TOPICS – 4 courses:

- the structure of DNA;
- basic genetic mechanisms, how the genetic information of the cell is:
    - maintained
    - replicated
    - expressed
    - occasionally improved.

Previous Courses

I. DNA and CHROMOSOMES:

- **I.A.** The Structure and Function of DNA
- **I.B.** Chromosomal DNA and Its Packaging in the Chromatin Fiber
- **I.C.** The Global Structure of Chromosomes

II. DNA Replication and Repair:

- **II.A.** The Maintenance of DNA Sequences
- **II.B.** DNA Replication Mechanisms
- **II.C.** The Initiation and Completion of DNA Replication in Chromosomes
- **II.D.** DNA Repair

Course structure

IV. How Cells Read the Genome: From DNA to Protein

- **IV.A.** From DNA to RNA
- **IV.B.** From RNA to Protein

how cells **decode and use the information** in their genomes

- 25.000 genes;
- encode for proteins/various types of RNA;
- 4 different “letters” (nucleotides).

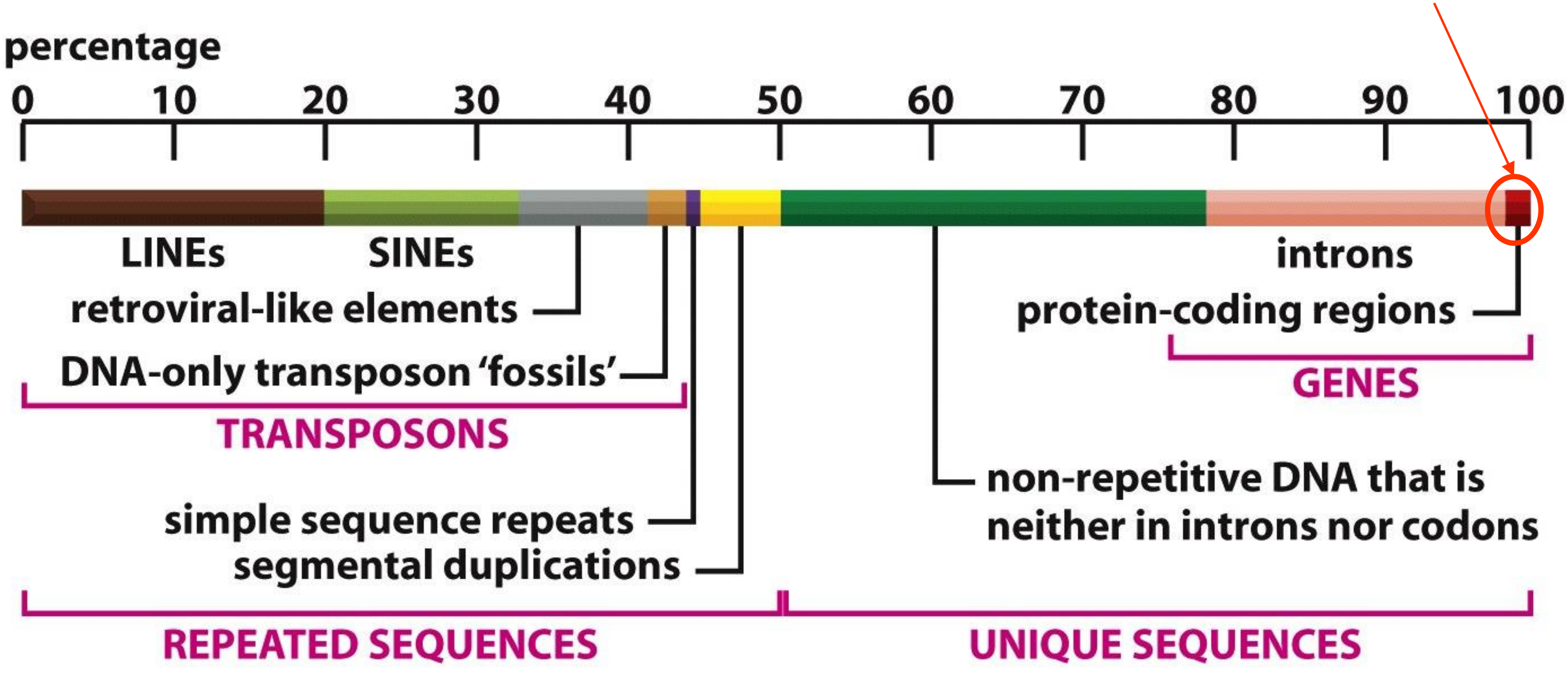
The information in eucaryotic cells genomes is not arranged in an orderly fashion (eg: dictionary or a telephone directory):

- Small bits of coding DNA are interspersed with large blocks of seemingly meaningless DNA.

- Some sections of the genome contain many genes and others lack genes altogether.

How Genes Are Arranged in Humans:

- only a few percent of Human genome codes for proteins or structural and catalytic RNAs



- Proteins that work closely with one another in the cell often have their genes located on different chromosomes;

- Adjacent genes typically encode proteins that have little to do with each other in the cell.

=> Decoding genomes is not a simple matter!!!

Even with the aid of powerful computers, it is still difficult to locate the beginning and end of genes!

The flow of genetic information in cells

the *central dogma* of molecular biology

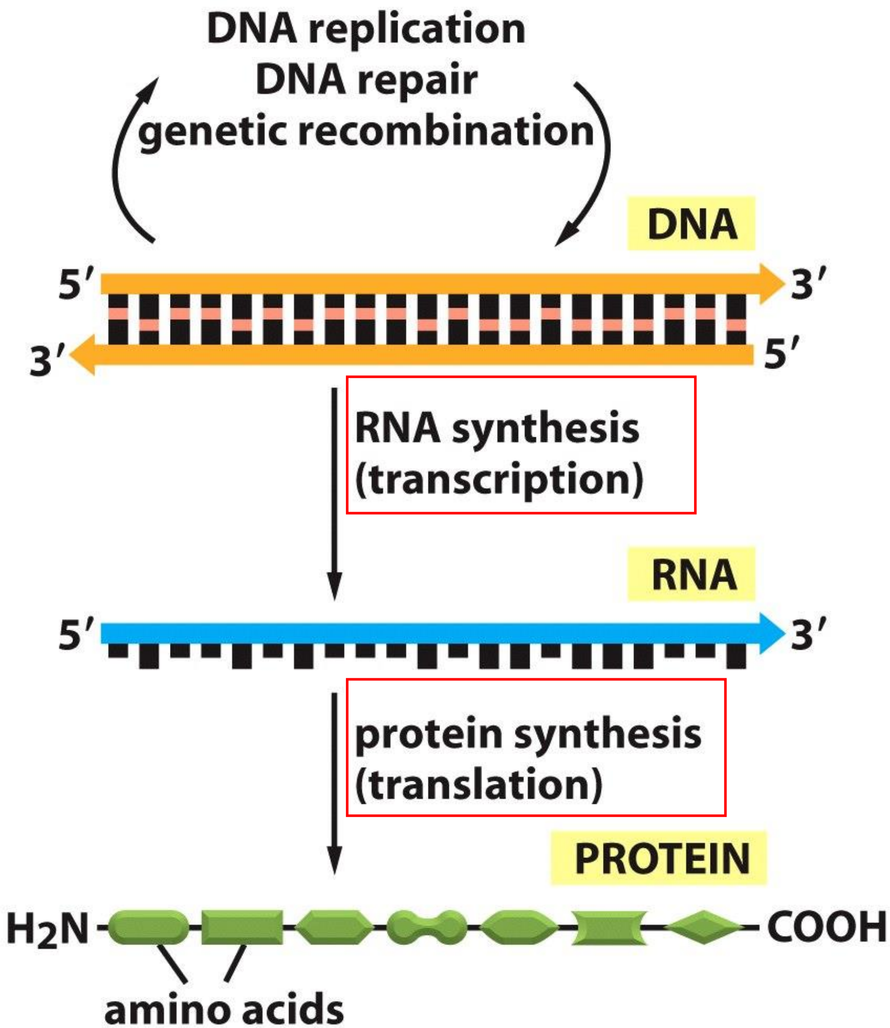
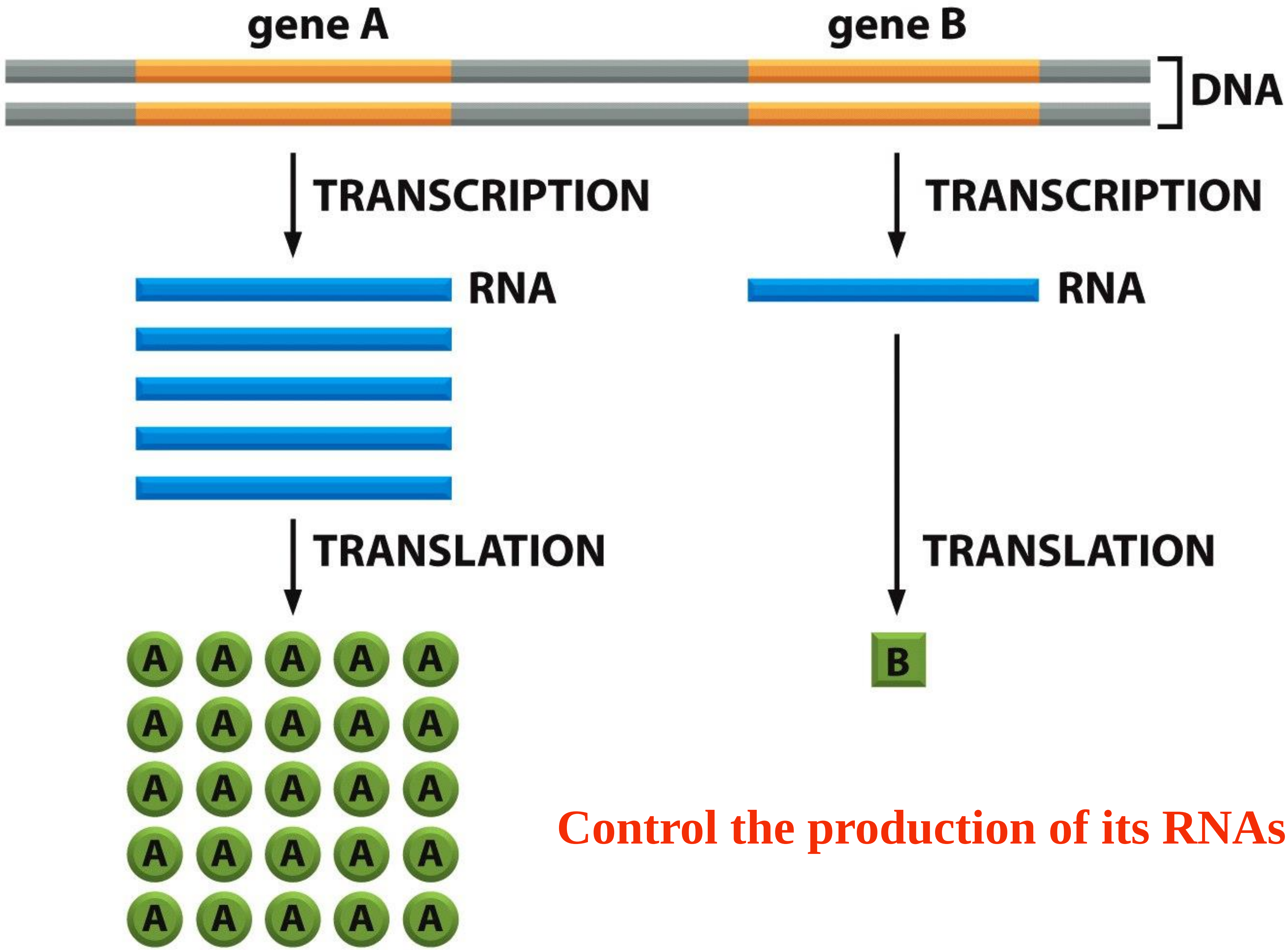


Figure 6-2 *Molecular Biology of the Cell* (© Garland Science 2008)

Transcription and **translation** are the means by which cells express the genetic instructions in their genes.

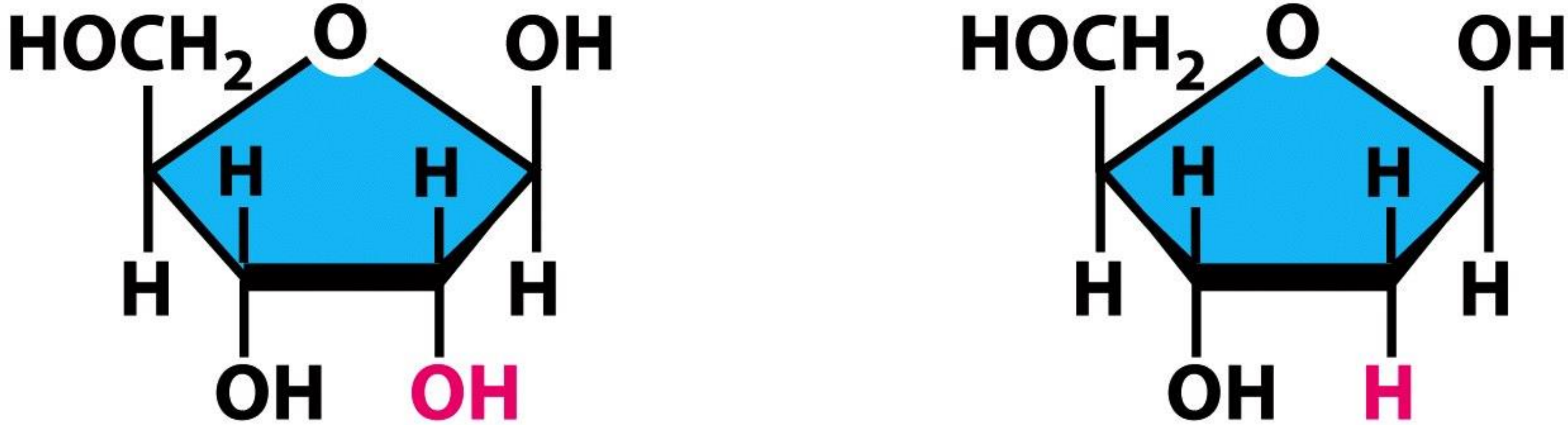


Control the production of its RNAs!!!

Figure 6-3 *Molecular Biology of the Cell* (© Garland Science 2008)

TRANSCRIPTION

- a particular portion of a DNA nucleotide sequence – gene – is copied into an RNA nucleotide sequence



ribose

used in ribonucleic acid (RNA)

deoxyribose

used in deoxyribonucleic acid (DNA)

Figure 6-4a *Molecular Biology of the Cell* (© Garland Science 2008)

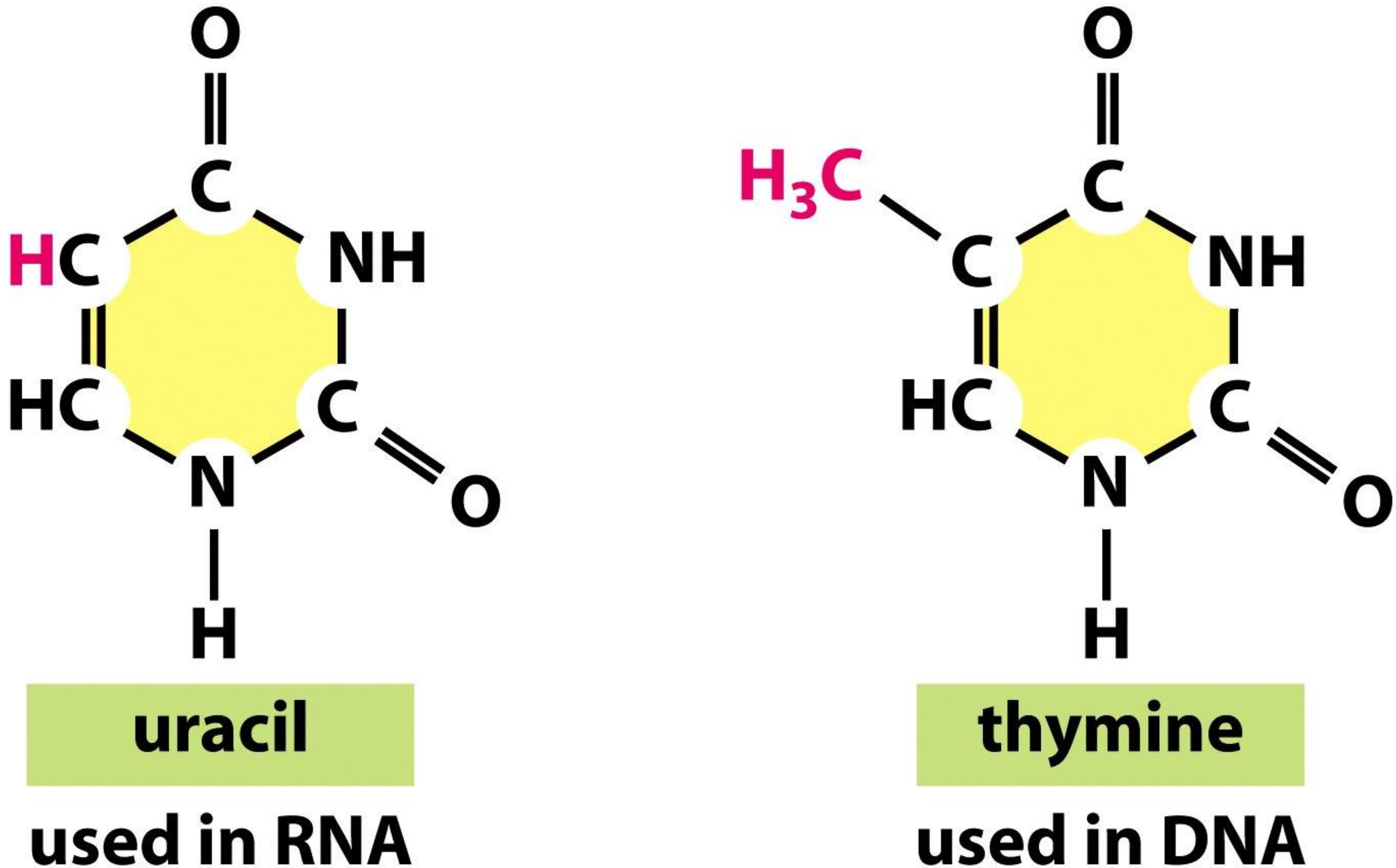


Figure 6-4b *Molecular Biology of the Cell* (© Garland Science 2008)

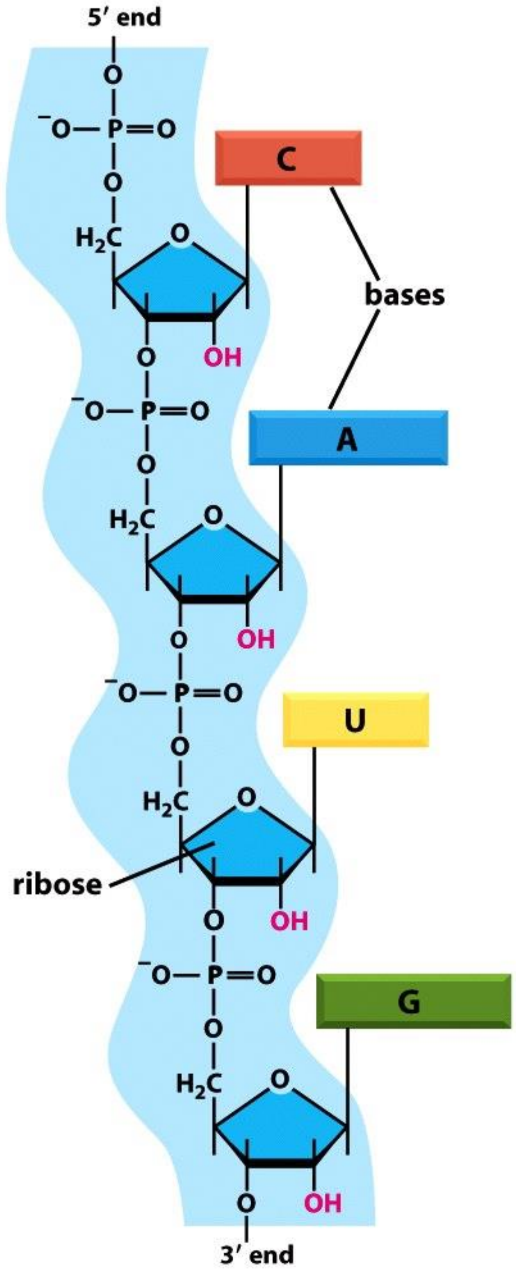


Figure 6-4c *Molecular Biology of the Cell* (© Garland Science 2008)

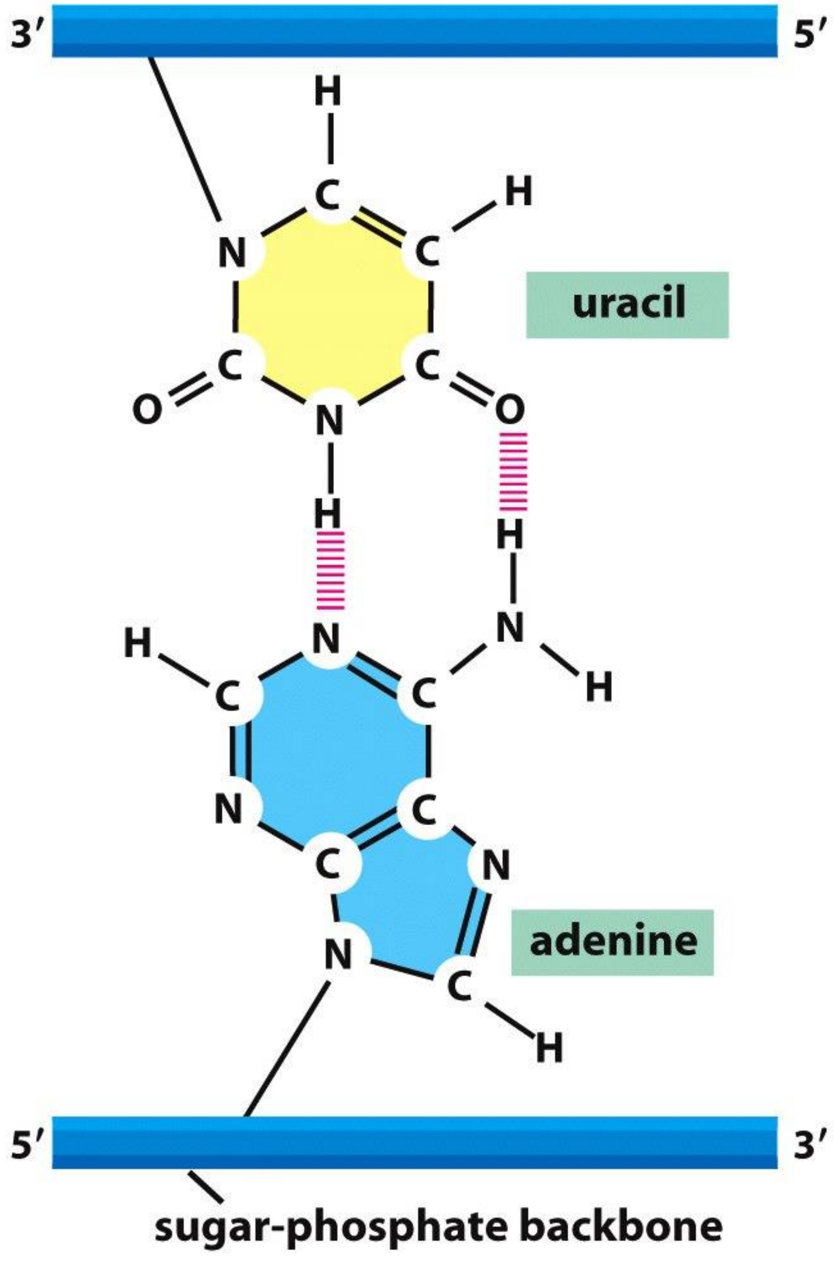


Figure 6-5 *Molecular Biology of the Cell* (© Garland Science 2008)

RNA can fold into specific structures

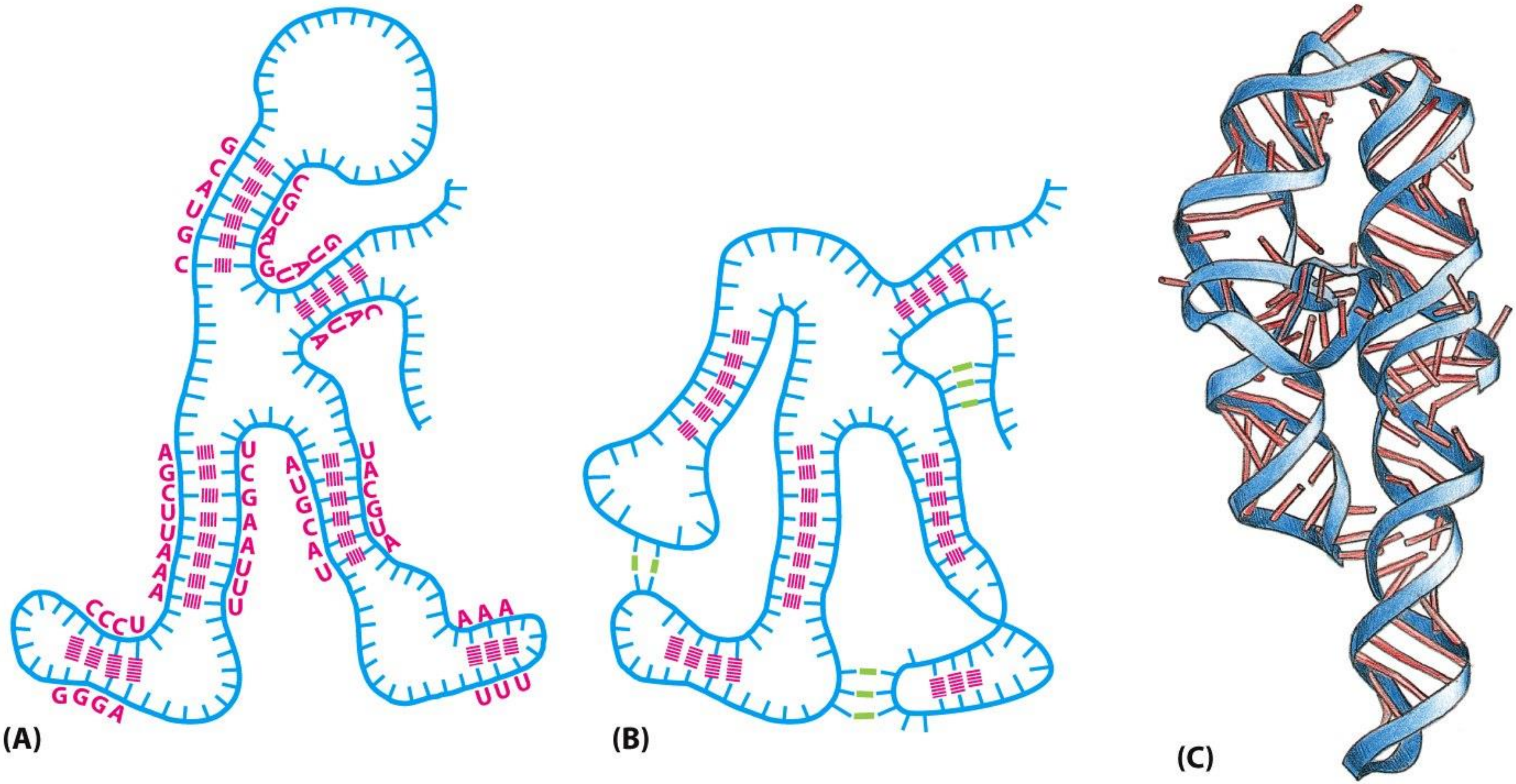


Figure 6-6 *Molecular Biology of the Cell* (© Garland Science 2008)

TRANSCRIPTION

- Produces RNA Complementary to One Strand of DNA

TRANSCRIPTION – steps:

I. opening and unwinding of a small portion of the DNA double helix (does not require the energy of ATP hydrolysis);

II. one of the two strands of the DNA double helix acts as a template - enzymatically catalyzed reaction

III. RNA molecules produced by transcription are released from the DNA template as single strand;

IV. The DNA helix re-forms

TRANSCRIPTION – enzymes - ***RNA polymerases***

- catalyze the formation of the phosphodiester

- growing RNA chain is extended by one nucleotide at a time in the 5′-to-3′ direction

- the substrates are nucleoside triphosphates (ATP, CTP, UTP, and GTP)

RNA and DNA polymerases - differences

- RNA polymerase catalyzes the linkage of ribonucleotides, not deoxyribonucleotides;

- RNA polymerases can start an RNA chain without a primer;

- transcription need not be as accurate as DNA replication:
  - one mistake for every 10^4 nucleotides copied into RNA compared
  - error rate for direct copying by DNA polymerase of about one in 10^7 nucleotides);

- RNA polymerases have a modest proofreading mechanism.

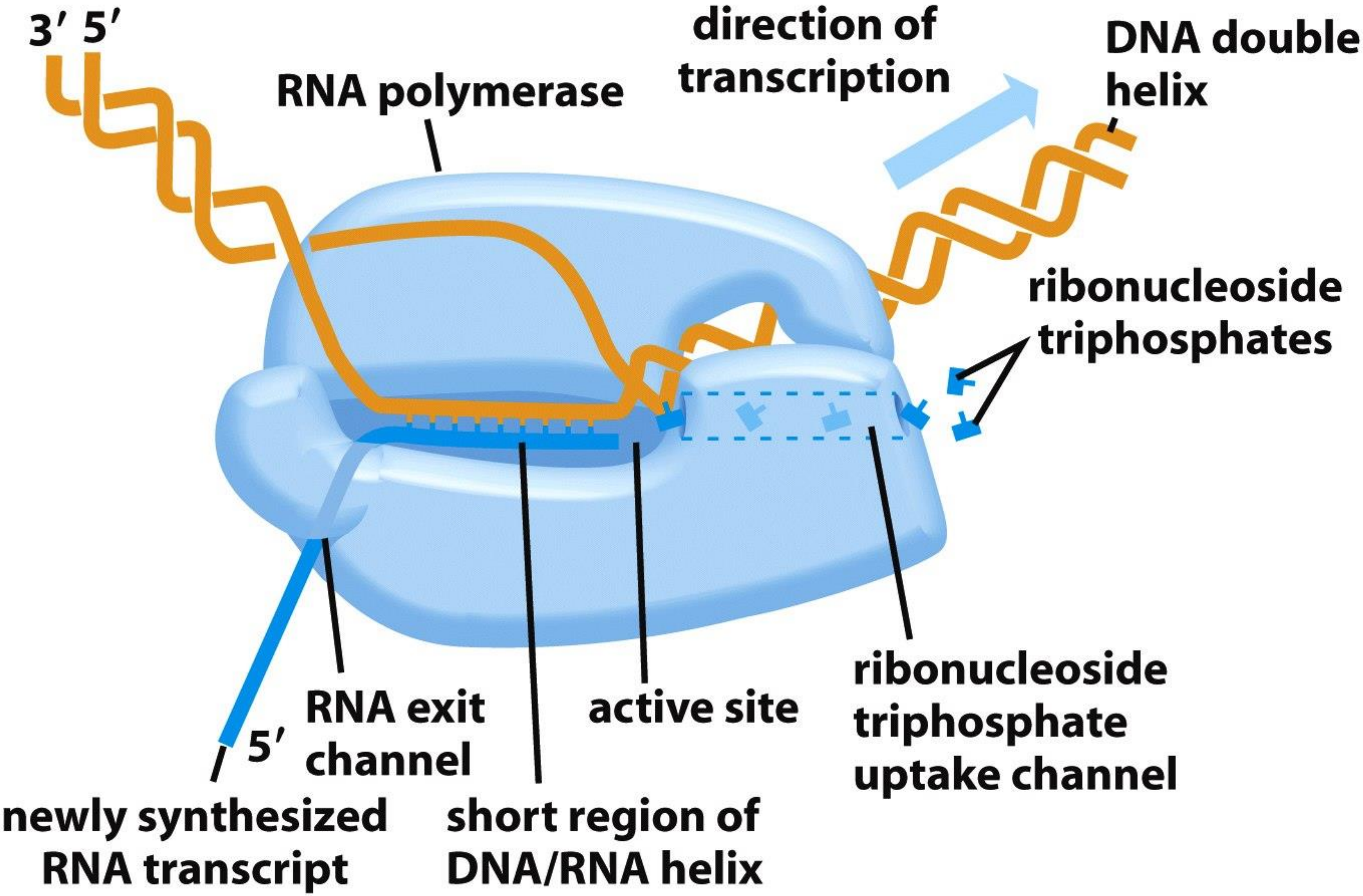


Figure 6-8a *Molecular Biology of the Cell* (© Garland Science 2008)

Cells Produce Several Types of RNA

- each transcribed segment of DNA - TRANSCRIPTION UNIT;

- RNA makes up a few percent of a cell's dry weight;

- most of the RNA in cells is rRNA;

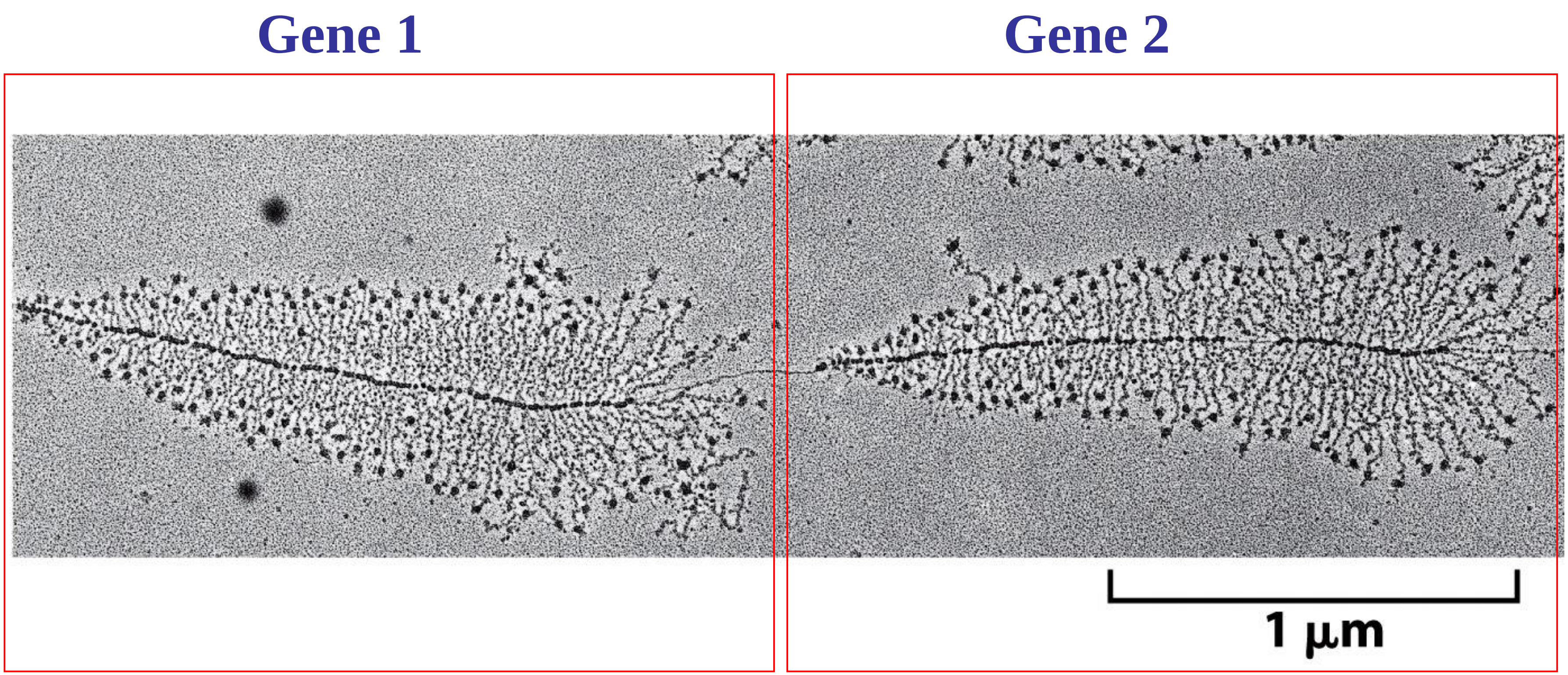
- mRNA comprises only 3–5% of the total RNA in a typical mammalian cell.

Table 6–1 Principal Types of RNAs Produced in Cells

TYPE OF RNA	FUNCTION
mRNAs	messenger RNAs, code for proteins
rRNAs	ribosomal RNAs, form the basic structure of the ribosome and catalyze protein synthesis
tRNAs	transfer RNAs, central to protein synthesis as adaptors between mRNA and amino acids
snRNAs	small nuclear RNAs, function in a variety of nuclear processes, including the splicing of pre-mRNA
snoRNAs	small nucleolar RNAs, used to process and chemically modify rRNAs
scaRNAs	small cajal RNAs, used to modify snoRNAs and snRNAs
miRNAs	microRNAs, regulate gene expression typically by blocking translation of selective mRNAs
siRNAs	small interfering RNAs, turn off gene expression by directing degradation of selective mRNAs and the establishment of compact chromatin structures
Other noncoding RNAs	function in diverse cell processes, including telomere synthesis, X-chromosome inactivation, and the transport of proteins into the ER

Table 6-1 *Molecular Biology of the Cell* (© Garland Science 2008)

Transcription of two genes as observed under the electron microscope.



- 20 nucleotides per second (the speed in eucaryotes)
- over a thousand transcripts can be synthesized in an hour from a single gene

Figure 6-9 *Molecular Biology of the Cell* (© Garland Science 2008)

Signals encoded in DNA tell RNA polymerase

where to START and STOP

Procaryotes
- one RNA polymerase

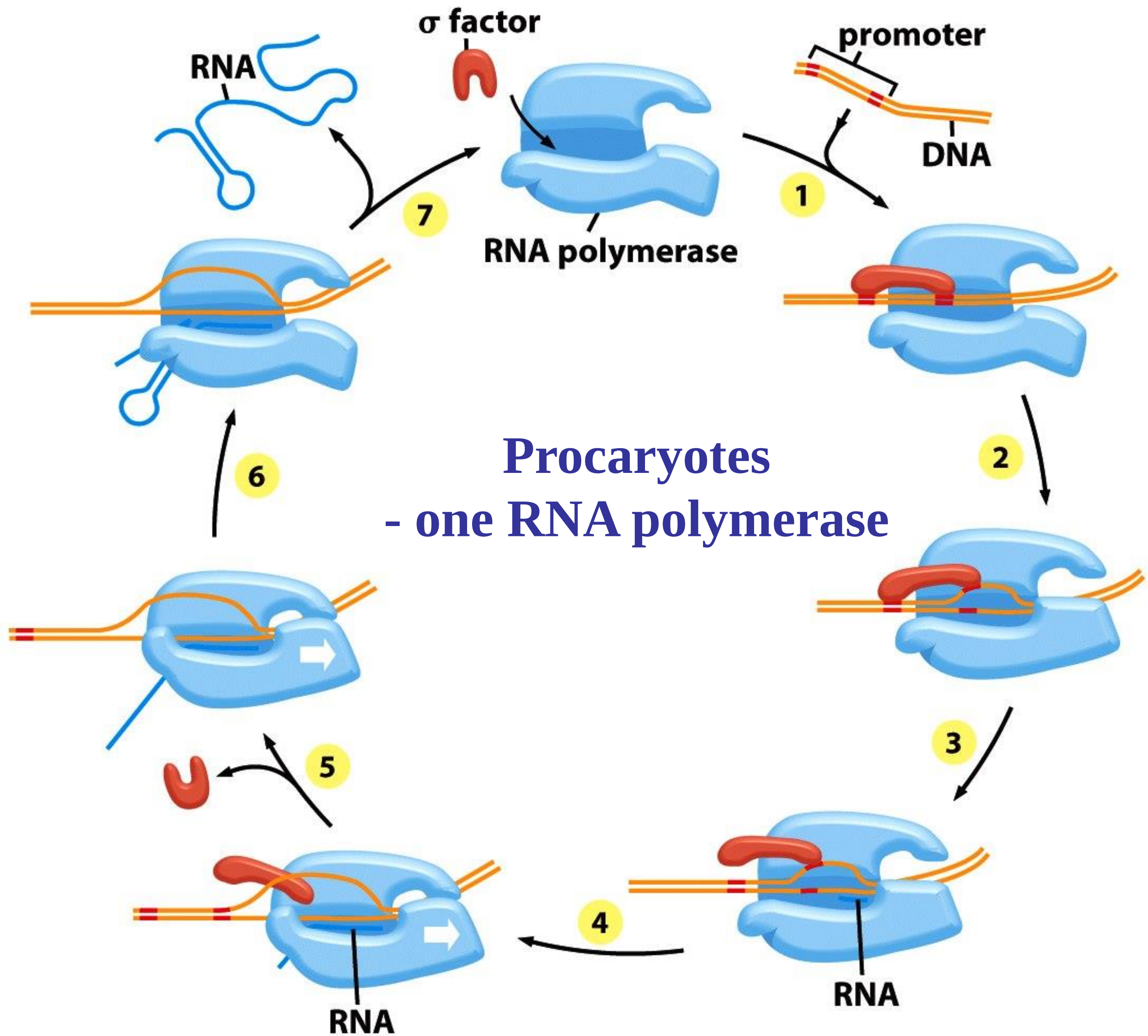


Figure 6-11 *Molecular Biology of the Cell* (© Garland Science 2008)

Table 6–2 The Three RNA Polymerases in Eucaryotic Cells

TYPE OF POLYMERASE	GENES TRANSCRIBED
RNA polymerase I	5.8S, 18S, and 28S rRNA genes
RNA polymerase II	all protein-coding genes, plus snoRNA genes, miRNA genes, siRNA genes, and most snRNA genes
RNA polymerase III	tRNA genes, 5S rRNA genes, some snRNA genes and genes for other small RNAs

The rRNAs are named according to their "S" values, which refer to their rate of sedimentation in an ultracentrifuge. The larger the S value, the larger the rRNA.

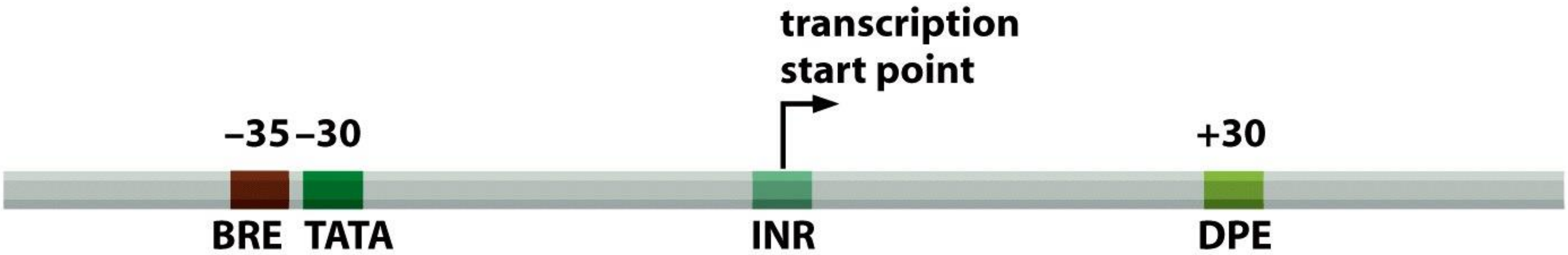
Bacterial and eucaryotic RNA polymerases - differences

- bacterial RNA polymerase requires only a single additional protein (sigma factor) for transcription initiation to occur in vitro;

- eucaryotic RNA polymerases require many additional proteins – general transcription factors;

- Eucaryotic transcription initiation must deal with the packing of DNA into nucleosomes and higher-order forms of chromatin structure.

Initiation of transcription of a eucaryotic gene by RNA polymerase II



element	consensus sequence	general transcription factor
BRE	G/C G/C G/A C G C C	TFIIB
TATA	T A T A A/T A A/T	TBP
INR	C/T C/T A N T/A C/T C/T	TFIID
DPE	A/G G A/T C G T G	TFIID

Figure 6-17 *Molecular Biology of the Cell* (© Garland Science 2008)

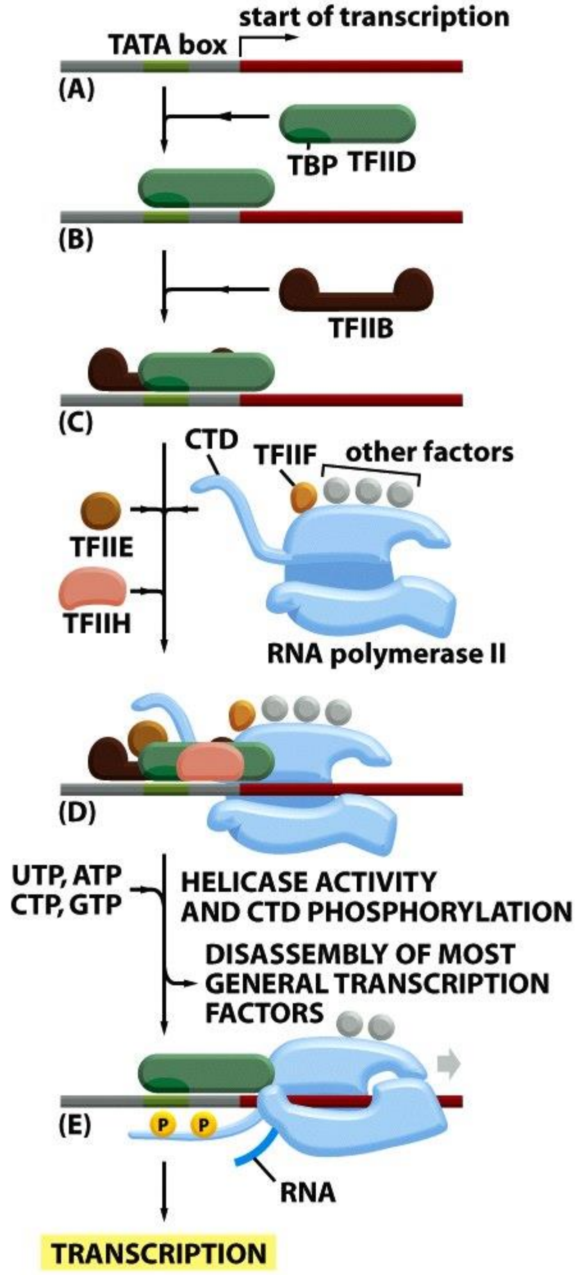


Figure 6-16 *Molecular Biology of the Cell* (© Garland Science 2008)

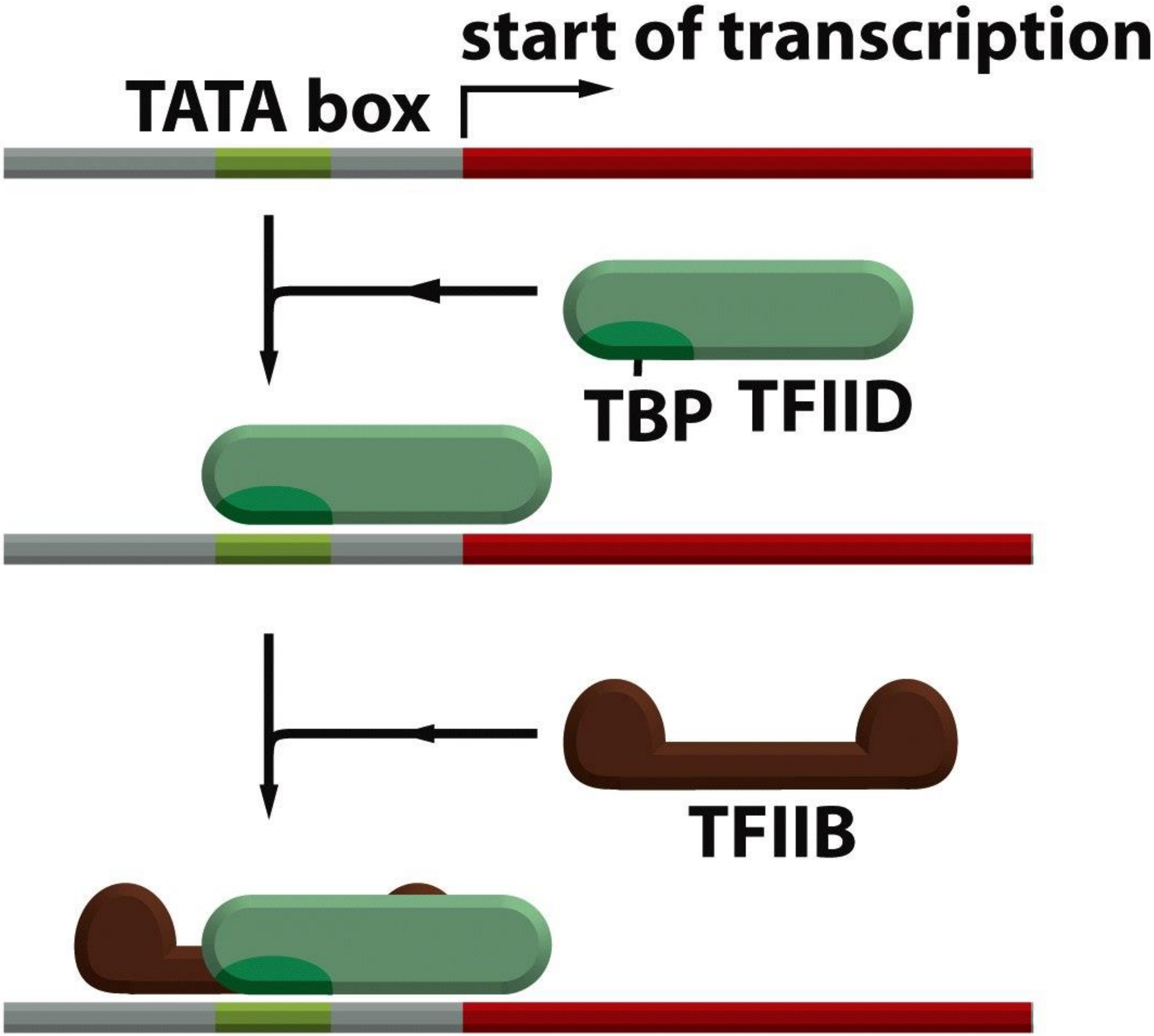


Figure 6-16 (part 1 of 3) *Molecular Biology of the Cell* (© Garland Science 2008)

TFIIH (ATP):

- pry apart the DNA double helix;
- locally expose the template strand;

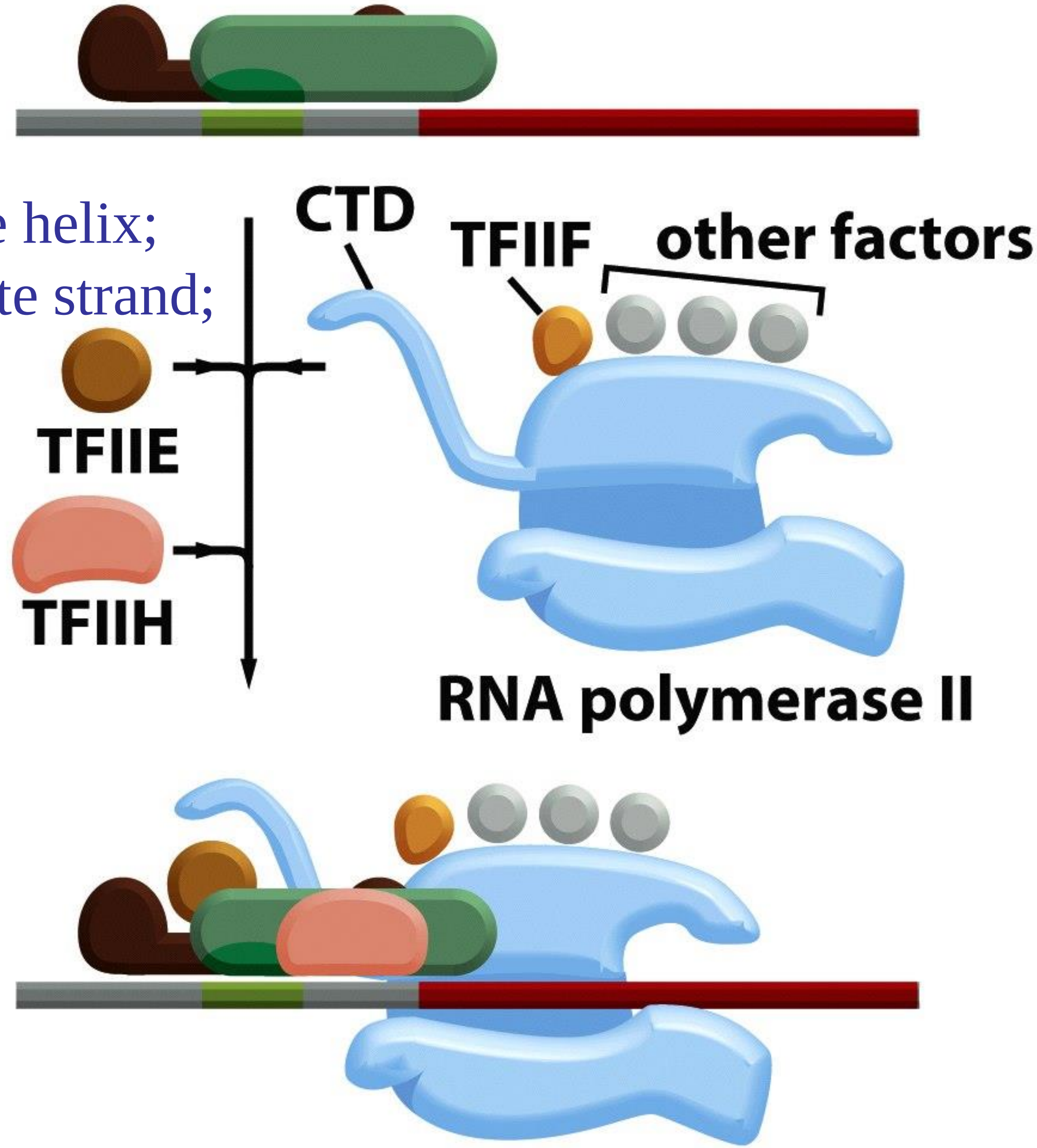


Figure 6-16 (part 2 of 3) *Molecular Biology of the Cell* (© Garland Science 2008)

TFIIH (ATP):
- phosphorylates RNA polymerase II (C-terminal domain);

- release RNA polymerase
 => elongation;

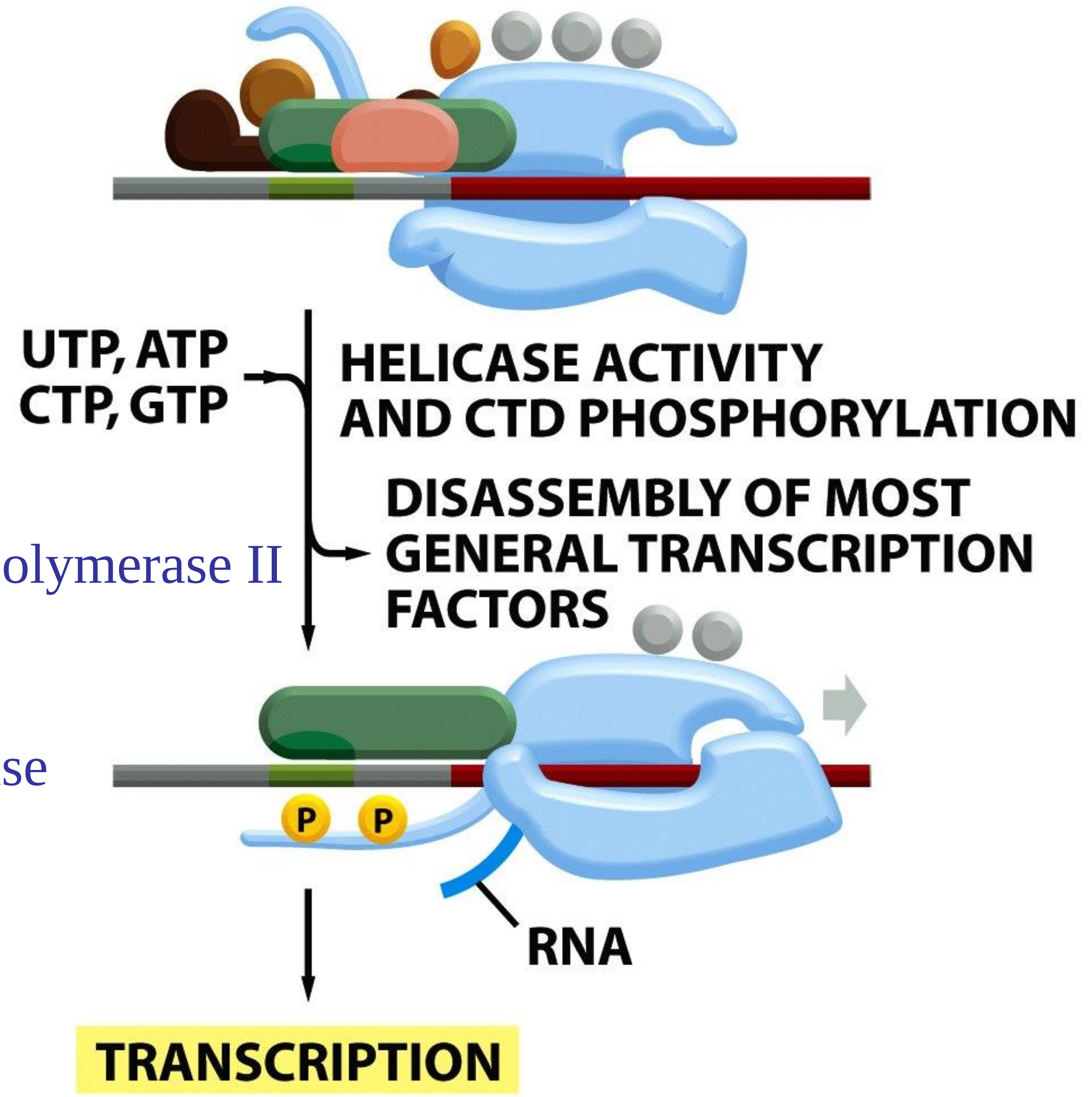


Figure 6-16 (part 3 of 3) *Molecular Biology of the Cell* (© Garland Science 2008)

Table 6–3 The General Transcription Factors Needed for Transcription Initiation by Eucaryotic RNA Polymerase II

NAME	NUMBER OF SUBUNITS	ROLES IN TRANSITION INITIATION
TFIID		
TBP subunit	1	recognizes TATA box
TAF subunits	~11	recognizes other DNA sequences near the transcription start point; regulates DNA-binding by TBP
TFIIB	1	recognizes BRE element in promoters; accurately positions RNA polymerase at the start site of transcription
TFIIF	3	stabilizes RNA polymerase interaction with TBP and TFIIB; helps attract TFIIE and TFIIH
TFIIE	2	attracts and regulates TFIIH
TFIIH	9	unwinds DNA at the transcription start point, phosphorylates Ser5 of the RNA polymerase CTD; releases RNA polymerase from the promoter

TFIID is composed of TBP and ~11 additional subunits called TAFs (TBP-associated factors); CTD, C-terminal domain.

Polymerase II Also Requires Activator, Mediator, and Chromatin-Modifying Proteins

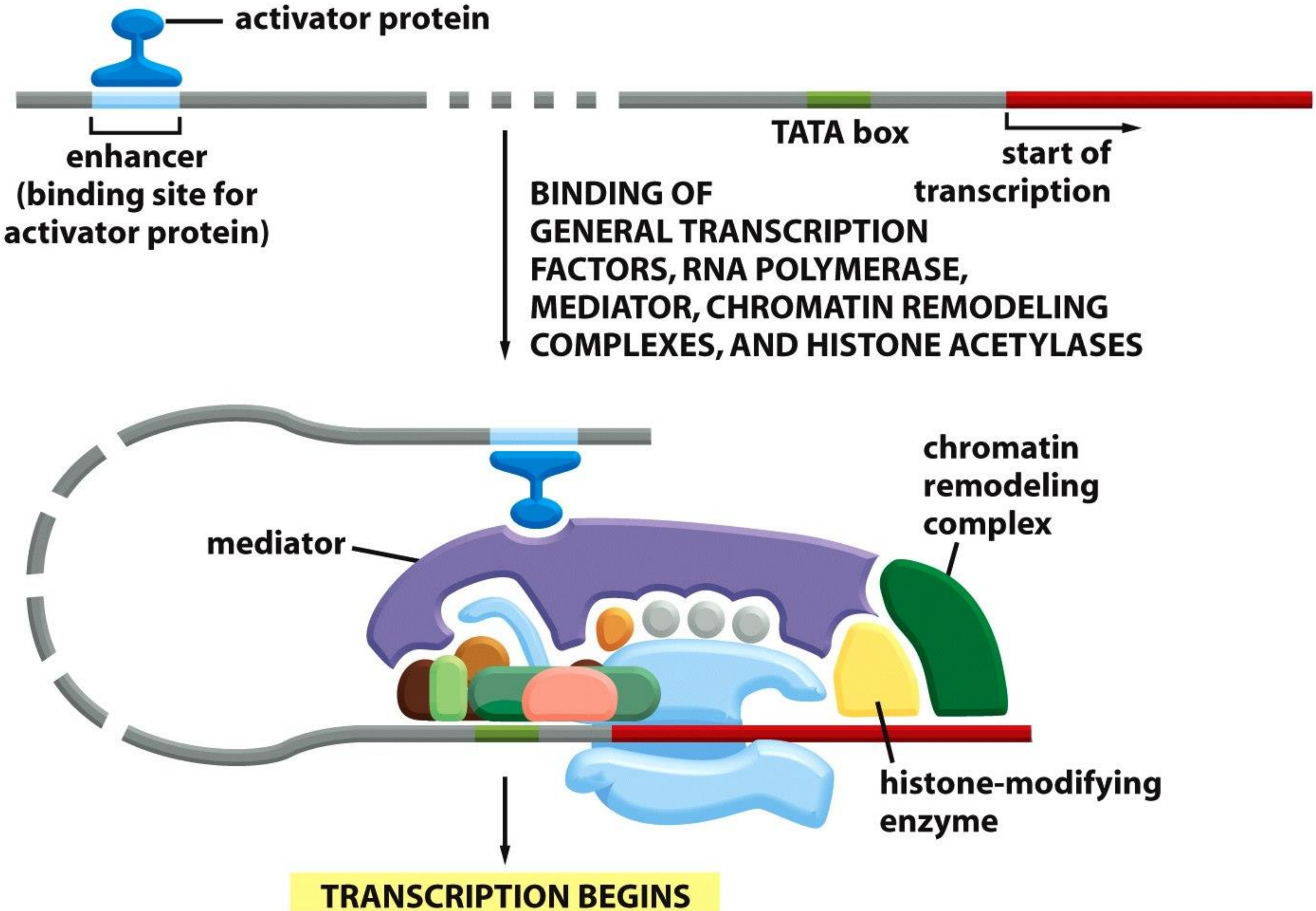


Figure 6-19 *Molecular Biology of the Cell* (© Garland Science 2008)

TRANSCRIPTION ELONGATION

- *elongation factors* - proteins that decrease the likelihood that RNA polymerase will dissociate before it reaches the end of a gene;

- DNA supercoiling issue

TRANSCRIPTION ELONGATION in eucaryotes is tightly coupled to RNA PROCESSING

- the removal of intron sequences – RNA splicing;
- Both ends of eucaryotic mRNAs are modified:
    - capping on the 5' end end
    - polyadenylation of the 3'end

5'-methyl cap signifies the 5' end of eucaryotic mRNAs, and this landmark helps the cell to distinguish mRNAs from the other types of RNA molecules present in the cell.

The cap binds a protein complex called CBC (cap-binding complex) - helps the RNA to be properly processed and exported

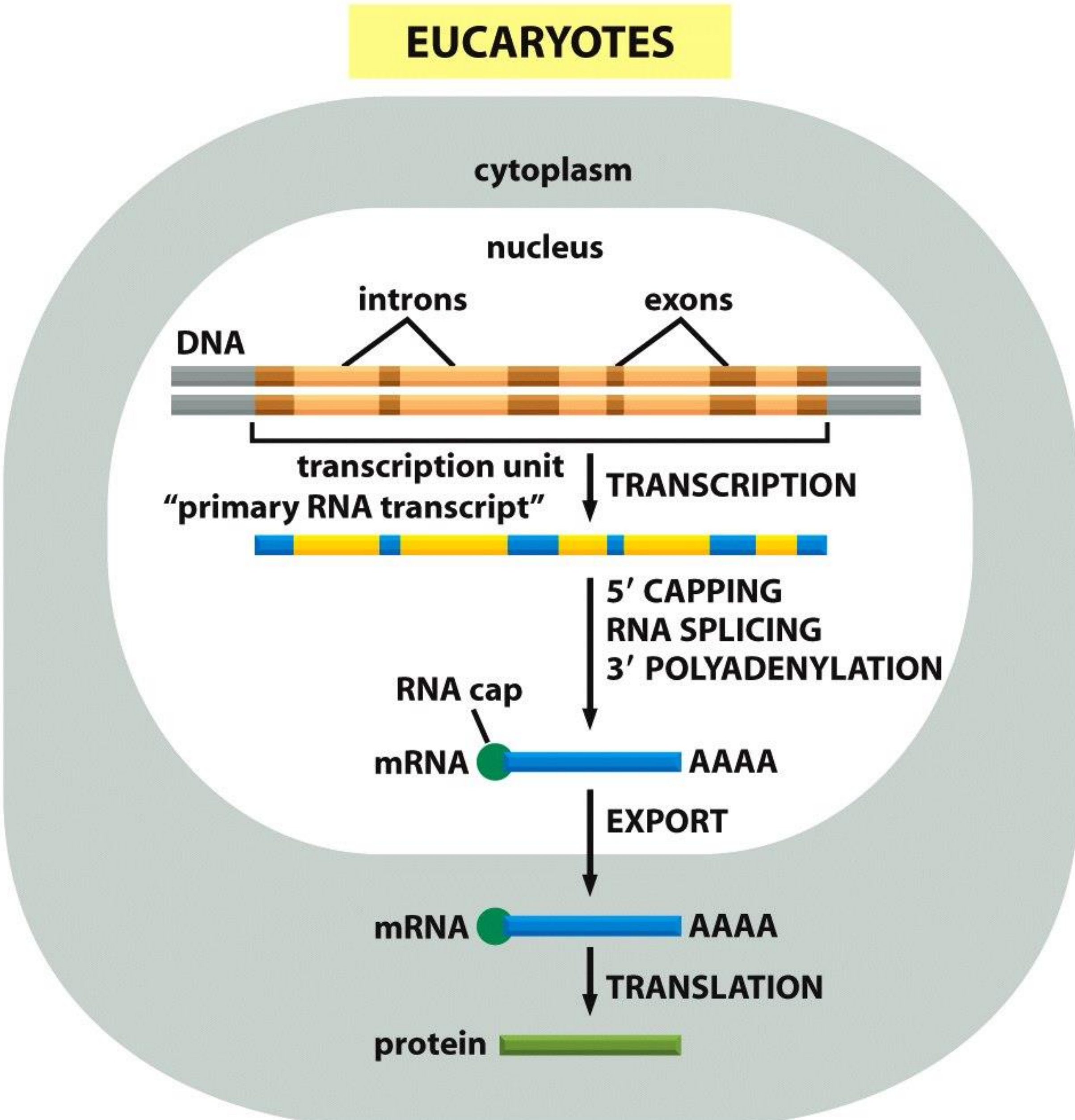


Figure 6-21a *Molecular Biology of the Cell* (© Garland Science 2008)

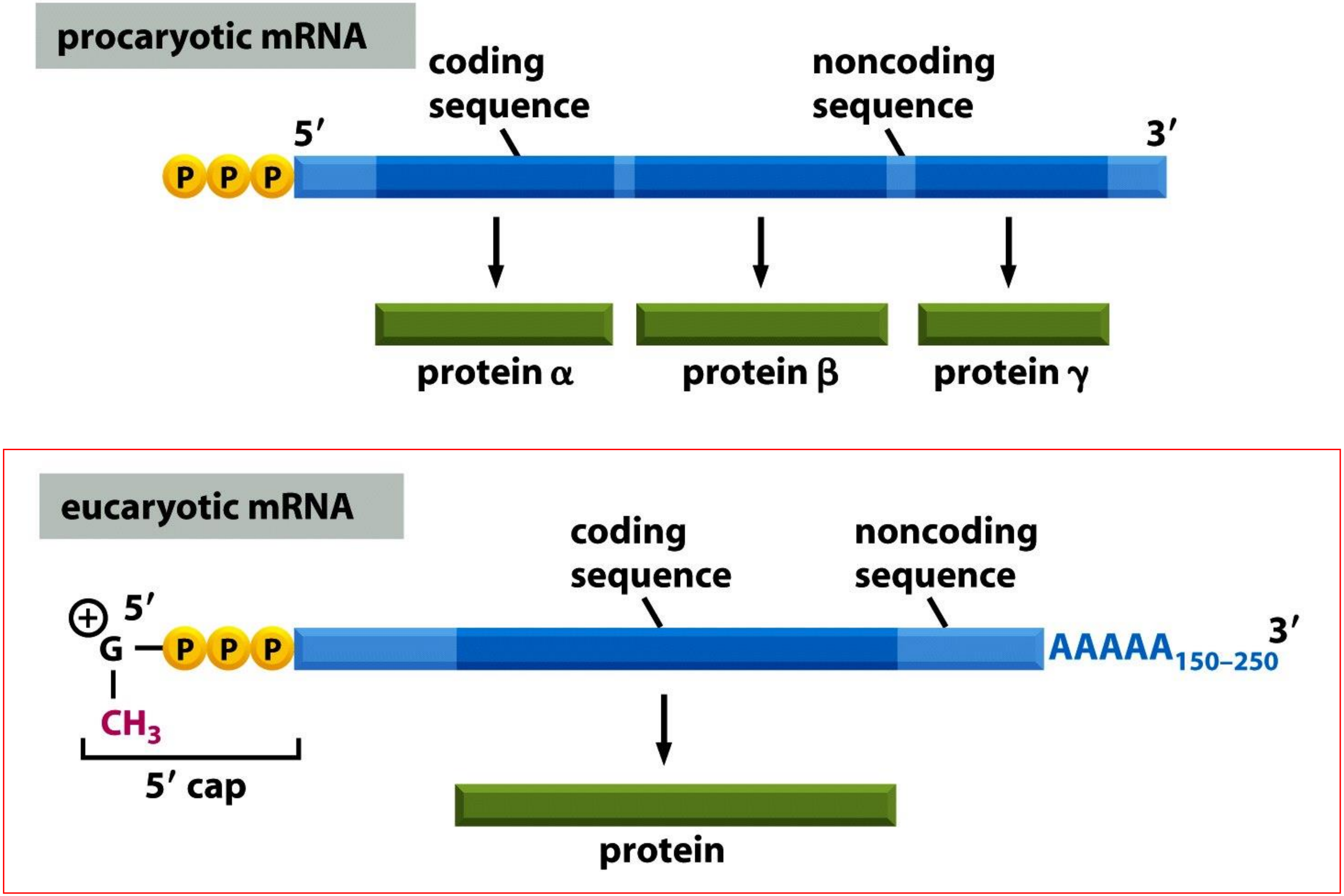


Figure 6-22a *Molecular Biology of the Cell* (© Garland Science 2008)

RNA PROCESSING - the removal of intron sequences – RNA splicing

-precursor-mRNA (or pre-mRNA);

- after splicing – mRNA;

- each splicing event removes one intron, proceeding through two sequential phosphoryl-transfer reactions known as transesterifications;

- these join two exons while removing the intron as a “lariat”

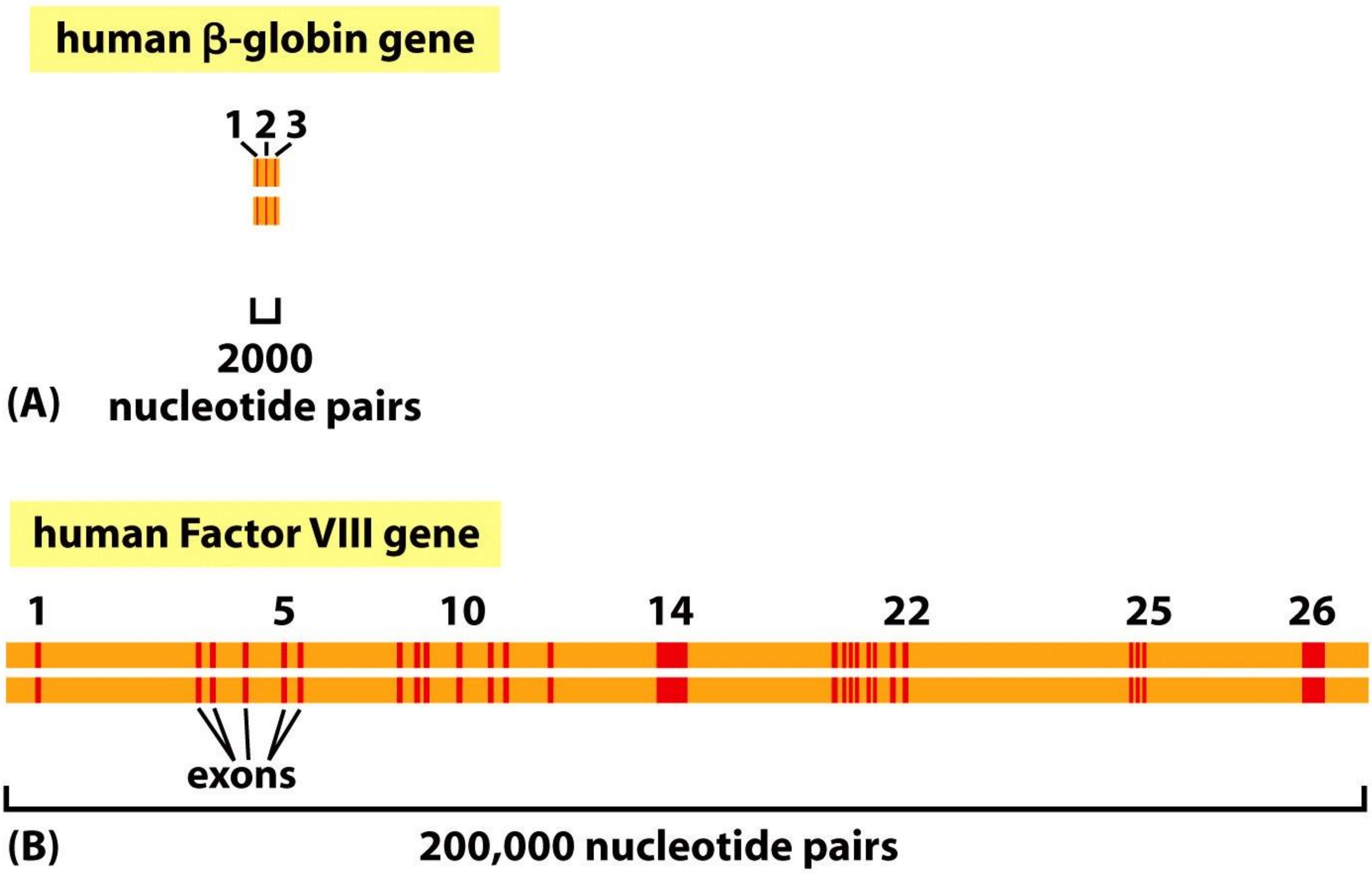


Figure 6-25 *Molecular Biology of the Cell* (© Garland Science 2008)

RNA splicing - advantages

-exon–intron arrangement would seem to facilitate the emergence of new and useful proteins over evolutionary time scales;

- many eucaryotic genes (estimated at 75% of genes in humans) are spliced in more than one way

=> eucaryotes increase the already enormous coding potential of their genomes.

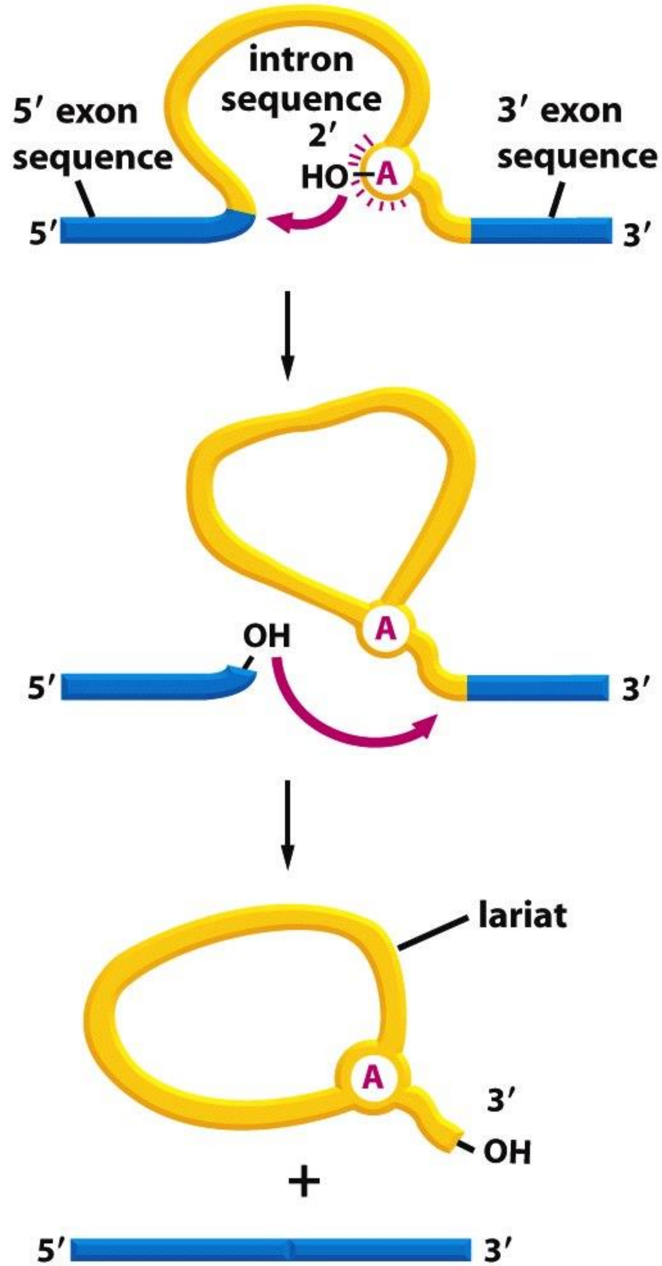


Figure 6-26a *Molecular Biology of the Cell* (© Garland Science 2008)

RNA Splicing Is Performed by the Spliceosome

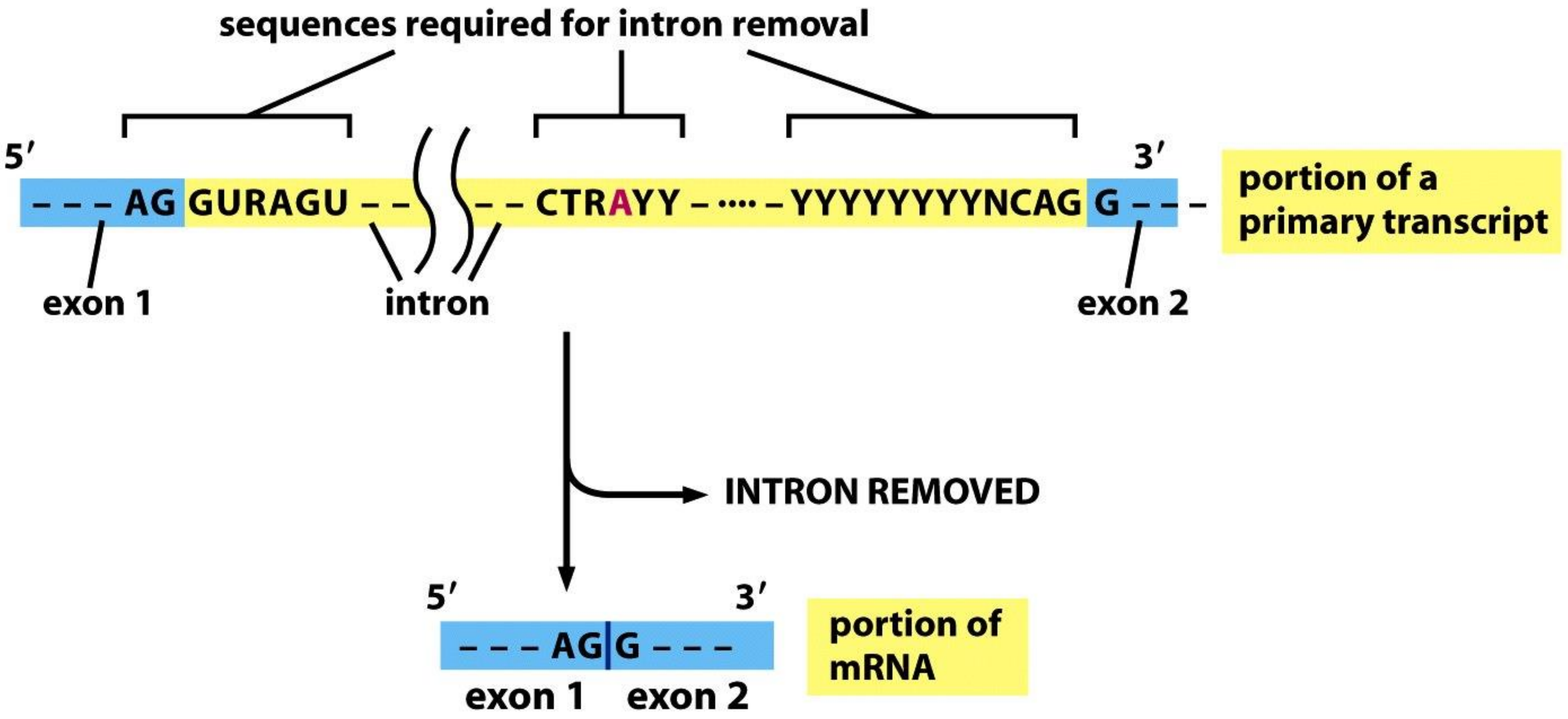


Figure 6-28 *Molecular Biology of the Cell* (© Garland Science 2008)

RNA Splicing Is Performed by the Spliceosome

- key steps in RNA splicing are performed by RNA molecules rather than proteins;

- Specialized RNA molecules recognize the nucleotide sequences;

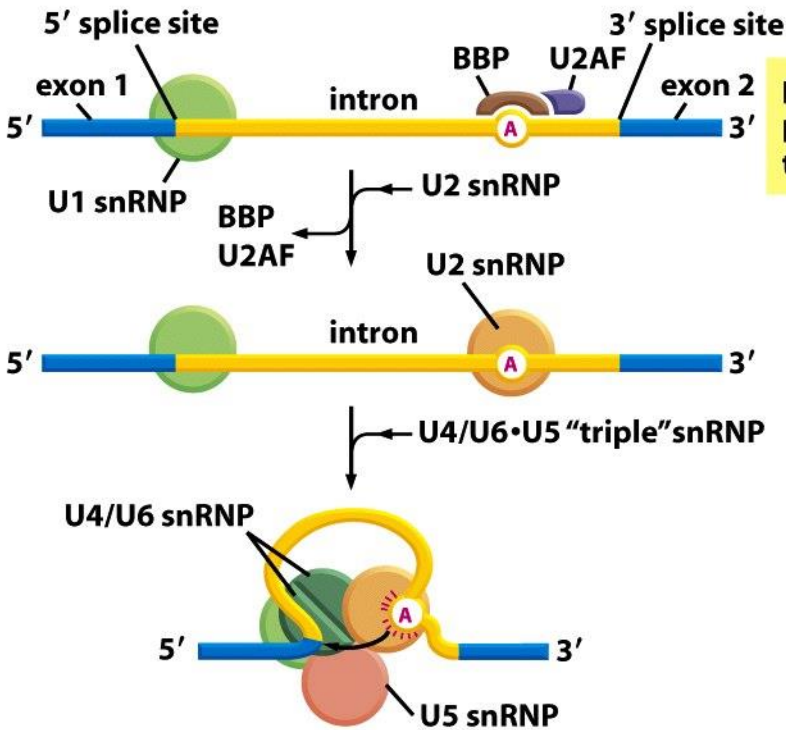
- specify where splicing is to occur;

RNA Splicing Is Performed by the Spliceosome

- participate in the chemistry of splicing;

- RNA molecules - relatively short (< 200 nucleotides);

- five of them (U1, U2, U4, U5, and U6) involved in the major form of pre-mRNA splicing.



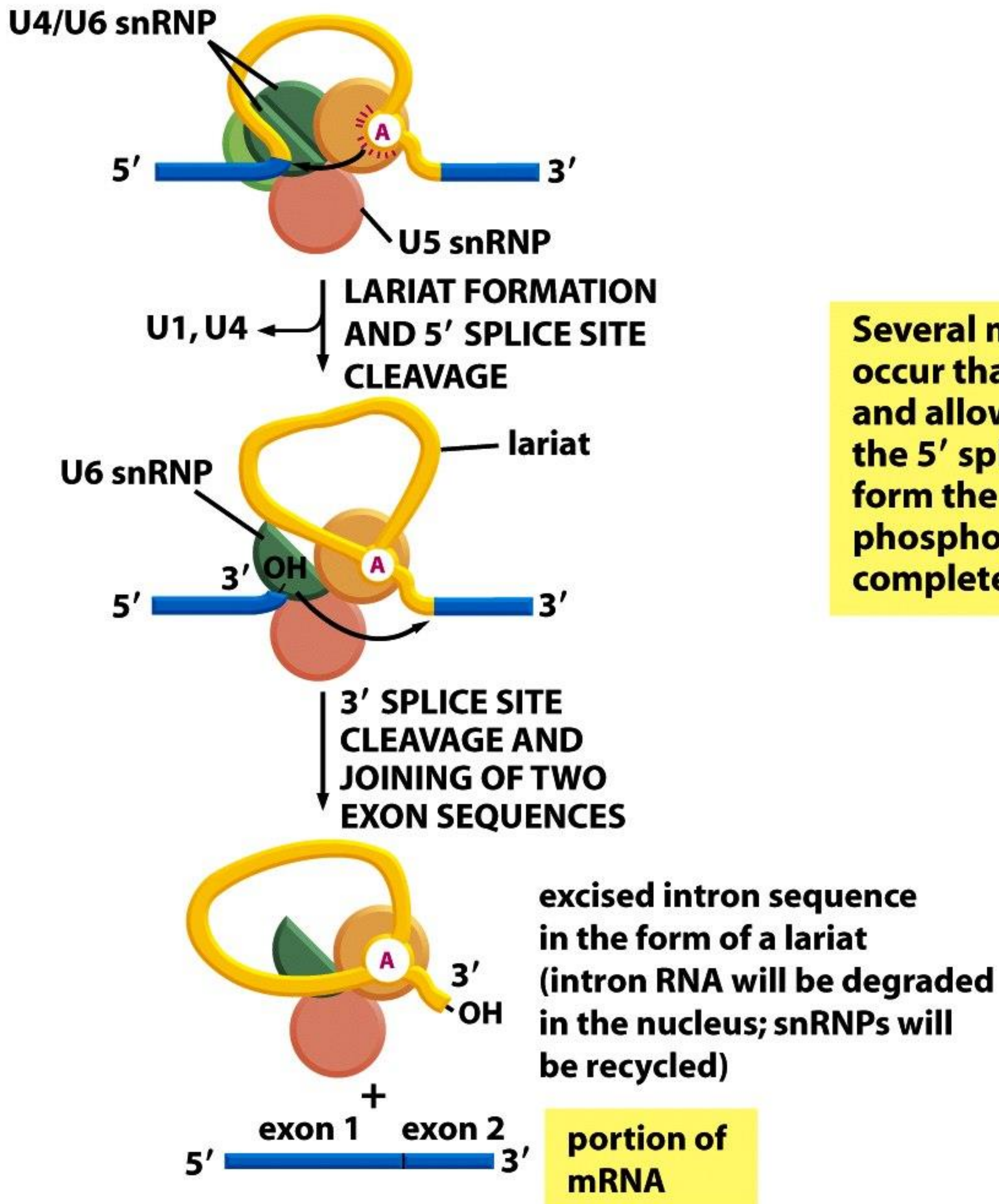
portion of a pre-mRNA transcript

The U1 snRNP forms base pairs with the 5′ splice junction (see Figure 6–30A) and the BBP (branch-point binding protein) and U2AF (U2 auxilliary factor) recognize the branch-point site.

The U2 snRNP displaces BBP and U2AF and forms base pairs with the branch-point site consensus sequence (see Figure 6–30B).

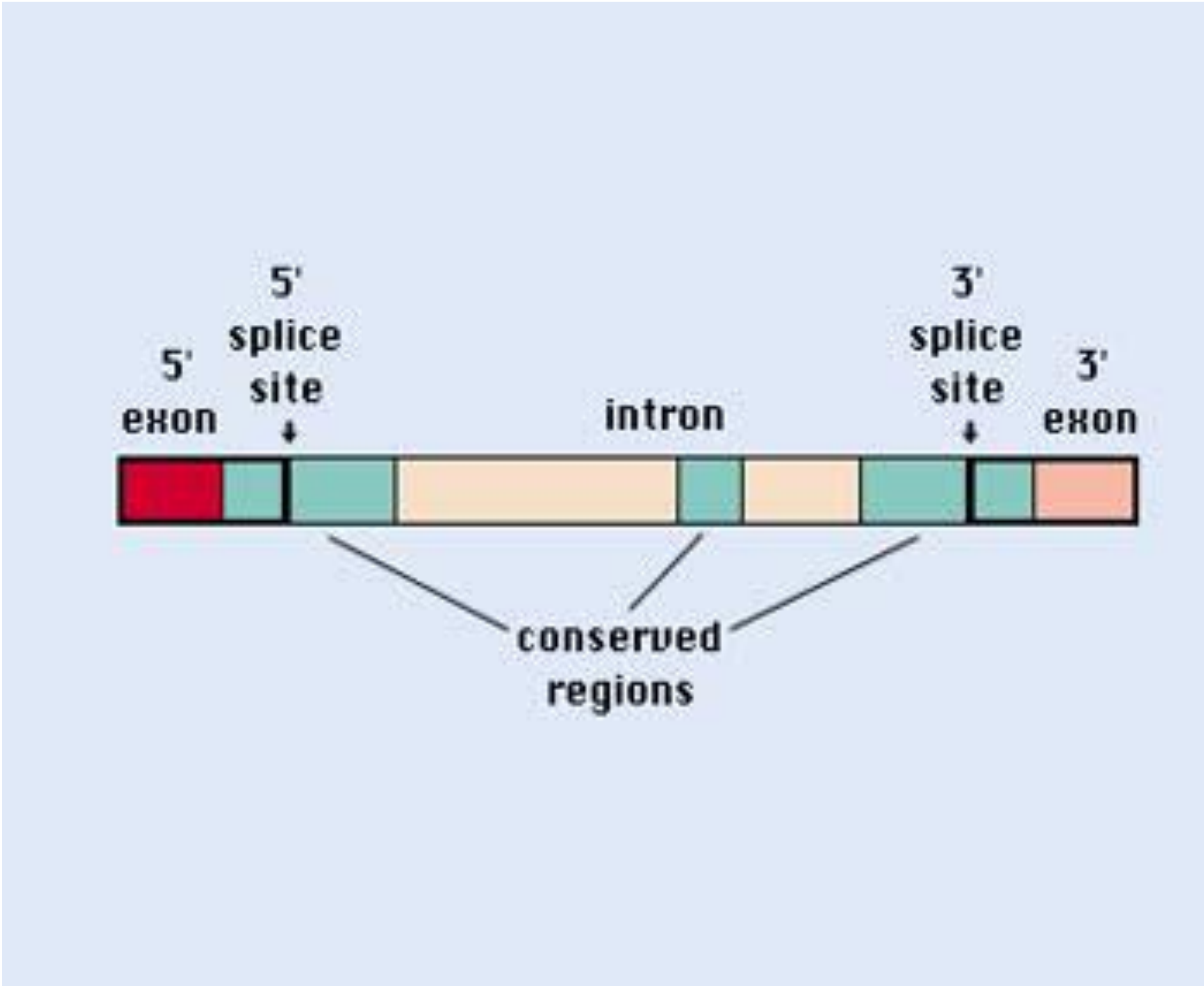
The U4/U6•U5 "triple" snRNP enters the reaction. In this triple snRNP, the U4 and U6 snRNAs are held firmly together by base-pair interactions. Subsequent rearrangements create the active site of the spliceosome and position the appropriate portions of the pre-mRNA substrate for the first phosphoryl-transferase reaction.

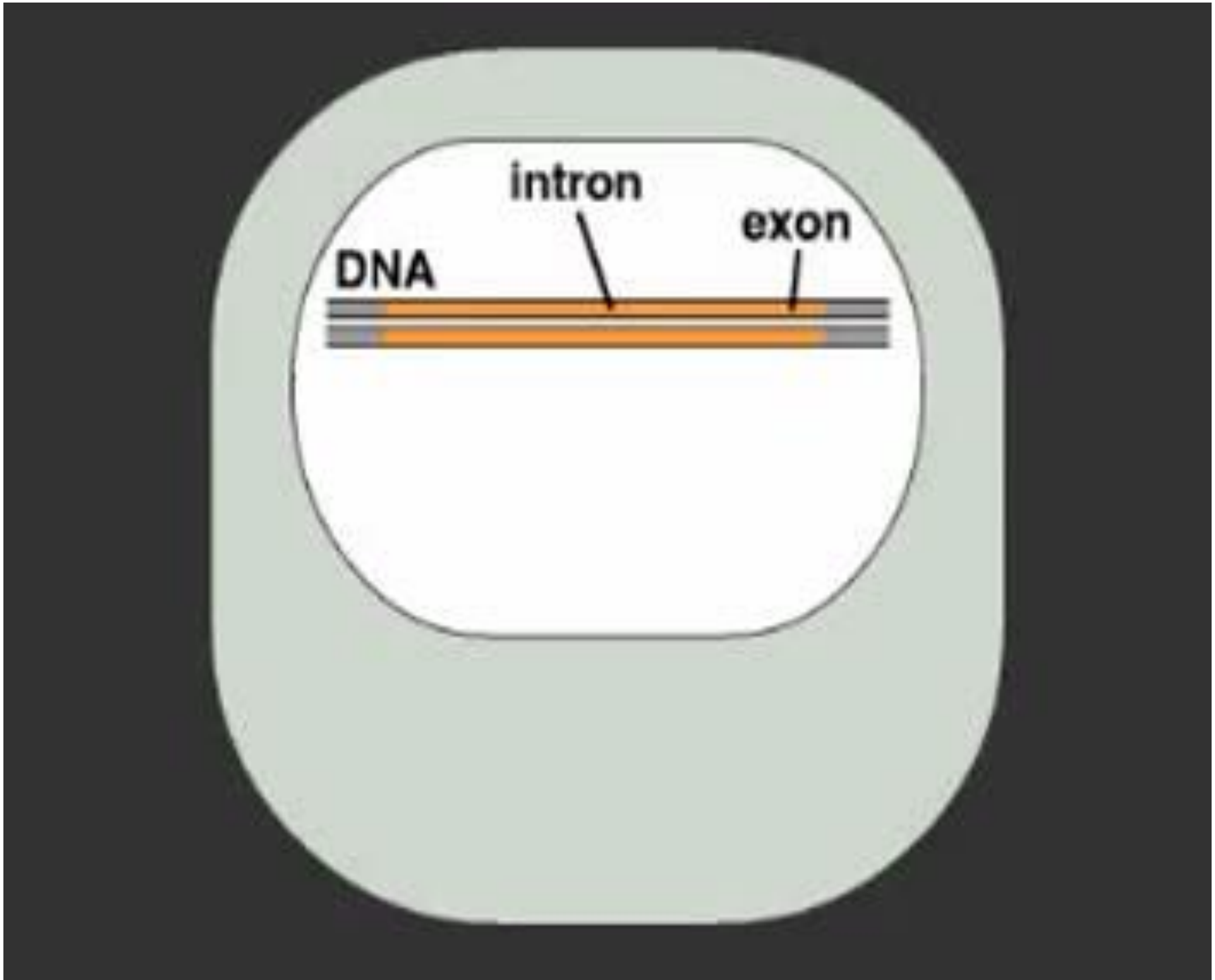
Figure 6-29 (part 1 of 2) *Molecular Biology of the Cell* (© Garland Science 2008)



Several more RNA–RNA rearrangements occur that break apart the U4/U6 base pairs and allow the U6 snRNP to displace U1 at the 5′ splice junction (see Figure 6–30A) to form the active site for the second phosphoryl-transferase reaction, which completes the splice.

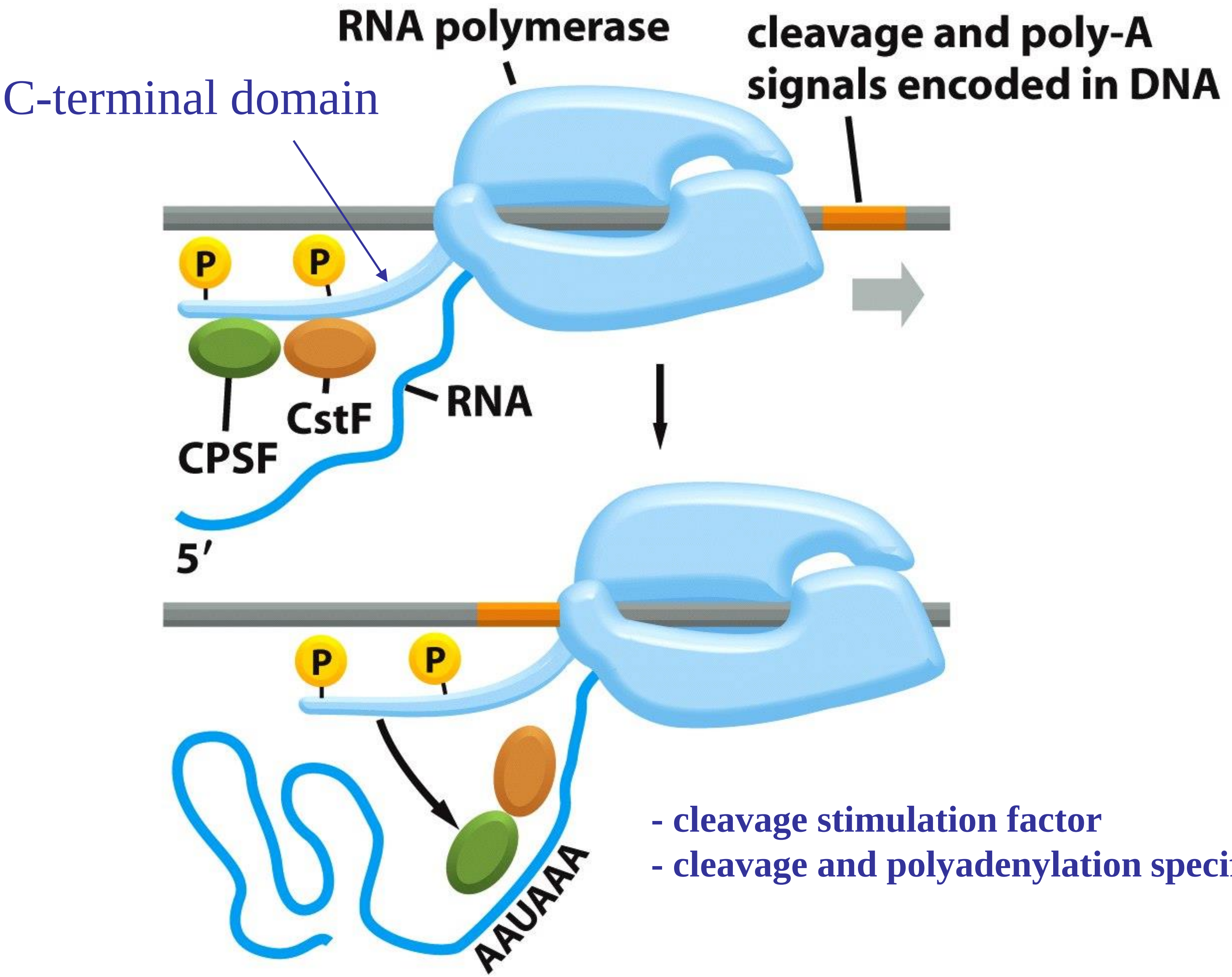
Figure 6-29 (part 2 of 2) *Molecular Biology of the Cell* (© Garland Science 2008)


5' exon
5' splice site
intron
3' splice site
3' exon
conserved regions


intron
exon
DNA

RNA-Processing Enzymes Generate the 3' end of Eucaryotic mRNAs

- Two multisubunit proteins: CstF (cleavage stimulation factor) and CPSF (cleavage and polyadenylation specificity factor);



- cleavage stimulation factor
- cleavage and polyadenylation specificity factor

Figure 6-38 (part 1 of 3) *Molecular Biology of the Cell* (© Garland Science 2008)

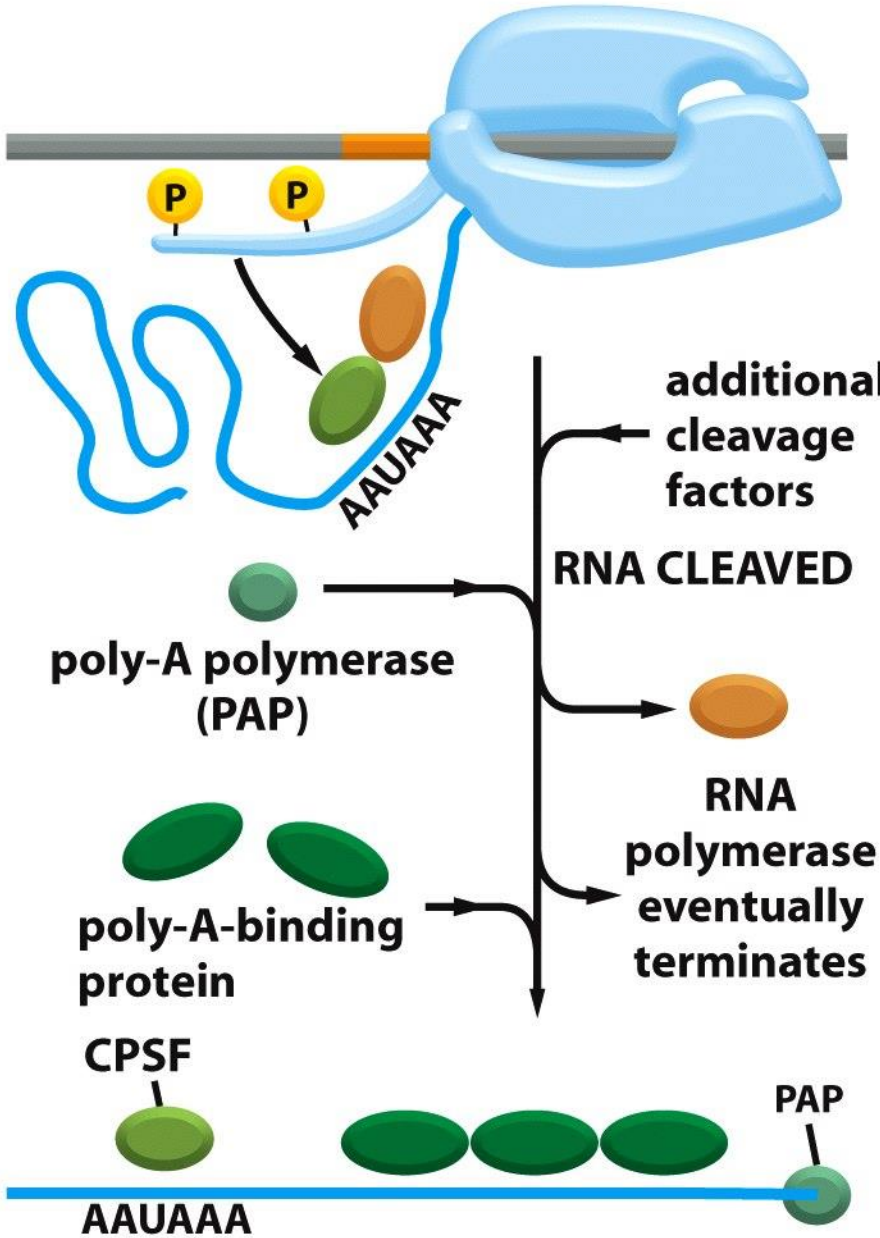


Figure 6-38 (part 2 of 3) *Molecular Biology of the Cell* (© Garland Science 2008)

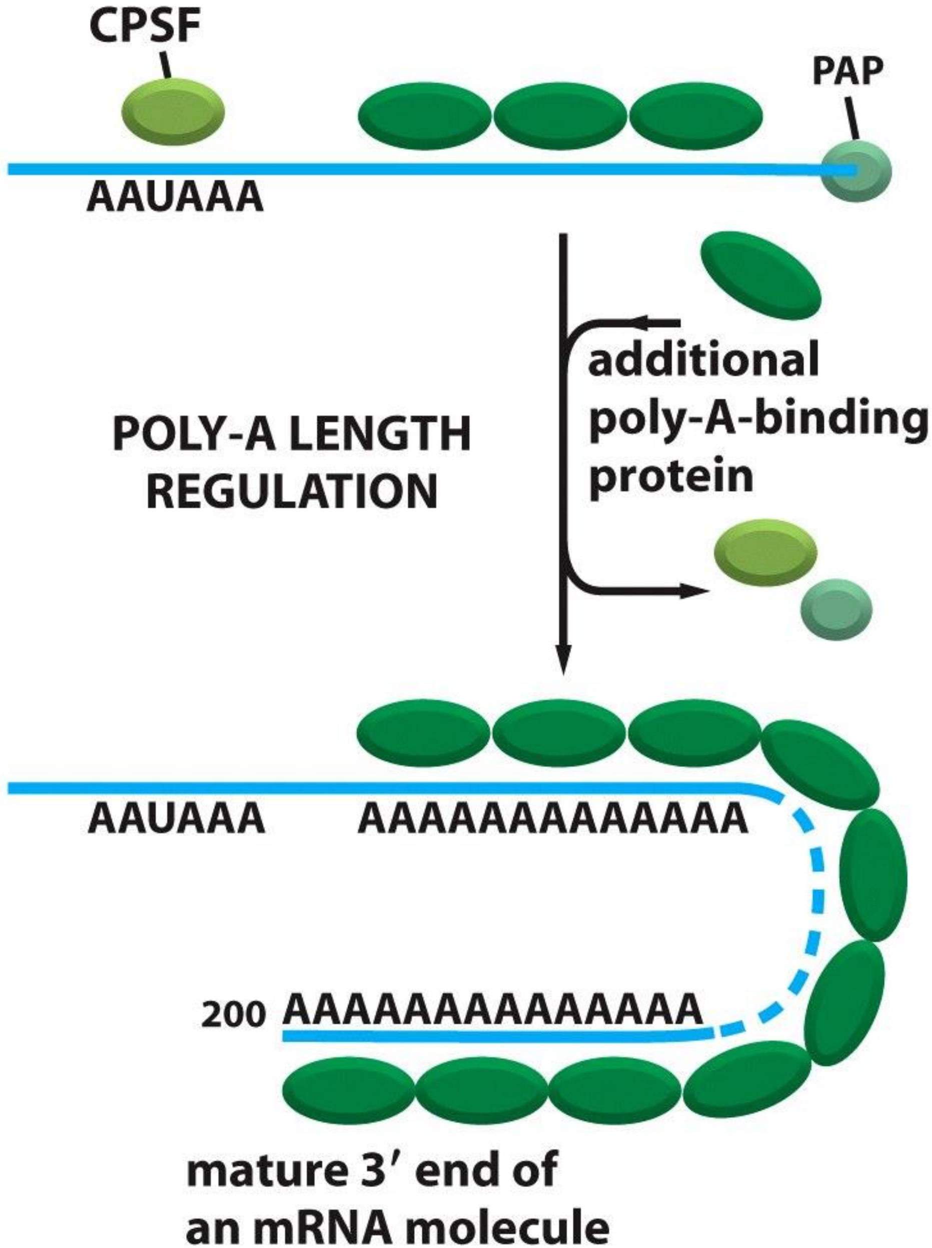


Figure 6-38 (part 3 of 3) *Molecular Biology of the Cell* (© Garland Science 2008)

Mature Eucaryotic mRNAs Are Selectively Exported from the Nucleus

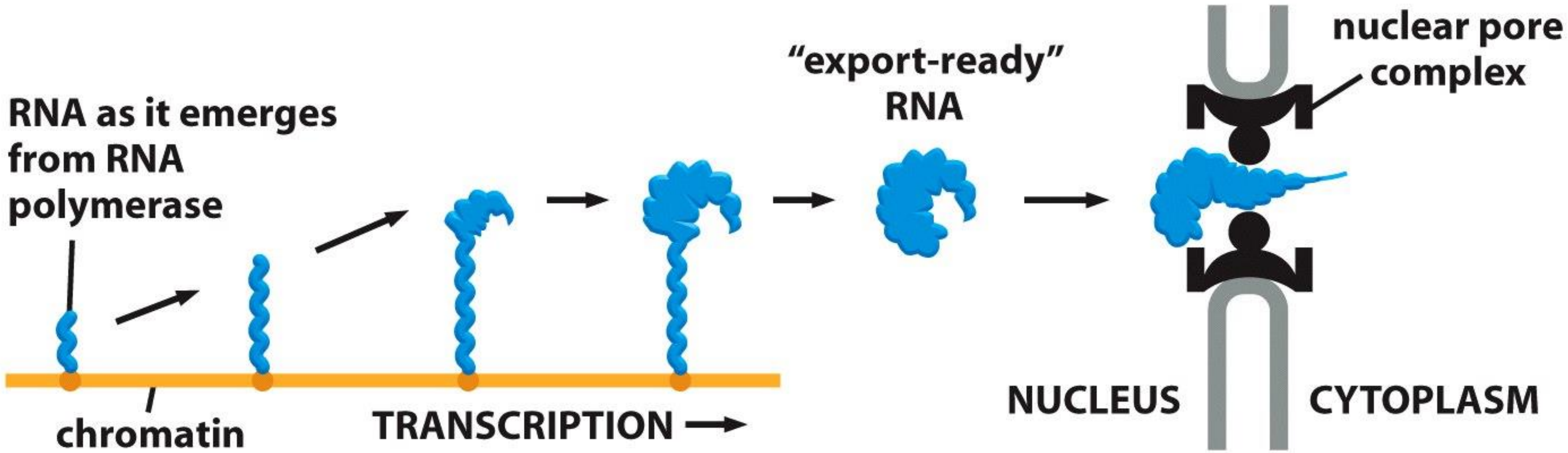


Figure 6-39a *Molecular Biology of the Cell* (© Garland Science 2008)

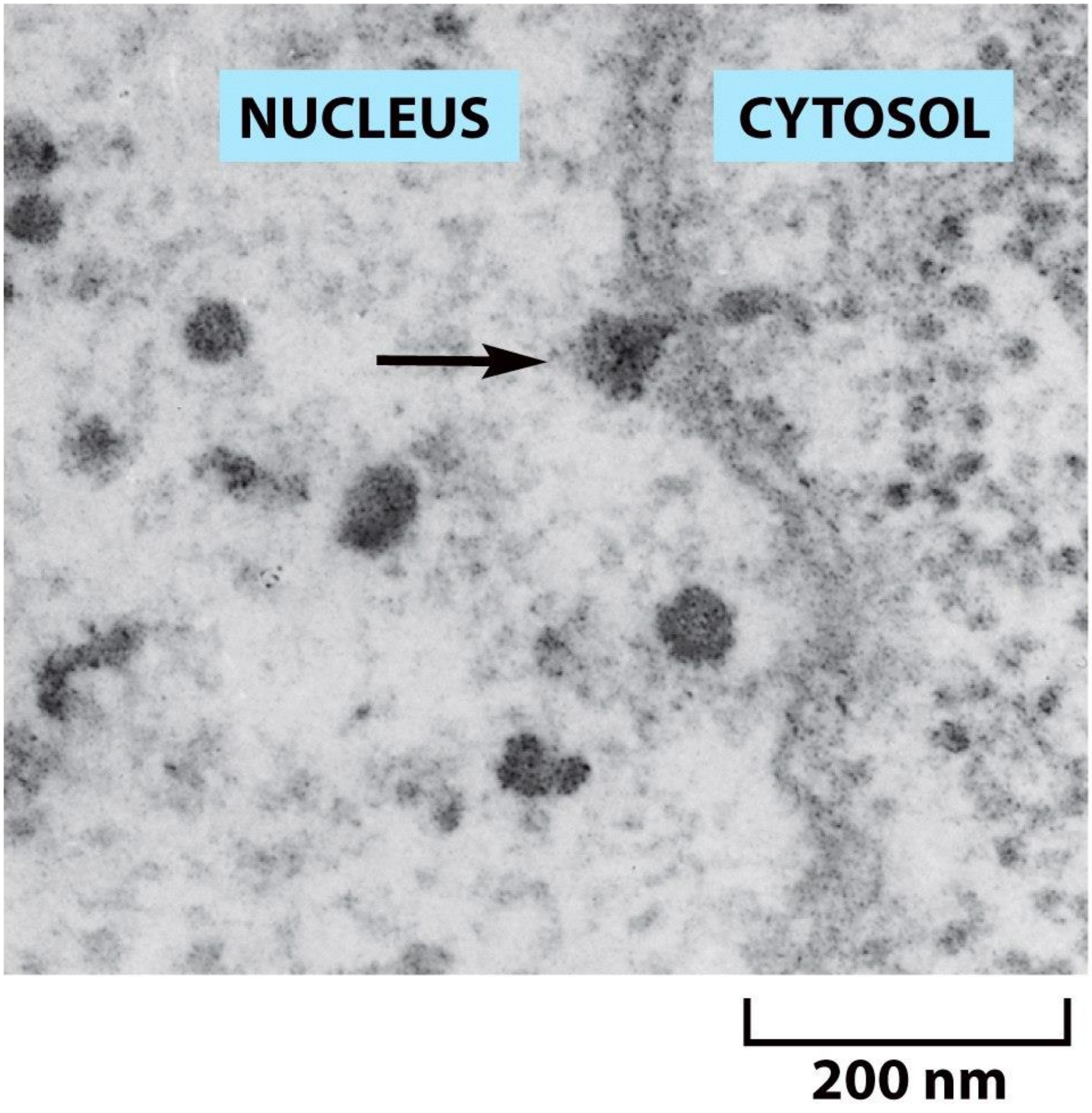


Figure 6-39b *Molecular Biology of the Cell* (© Garland Science 2008)

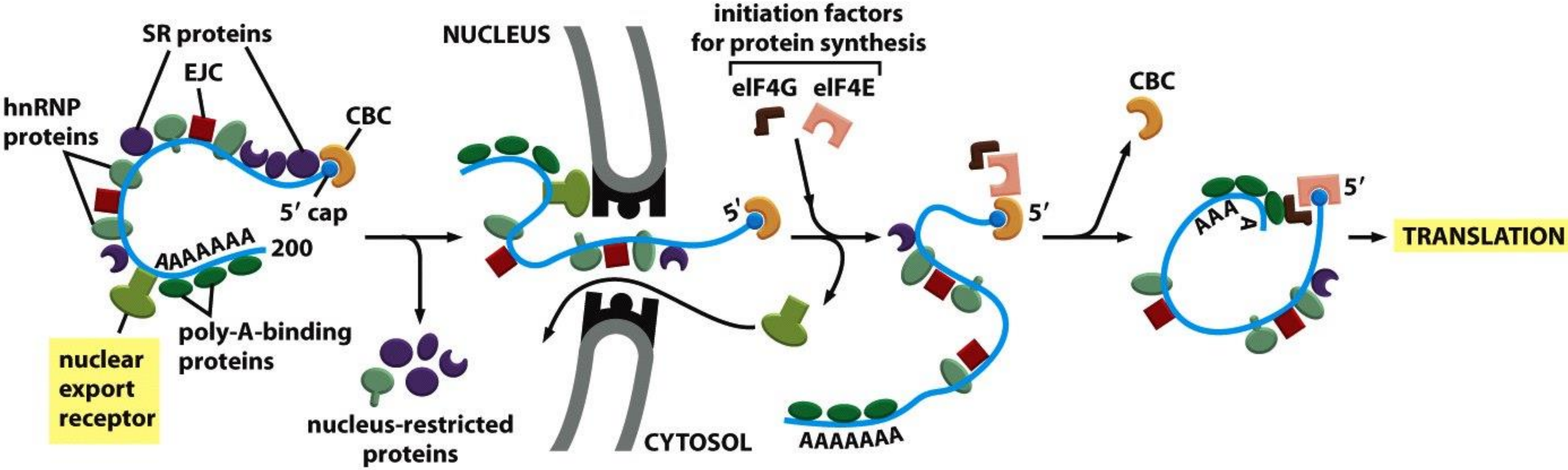


Figure 6-40 *Molecular Biology of the Cell* (© Garland Science 2008)

FROM RNA TO PROTEIN

An mRNA Sequence Is Decoded in Sets of Three Nucleotides

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	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
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
A	R	D	N	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V	

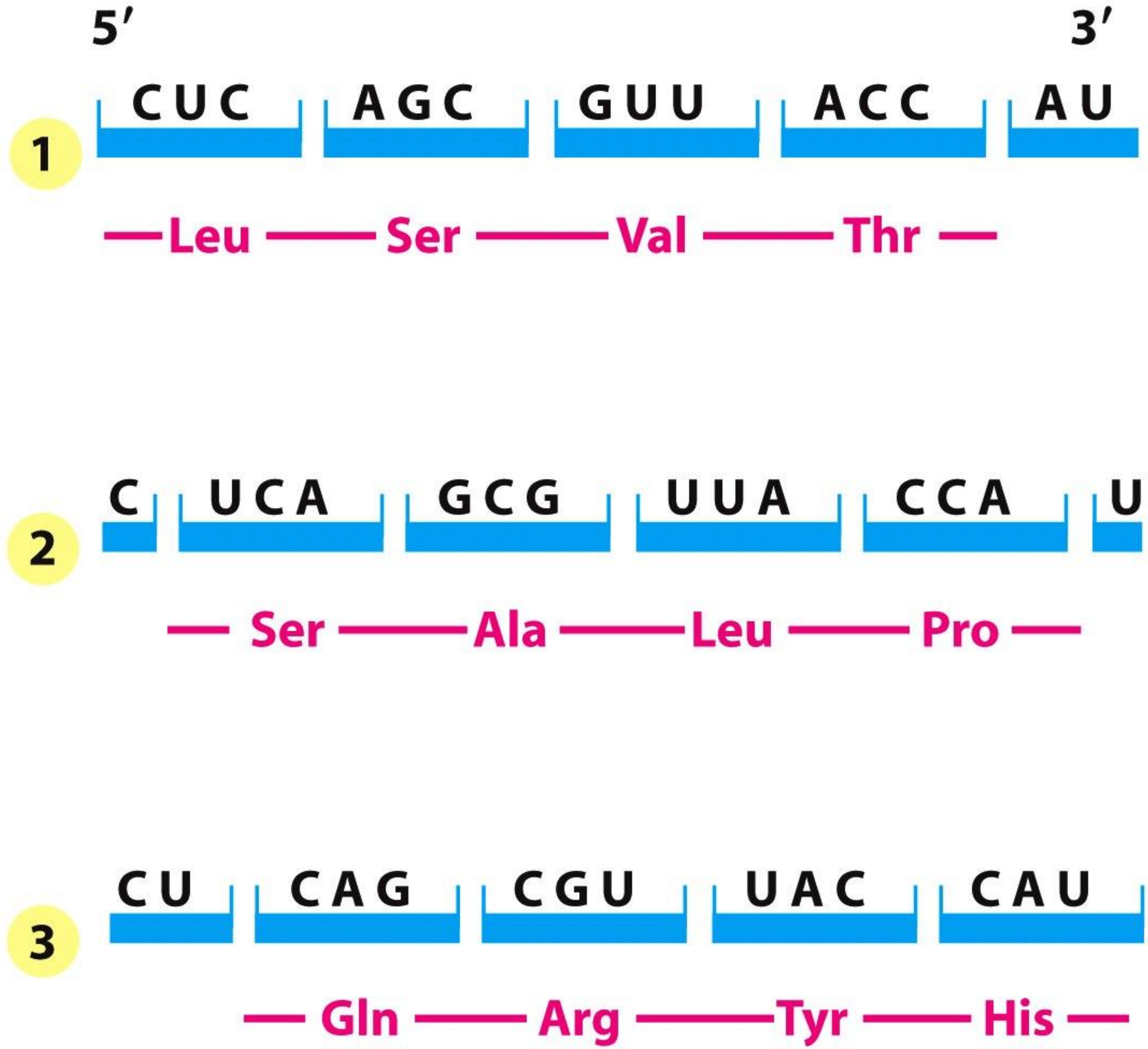


Figure 6-51 *Molecular Biology of the Cell* (© Garland Science 2008)

Key “actors” - Translation

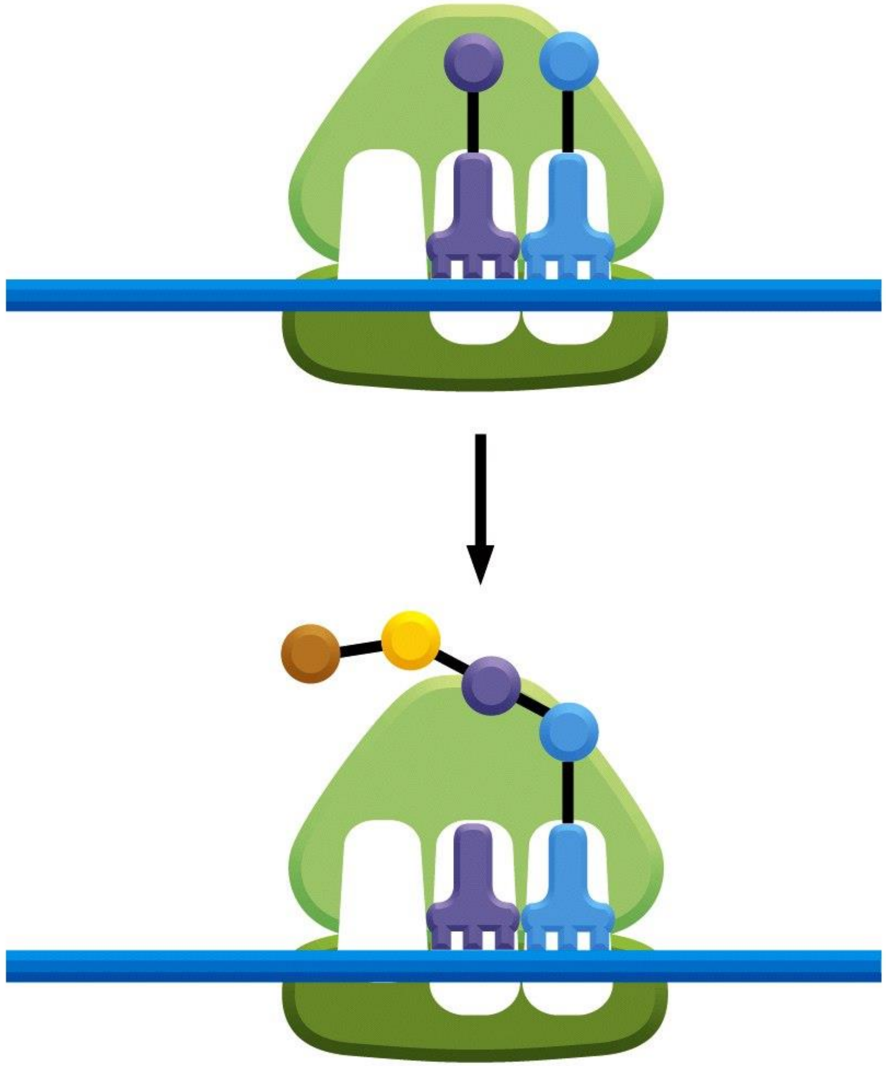


Figure 6-67 (part 5 of 7) *Molecular Biology of the Cell* (© Garland Science 2008)

tRNA Molecules Match Amino Acids to Codons in mRNA

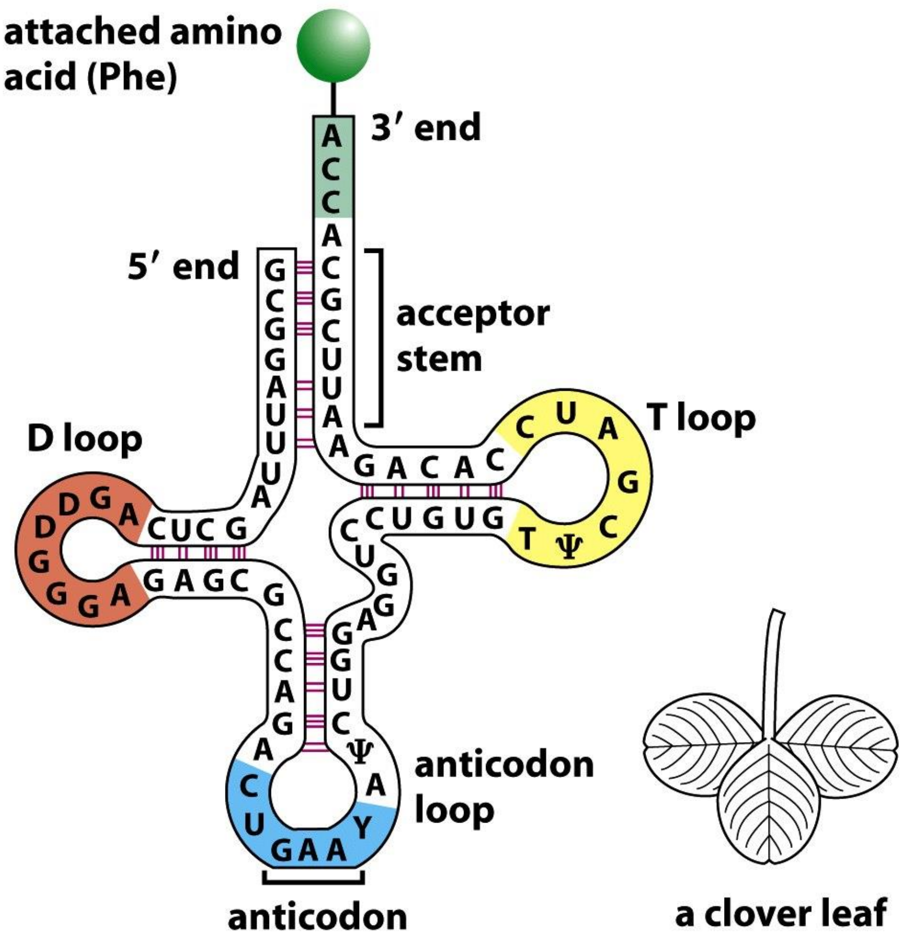


Figure 6-52a *Molecular Biology of the Cell* (© Garland Science 2008)

tRNA

Figure 6-52d *Molecular Biology of the Cell* (© Garland Science 2008)

tRNA - Phe

5′ GCGGAUUUAGCUCAGDDGGGAGAGCGCCAGACUGAAYAΨCUGGAGGUCCUGUGTΨCGAUCCACAGAAUUCGCACCA 3′

anticodon

Figure 6-52d *Molecular Biology of the Cell* (© Garland Science 2008)

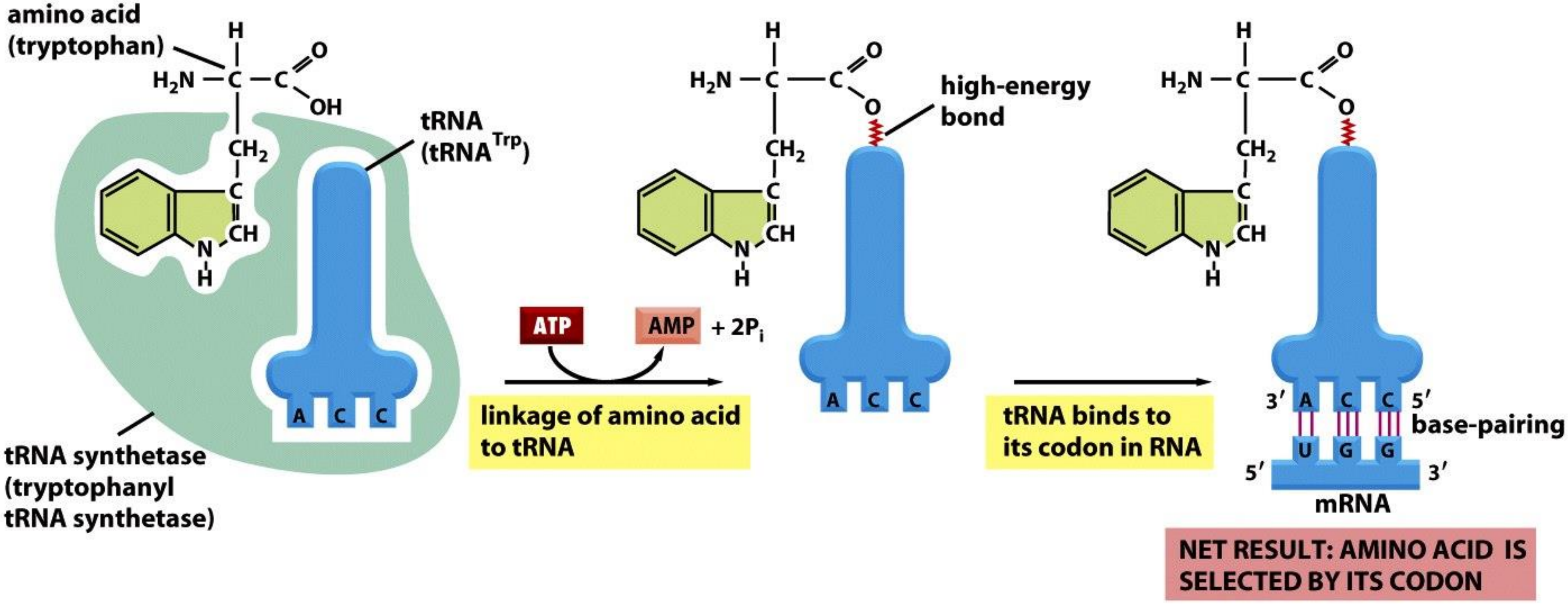
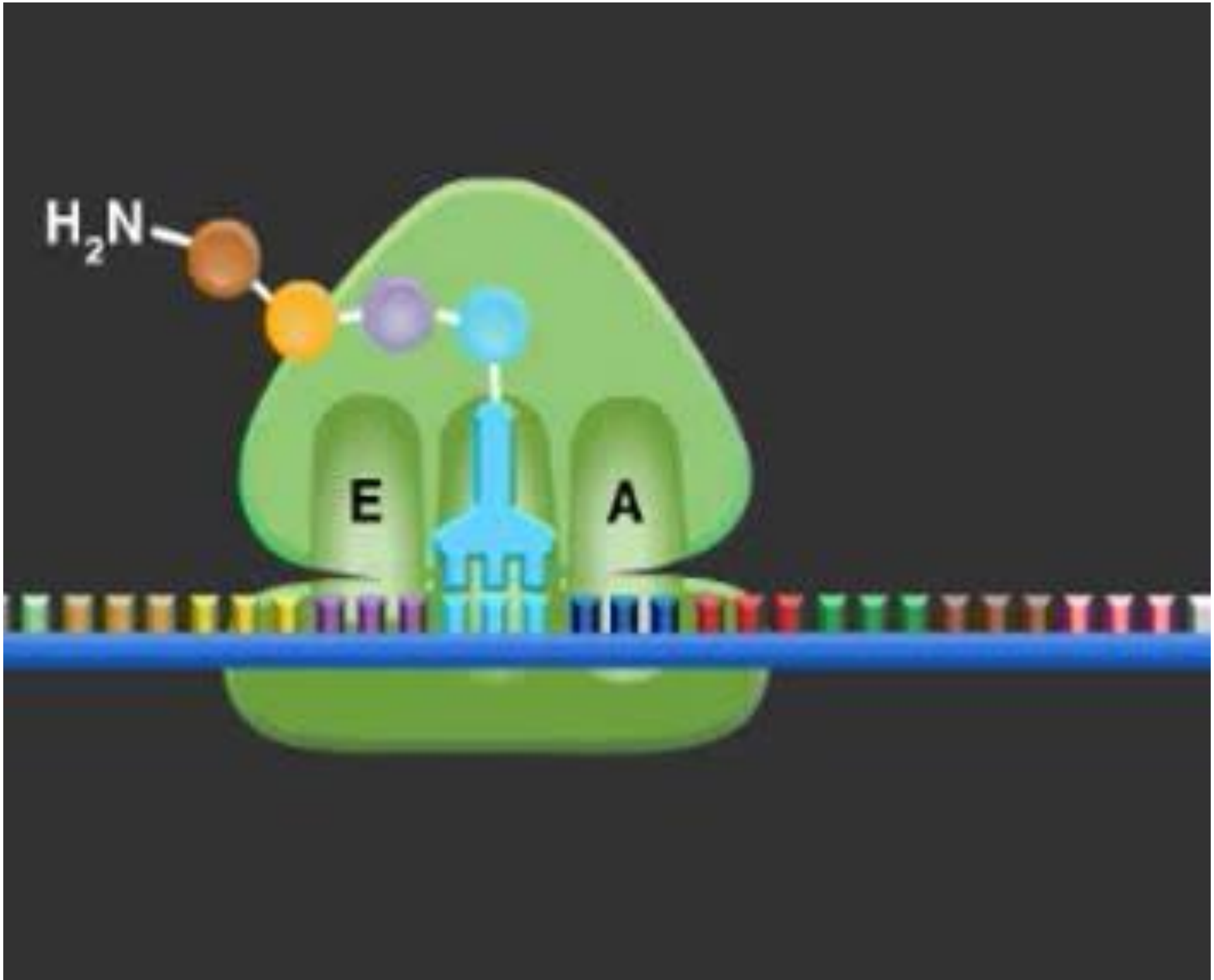
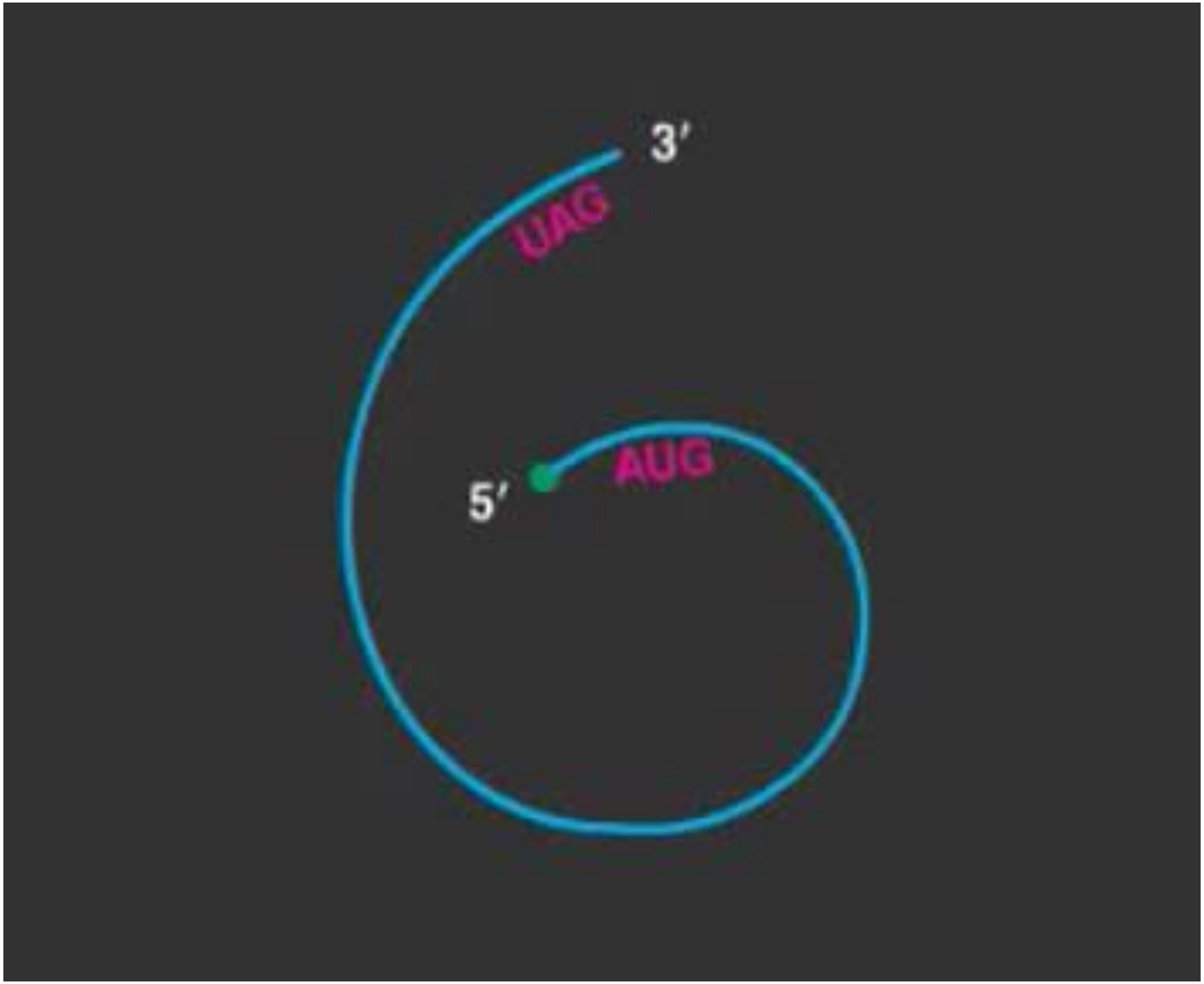
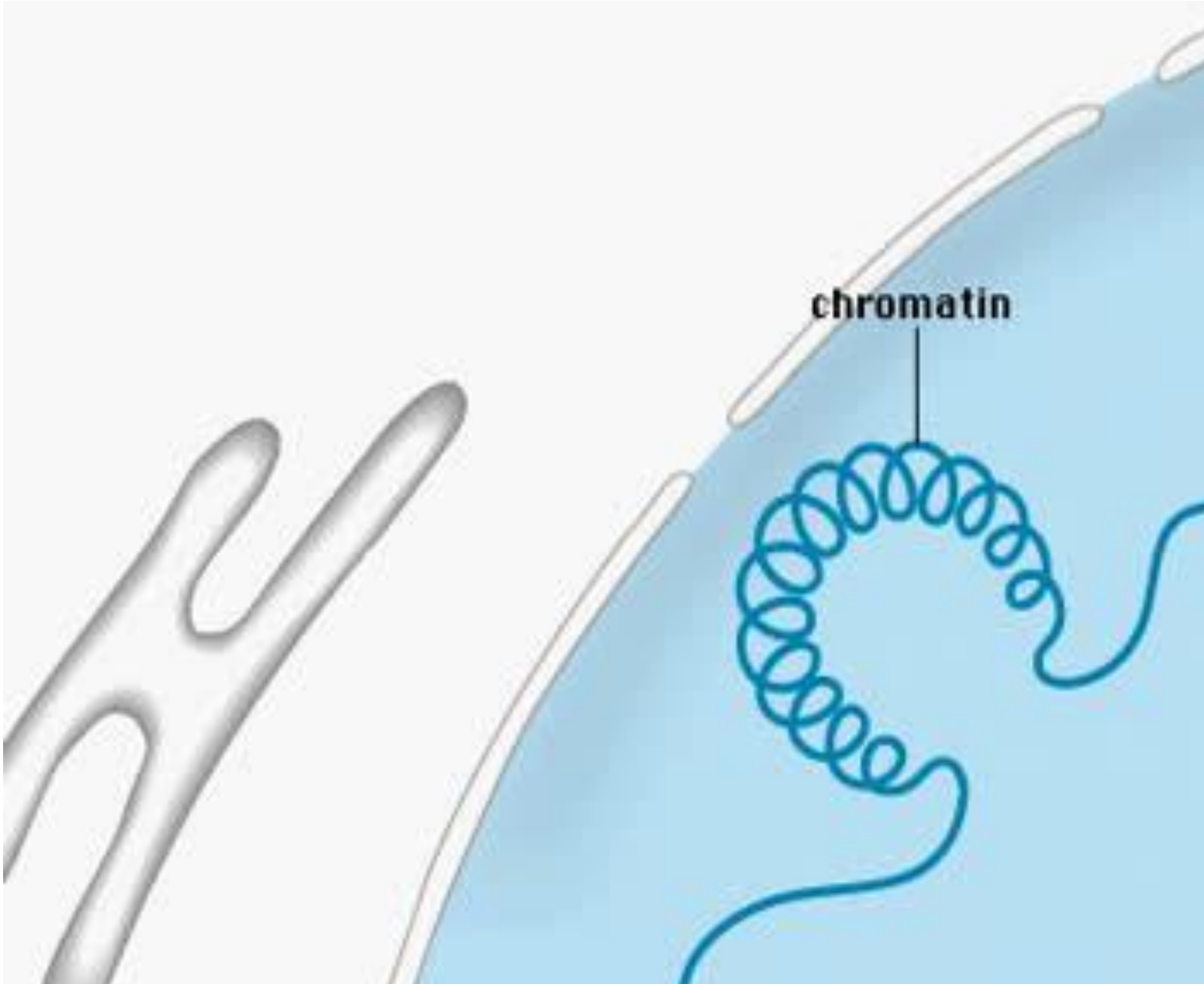


Figure 6-58 *Molecular Biology of the Cell* (© Garland Science 2008)

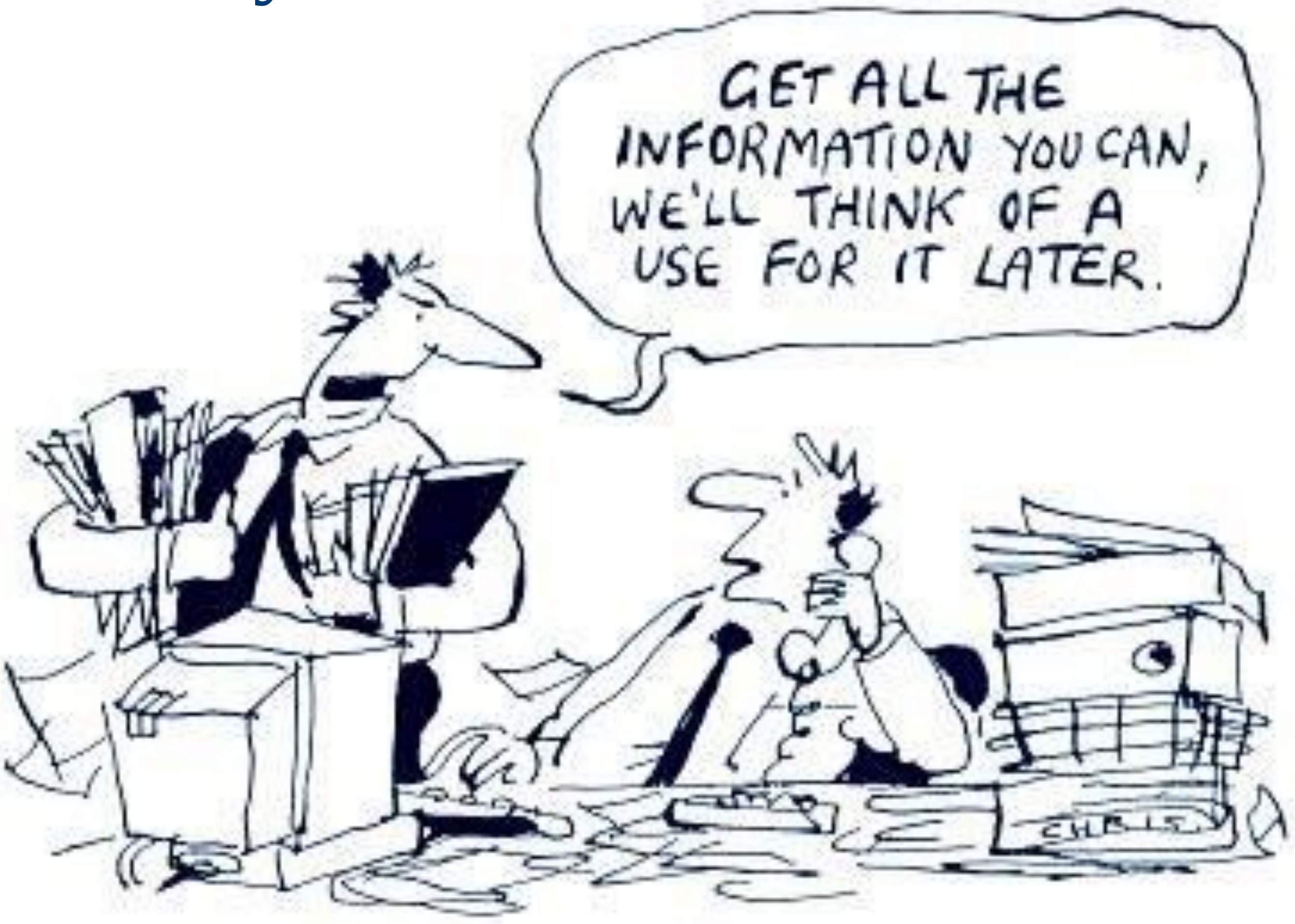

H2N
E
A

[redacted]


3′
UAG
5′
AUG


chromatin

Thank you!


GET ALL THE INFORMATION YOU CAN, WE'LL THINK OF A USE FOR IT LATER.