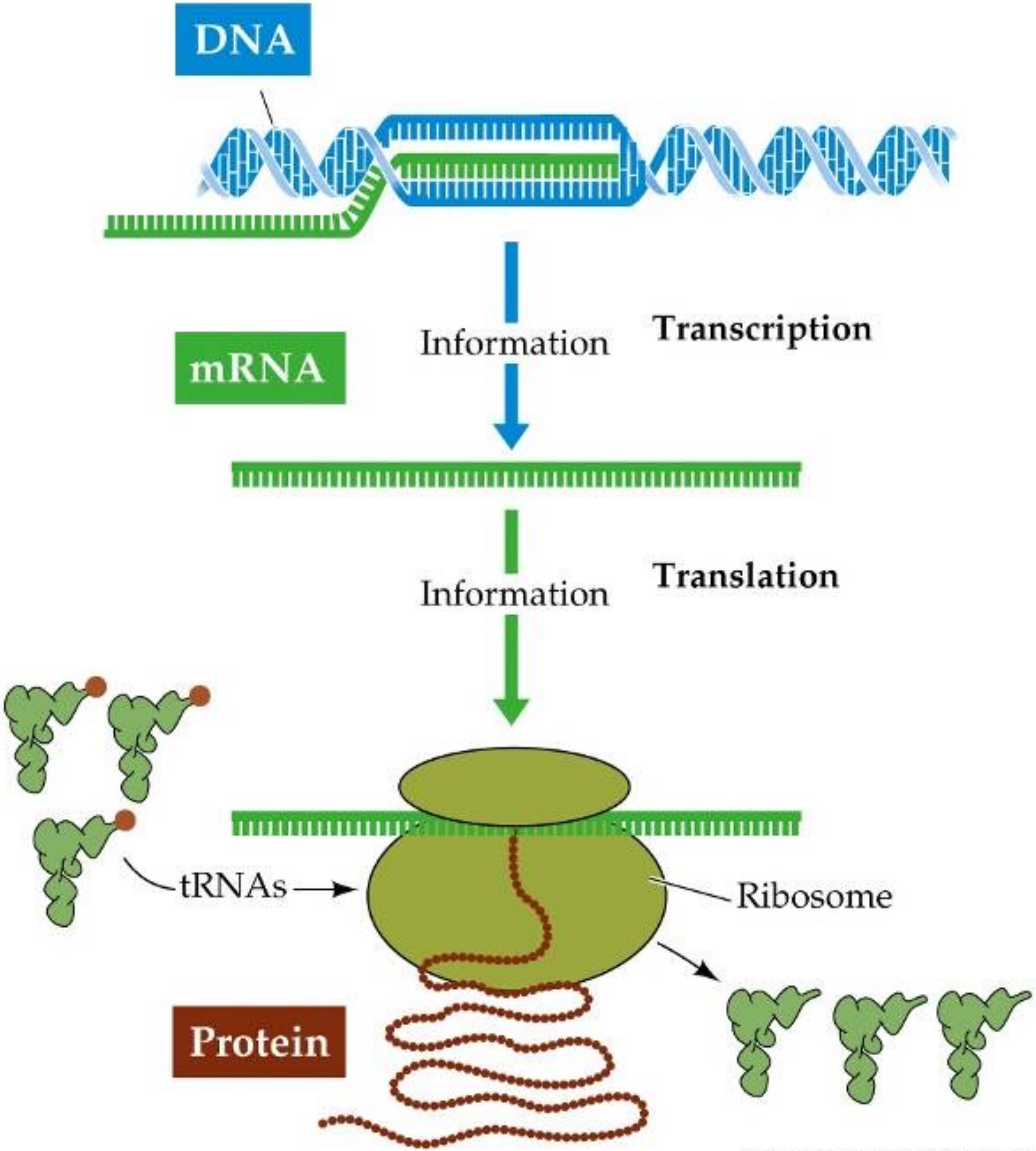
TRASCRIZIONE

- Il DNA è il depositario dell’informazione genetica

- L’informazione genetica controlla la sequenza degli amino acidi nelle proteine ma il DNA non è lo stampo diretto per questa sintesi

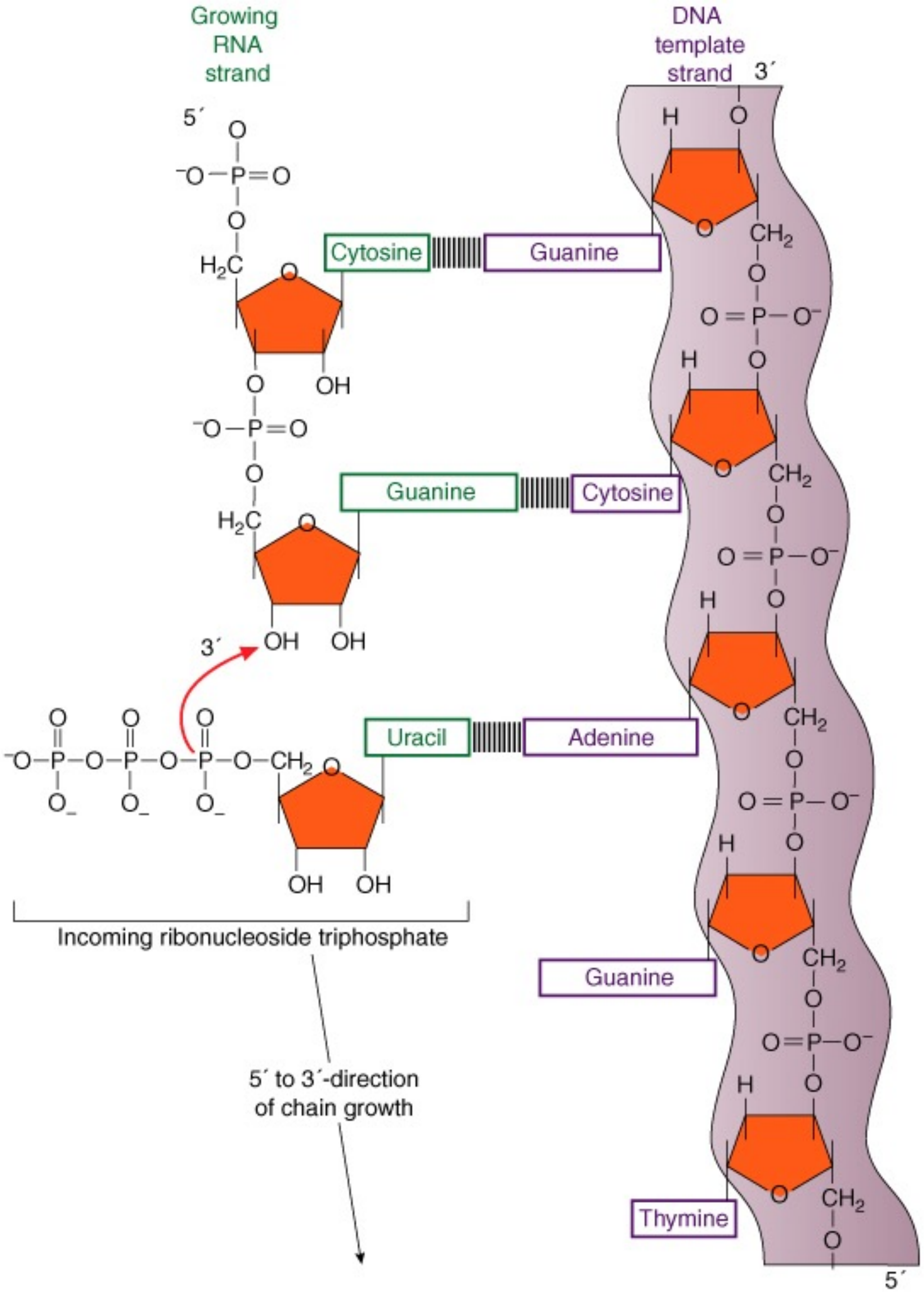
- Esiste una seconda molecola, l’ RNA che: copia l’informazione genetica, la trasferisce e dirige la sintesi delle proteine


DNA
Information
Transcription
mRNA
Information
Translation
tRNAs
Ribosome
Protein

Trascrizione

DNA ----> RNA

- E’ il processo in cui la sequenza nucleotidica del DNA che porta l’informazione per una funzione viene trascritta in una sequenza nucleotidica dell’RNA
- La sequenza dell’RNA è complementare alla sequenza del DNA stampo


Growing RNA strand
DNA template strand
5´
3´
Cytosine
Guanine
Guanine
Cytosine
Uracil
Adenine
Guanine
Thymine
3´
5´
Incoming ribonucleoside triphosphate
5´ to 3´-direction of chain growth

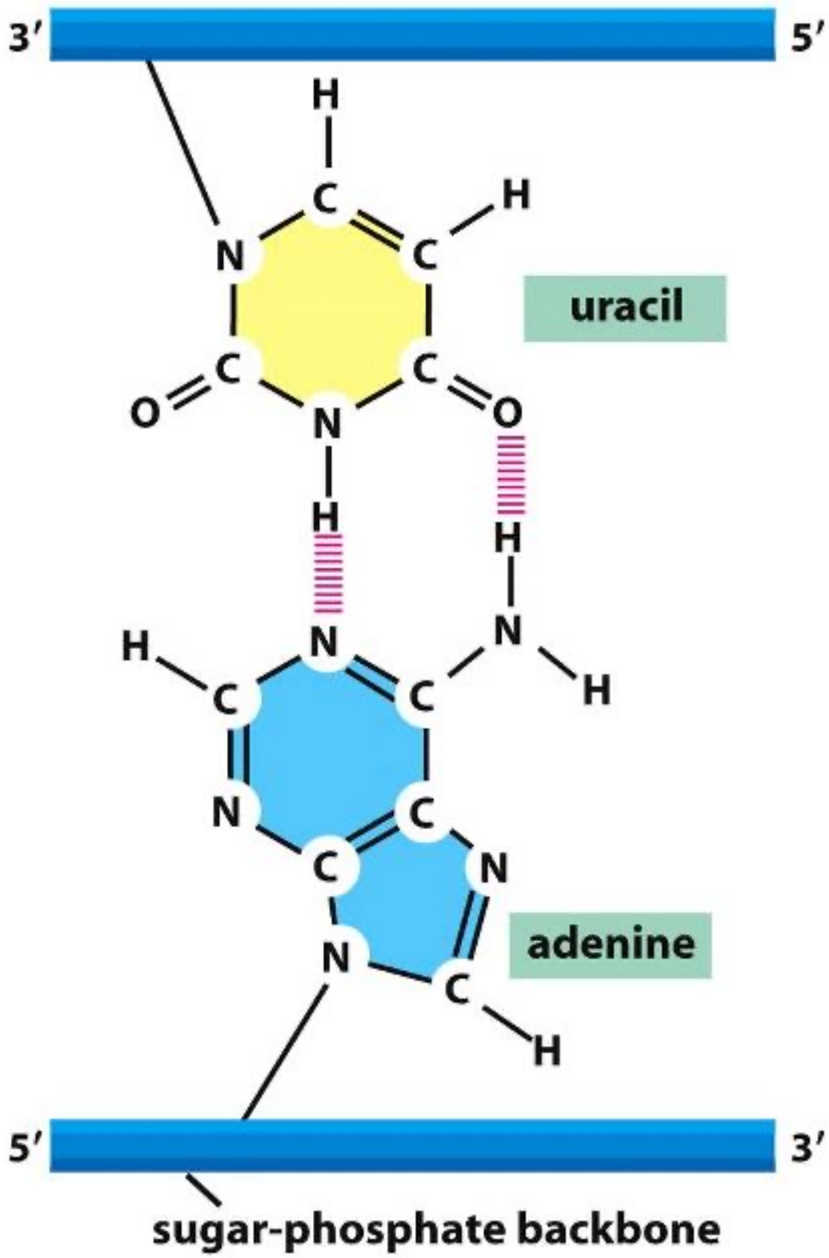


Figure 6-5 *Molecular Biology of the Cell* (© Garland Science 2008)

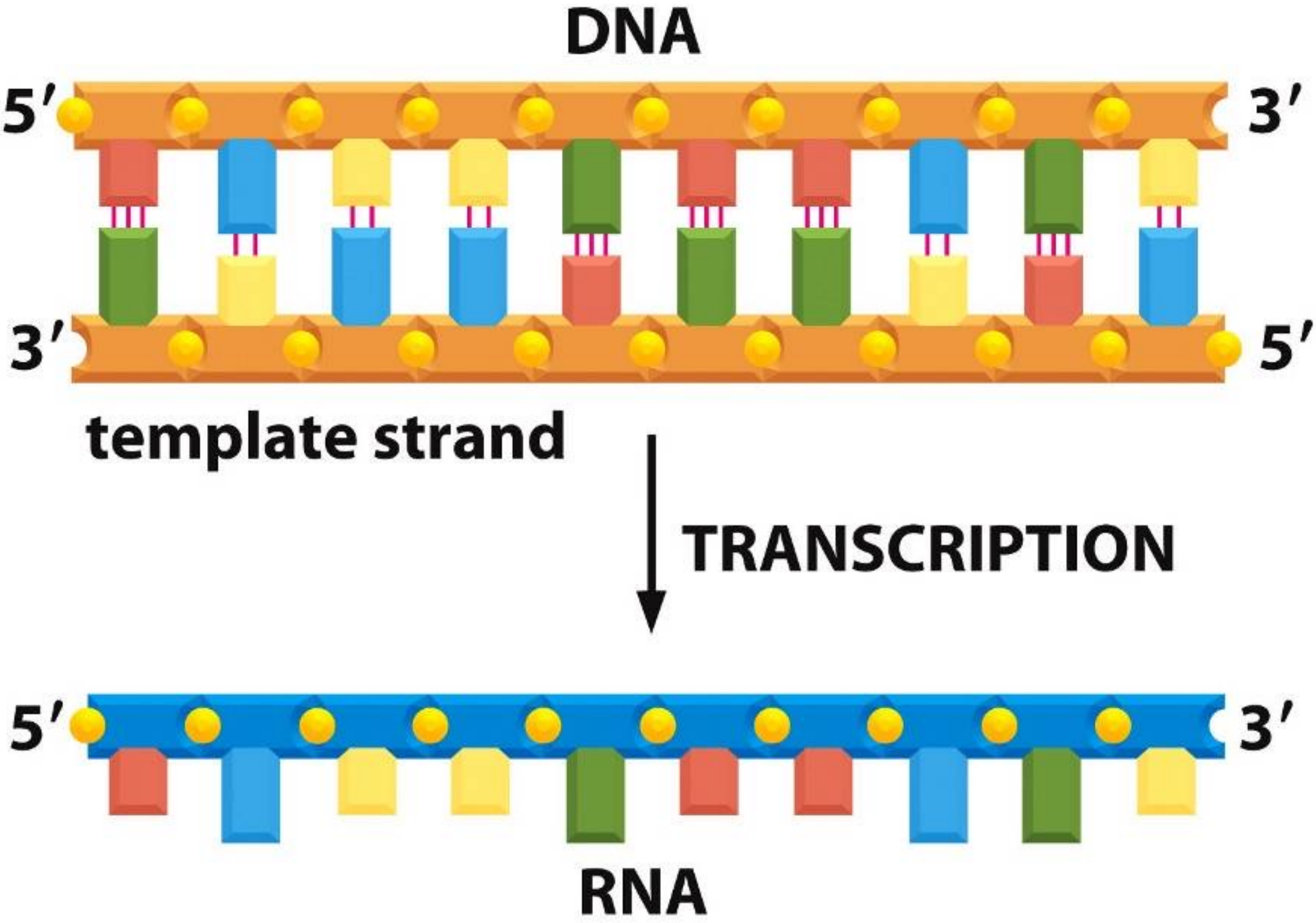


Figure 6-7 *Molecular Biology of the Cell* (© Garland Science 2008)

DNA:

5’-ATGTTACCATTCTAGGGGGACCGA-3’ filamento senso
coding

3’-TACAATGGTAAGATCCCCCTGGCT-5’ filamento antisenso
stampo

RNA polimerasi erasi

TRASCRIZIONE

5’-AUGUUACCAUUCUAGGGGGACCGA-3’ RNA

TRADUZIONE

NH2-Met-Leu-Pro-Cys-Trp-Asp-Asp-COOH Proteina

Quali molecole sono trascritte?

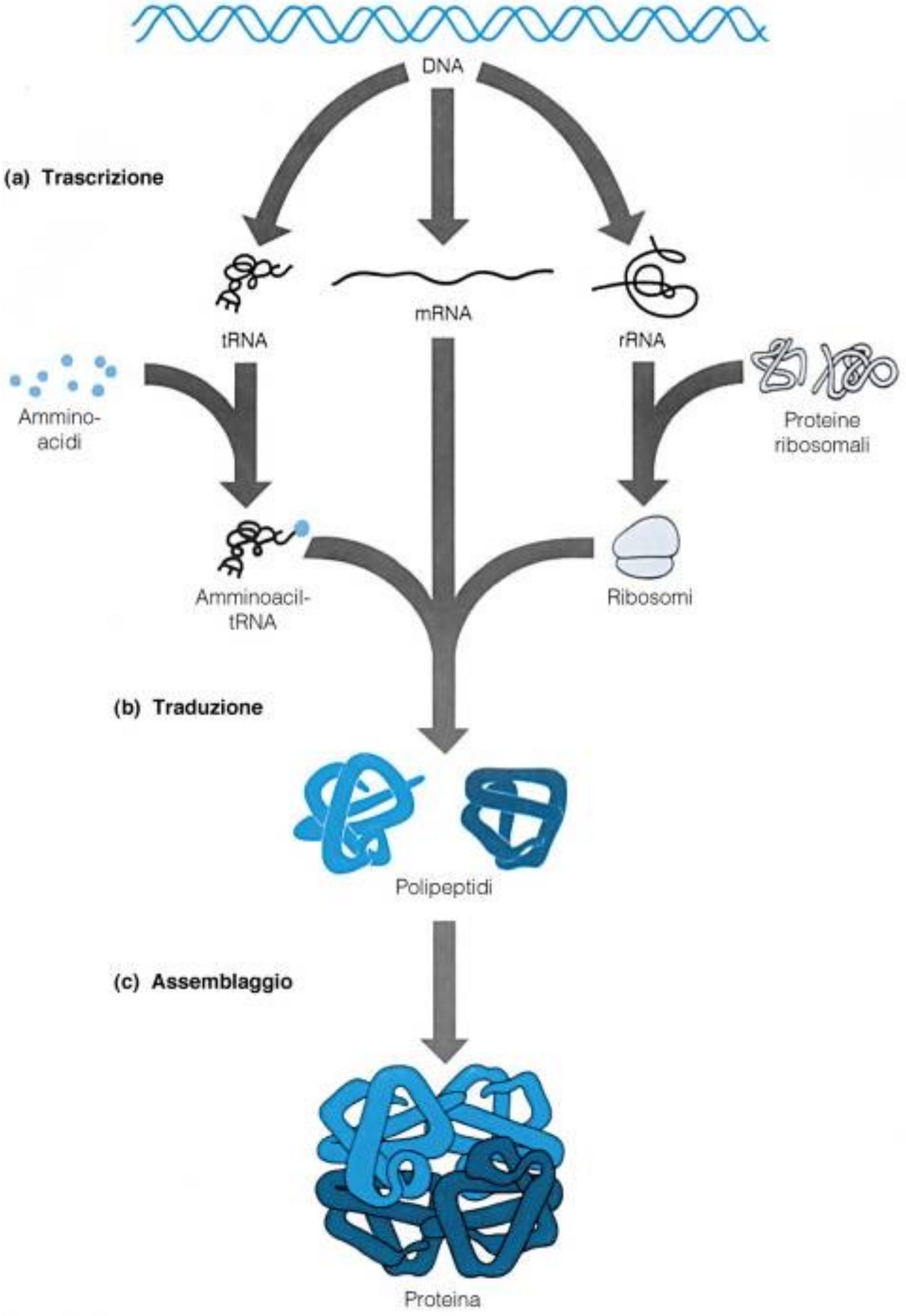


Figura 19-1

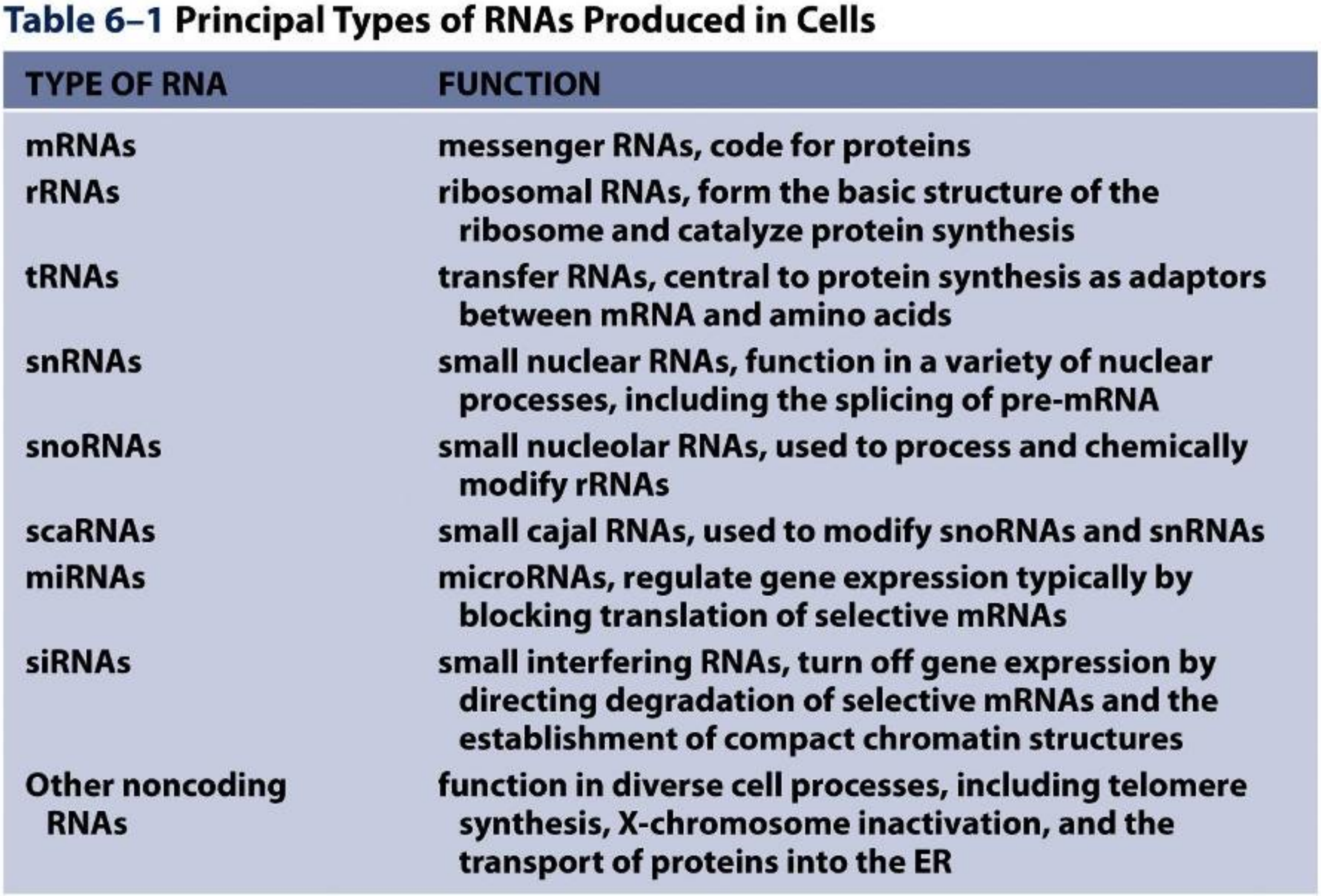


Table 6–1 Principal Types of RNAs Produced in Cells

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)

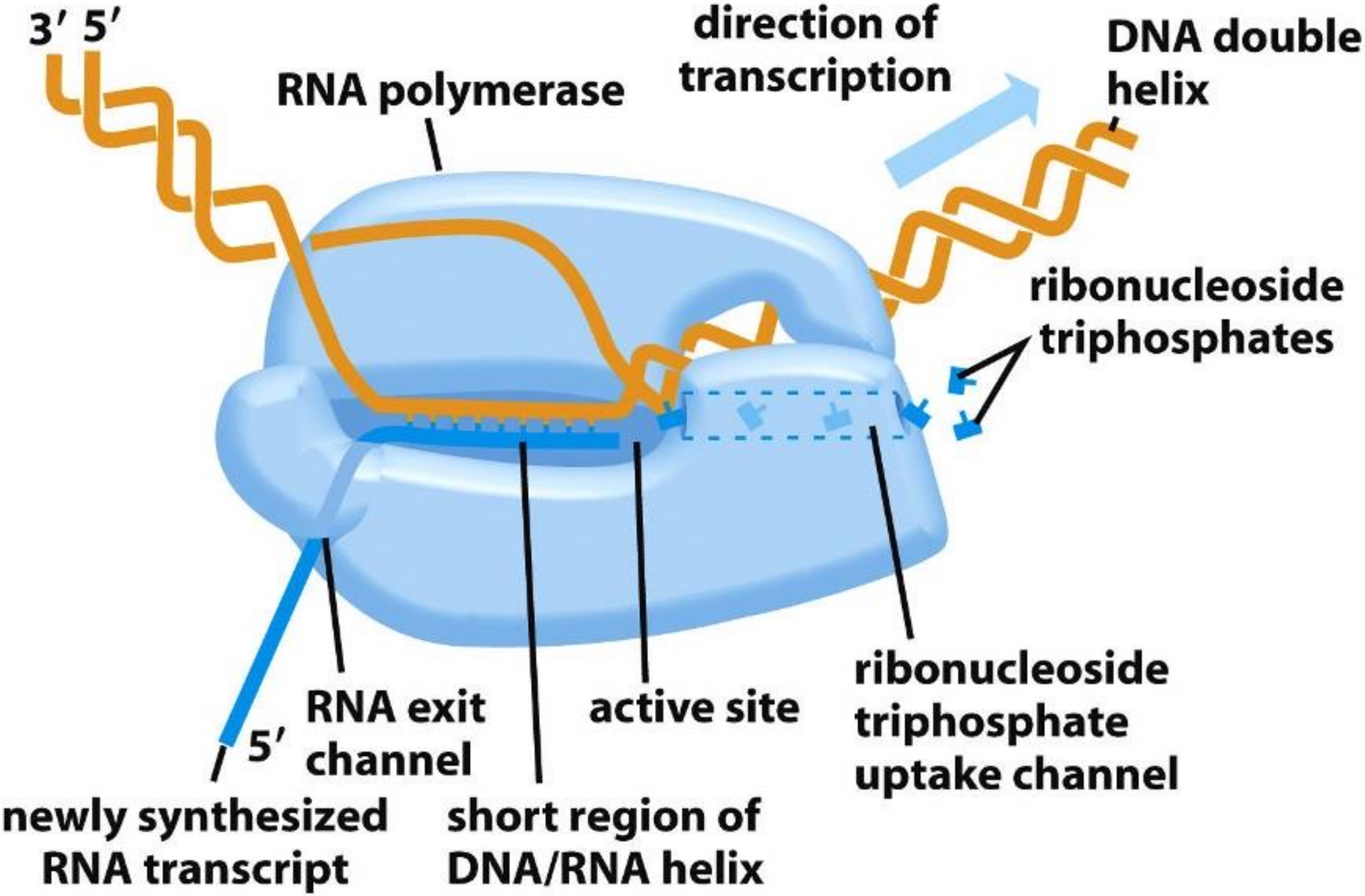


Figure 6-8a *Molecular Biology of the Cell* (© Garland Science 2008)

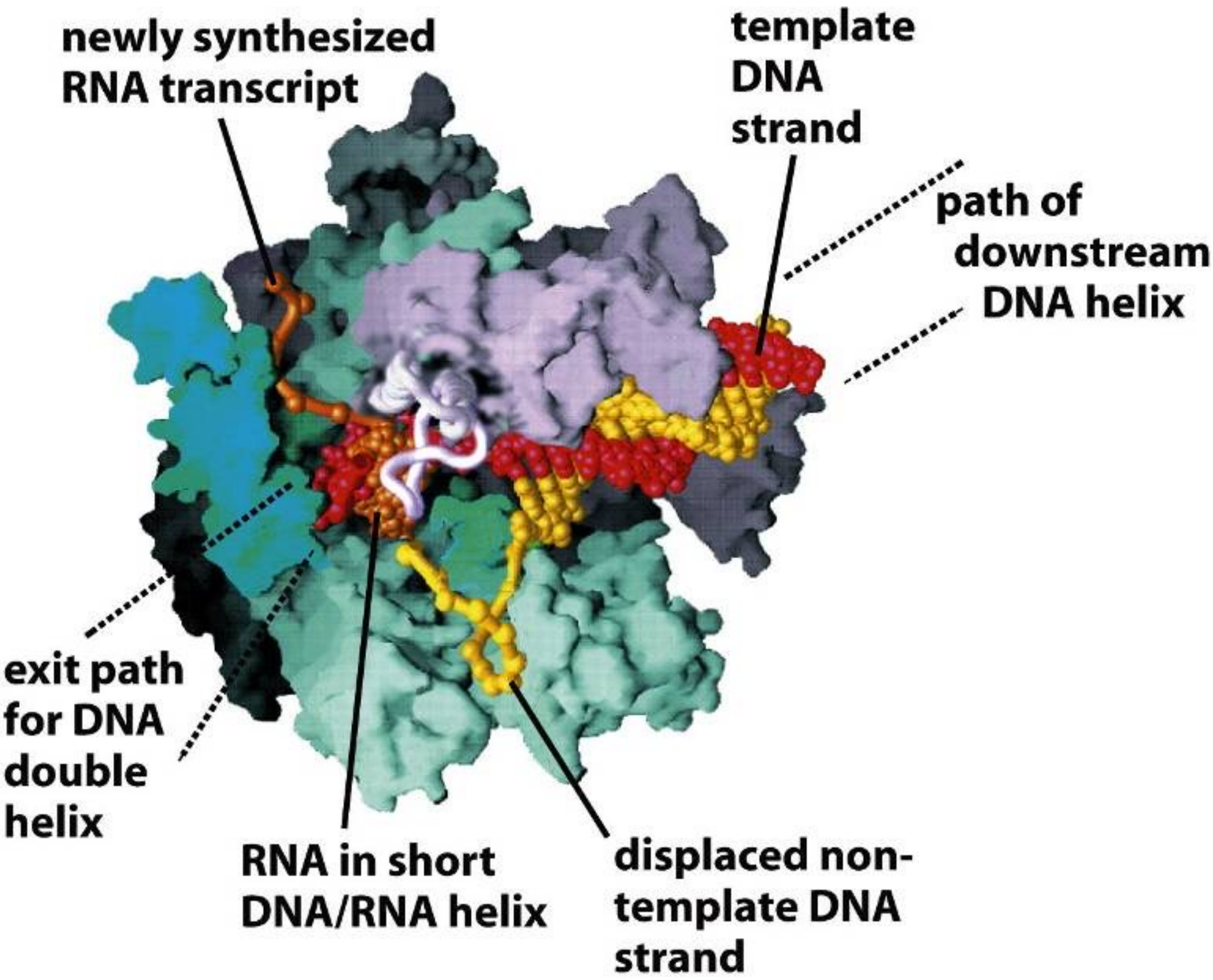


Figure 6-8b *Molecular Biology of the Cell* (© Garland Science 2008)

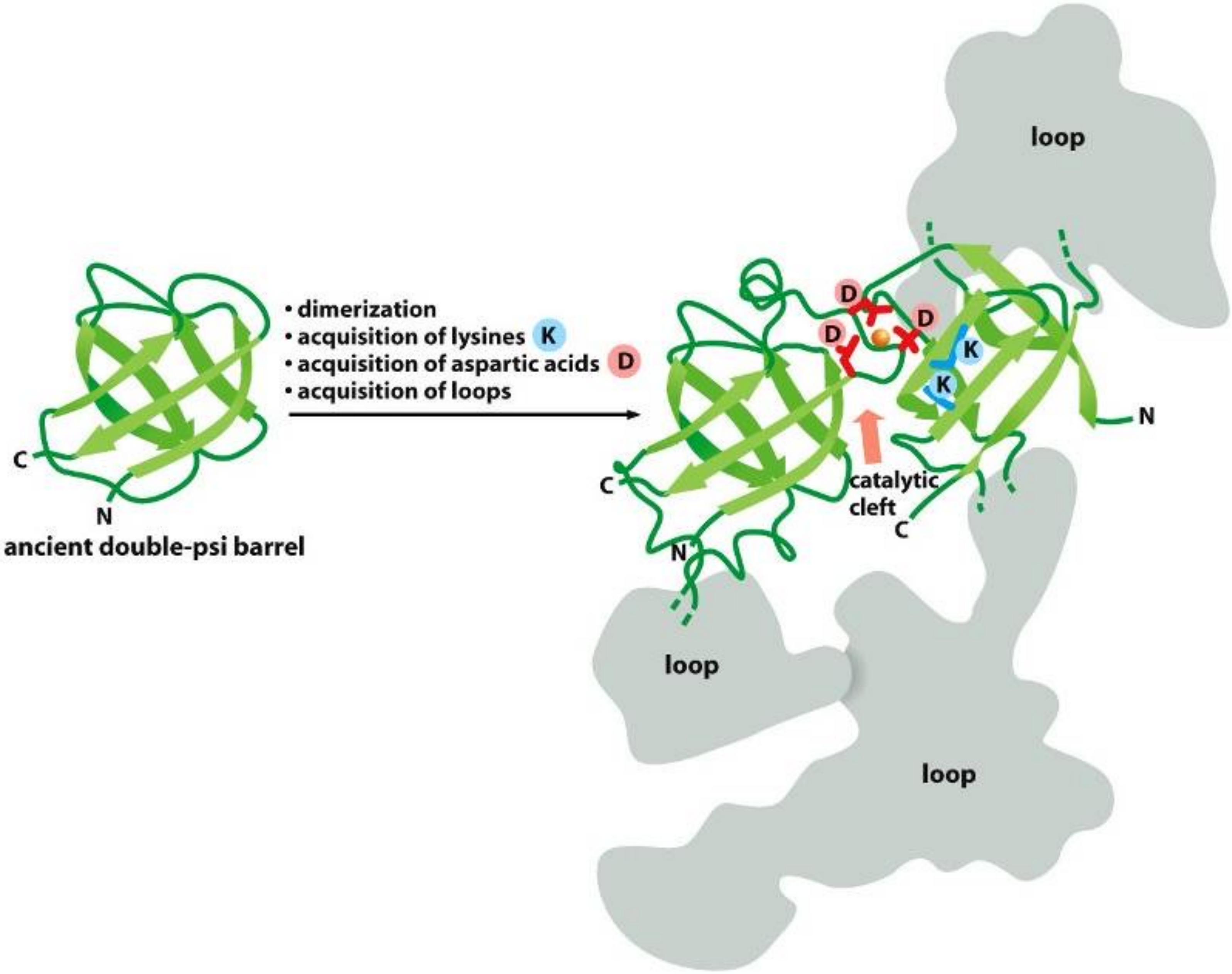


Figure 6-10 *Molecular Biology of the Cell* (© Garland Science 2008)

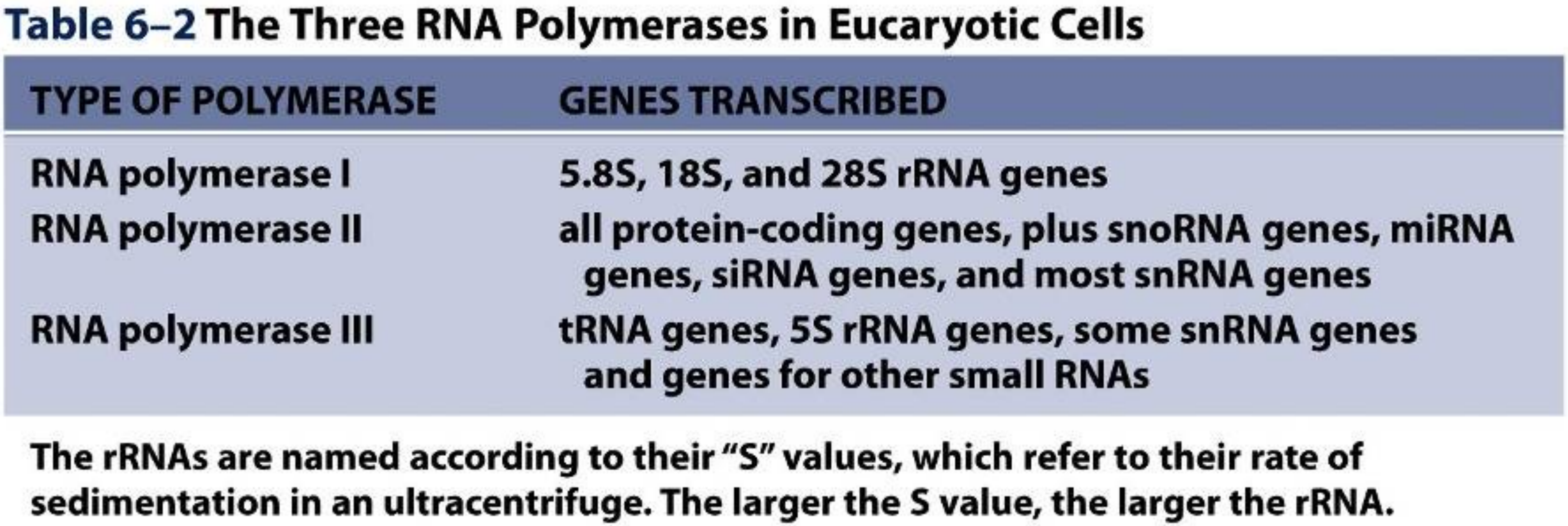


Table 6–2 The Three RNA Polymerases in Eucaryotic Cells

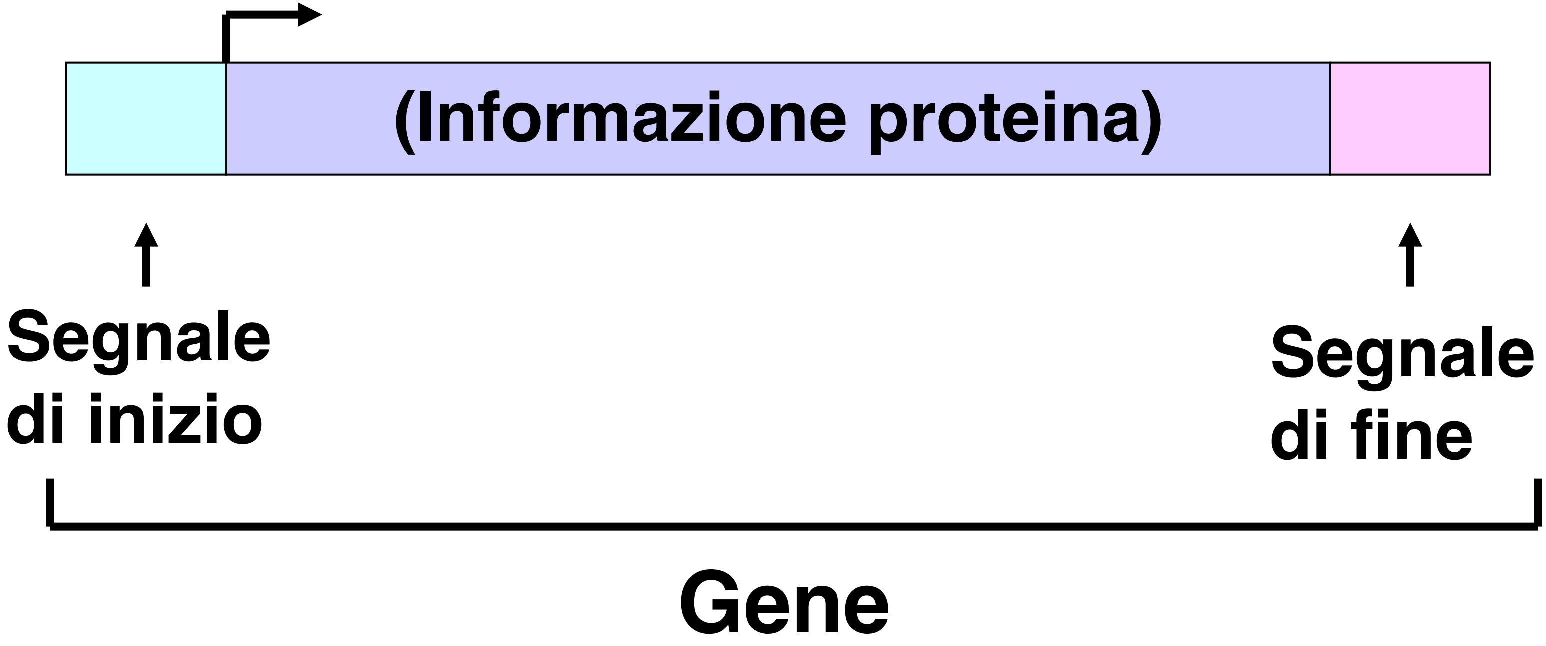
TYPE OF POLYMERASE	GENES TRANSCRIBED
RNA polymerase I	5.8S, 18S, and 28S rRNA genes
RNA polymerase II	all protein-coding genes, plus snoRNA genes, miRNA genes, siRNA genes, and most snRNA genes
RNA polymerase III	tRNA genes, 5S rRNA genes, some snRNA genes and genes for other small RNAs

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.

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

Struttura dell'informazione

Il DNA deve contenere, oltre all'informazione genetica per una data proteina, anche dei segnali che indichino dove questa informazione inizia e finisce, cioè dove inizia e finisce anche la trascrizione



(Informazione proteina)

Segnale di inizio

Segnale di fine

Gene

PROMOTORE

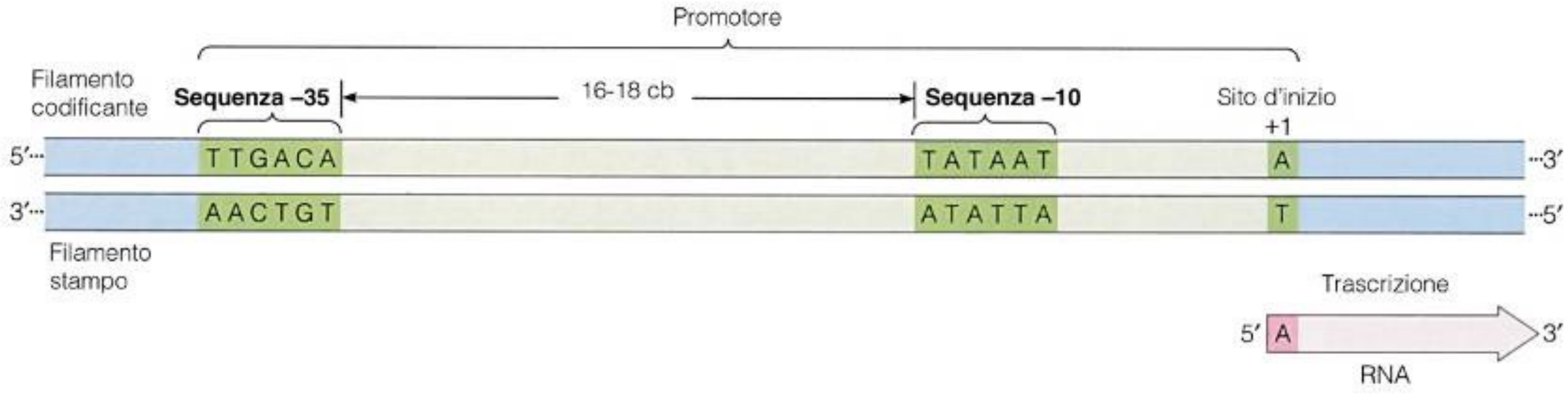
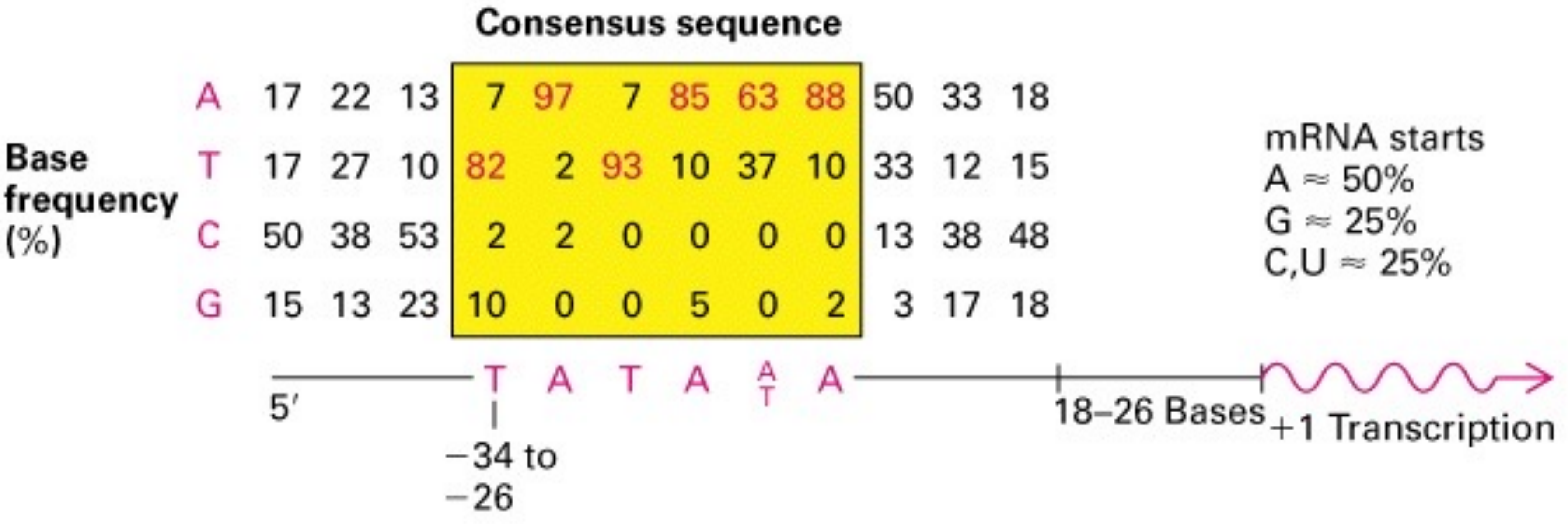


Figura 19-10

- Sequenza situata immediatamente “a monte” del sito di inizio della trascrizione.
- Consente l’attacco dell’RNA polimerasi e di altri fattori che facilitano l’inizio della trascrizione
- Il sito di attacco dell’RNA polimerasi è sull’elica stampo

10.4 The TATA box is a highly conserved promoter in eukaryotic DNA



Base frequency (%)				Consensus sequence								
A	17	22	13	7	97	7	85	63	88	50	33	18
T	17	27	10	82	2	93	10	37	10	33	12	15
C	50	38	53	2	2	0	0	0	0	13	38	48
G	15	13	23	10	0	0	5	0	2	3	17	18

Alternative promoters in eukaryotes include initiators and CpG islands

Figure 10-30

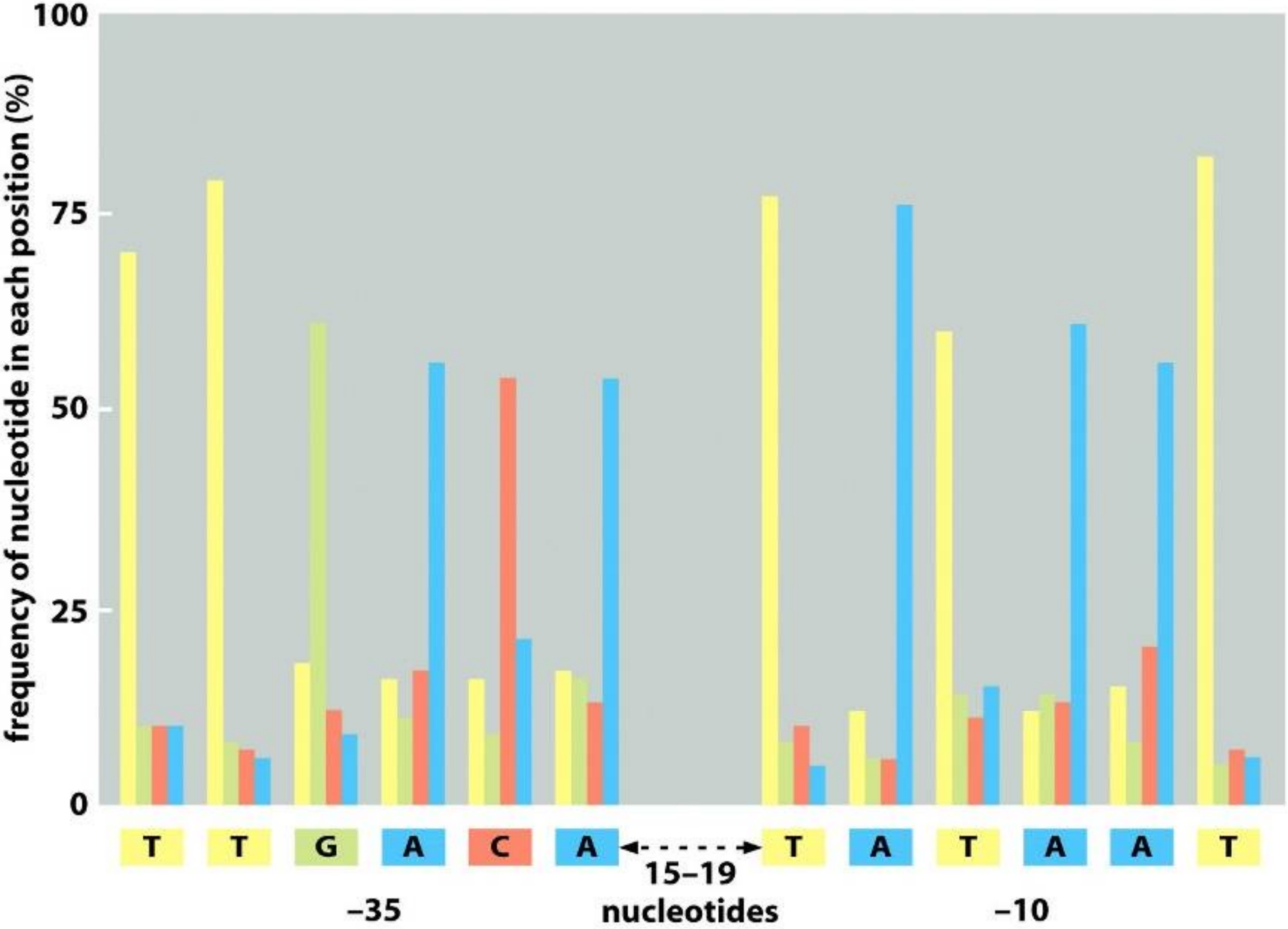


Figure 6-12a *Molecular Biology of the Cell* (© Garland Science 2008)

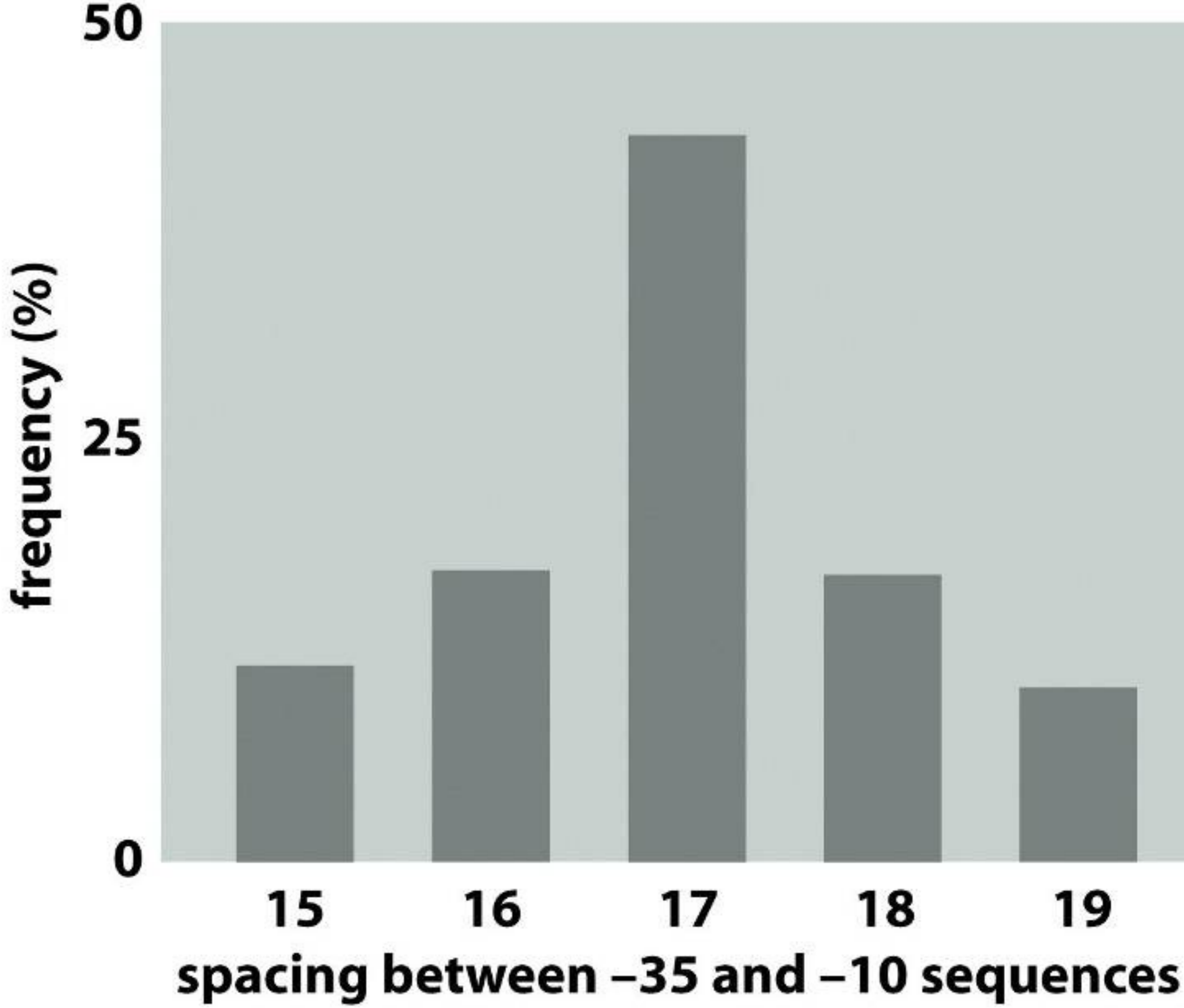
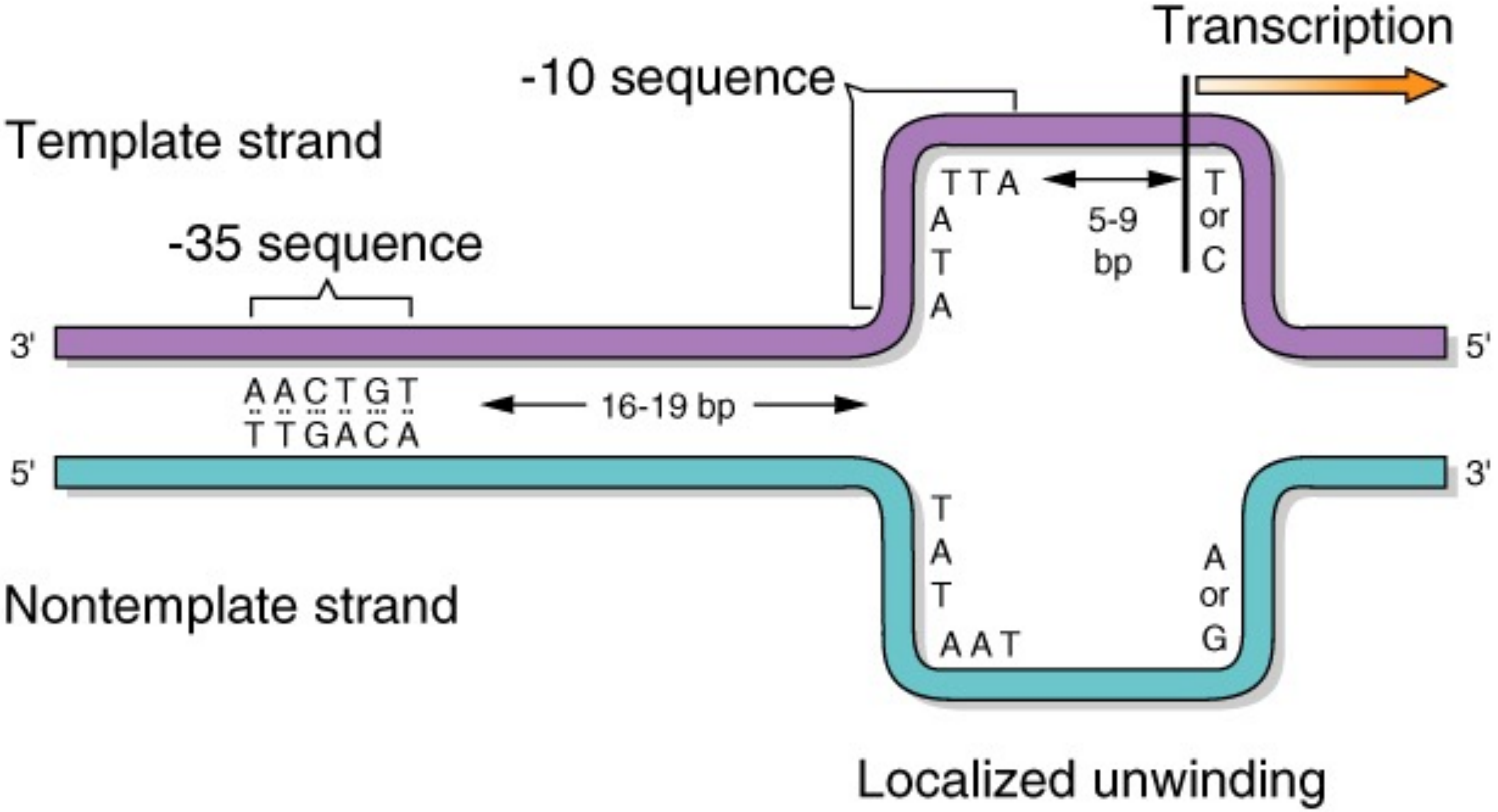


Figure 6-12b *Molecular Biology of the Cell* (© Garland Science 2008)


Transcription
-10 sequence
Template strand
-35 sequence
T T A
A
T
A
5-9
bp
T
or
C
3'
5'
A A C T G T
T T G A C A
16-19 bp
5'
3'
T
A
T
A A T
A
or
G
Nontemplate strand
Localized unwinding

Trascrizione nei procarioti

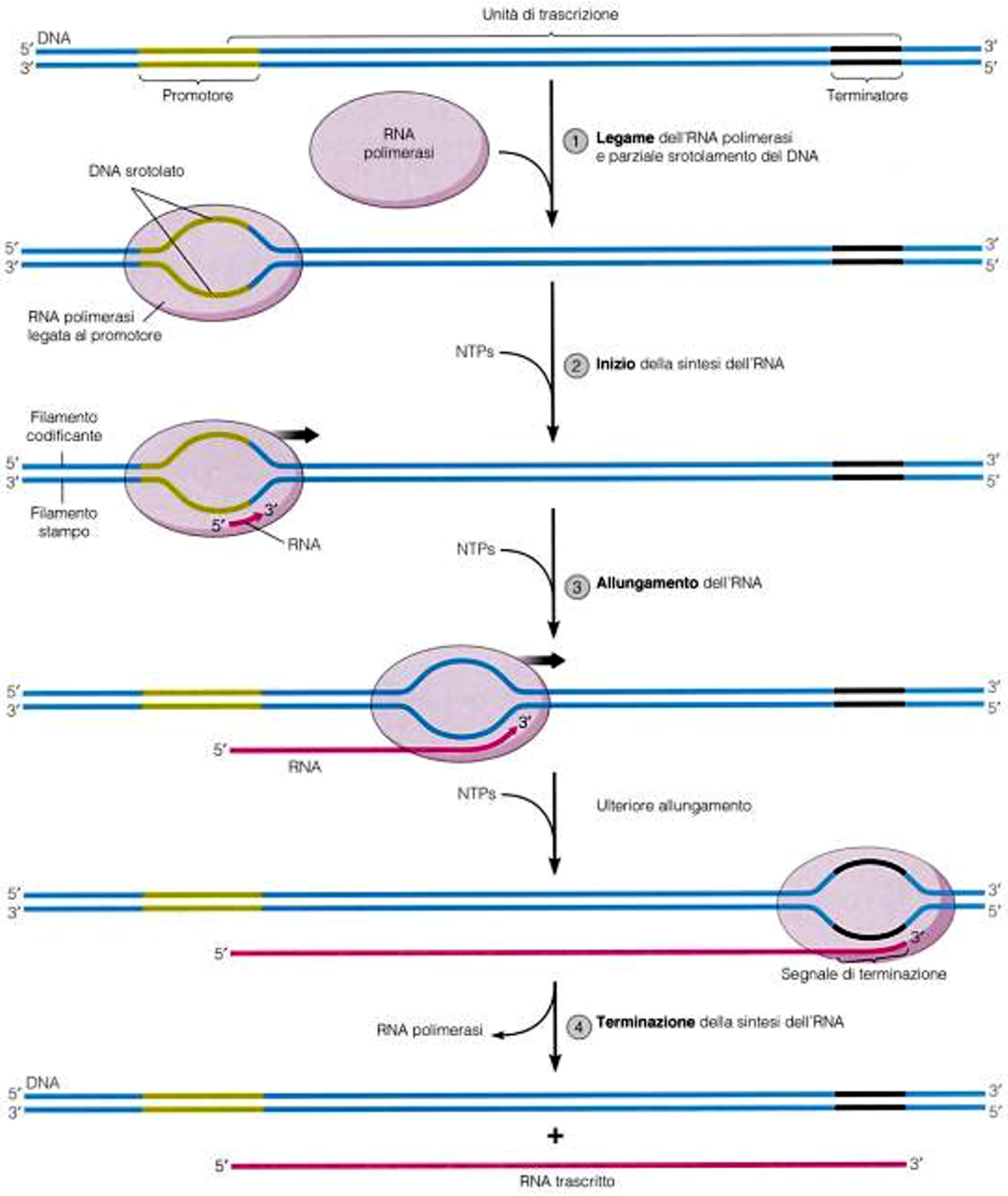
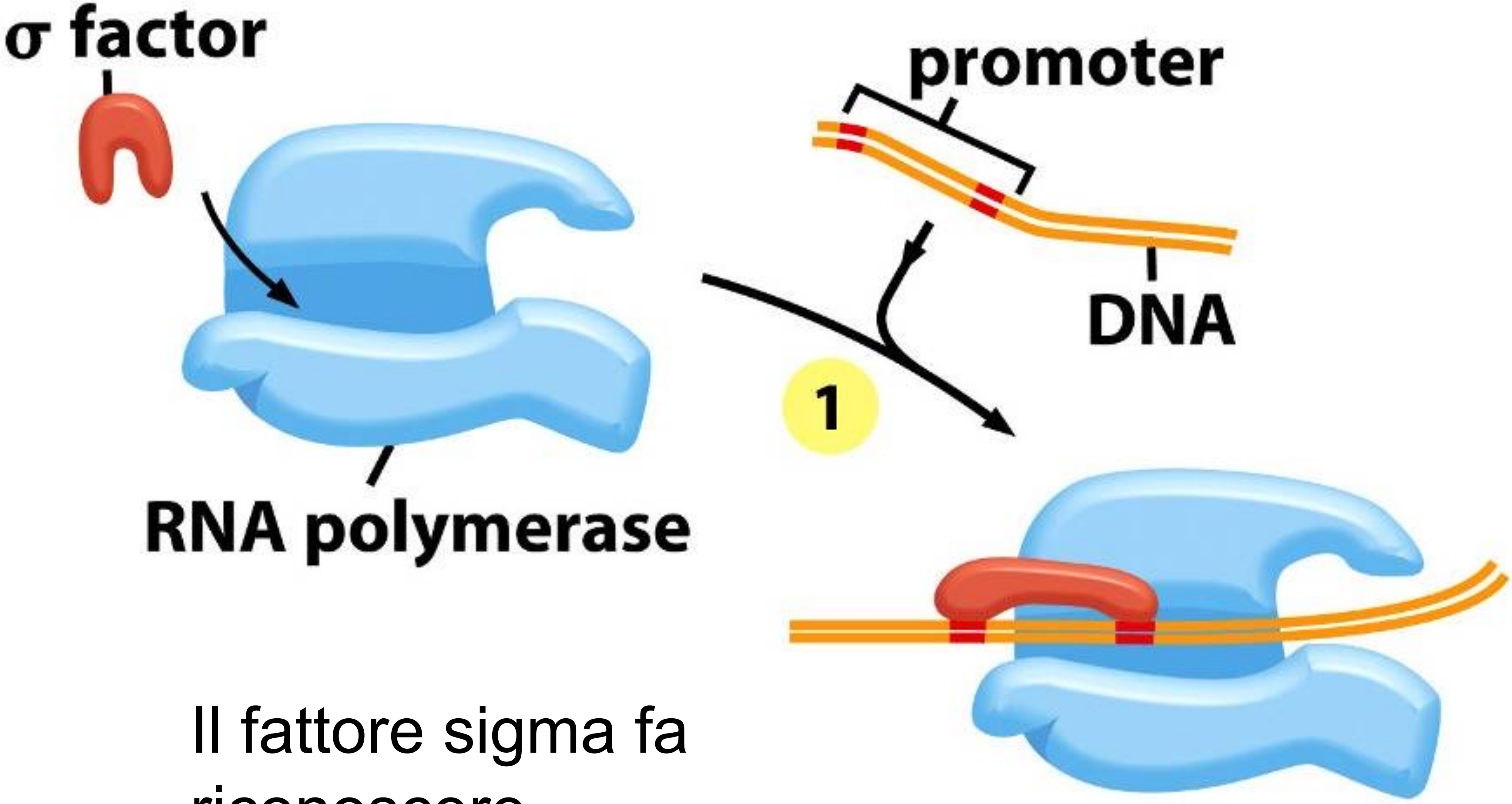
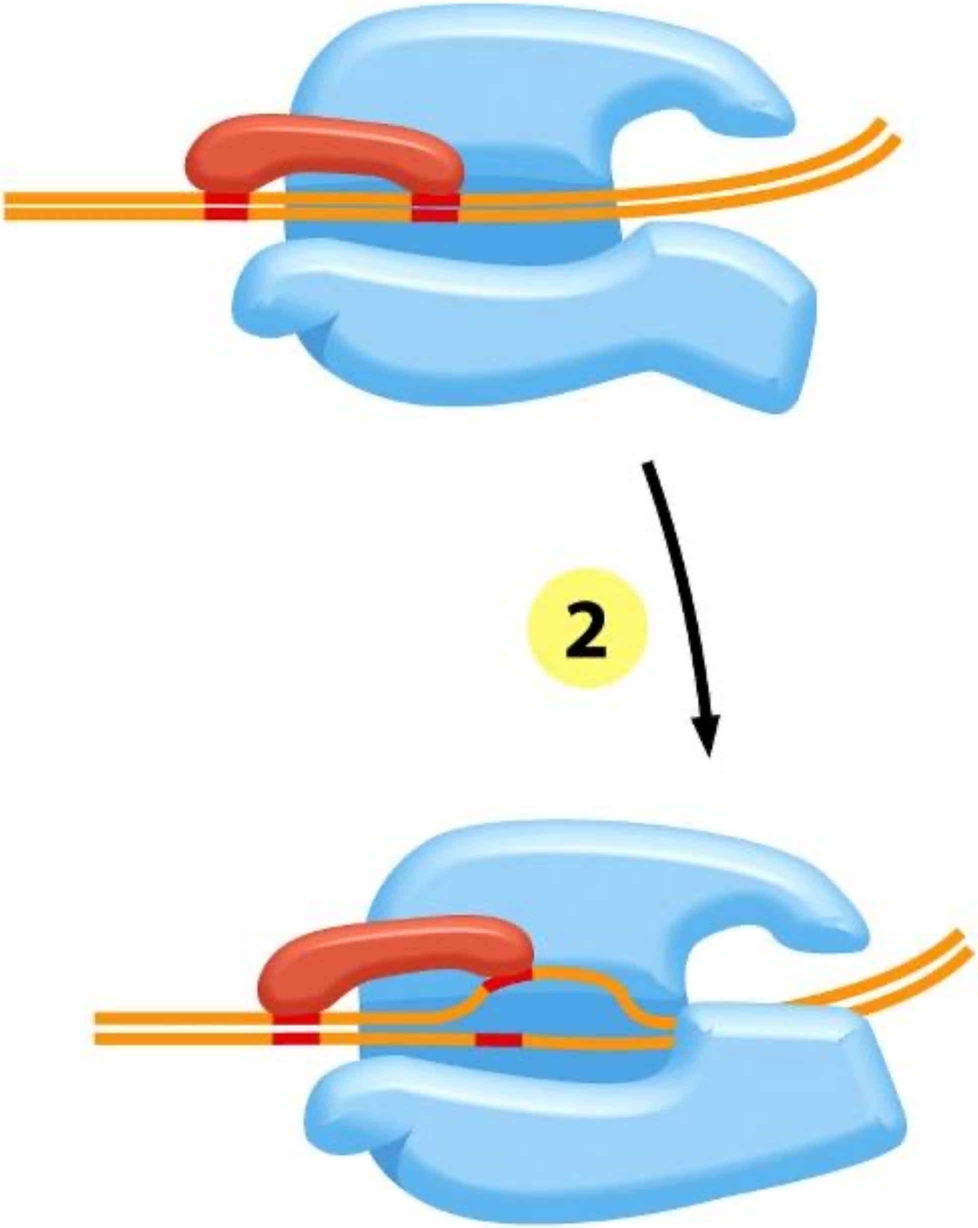


Figura 19-9



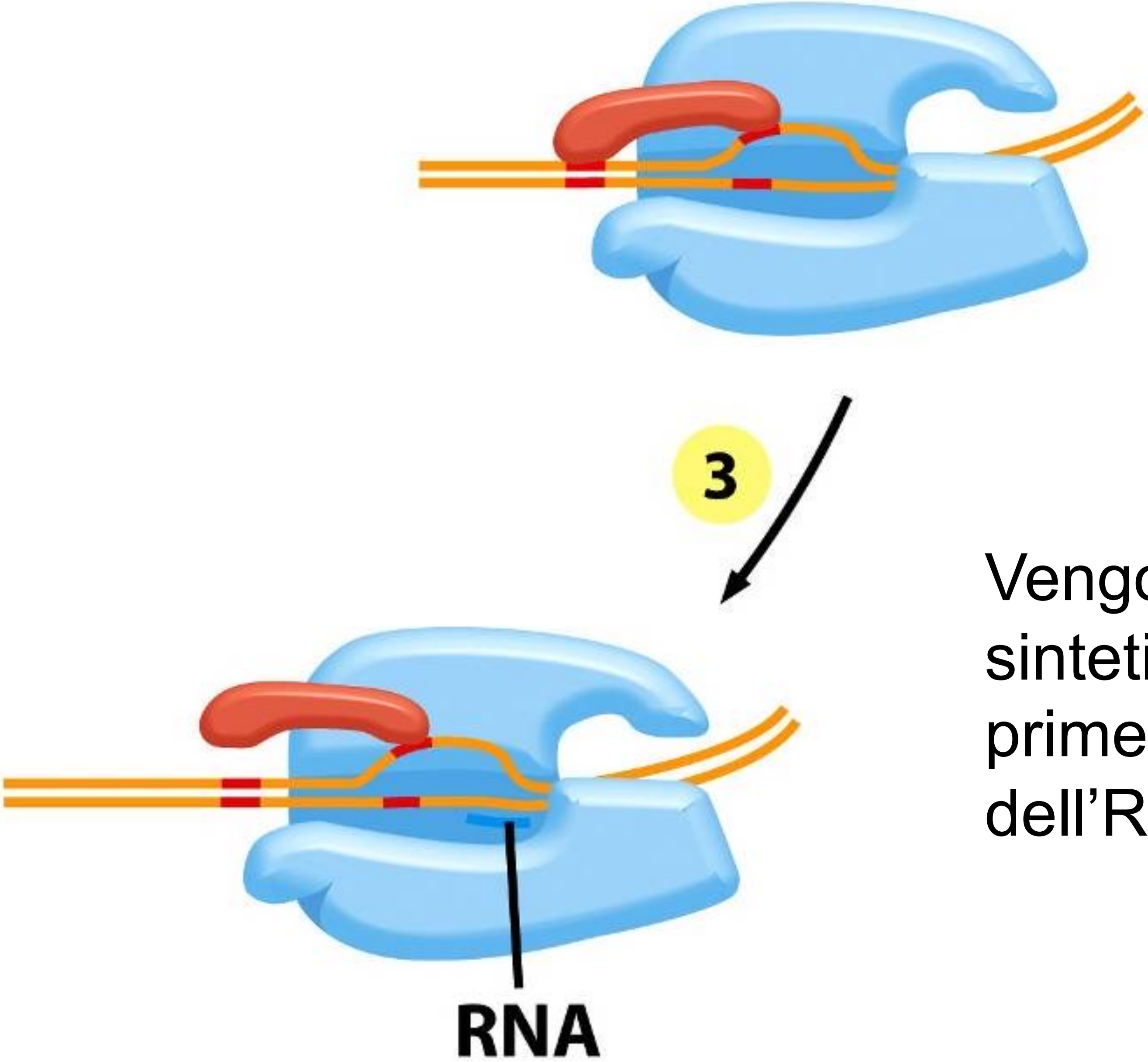
Il fattore sigma fa
riconoscere
il promotore (porzione
delle basi esposte
all’esterno dell’elica)
alla polimerasi

Figure 6-11 (part 1 of 7) *Molecular Biology of the Cell* (© Garland Science 2008)



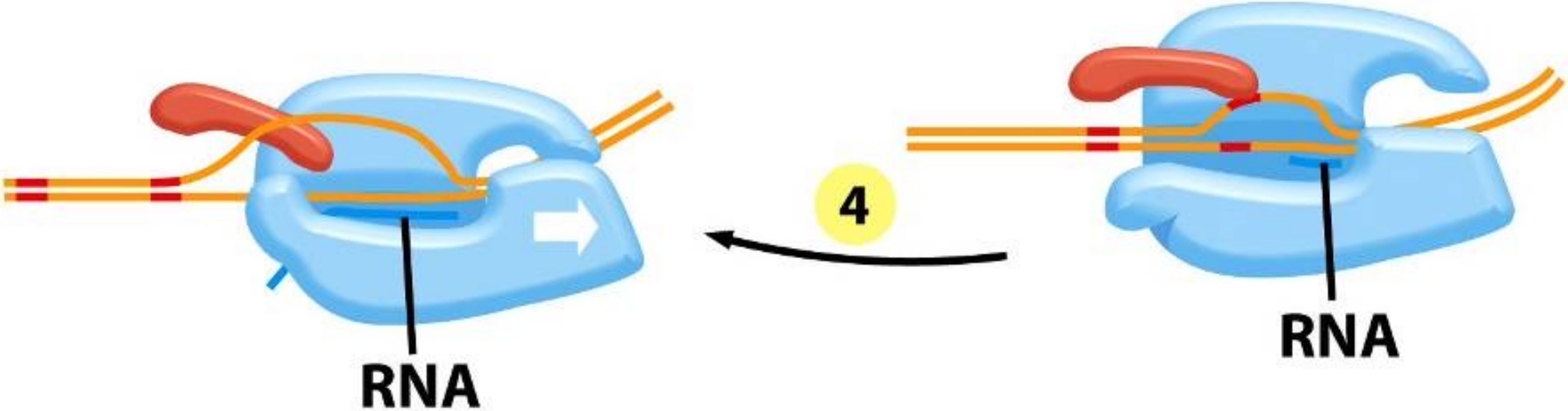
La polimerasi apre il doppio filamento di DNA (non richiede energia)

Figure 6-11 (part 2 of 7) *Molecular Biology of the Cell* (© Garland Science 2008)



Vengono sintetizzate le prime basi dell’RNA

Figure 6-11 (part 3 of 7) *Molecular Biology of the Cell* (© Garland Science 2008)



Dopo I primi nucleotidi (circa 10) la polimerasi indebolisce legame con sigma e scorre lungo DNA

Figure 6-11 (part 4 of 7) *Molecular Biology of the Cell* (© Garland Science 2008)

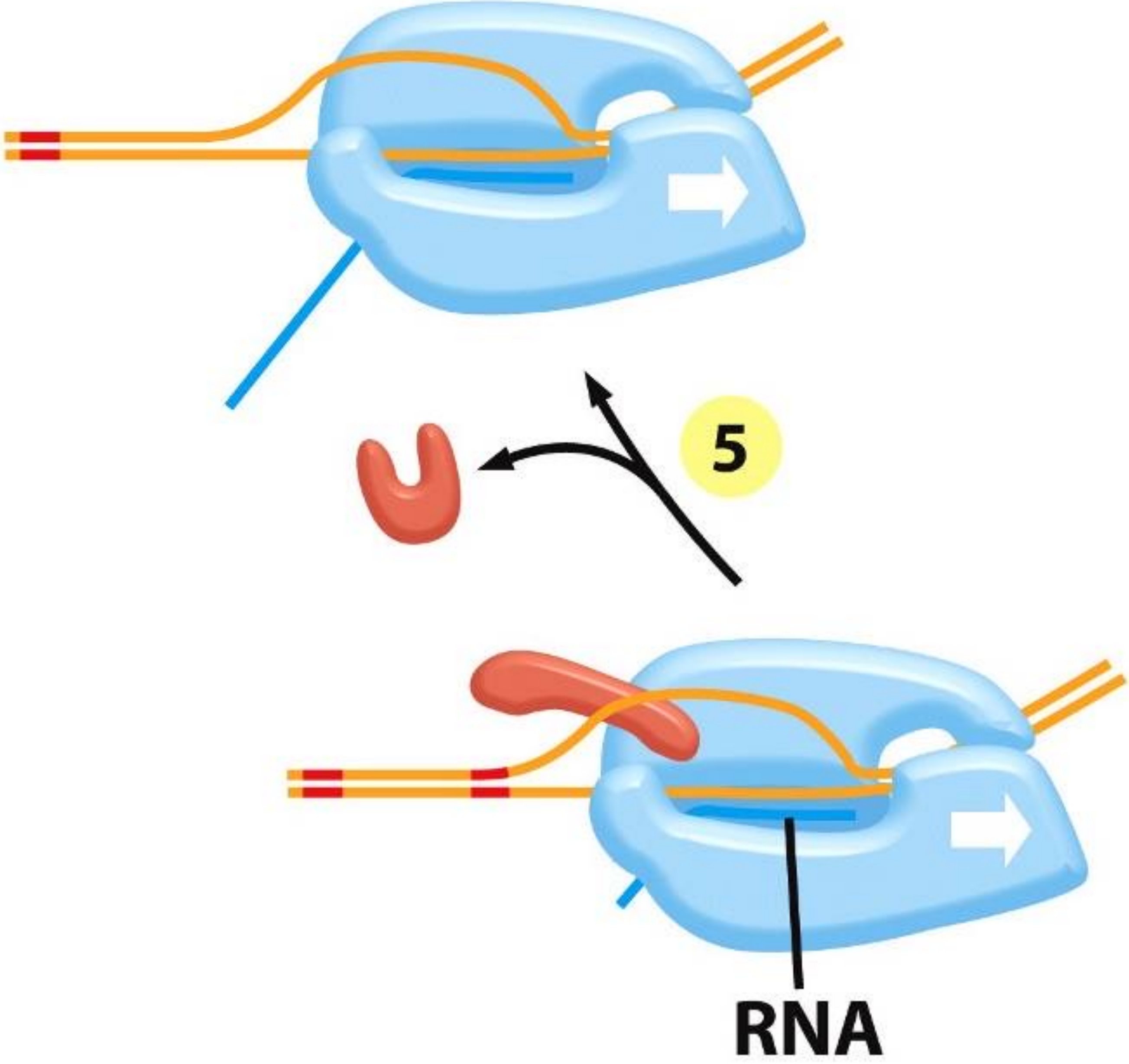


Figure 6-11 (part 5 of 7) *Molecular Biology of the Cell* (© Garland Science 2008)

Fase di allungamento

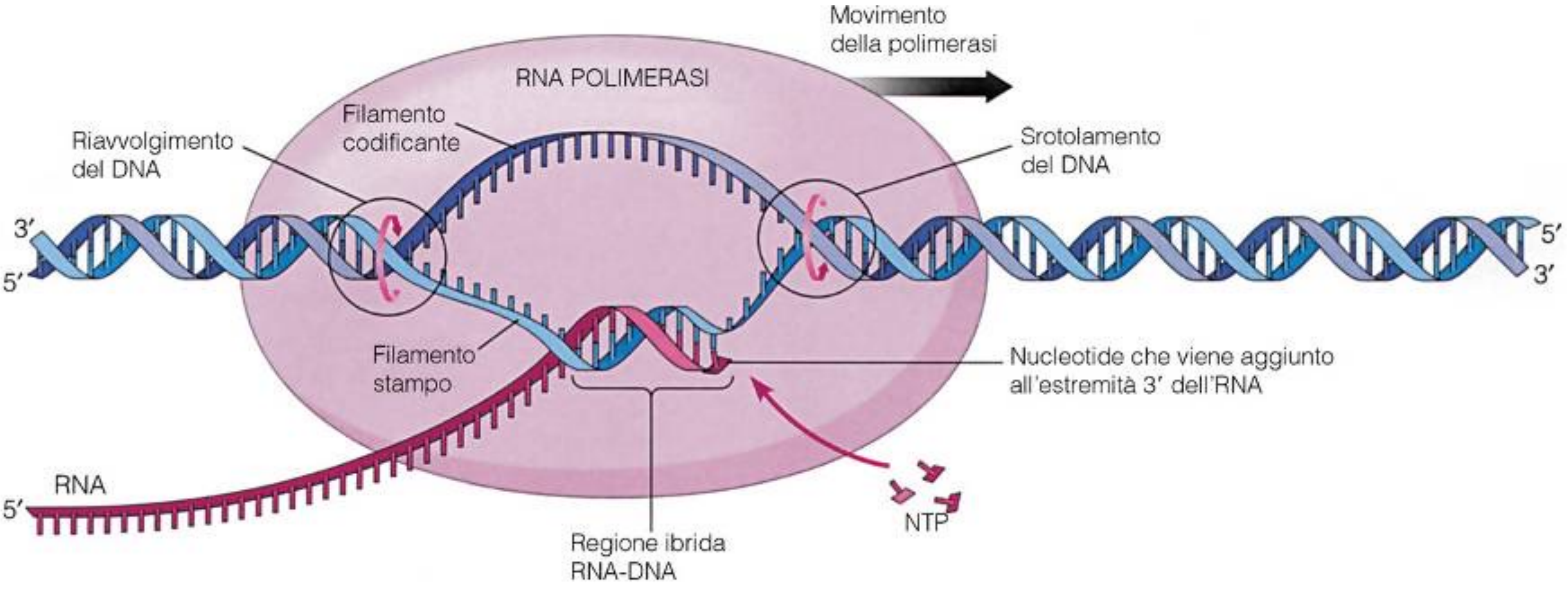
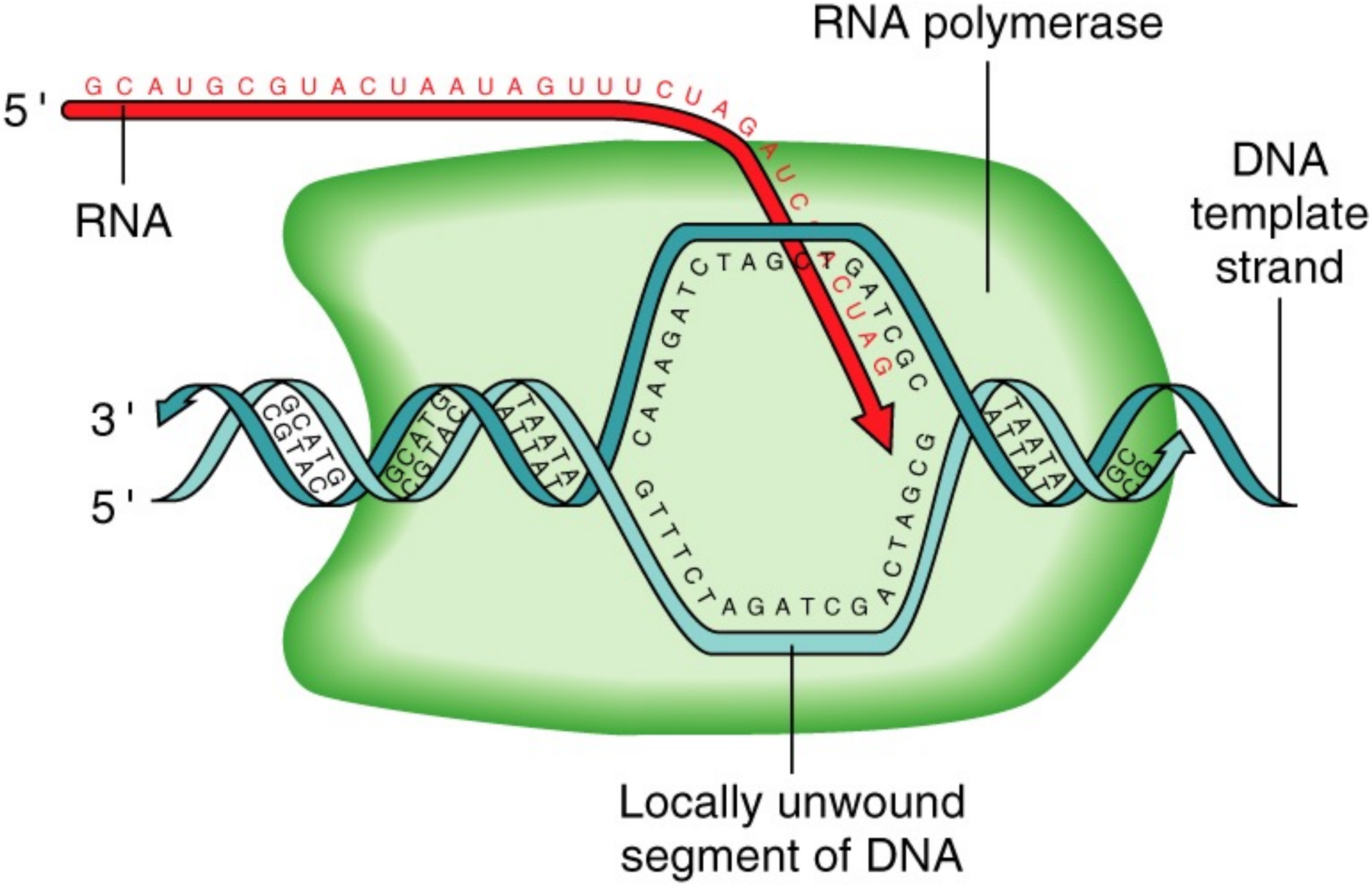
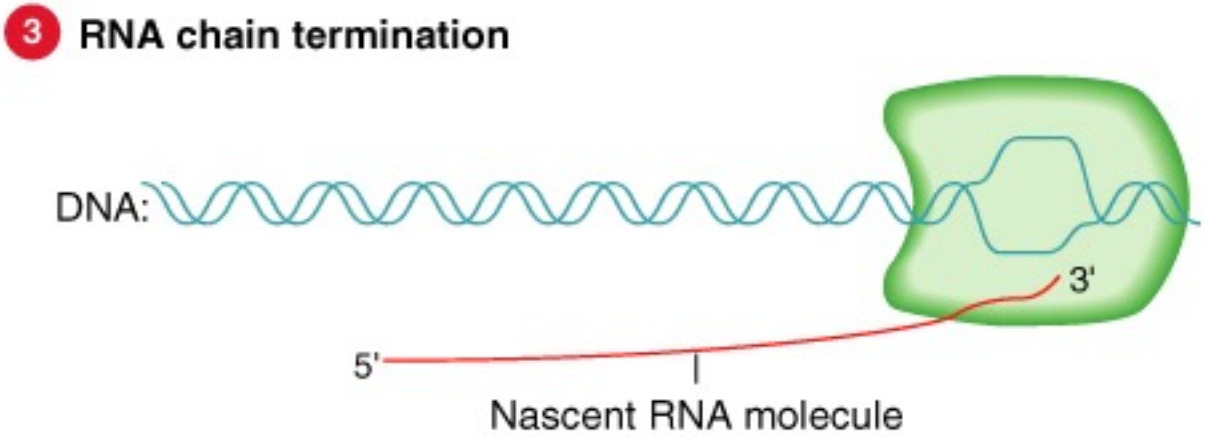


Figura 19-11

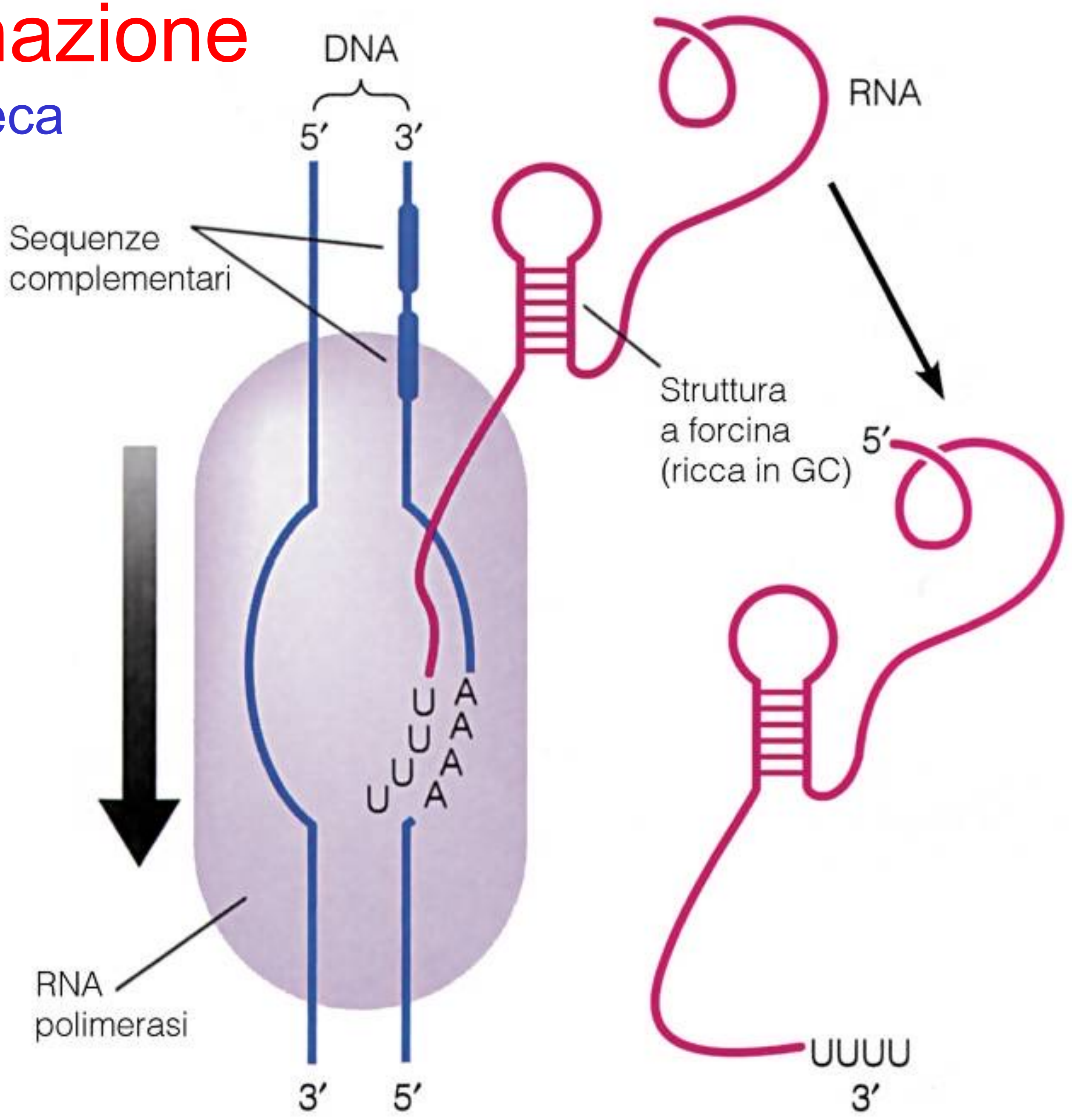

5'
G C A U G C G U A C U A A U A G U U U C U A G A U C
RNA
RNA polymerase
DNA template strand
3'
5'
Locally unwound segment of DNA

Fase di terminazione



Fase di terminazione

Terminazione intrinseca



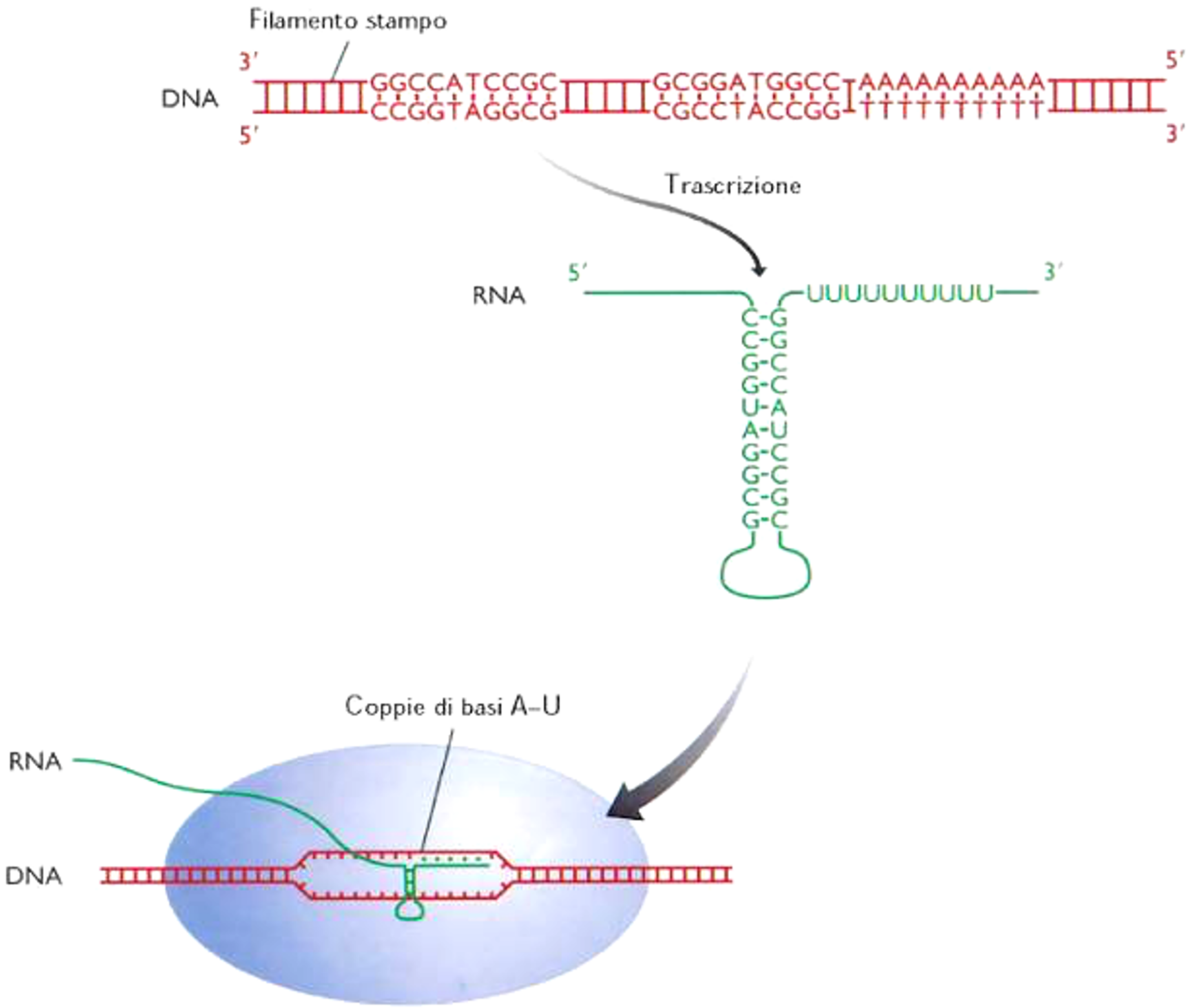


Figura 10.3 La terminazione della trascrizione a un terminatore intrinseco.

T.A. Brown
Genomi
EdiSES

Terminazione Rho dipendente

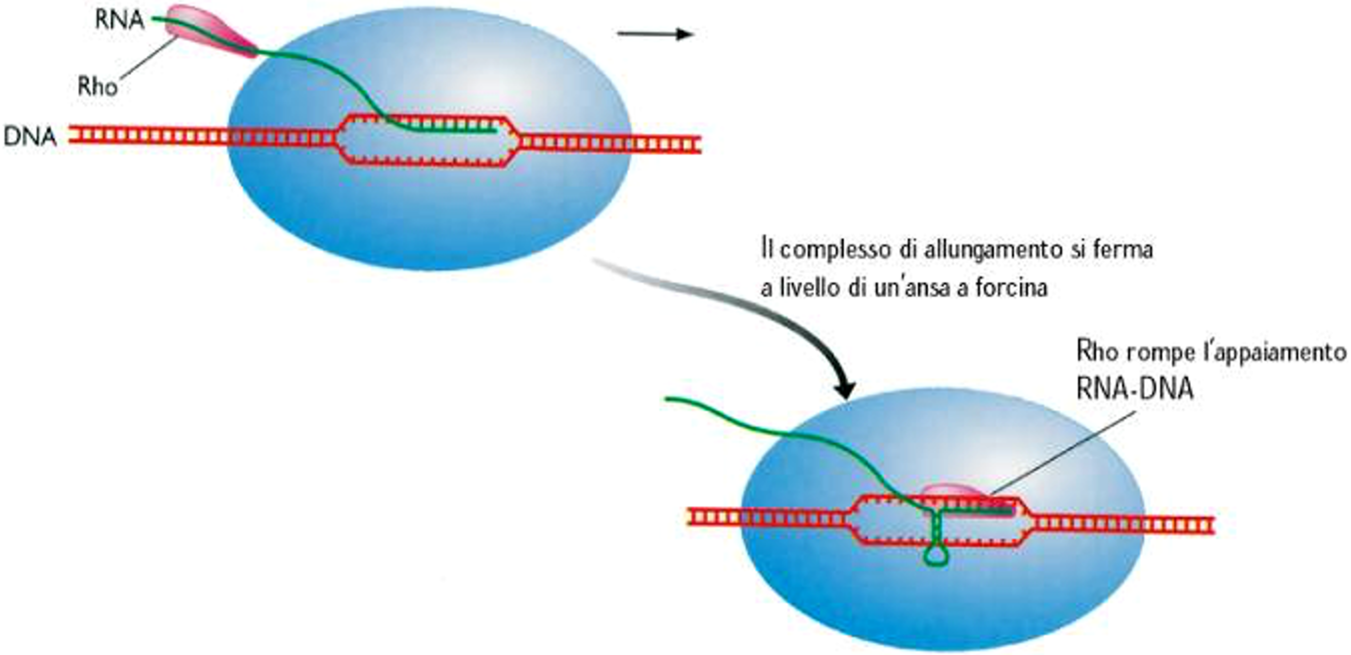
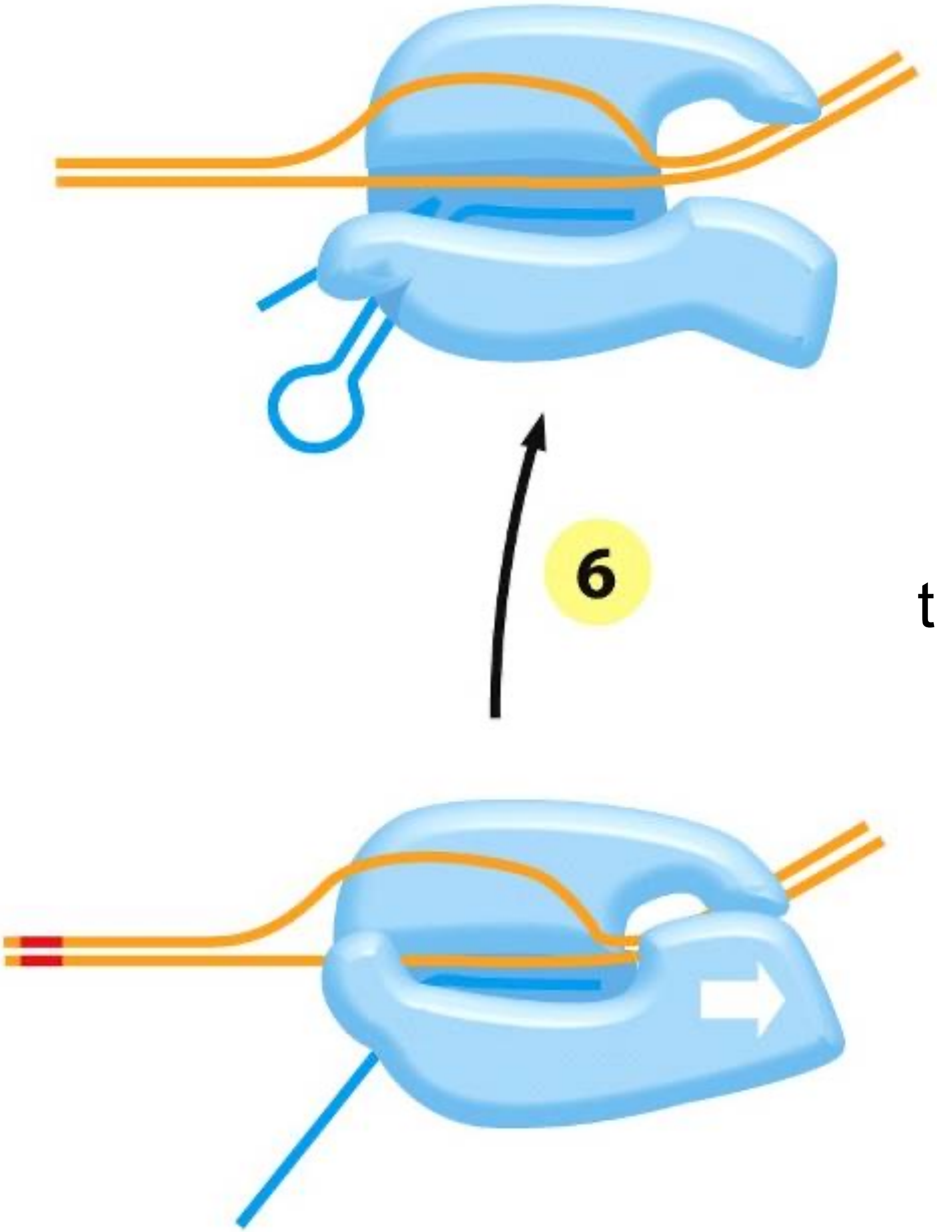


Figura 10.4 La terminazione Rho-dipendente.

EdiSES
T.A. Brown
Genomi
EdiSES

Rho è una elicasi che segue la RNA pol lungo il trascritto.
Quando la pol si ferma a livello di una forcina la elicasi la raggiunge e rompe l'appaiamento RNA-DNA



terminazione

Figure 6-11 (part 6 of 7) *Molecular Biology of the Cell* (© Garland Science 2008)

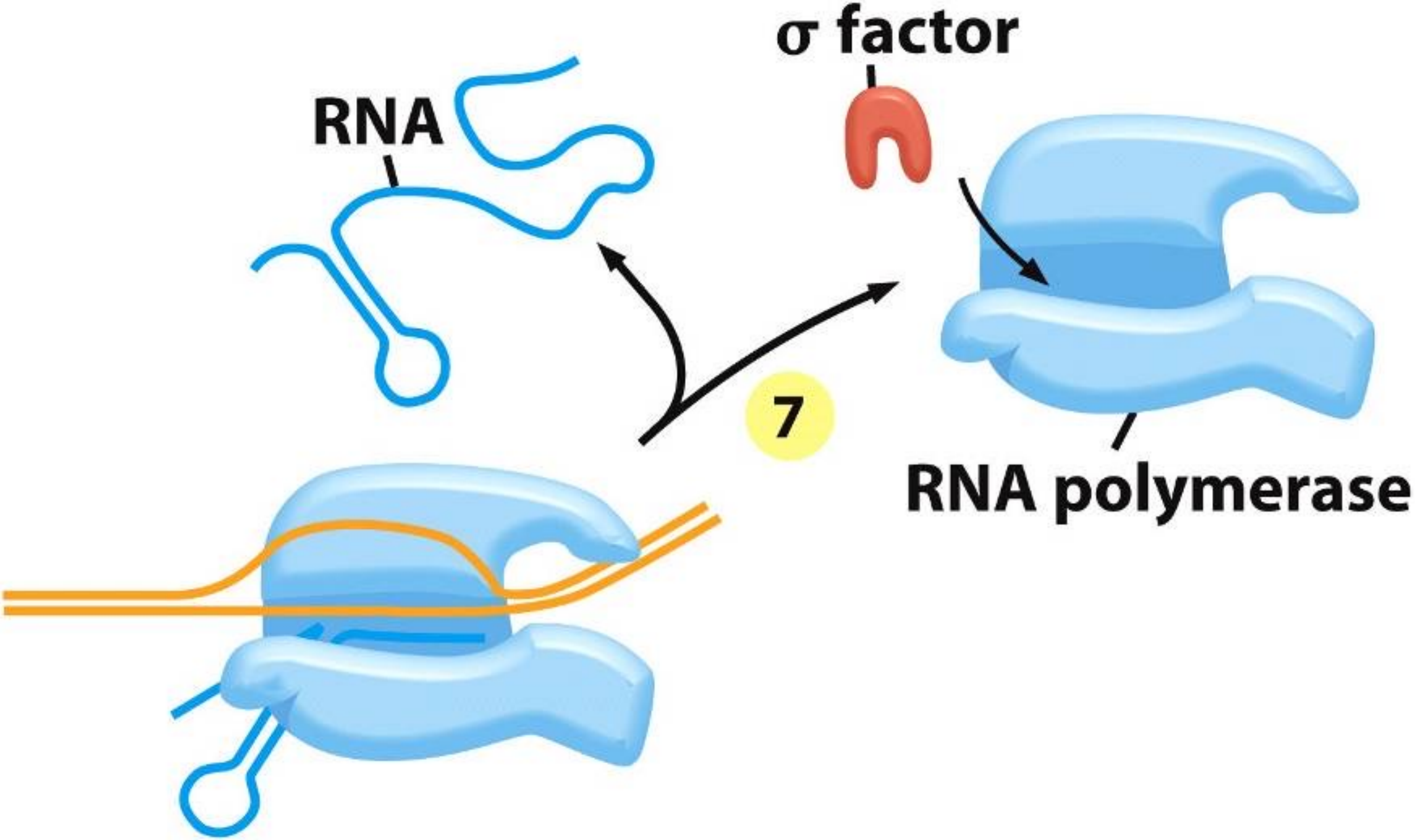


Figure 6-11 (part 7 of 7) *Molecular Biology of the Cell* (© Garland Science 2008)

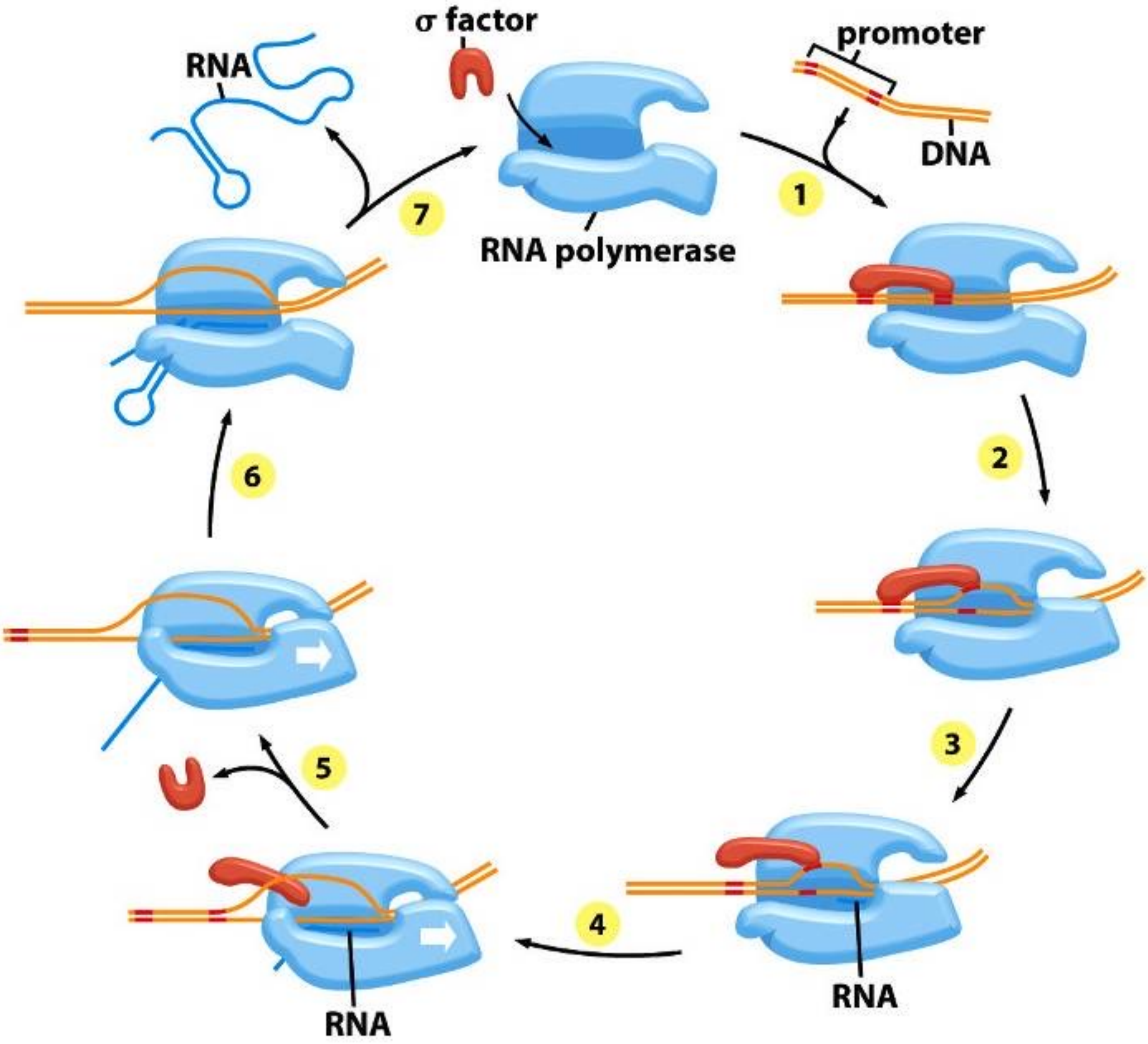


Figure 6-11 *Molecular Biology of the Cell* (© Garland Science 2008)

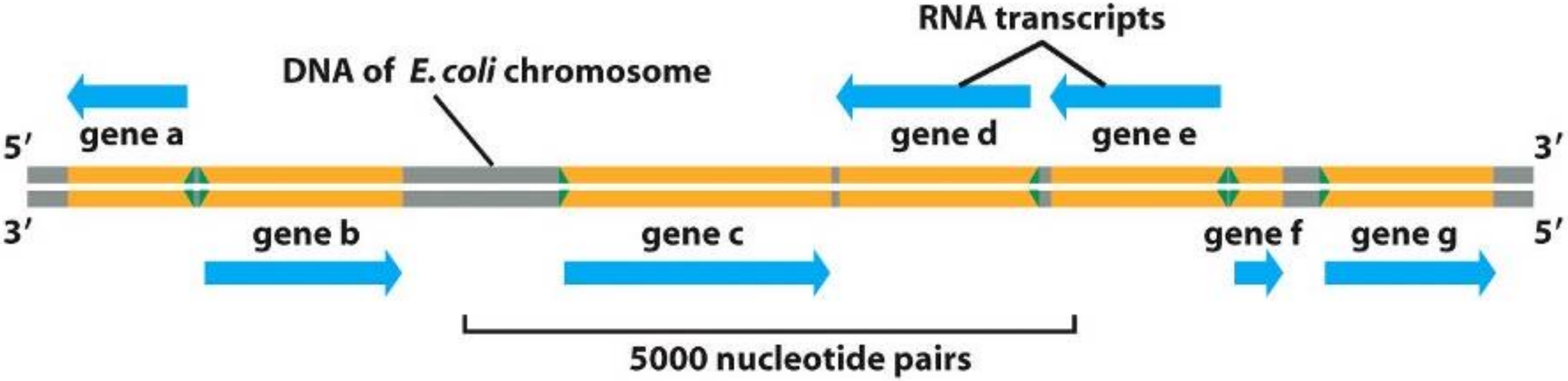
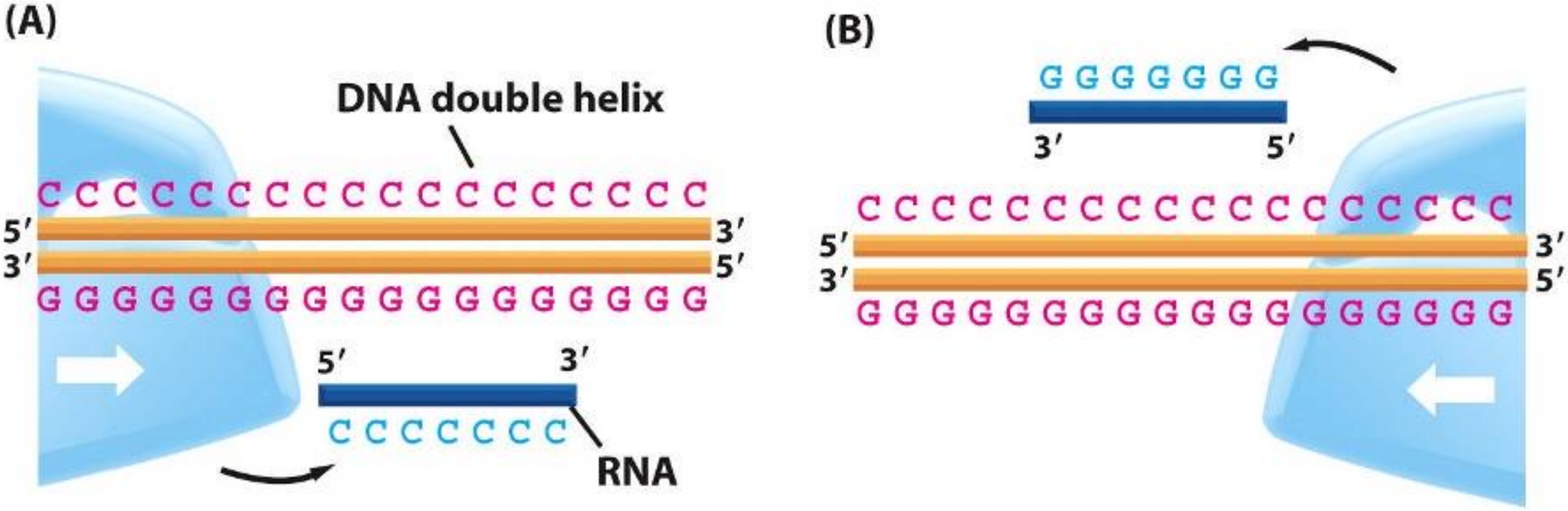


Figure 6-14 *Molecular Biology of the Cell* (© Garland Science 2008)

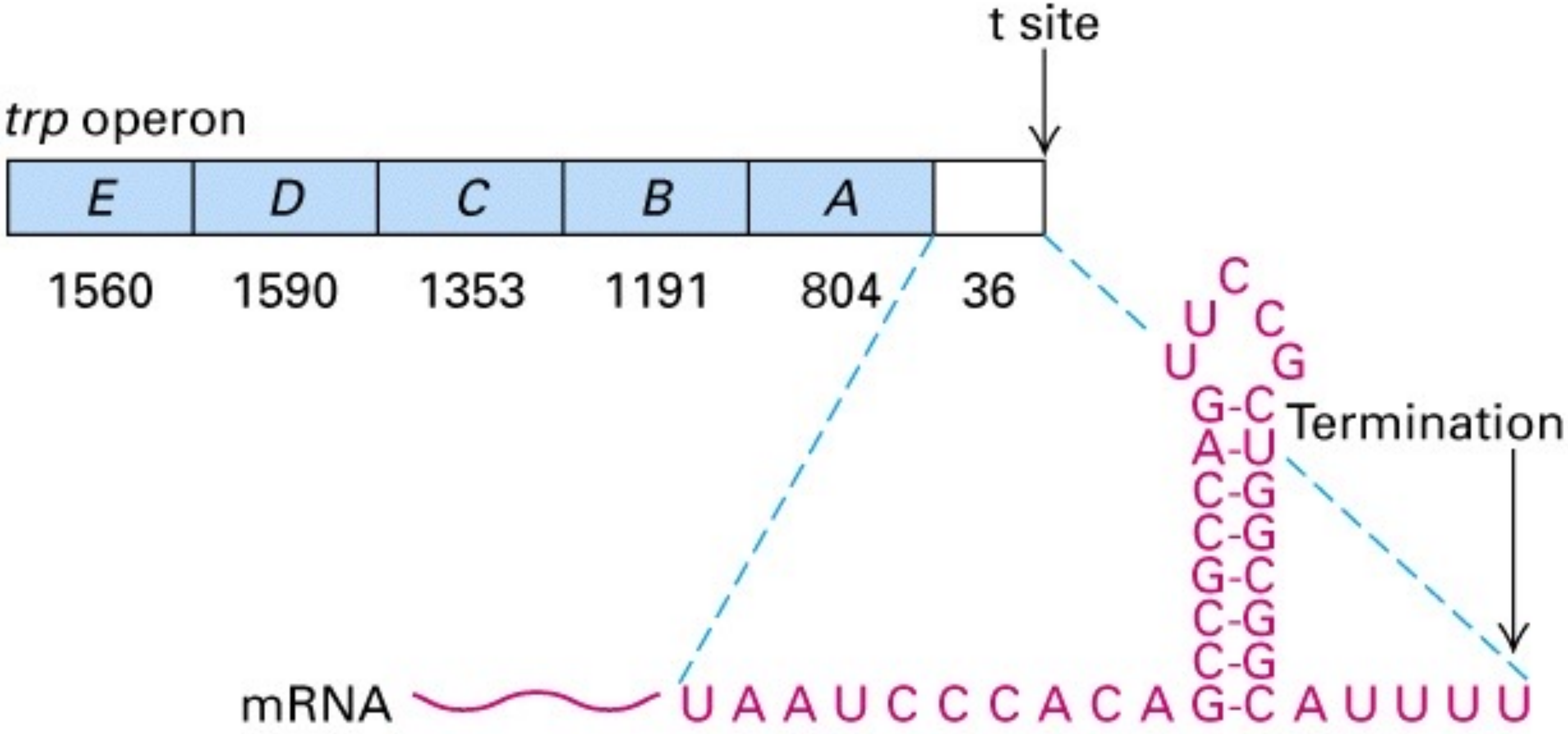


an RNA polymerase that moves from left to right makes RNA by using the bottom strand as a template

an RNA polymerase that moves from right to left makes RNA by using the top strand as a template

Figure 6-13 *Molecular Biology of the Cell* (© Garland Science 2008)

Geni policistronici dei procarioti



4.3 Gene organization, transcription, and translation in prokaryotes

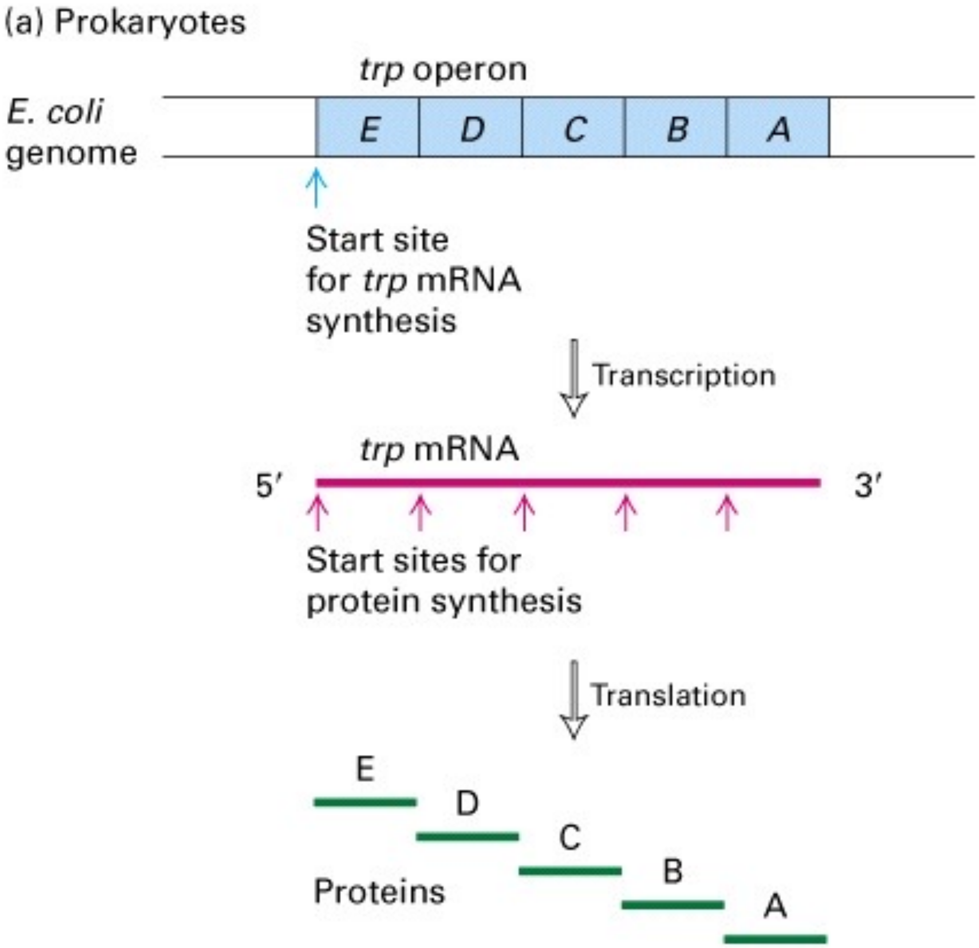


Figure 4-17a

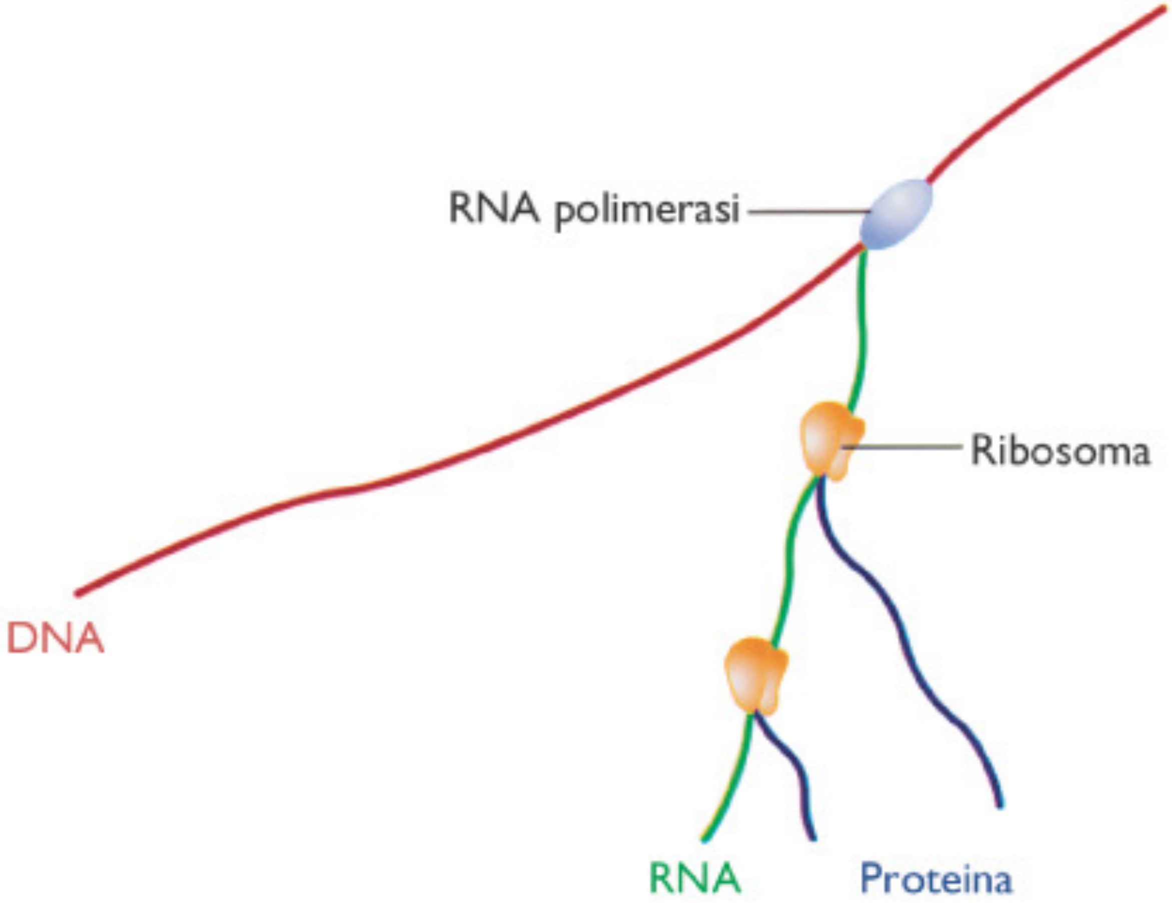


Figura 10.1 Nei batteri, la trascrizione e la traduzione sono spesso accoppiate.

T.A. Brown
Genomi
EdiSES

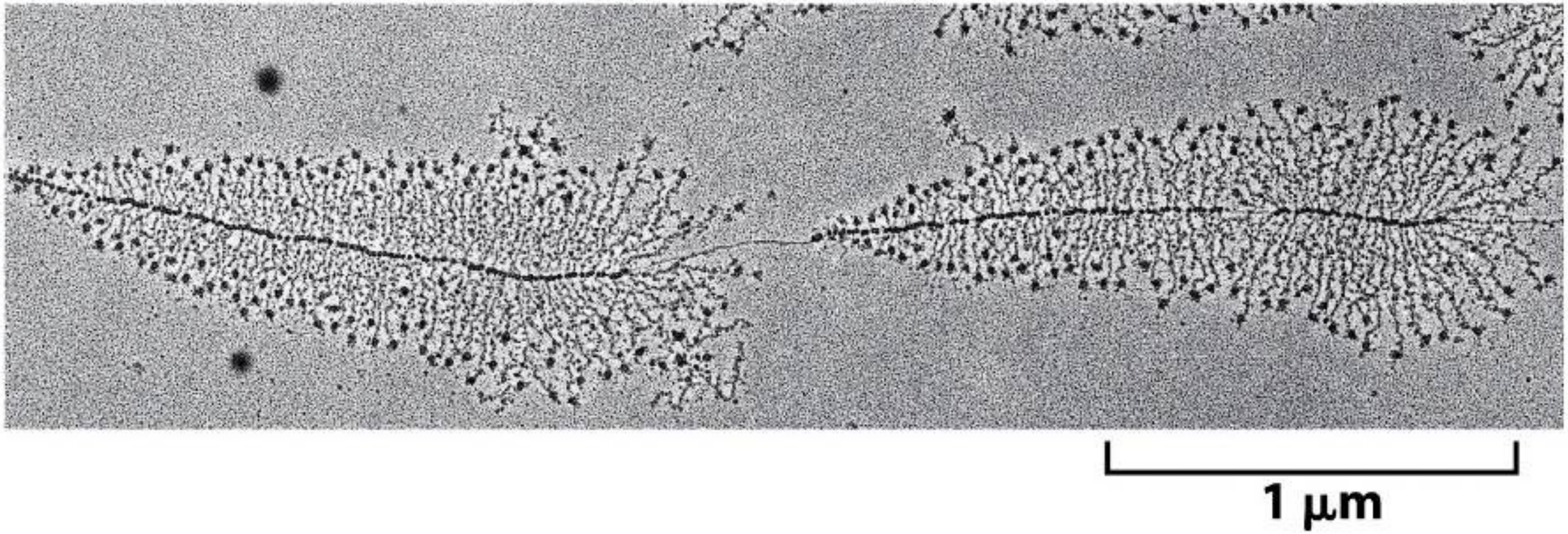
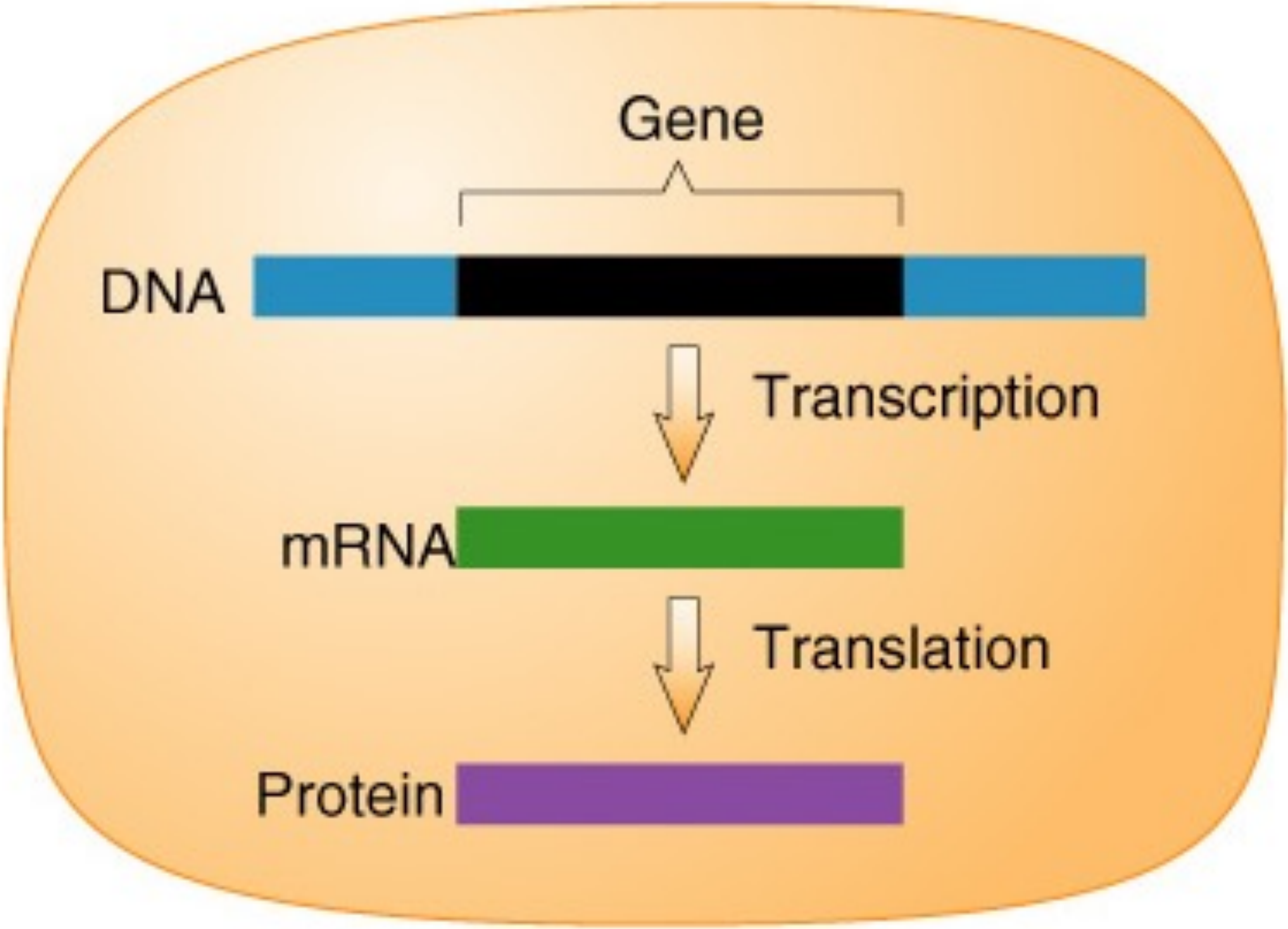


Figure 6-9 *Molecular Biology of the Cell* (© Garland Science 2008)

Procarioti



EUCARIOTI

trascrizione degli mRNA

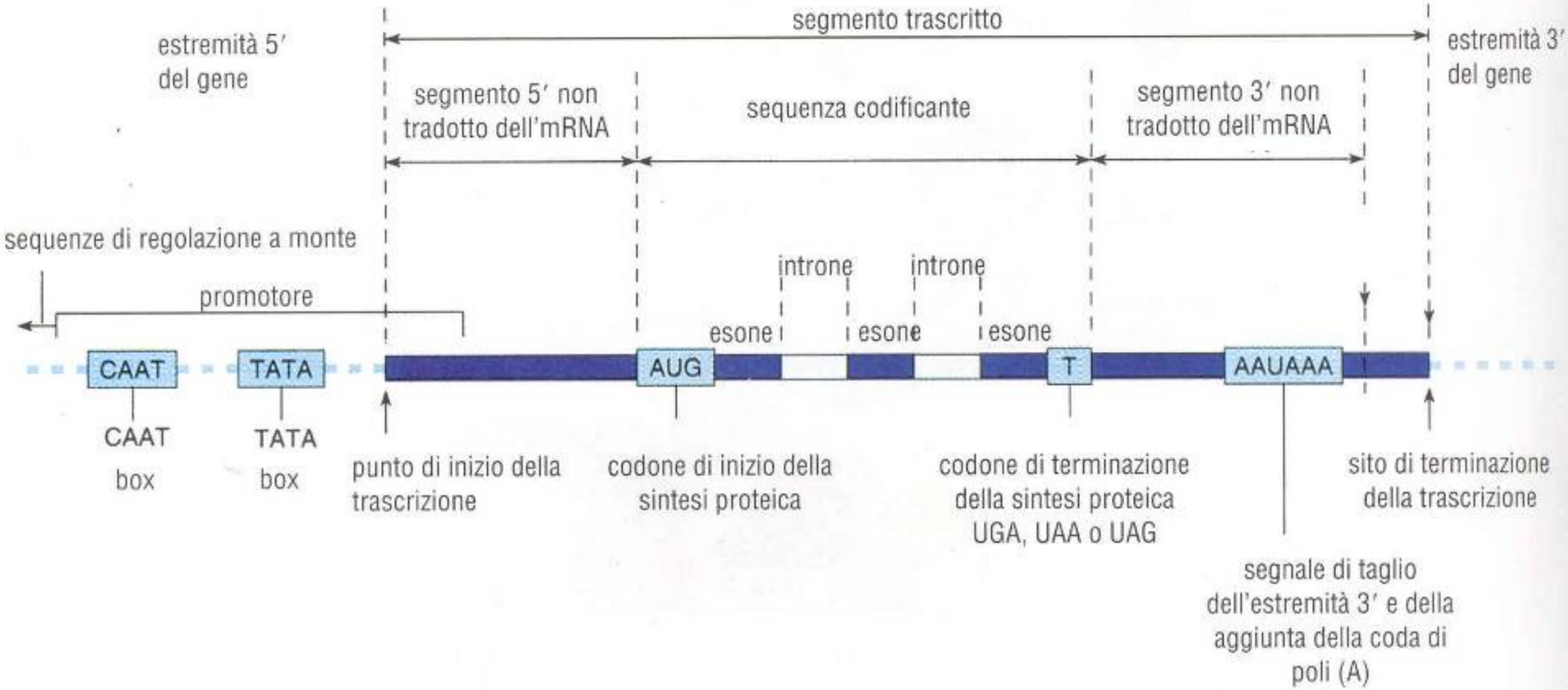


FIGURA 6.15

L'organizzazione degli elementi che costituiscono i geni codificanti per gli mRNA negli eucarioti (vedi testo).

4.3 Gene organization, transcription, and translation in eukaryotes

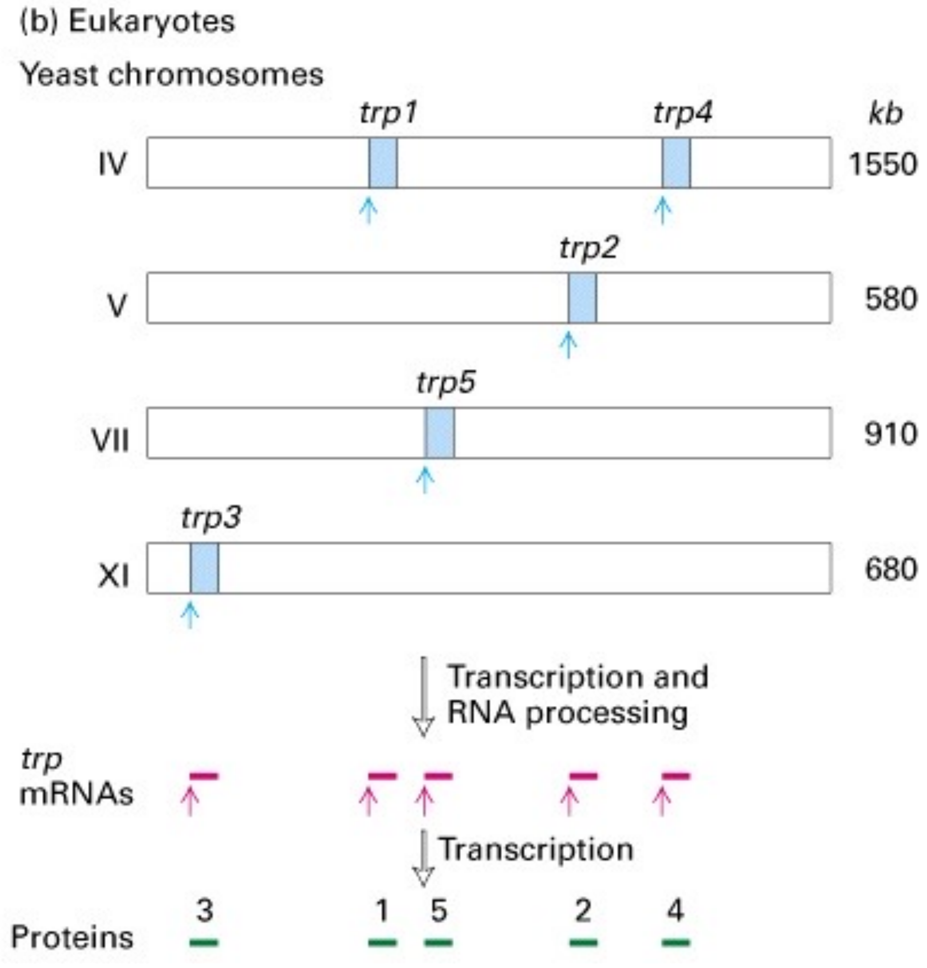
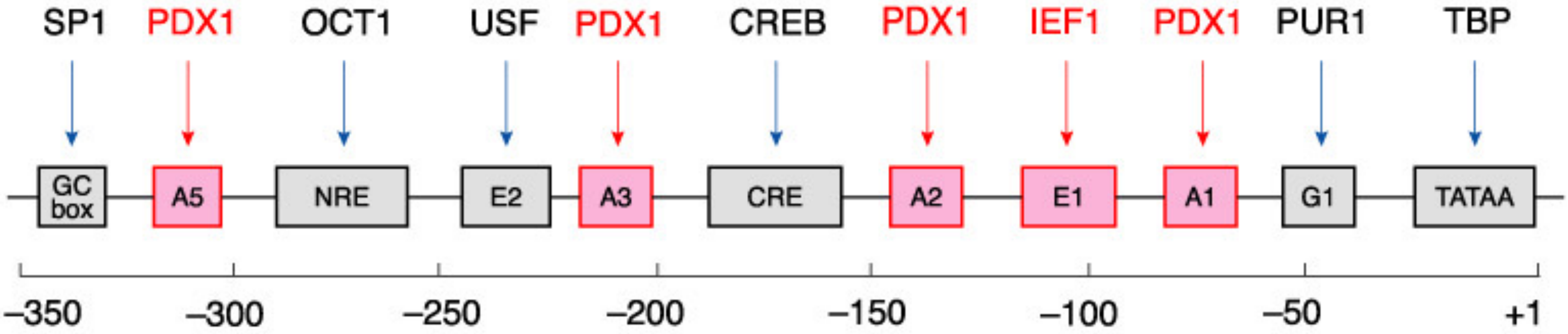


Figure 4-17b

Il promotore del gene umano dell'insulina



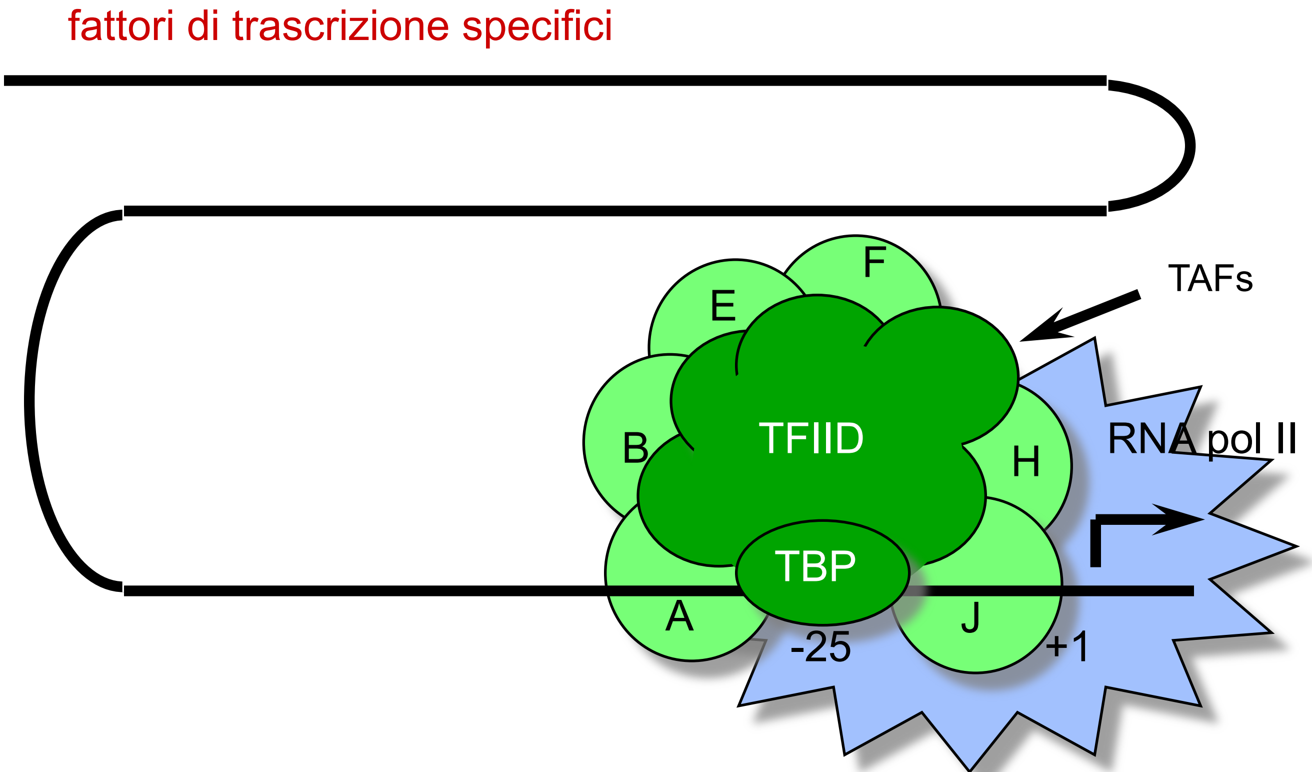
Nero: fattori ubiquitari
Rossi: fattori specifici delle cellule beta pancreatiche

Figure 10-6 Human Molecular Genetics, 3/e. (© Garland Science 2004)

Le proteine che regolano la sintesi degli mRNA eucariotici sono classificate come

fattori di trascrizione generali

- TFIID (complesso multi-proteico) si lega alla TATA box iniziando il processo di assemblaggio dell'apparato fondamentale della trascrizione
- La proteina TBP che si lega direttamente alla TATA box)
- I fattori associati a TBP (TAFs) che si legano a TBP
- TFIIA, TFIIB, TFIIE, TFIIF, TFIIH, TFIIJ si associano TFIID
- RNA polimerasi II si lega al promotore attraverso i TF



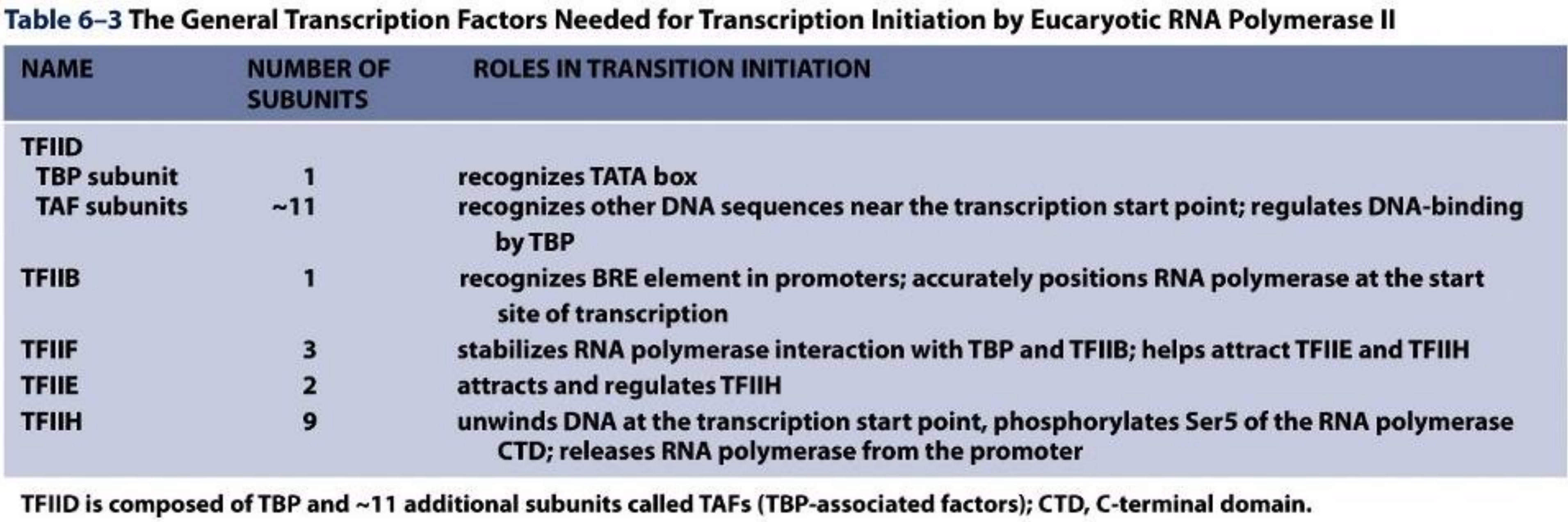


Table 6–3 The General Transcription Factors Needed for Transcription Initiation by Eucaryotic RNA Polymerase II

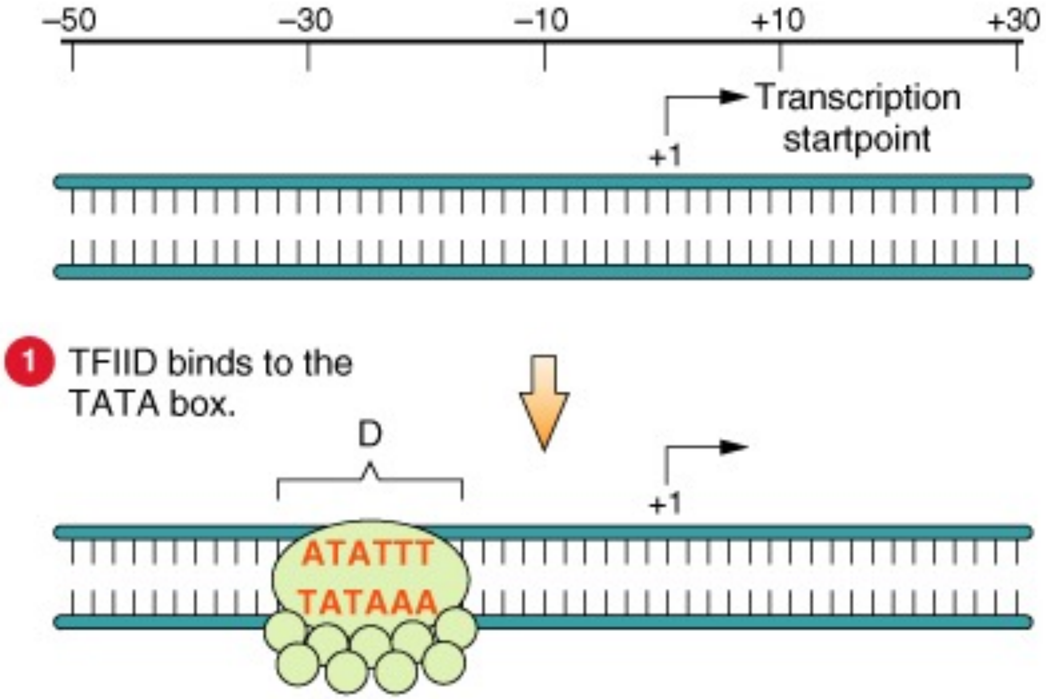
NAME	NUMBER OF SUBUNITS	ROLES IN TRANSITION INITIATION
TFIID		
TBP subunit	1	recognizes TATA box
TAF subunits	~11	recognizes other DNA sequences near the transcription start point; regulates DNA-binding by TBP
TFIIB	1	recognizes BRE element in promoters; accurately positions RNA polymerase at the start 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.

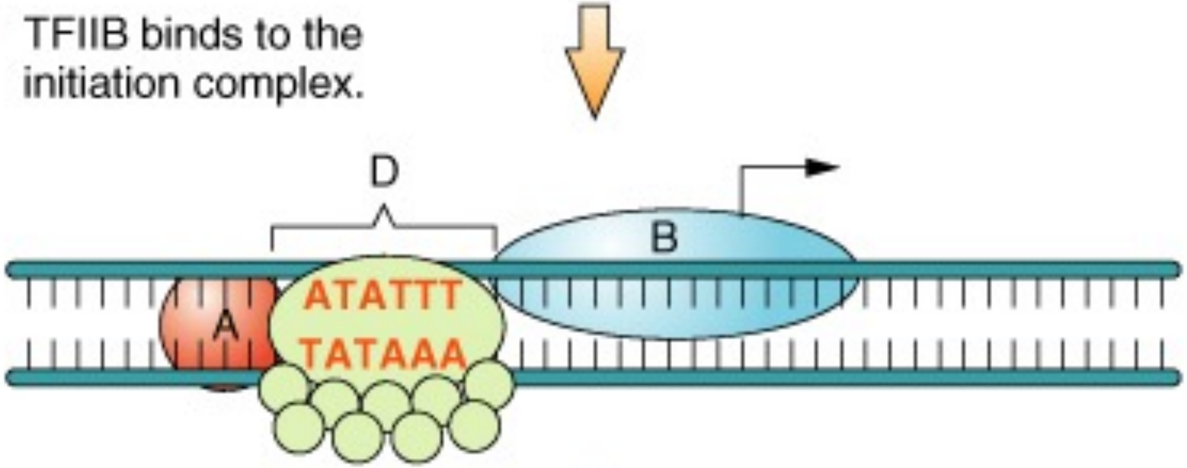
Table 6-3 *Molecular Biology of the Cell* (© Garland Science 2008)

Fattori di trascrizione

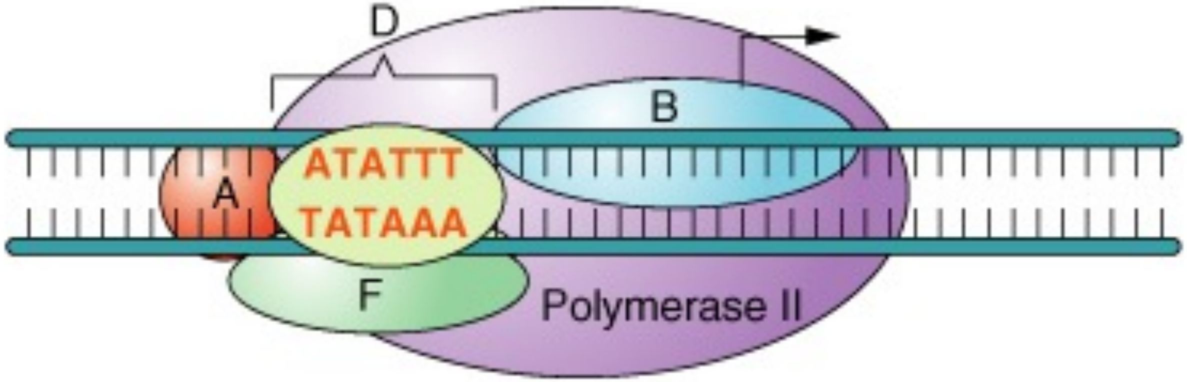
TFIID riconosce la TATA box



TFIIB binds to the initiation complex.



TFIIF, which has unwinding activity, joins the complex; polymerase II also binds here.



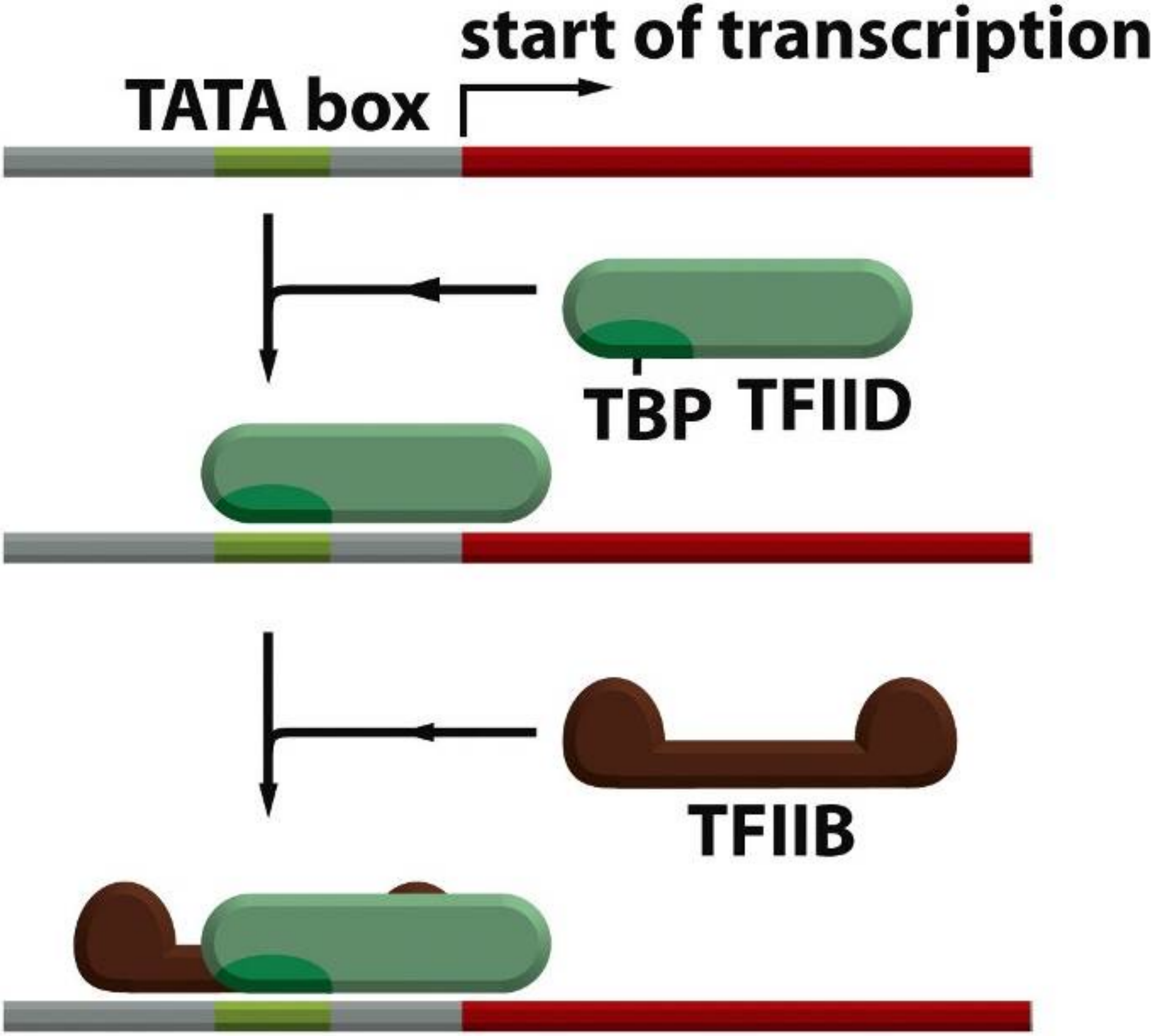


Figure 6-16 (part 1 of 3) *Molecular Biology of the Cell* (© Garland Science 2008)

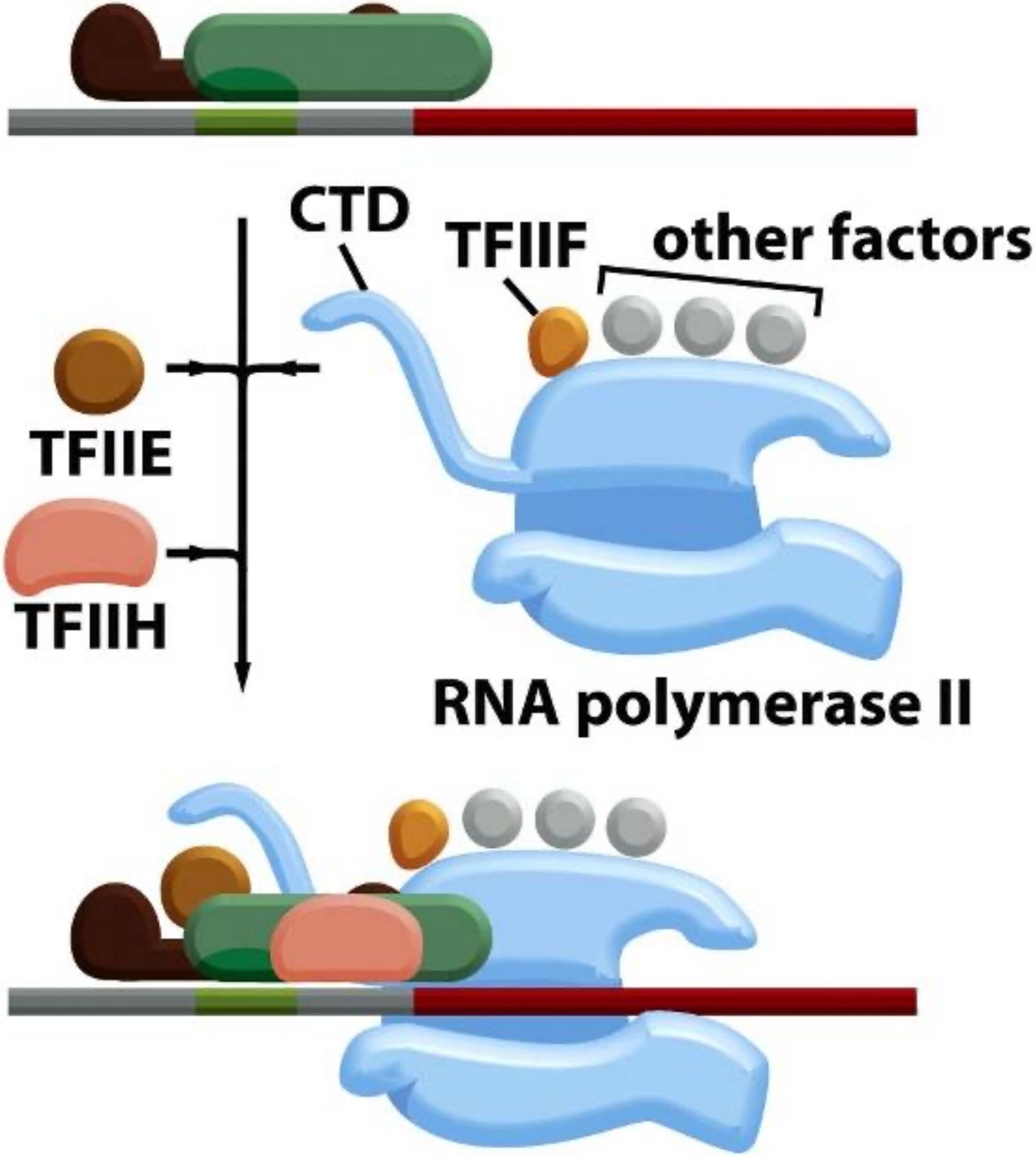
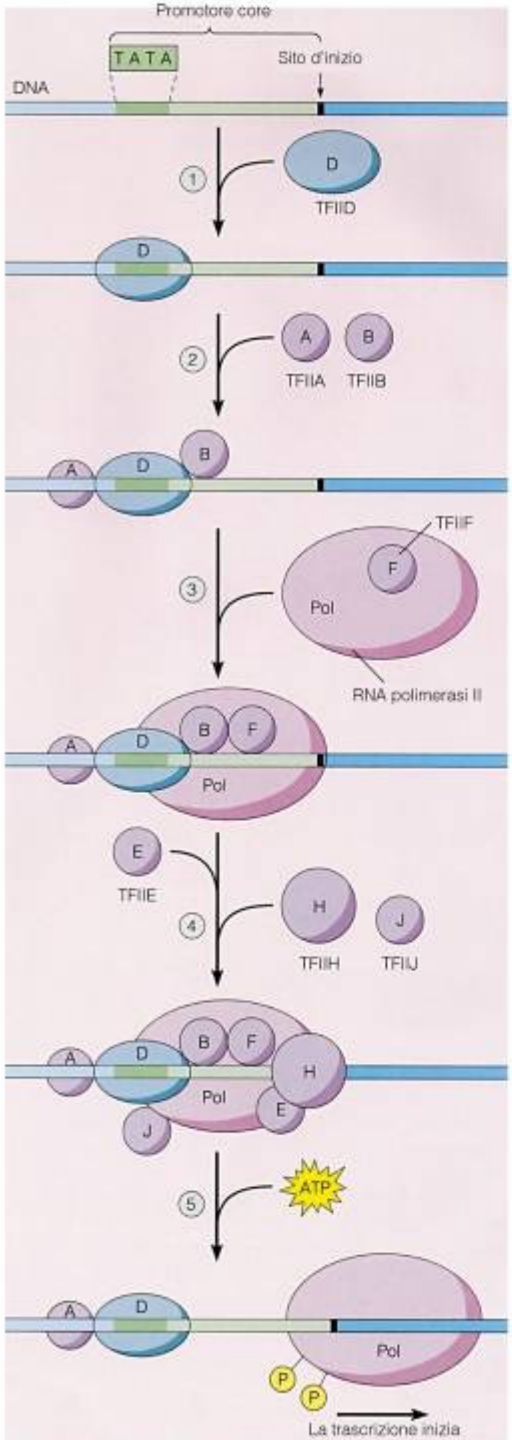
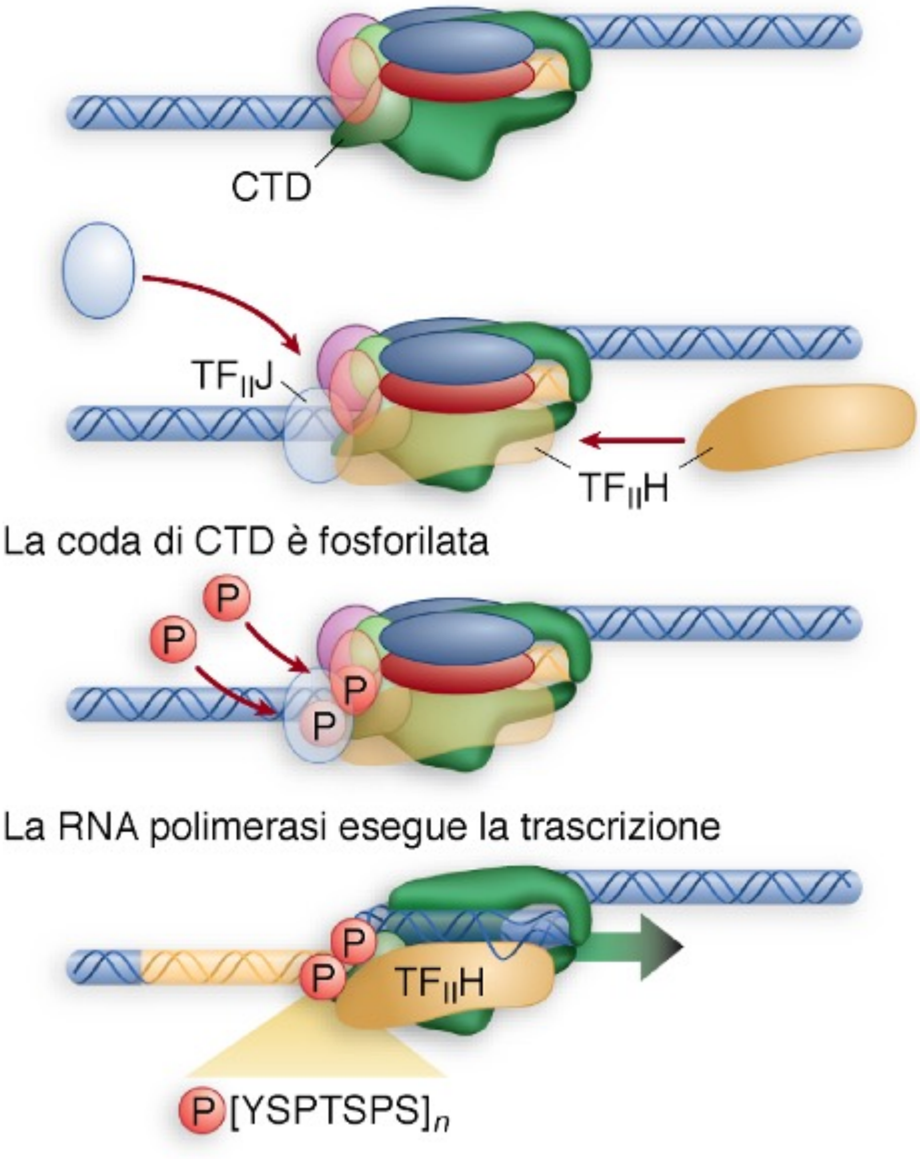


Figure 6-16 (part 2 of 3) *Molecular Biology of the Cell* (© Garland Science 2008)

Una volta assemblato il complesso di inizio, la separazione dei due filamenti di DNA, allo “start site”, richiede idrolisi di ATP.
La fosforilazione del CTD (C-terminal domain) della pol II, determina la dissociazione dei fattori di trascrizione generali e l’inizio della trascrizione.

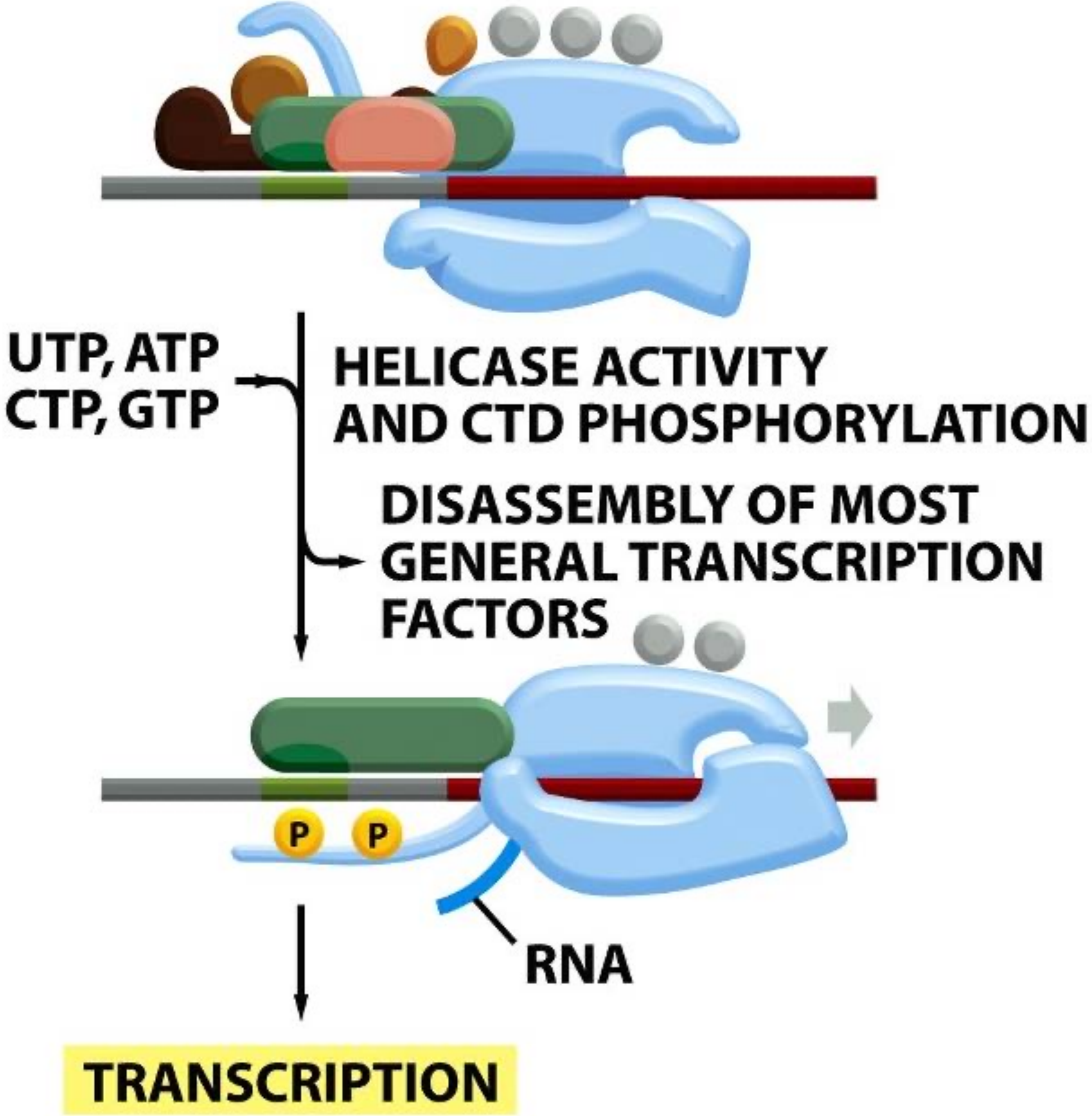


Il CTD è fosforilato durante l'inizio della trascrizione



La coda di CTD è fosforilata

La RNA polimerasi esegue la trascrizione


UTP, ATP
CTP, GTP

HELICASE ACTIVITY
AND CTD PHOSPHORYLATION

DISASSEMBLY OF MOST
GENERAL TRANSCRIPTION
FACTORS

TRANSCRIPTION

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

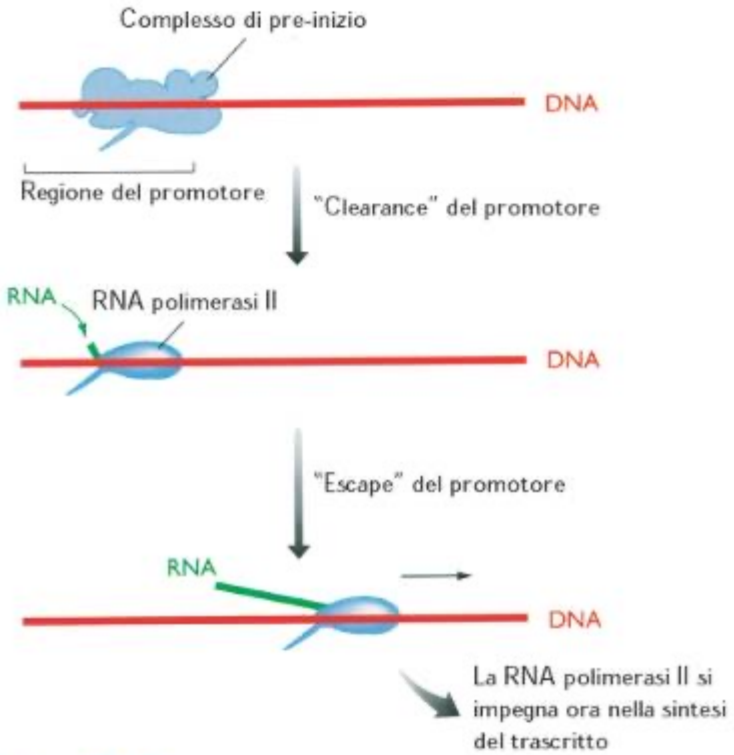
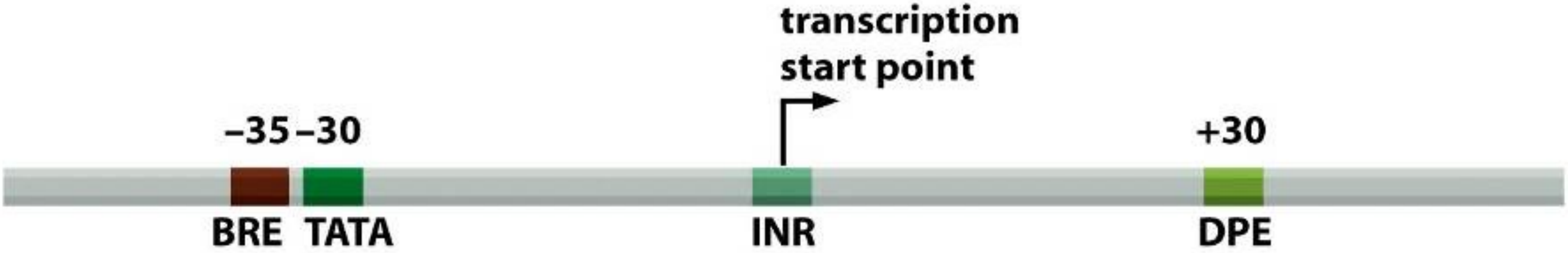


Figura 10.8 "Clearance" e "escape" del promotore.

T.A. Brown
Genomi
EdiSES

Il pre-mRNA del gene umano della distrofina è lungo 2400 kb, la RNA pol II impiega circa 20 ore per la sua sintesi

L'estrema lunghezza dei geni eucariotici richiede requisiti di stabilità per il complesso di trascrizione. Esistono quindi dei "fattori di allungamento" che stabilizzano la RNA polII, quando questa ha lasciato il promotore e si è dissociata dai fattori di trascrizione.



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)

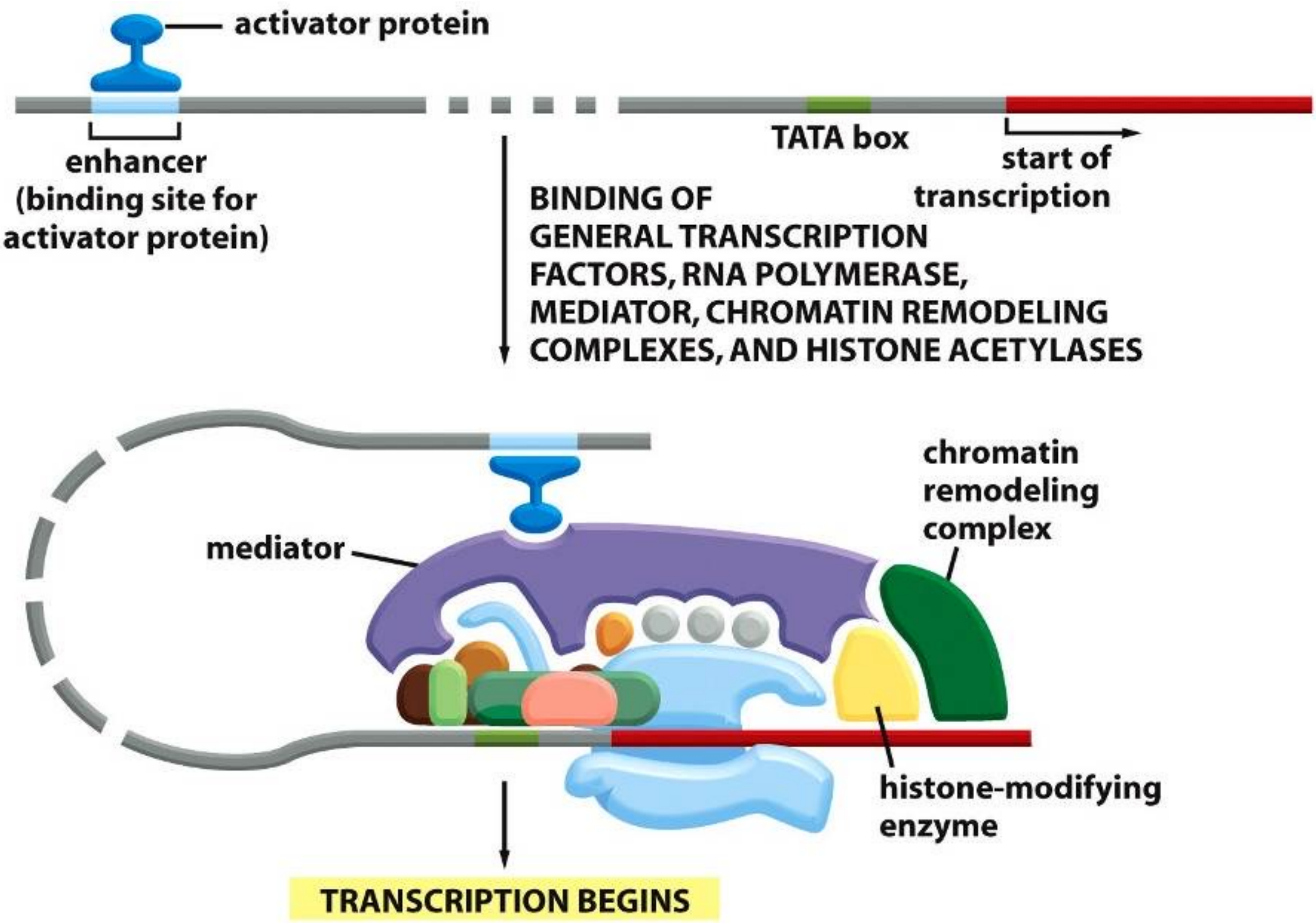
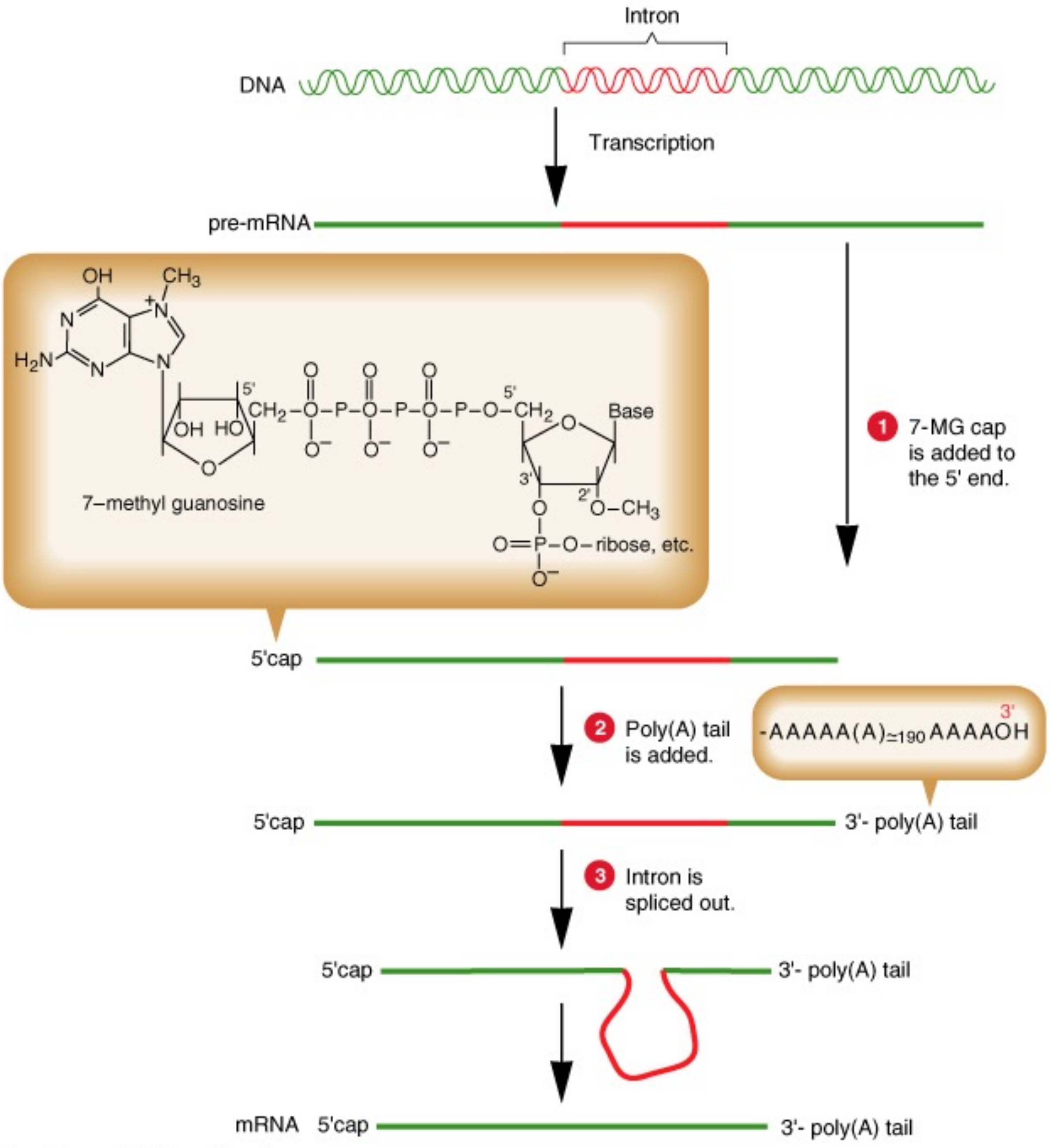


Figure 6-19 *Molecular Biology of the Cell* (© Garland Science 2008)

Maturazione dell’mRNA

Serve a produrre, nelle cellule eucariotiche, un mRNA pronto ad essere tradotto

- Processi che aumentano la stabilità dell’ mRNA
  - Cap al 5’
  - Coda di poliA al 3’
- Processi di rimozione di sequenze non codificanti (introni, non portano informazione per la sequenza proteina)
  - Splicing
  - Splicing alternativo



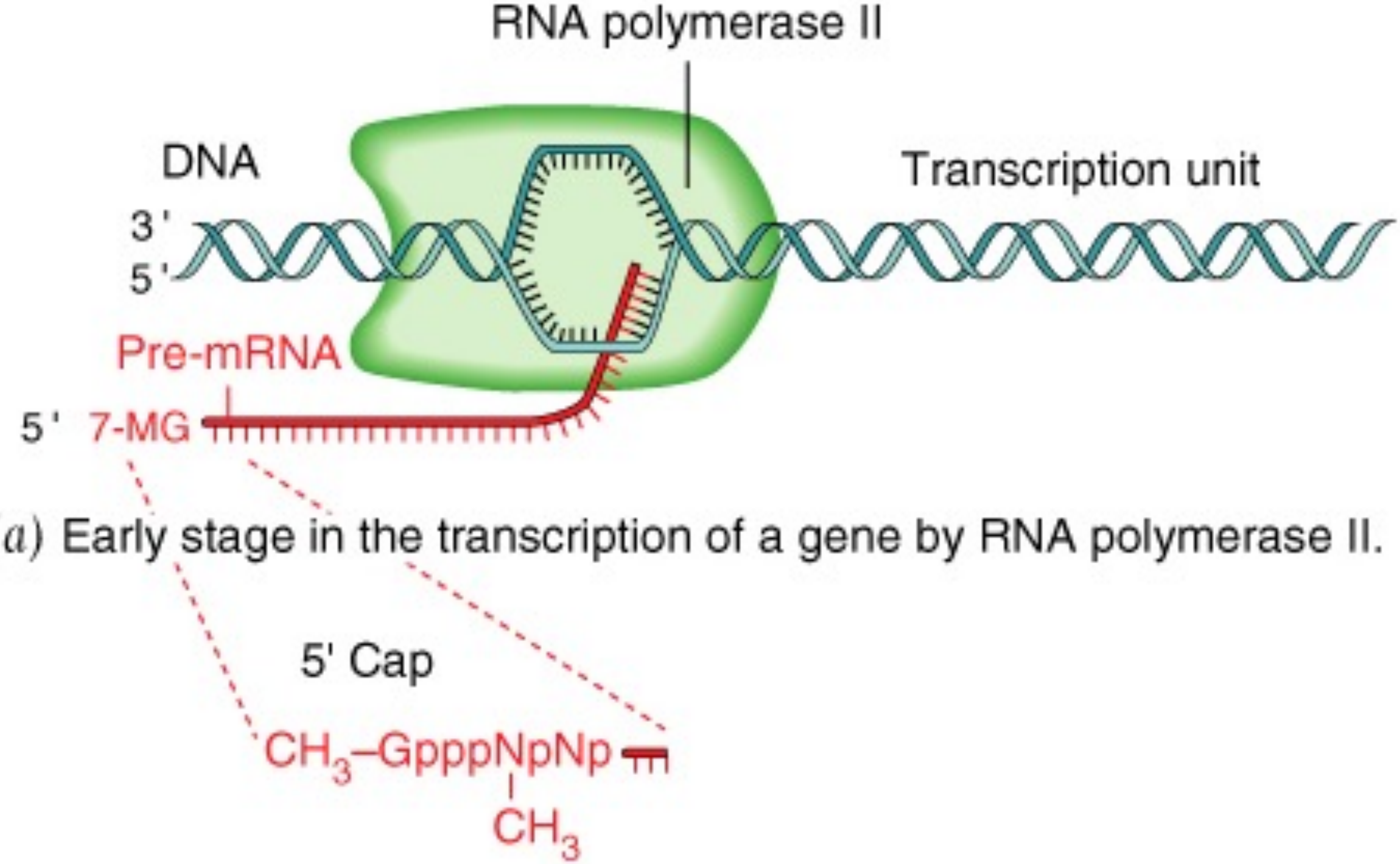
① 7-MG cap is added to the 5' end.

② Poly(A) tail is added.

③ Intron is spliced out.

Cap (cappuccio) al 5’

- E’ l’aggiunta all’estremità 5’ dell’mRNA che viene trascritto di una guanosina metilata.
- Tale guanosina viene aggiunta però mediante un legame 5’ – 5’
- Serve a rendere l’mRNA più resistente all’attacco delle nucleasi e a farlo riconoscere come un mRNA da tradurre



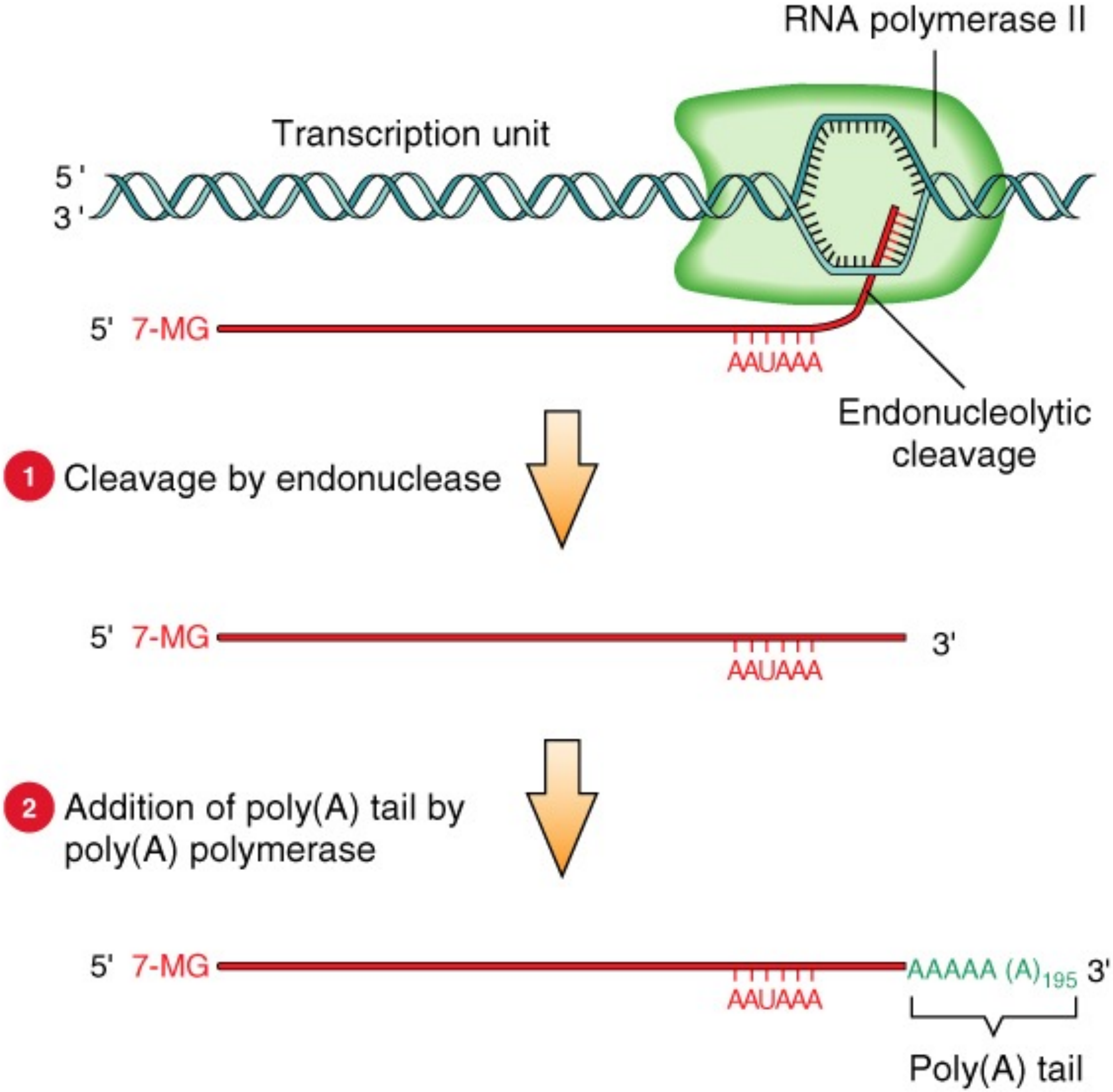
(*a*) Early stage in the transcription of a gene by RNA polymerase II.

Coda di PoliA

- Consiste nell'aggiunta all'estremità 3' di una coda di A di lunghezza variabile (50-250 nucleotidi)

- La coda viene aggiunta dalla poli(A) polimerasi che riconosce uno specifico segnale sull'mRNA, taglia l'mRNA a valle di tale segnale e attacca la coda di poliA

- La coda protegge l'mRNA dall'attacco delle nucleasi



① Cleavage by endonuclease

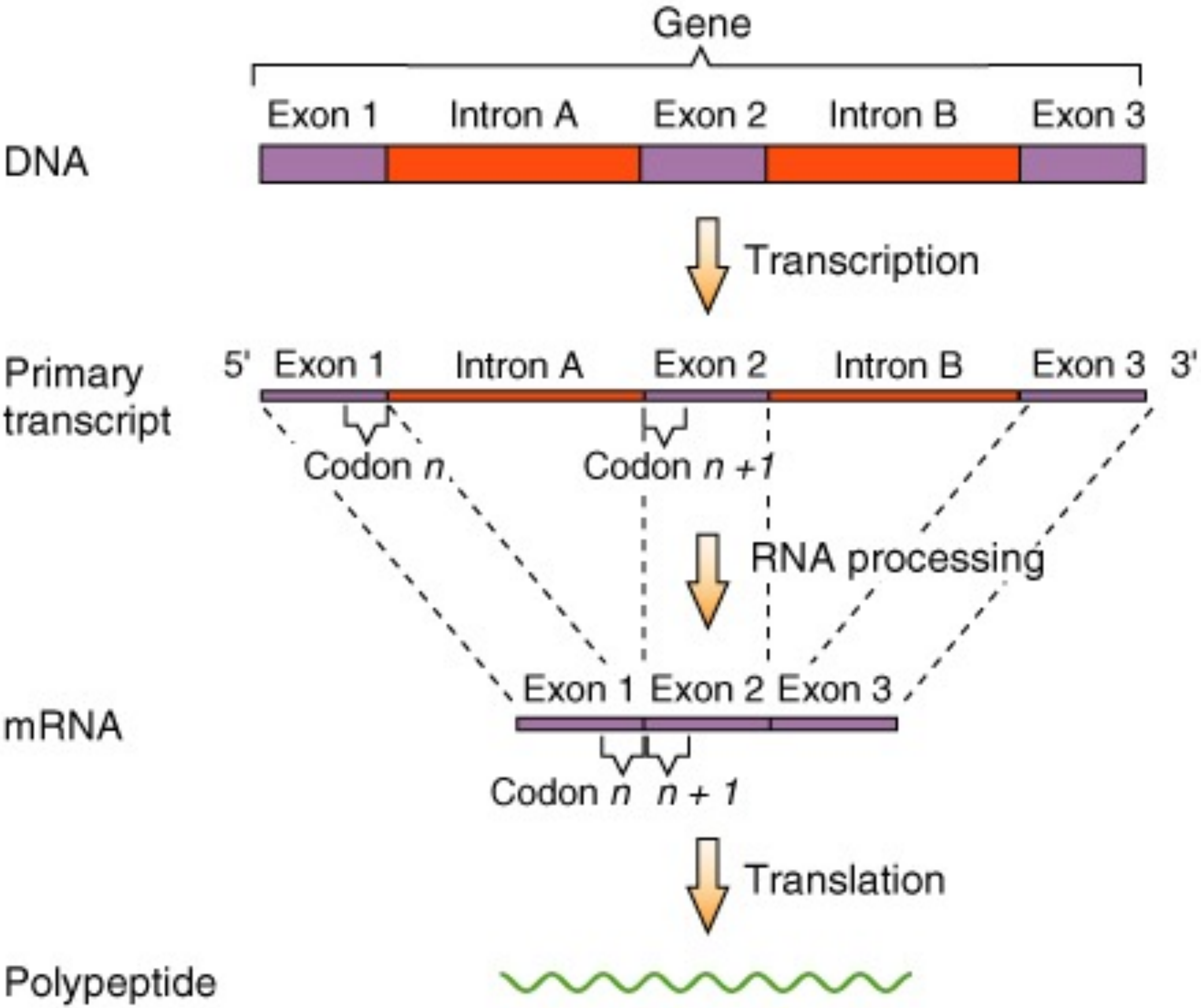
② Addition of poly(A) tail by poly(A) polymerase

Splicing

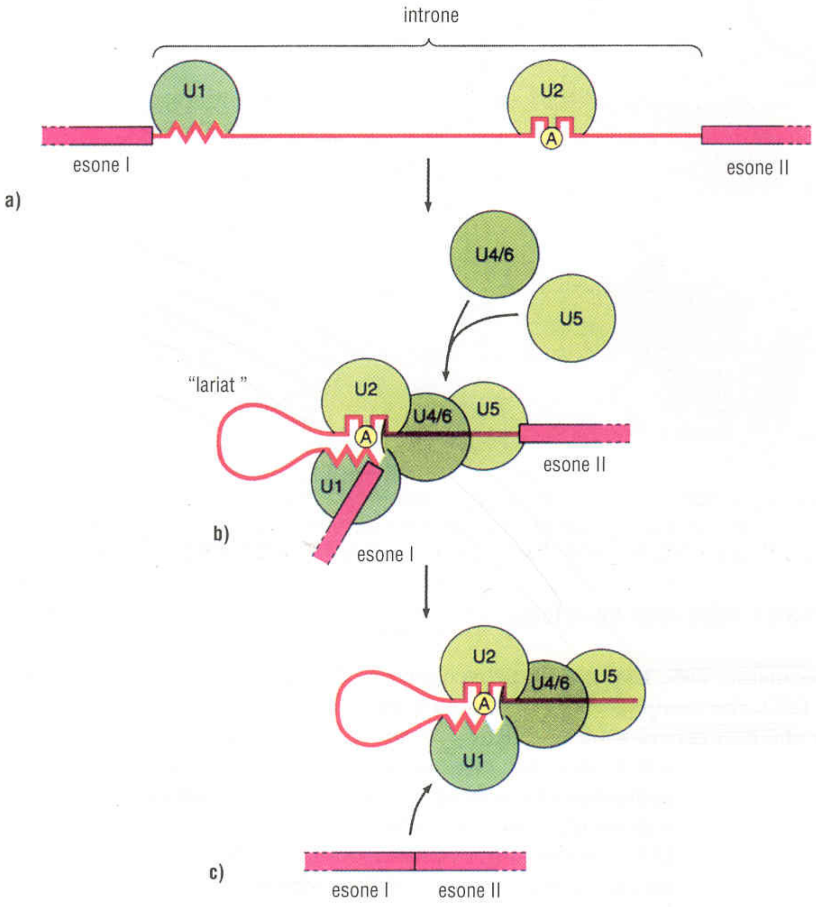
- L’RNA appena trascritto contiene gli introni, cioè sequenze che non portano l’informazione per una proteina e che non sono presenti nell’RNA maturo

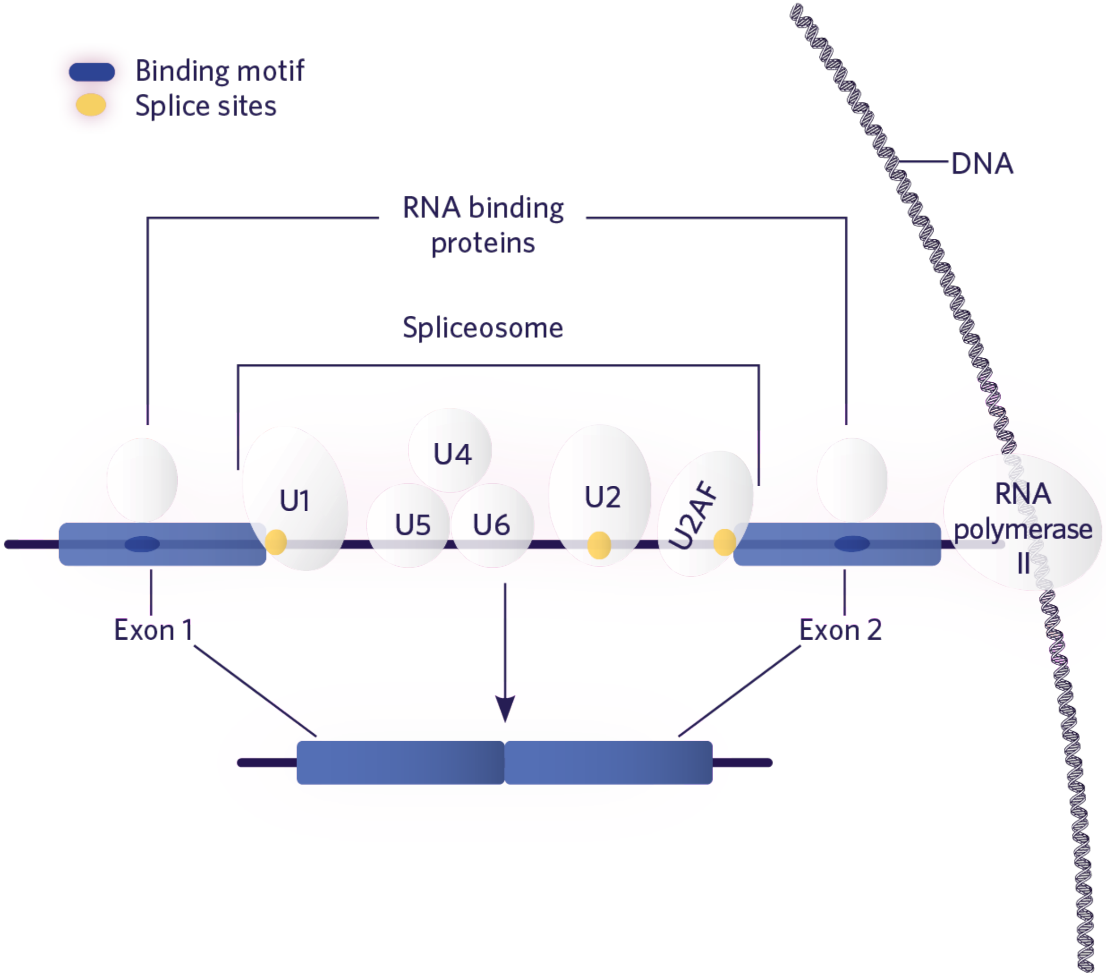
- Queste sequenze vengono rimosse prima che l’RNA passi nel citoplasma (splicing)

- Lo splicing avviene mediante l’utilizzo di particolari proteine ed RNA che riconoscono i siti di taglio


Gene
Exon 1
Intron A
Exon 2
Intron B
Exon 3
DNA
Transcription
Primary transcript
5'
Exon 1
Intron A
Exon 2
Intron B
Exon 3
3'
Codon *n*
Codon *n* +1
RNA processing
Exon 1
Exon 2
Exon 3
mRNA
Codon *n*
n + 1
Translation
Polypeptide

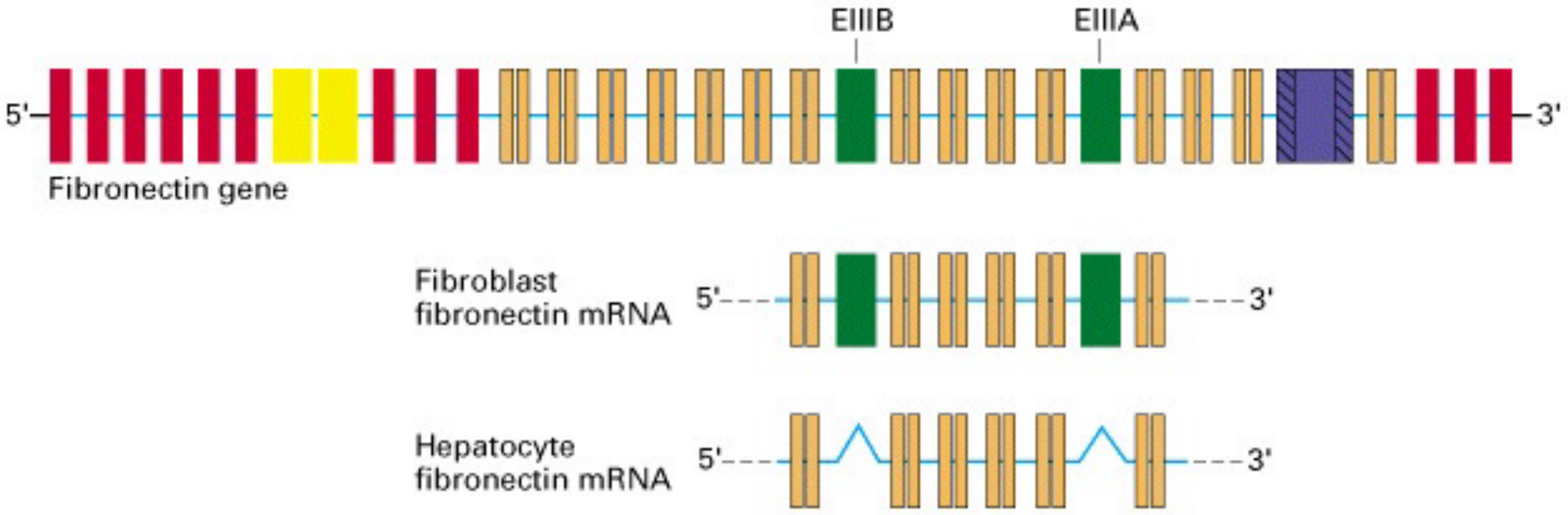
Per le sue reazioni lo spliceosoma idrolizza diverse molecole di ATP. I cinque RNA (U1, U2, U4, U5 e U6) sono chiamati piccoli RNA nucleari (snRNA, small nuclear RNA). Ognuno di questi si complessa con diverse proteine del macchinario, formando complessi, chiamati ribonucleoproteine nucleari (snRNP, small nuclear ribonuclear protein).

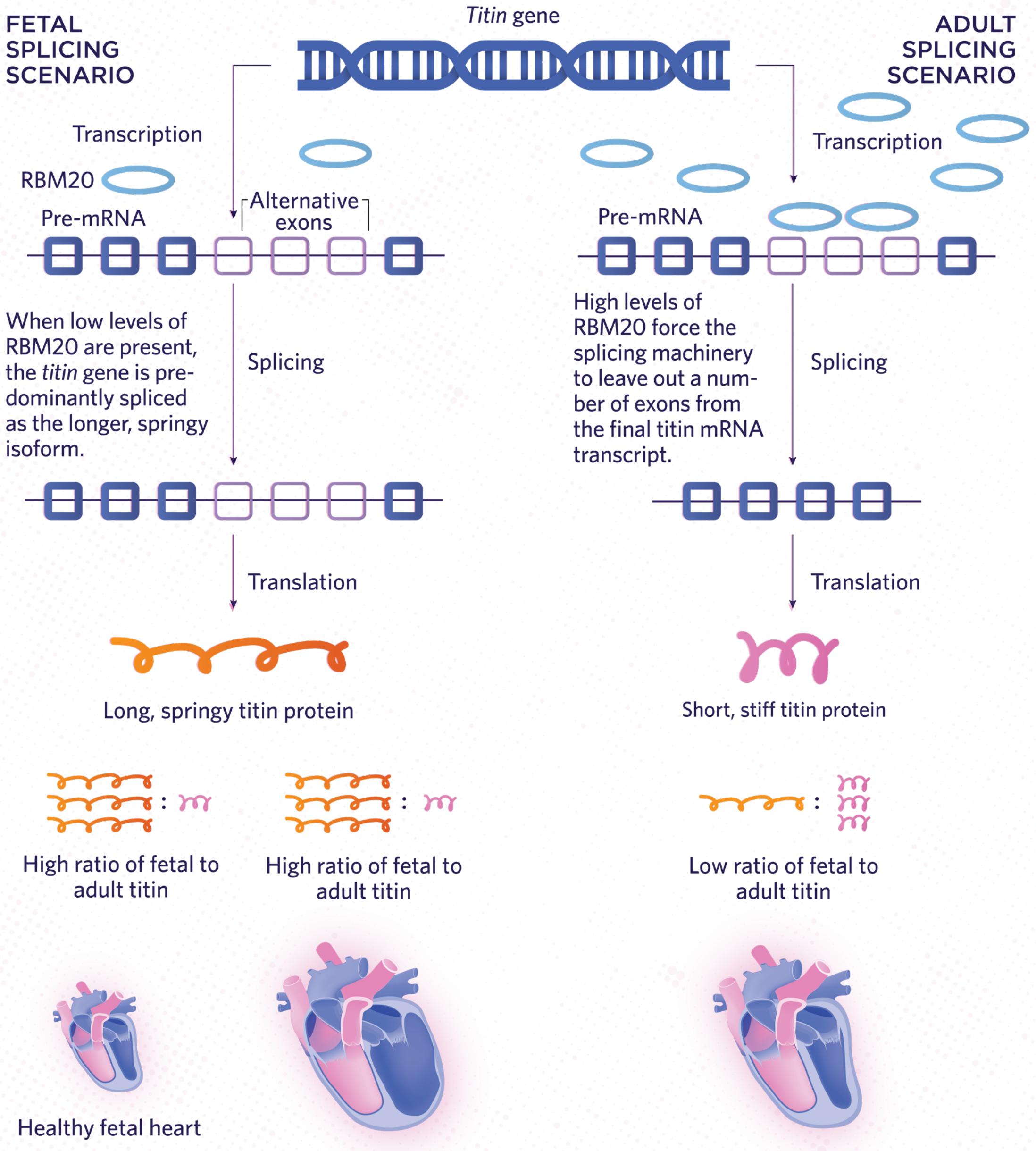



Binding motif
Splice sites
DNA
RNA binding proteins
Spliceosome
U4
U1
U5
U6
U2
U2AF
RNA polymerase II
Exon 1
Exon 2

Splicing alternativo

splicing alternativo, tessuto-specifico degli mRNA delle fibronectine





When low levels of RBM20 are present, the *titin* gene is predominantly spliced as the longer, springy isoform.

High levels of RBM20 force the splicing machinery to leave out a number of exons from the final titin mRNA transcript.

Long, springy titin protein

Short, stiff titin protein

High ratio of fetal to adult titin

High ratio of fetal to adult titin

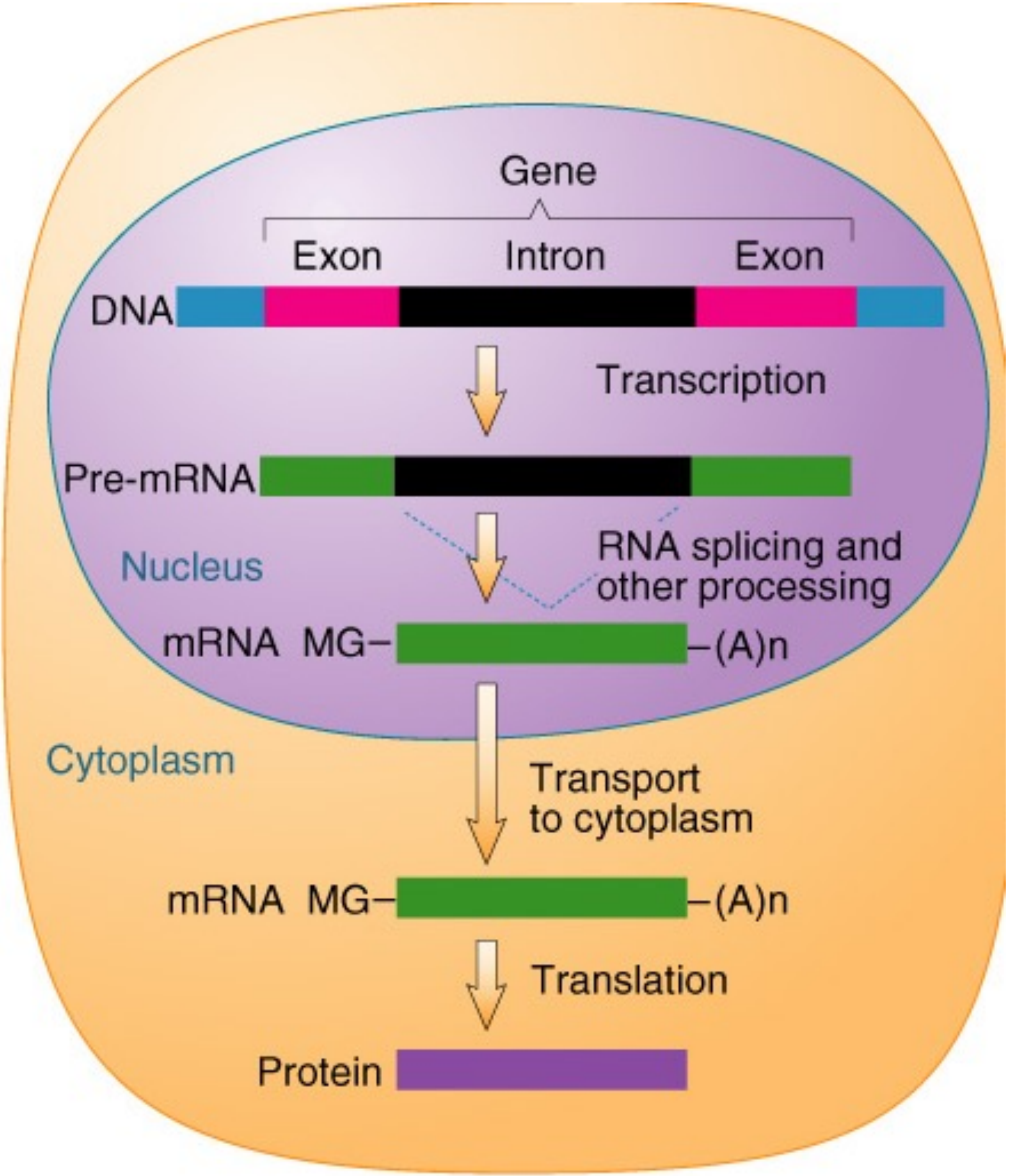
Low ratio of fetal to adult titin

Healthy fetal heart

A higher-than-normal proportion of fetal titin in an adult heart can contribute to a heart that's too elastic, a condition called dilated cardiomyopathy.

Healthy adult heart

Eucarioti


Gene
Exon
Intron
Exon
DNA
Transcription
Pre-mRNA
Nucleus
RNA splicing and other processing
mRNA MG–
–(A)n
Cytoplasm
Transport to cytoplasm
mRNA MG–
–(A)n
Translation
Protein

Trascrizione dei geni per l’ rRNA

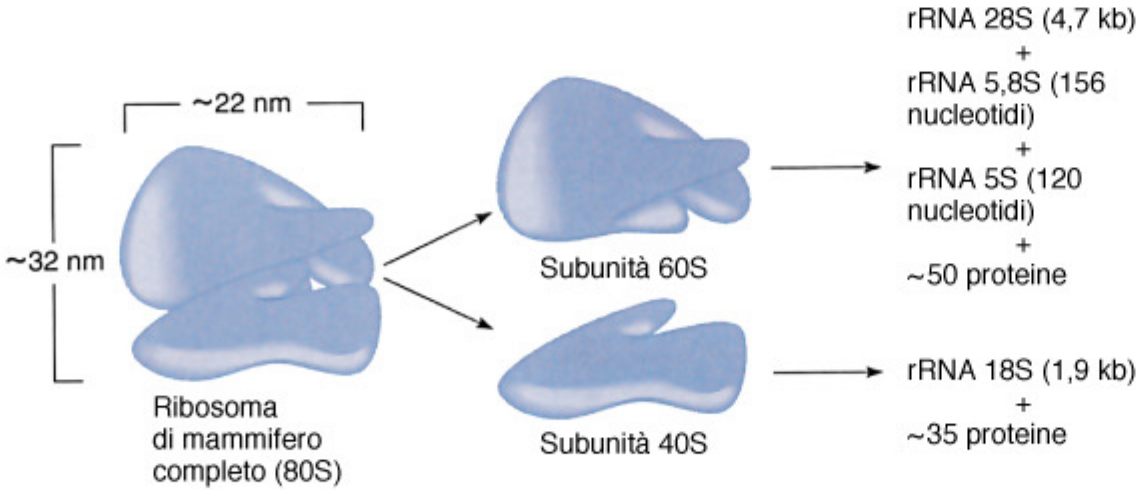


Figura 5.16

Composizione dei ribosomi interi e delle subunità ribosomali delle cellule di mammifero.

I geni per gli rRNA-eucariotici

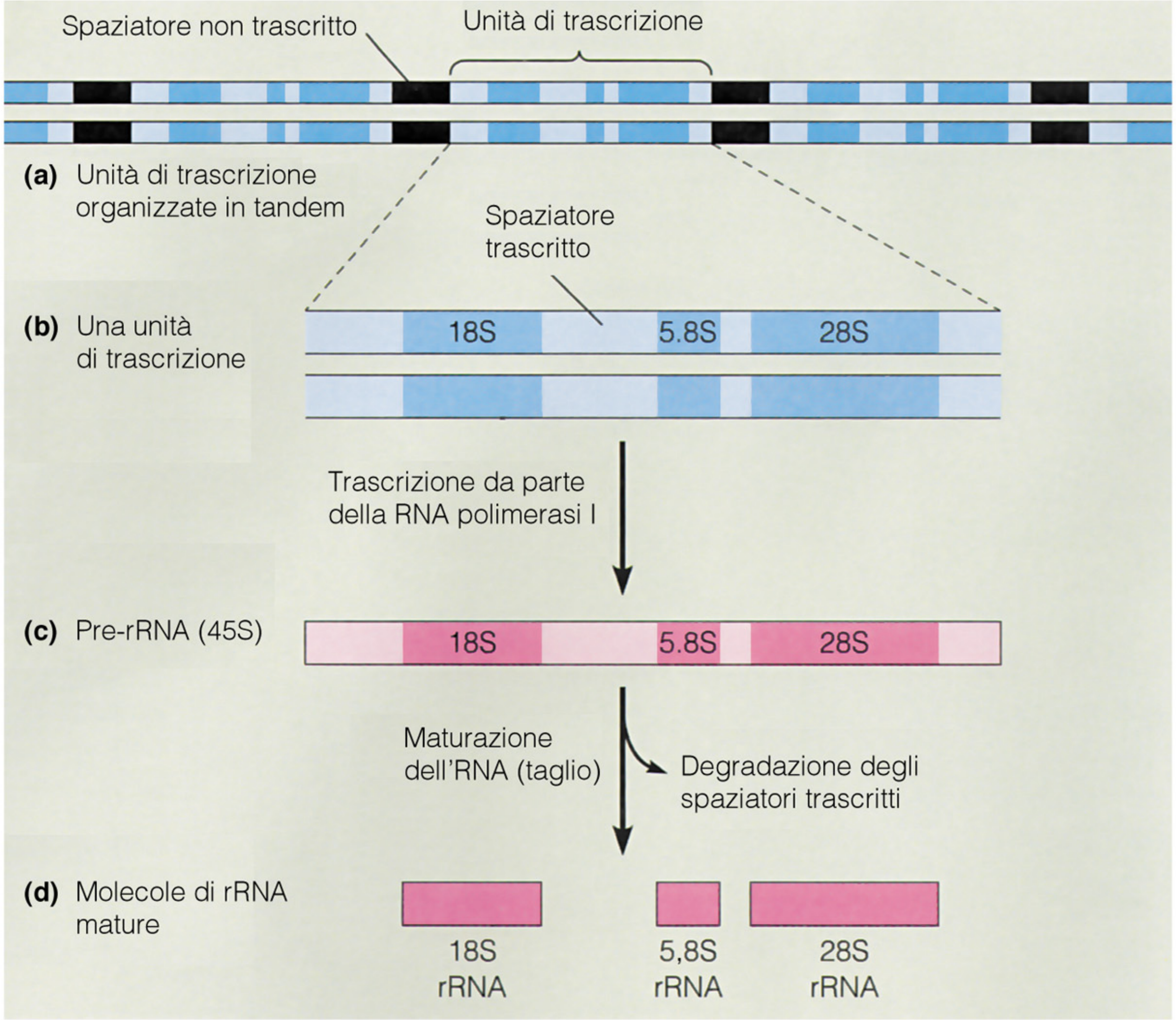
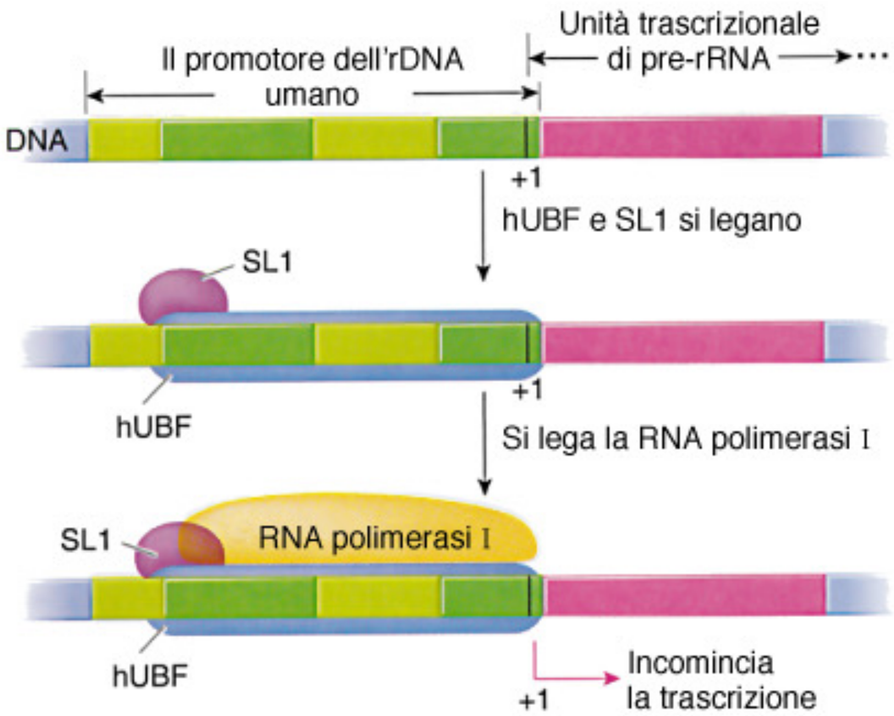


Figura 19-16

cluster di geni ripetuti

Figura 5.19

Fattori di trascrizione coinvolti nell'inizio della trascrizione dell'rDNA umano da parte della RNA polimerasi I. (Per la spiegazione si rimanda al testo).

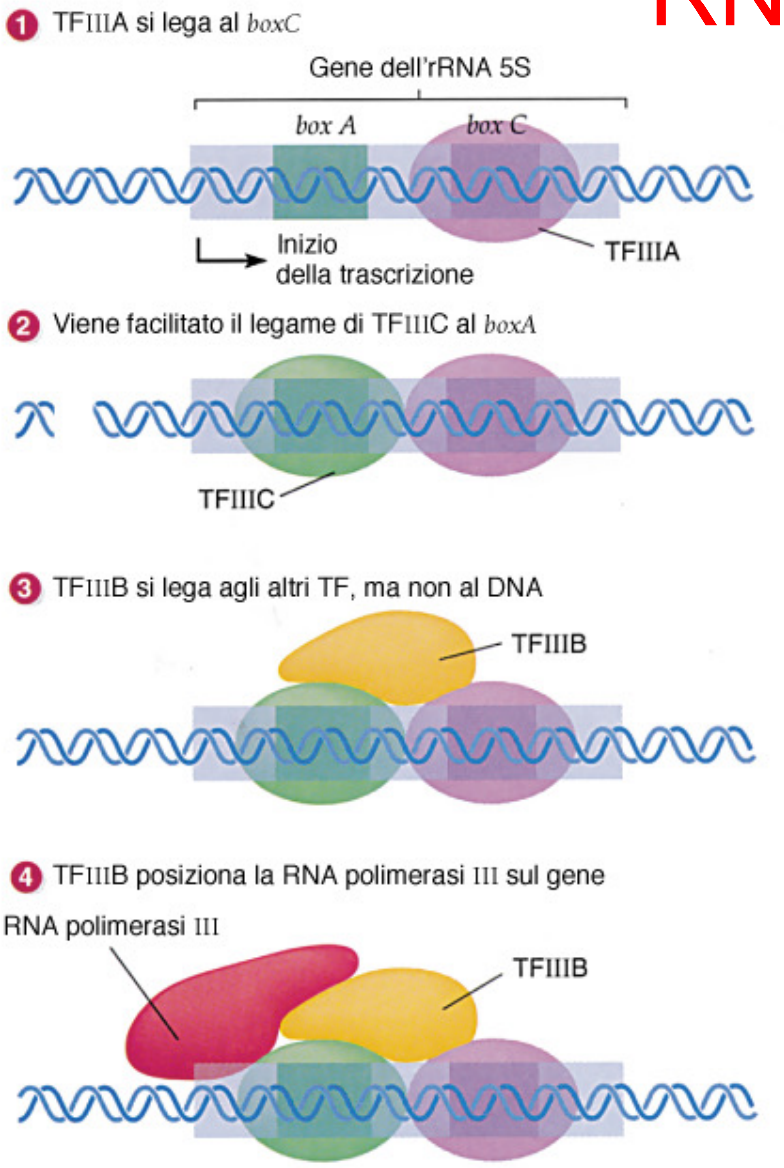


I fattori di trascrizione:

hUBF (human upstream binding factor) lega due sequenze specifiche del promotore;

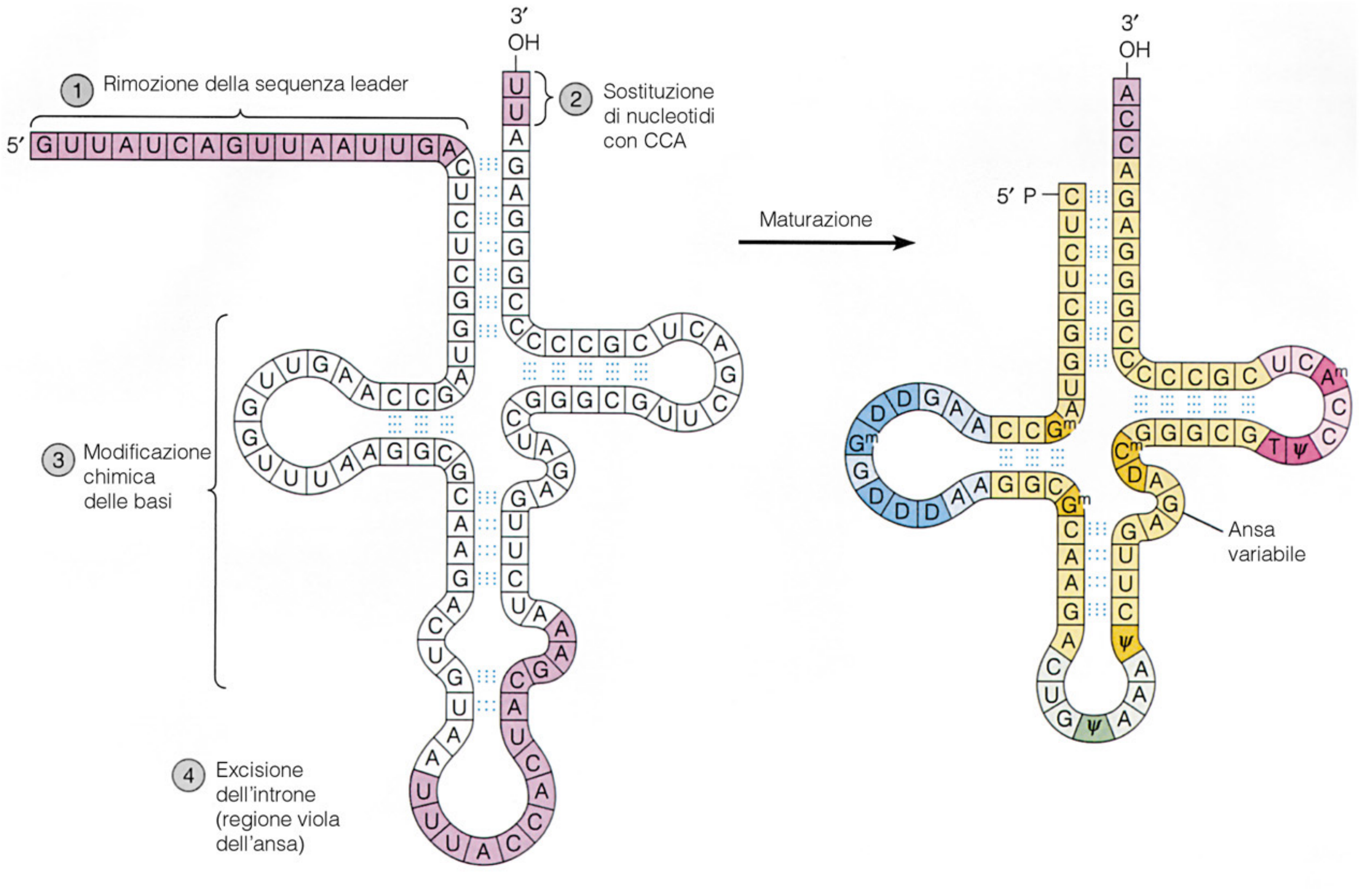
SL1 si lega al complesso

Trascrizione dei geni da parte della RNA polIII



Trascrive i geni per l’ rRNA 5S, i tRNA e alcuni geni per altri piccoli RNA (snRNA).
Il promotore è interno all’ unità di trascrizione.

La maturazione dei tRNA



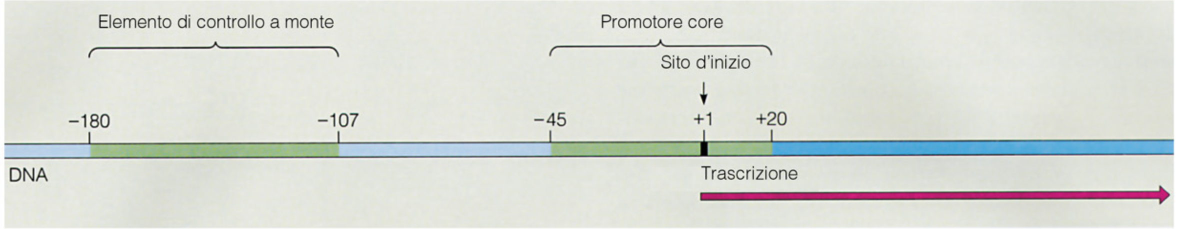
(a) Transcritto primario (precursore) per il tRNA di lievito per la tirosina

(b) tRNA maturo, struttura secondaria

Figura 19-17

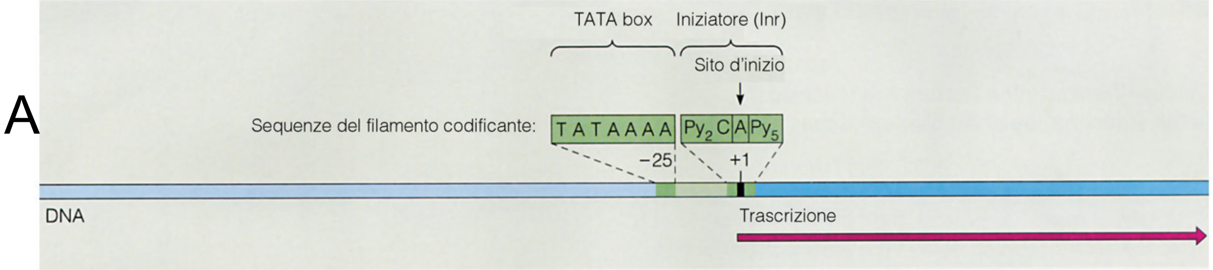
Promotori eucariotici

RNA pol I trascrive rRNA



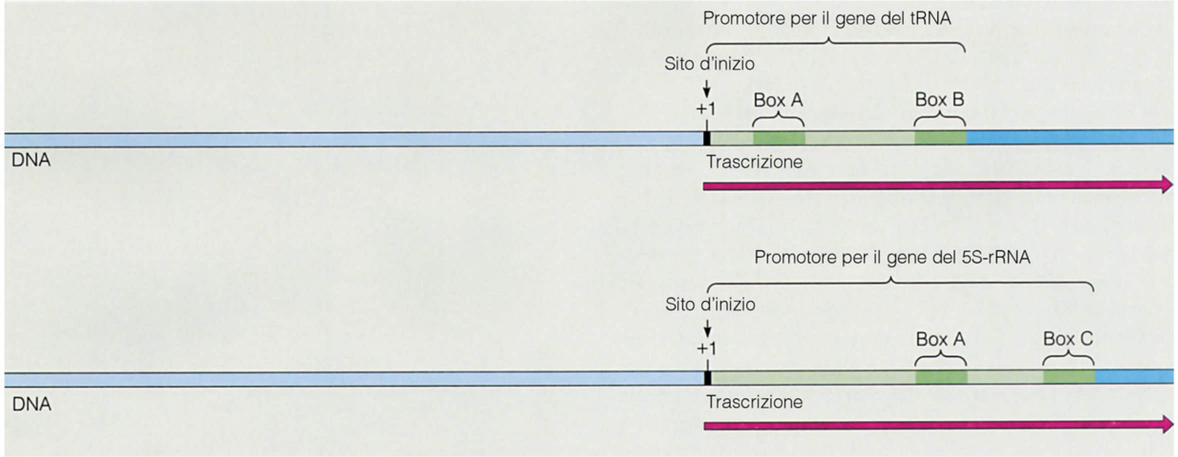
(a) Promotore per la RNA polimerasi I

RNA pol II trascrive mRNA per proteine e miRNA



(b) Promotore core per la RNA polimerasi II

RNA pol III trascrive tRNA e 5S rRNA



(c) Due tipi di promotore per la RNA polimerasi III

Figura 19-13

Meccanismi di terminazione della trascrizione negli eucarioti

- RNA polymerase I termina la trascrizione attraverso un meccanismo che richiede un fattore di terminazione polimerasi-specifico, che si lega a valle dell'unità di trascrizione

- RNA polymerase II termina la trascrizione in una regione posta 0.5-2 kb a valle del sito di poliadenilazione grazie ad un meccanismo che accoppia il taglio del pre-mRNA e la sua poliadenilazione all'estremità 3′

- RNA polymerase III termina la trascrizione dopo aver polimerizzato una serie di residui U

New Roles of RNA

- ncRNAs - functional RNA molecules rather than proteins; RNA other than mRNA (ex.XIST)
- RNAi - RNA interference
- siRNA- active molecules in RNA interference; degrades mRNA (act where they originate)
- miRNAs - tiny 21–24-nucleotide RNAs; probably acting as translational regulators of protein-coding mRNAs (regulate elsewhere)

Micro RNA (miRNA)

- ***Piccole molecole di RNA (20-22 nt)***

- ***Prodotte da precursori di 90-100 nt trascritti autonomamamente (anche policistronici) o maturati da introni***

- ***Si legano a mRNA che hanno sequenze complementari***

- ***Presenti in tutti gli eucarioti***

Table II. **miRNAs conserved across phyla**

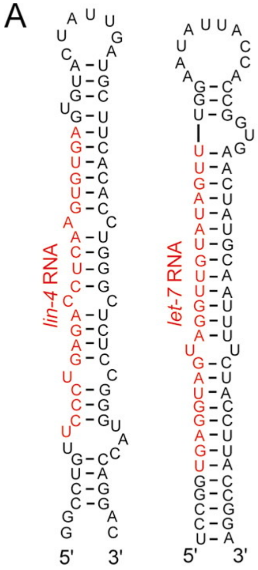
Name	**Sequence**[1]	**Homologues**[2]
miR-1	UGGAAUGUAAAGAAGUAUGGAG	C, D, H
miR-2	UAUCACAGCCAGCUUUGA(G/U)G(U/A)GC[3]	C, D
miR-7	UGGAAGACUAGUGAUUUUGUUGU	D, H
miR-34	AGGCAGUGUGGUUAGCUGGUUG	C, D, H
miR-60	UAUUAUGCACAUUUUCUAGUUCA	C, D, H
miR-79	AUAAAGCUAGGUUACCAAAGCU	C, D
miR-84	UGAGGUAGUAUGUAAUAUUGUA	C, D, H
miR-87	GUGAGCAAAGUUUCAGG(U/A)GU[3]	C, D, H

[1] RNA sequences are deduced from cDNA sequencing; no RNAs have yet been sequenced.

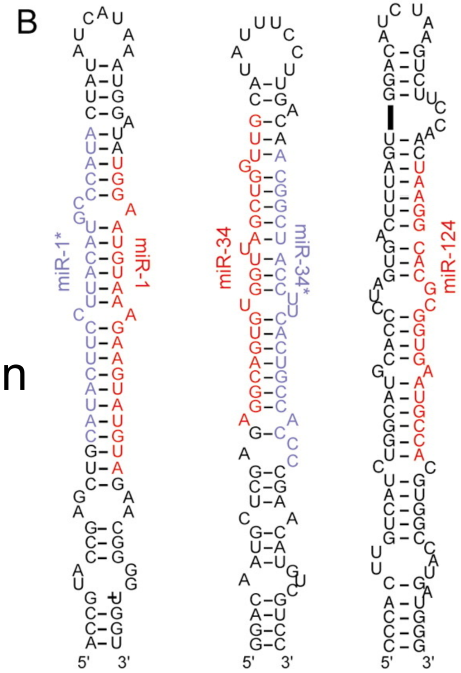
[2] C, *C. elegans*; D, *D. melanogaster*; H, *H. sapiens*.

[3] Letters in parentheses indicate variations in otherwise identical miRNAs from different organisms or variant genes within one organism.

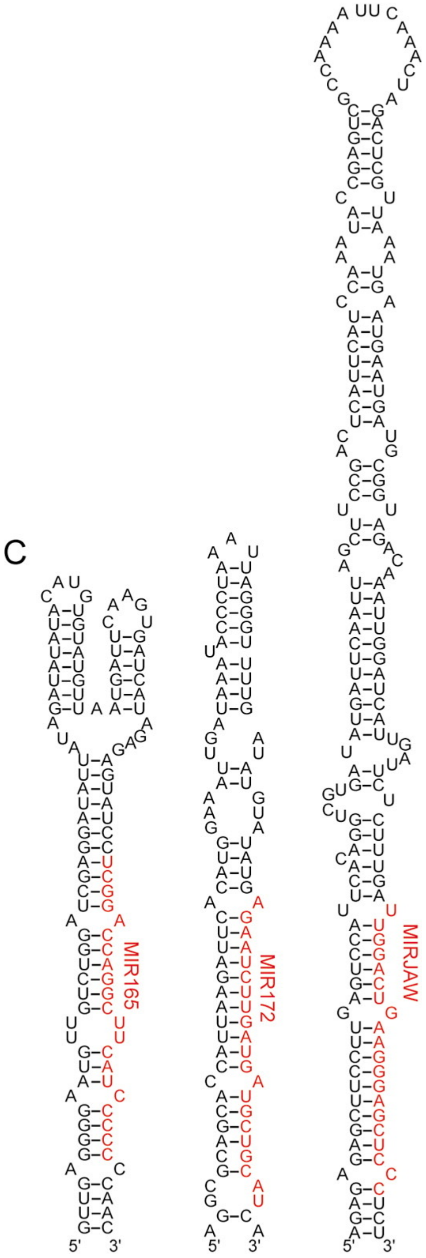
First miRNA
in *C.elegans*



miRNA
in *C.elegans*
with homologs
In flies and human



miRNA
in Plants



How Many Different MicroRNA genes are There?

C. elegance ~ 40,000 pairs of hairpins

35,697 had the minimal conservation

to receive *MiRscan* score.

53 miRNAs were cloned

Drosophila melanogaster ~ 436,000 pairs of hairpins

118,000 structure with high score

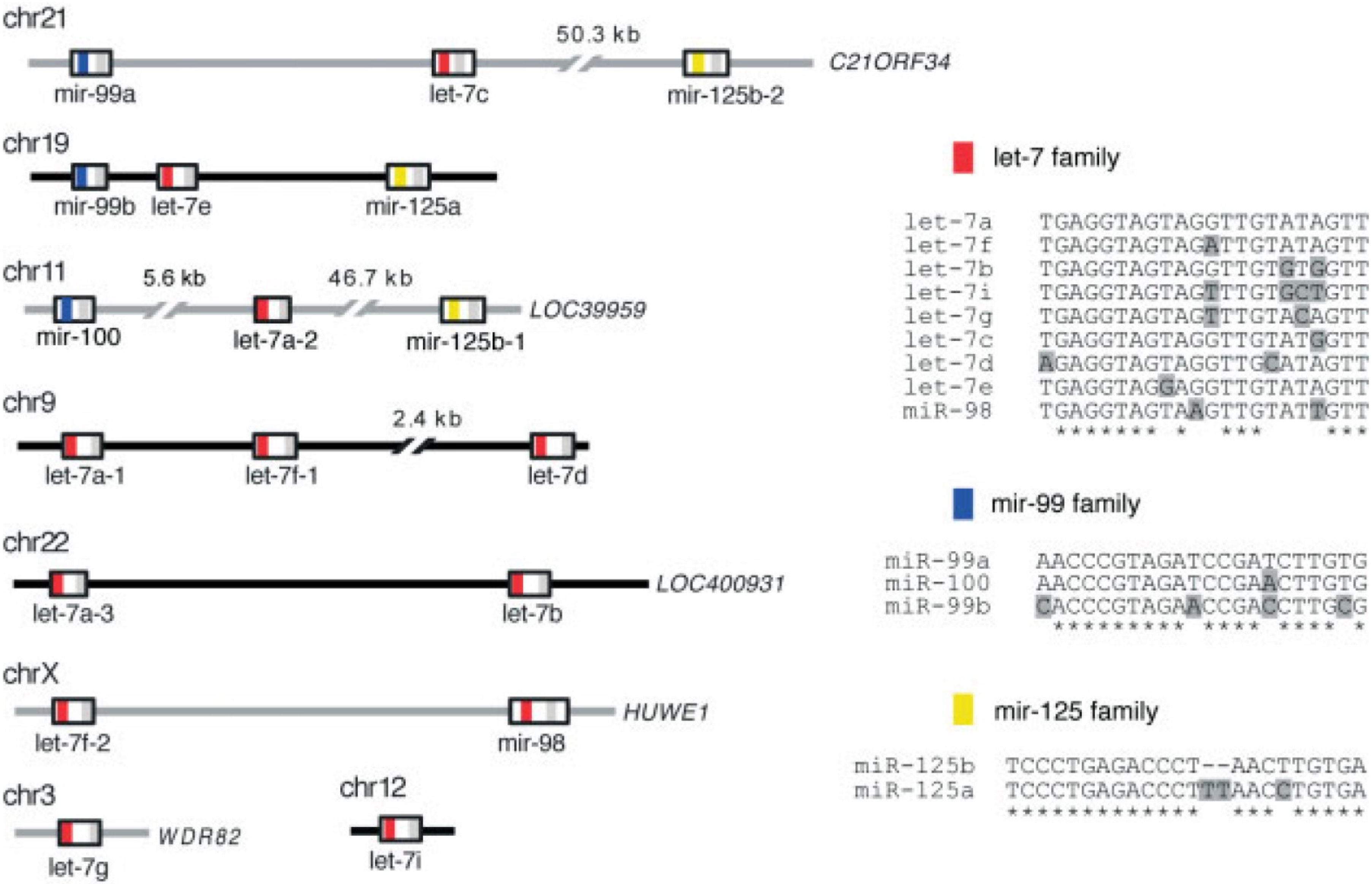
124 miRNA genes

Human ~ 800,000 pairs of hairpins

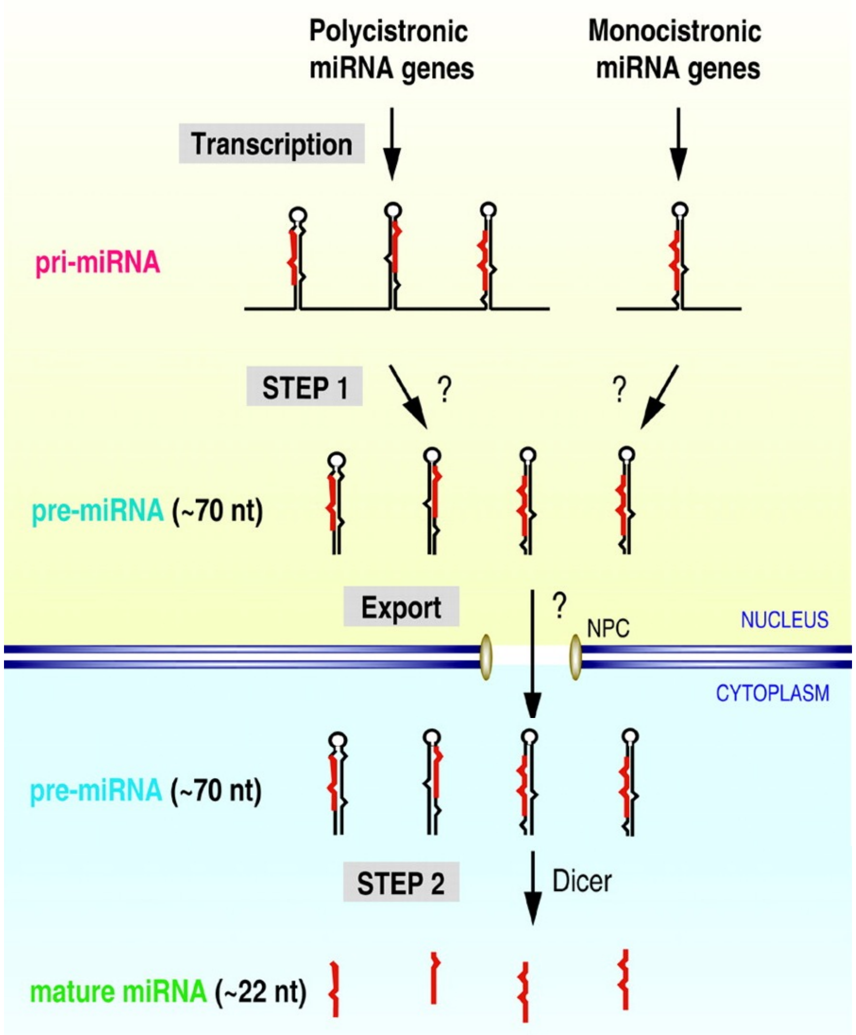
15,000 have a minimal conservation

to receive *MIRscan* score (non-coding regions)

255 miRNA genes


chr21
50.3 kb
mir-99a
let-7c
mir-125b-2
C21ORF34
chr19
mir-99b
let-7e
mir-125a
chr11
5.6 kb
46.7 kb
mir-100
let-7a-2
mir-125b-1
LOC39959
chr9
2.4 kb
let-7a-1
let-7f-1
let-7d
chr22
let-7a-3
let-7b
LOC400931
chrX
let-7f-2
mir-98
HUWE1
chr3
let-7g
WDR82
chr12
let-7i
let-7 family
let-7a TGAGGTAGTAGGTTGTATAGTT
let-7f TGAGGTAGTAGATTGTATAGTT
let-7b TGAGGTAGTAGGTTGTGTGGTT
let-7i TGAGGTAGTAGTTTGTGCTGTT
let-7g TGAGGTAGTAGTTTGTACAGTT
let-7c TGAGGTAGTAGGTTGTATGGTT
let-7d AGAGGTAGTAGGTTGCATAGTT
let-7e TGAGGTAGGAGGTTGTATAGTT
miR-98 TGAGGTAGTAAGTTGTATTGTT
mir-99 family
miR-99a AACCCGTAGATCCGATCTTGTG
miR-100 AACCCGTAGATCCGAACTTGTG
miR-99b CACCCGTAGAACCGACCTTGCG
mir-125 family
miR-125b TCCCTGAGACCCT--AACTTGTGA
miR-125a TCCCTGAGACCCTTTAACCTGTGA

MicroRNA biogenesis



Differences in miRNA Mode of Action