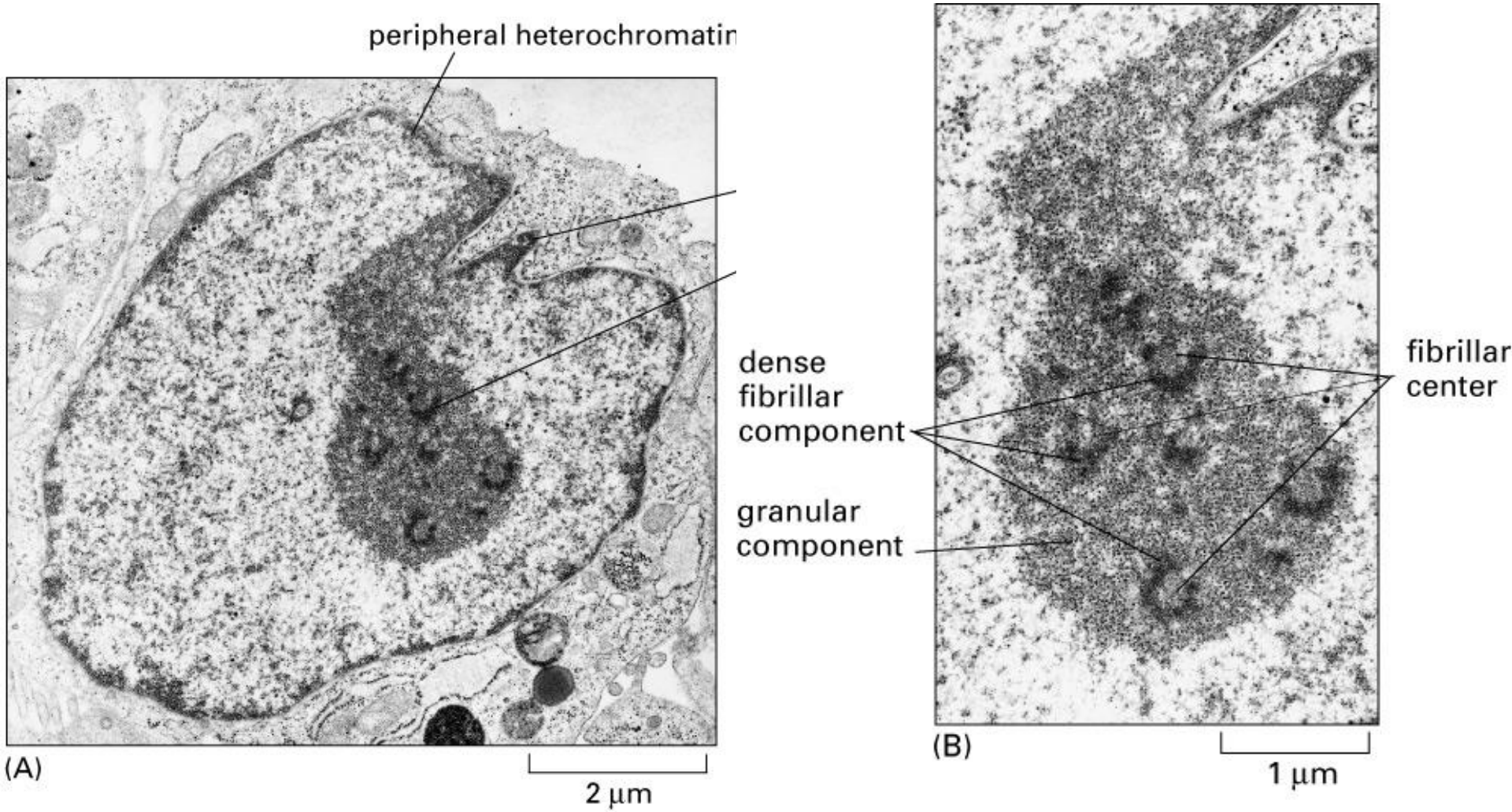


Figure 6–44 part 1 of 2. Molecular Biology of the Cell, 4th Edit

Figure 6–44 part 2 of 2. Molecular Biology of the Cell, 4th Ed

Nucleolus and other subcompartments
Cajal bodies, GEMS (Gemini of coiled bodies), interchromatin granule clusters

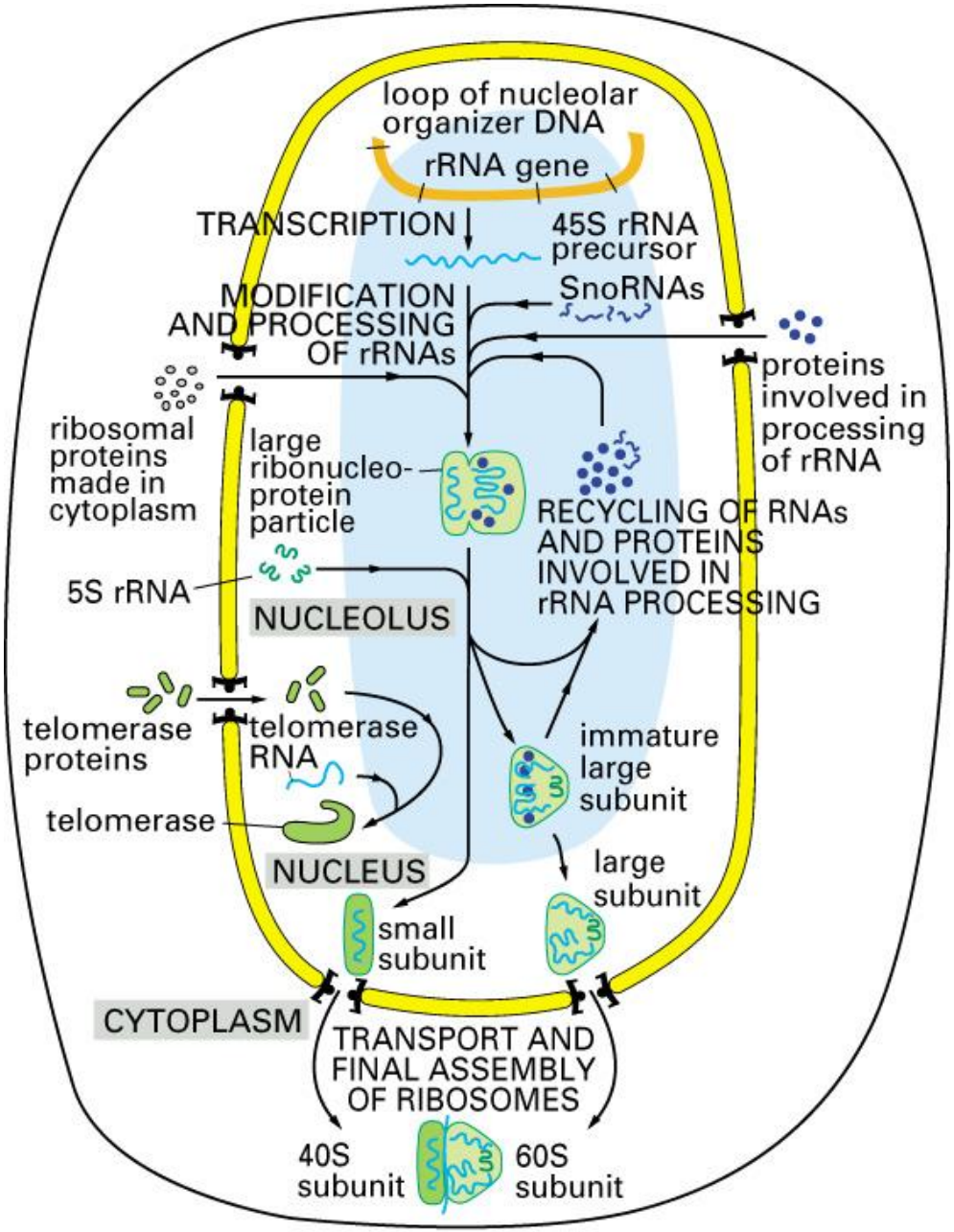


Figure 6–47. Molecular Biology of the Cell, 4th Edition.

Summary

- Transcription: RNA Polymerase, Promoter, enhancer, transcription factor
- 5’ capping, splicing, 3’ cleavage and polyadenylation
- rRNA needs chemical modifications before maturation
- Nucleolus with sub-compartments

From RNA to Protein

Protein synthesis

Protein Folding and regulation

The Genetic Code

GCA GCC GCG GCU	AGA AGG CGA CGC CGG CGU	GAC GAU	AAC AAU	UGC UGU	GAA GAG	CAA CAG	GGA GGC GGG GGU	CAC CAU	AUA AUC AUU	
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	
A	R	D	N	C	E	Q	G	H	I	
UUA UUG CUA CUC CUG CUU	AAA AAG	AUG	UUC UUU	CCA CCC CCG CCU	AGC AGU UCA UCC UCG UCU	ACA ACC ACG ACU	UGG	UAC UAU	GUA GUC GUG GUU	UAA UAG UGA
Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
L	K	M	F	P	S	T	W	Y	V	

Figure 6–50. Molecular Biology of the Cell, 4th Edition.

The Reading Frames

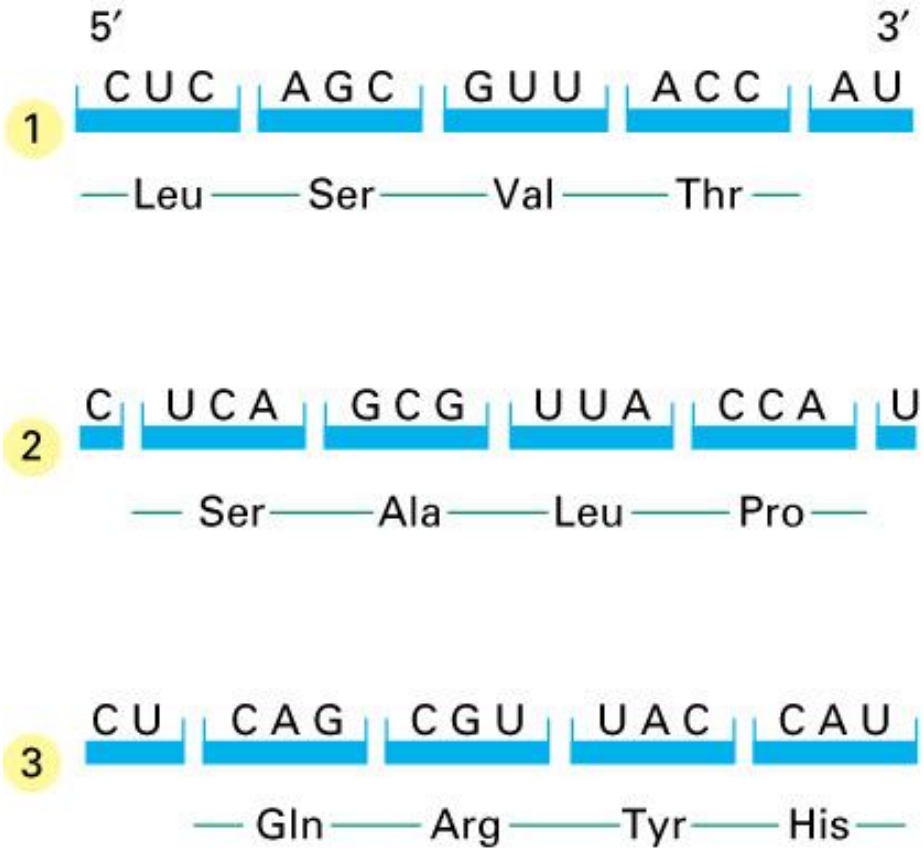
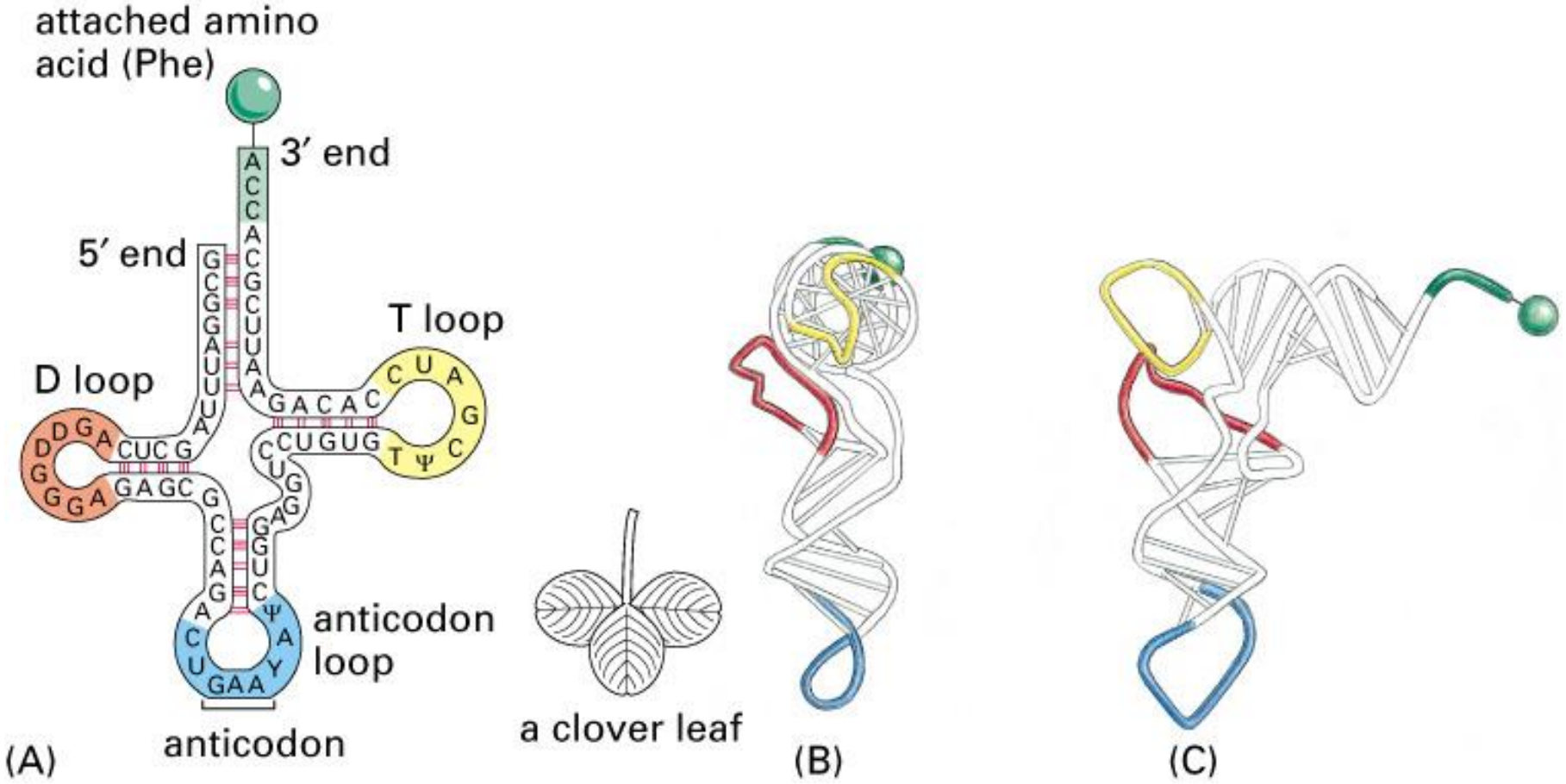


Figure 6–51. Molecular Biology of the Cell, 4th Edition.

tRNA (clover leaf shape with four strands folded, finally L-shape)



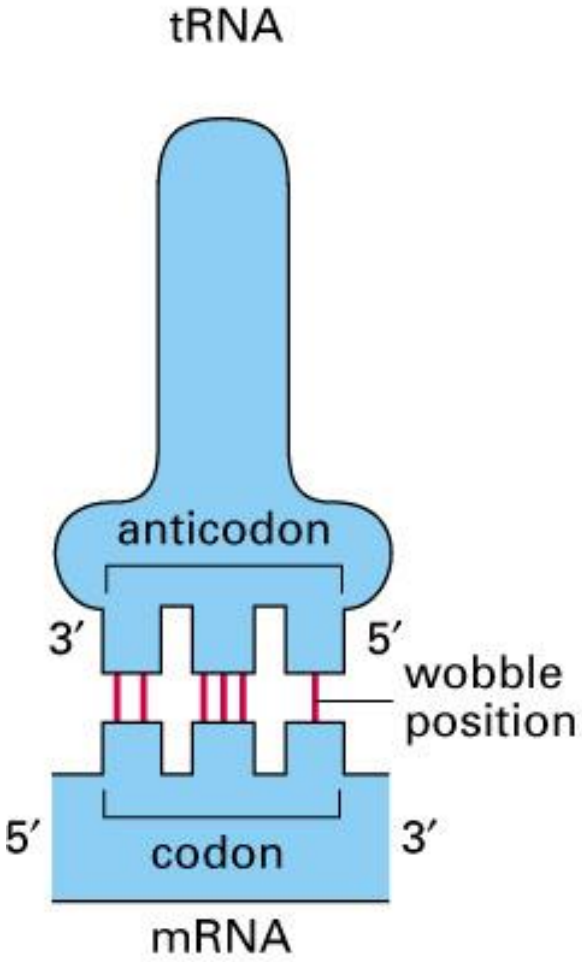
5′ GCGGAUUUAGCUCAGDDGGGAGAGCGCCAGACUGAAYAΨCUGGAGGUCCUGUGTΨCGAUCCACAGAAUUCGCACCA 3′

(D)

anticodon

Figure 6–52. Molecular Biology of the Cell, 4th Edition.

tRNA and mRNA pairing



bacteria

wobble codon base	possible anticodon bases
U	A, G, or I
C	G or I
A	U or I
G	C or U

eucaryotes

wobble codon base	possible anticodon bases
U	G or I
C	G or I
A	U
G	C

Figure 6–53. Molecular Biology of the Cell, 4th Edition.

Amino Acid attachment to tRNA
Aminoacyl-tRNA synthetases

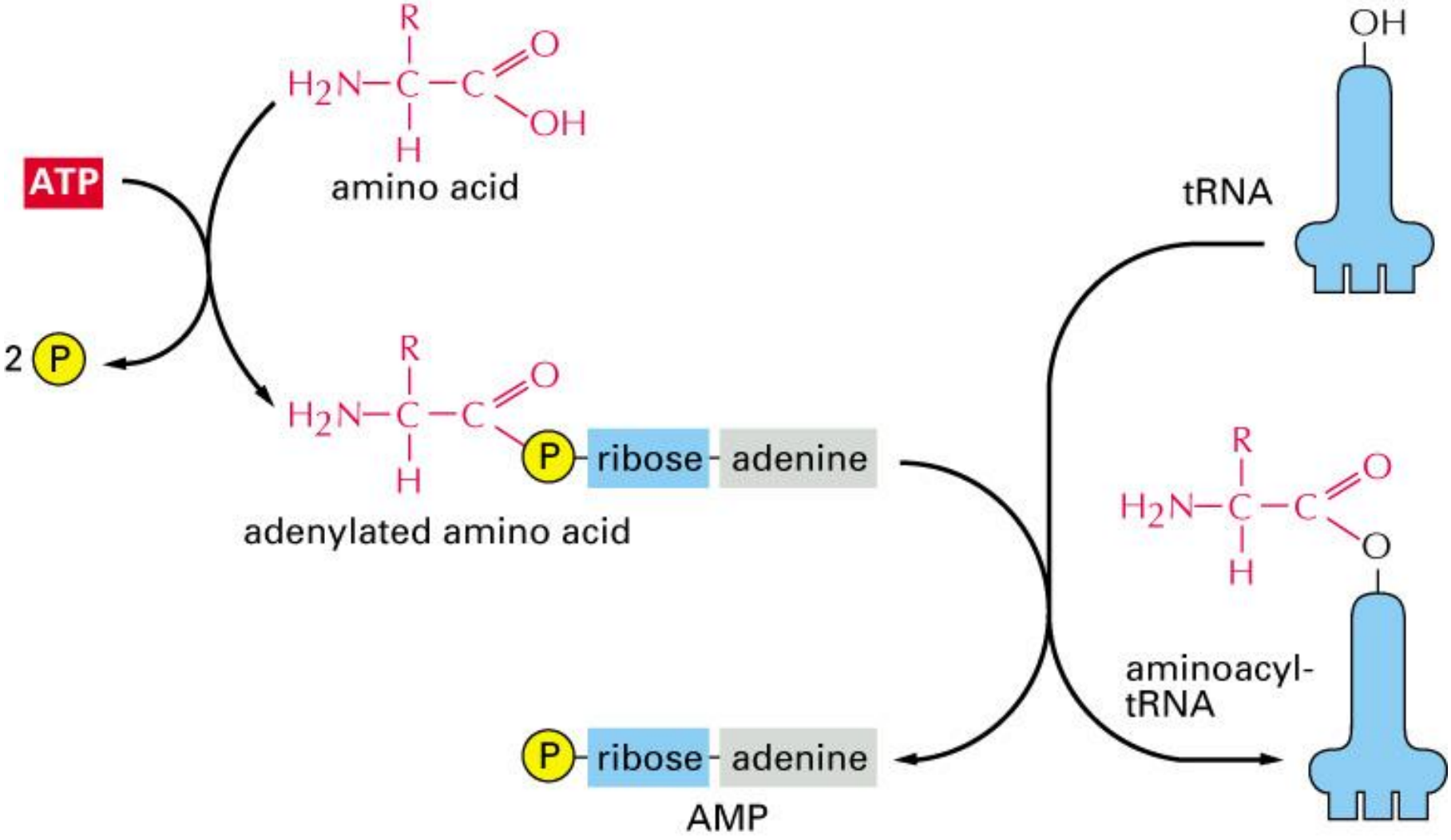


Figure 6–56. Molecular Biology of the Cell, 4th Edition.

Structure View (ester bond between amino acid and 3' of tRNA)

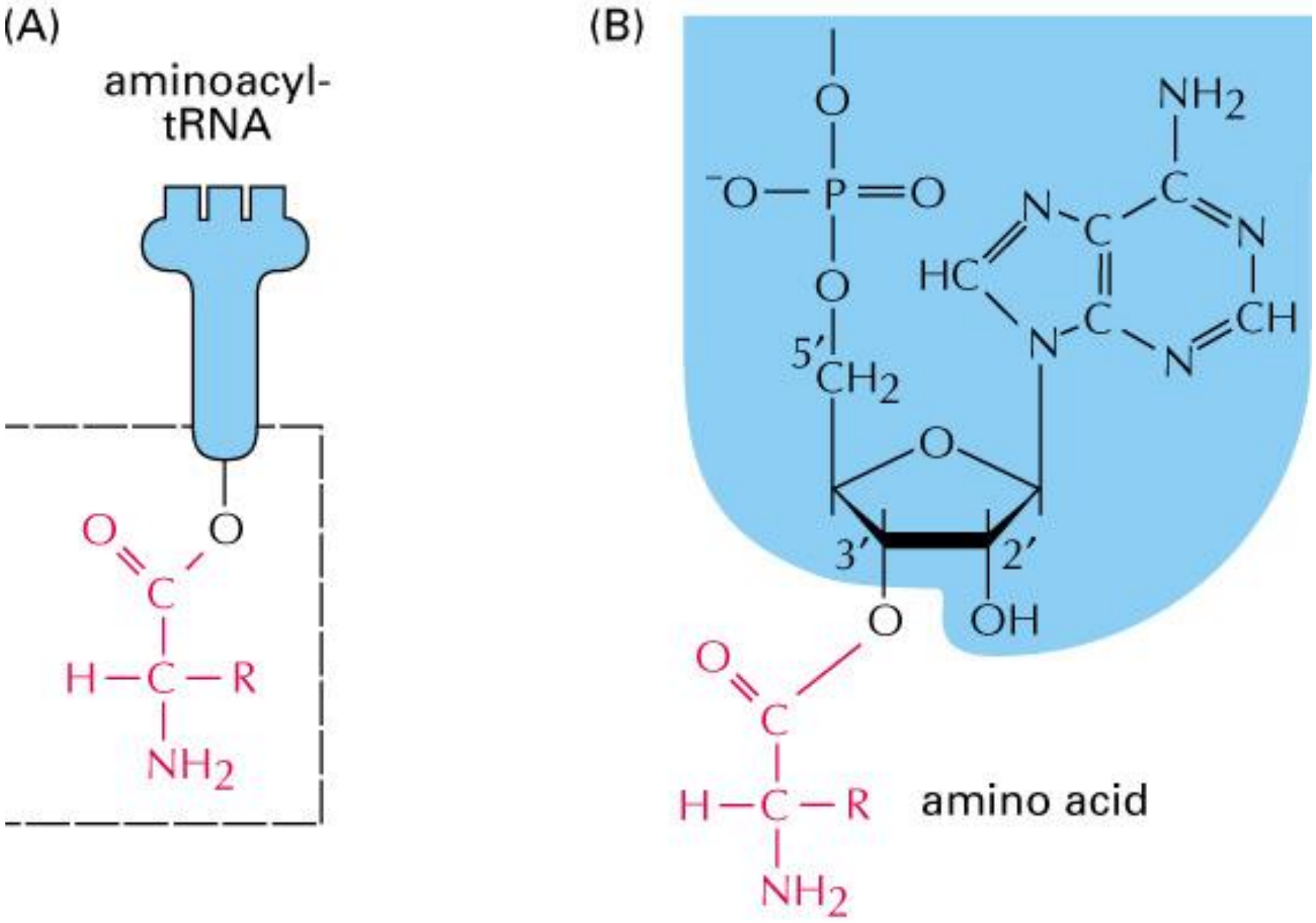


Figure 6–57. Molecular Biology of the Cell, 4th Edition.

Two Steps

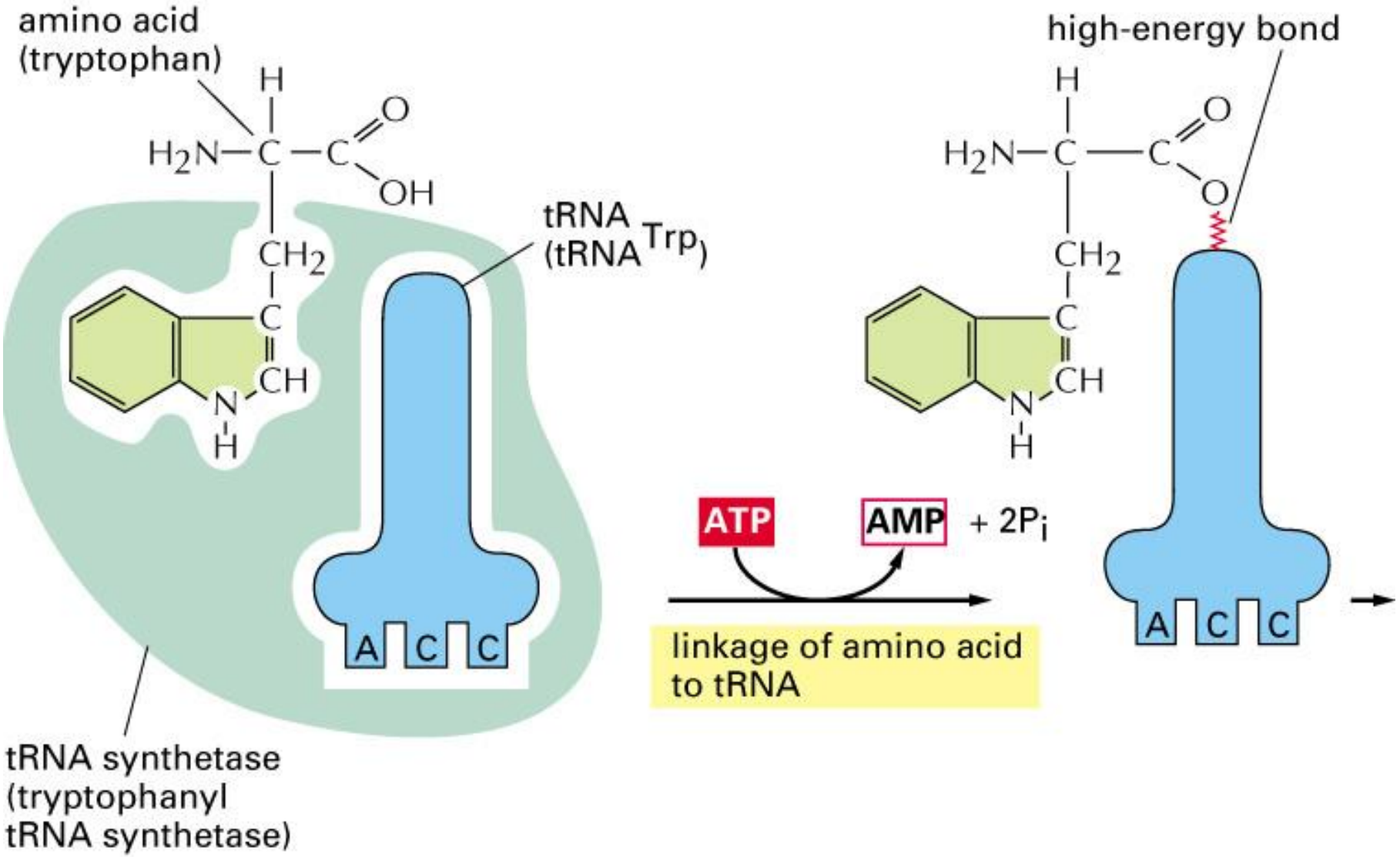


Figure 6–58 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

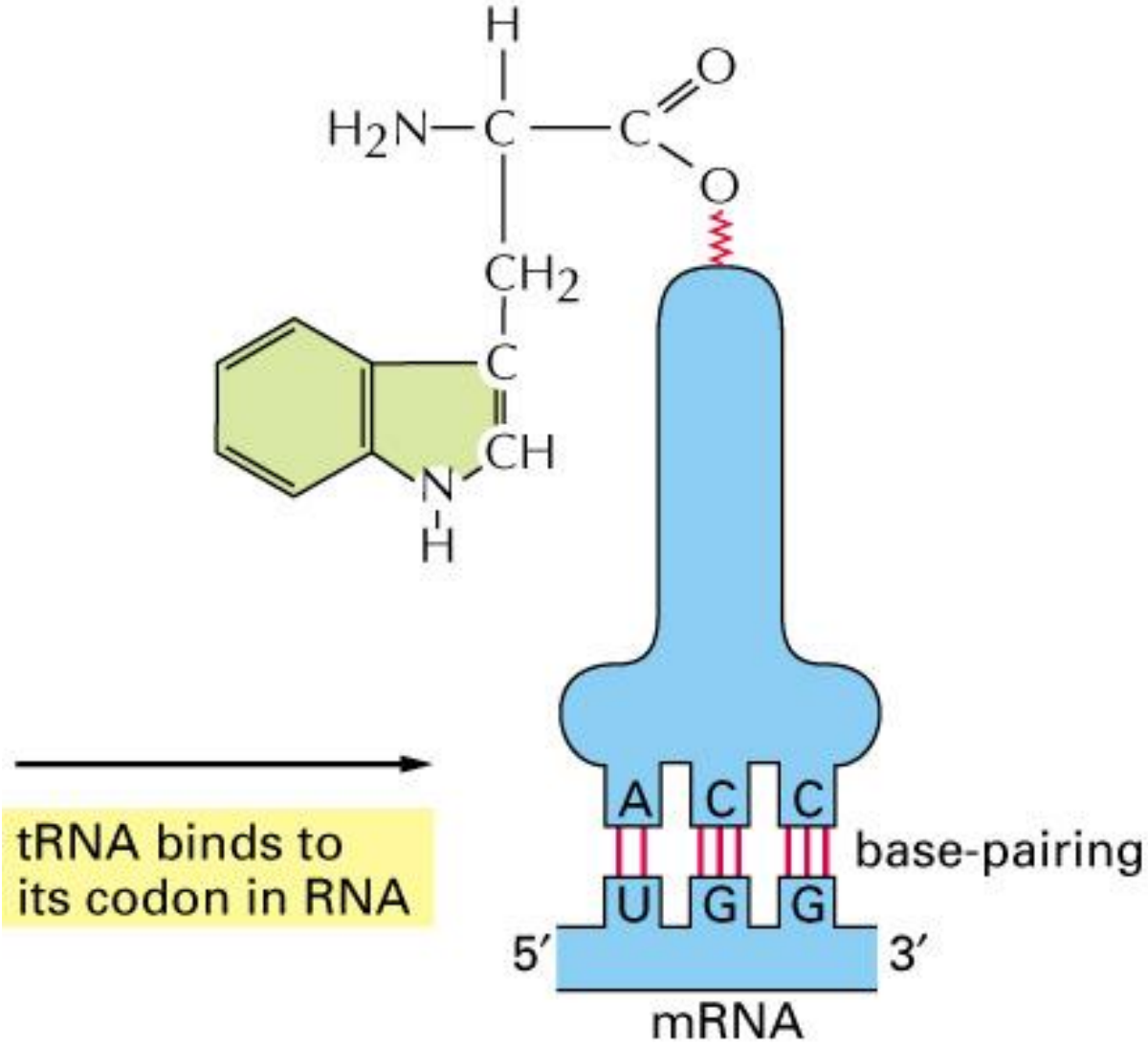
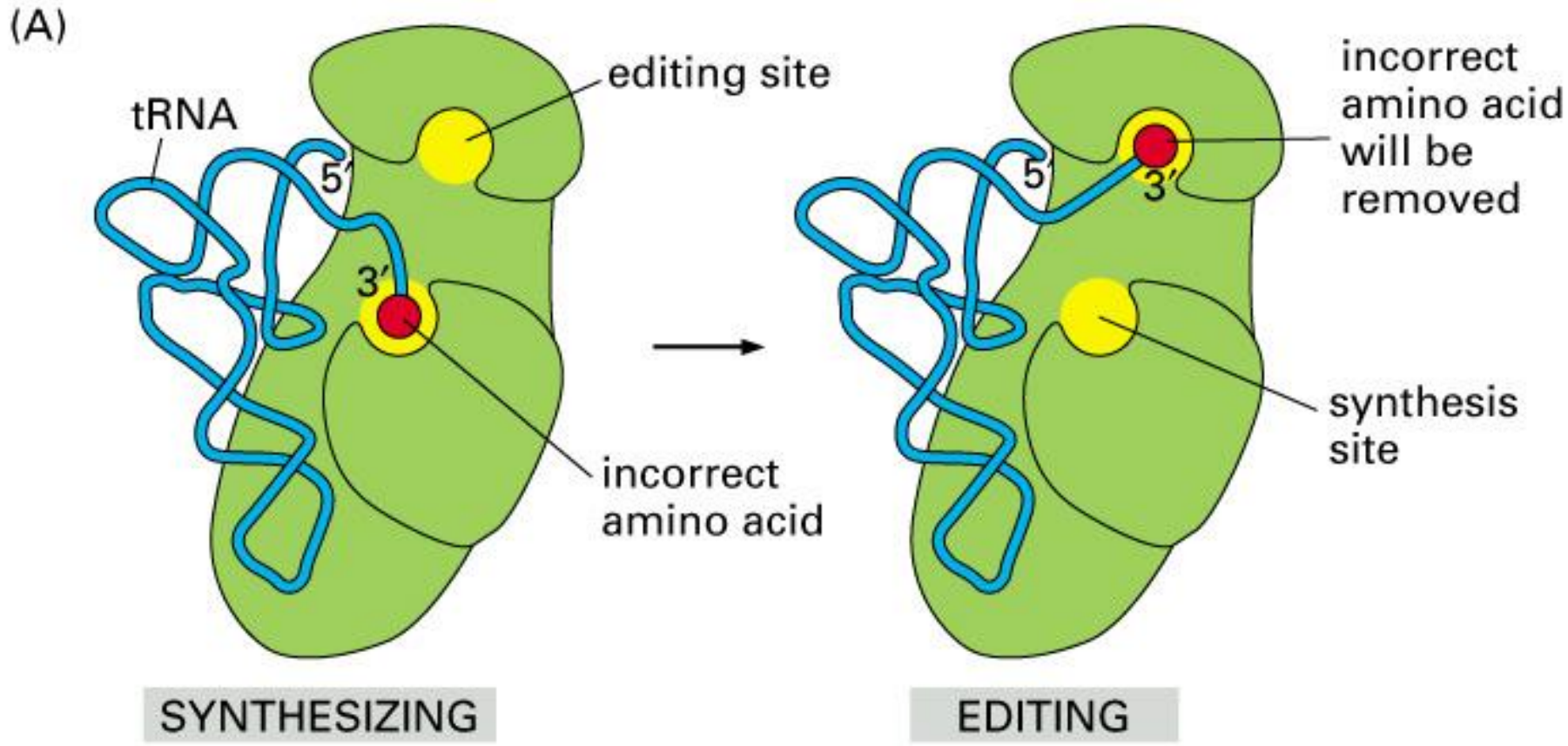


Figure 6–58 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Hydrolytic Editing
tRNA synthetases



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Hydrolytic Editing
DNA polymerase

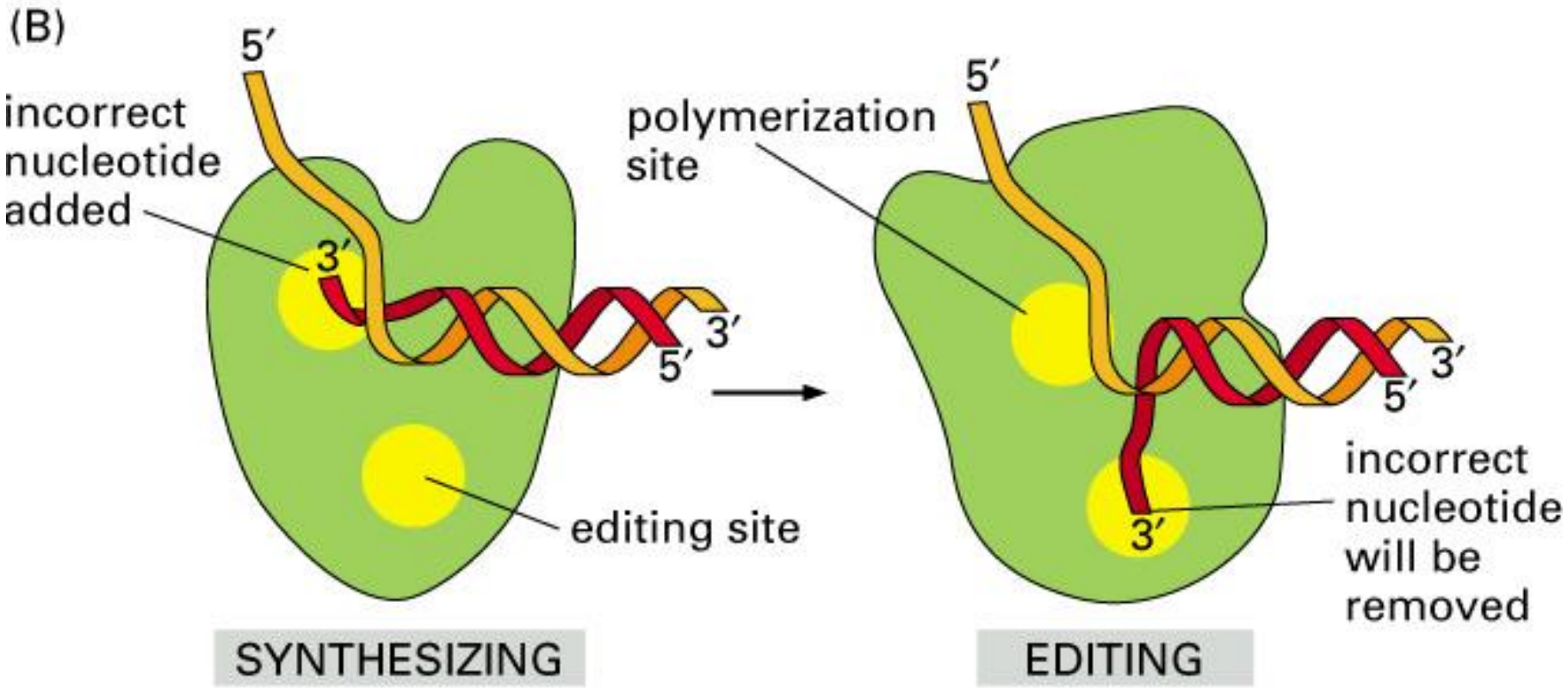


Figure 6–59 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Protein synthesis

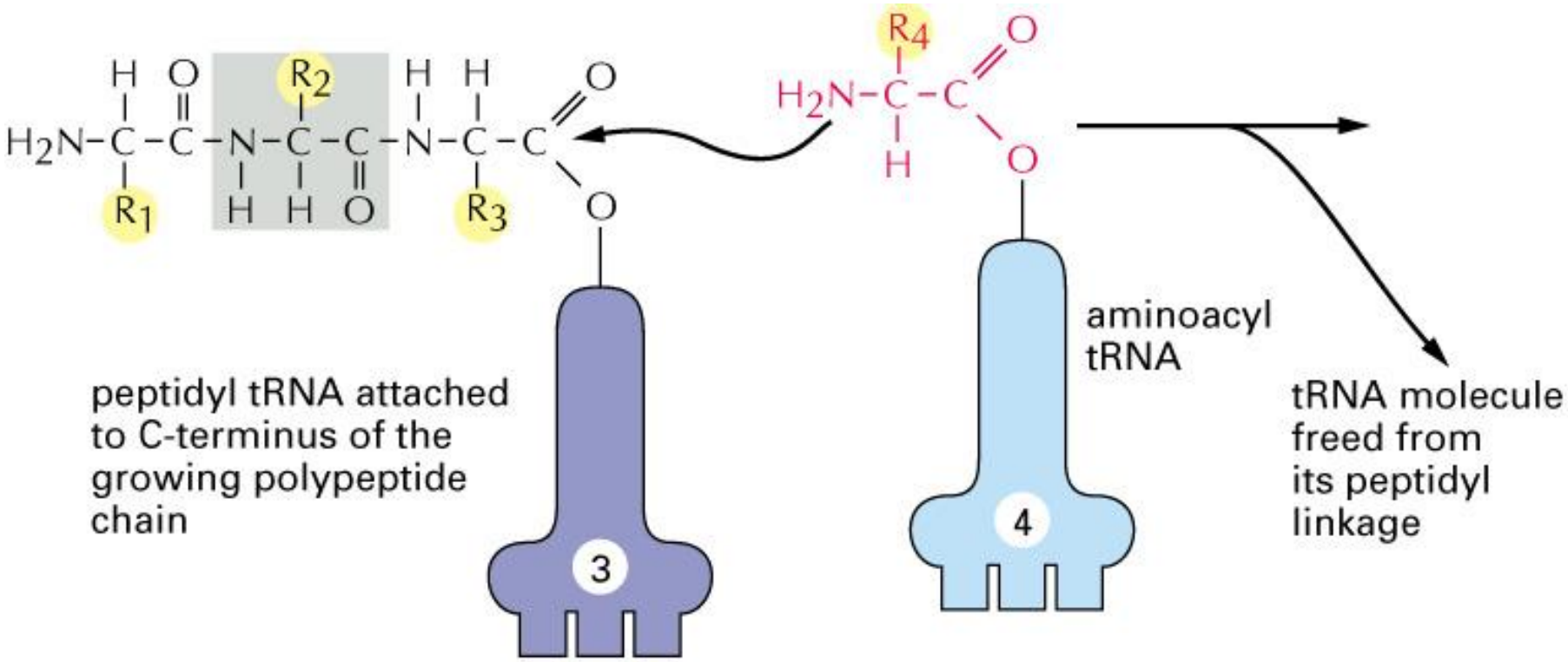


Figure 6–61 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

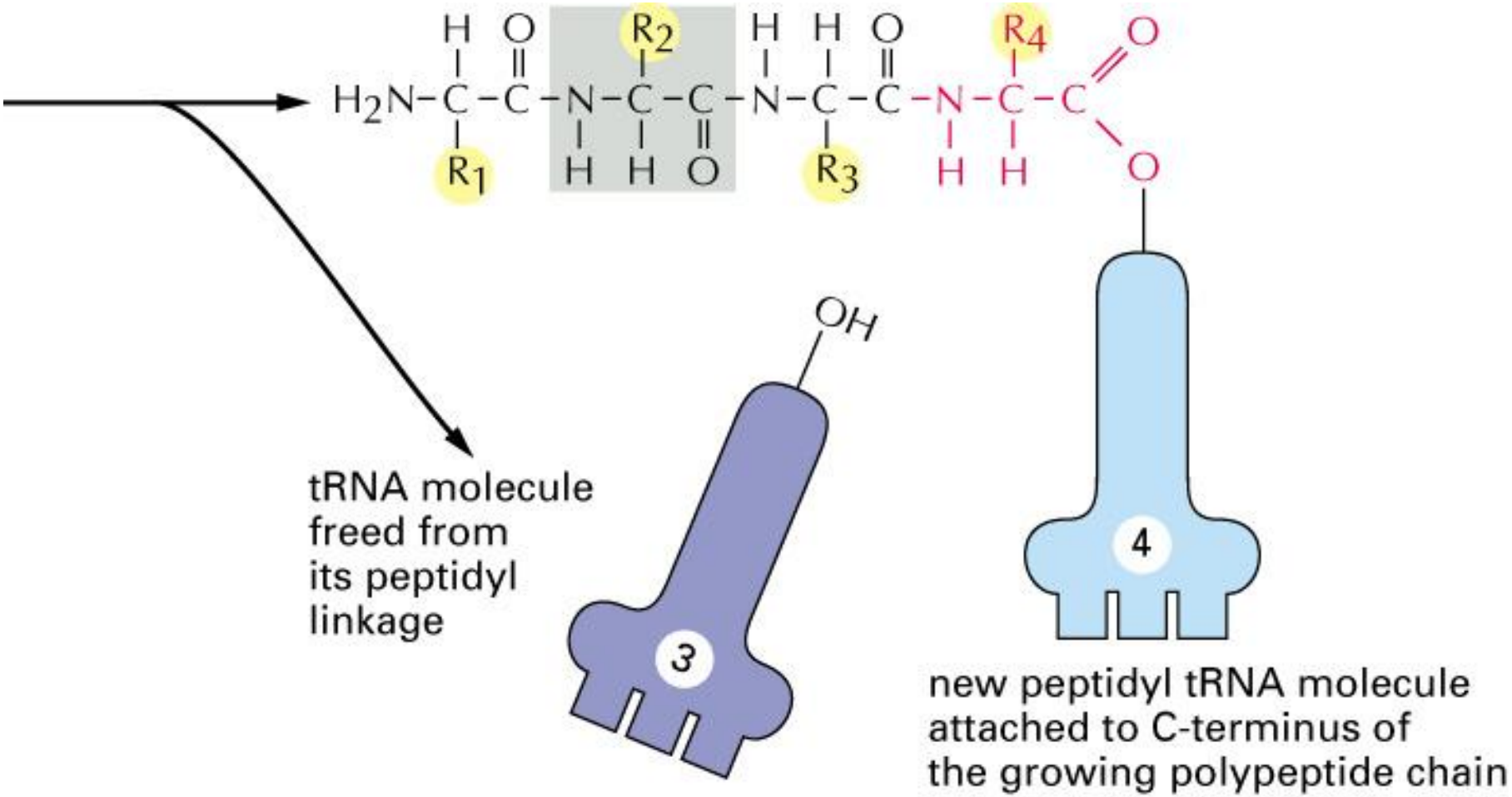


Figure 6–61 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Ribosome

Some on endoplasmic reticulum, Some are free

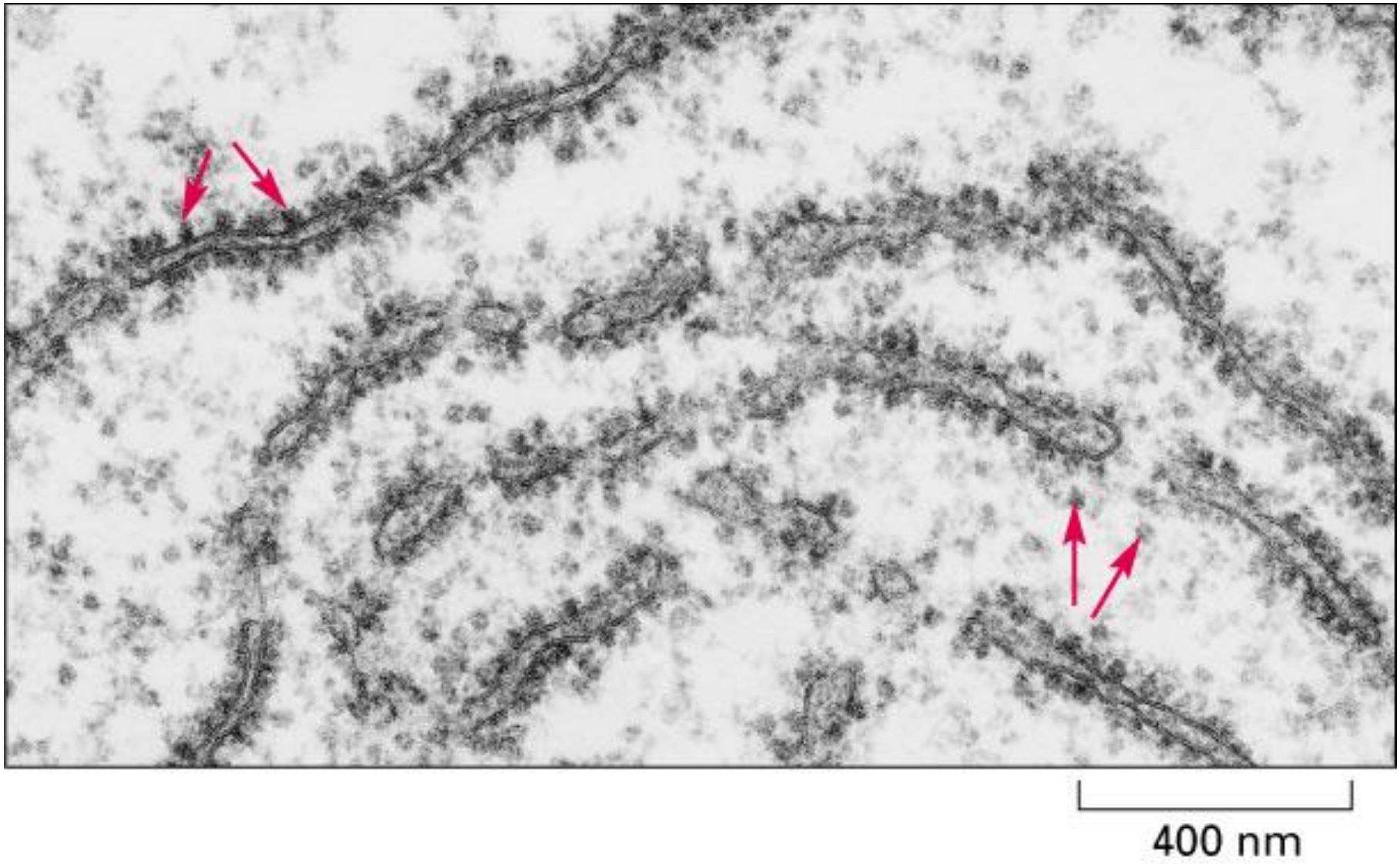


Figure 6–62. Molecular Biology of the Cell, 4th Edition.

Ribosome binding sites
2 subunits: large and small
4 binding sites: 1 for mRNA at small subunit, 3 for tRNA in large subunit

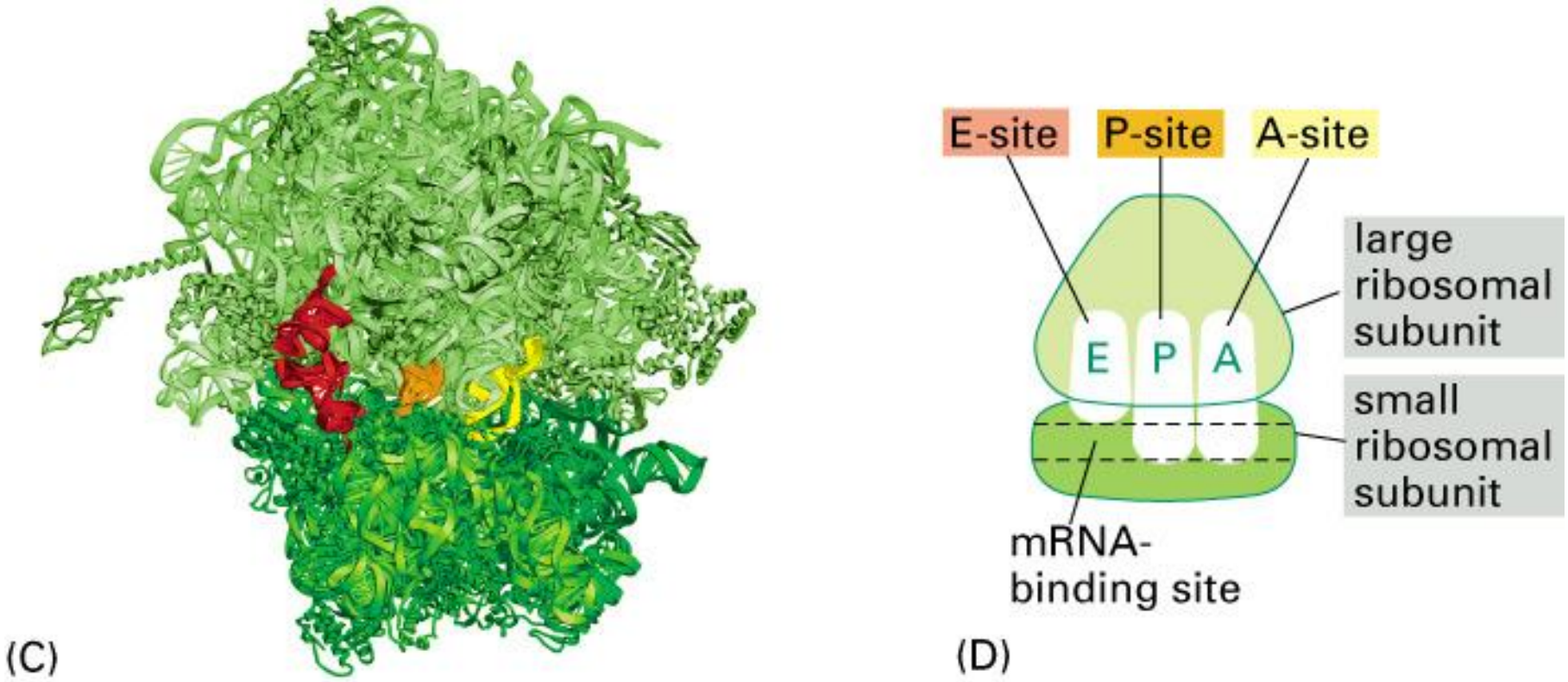


Figure 6–64 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

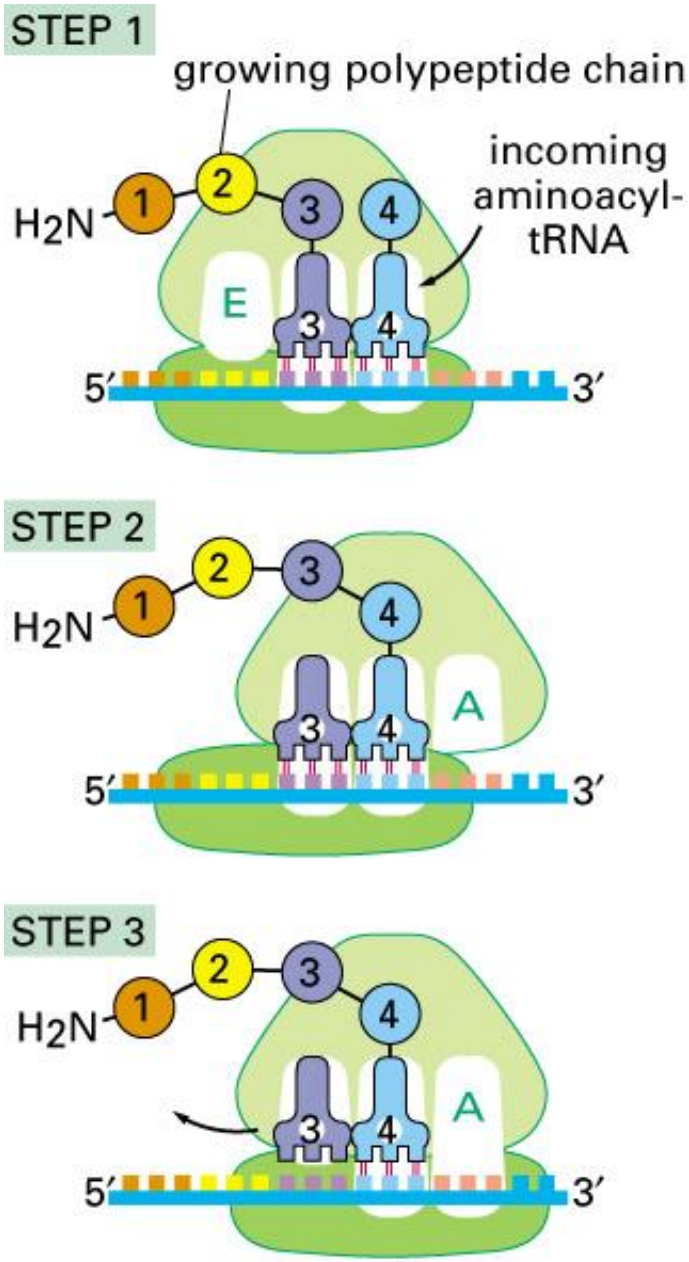


Figure 6–65 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Translation:

1. Position at A
2. Peptidyl transferase to transfer peptide to tRNA at A site
3. Conformational change of large unit and mRNA on small unit.

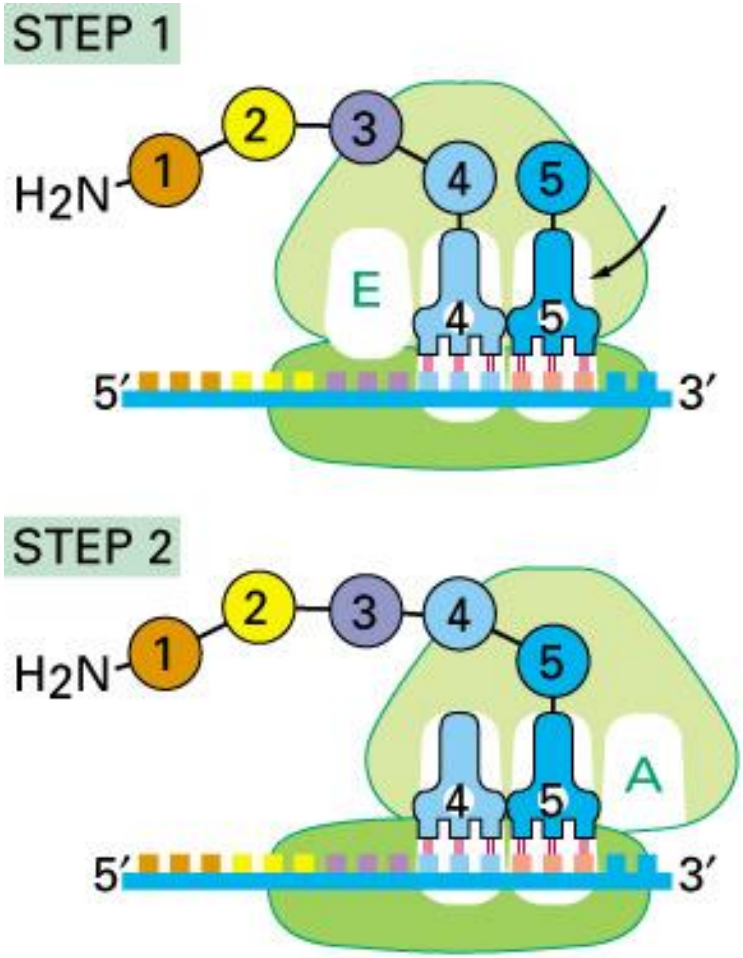
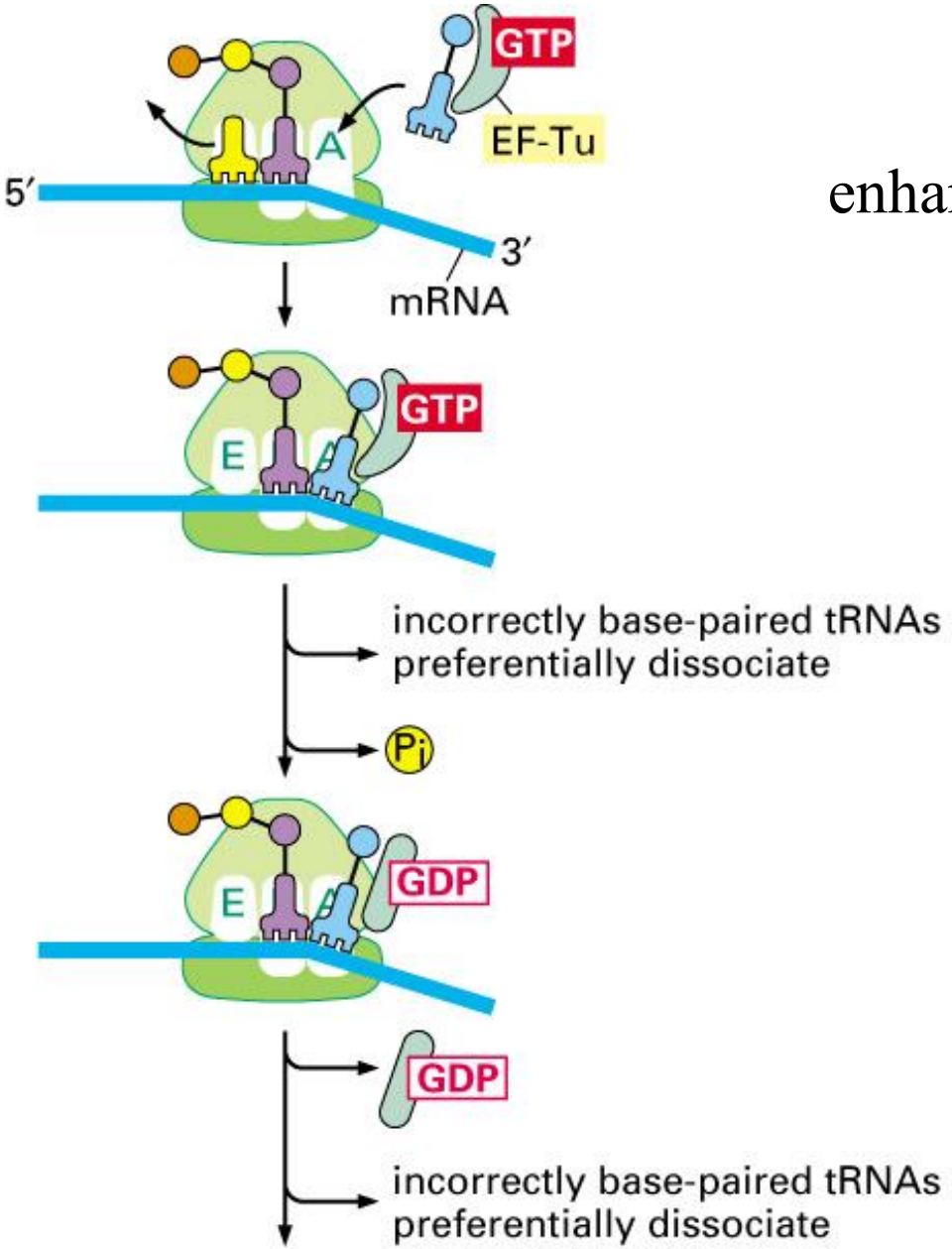
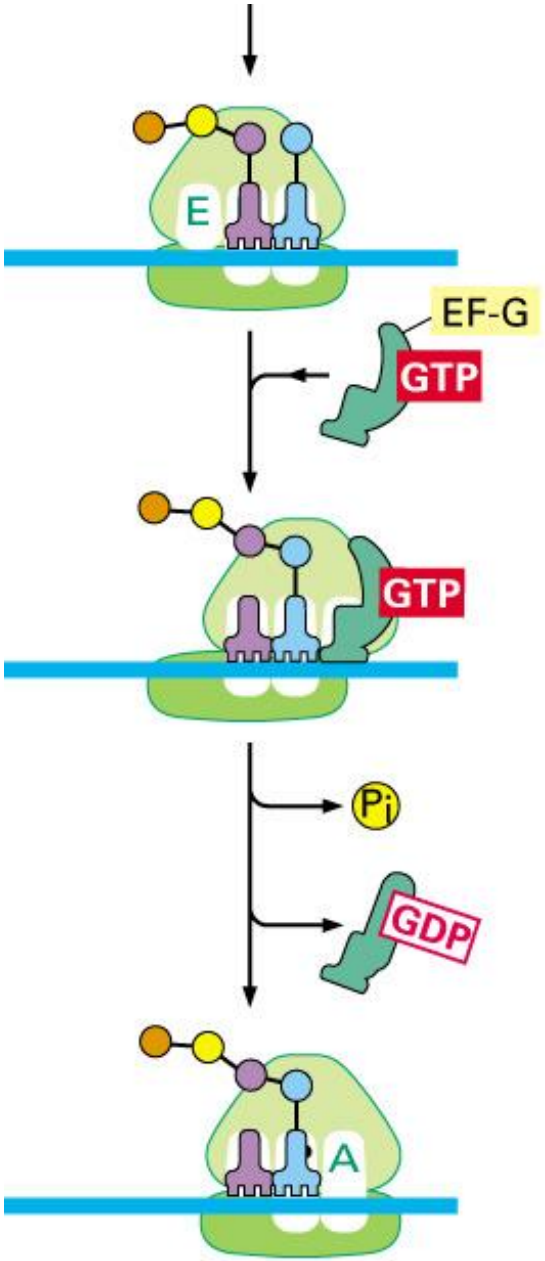


Figure 6–65 part 2 of 2. Molecular Biology of the Cell, 4th Edition.



Elongation Factor
enhances accuracy and efficiency

Figure 6–66 part 1 of 2. Molecular Biology of the Cell, 4th Edition.



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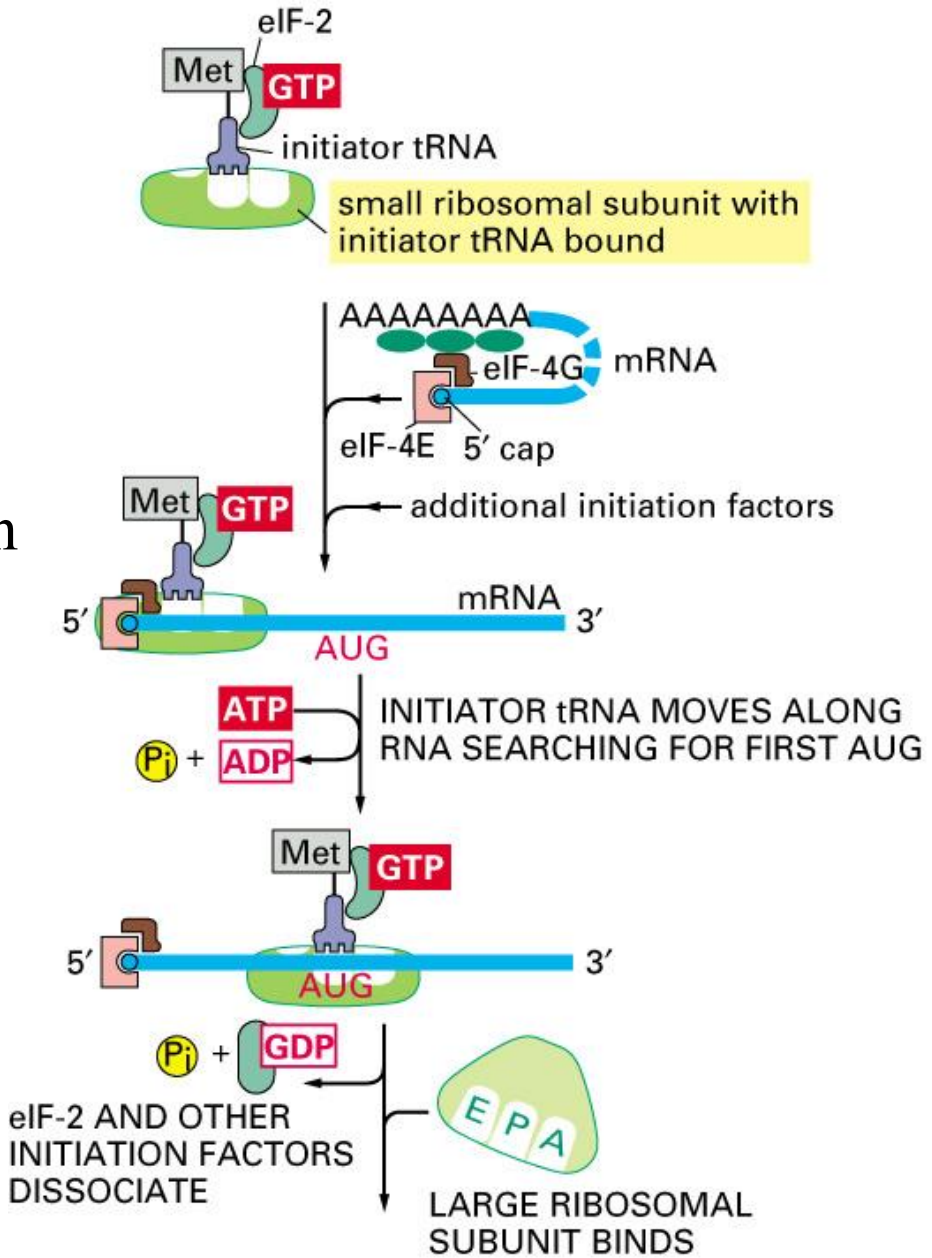
The Initiation of protein synthesis in eucaryotes

Eucaryotic initiation factors (eIFs)

AUG encodes Met



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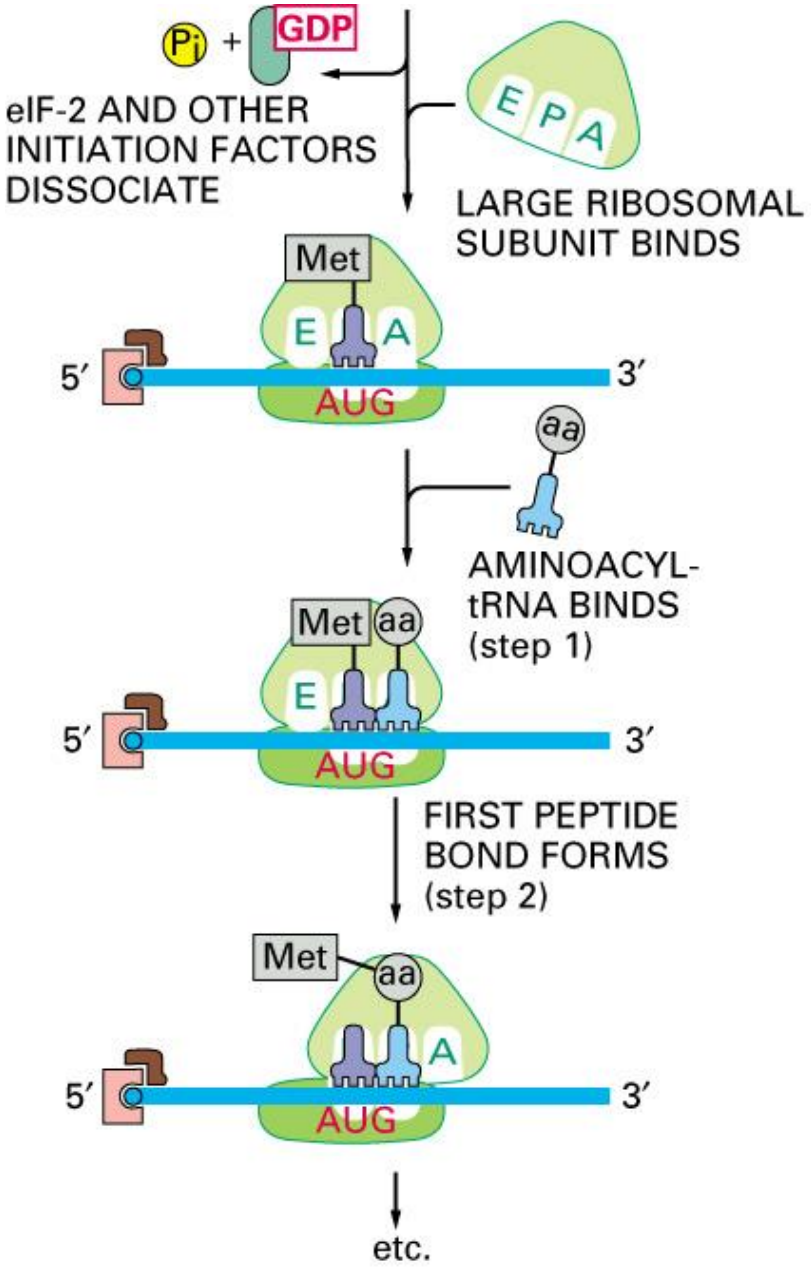


Figure 6–71 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Stop codons
UAA, UAG, UGA
Releasing factor, coupling a water molecule

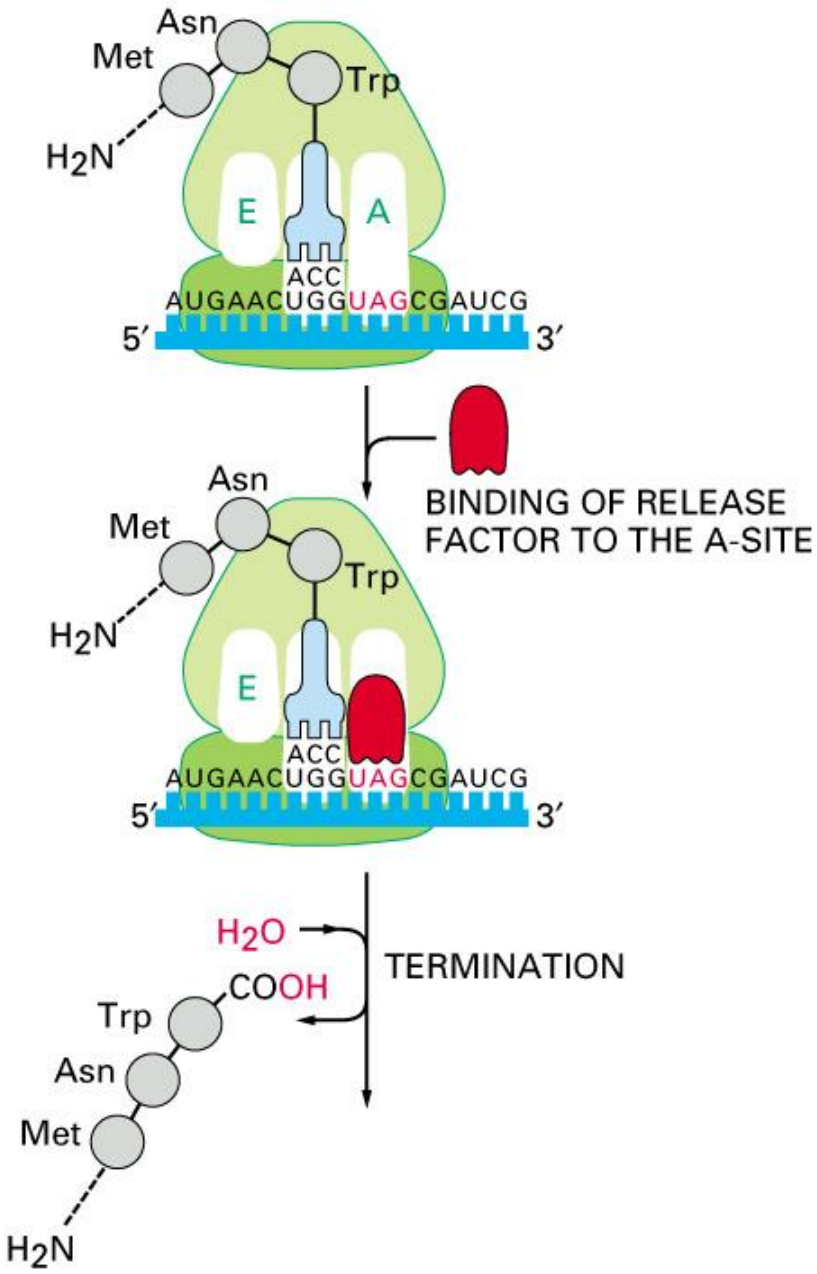
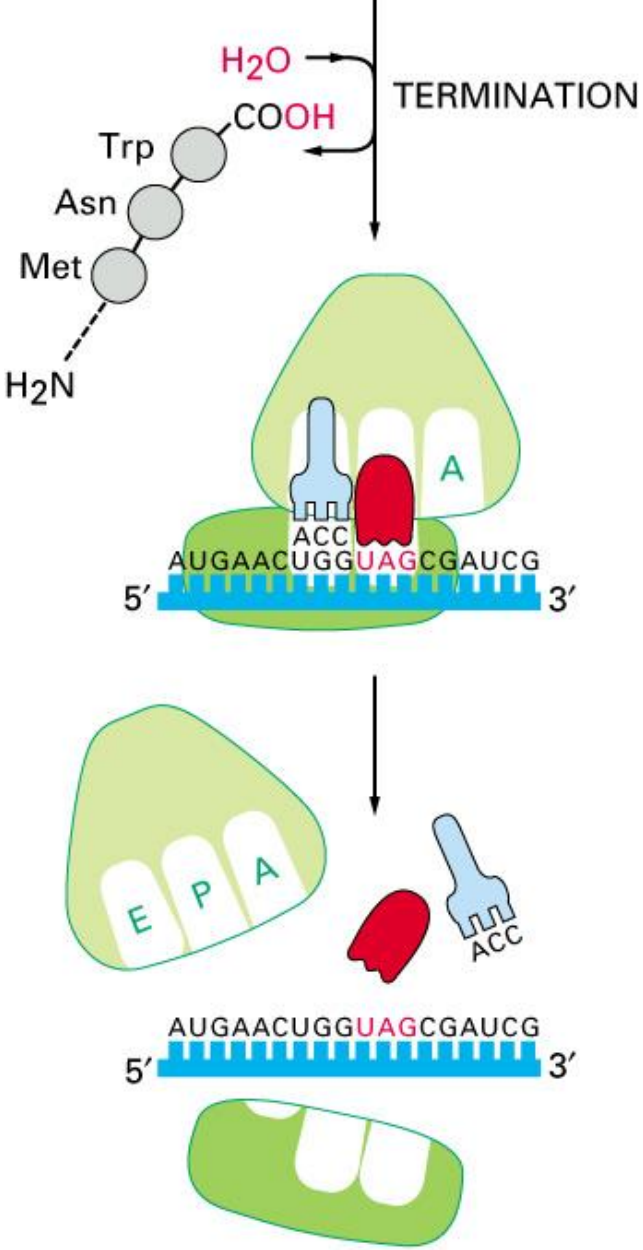


Figure 6–73 part 1 of 2. Molecular Biology of the Cell, 4th Edition.



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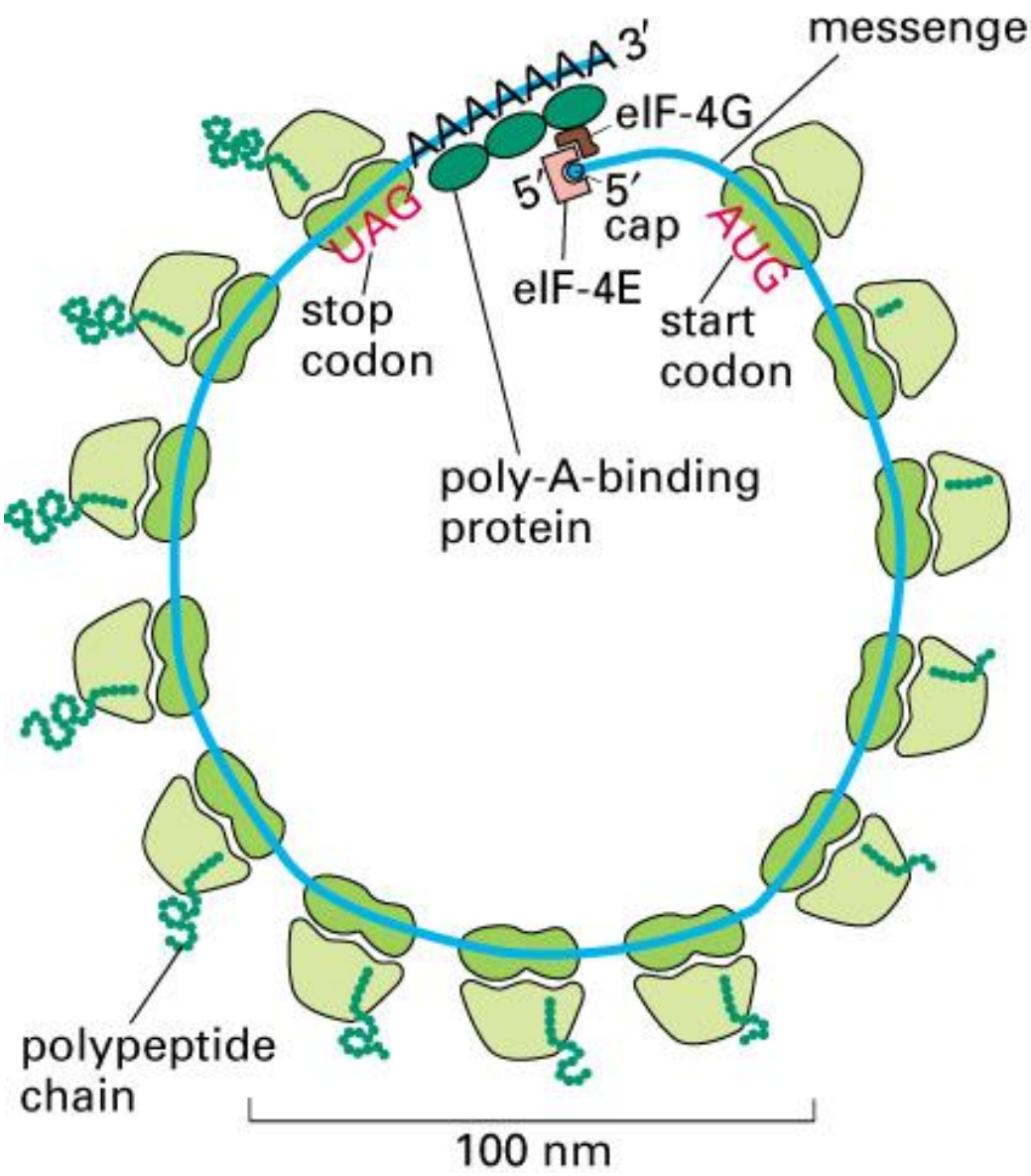
Multiple Copies on the Same mRNA (polysomes)
Most proteins are synthesized in 20 sec or minutes



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