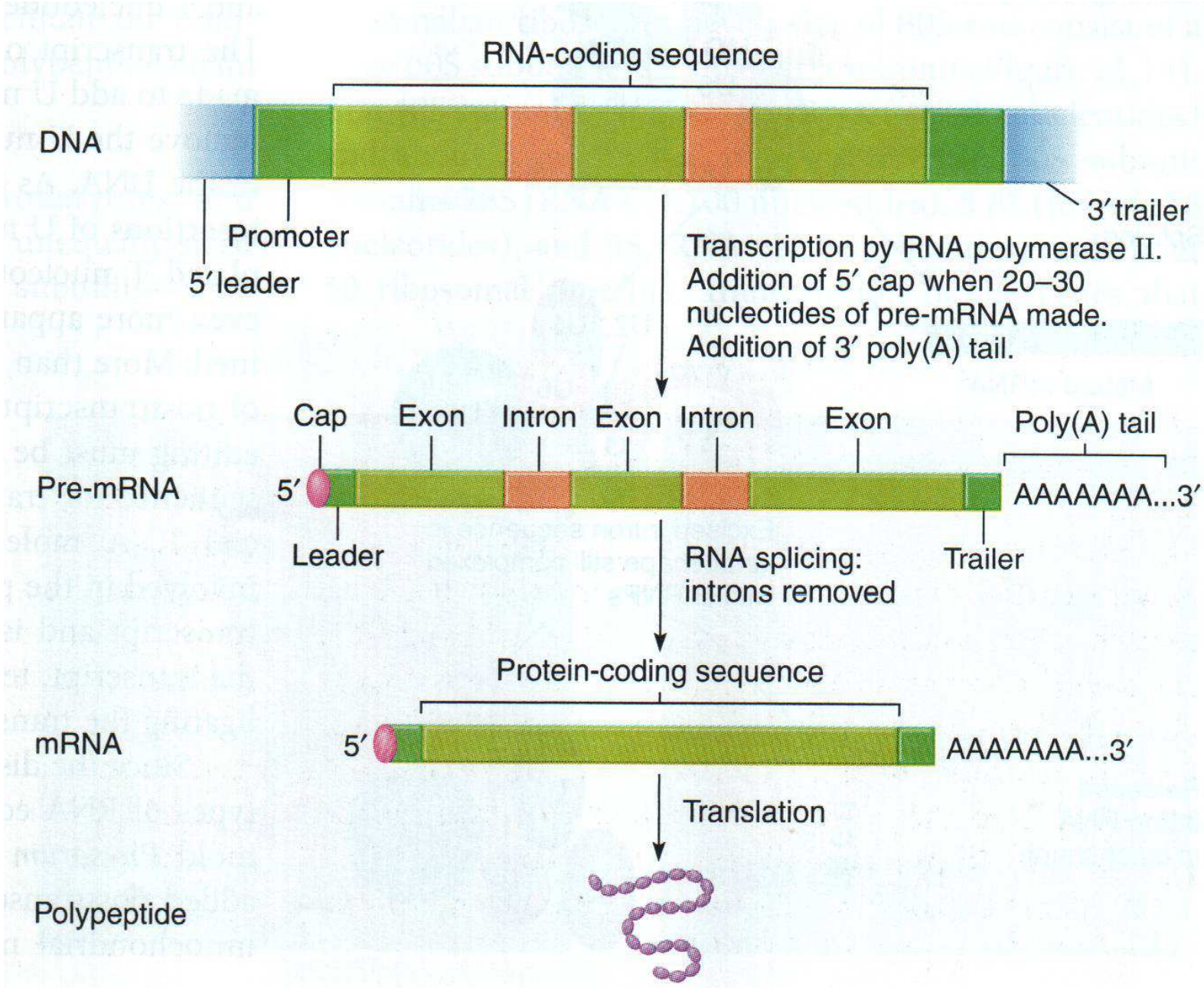
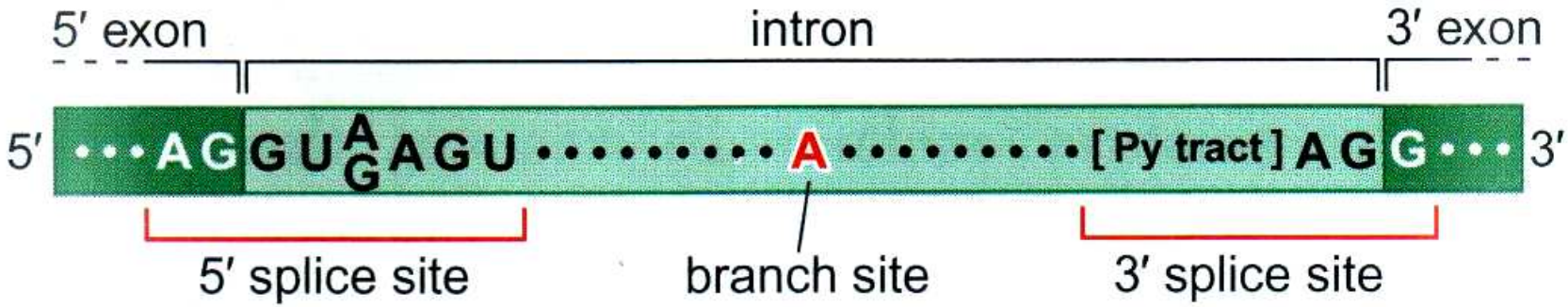
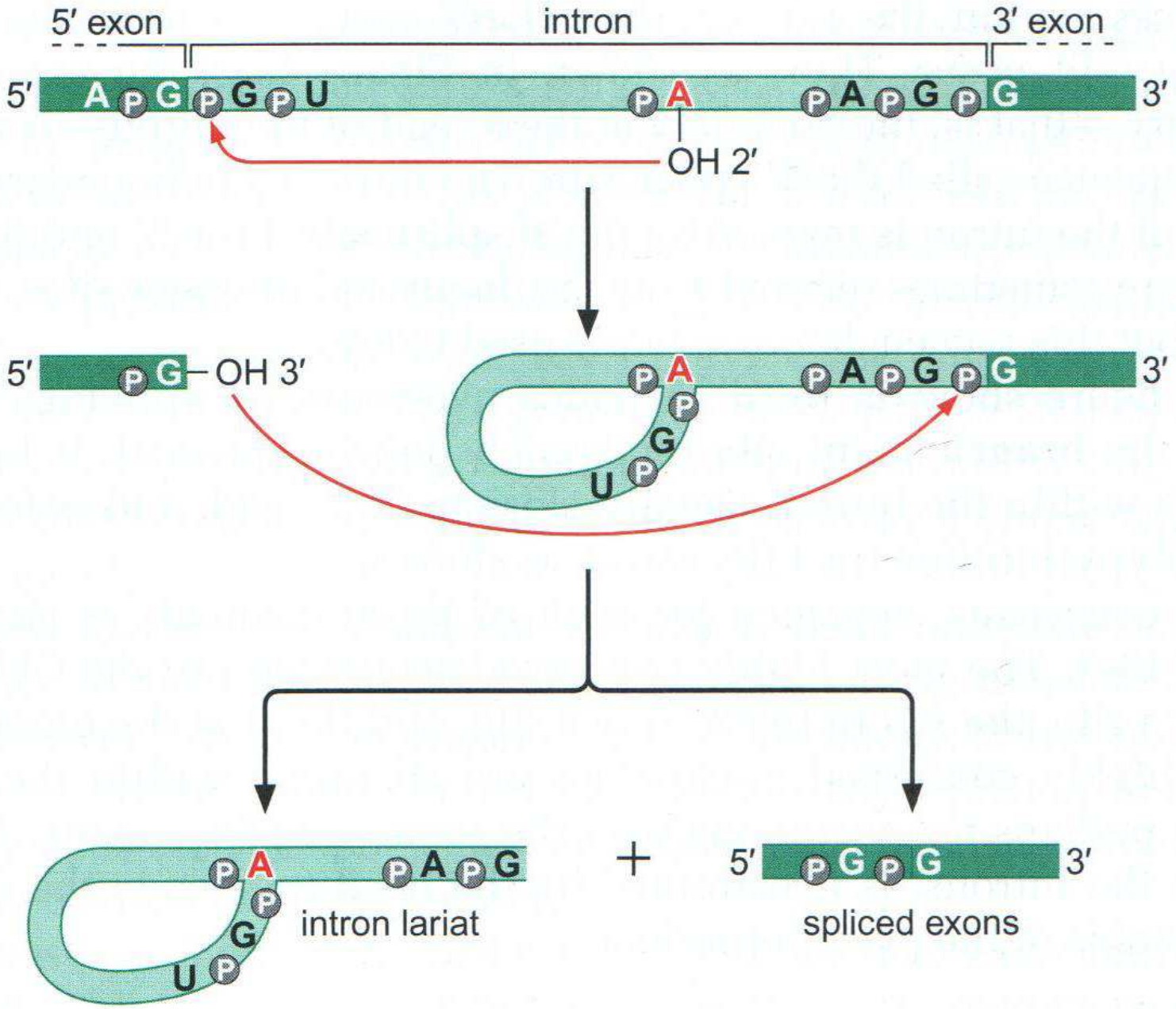
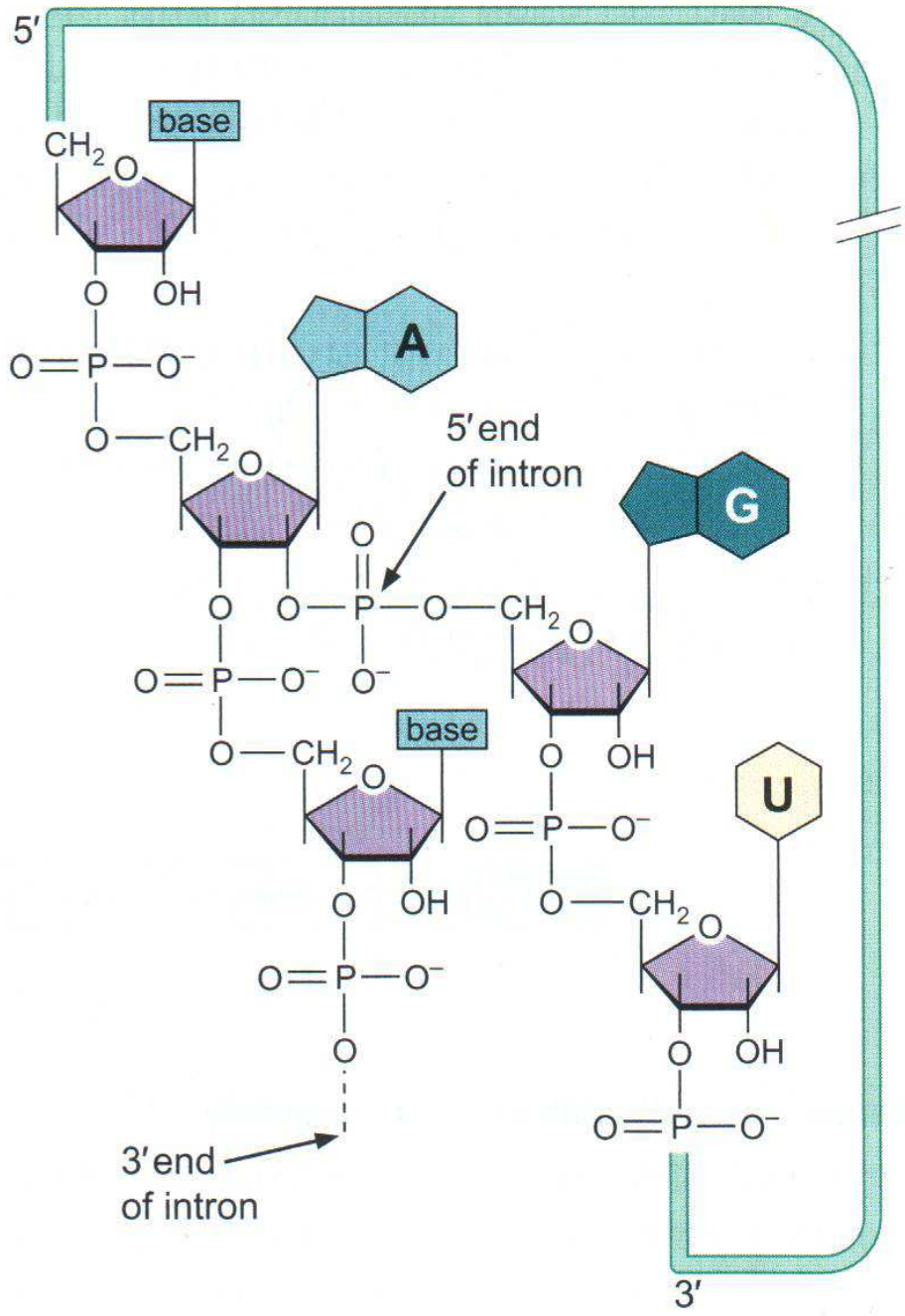
Espressione Genica I:
Lo splicing


RNA-coding sequence
DNA
3′ trailer
Promoter
5′ leader
Transcription by RNA polymerase II.
Addition of 5′ cap when 20–30
nucleotides of pre-mRNA made.
Addition of 3′ poly(A) tail.
Cap
Exon
Intron
Exon
Intron
Exon
Poly(A) tail
Pre-mRNA
5′
AAAAAAA...3′
Leader
Trailer
RNA splicing:
introns removed
Protein-coding sequence
mRNA
5′
AAAAAAA...3′
Translation
Polypeptide


5′ exon
intron
3′ exon
5′
• • • A G G U A/G A G U • • • • • • • • • A • • • • • • • • • [Py tract] A G G • • • 3′
5′ splice site
branch site
3′ splice site

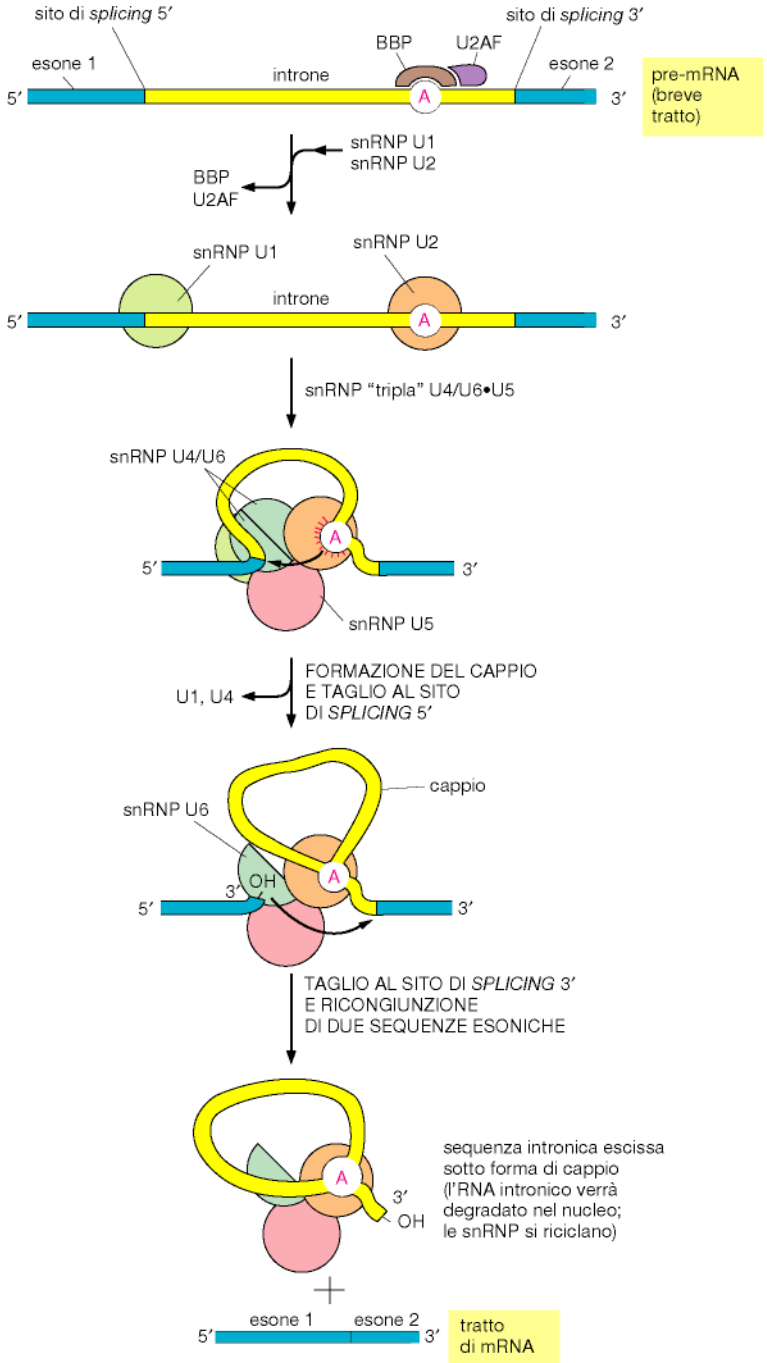

5′ exon
intron
3′ exon
5′
A P G P G P U
P A
P A P G P G
3′
OH 2′
5′
P G
OH 3′
P A
P A P G P G
3′
P
G
U
P A P A G
intron lariat
+
5′
P G P G
3′
spliced exons

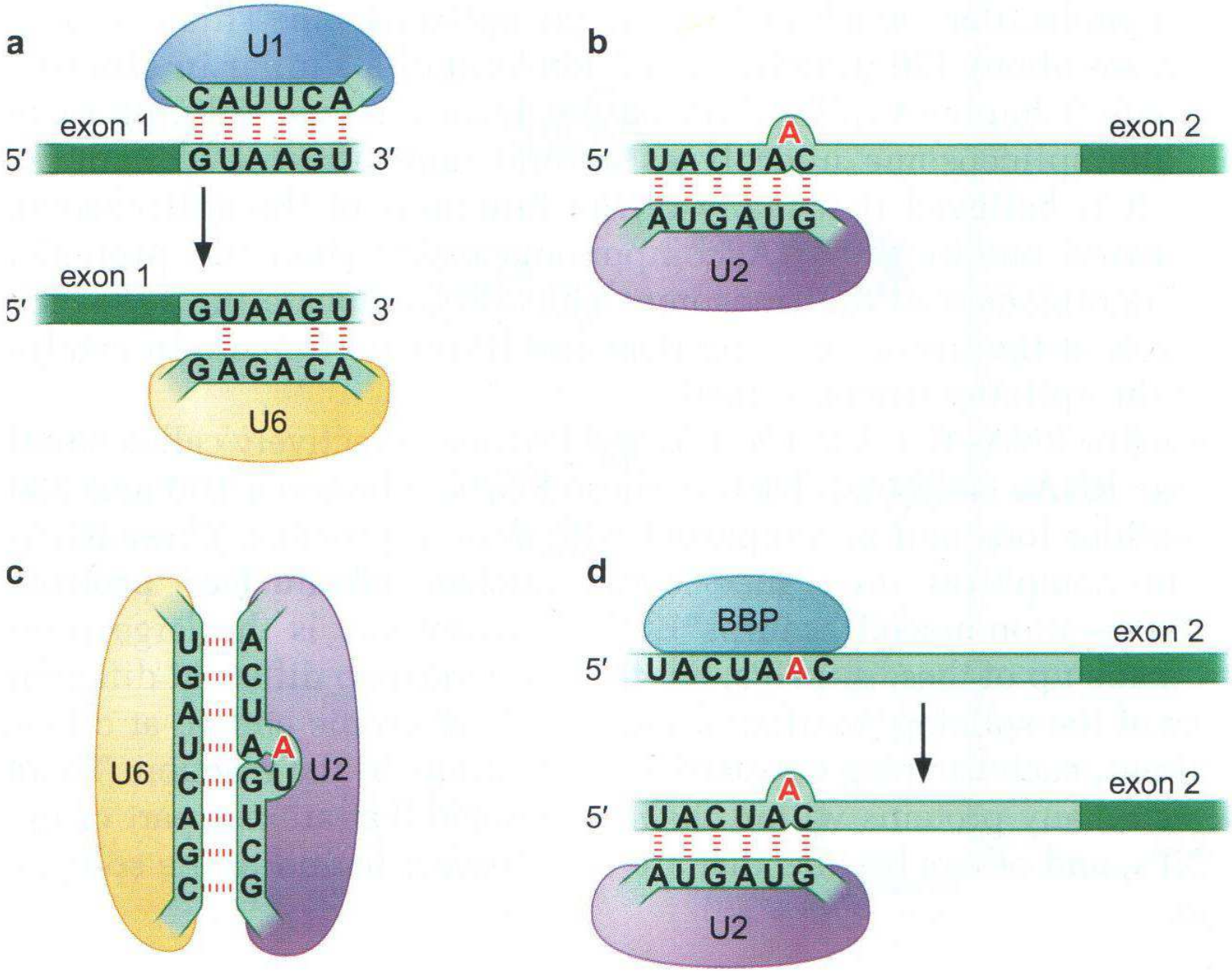

5′
base
CH_2
O
OH
O=P—O⁻
O—CH_2
A
5′ end
of intron
G
O
O—P—O—CH_2
O=P—O⁻
O⁻
base
U
3′ end
of intron
3′

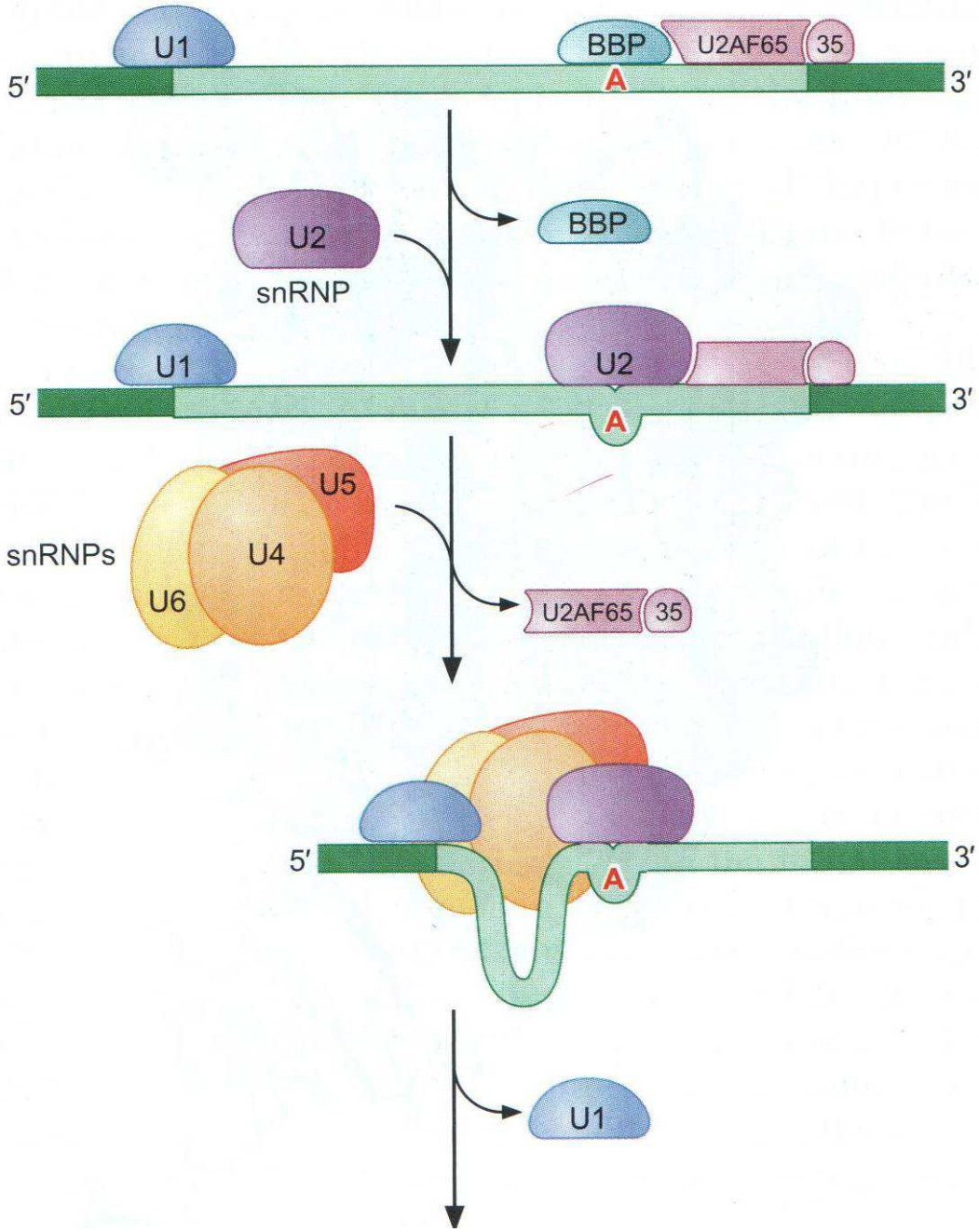
SnRNA U1, U2, U4, U5, U6. Hanno una lunghezza di 100 - 300 nucleotidi.

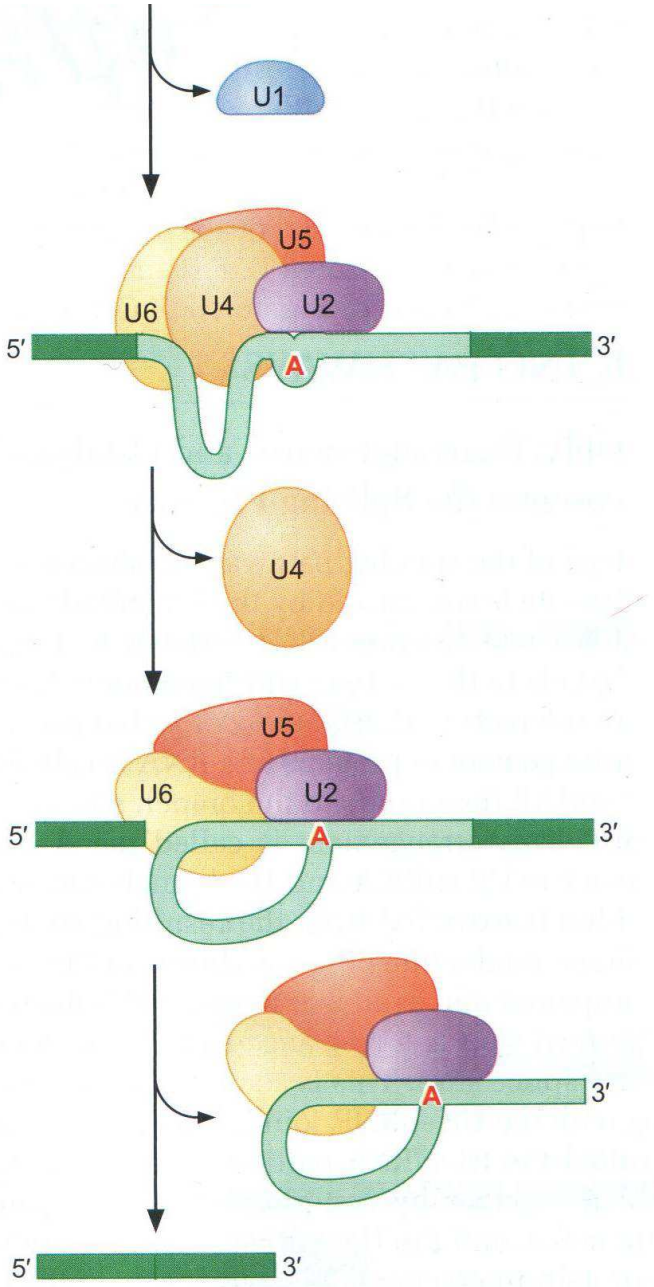
Gli snRNA si associano a diverse proteine e formano le **snRNP** (es: snRNP-U1, snRNP-U4)

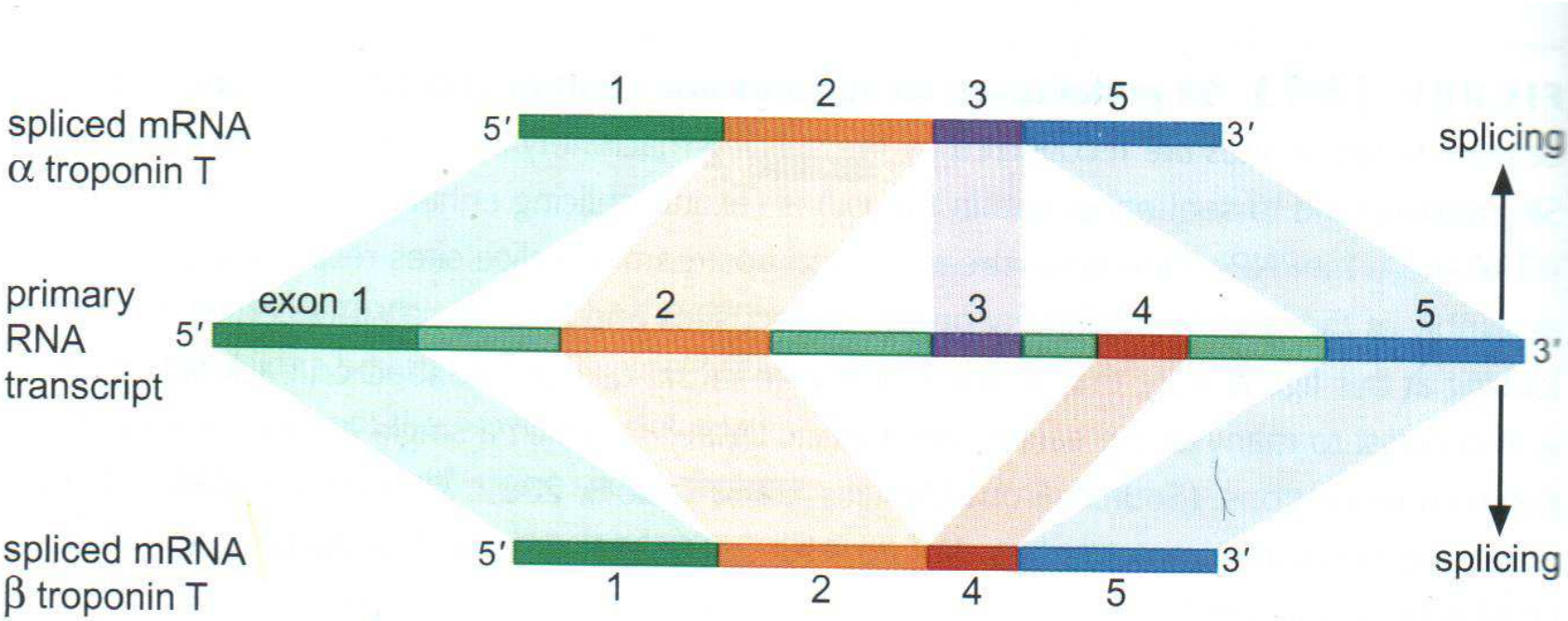
Spliceosoma (150 proteine + 5 RNA)


sito di *splicing* 5′
sito di *splicing* 3′
BBP
U2AF
esone 1
introne
esone 2
5′
A
3′
pre-mRNA (breve tratto)
snRNP U1
snRNP U2
BBP
U2AF
snRNP U1
snRNP U2
introne
5′
A
3′
snRNP "tripla" U4/U6•U5
snRNP U4/U6
A
5′
3′
snRNP U5
FORMAZIONE DEL CAPPIO
E TAGLIO AL SITO
DI *SPLICING* 5′
U1, U4
cappio
snRNP U6
3′
OH
A
5′
3′
TAGLIO AL SITO DI *SPLICING* 3′
E RICONGIUNZIONE
DI DUE SEQUENZE ESONICHE
A
3′
OH
sequenza intronica escissa sotto forma di cappio (l'RNA intronico verrà degradato nel nucleo; le snRNP si riciclano)
esone 1
esone 2
5′
3′
tratto di mRNA


a
U1
CAUUCA
exon 1
5′
GUAAGU
3′
exon 1
5′
GUAAGU
3′
GAGACA
U6
b
A
exon 2
5′
UACUAC
AUGAUG
U2
c
U6
U2
d
BBP
exon 2
5′
UACUAAC
A
exon 2
5′
UACUAC
AUGAUG
U2


U1
BBP
U2AF65
35
5′
A
3′
U2
snRNP
BBP
U1
U2
5′
A
3′
snRNPs
U5
U4
U6
U2AF65
35
5′
A
3′
U1

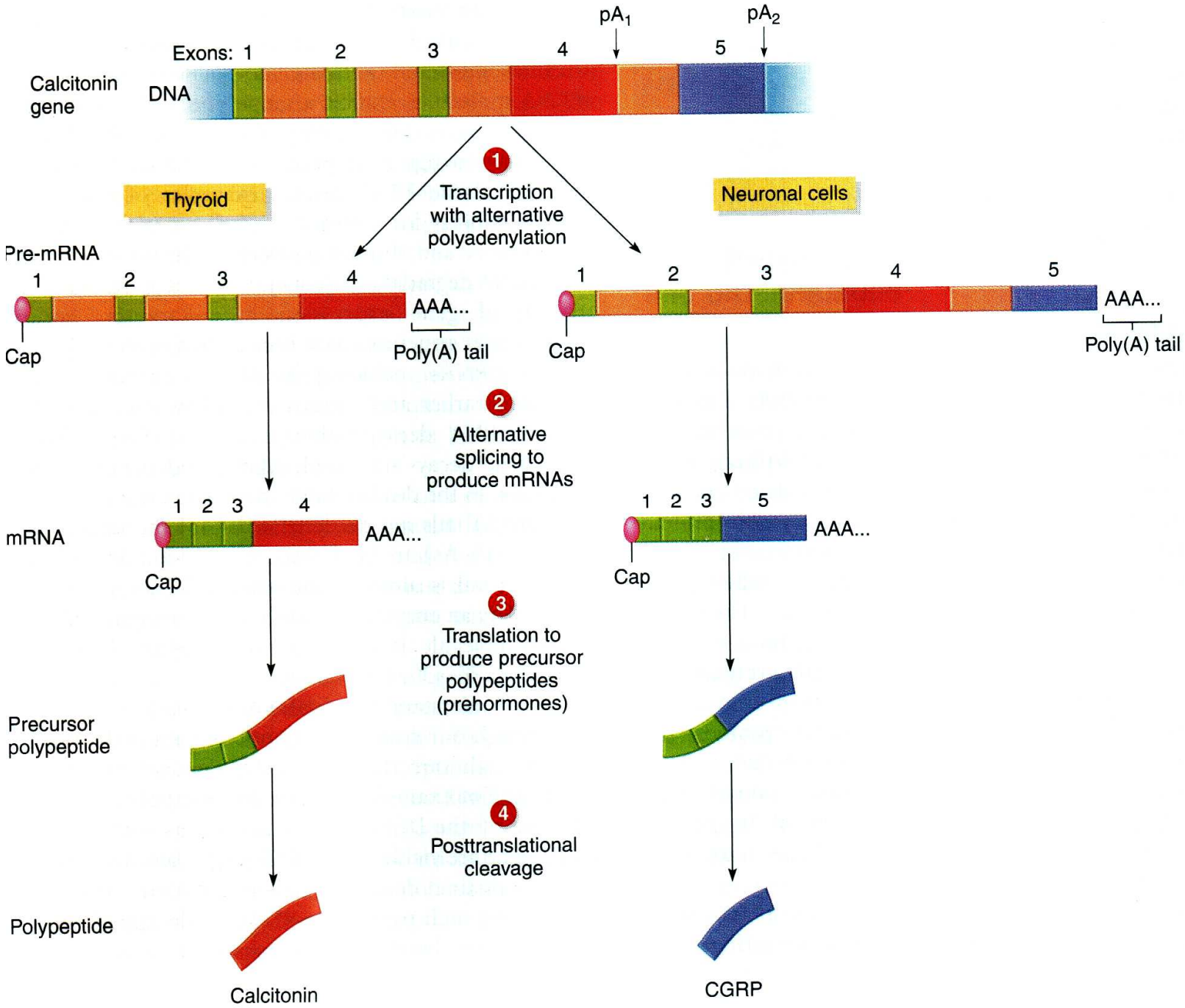

U1
U5
U6
U4
U2
5′
3′
A
U4
U5
U6
U2
5′
A
3′
A
3′
5′
3′

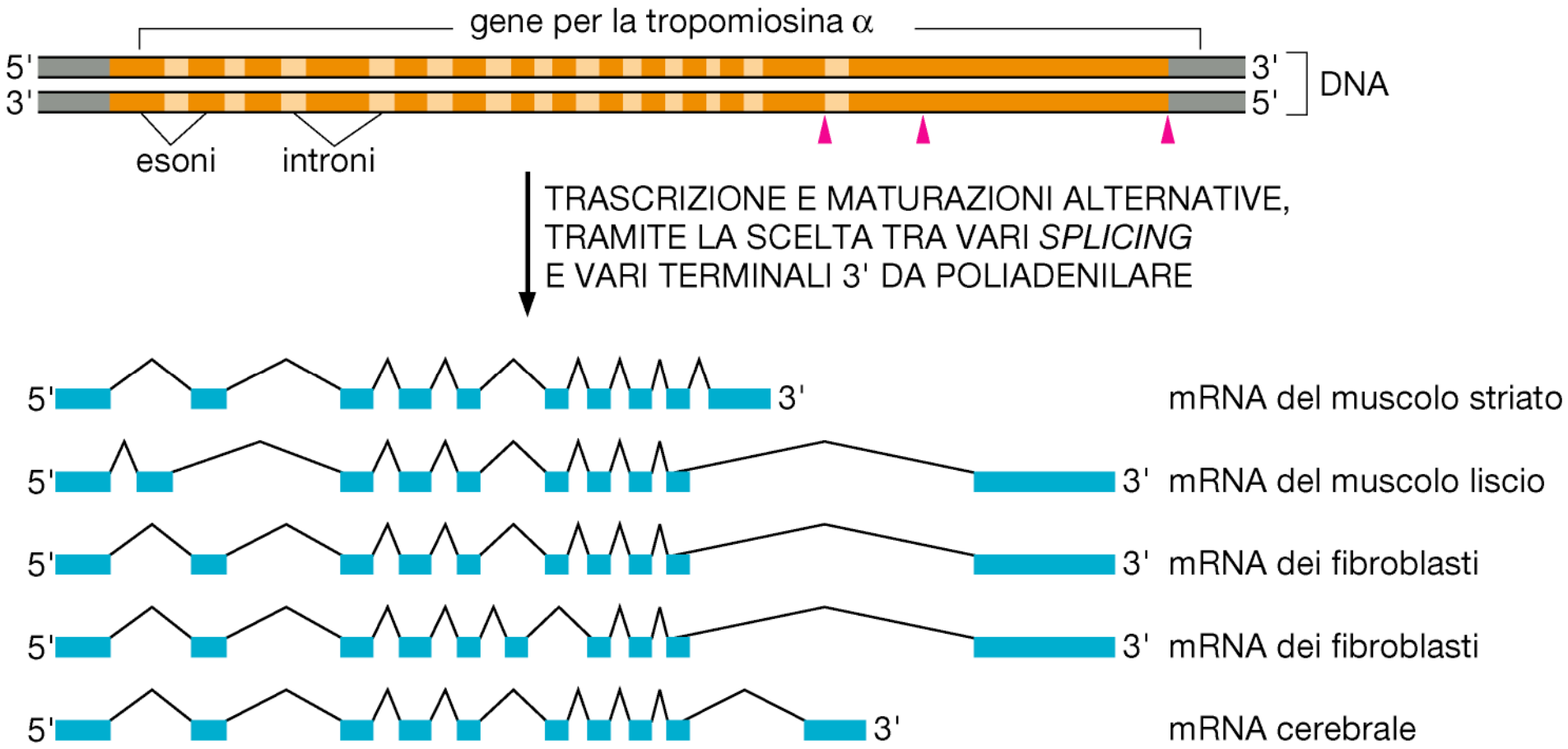

spliced mRNA
α troponin T
1
2
3
5
5′
3′
splicing
primary
RNA
transcript
exon 1
2
3
4
5
5′
3′
spliced mRNA
β troponin T
5′
1
2
4
5
3′
splicing

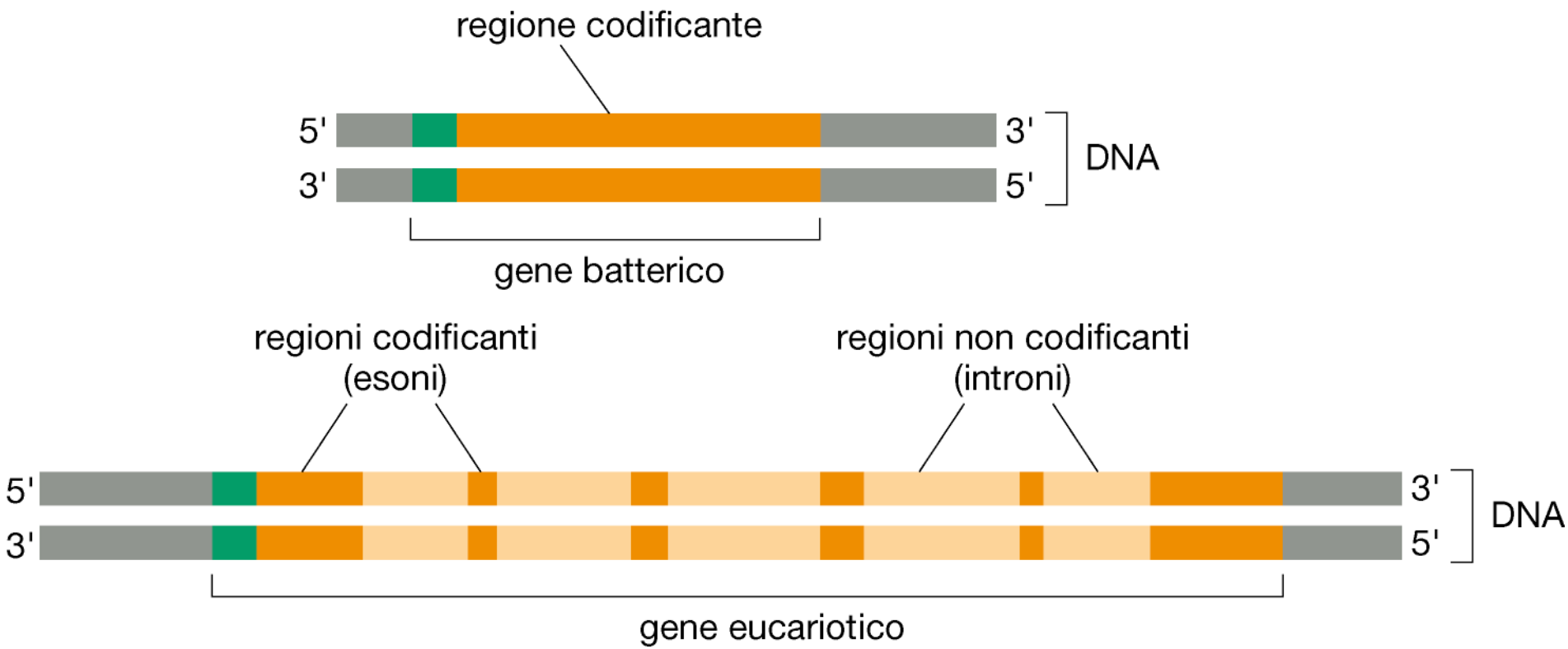
Splicing alternativo

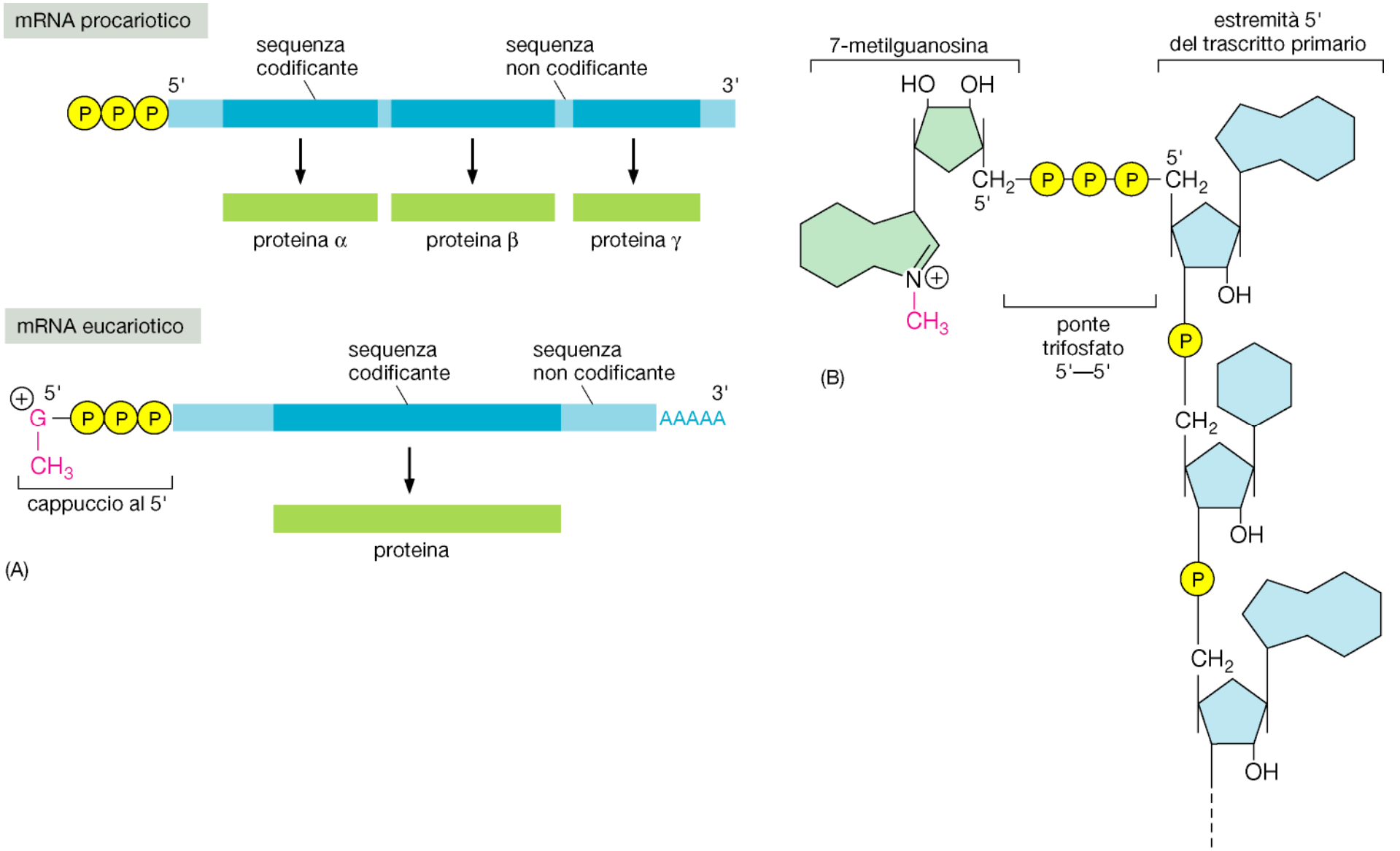
Si stima che circa il 60% dei geni umani mostra il fenomeno dello splicing alternativo.

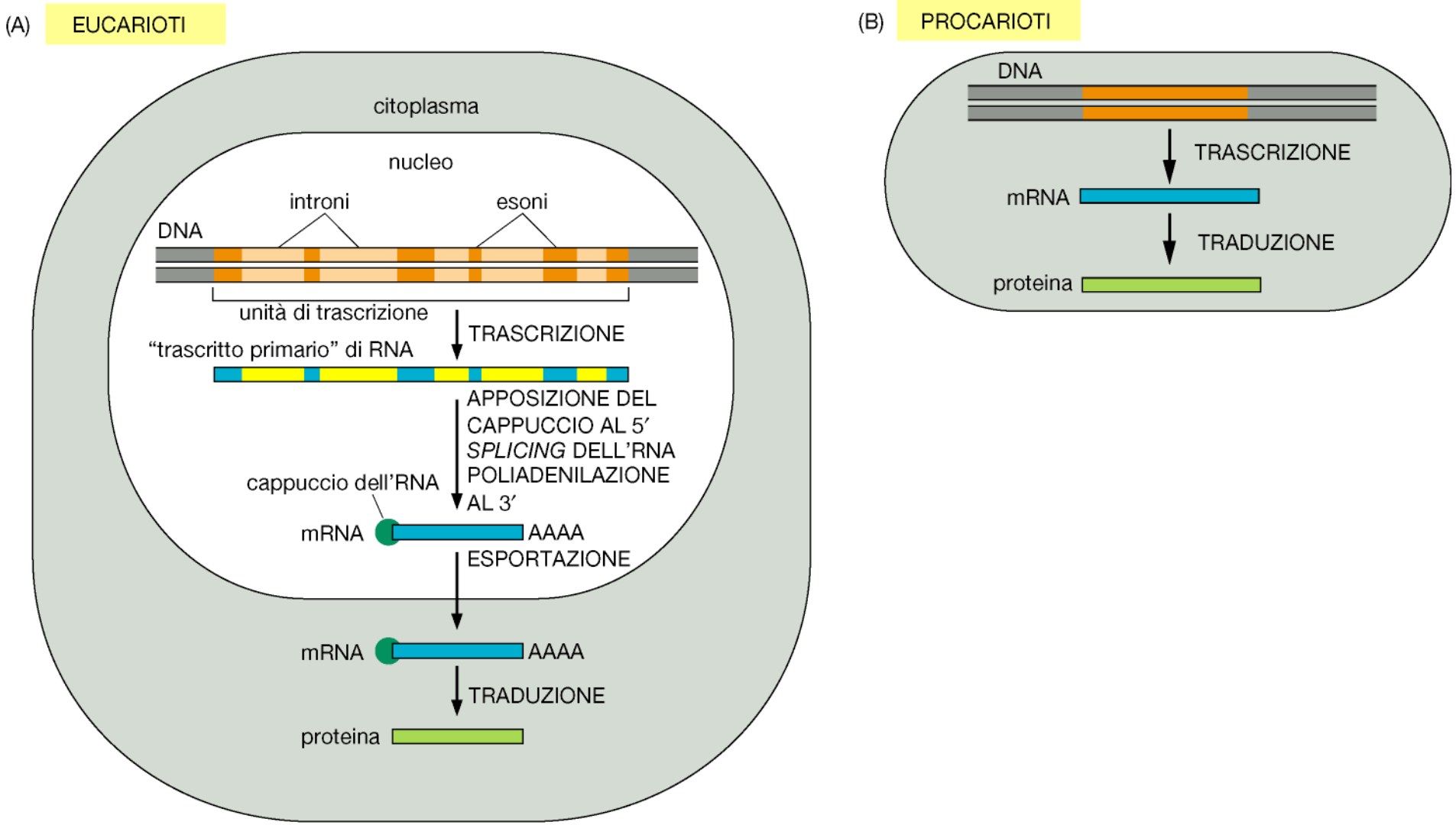
Regolato Diversi messaggeri maturi vengono prodotti da tipi cellulari diversi, oppure in diversi stadi dello sviluppo

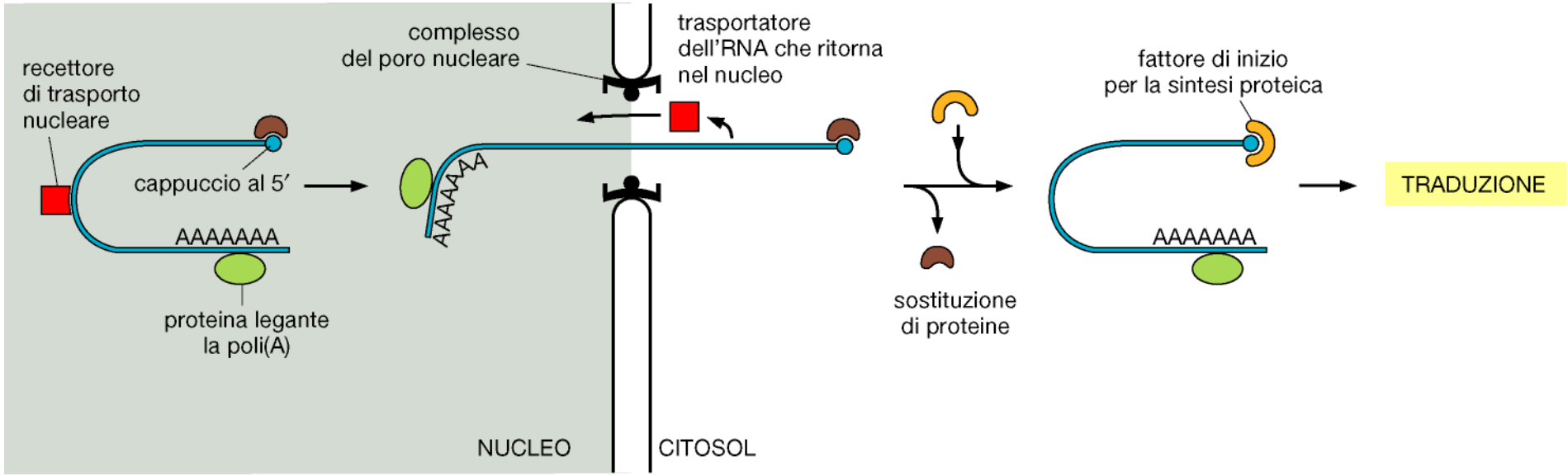

Calcitonin gene
DNA
Exons: 1 2 3 4 5
pA1
pA2
1
Transcription with alternative polyadenylation
Thyroid
Neuronal cells
Pre-mRNA
1 2 3 4
AAA...
Cap
Poly(A) tail
1 2 3 4 5
AAA...
Cap
Poly(A) tail
2
Alternative splicing to produce mRNAs
mRNA
1 2 3 4
AAA...
Cap
1 2 3 5
AAA...
Cap
3
Translation to produce precursor polypeptides (prehormones)
Precursor polypeptide
4
Posttranslational cleavage
Polypeptide
Calcitonin
CGRP

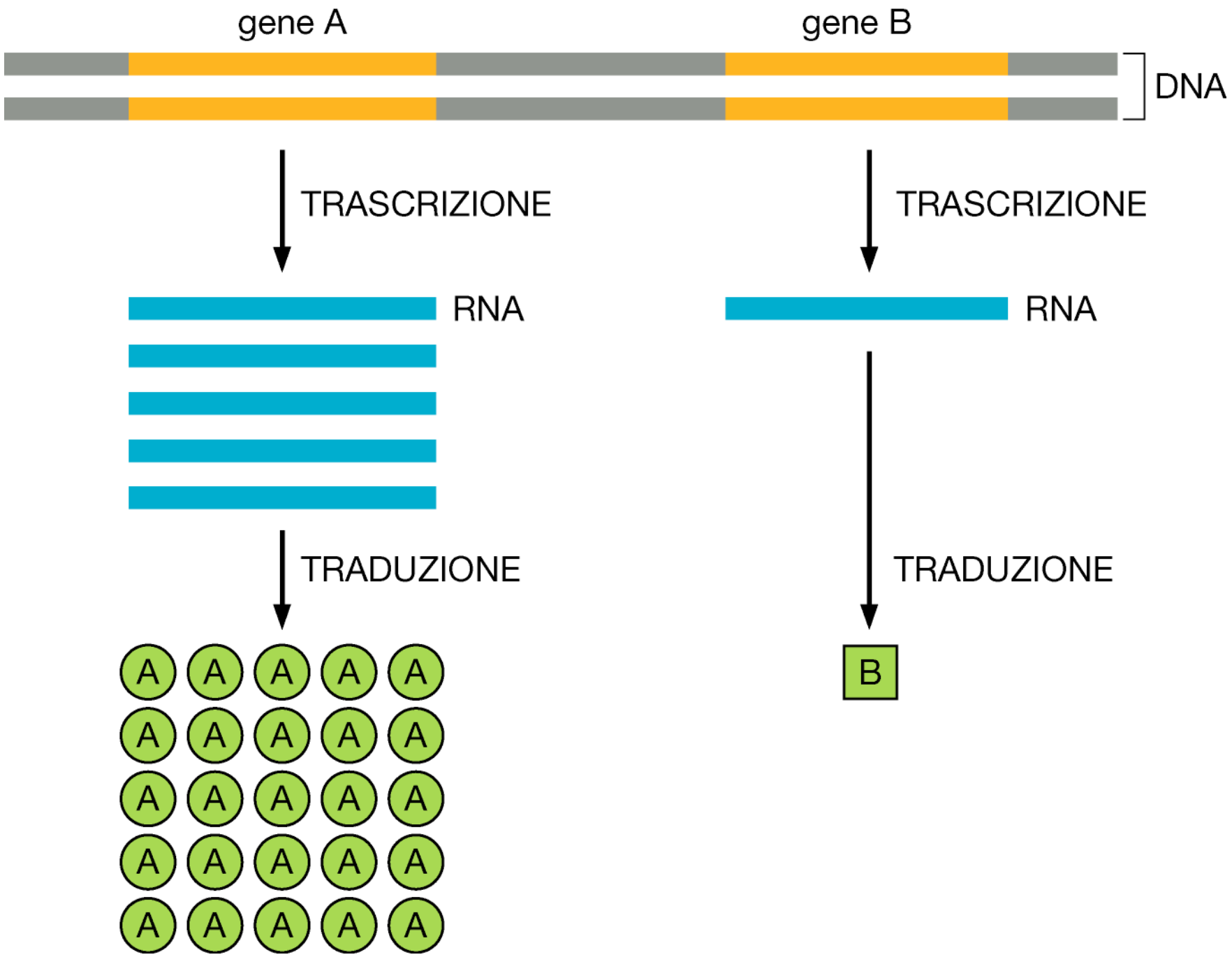

gene per la tropomiosina α
5'
3'
3'
5'
DNA
esoni
introni
TRASCRIZIONE E MATURAZIONI ALTERNATIVE,
TRAMITE LA SCELTA TRA VARI SPLICING
E VARI TERMINALI 3' DA POLIADENILARE
5'
3'
mRNA del muscolo striato
5'
3'
mRNA del muscolo liscio
5'
3'
mRNA dei fibroblasti
5'
3'
mRNA dei fibroblasti
5'
3'
mRNA cerebrale

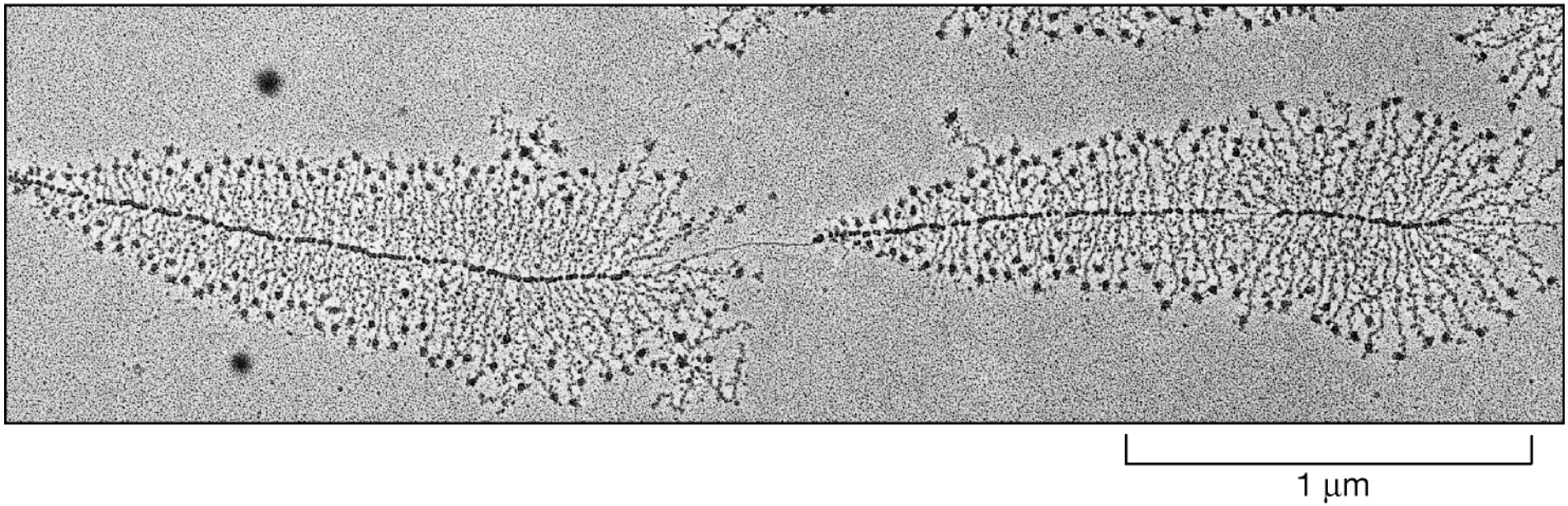

regione codificante
5'
3'
3'
5'
DNA
gene batterico
regioni codificanti
(esoni)
regioni non codificanti
(introni)
5'
3'
3'
5'
DNA
gene eucariotico


mRNA procariotico
sequenza codificante
sequenza non codificante
5'
3'
P P P
proteina α
proteina β
proteina γ
mRNA eucariotico
sequenza codificante
sequenza non codificante
5'
3'
G
CH3
P P P
AAAAA
cappuccio al 5'
proteina
(A)
7-metilguanosina
estremità 5' del trascritto primario
HO OH
CH2
5'
P P P
5'
CH2
N
CH3
OH
ponte trifosfato 5'—5'
(B)
P
CH2
OH
P
CH2
OH


(A)
EUCARIOTI
citoplasma
nucleo
introni
esoni
DNA
unità di trascrizione
TRASCRIZIONE
"trascritto primario" di RNA
APPOSIZIONE DEL
CAPPUCCIO AL 5′
SPLICING DELL'RNA
POLIADENILAZIONE
AL 3′
cappuccio dell'RNA
mRNA
AAAA
ESPORTAZIONE
mRNA
AAAA
TRADUZIONE
proteina
(B)
PROCARIOTI
DNA
TRASCRIZIONE
mRNA
TRADUZIONE
proteina


recettore
di trasporto
nucleare
cappuccio al 5′
AAAAAAA
proteina legante
la poli(A)
complesso
del poro nucleare
trasportatore
dell'RNA che ritorna
nel nucleo
AAAAAAA
NUCLEO
CITOSOL
sostituzione
di proteine
fattore di inizio
per la sintesi proteica
AAAAAAA
TRADUZIONE


gene A
gene B
DNA
TRASCRIZIONE
TRASCRIZIONE
RNA
RNA
TRADUZIONE
TRADUZIONE
A
A
A
A
A
A
A
A
A
A
A
A
A
A
A
A
A
A
A
A
A
A
A
A
B


1 μm

RNA Polimerasi eucariotiche

- RNA Pol I — 28S, 18S, 5.8S rRNA
- RNA Pol II — mRNA; alcuni snRNA
- RNA Pol III — tRNA; 5S rRNA; altri snRNA

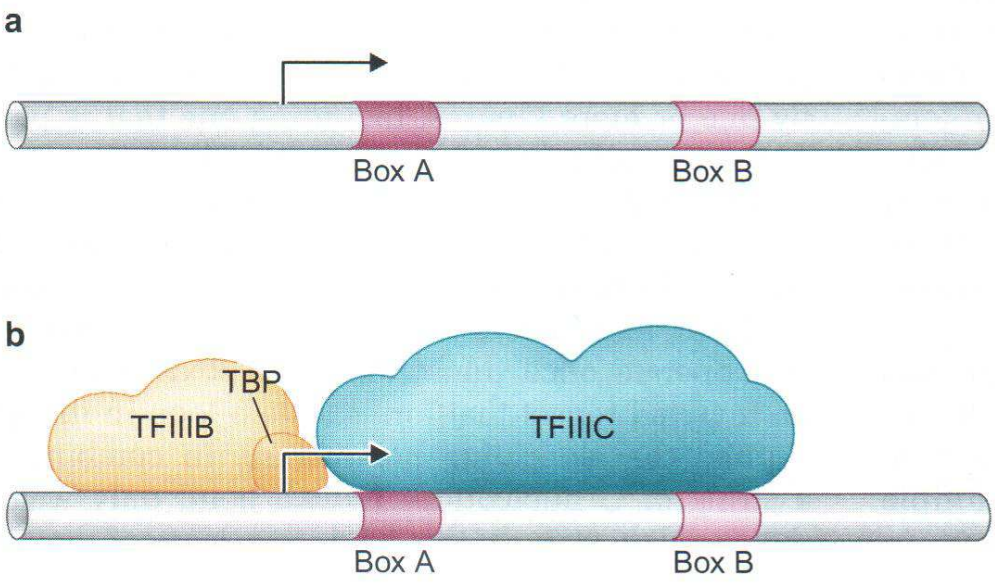


FIGURE 12-22 Pol III core promoter. Shown here is the promoter for a yeast tRNA gene. The order of events leading to transcription initiation is described in the text.

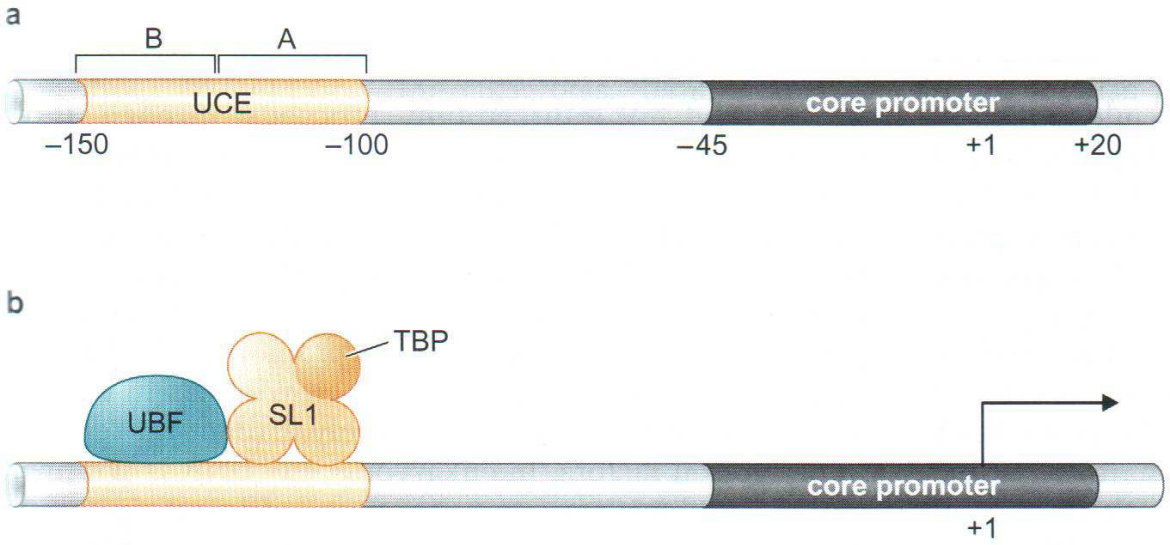
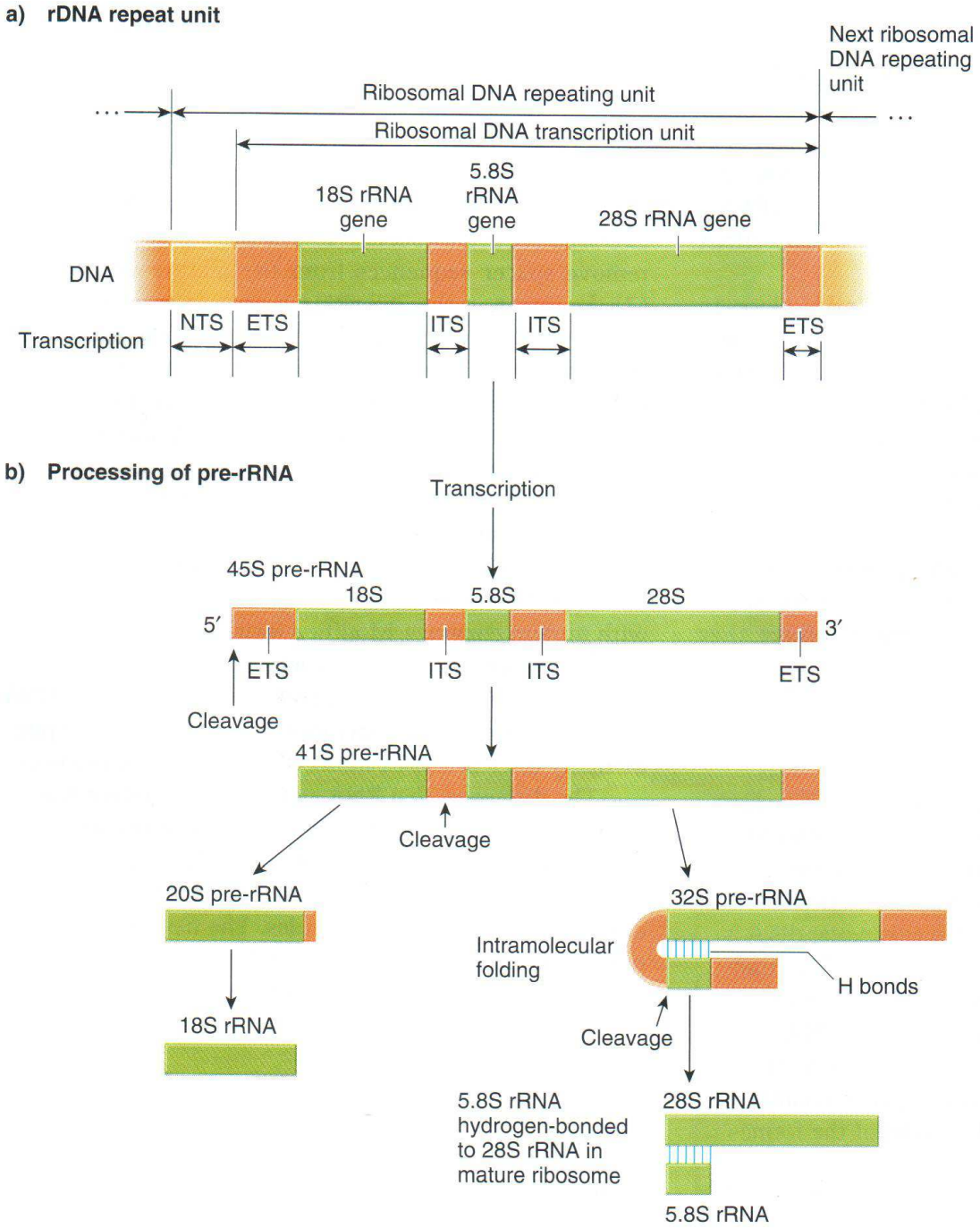
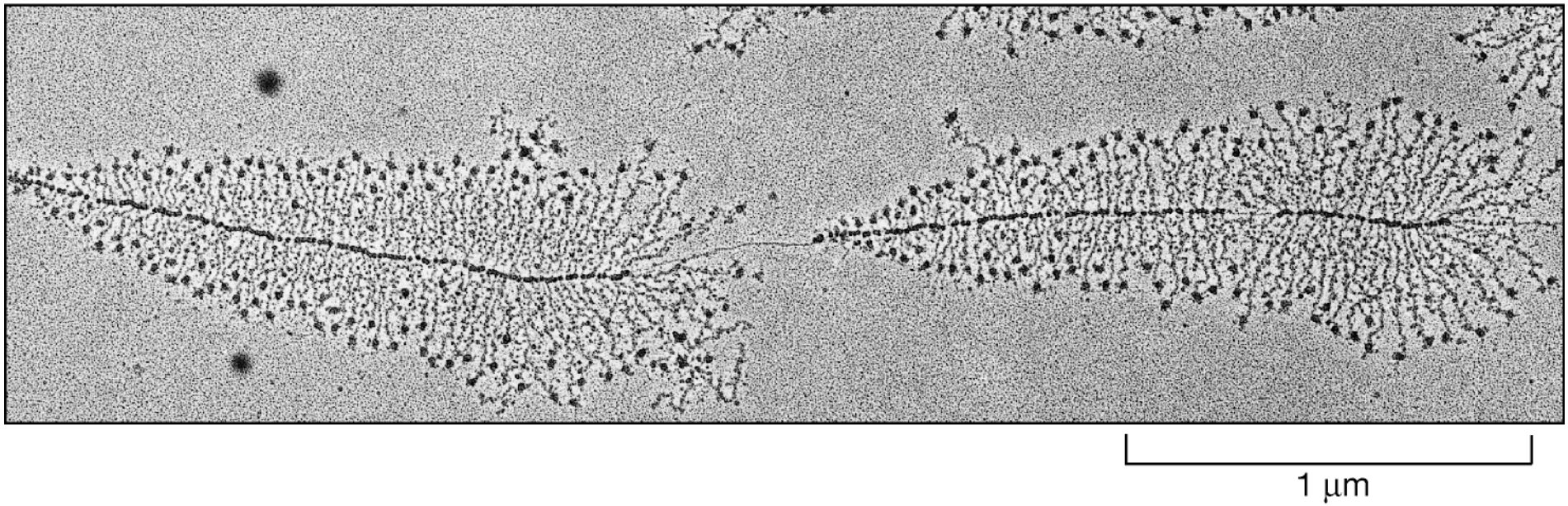


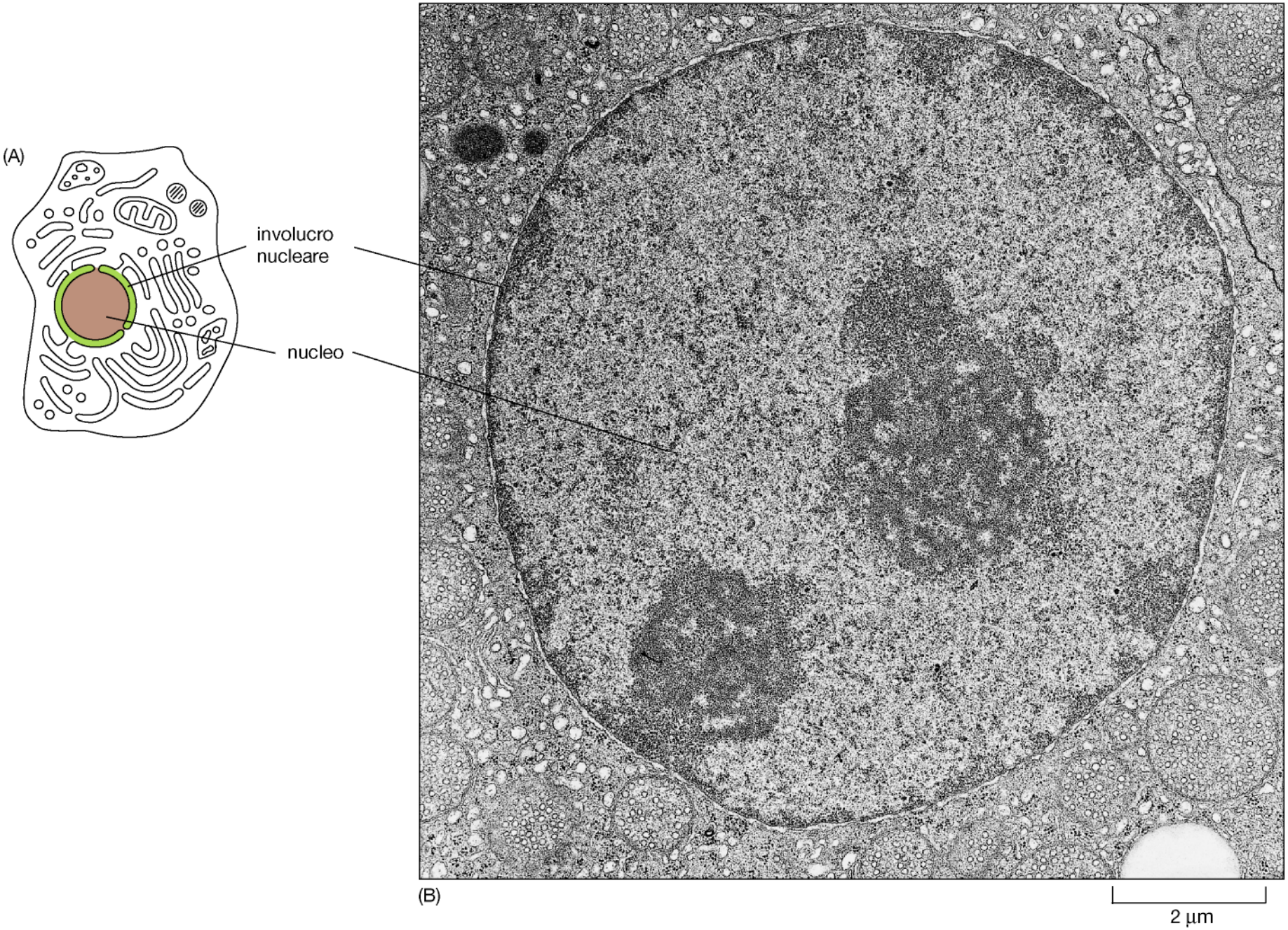
FIGURE 12-21 Pol I promoter region. (a) Structure of the Pol I promoter. (b) Pol I txn factors. The case shown here is the vertebrate system. The set of proteins involved in helping Pol I transcription in yeast is rather different.


a) rDNA repeat unit
Next ribosomal DNA repeating unit
Ribosomal DNA repeating unit
Ribosomal DNA transcription unit
18S rRNA gene
5.8S rRNA gene
28S rRNA gene
DNA
Transcription
NTS
ETS
ITS
ITS
ETS
b) Processing of pre-rRNA
Transcription
45S pre-rRNA
18S
5.8S
28S
5′
3′
ETS
ITS
ITS
ETS
Cleavage
41S pre-rRNA
Cleavage
20S pre-rRNA
18S rRNA
32S pre-rRNA
Intramolecular folding
H bonds
Cleavage
5.8S rRNA hydrogen-bonded to 28S rRNA in mature ribosome
28S rRNA
5.8S rRNA

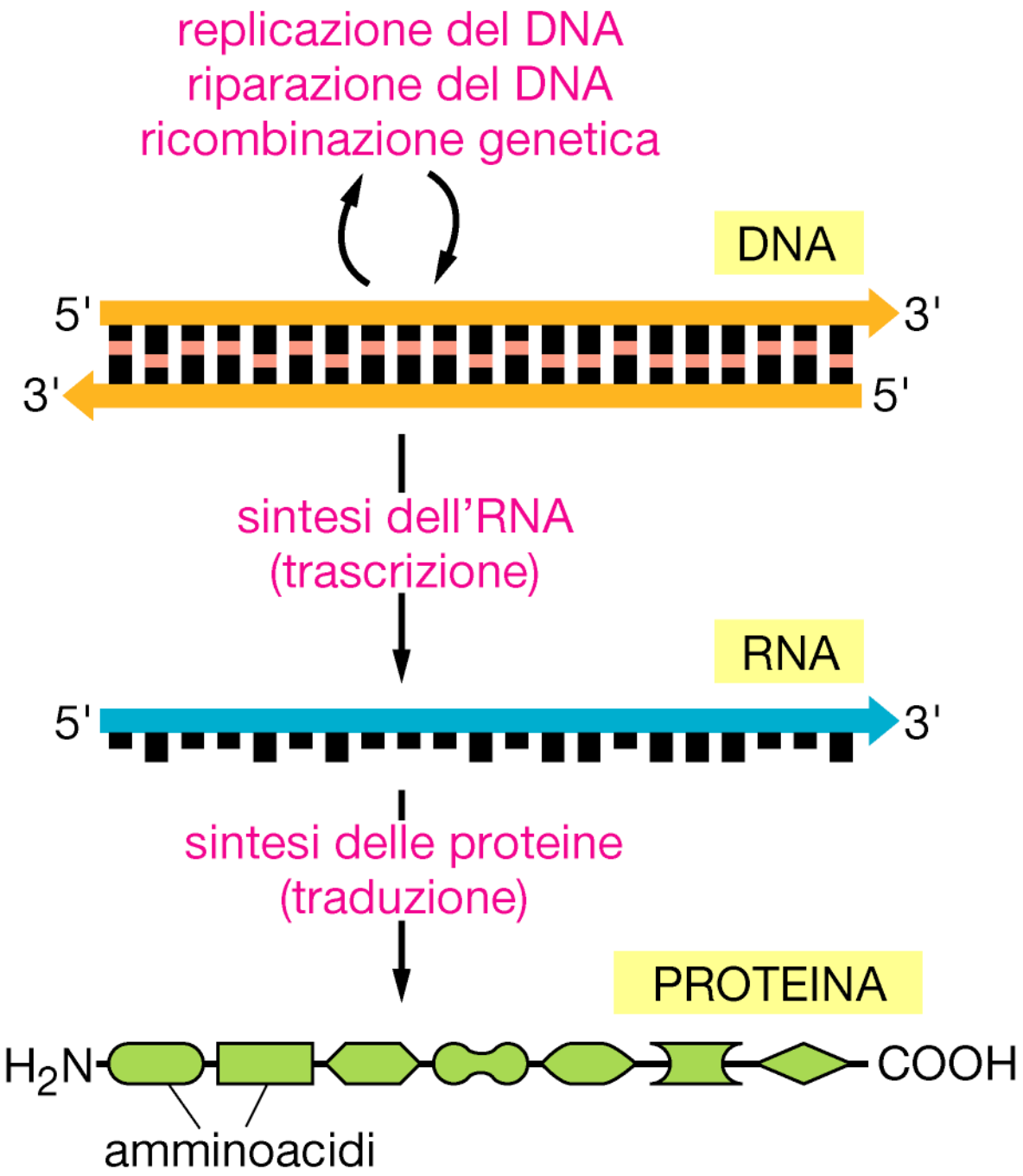
RNA Polimerasi eucariotiche

- RNA Pol I — 28S, 18S, 5.8S rRNA
- RNA Pol II — mRNA; alcuni snRNA
- RNA Pol III — tRNA; 5S rRNA; altri snRNA

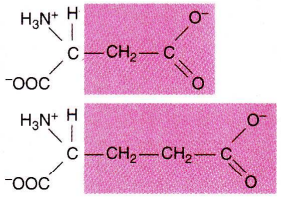

1 μm


(A)
involucro
nucleare
nucleo
(B)
2 µm

Espressione Genica II:
la Traduzione


replicazione del DNA
riparazione del DNA
ricombinazione genetica
DNA
5'
3'
3'
5'
sintesi dell'RNA
(trascrizione)
RNA
5'
3'
sintesi delle proteine
(traduzione)
PROTEINA
H_2N
COOH
amminoacidi

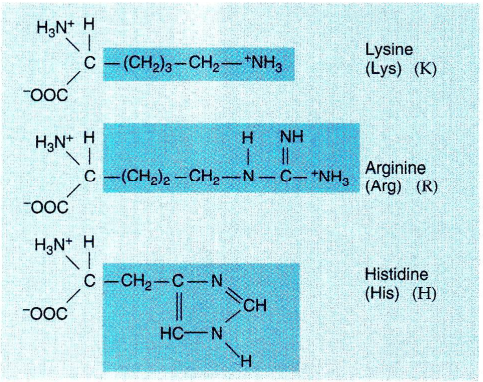
Acidic



Aspartic acid (Asp) (D)

Glutamic acid (Glu) (E)

Basic

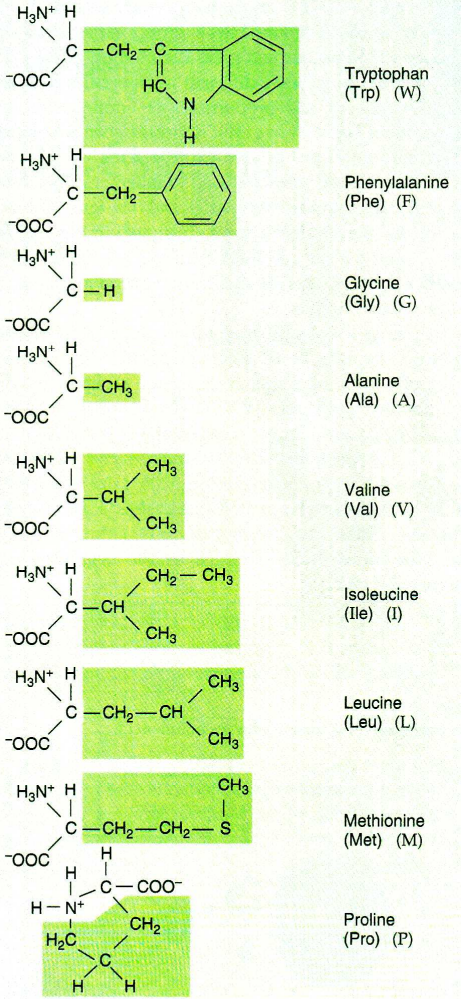


Lysine (Lys) (K)

Arginine (Arg) (R)

Histidine (His) (H)

Neutral, nonpolar



Tryptophan (Trp) (W)

Phenylalanine (Phe) (F)

Glycine (Gly) (G)

Alanine (Ala) (A)

Valine (Val) (V)

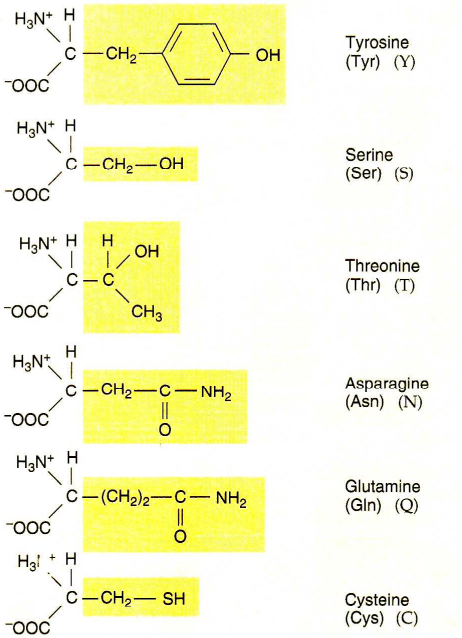
Isoleucine (Ile) (I)

Leucine (Leu) (L)

Methionine (Met) (M)

Proline (Pro) (P)

Neutral, polar



Tyrosine (Tyr) (Y)

Serine (Ser) (S)

Threonine (Thr) (T)

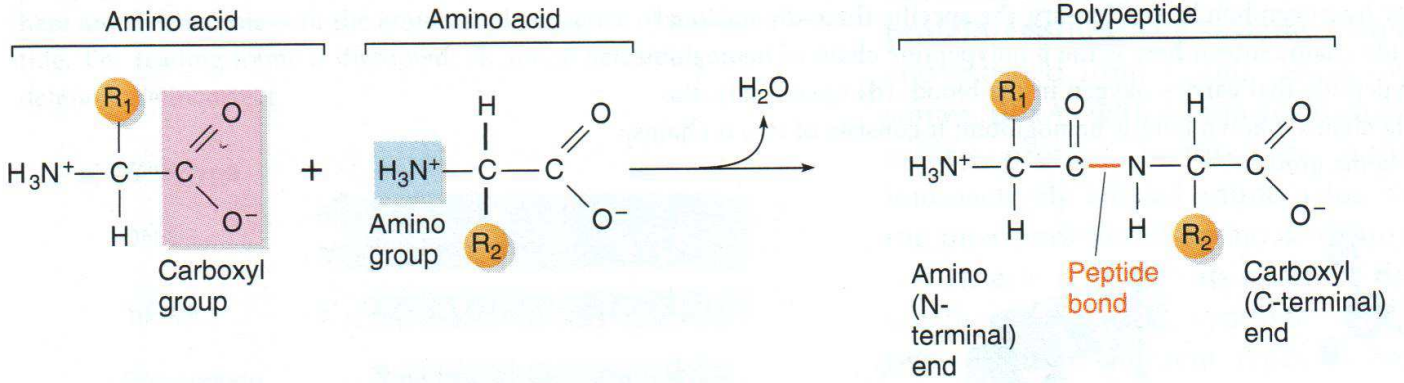
Asparagine (Asn) (N)

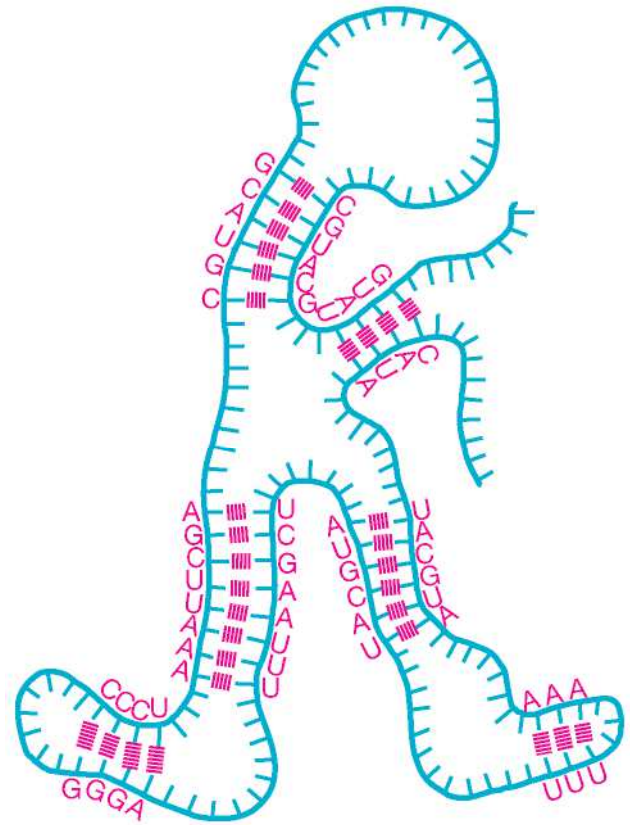
Glutamine (Gln) (Q)

Cysteine (Cys) (C)

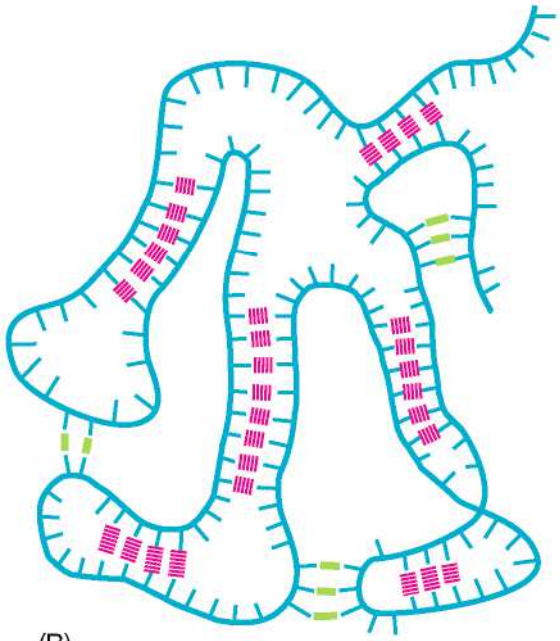
Figure 12.3

Mechanism for peptide bond formation between the carboxyl group of one amino acid and the amino group of another amino acid.

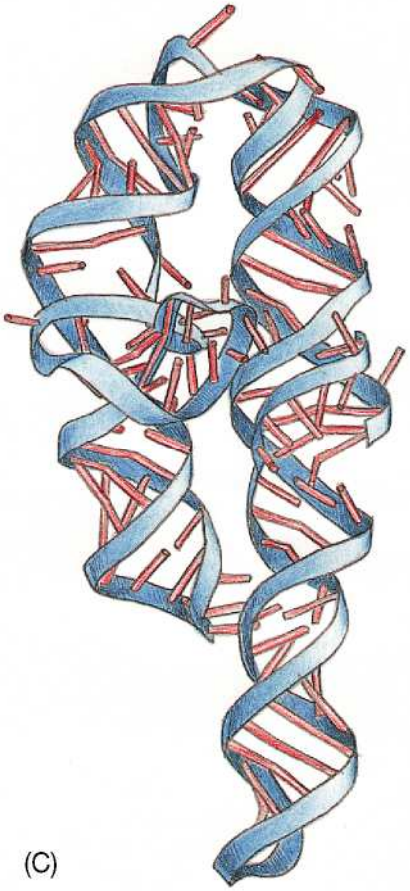




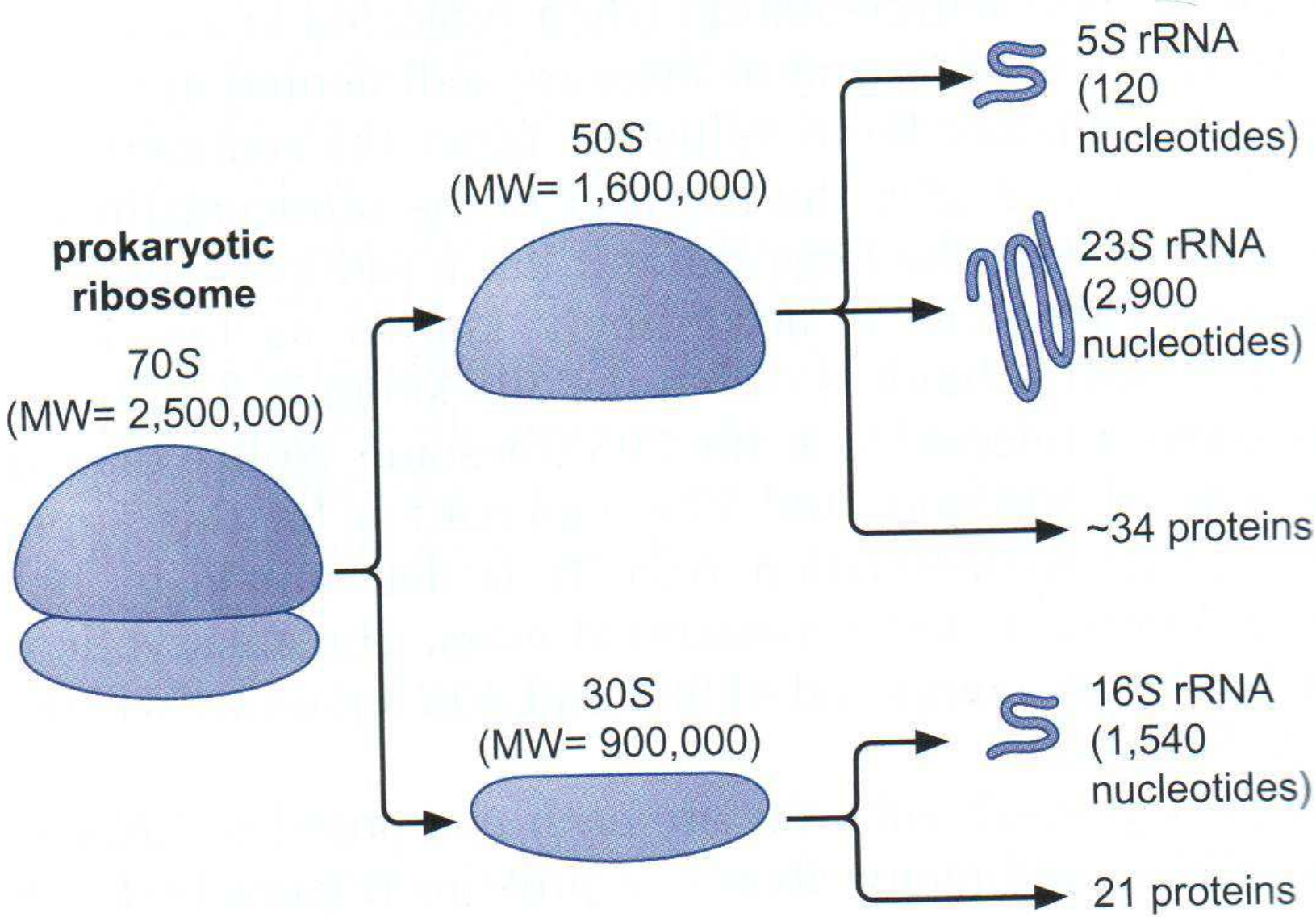
(A)

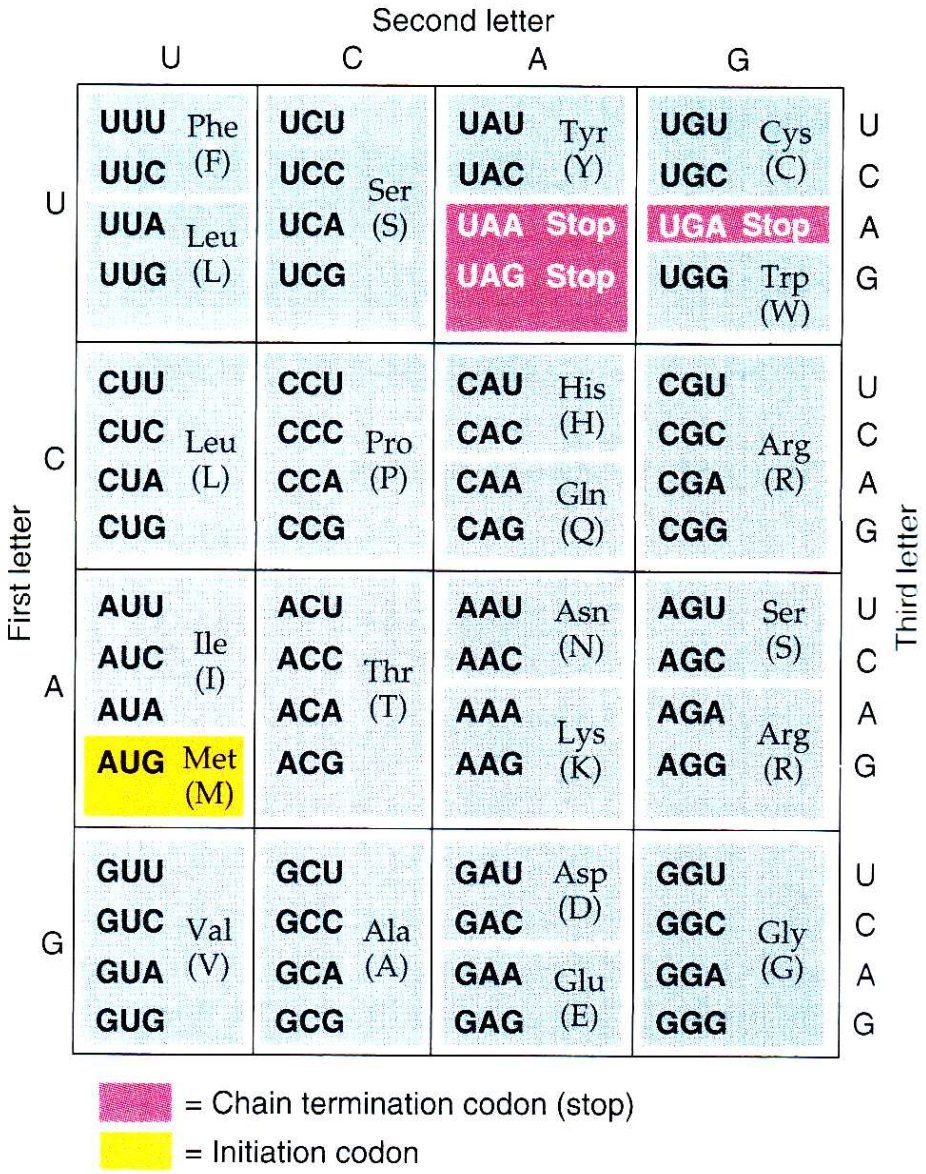


(B)



(C)


prokaryotic ribosome
70S
(MW= 2,500,000)
50S
(MW= 1,600,000)
30S
(MW= 900,000)
5S rRNA
(120
nucleotides)
23S rRNA
(2,900
nucleotides)
~34 proteins
16S rRNA
(1,540
nucleotides)
21 proteins


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

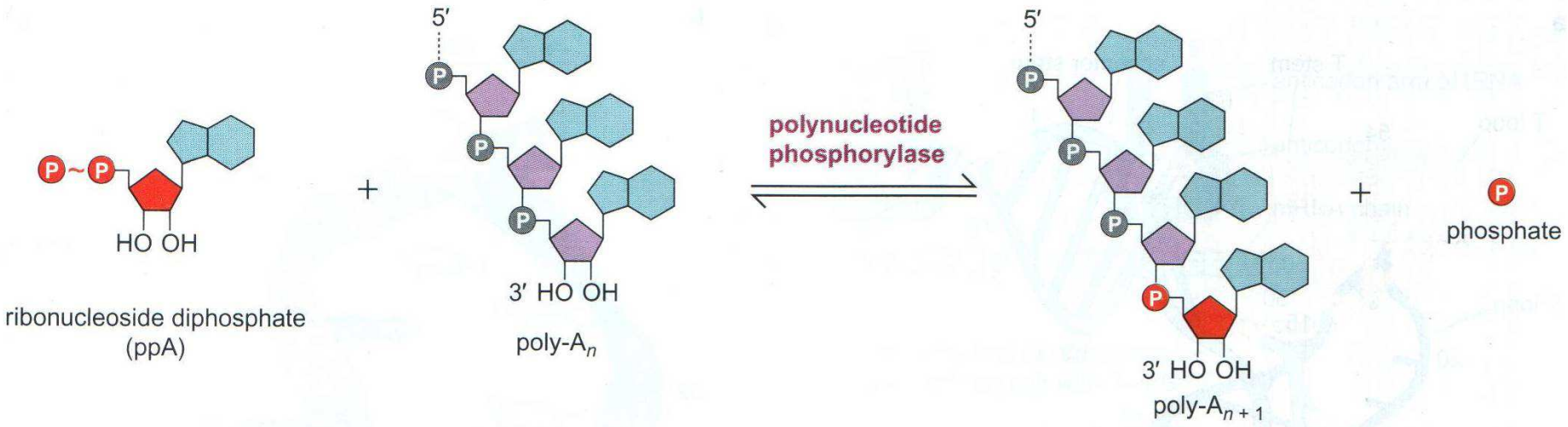
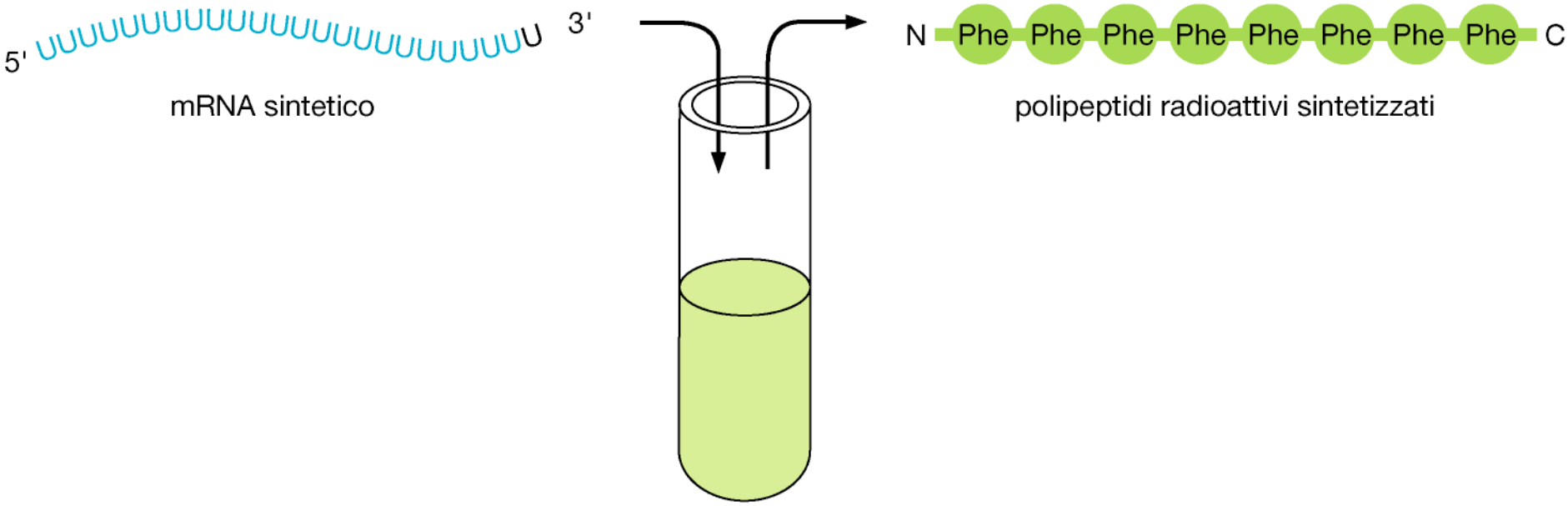


FIGURE 15-4 Polynucleotide phosphorylase reaction. The figure shows the reversible reactions of synthesis or degradation of polyadenylic acid catalyzed by the enzyme polynucleotide phosphorylase.

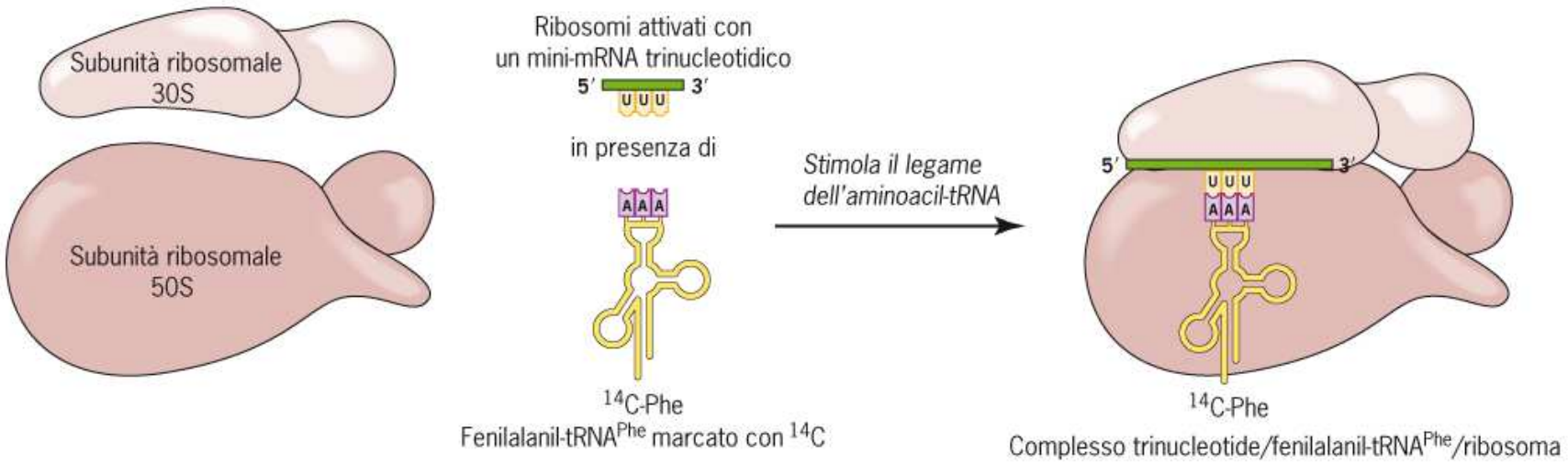
$$[XMP]_n + XDP = [XMP]_{n+1} + P$$


5' UUUUUUUUUUUUUUUUUUUUUUU 3'
mRNA sintetico
N Phe Phe Phe Phe Phe Phe Phe Phe C
polipeptidi radioattivi sintetizzati
traduzione in sistema
acellulare in presenza
di amminoacidi radioattivi

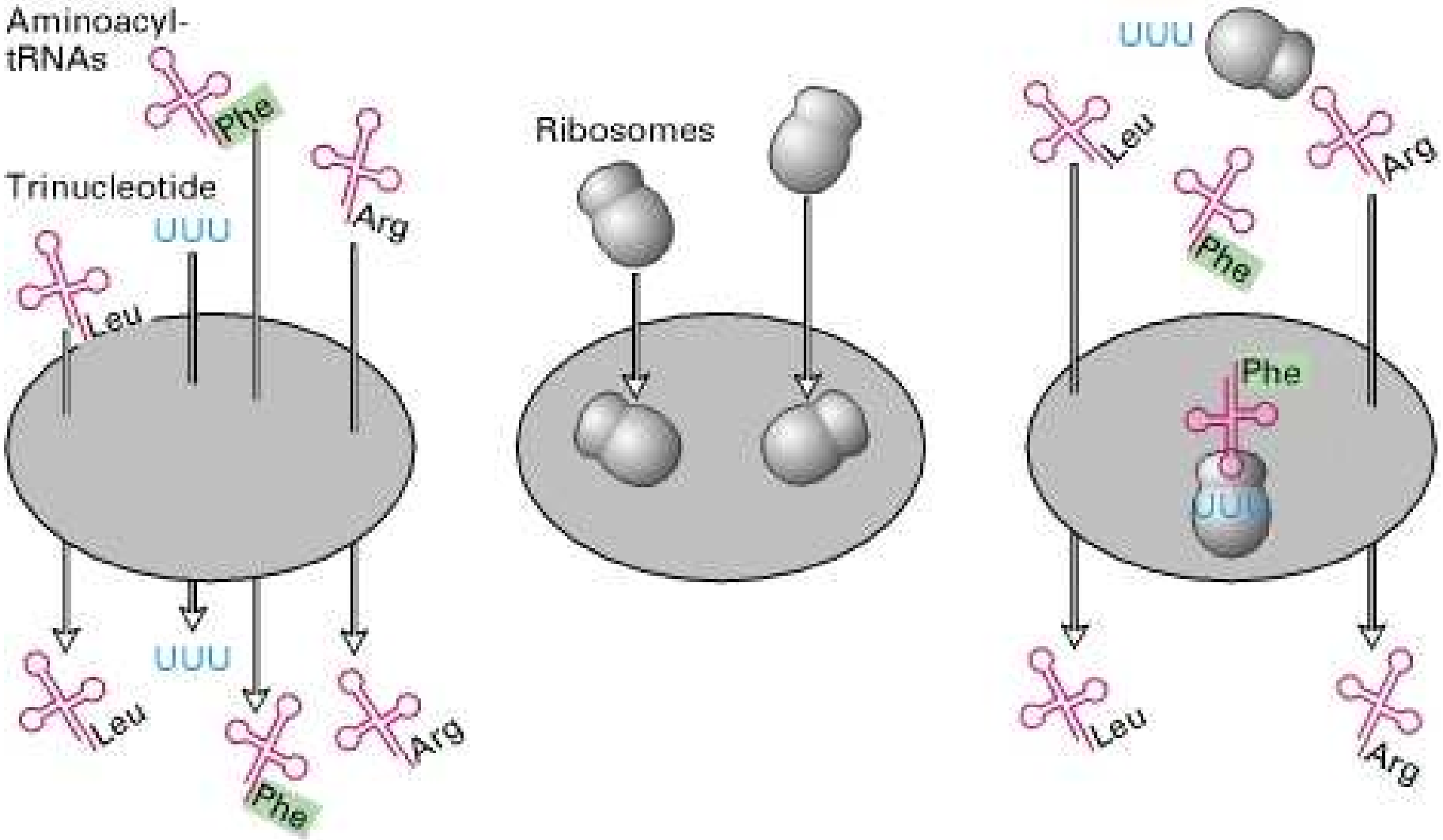
Amino Acid	Observed Amino Acid Incorporation	Tentative Codon Assignments	Calculated Triplet Frequency				Sum of Calculated Triplet Frequencies
			3A	2A1C	1A1C	3C	
Poly-AC (5:1)							
Asparagine	24	2A1C		20			20
Glutamine	24	2A1C		20			20
Histidine	6	1A2C			4.0		4
Lysine	100	3A	100				100
Proline	7	1A2C, 3C			4.0	0.8	4.8
Threonine	26	2A1C, 1A2C		20	4.0		24

Amino Acid	Observed Amino Acid Incorporation	Tentative Codon Assignments	Calculated Triplet Frequency				Sum of Calculated Triplet Frequencies
			3A	2A1C	1A1C	3C	
Poly-AC (5:1)							
Asparagine	24	2A1C		20			20
Glutamine	24	2A1C		20			20
Histidine	6	1A2C			4.0		4
Lysine	100	3A	100				100
Proline	7	1A2C, 3C			4.0	0.8	4.8
Threonine	26	2A1C, 1A2C		20	4.0		24
Poly-AC (1:5)							
Asparagine	5	2A1C		3.3			3.3
Glutamine	5	2A1C		3.3			3.3
Histidine	23	1A2C			16.7		16.7
Lysine	1	3A	0.7				0.7
Proline	100	1A2C, 3C			16.7	83.3	100
Threonine	21	2A1C, 1A2C		3.3	16.7		20

Copolymer	Codons Recognized	Amino Acids Incorporated or Polypeptide Made	Codon Assignment
$(CU)_n$	CUC|UCU|CUC . . .	Leucine	5′-CUC-3′
		Serine	UCU
$(UG)_n$	UGU|GUG|UGU . . .	Cysteine	UGU
		Valine	GUG
$(AC)_n$	ACA|CAC|ACA . . .	Threonine	ACA
		Histidine	CAC
$(AG)_n$	AGA|GAG|AGA . . .	Arginine	AGA
		Glutamine	GAG



■ **FIGURA 12.22** Induzione del legame dell'aminoacil-tRNA ai ribosomi da parte di mini-RNA sintetici trinucleotidici. I risultati di questi saggi di legame indotto da trinucleotidi hanno aiutato gli scienziati a decifrare il codice.



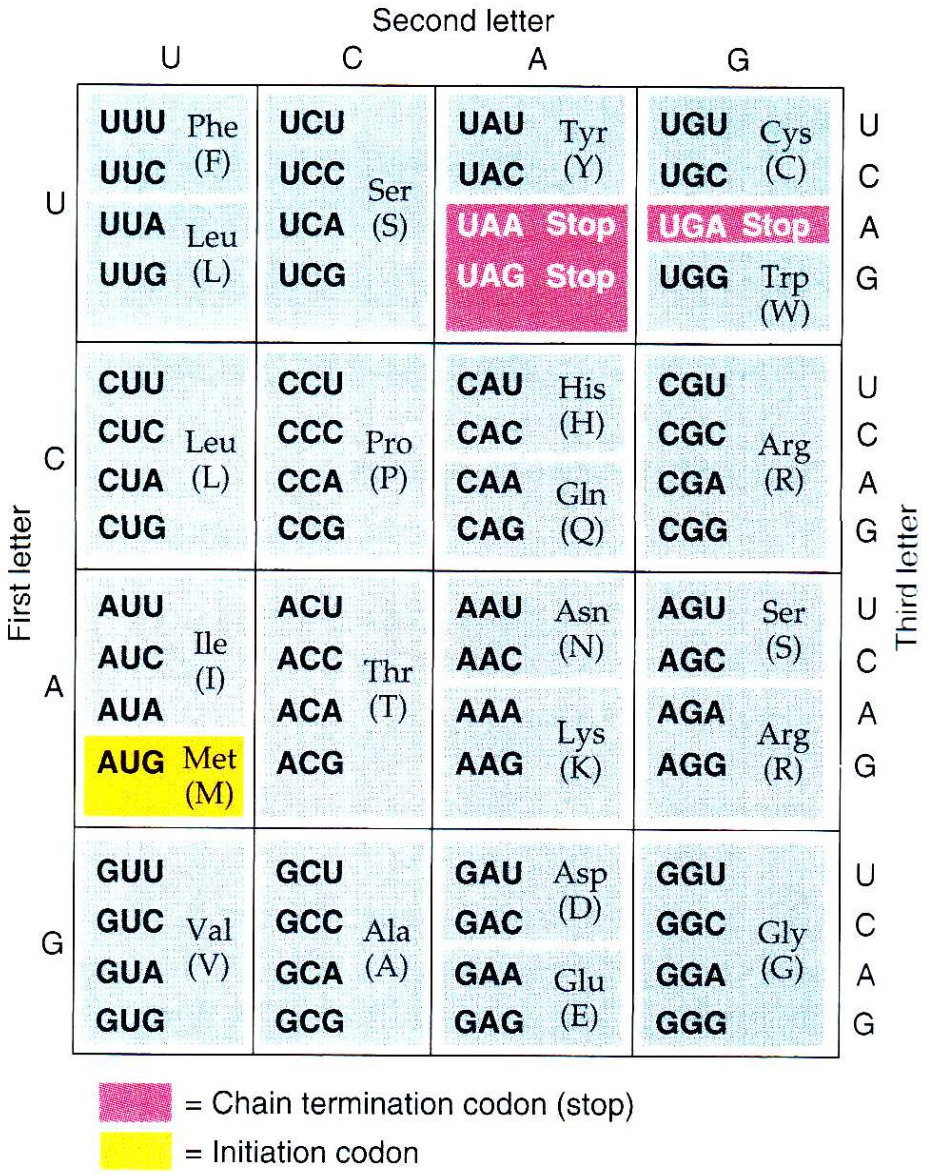
Trinucleotide and all tRNAs pass through filter

Ribosomes stick to filter

Complex of ribosome, UUU, and Phe-tRNA sticks to filter

TABLE 15-4 Binding of Aminoacyl tRNA Molecules to Trinucleotide-Ribosome Complexes

Trinucleotide						AA-tRNA Bound
5′-UUU-3′	UUC					Phenylalanine
UUA	UUG	CUU	CUC	CUA	CUG	Leucine
AAU	AUC	AUA				Isoleucine
AUG						Methionine
GUU	GUC	GUA	GUG	UCU*		Valine
UCU	UCC	UCA	UCG			Serine
CCU	CCC	CCA	CCG			Proline
AAA	AAG					Lysine
UGU	UGC					Cysteine
GAA	GAG					Glutamic acid


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