Chapter II

Protein Synthesis: From DNA to Protein

Protein Synthesis: From DNA to Protein

I. Transcription

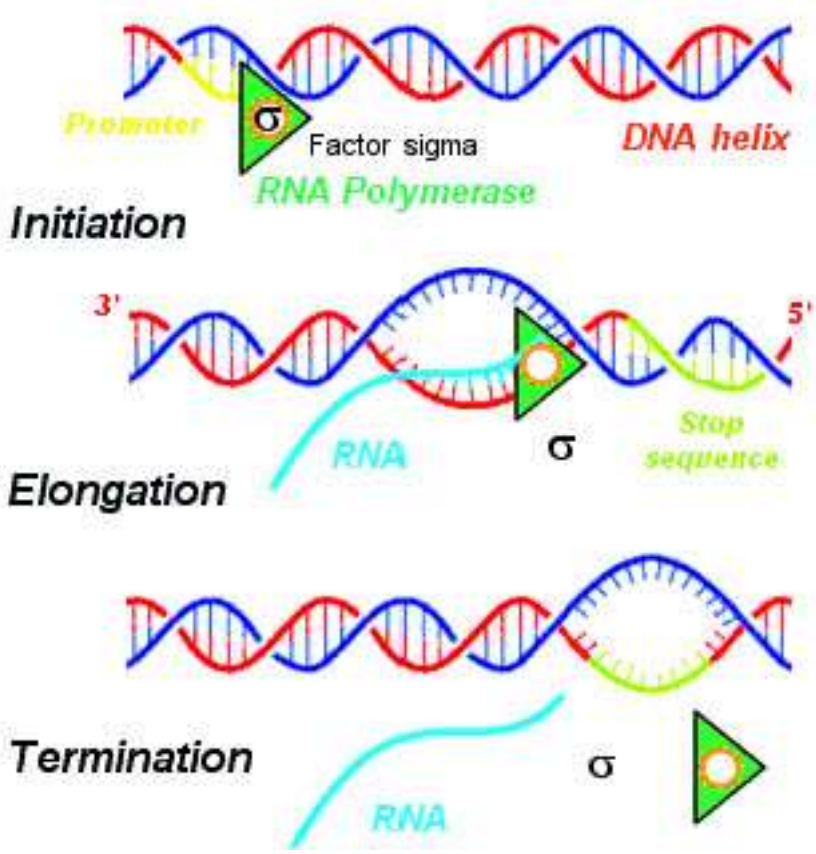
Similarities between Replication and Transcription

The processes of DNA and RNA synthesis are similar in that they involve-

(1) the general steps of initiation, elongation, and termination with 5' to 3' polarity;

(2) large, multicomponent initiation complexes; and

(3) adherence to Watson-Crick base-pairing rules.



Similarities between Replication and Transcription

(1) Ribonucleotides are used in RNA synthesis rather than deoxy ribonucleotides;

(2) U replaces T as the complementary base pair for A in RNA;

(3) A primer is not involved in RNA synthesis;

(4) Only a portion of the genome is transcribed or copied into RNA, whereas the entire genome must be copied during DNA replication; and

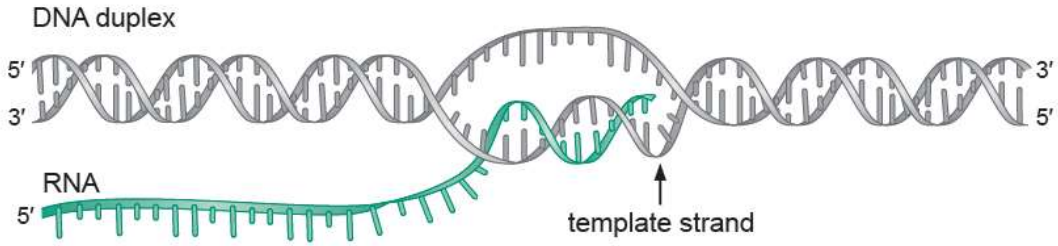
(5) There is no proofreading function during RNA transcription.

I. Transcription

Overview

- RNA synthesis is a DNA-dependent operation:
  - Starts at a “promoter” sequence, and ends at the termination signal
  - Often only one of the two strands is transcribed
  - Proceeds in the 5' to 3' direction
  - The DNA template is copied in the 3'-5'
  - The new nucleotides are added to the 3' OH end
  - A DNA-RNA hybrid is temporarily formed
  - Transcription speed rate (50 to 90 Nts / sec)
  - Complete RNA polymerase has processivity
    - RNA Pol processivity is the ability of RNA Pol to travel the entire length of the gene and thus controls the production of full-length transcripts.

La Transcription est Importante



transcription

(RNA)

mRNAs

translation

proteins

rRNAs

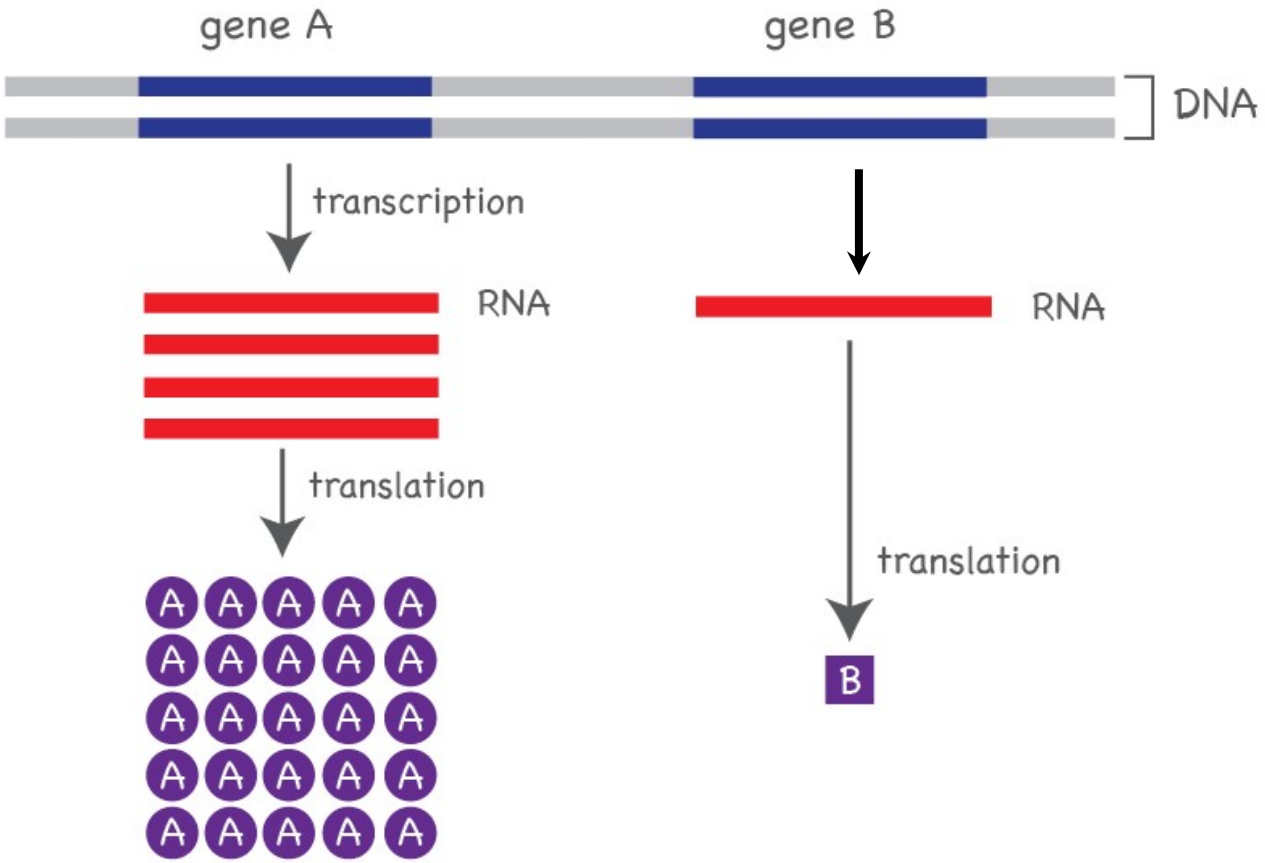
snRNAs

tRNAs

other non-coding RNAs
(e.g. RNA of telomerases)

I. Transcription

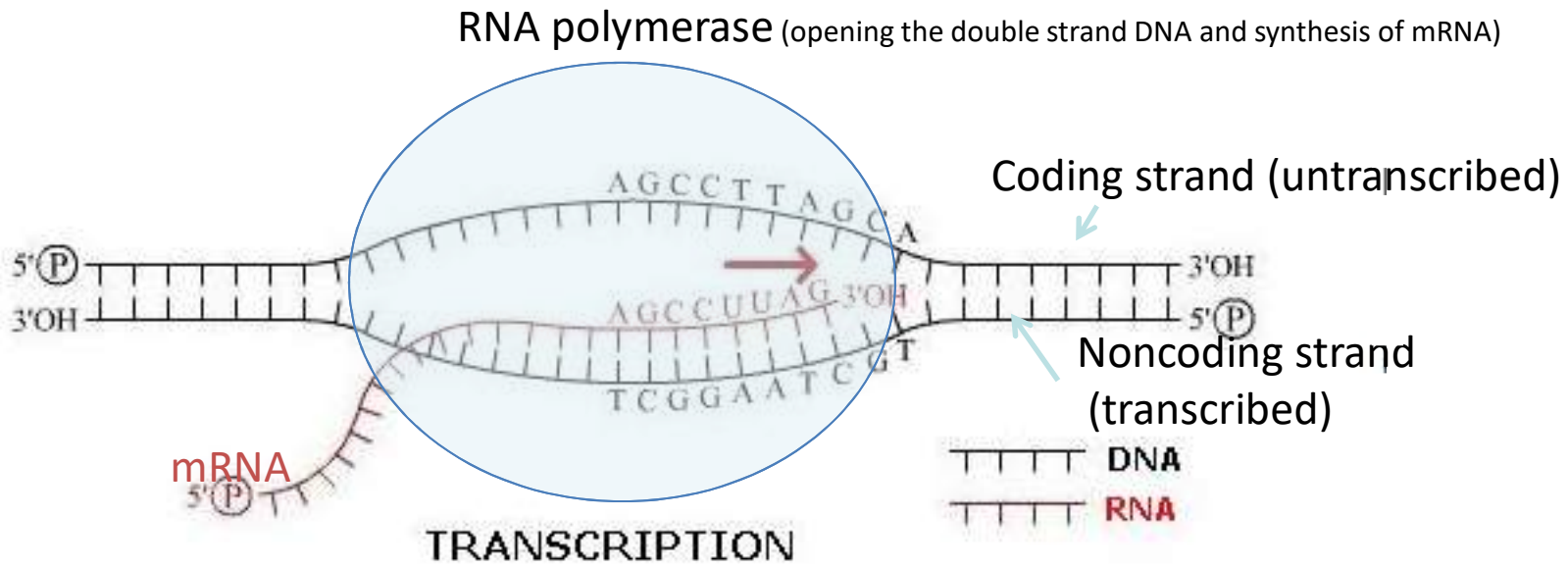
Proteins are differently expressed



- Gene A is transcribed and translated much more efficiently than gene B. This allows the amount of protein A in the cell to be much greater than that of protein B.

I. Transcription

- Transcription of a DNA fragment into mRNA:
  - Often only one of the two strands is transcribed (non-coding strand),



I. Transcription

Transcription of a DNA into RNA

Several RNA polymerases II can "work" at the same time, one after the other, on the same strand of DNA.

So several copies of RNA can be formed at the same time

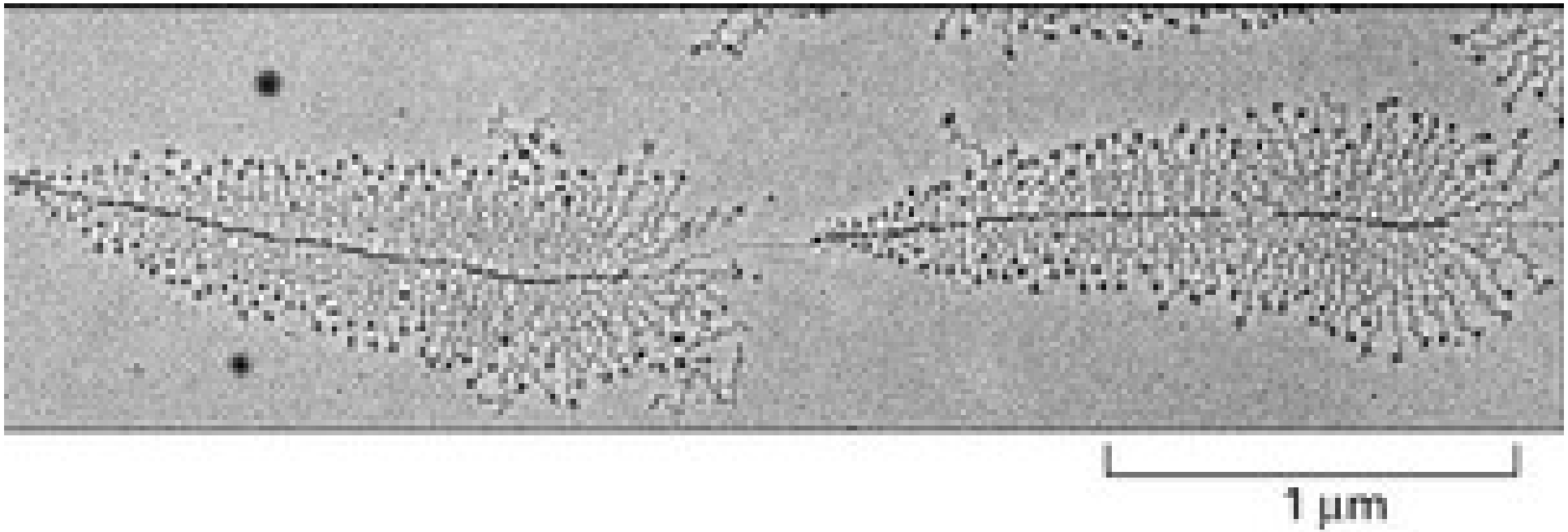


Figure 6–9. Molecular Biology of the Cell, 4th Edition.

General mechanism of RNA Synthesis

RNA synthesis reaction

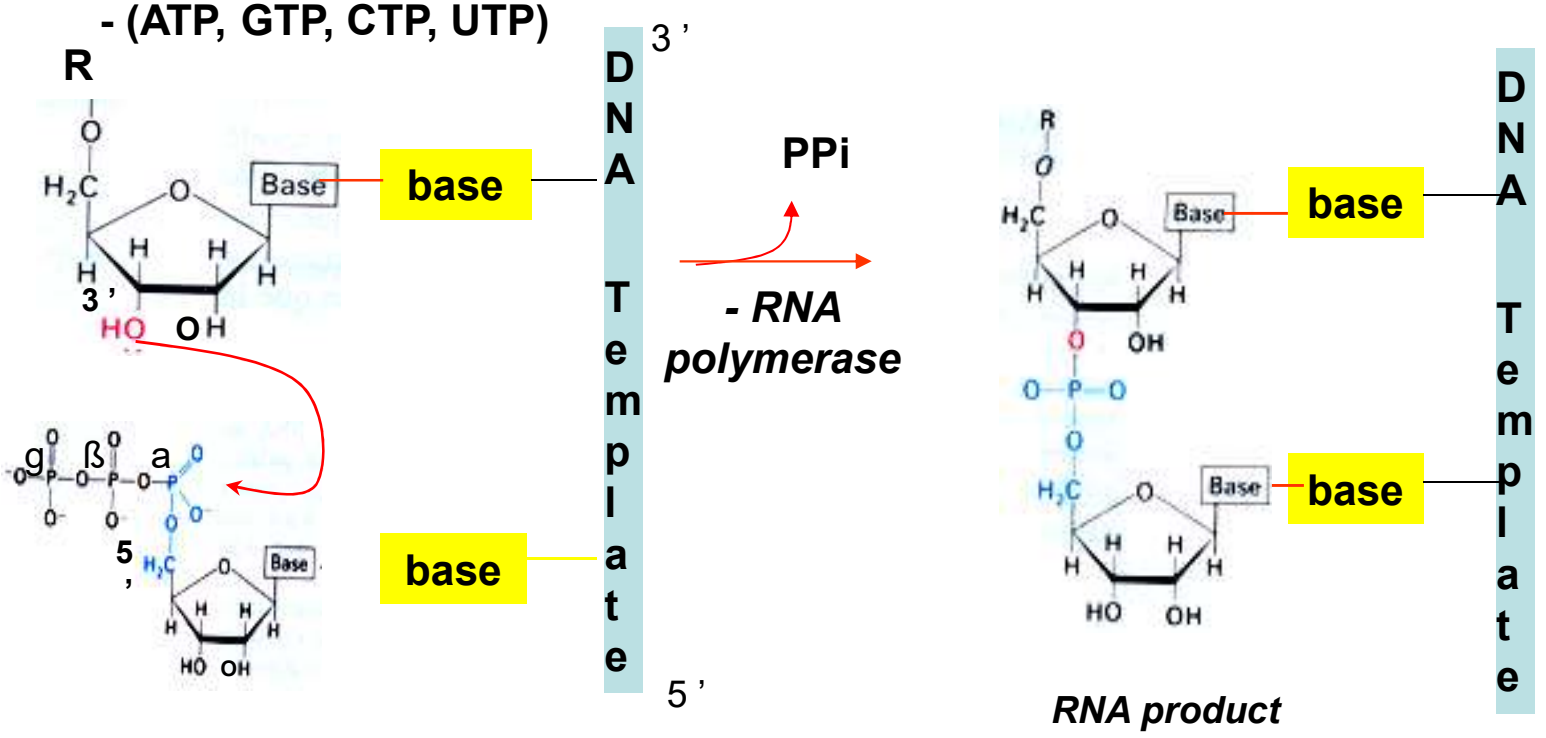
DNA-dependent RNA polymerase

RNA $_{(n)\text{residues}}$ + ribonucleotides triphosphate ⟺ RNA $_{(n+1)\text{ residues}}$ + Pyrophosphatase

2 Pi

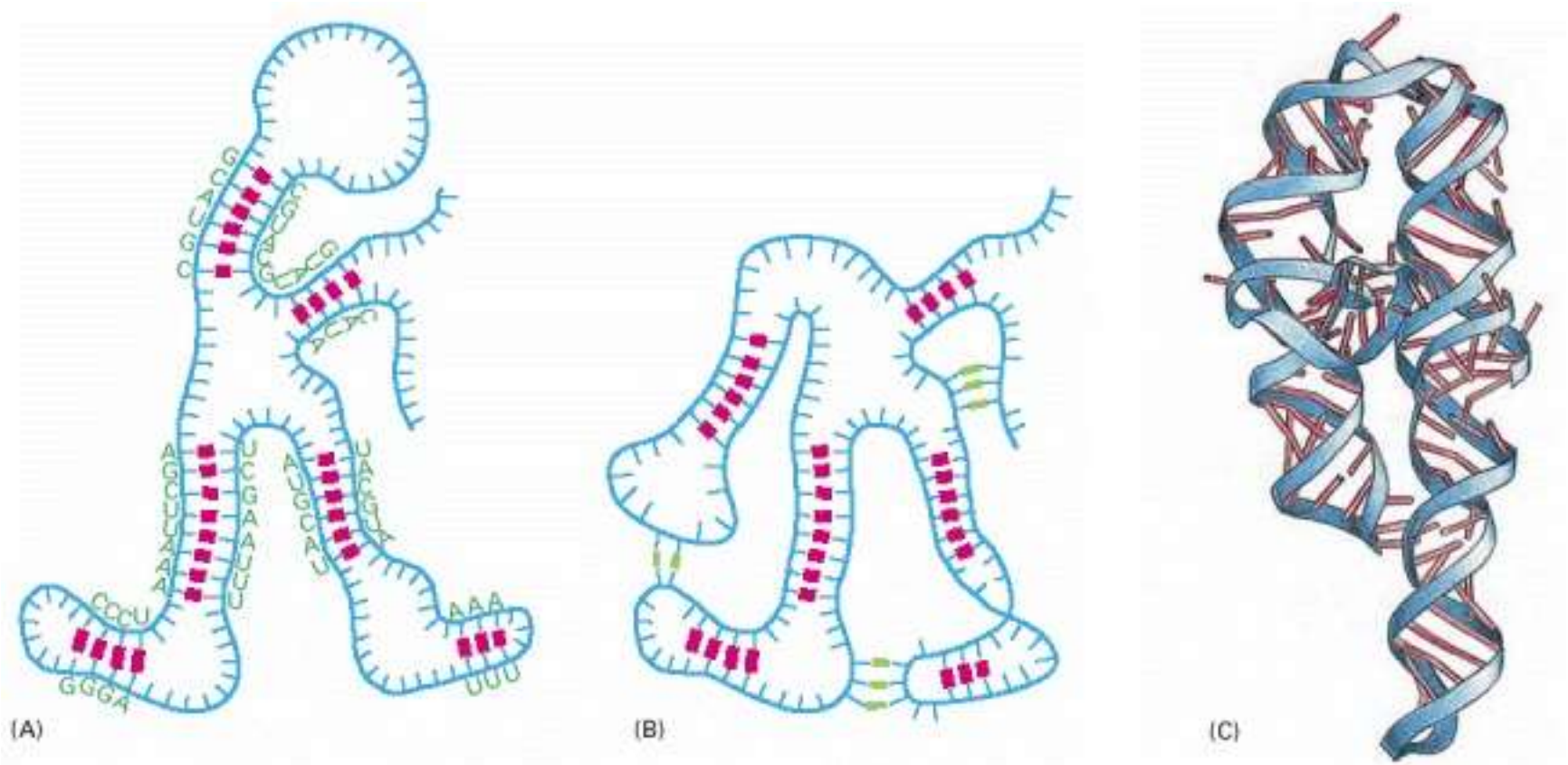
- ADN matrice

- (ATP, GTP, CTP, UTP)



ADN matriciel

I. Transcription



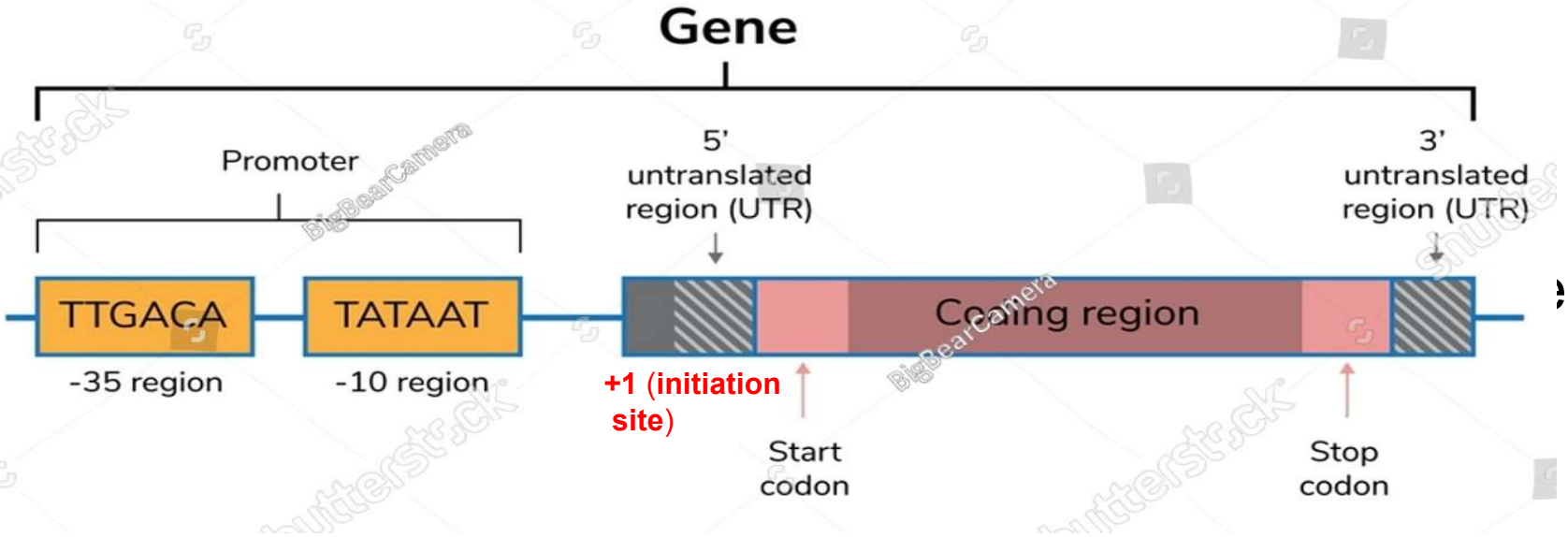
RNA is largely single-stranded, but it often contains short stretches of nucleotides that can form conventional base-pairs with complementary sequences found elsewhere on the same molecule.

These interactions, along with additional “nonconventional” base-pair interactions, allow an RNA molecule to fold into a three-dimensional structure that is determined by its sequence of nucleotides.

I.I Transcription in prokaryotes

Prokaryotic gene structure

Gene Promoter : A promoter contains DNA sequences that let RNA polymerase or its helper proteins attach to the DNA.

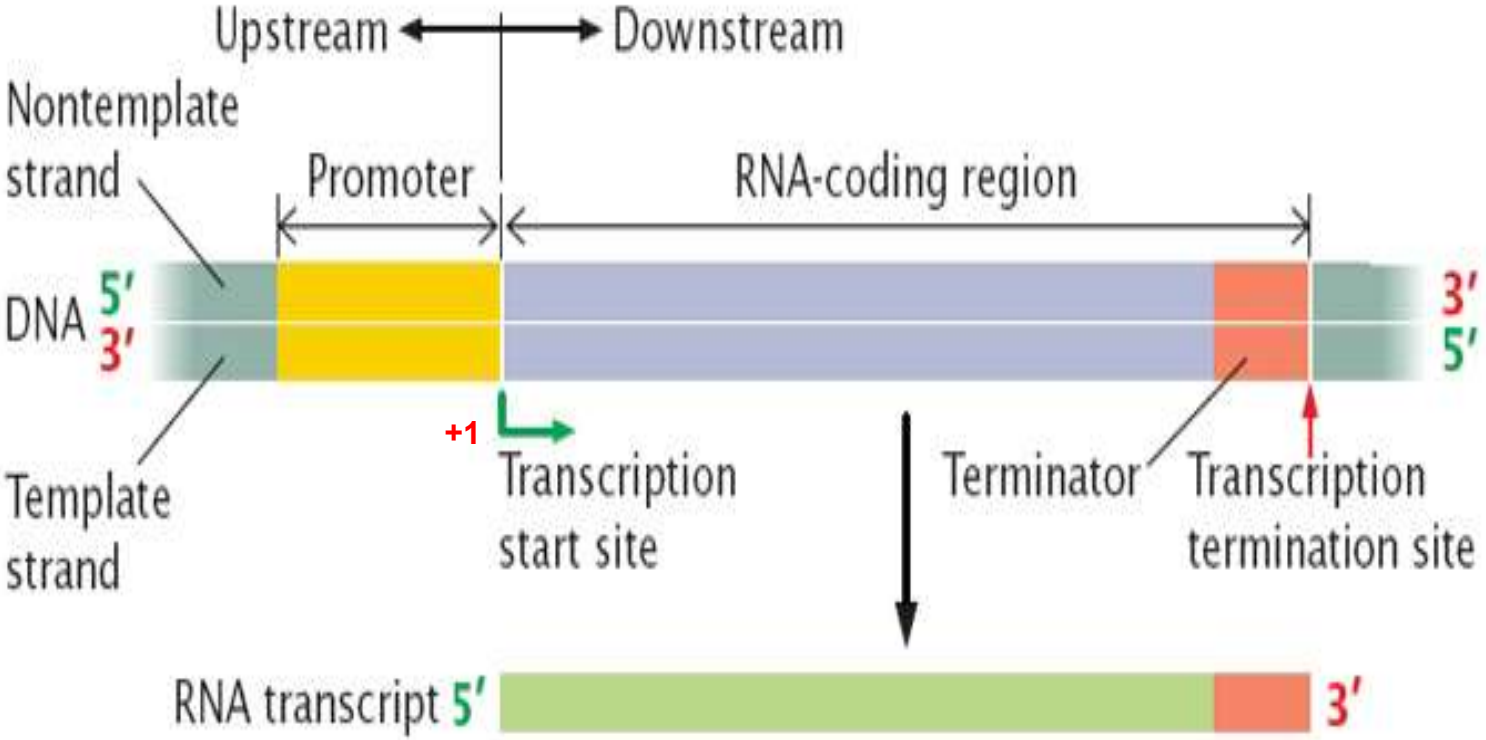


+1 : Starting the transcription

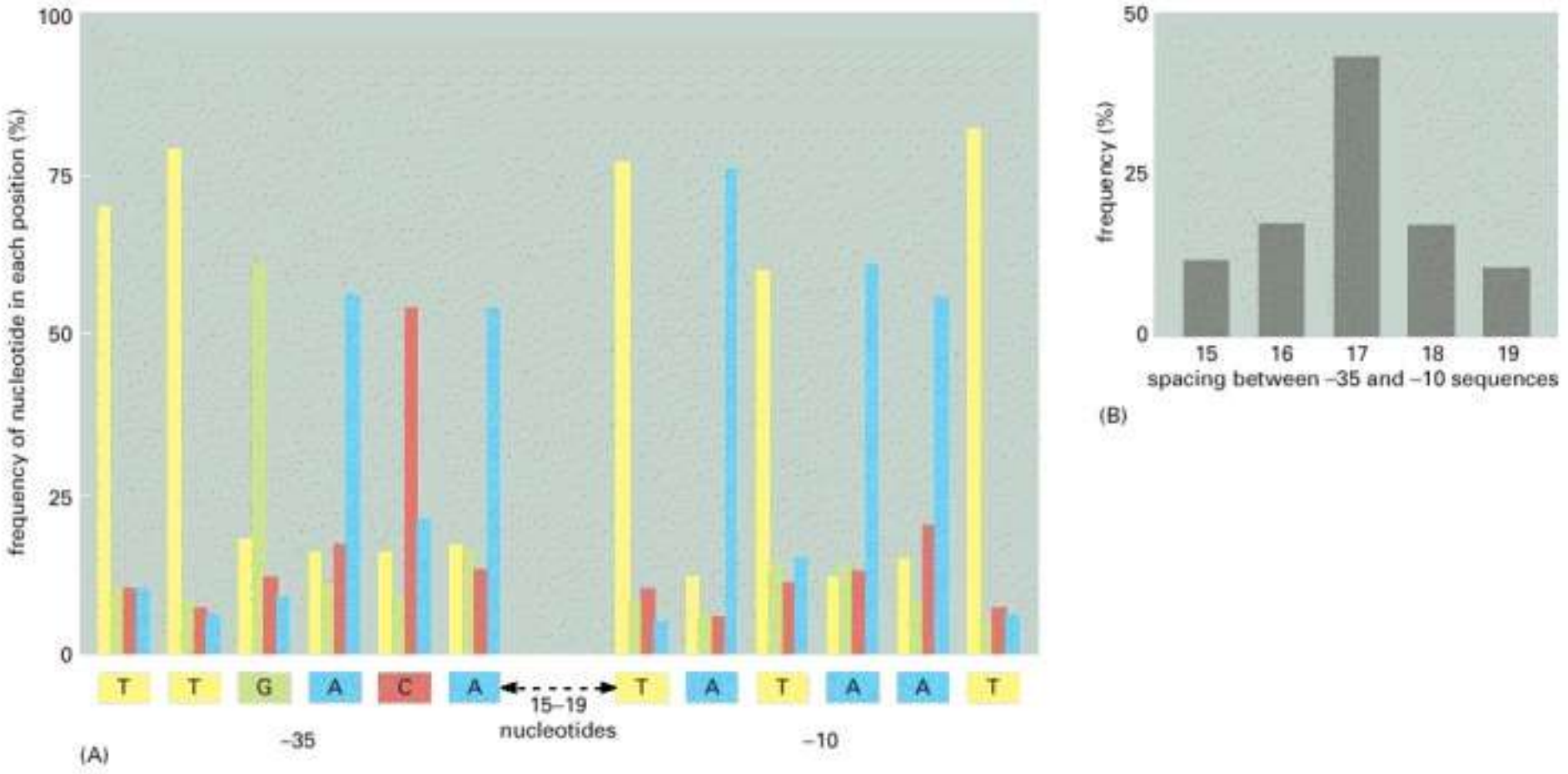
Box -35	5'-TTGACA-3'
Box -10 (Pribnow box)	5'-TATAAT-3'

The promoters are characterized by two hexameric DNA sequences, the -35 sequence and the -10 sequence named for their approximate location relative to the start point of transcription (designated +1).

Transcription unit



Consensus sequence for the major class of *E. coli* promoters



Promoters for genes that code for abundant proteins are much stronger than those associated with genes that encode rare proteins, and their nucleotide sequences are responsible for these differences.

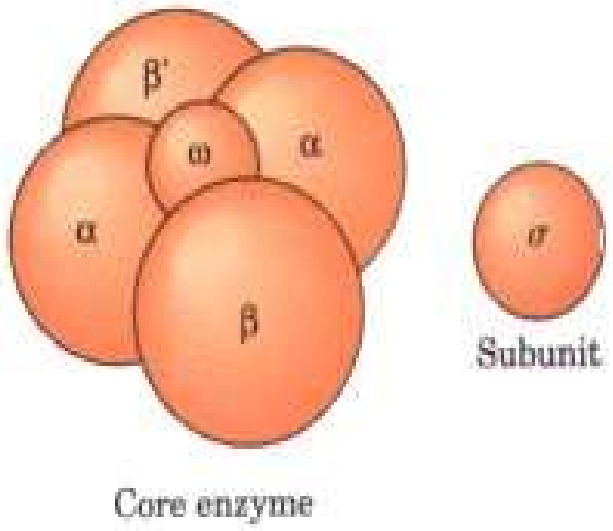
Primary transcript

- This is designated position +1, as is the corresponding nucleotide in the DNA
- The numbers increase as the sequence proceeds *downstream.*
- The nucleotide in the promoter adjacent to the transcription initiation site is designated -1,
- These negative numbers increase as the sequence proceeds *upstream,* away from the initiation site.
- This provides a conventional way of defining the location of regulatory elements in the promoter.

Bacterial DNA-Dependent RNA Polymerase

The DNA-dependent RNA polymerase (RNA Pol) of the bacterium *Escherichia coli* exists as an approximately 400 kDa core complex consisting of-

- two identical α subunits,
- similar but not identical β and β ' subunits, and
- an ω subunit and a
- A sigma subunit (σ)



Prokaryotic transcription

Steps of RNA Synthesis-

The process of transcription of a typical gene of E. Coli can be divided in to three phases-

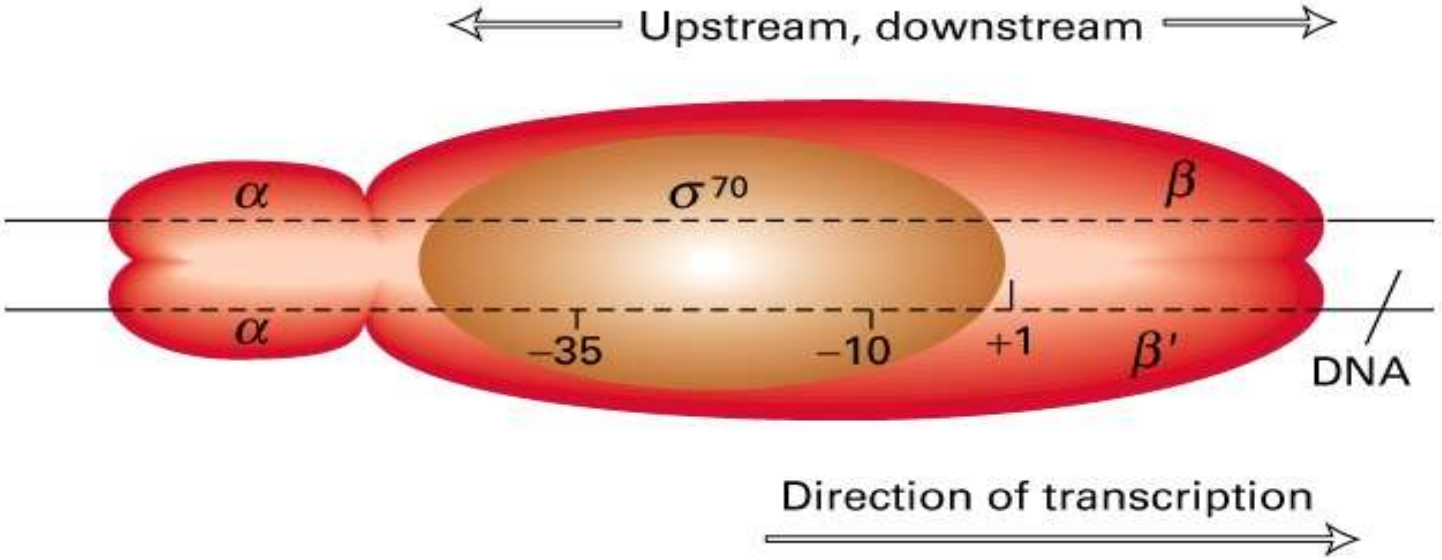
i) Initiation

ii) Elongation

iii) Termination

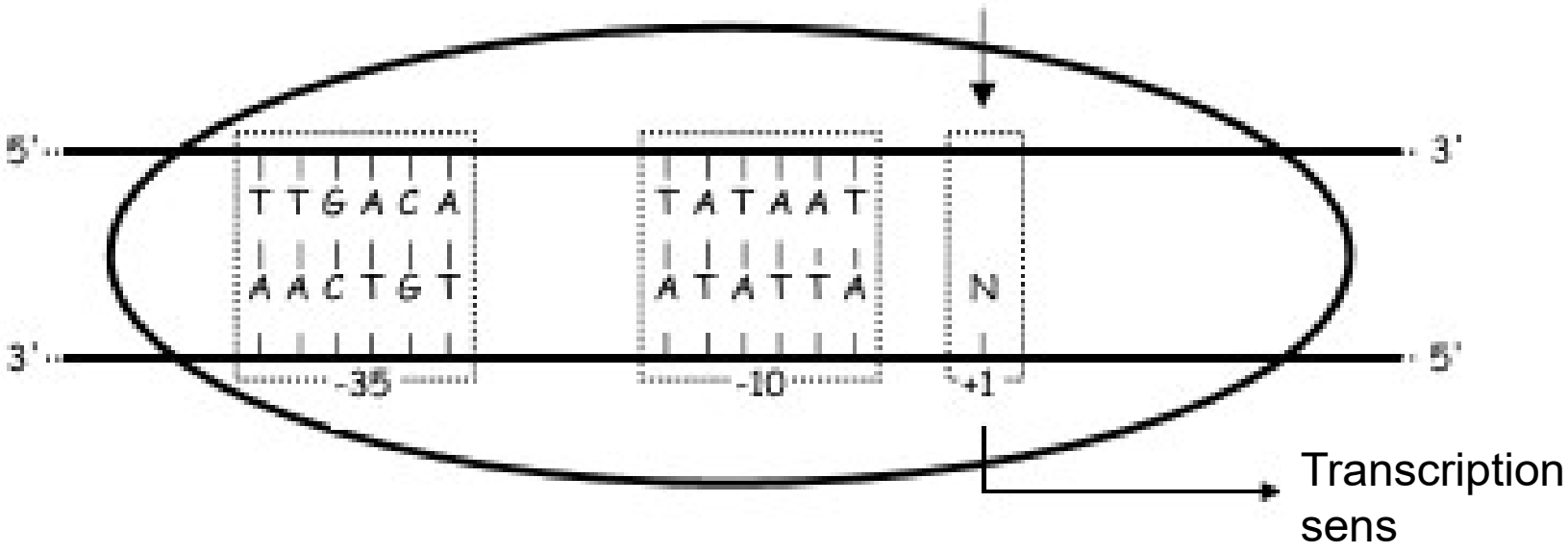
i) Initiation of Transcription

- Initiation of transcription involves the binding of the RNA polymerase holoenzyme to the promoter region on the DNA to form a **preinitiation complex, or PIC**
- "Consensus" nucleotide sequence of the prokaryotic promoter region are highly conserved.



Initiation of Transcription

Promoter



40 Nucleotides

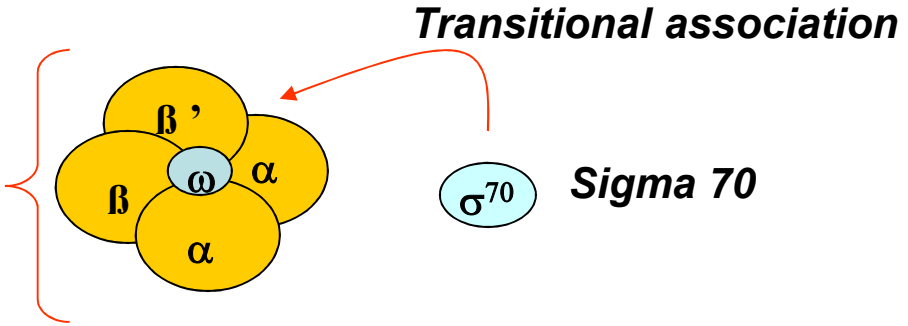
20 Nucleotides

60 Nucleotides

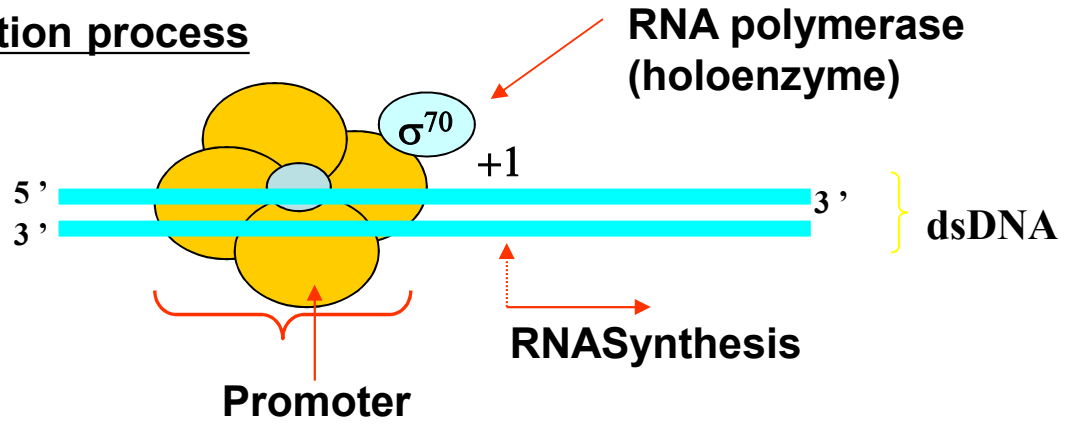
Initiation of Transcription

DNA dependant RNA polymerase

In *E.coli (390 000 Da)*

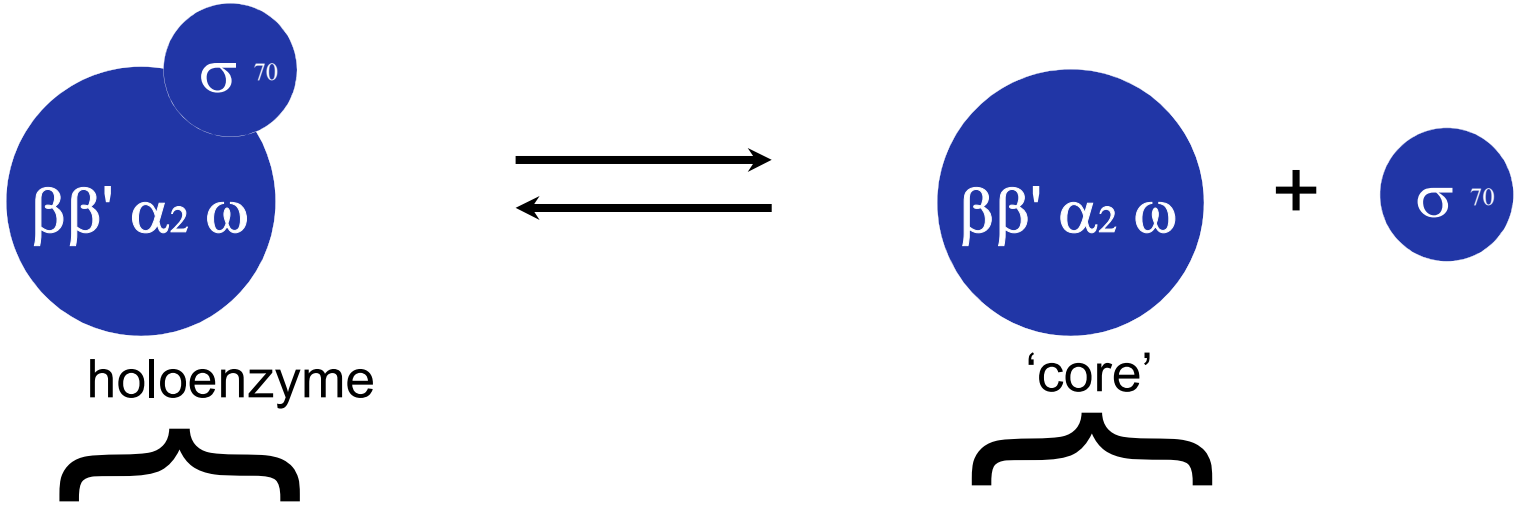


Transcription initiation process



Initiation of Transcription

σ factor is required for bacterial RNA polymerase to initiate transcription at promoters



holoenzyme

Can initiate transcription at promoters and can proceed to elongation

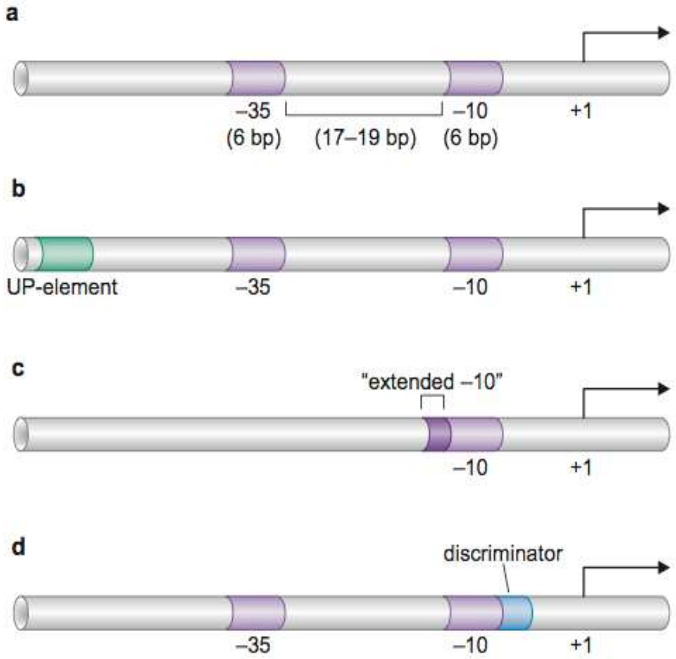
'core'

Can proceed with elongation but cannot start transcription at promoters

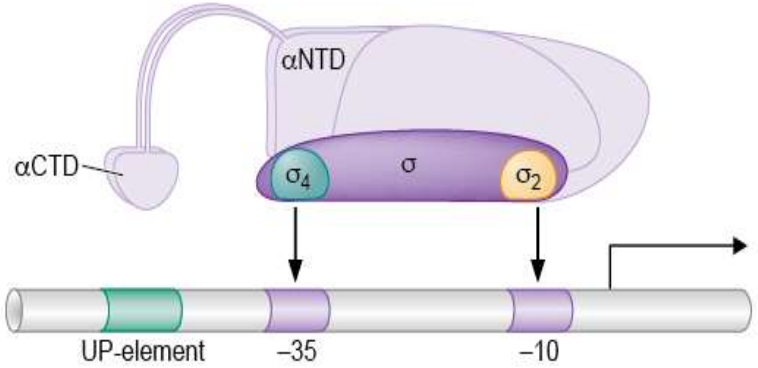
Initiation of Transcription

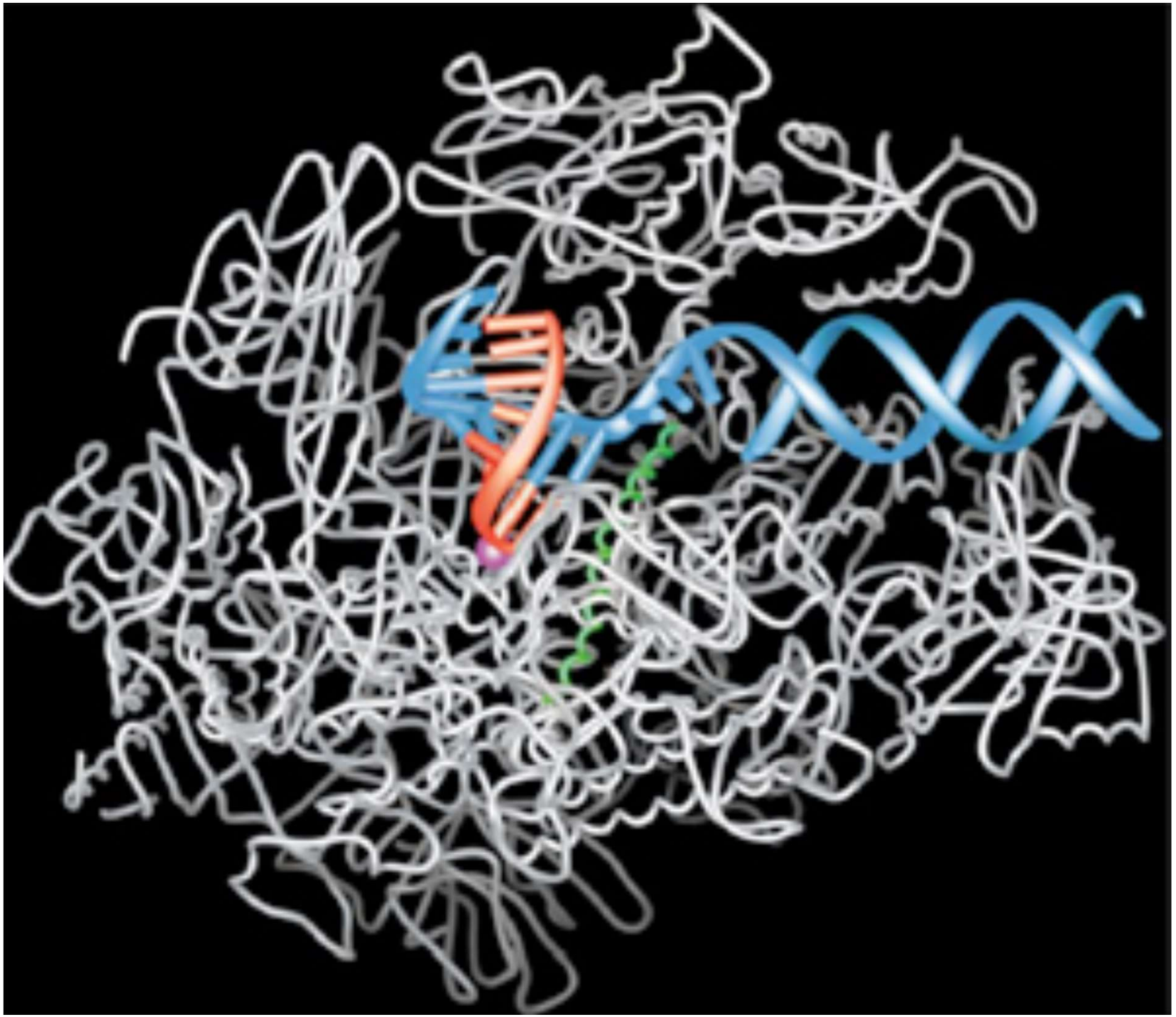
Promoteurs Bactérien

Il y’a plusieurs promoteurs bactériens



σ and α lie ARN Pol au promoteur



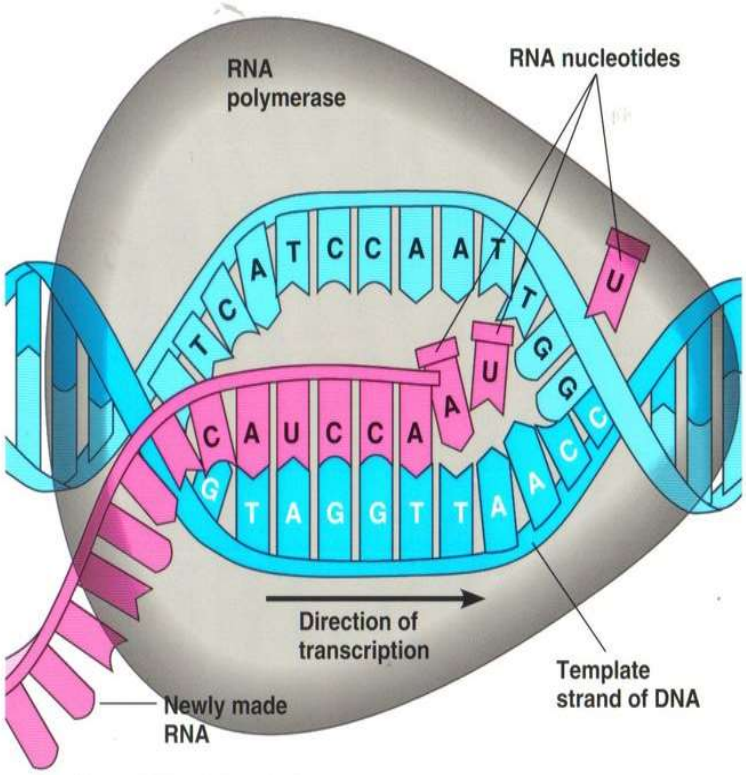


II) Elongation of Transcription

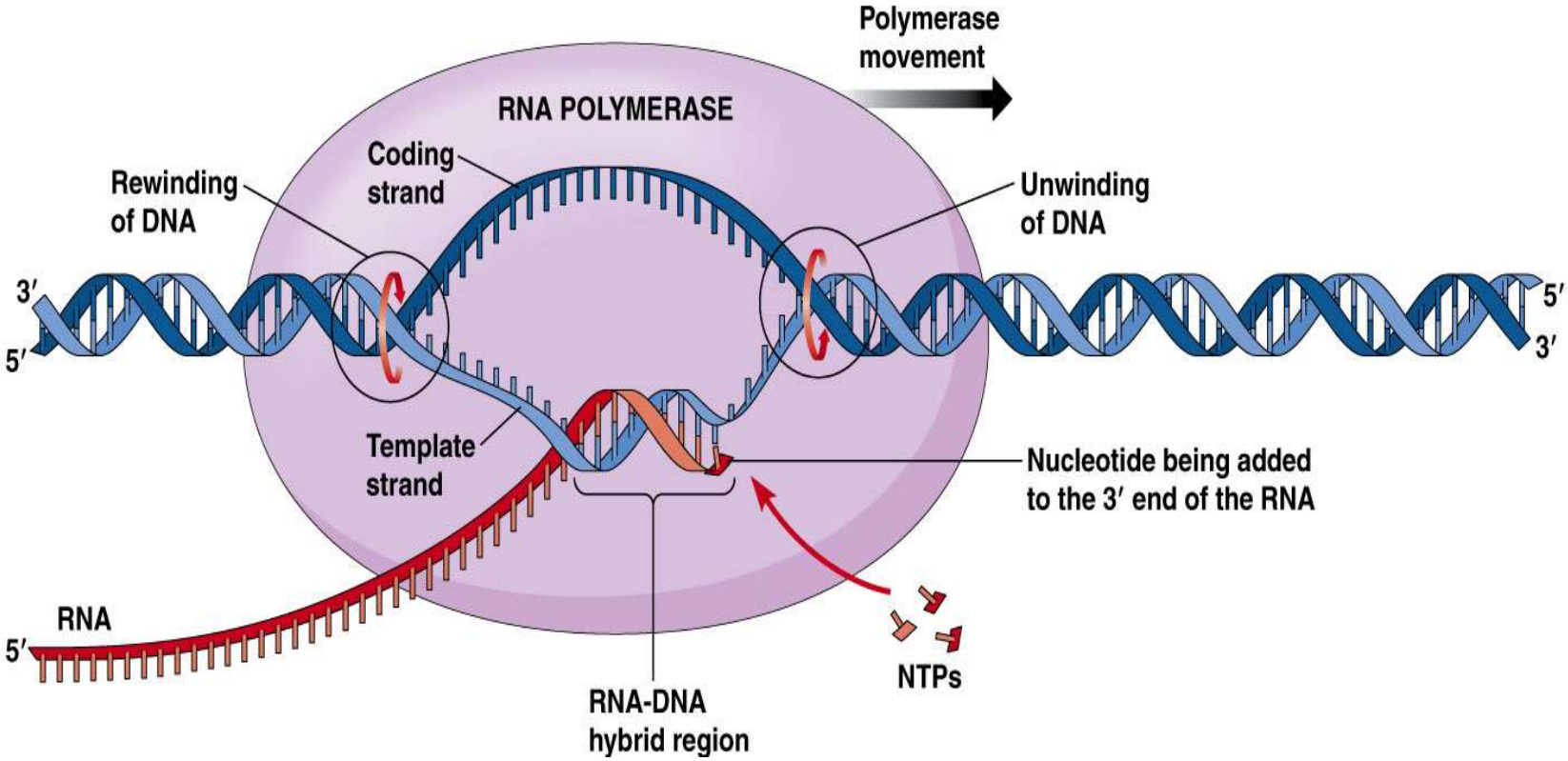
- As the elongation complex containing the core RNA polymerase progresses along the DNA molecule, DNA unwinding must occur in order to provide access for the appropriate base pairing to the nucleotides of the template strand.
- The extent of this transcription bubble (i.e., DNA unwinding) is constant throughout and is about 20 base pairs per polymerase molecule.

Elongation of Transcription

- RNA polymerase has associated with it an "unwindase" activity that opens the DNA helix.
- Topo isomerase both precedes and follows the progressing RNAP to prevent the formation of super helical complexes.
- Base pairing rule is followed during the incorporation of ribonucleotides



Elongation of Transcription

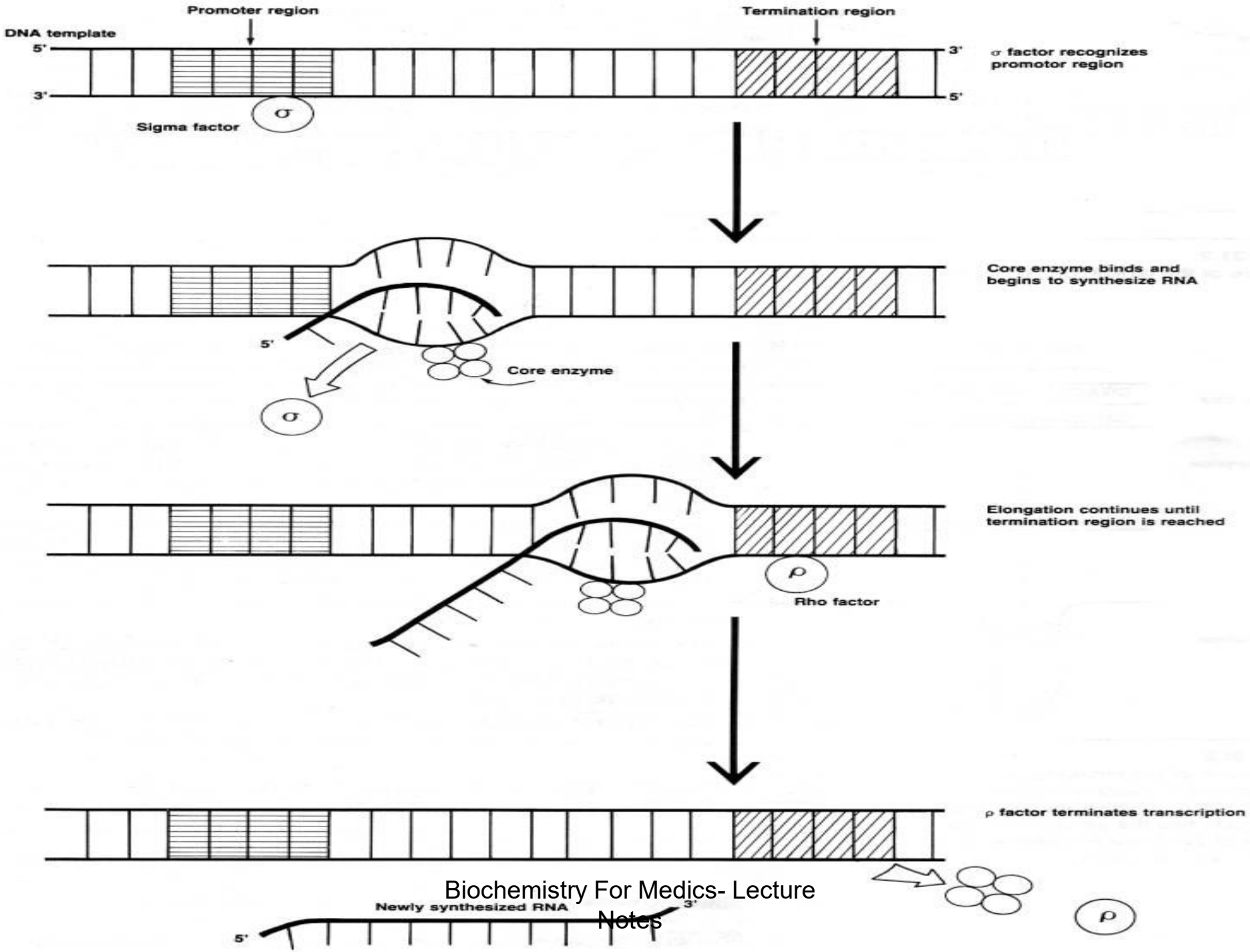


III) Termination of transcription

Termination of the synthesis of the RNA molecule in bacteria is of two types-

a)Rho (ρ) dependent termination-

- The termination process is signaled by a sequence in the template strand of the DNA molecule—a signal that is recognized by a termination protein, the rho (ρ) factor.
- Rho is an ATP-dependent RNA-stimulated helicase that disrupts the nascent RNA-DNA complex.


Promoter region
Termination region
DNA template
5'
3'
3'
5'
Sigma factor
σ factor recognizes promotor region
Core enzyme binds and begins to synthesize RNA
5'
Core enzyme
Elongation continues until termination region is reached
Rho factor
ρ factor terminates transcription
Newly synthesized RNA
3'
5'

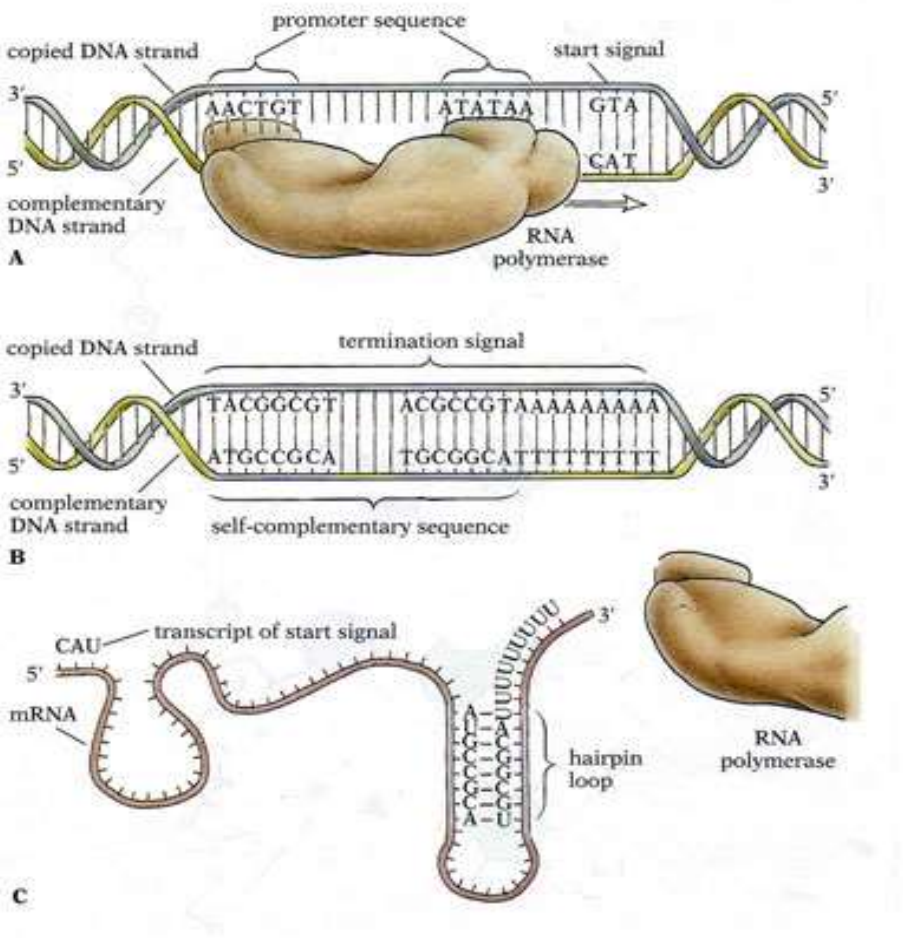
Termination of transcription

b) Rho independent termination

- This process requires the presence of intrachain self complementary sequences in the newly formed primary transcript so that it can acquire a stable hair pin turn that slows down the progress of the RNA polymerase and causes it to pause temporarily.
- Near the stem of the hairpin, a sequence occurs that is rich in G and C.
- This stabilizes the secondary structure of the hair pin.

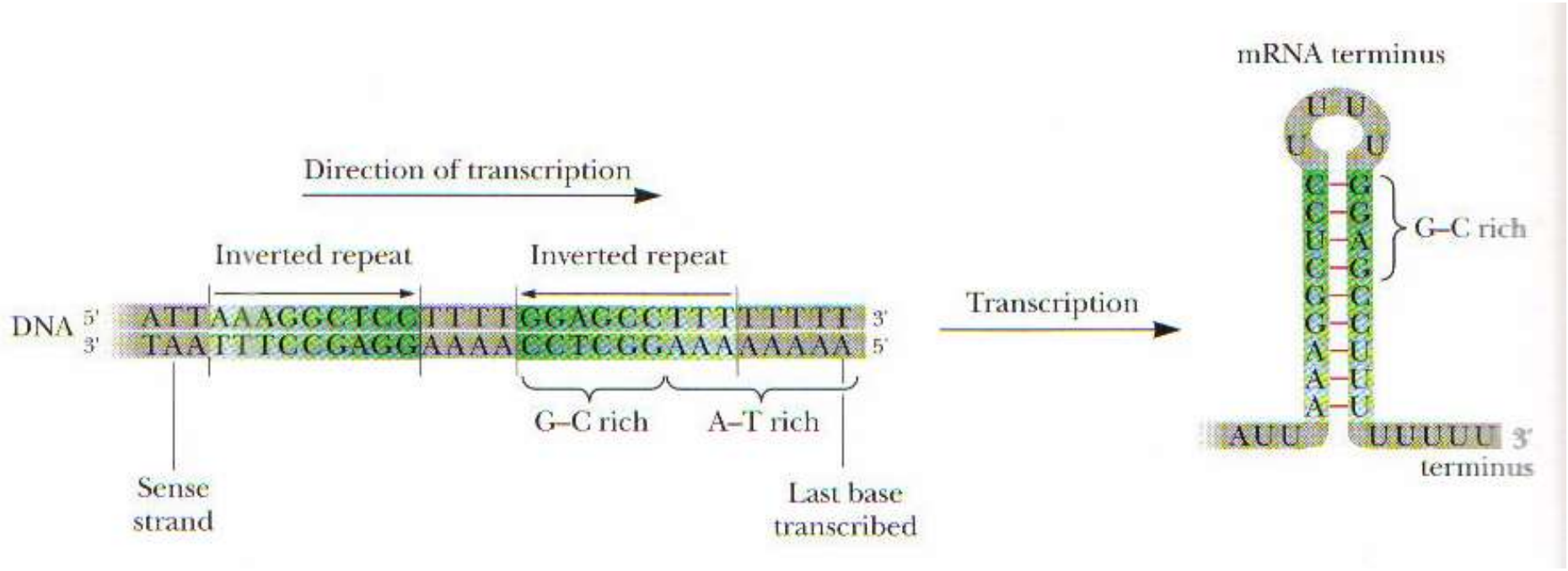
Termination of transcription

- Beyond the hair pin, the RNA transcript contains a strings of Us, the bonding of Us to the corresponding As is weak.
- This facilitates the dissociation of the primary transcript from DNA.



Termination of transcription

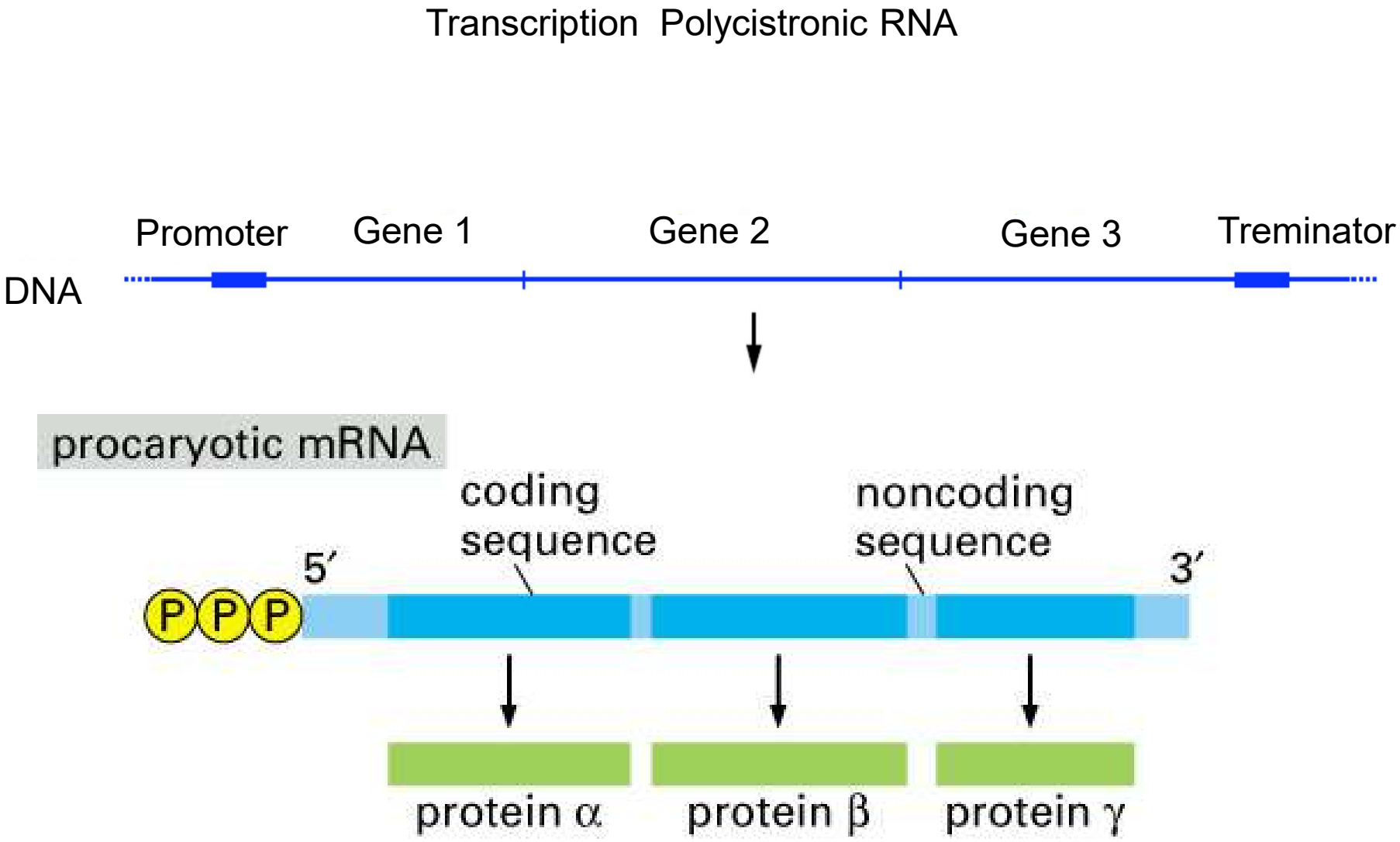
- **b) Rho independent termination :**
  - sites on DNA with a specific structure
  - DNA template contains inverted repeats (palindromic sequences)
  - Formation of a “hairpin” structure that destabilizes RNA polymer.



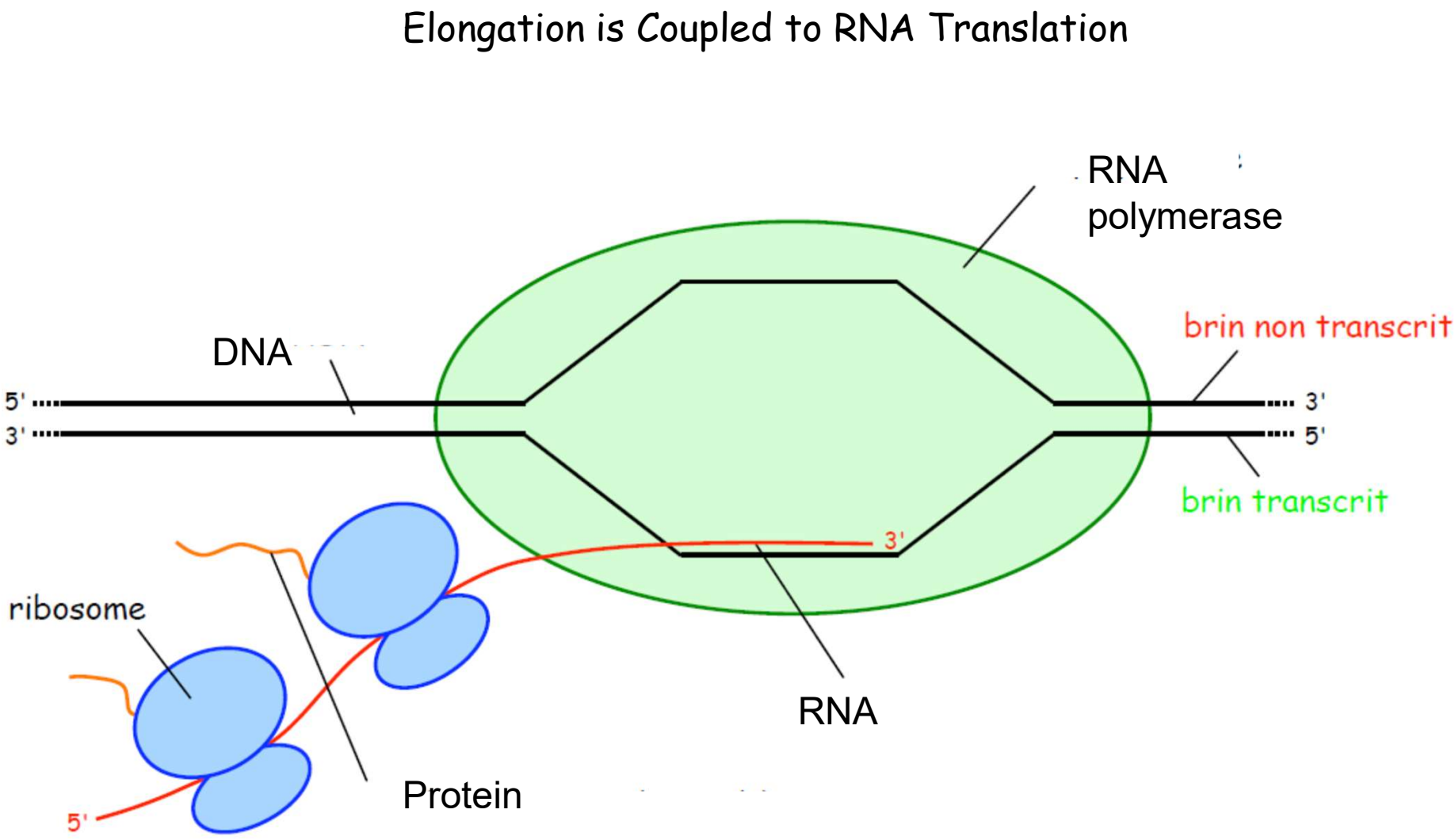
Termination of transcription

- After termination of synthesis of the RNA molecule, the enzyme separates from the DNA template.
- With the assistance of another factor, the core enzyme then recognizes a promoter at which the synthesis of a new RNA molecule commences.

Particularities of prokaryotic transcription



Particularities of prokaryotic transcription



I. II. Transcription in eukaryotes

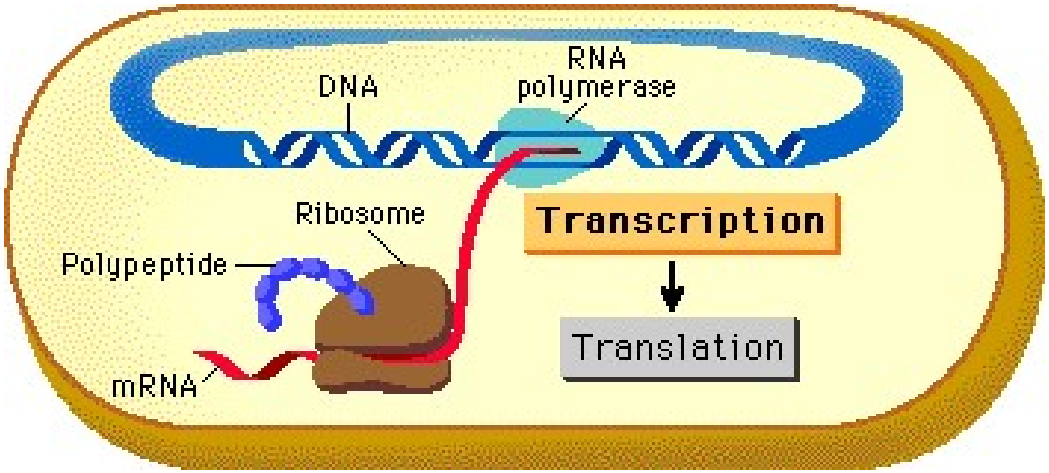
Eukaryotic transcription

- The general process of transcription can be applied to both prokaryotic cells and eukaryotic cells.
- The basic biochemistry for each is the same; however, the specific mechanisms and regulation of transcription differ between prokaryotes and eukaryotes.
- Transcription of eukaryotic genes is far more a complicated process than prokaryotes.

Prokaryotic versus Eukaryotic Transcription

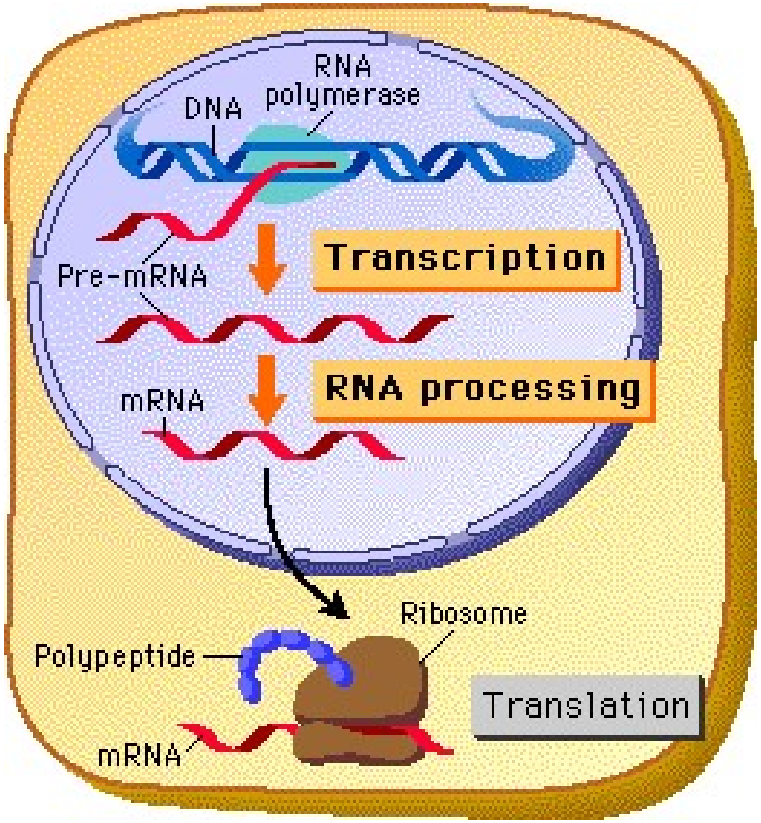
1) Location

- In prokaryotes (bacteria), transcription occurs in the cytoplasm.
- Translation of the mRNA into proteins also occurs in the cytoplasm



Prokaryotic versus Eukaryotic Transcription

- In eukaryotes, transcription occurs in the cell's nucleus, mRNA then moves to the cytoplasm for translation.



Prokaryotic versus Eukaryotic Transcription

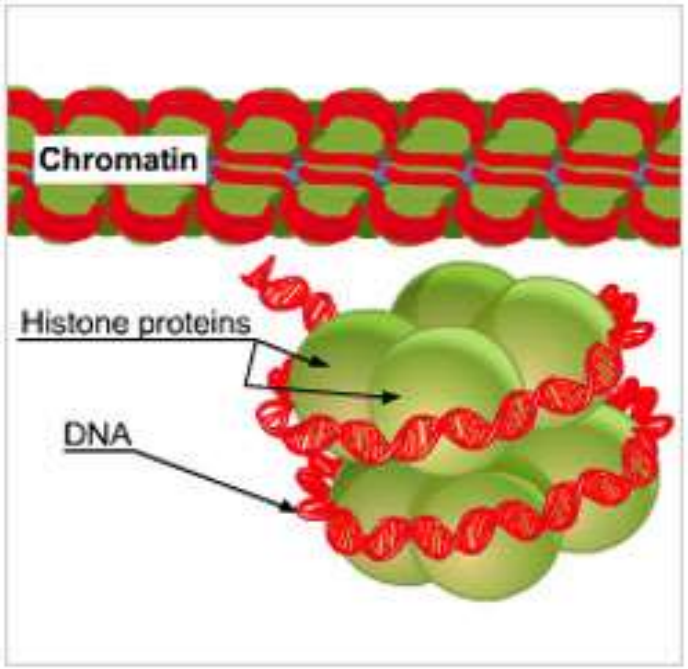
2) Genome size

- The genome size is much larger in eukaryotes,
- Greater specificity is needed for the transcription of eukaryotic genes.

Prokaryotic versus Eukaryotic Transcription

3) Chromatin Structure

- DNA in prokaryotes is much more accessible to RNA polymerase than DNA in eukaryotes.
- Eukaryotic DNA is wrapped around proteins called histones to form structures called nucleosomes
- Eukaryotic DNA is packed to form chromatin .
- While RNA polymerase interacts directly with prokaryotic DNA, other proteins mediate the interaction between RNA polymerase and DNA in eukaryotes



Prokaryotic versus Eukaryotic Transcription

4) RNA polymerases

- There are three distinct classes of RNA polymerases in eukaryotic cells. All are large enzymes with multiple subunits. Each class of RNA polymerase recognizes particular types of genes.
- RNA polymerase I- Synthesizes the precursor of the large ribosomal RNAs (28S, 18S and 5.8S).
- RNA polymerase II - Synthesizes the precursors of messenger RNA and small nuclear RNAs(snRNAs).
- RNA polymerase III- Synthesizes small RNA, including t RNAs, small 5S RNA and some snRNAs.

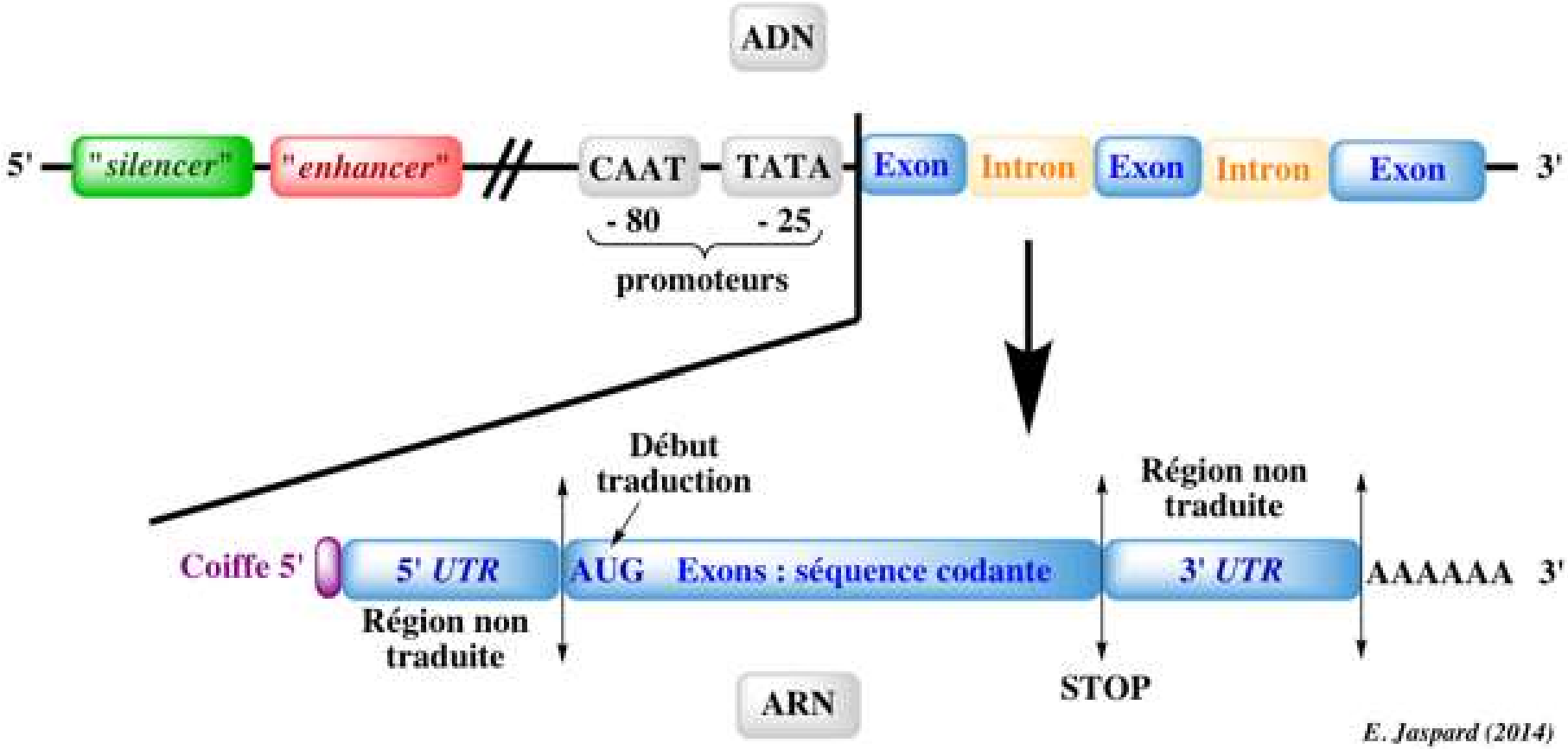
Prokaryotic versus Eukaryotic Transcription

5) Promoter regions

- Eukaryotic promoters are more complex.
- Two types of sequence elements are promoter-proximal and distal regulatory elements.
- There are two elements in promoter proximal ,One of these defines **where transcription is to commence** along the DNA, and the other contributes to the mechanisms that control **how frequently** this event is to occur.
- Most mammalian genes have a TATA box that is usually located 25–30 bp upstream from the transcription start site.

Transcription

Structure of eukaryotic gene



Regulation of transcription: presence of regulatory sequences on DNA

Prokaryotic versus Eukaryotic Transcription

- The consensus sequence for a TATA box is TATAAA, though numerous variations have been characterized.
- Sequences farther upstream from the start site determine how frequently the transcription event occurs.
- Typical of these DNA elements are the GC and CAAT boxes, so named because of the DNA sequences involved.
- Each of these boxes binds a specific protein.
- Distal regulatory elements enhance or decrease the rate of transcription.
- They include the enhancer/ silencer regions and other regulatory elements.

Prokaryotic versus Eukaryotic Transcription

6) Promoter identification

- In contrast to the situation in prokaryotes, eukaryotic RNA polymerases alone are not able to discriminate between promoter sequences and other regions of DNA
- The TATA box is bound by 34 kDa **TATA binding protein (TBP),** which in turn binds several other proteins called **TBP-associated factors (TAFs).**
- This complex of TBP and TAFs is referred to as TFIID

Prokaryotic versus Eukaryotic Transcription

- Binding of TFIID to the TATA box sequence is thought to represent the first step in the formation of the transcription complex on the promoter.
- Another set of proteins—co activators—help regulate the rate of transcription initiation by interacting with transcription activators that bind to upstream DNA elements

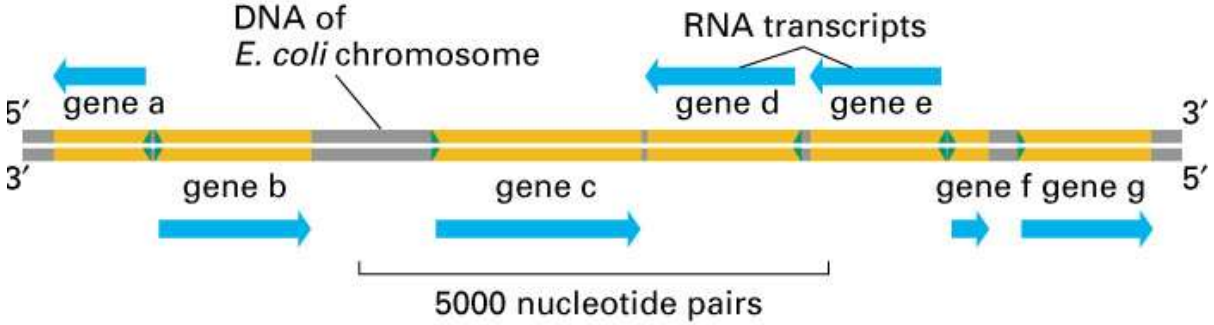
Prokaryotic versus Eukaryotic Transcription

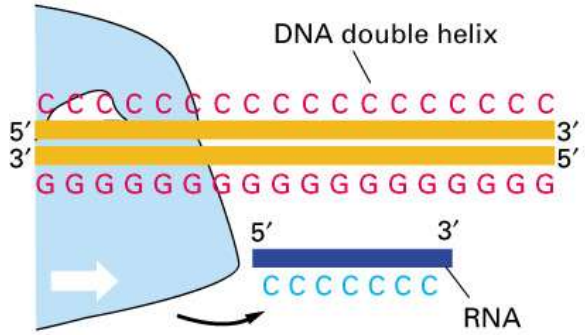
7) Enhancers and Repressors

- A third class of sequence elements can either increase or decrease the rate of transcription initiation of eukaryotic genes
- These elements are called either enhancers or repressors (or silencers), depending on which effect they have.

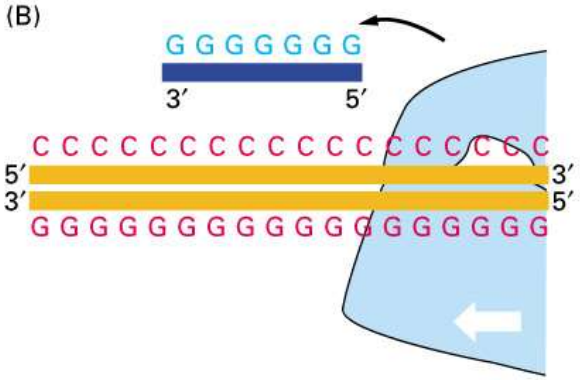
Prokaryotic versus Eukaryotic Transcription

- They have been found in a variety of locations both upstream and downstream of the transcription start site and even within the transcribed portions of some genes.
- In contrast to proximal and upstream promoter elements, enhancers and silencers can exert their effects when located hundreds or even thousands of bases away from transcription units located on the same chromosome.
- **Hormone response elements** (for steroids, T_3, retinoic acid, peptides, etc) act as—or in conjunction with—enhancers or silencers





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

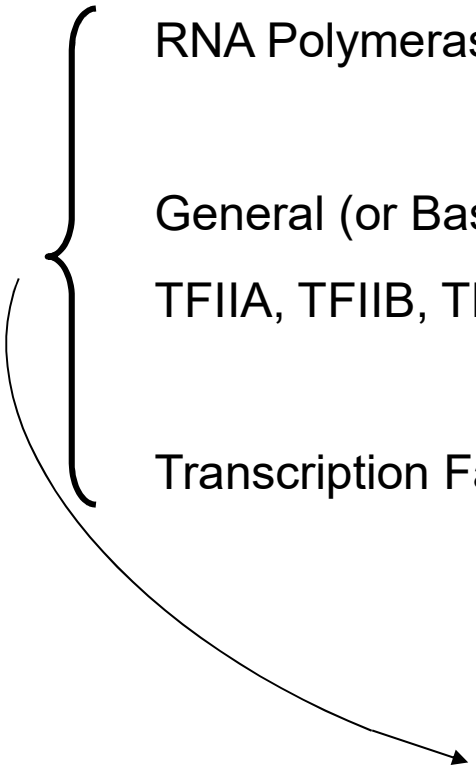
Initiation of the transcription

Initiation of the transcription

- A protein, called a transcription factor, recognizes the TATA box and binds to it. A protein complex is then formed by the addition of other proteins including RNA polymerase II which can then begin its transcription work.

- Initiation of transcription by RNA Pol II requires the recruitment of transcription factors (TFIIA, B, etc.) and then RNA Pol II constituting the transcription initiation complex.

Proteins Involved in Transcription



RNA Polymerase

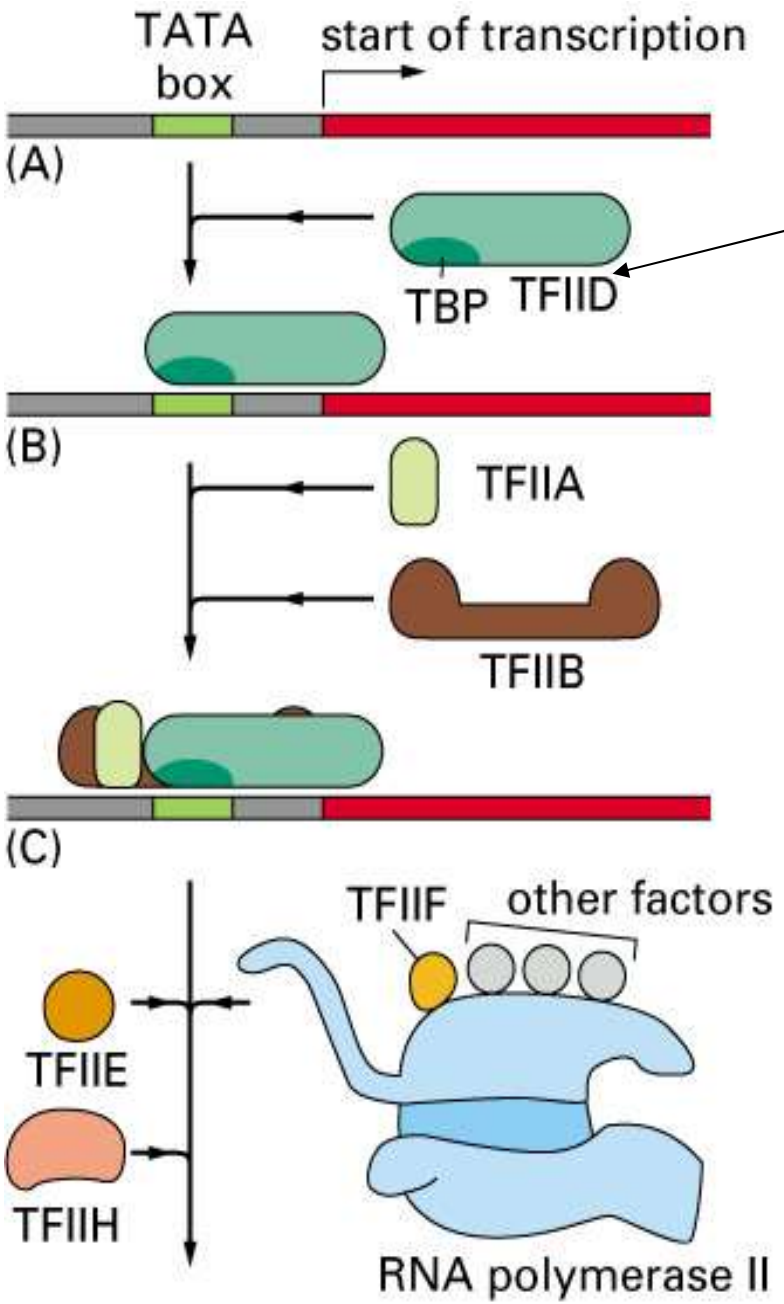
General (or Basal) Transcription Factors:

TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH

Transcription Factors that Bind to Regulatory Elements

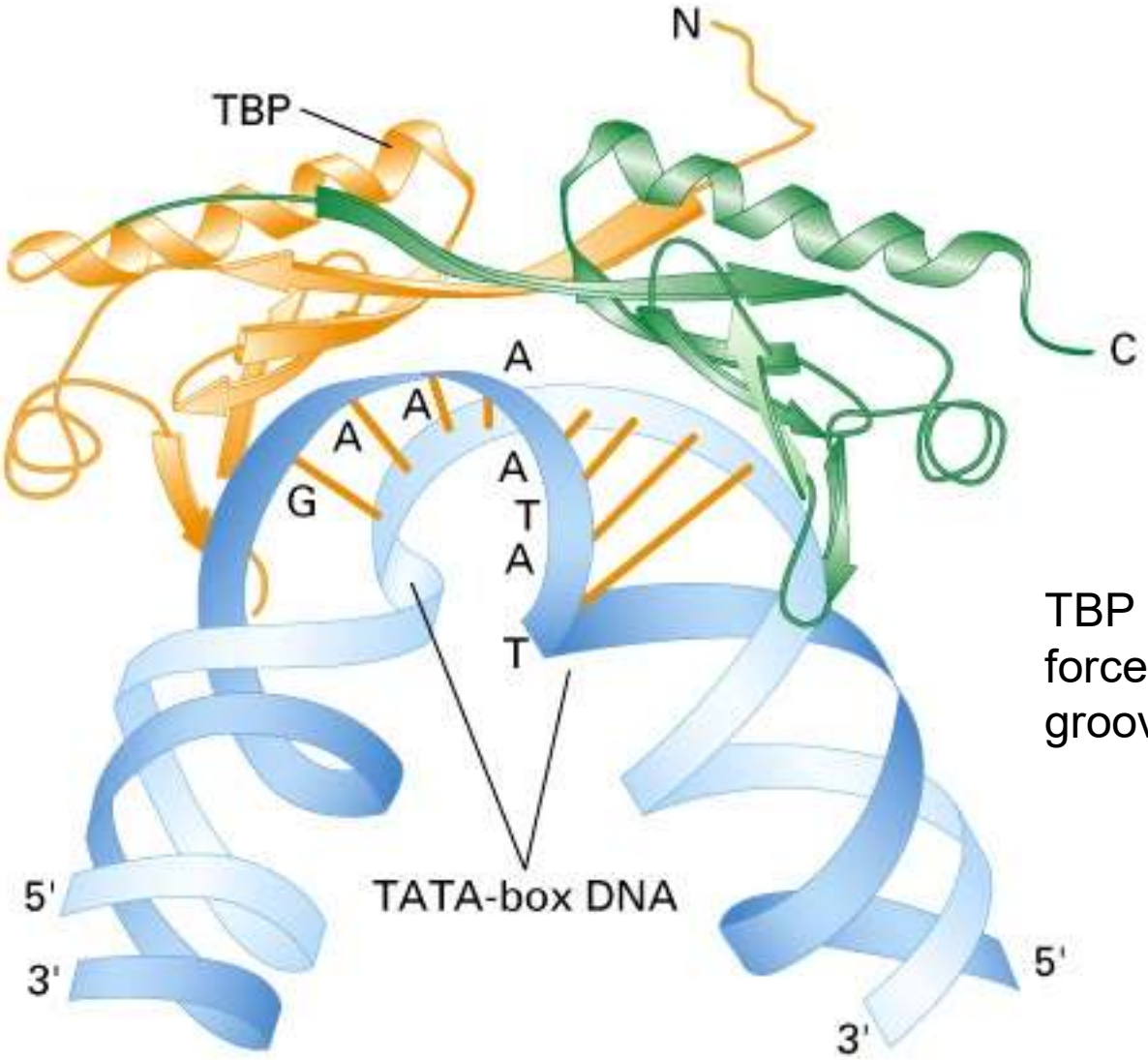
Holoenzyme or

Initiation Complex

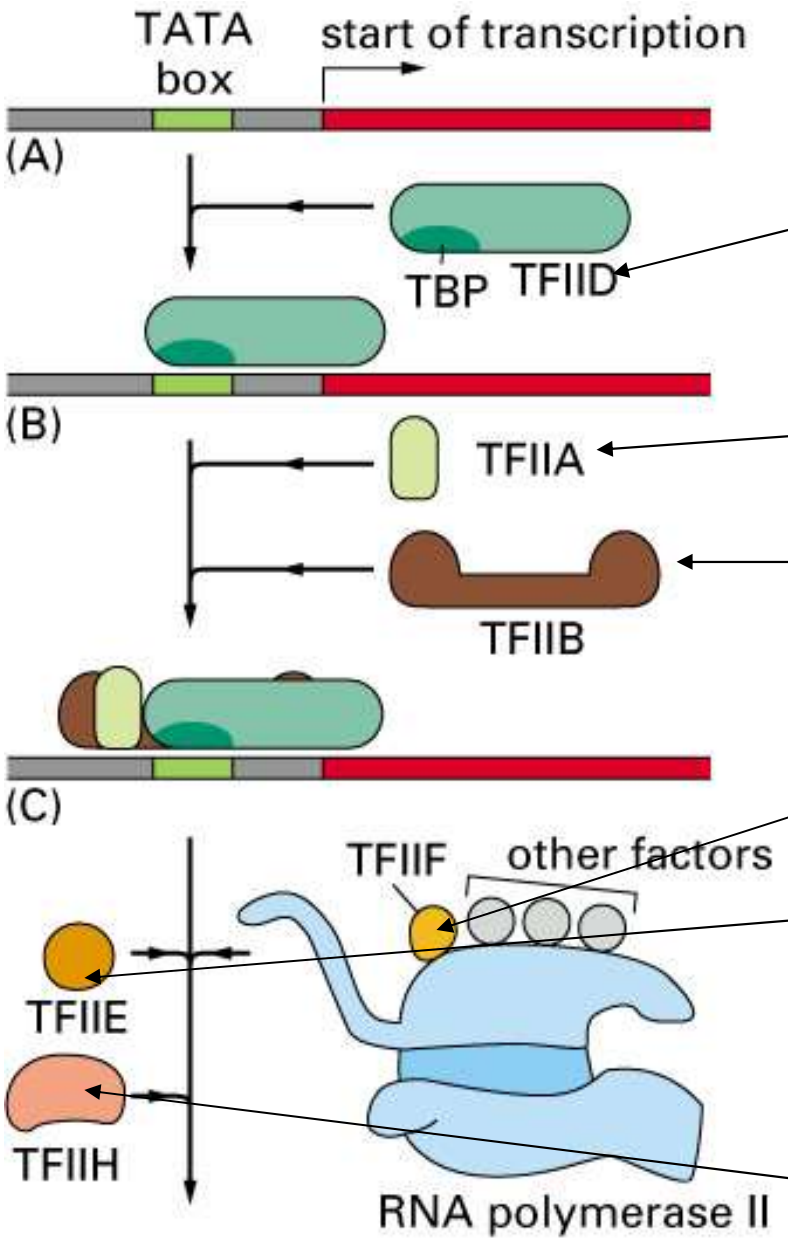


Recognizes and binds to TATA box; TBP + TFIID 10 TBP associated factors; position set

Figure 6–16 part 1 of 2 Molecular Biology of the Cell, 4th Edition



TBP bends DNA ~80^{o} and forces open the minor groove.



Recognizes and binds to TATA box; TBP + 10 TBP associated factors

Binds and stabilizes the TFIID complex

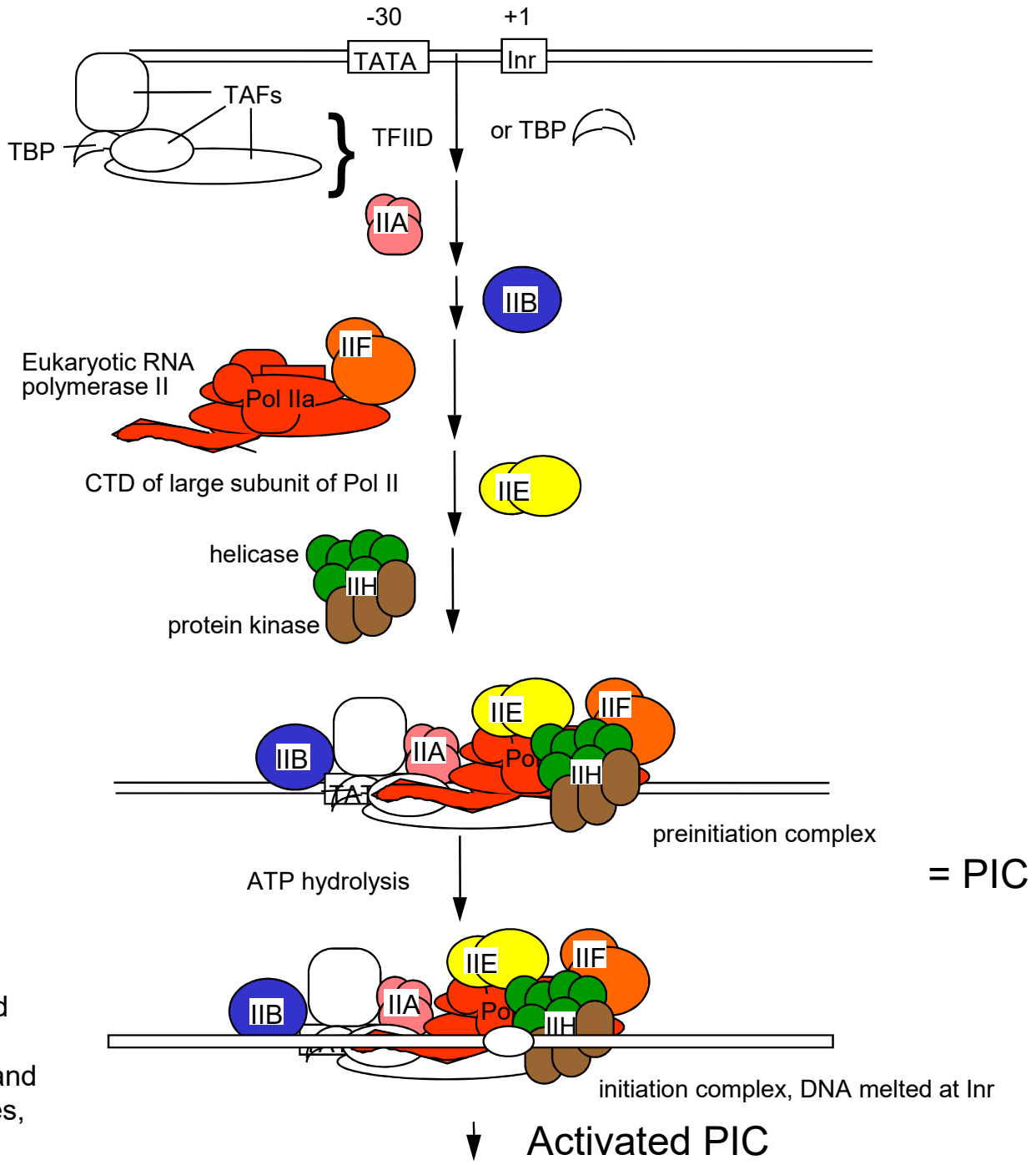
Recruits RNA pol II + TFIIF to the location
-- enzyme activity (DNA-unwinding)

Two subunits - **RAP38** & **RAP74.** Rap74 has a **helicase activity**; RAP38 binds RNAPolII

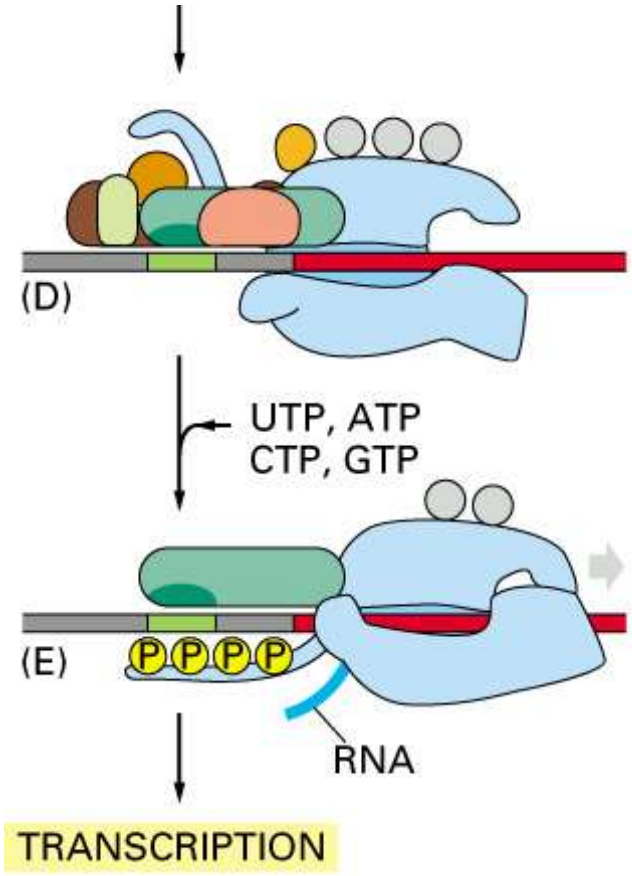
Two subunits - recruits **TFIIH** to the complex thereby priming the initiation complex for promoter clearance and elongation

complex of 9 subunits. One w/ kinase activity; one w/ helicase activity; one is a cyclin (cdk7)

Sequential Binding Model for assembly of preinitiation complex



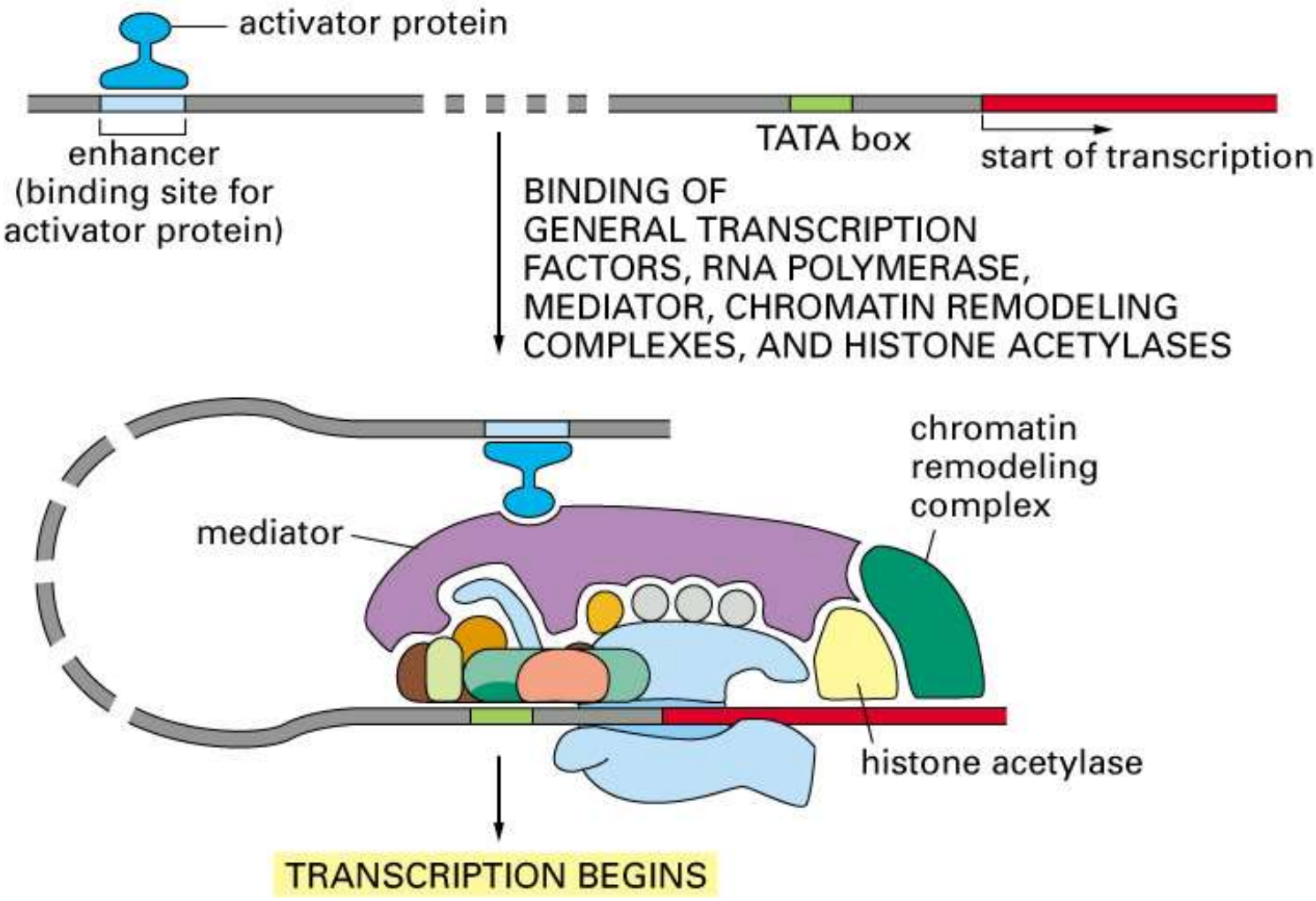
Polymerization of 1st few NTPs and phosphorylation of CTD leads to promoter clearance. TFIIB, TFIIE and TFIIH dissociate, PolII+IIF elongates, and TFIID + TFIIA stays at TATA.



Phosphorylation of the carboxy terminal domain (CTD) of one of the subunits of RNA PolII;

RNA polymerase II dissociates from the transcription factors and other protein complexes that were required for assembly and elongation begins

Phosphorylation also promotes the accumulation of elongation factors – other proteins that arrest transcription long enough to recruiting RNA processing enzymes



Transcription initiation in the cell often requires the local recruitment of chromatin-modifying enzymes, including chromatin remodeling complexes and histone acetylases - greater accessibility to the DNA present in chromatin

Control of transcription

Transcription initiation also requires:

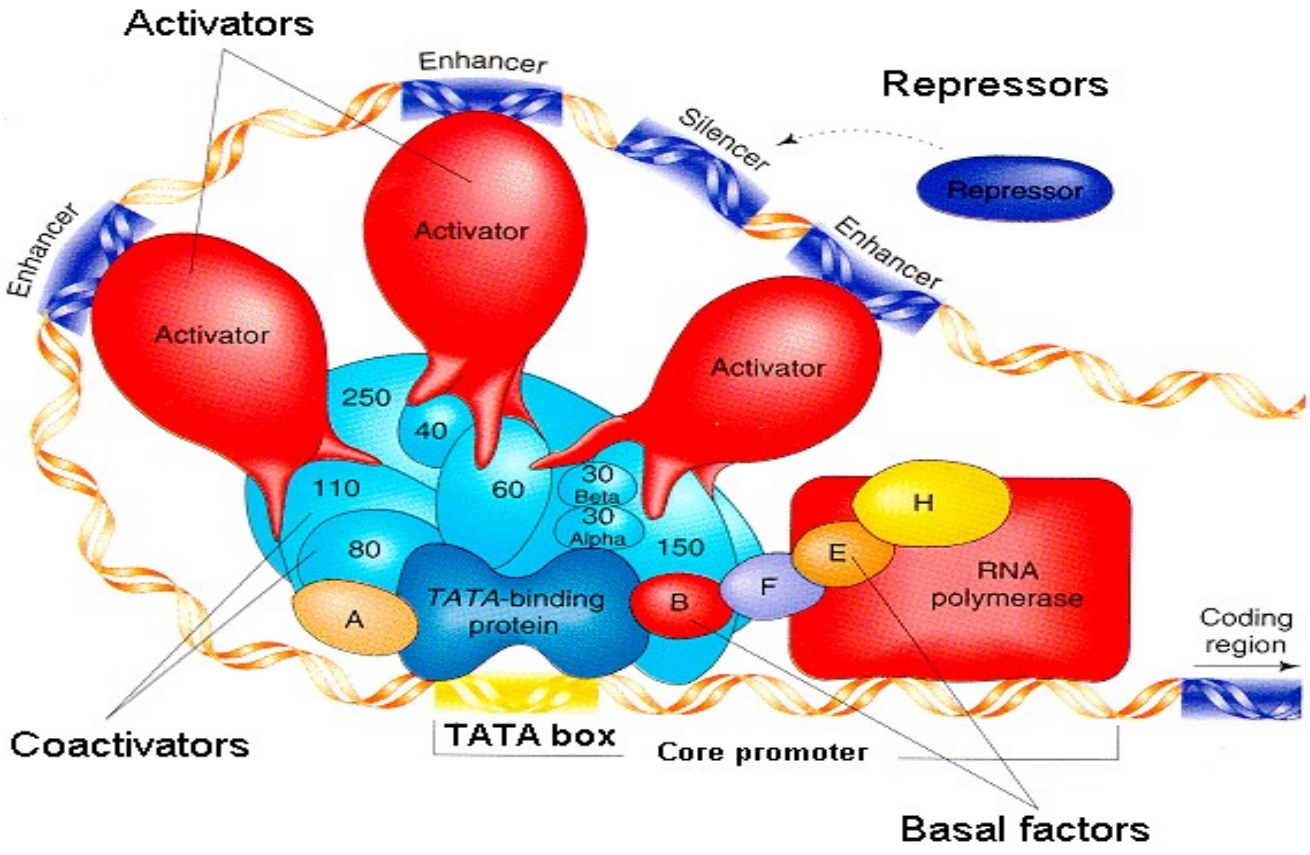
- activators
- mediators (or co-activators),
- chromatin-remodeling proteins

Activators increase the likelihood of successful transcription initiation

Silencers - upon binding with transcription factors (**repressors**), can repress transcription.

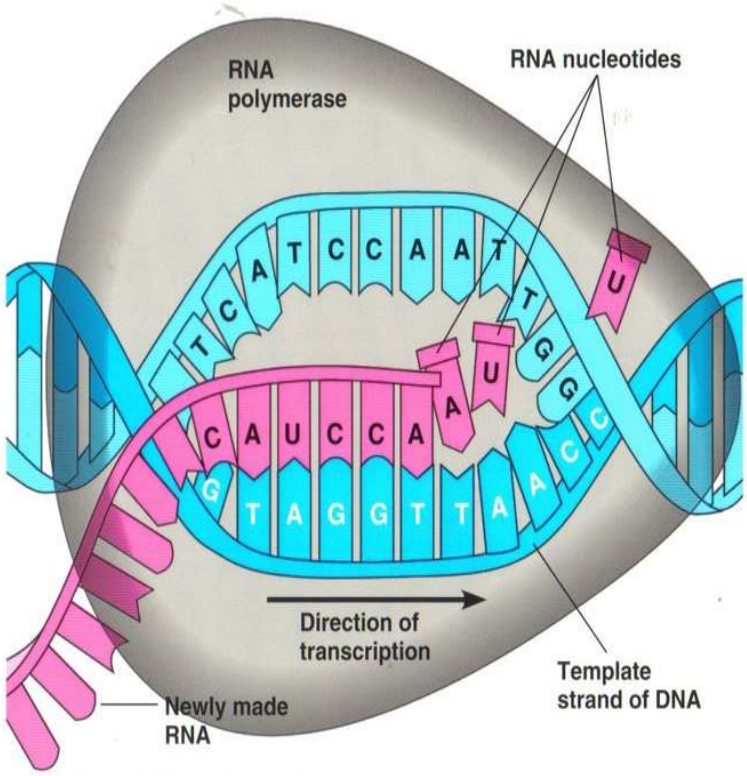
Mediators allow activators to communicate with RNA Pol II

Initiation of transcription



Elongation de la Transcription

- The elongation complex containing basic RNA polymerase progresses along the DNA molecule, and DNA unwinding must occur
- The extent of the transcription eye (unwound DNA) is constant and is approximately 20 base pairs per polymerase.
- RNA polymerase is associated with an activity that opens the DNA helix.
- Topoisomerase precedes and follows the progression of RNA pol to prevent the formation of superturn complexes.
- The incorporation of ribonucleotides is followed according to the base pairing rule



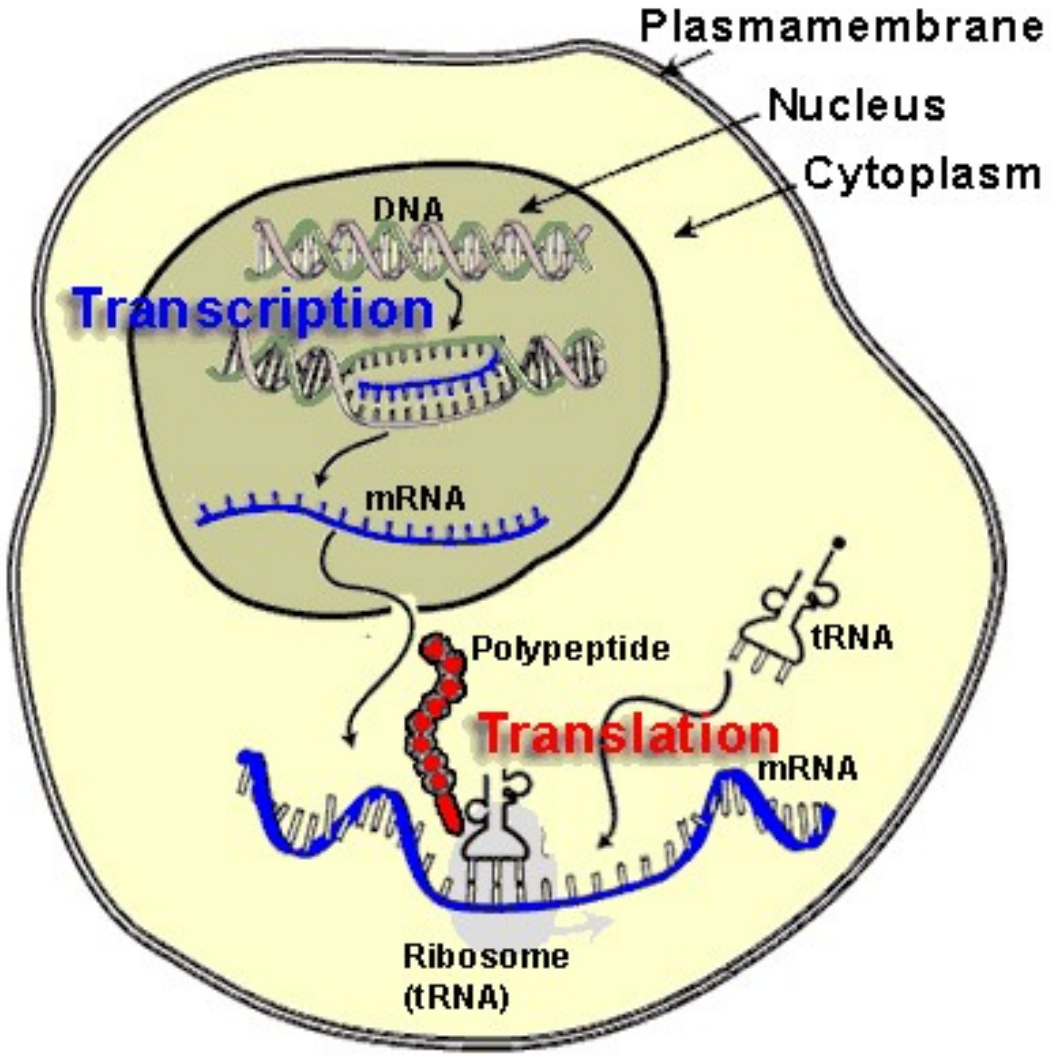
Termination of the transcription

- In eukaryotes, the termination mechanism is not the same as in prokaryotes.

- Termination is ensured by specific signals including the polyadenylation signal AAUAAA. RNA polymerase continues its transcription a little after this motif and is then released under the action of various factors.

- The signals for transcription termination by eukaryotic RNA polymerase II are poorly understood.

RNA Maturation



RNA processing

Newly synthesized transcripts (mRNA) are processed :

- Splice out intervening sequences (=introns) leaving expressed sequences (exons)
  - Introns are removed in spliceosomes (a complex of proteins and snRNA)
  - Cut and paste RNA at specific sites
  - Requires ATP
- “Cap” 5’ end of RNA
- Poly-adenylate 3’ end (Poly A^+ tail)

Post-transcriptional modification and RNA processing

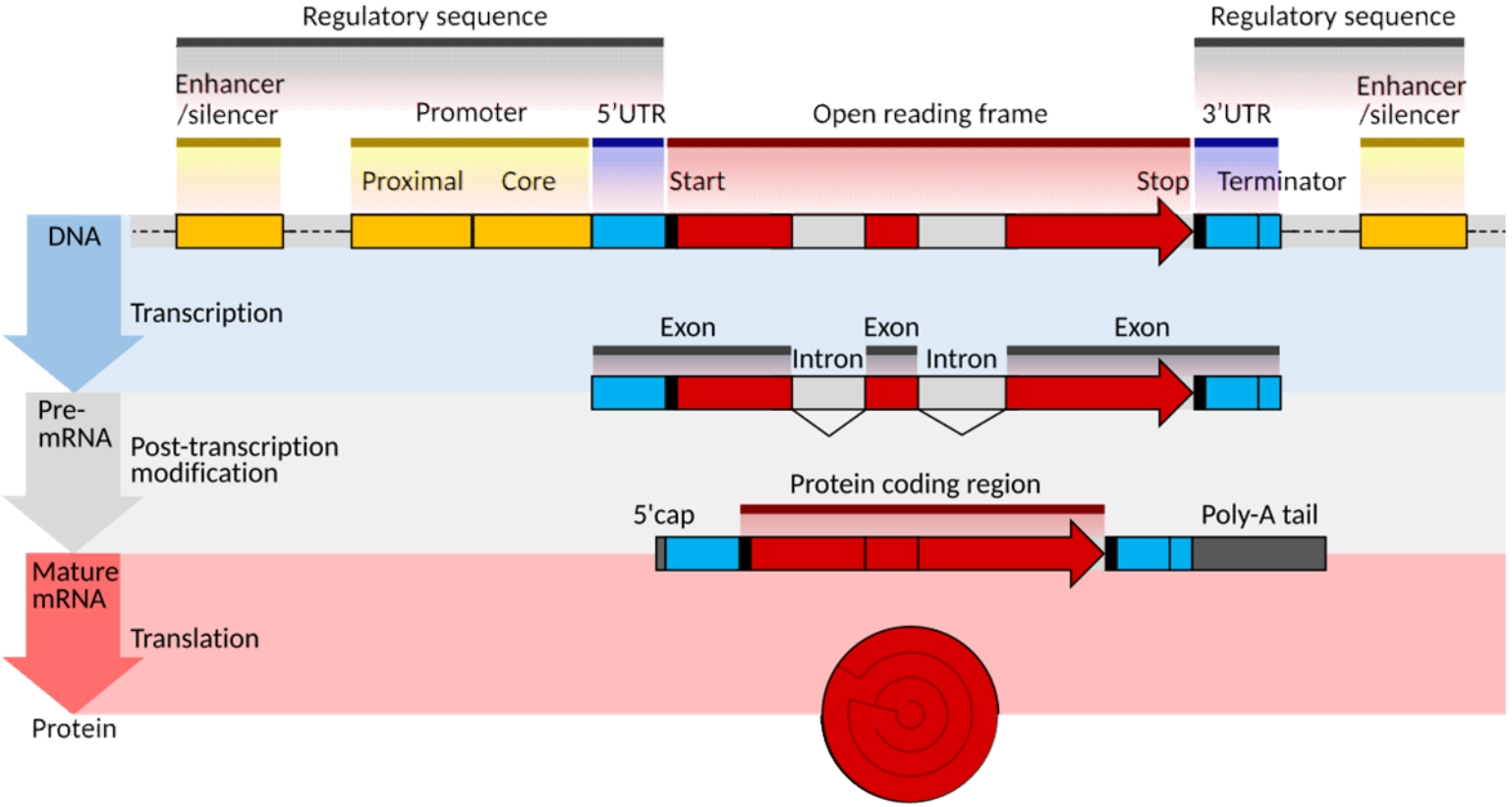
Maturation de l'ARNm

I- 5' and 3' ends processing

5' end processing : " Cap"

3' end processing : poly (A) tail

II- RNA Splicing



Post-transcriptional modification and RNA processing

I- 5’ and 3’ ends processing

5’ end processing : “ Cap”

3’ end processing : poly (A) tail

RNA + n ATP → **RNA-(AMP)n + nPPi**

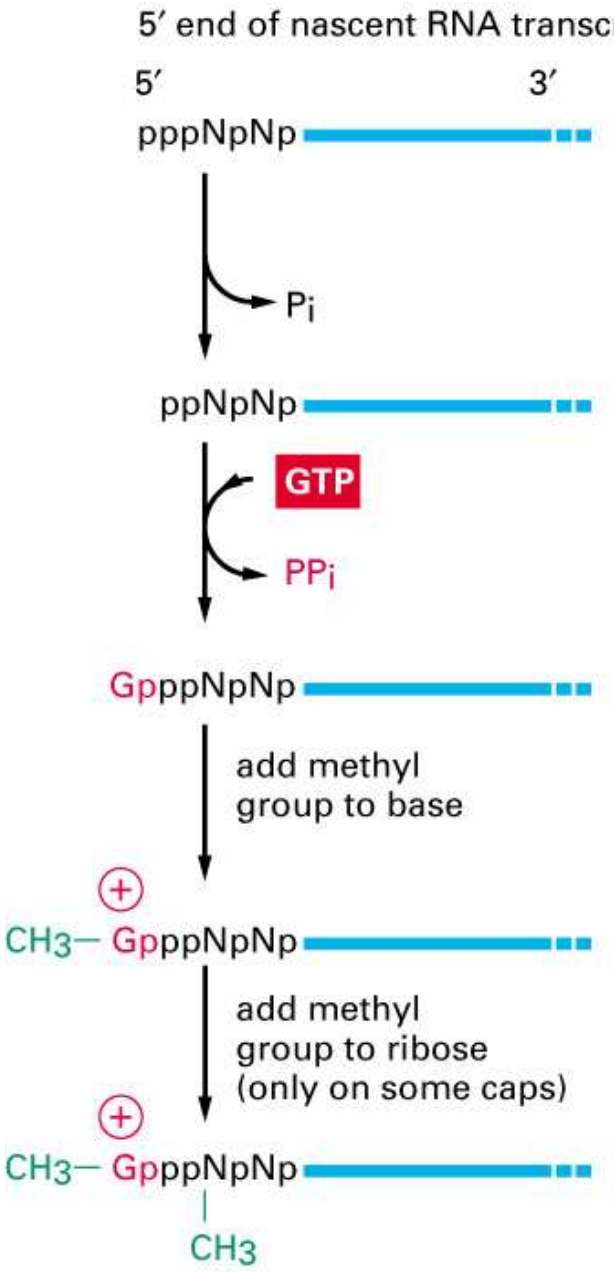
Polyadenylate polymerase

Biological significance:

-binding of mRNA to the ribosome

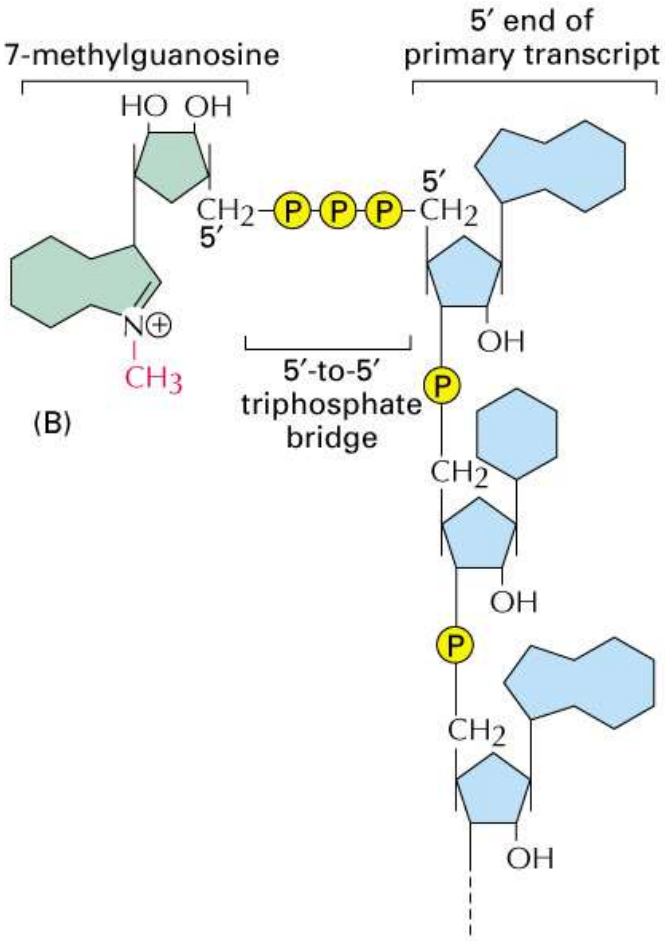
-protection of mRNA

-transport



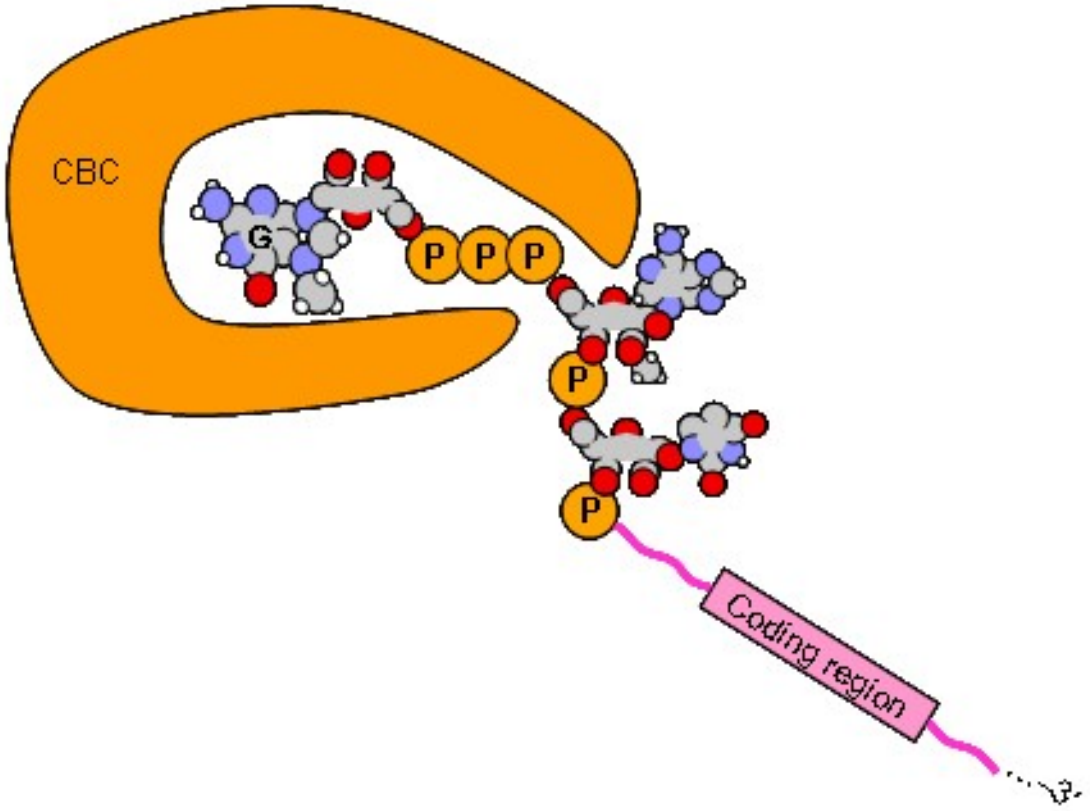
Enzymes Capping de ARN:

- Phosphatase
- Guanyl transferase – adding GMP in 5’ to 5’ liaison
- Methyltransferase



CBC – cap binding complex proteins also associate and protect the cap;

Later they will direct transcript in its exit from the nucleus



Post-transcriptional modification and RNA processing

-3’ end is also processed:

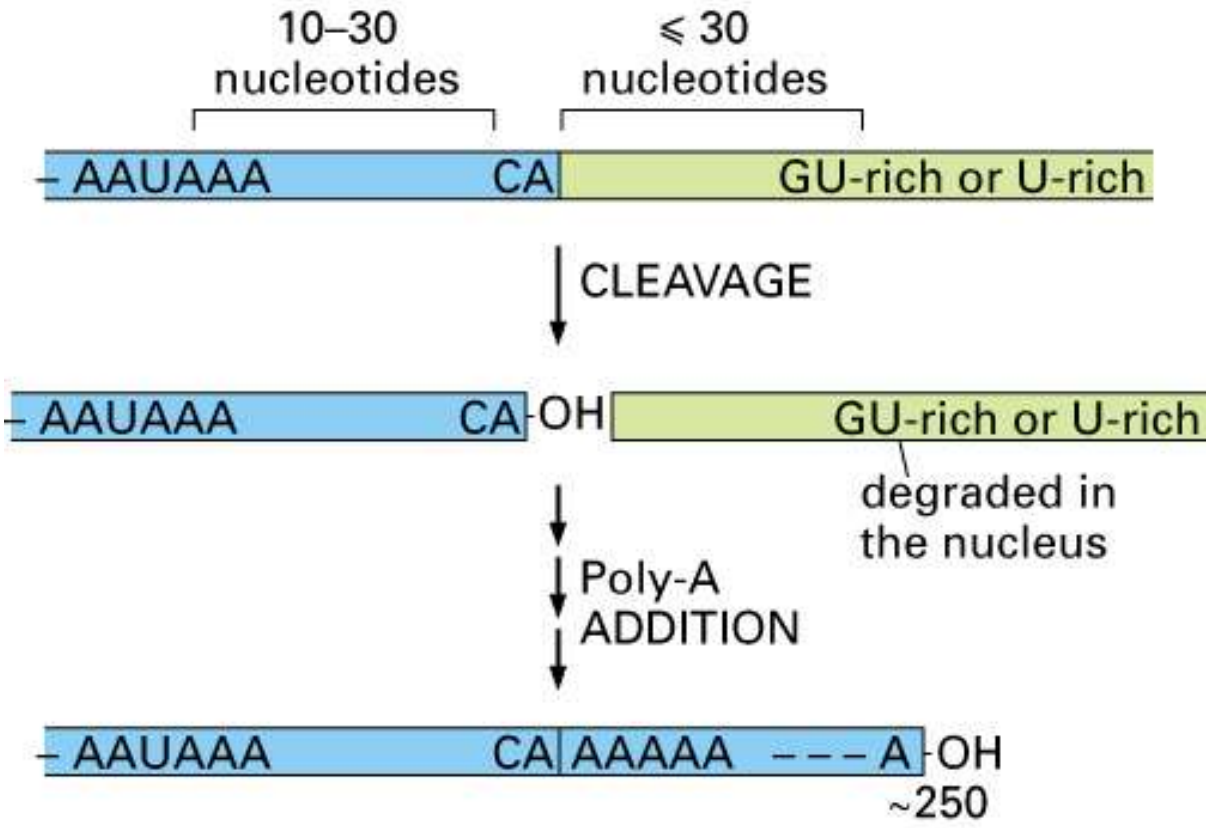
- An enzyme cuts downstream of the polyadenylation site (AAUAAA region)

- Clivage Site (CA) – nearby 10-30 nucleotides in the upstream région AAUAAA

- Poly A (PAP) adds 100 to 200 ATPs

- Poly A tail length influences half-life (degradation rate)

III- Modifications post-transcriptionnelles et régulation de la transcription



CPSF = **C**leavage and **P**olyadenylation **S**pecificity **F**actor

CstF = **C**leavage **St**imulation **F**actor

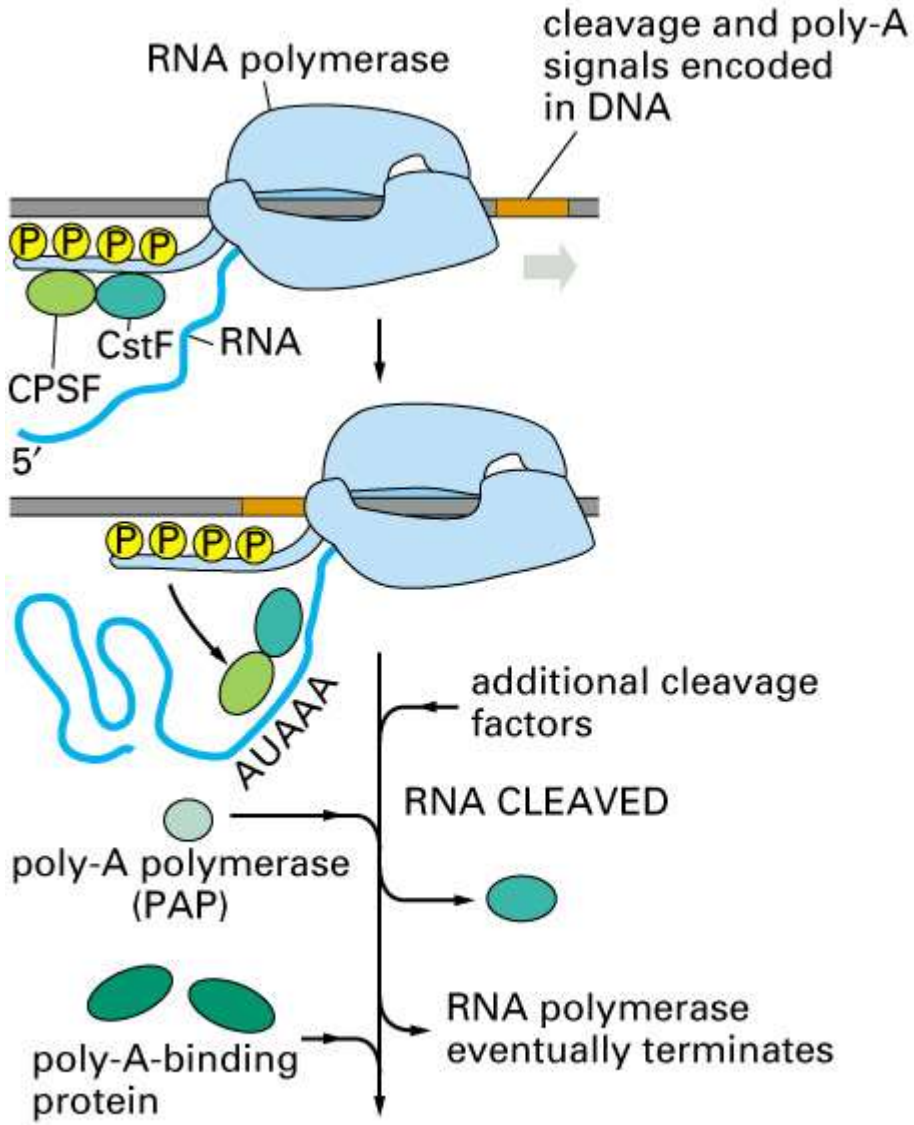


Figure 6–38 part 1 of 2. Molecular Biology of the Cell, 4th Edi

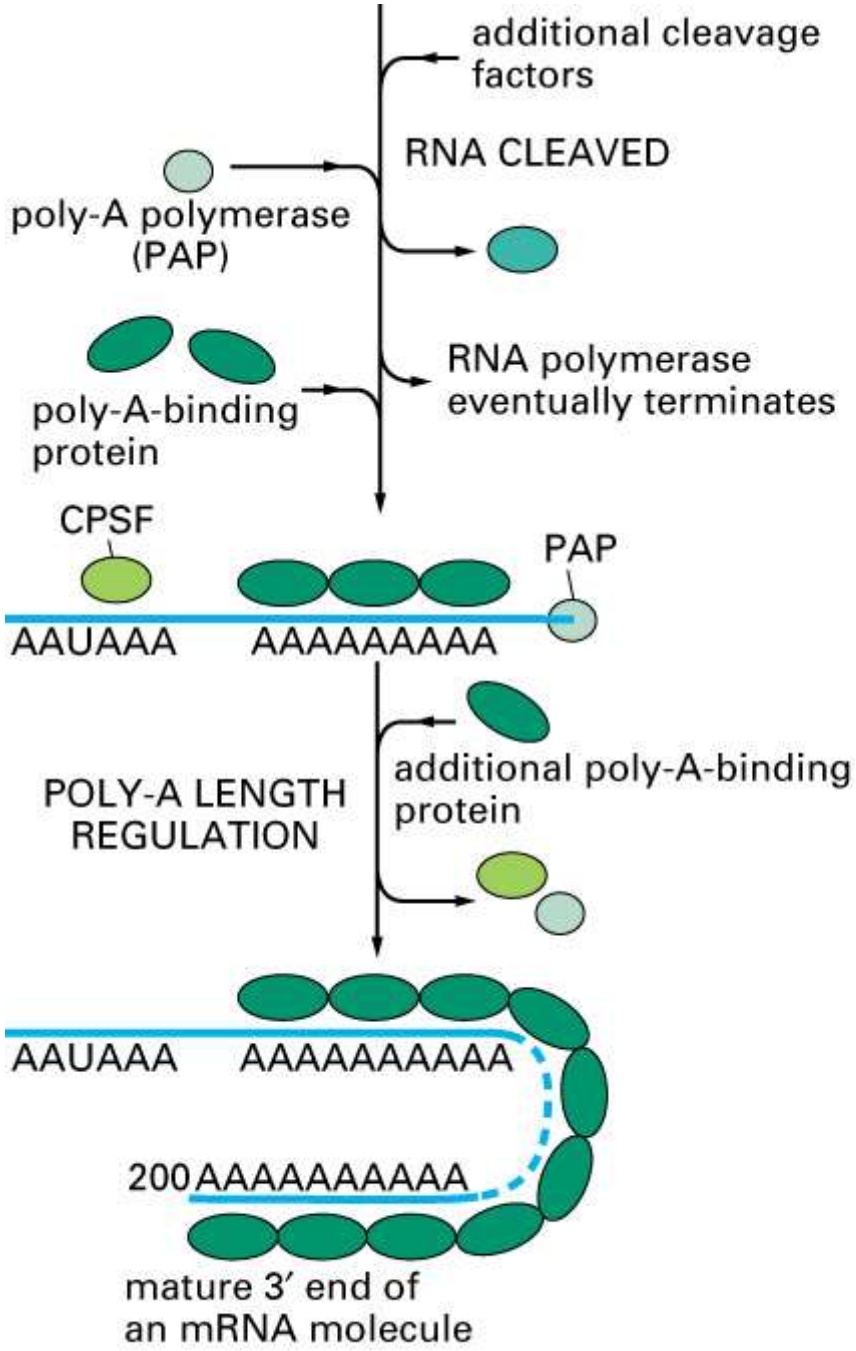


Figure 6–38 part 2 of 2. Molecular Biology of the Cell, 4

2. RNA Splicing

Exons and Introns

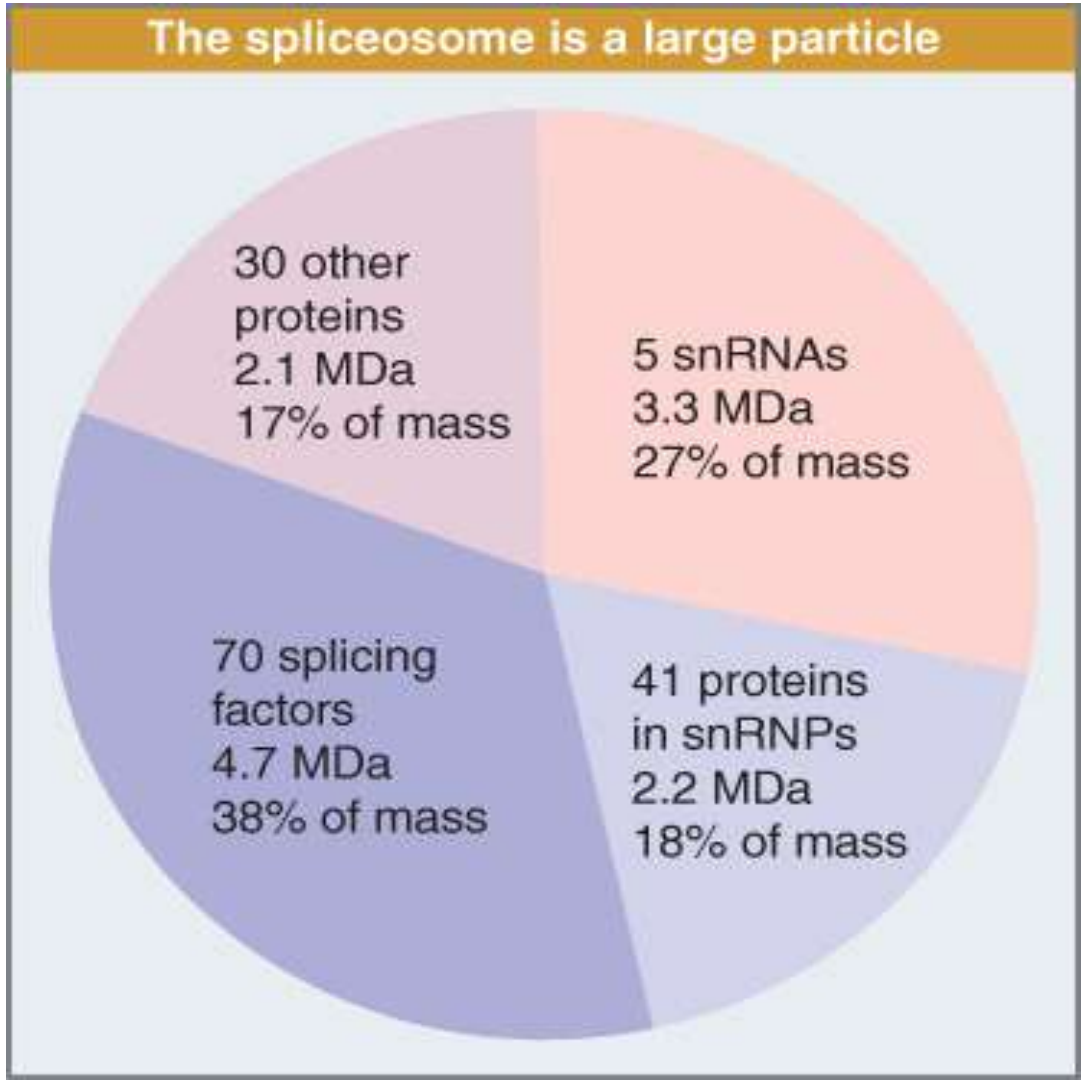
- Most eukaryotic genes contain noncoding sequences called introns that interrupt the coding sequences of exons.

- Introns are excised from RNA before transport to the cytoplasm.

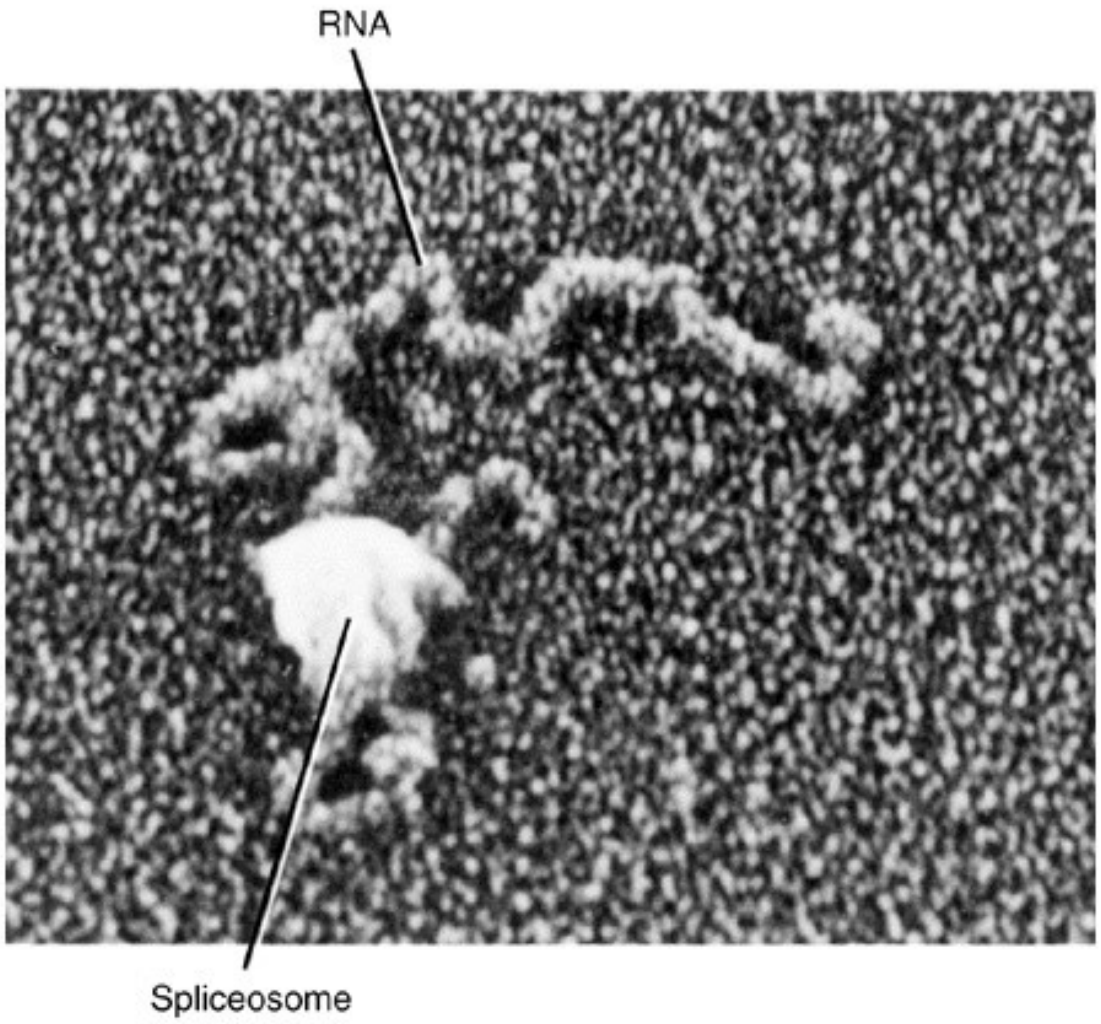
- Pre-mRNA introns are excised by complex ribonucleoprotein structures called spliceosomes.

Spliceosomes:
snRNA plus ~40 proteins

Pre-mRNA introns are excised by complex ribonucleoprotein structures called **spliceosomes**.



Spliceosome



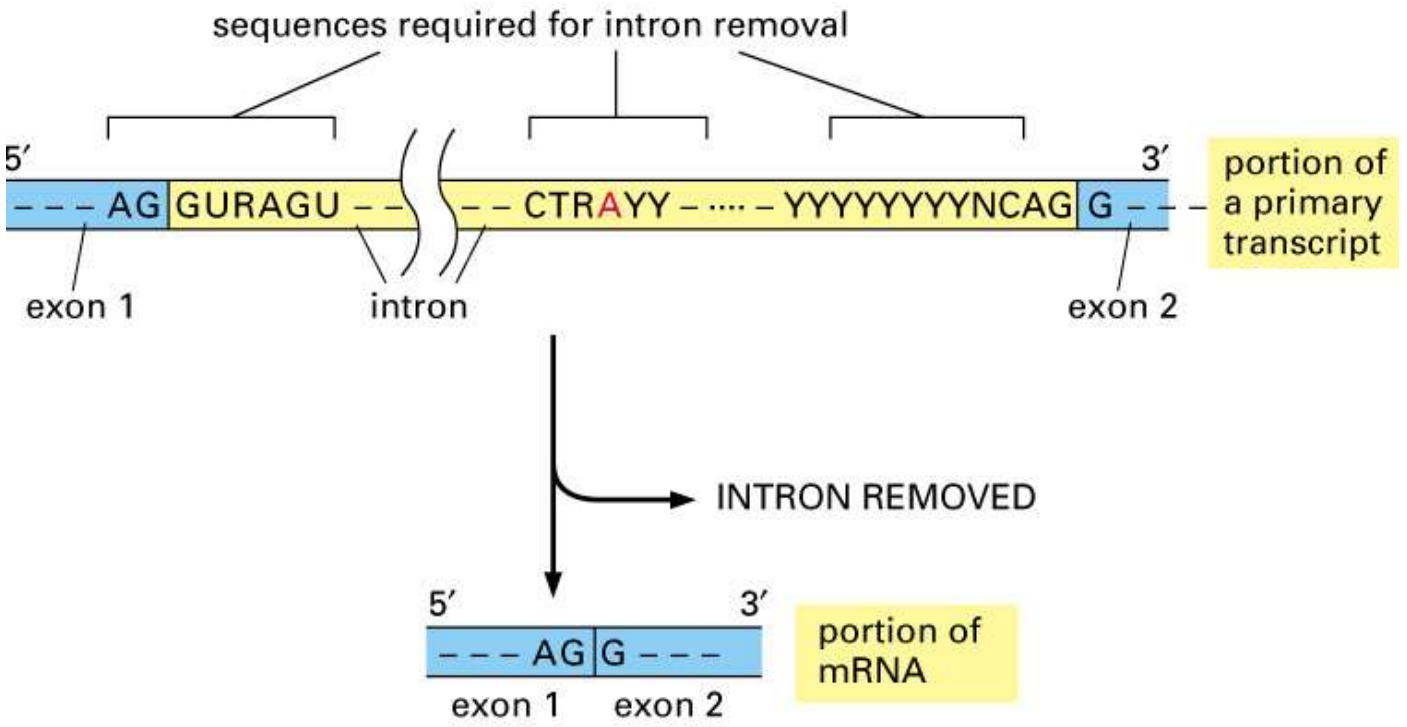
How Introns Are Identified:

Consensus sequences at (5’ to 3’ direction)

•5’ splice site

•Lariate loop closure site of the intron sequence

•3’ splice site



R=A or G,Y=C or U

The Spliceosome Forms

snRNAs (U1, U2, U4, U5 and U6) and associated proteins = snRNPs

- U1 binds to the GU sequence at the 5' splice site, along with accessory proteins/enzymes,
- U2 binds to the branch site, and ATP is hydrolyzed;
- U5/U4/U6 trimer binds, and the U5 binds exons at the 5' site, with U6 binding to U2;
- U1 is released, U5 shifts from exon to intron and the U6 binds at the 5' splice site;
- U4 is released, U6/U2 catalyzes transesterification, U5 binds exon at 3' splice site, and the 5' site is cleaved, resulting in the formation of the lariat;
- U2/U5/U6 remain bound to the lariat, and the 3' site is cleaved and exons are ligated using ATP hydrolysis. The spliced RNA is released and the lariat debranches.

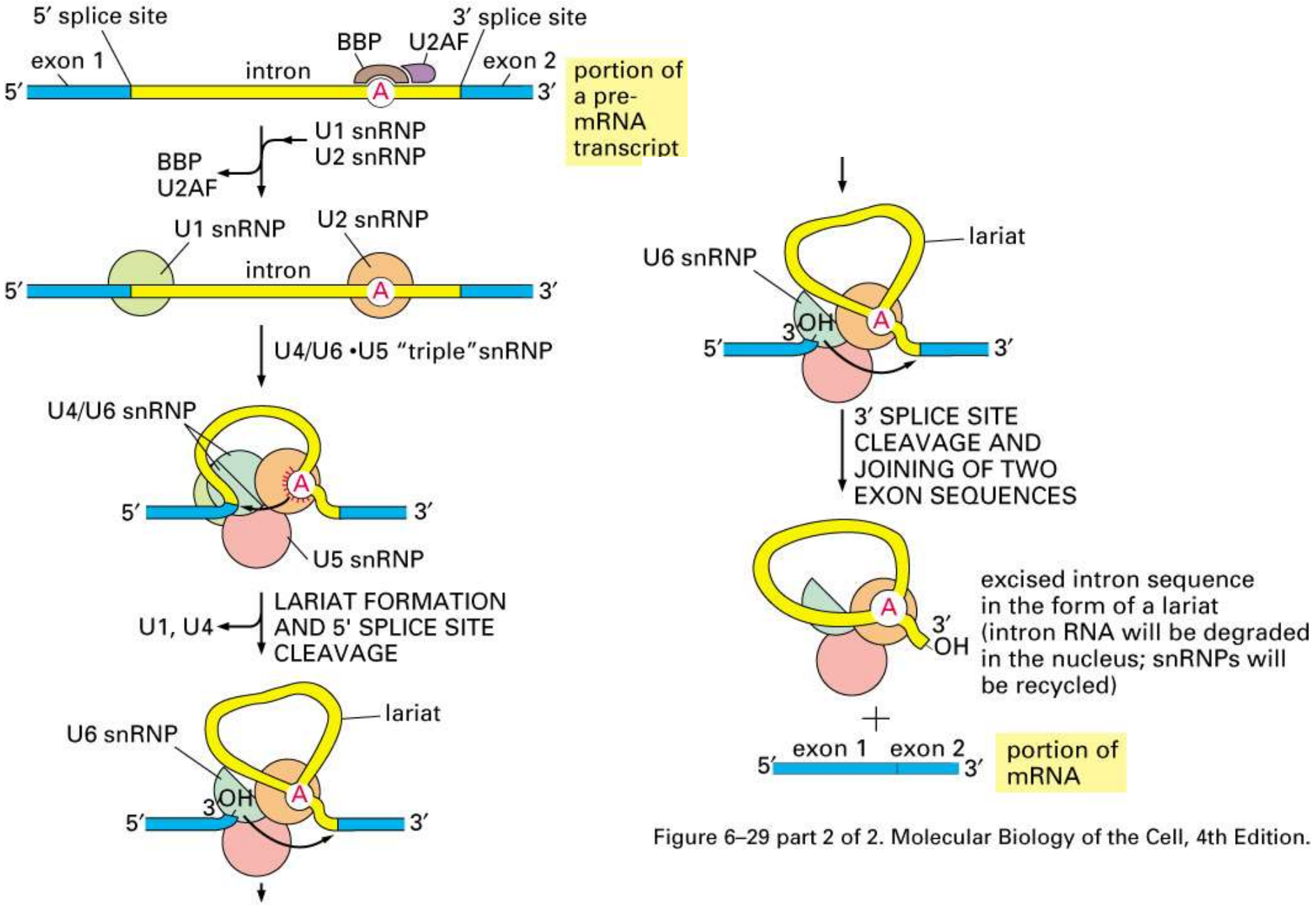
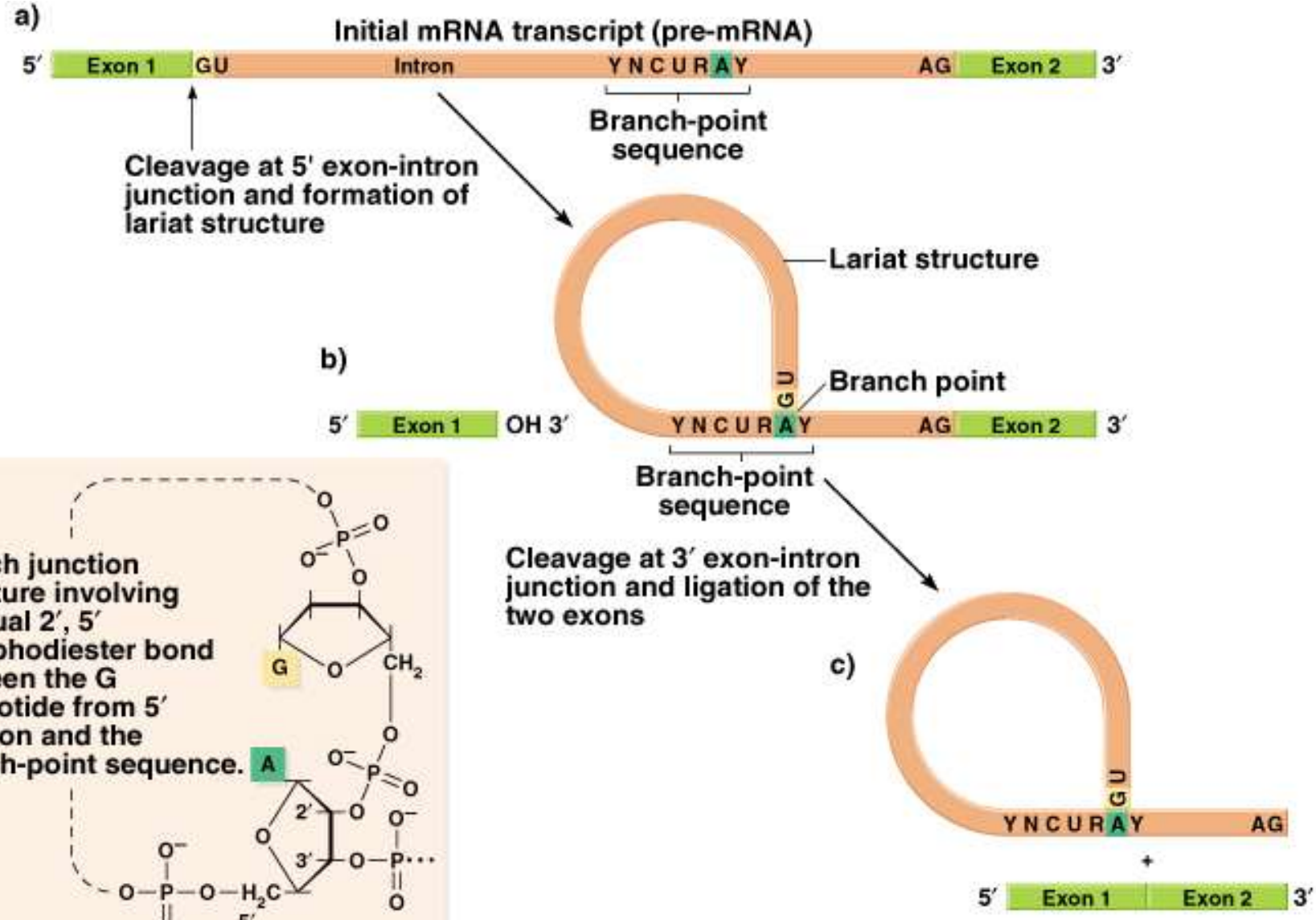


Figure 6–29 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

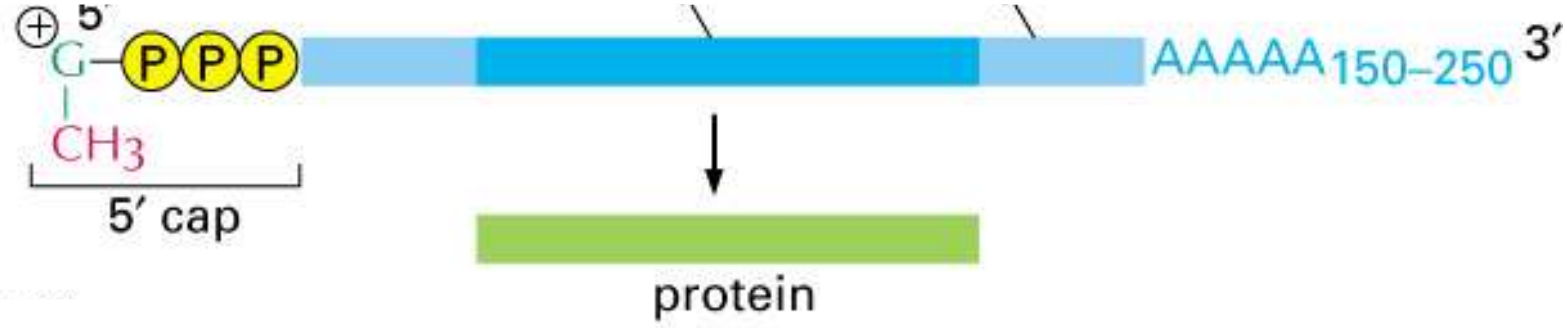
Figure 6–29 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

2. Excision-Epissage



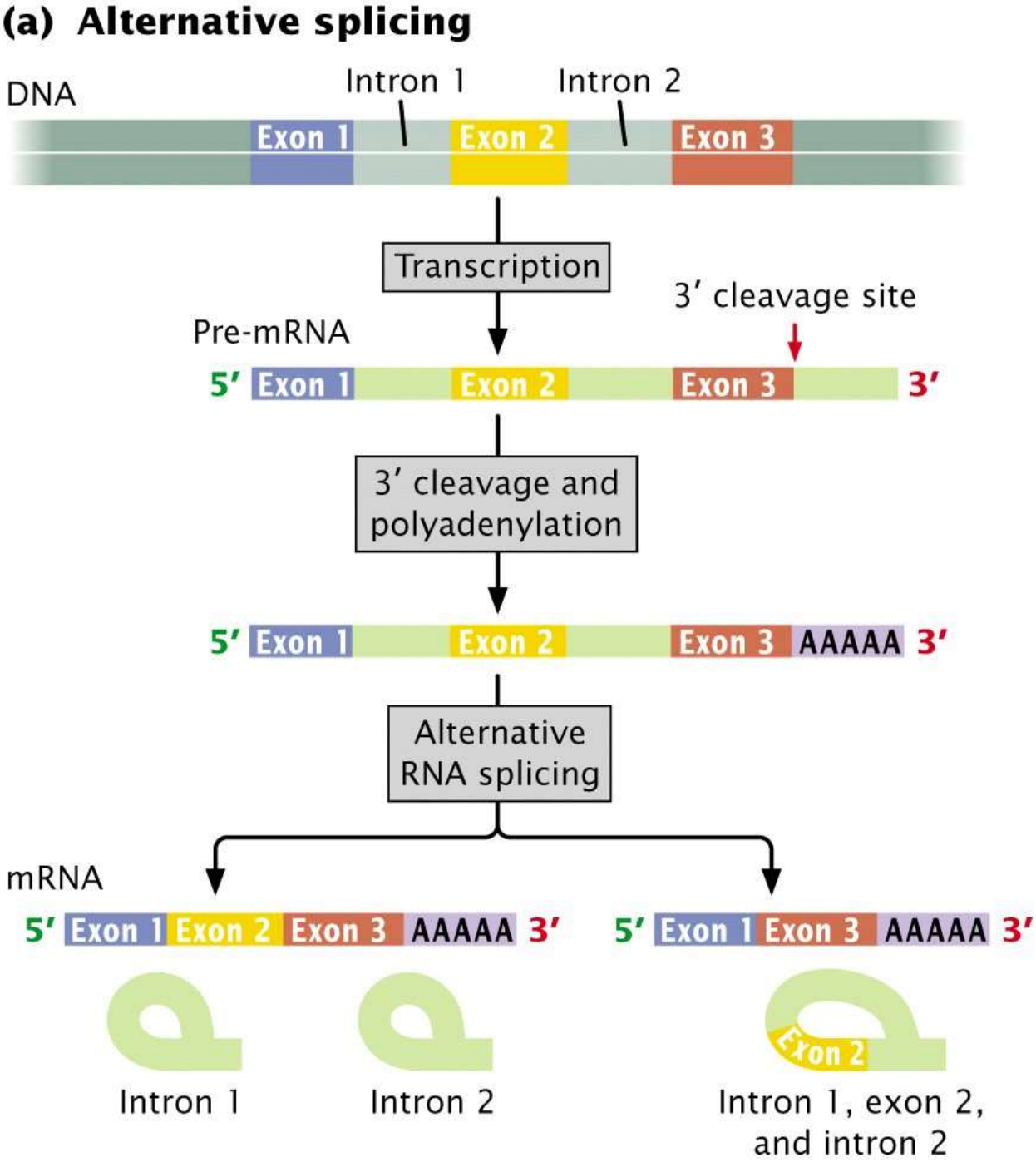
L'épissage implique la transestérification

III- Modifications post-transcriptionnelles et régulation de la transcription



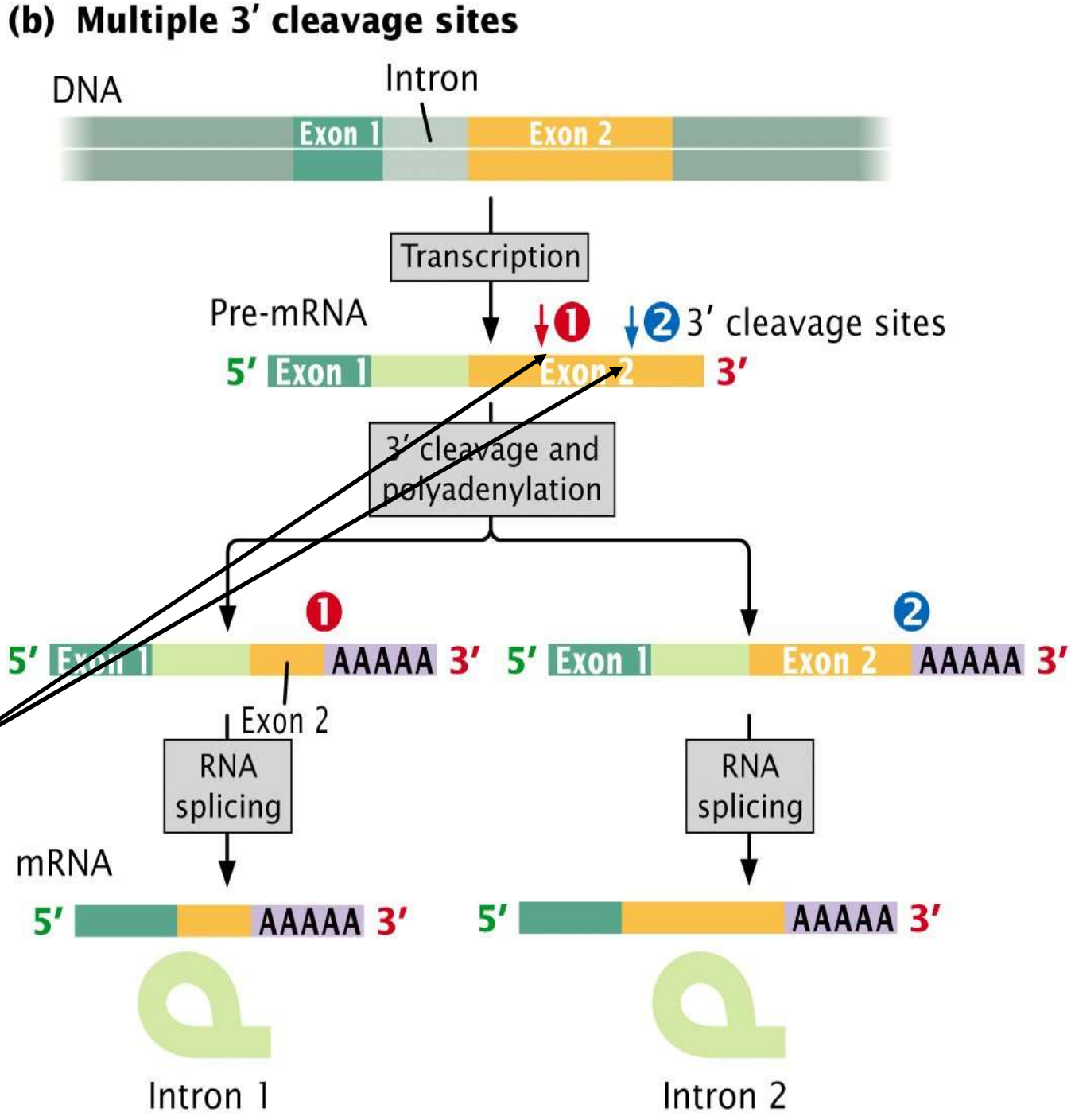
Alternative Splicing

- Exon removed with intron



Alternative Splicing

- Multiple 3’ cleavage sites
- EX. AG found at 5’ end of exon 2 and inside exon 2



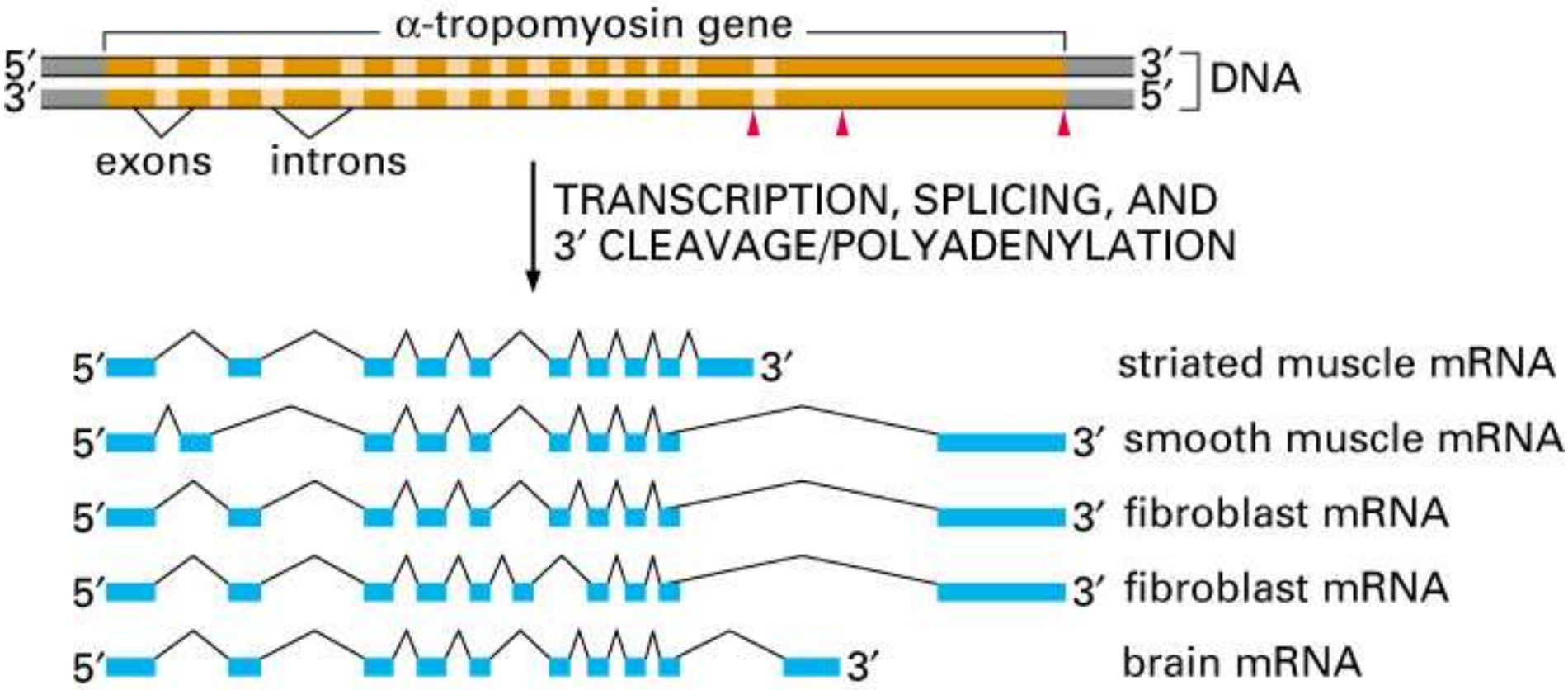


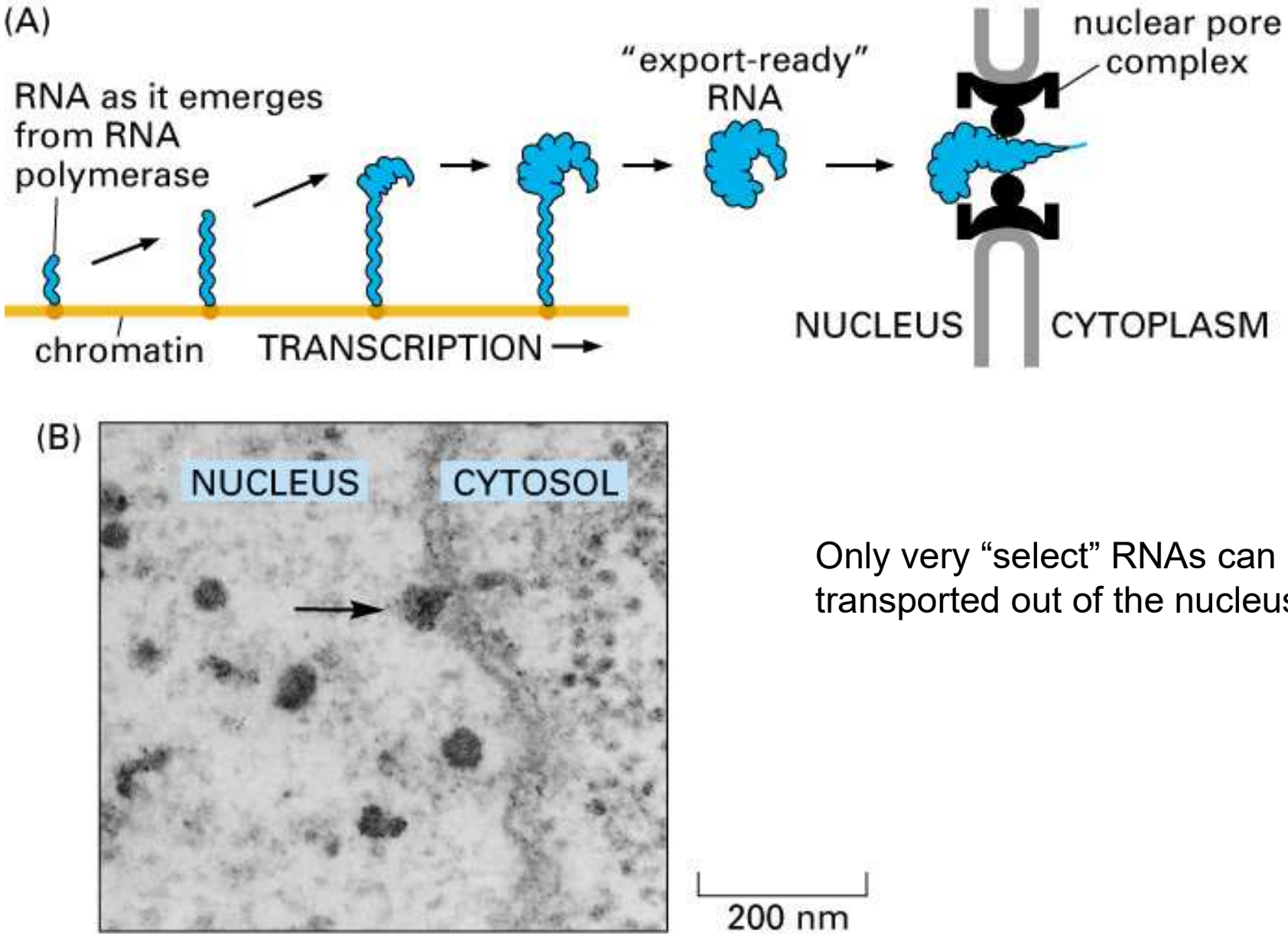
Figure 6–27. Molecular Biology of the Cell, 4th Edition.

Transcript export

Proteins associated with mRNA mark it for export

Only mature mRNA is exported from nucleus

Exit via *nuclear pore complexes*



Only very "select" RNAs can be transported out of the nucleus

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

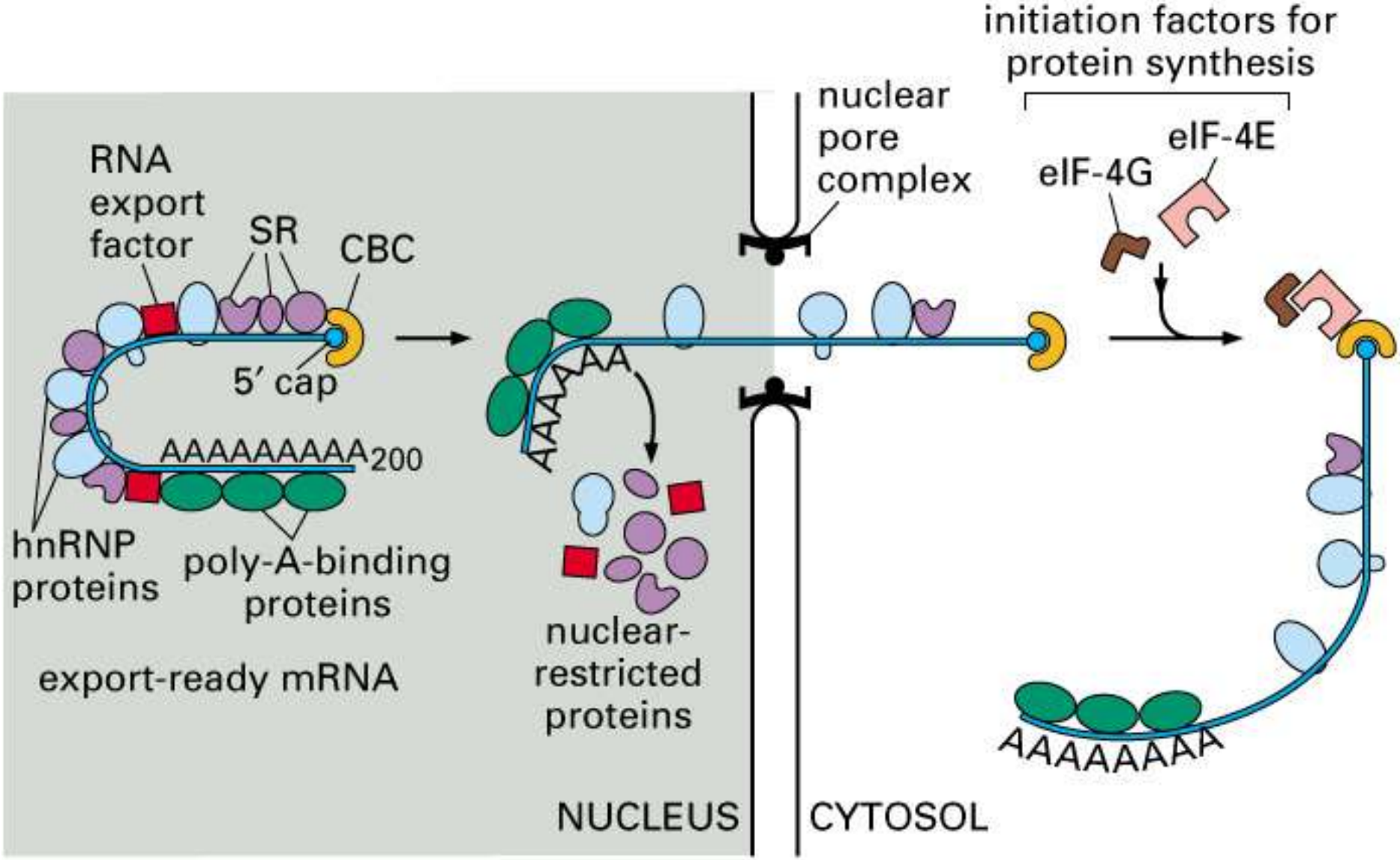


Figure 6–40 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

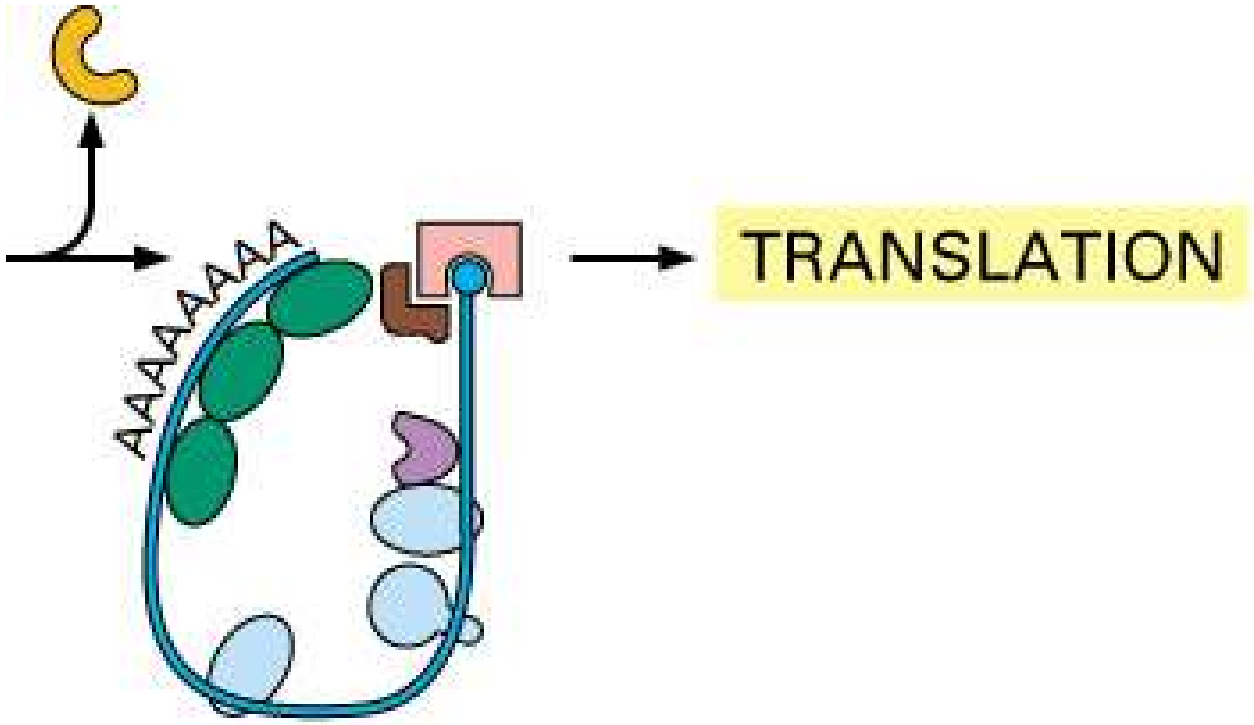
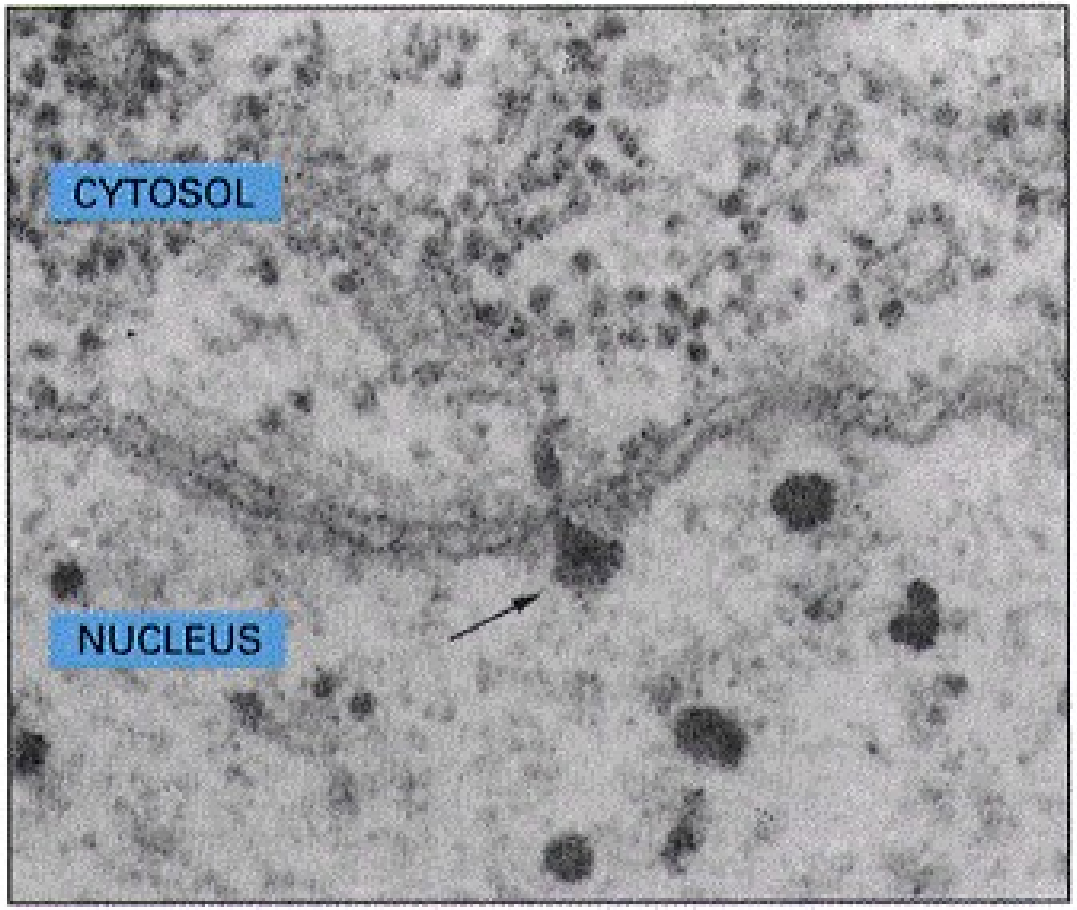
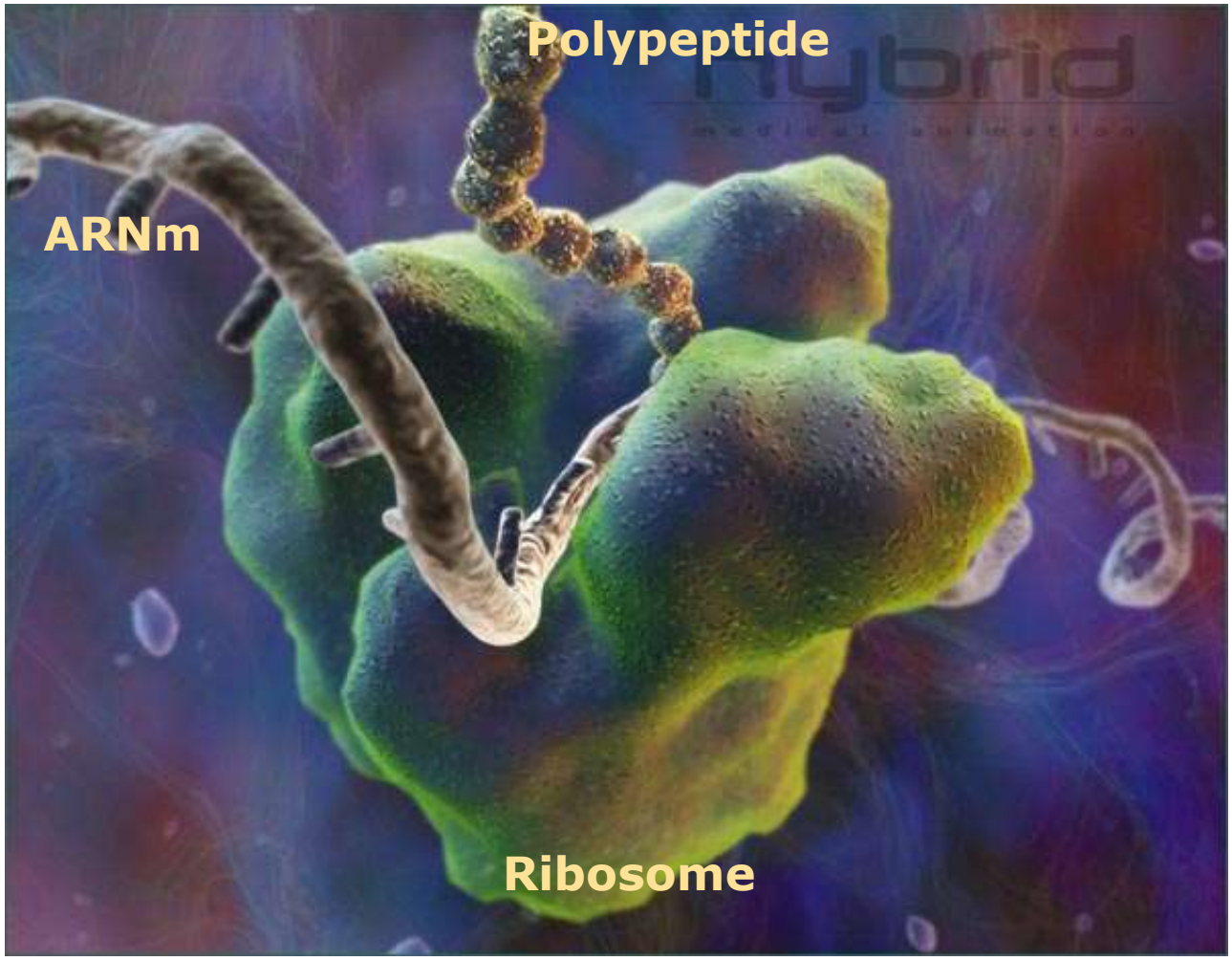


Figure 6–40 part 2 of 2. Molecular Biology of the Cell, 4th Edition.



(B)

II. TRANSLATION



TRANSLATION

- This is the mechanism by which information will pass from the nucleic acid form (4-letter alphabet) to the protein form (20-letter alphabet) according to a universal or almost universal code.
- Translation involves matching the nucleotide sequence of the mRNA to the amino acid sequence of the protein.

Genetic code

- The genetic code is the system of correspondence between the nucleotide sequences of RNA and the amino acid sequences of the manufactured protein.

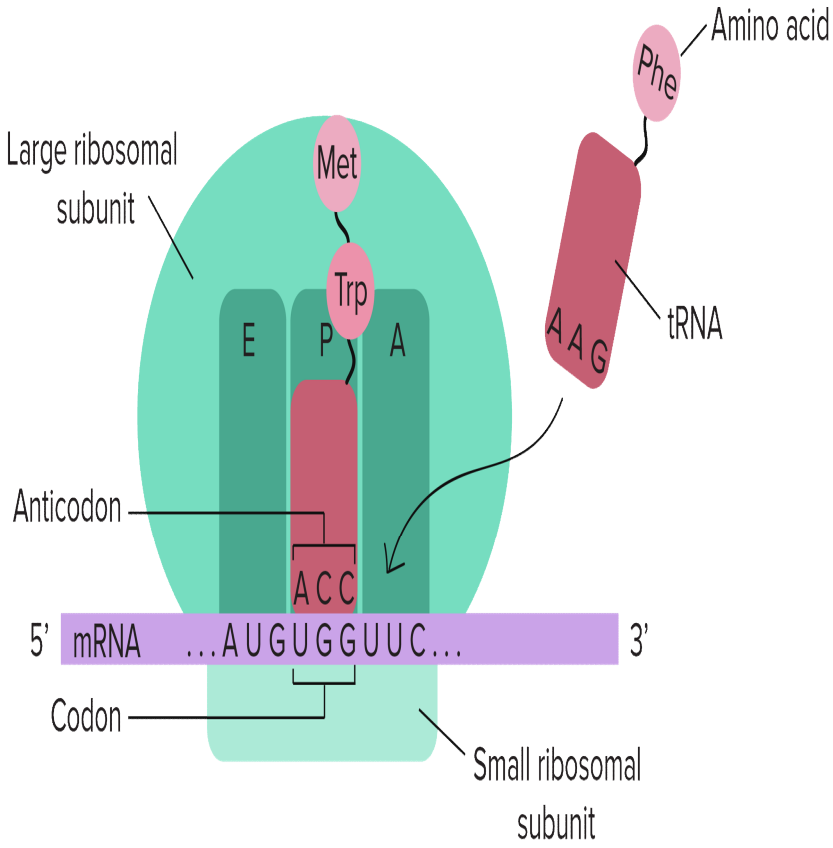
Genetic code

Each triplet of nucleotides on DNA corresponds to a codon in mRNA.

Each codon of the mRNA corresponds to a specific anti-codon of the tRNA.

Each anticodon corresponds to a specific amino acid.

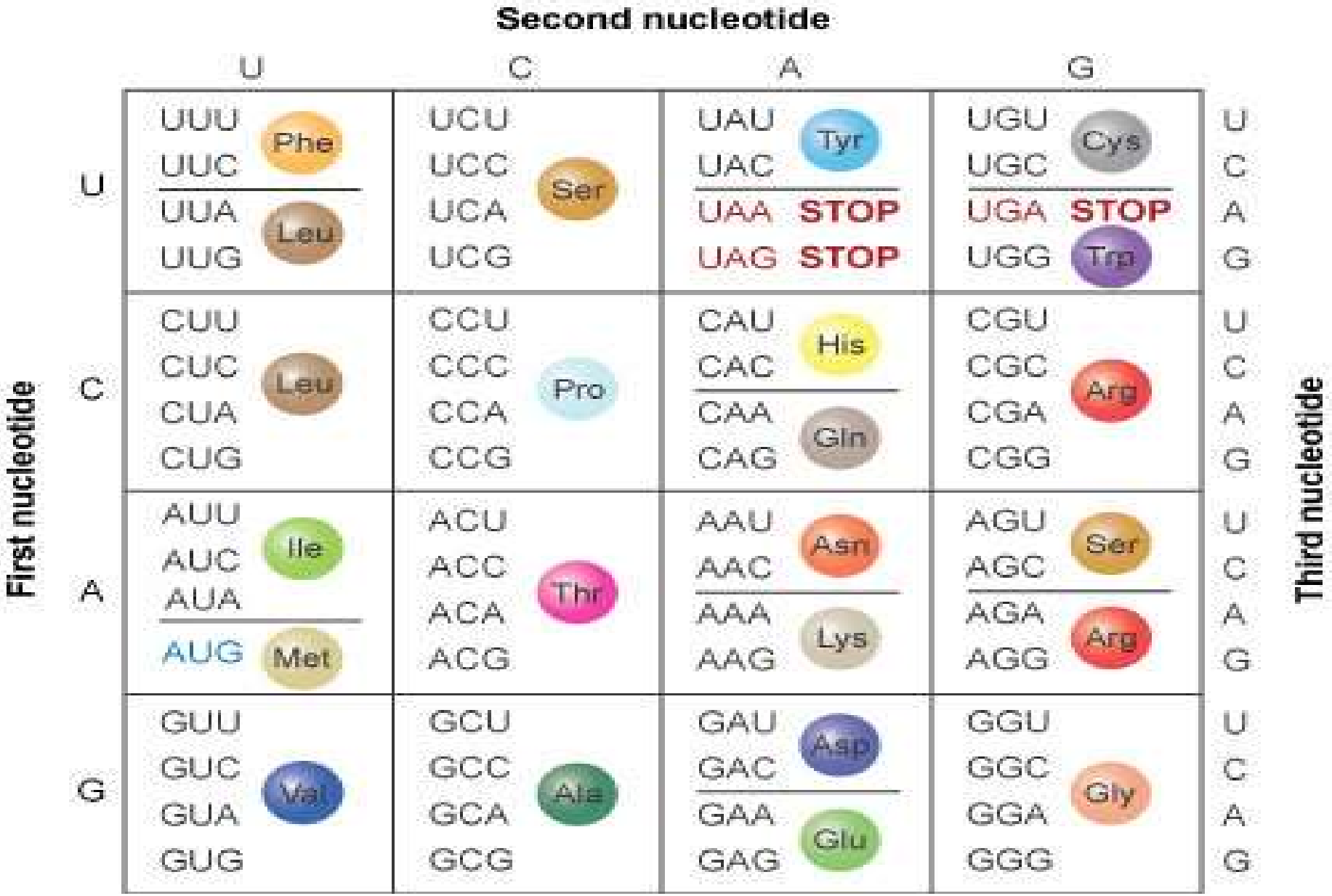
SO: each triplet of nucleotides on DNA corresponds to an amino acid.



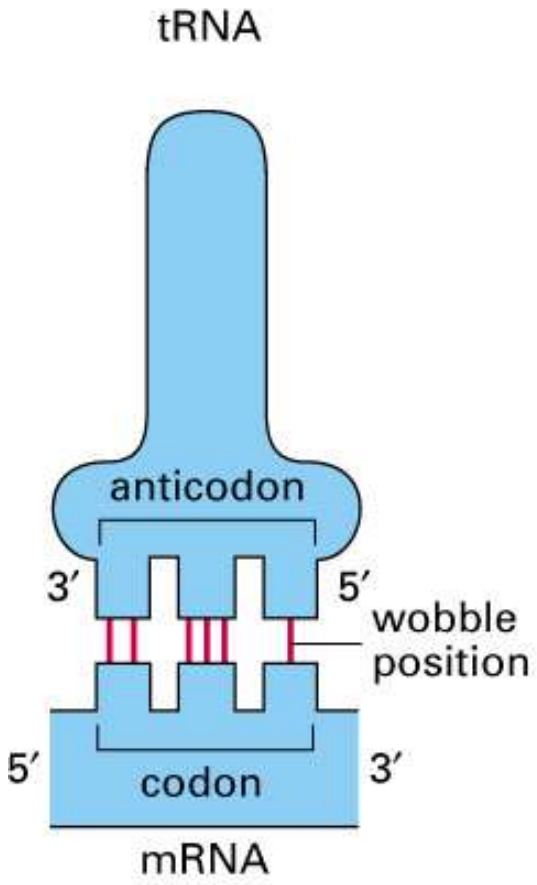
Genetic code

- Genetic code has four characteristics :
  - The genetic code is degenerate: most amino acids are defined by one or more than one codon.
  - The genetic code is not overlapping: a nucleotide only belongs to a single codon and reading is done codon by codon.
  - The genetic code is, with a few exceptions, universal.
  - Three codons do not define any amino acid and are thus called stop codons or nonsense codons. These are the codons UAA, UAG and UGA.

Genetic Code



Wobble Hypothesis



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

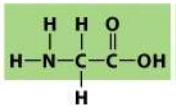
wobble codon base	possible anticodon bases
U	G or I
C	G or I
A	U
G	C

The one-letter genetic code is used in bioinformatics

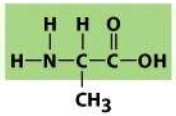
Amino Acid	3 Letter Abbreviation	1 Letter Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

1. Hydrophobic or nonpolar side groups

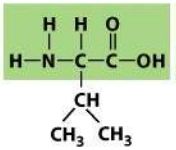
Glycine (Gly) [G]



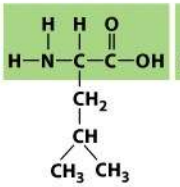
L-Alanine (Ala) [A]



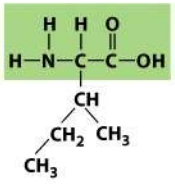
L-Valine (Val) [V]



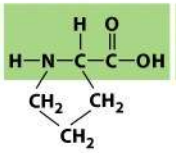
L-Leucine (Leu) [L]



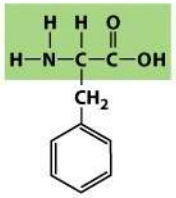
L-Isoleucine (Ile) [I]



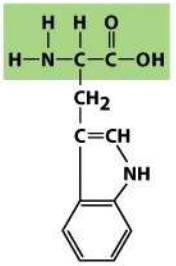
L-Proline (Pro) [P]



L-Phenylalanine (Phe) [F]

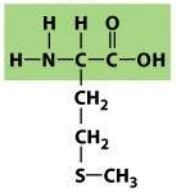


L-Tryptophan (Trp) [W]

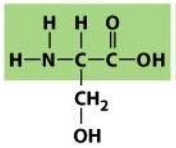


2. Hydrophilic or polar side groups

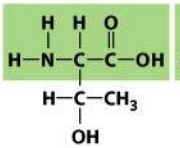
L-Methionine (Met) [M]



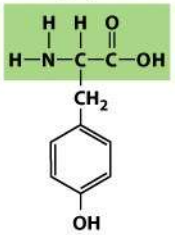
L-Serine (Ser) [S]



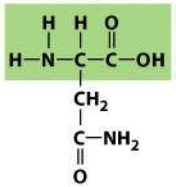
L-Threonine (Thr) [T]



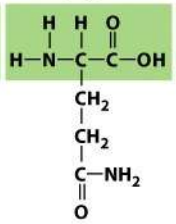
L-Tyrosine (Tyr) [Y]



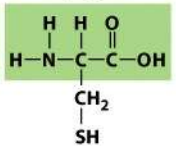
L-Asparagine (Asn) [N]



L-Glutamine (Gln) [Q]

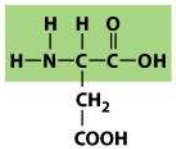


L-Cysteine (Cys) [C]

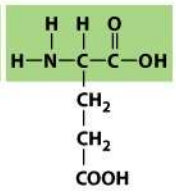


3. Acidic side groups

L-Aspartic acid (Asp) [D]

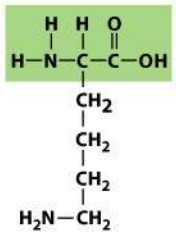


L-Glutamic acid (Glu) [E]

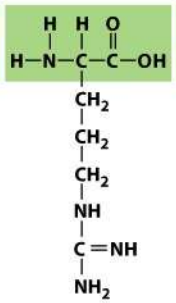


4. Basic side groups

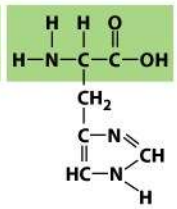
L-Lysine (Lys) [K]



L-Arginine (Arg) [R]



L-Histidine (His) [H]



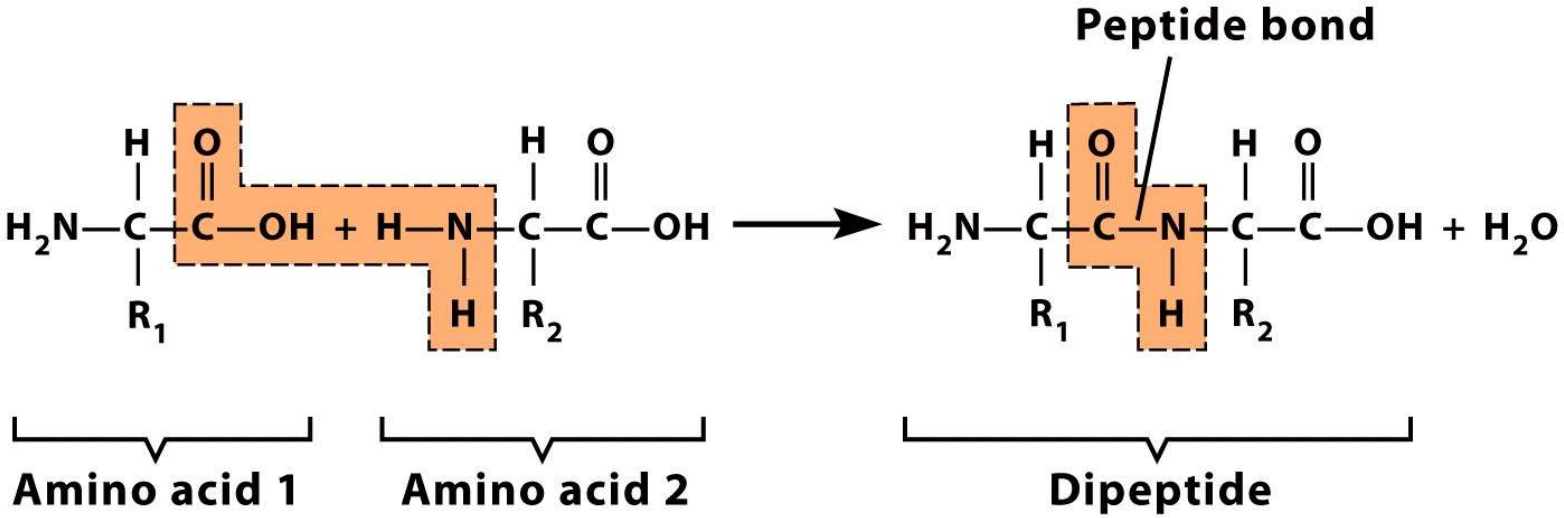
Amino acids are the building blocks of life. There are 22 aa in the living world.

Genetic Code

- The 21st proteinogenic amino acid is selenocysteine (codon UGA usually stop codon).

- In 2002, it was discovered that the UAG codon (which is usually a stop codon) present in the monomethylamine methyltransferase gene of *Methanosarcina barkeri* (a methanogenic archaea) codes for a modified 22 amino acid: pyrrolysine.

Peptide bonds



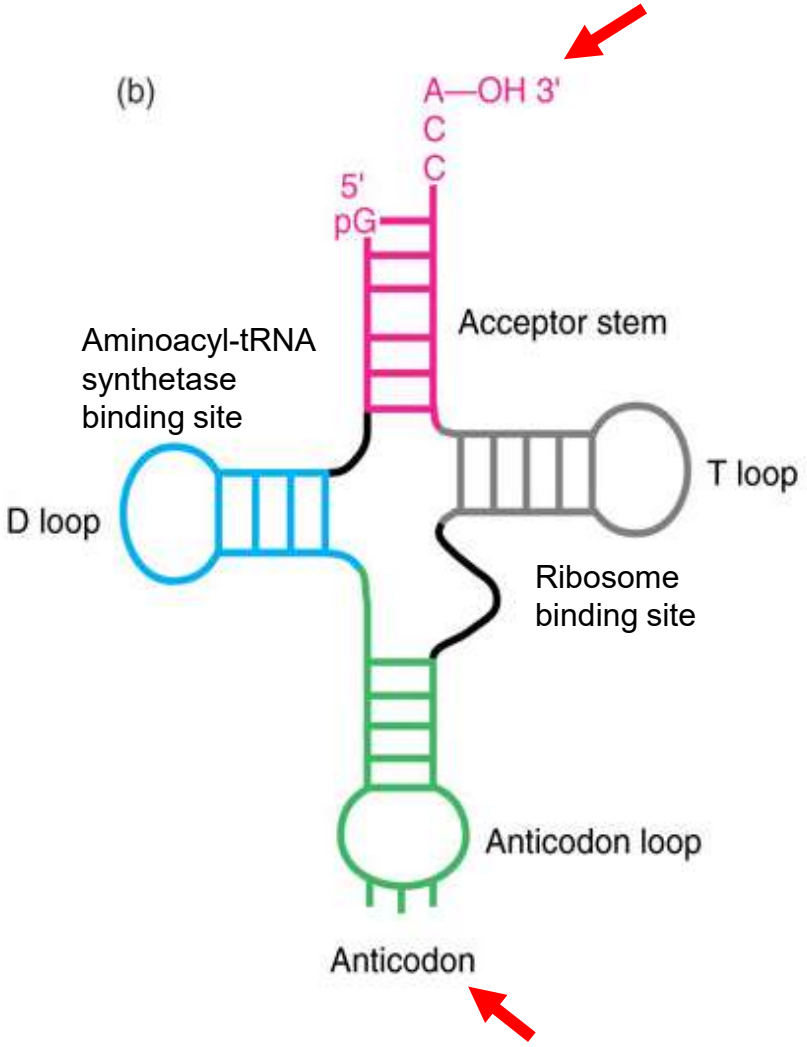
- Amino acids are joined together by peptide bonds.
- The carboxyl group of one amino acid is covalently attached to the amino group of the next amino acid.

Translation

- To translate an mRNA into a protein, we need the following ingredients:
  - A messenger RNA (mRNA)
  - Amino acids
  - A transfer RNA (tRNA):
    - Adapter between mRNA and amino acids
    - Responsible for deciphering the nucleotide code of the mRNA into an amino acid sequence.
  - Ribosomes:
    - organelles that direct the translation process.

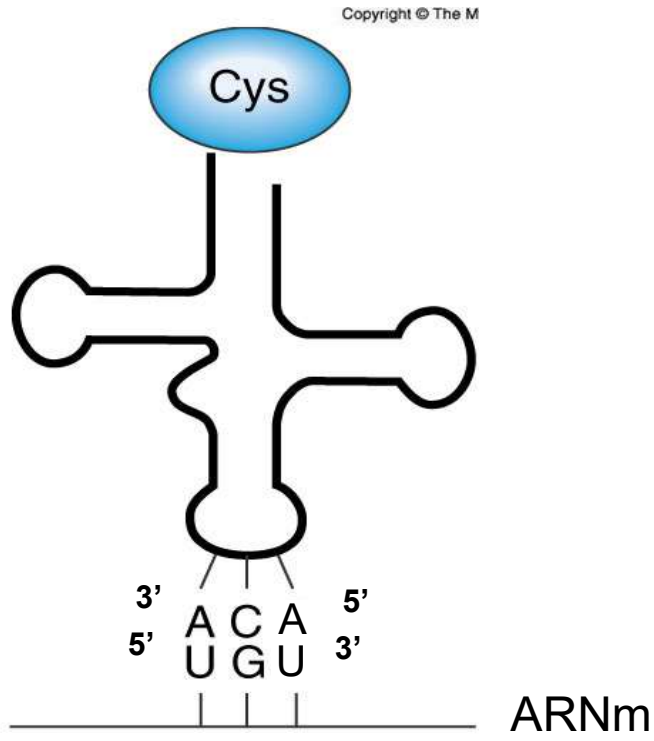
Transfert RNA (tRNA)

- Small RNA molecules (between 73 and 95 nucleotides in length)
- Have two major characteristics :
  - An acceptor arm: where a specific amino acid is directly coupled to the tRNA;
  - The anticodon arm:
    - Has a particular sequence of 3 nucleotides: the **anticodon**;
    - The anticodon forms complementary and antiparallel base pairs with a 3-nucleotide sequence of the mRNA: the **codon**;
- The genetic code is the relationship between the sequence of a **codon** and a specific **amino acid**.

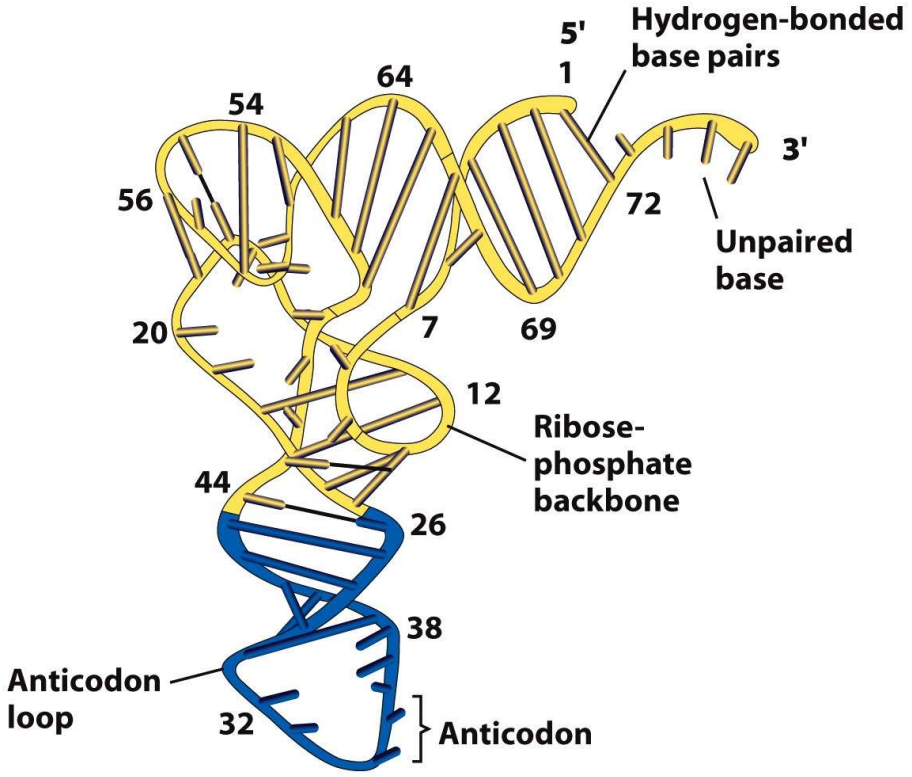


Transfert RNA (tRNA)

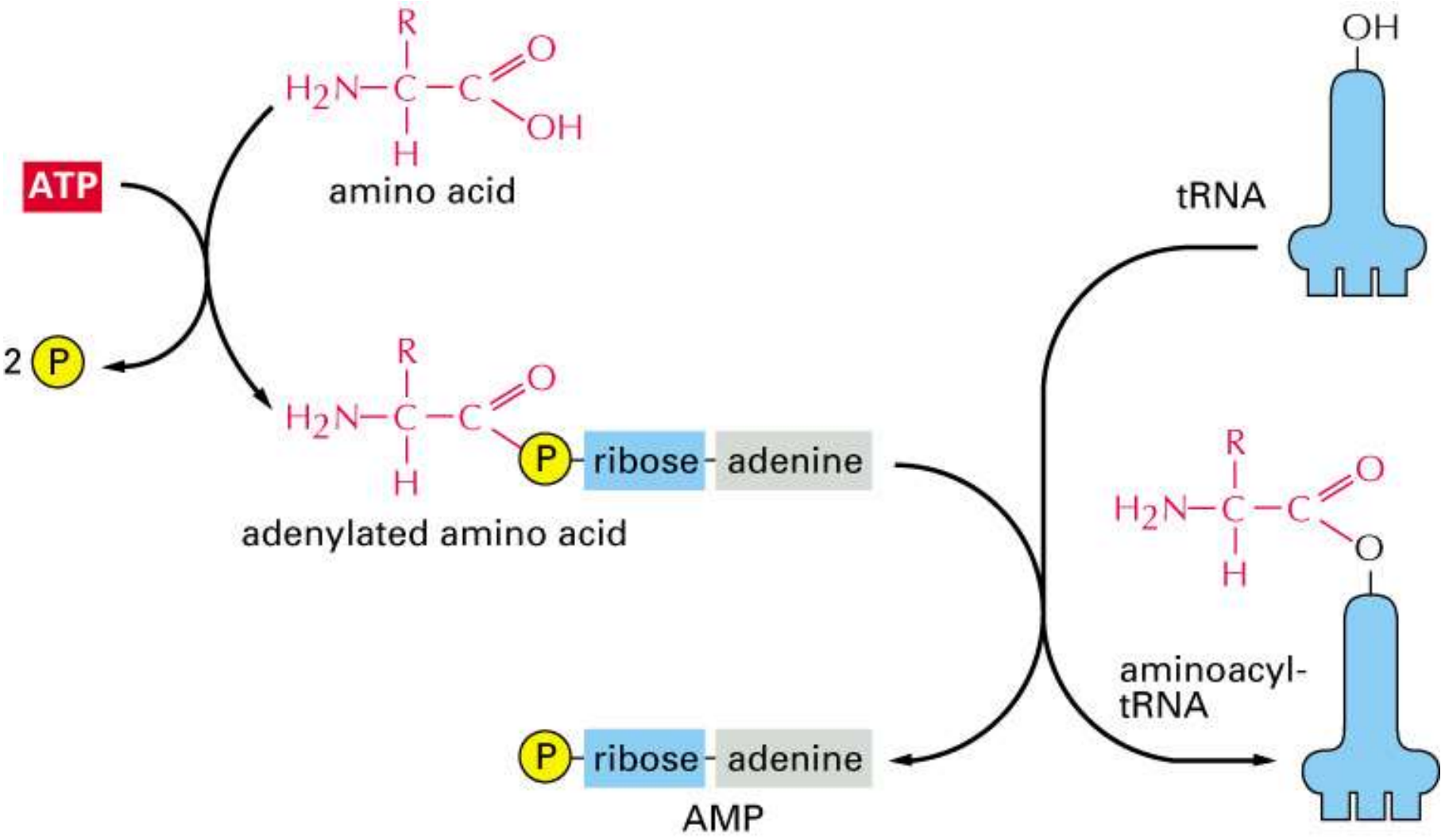
- Each tRNA is linked to a single amino acid specific to it;
- The nature of this amino acid depends on the sequence of the anticodon;



RNAt Structure



Covalent ester bond at 3'OH of the CCA tail of tRNA



A different aminoacyl-tRNA synthetase for each amino acid

Ribosomes

- Ribosomes are universal ribonucleoprotein complexes: they are present in the cells of both Eukaryotes and Prokaryotes.

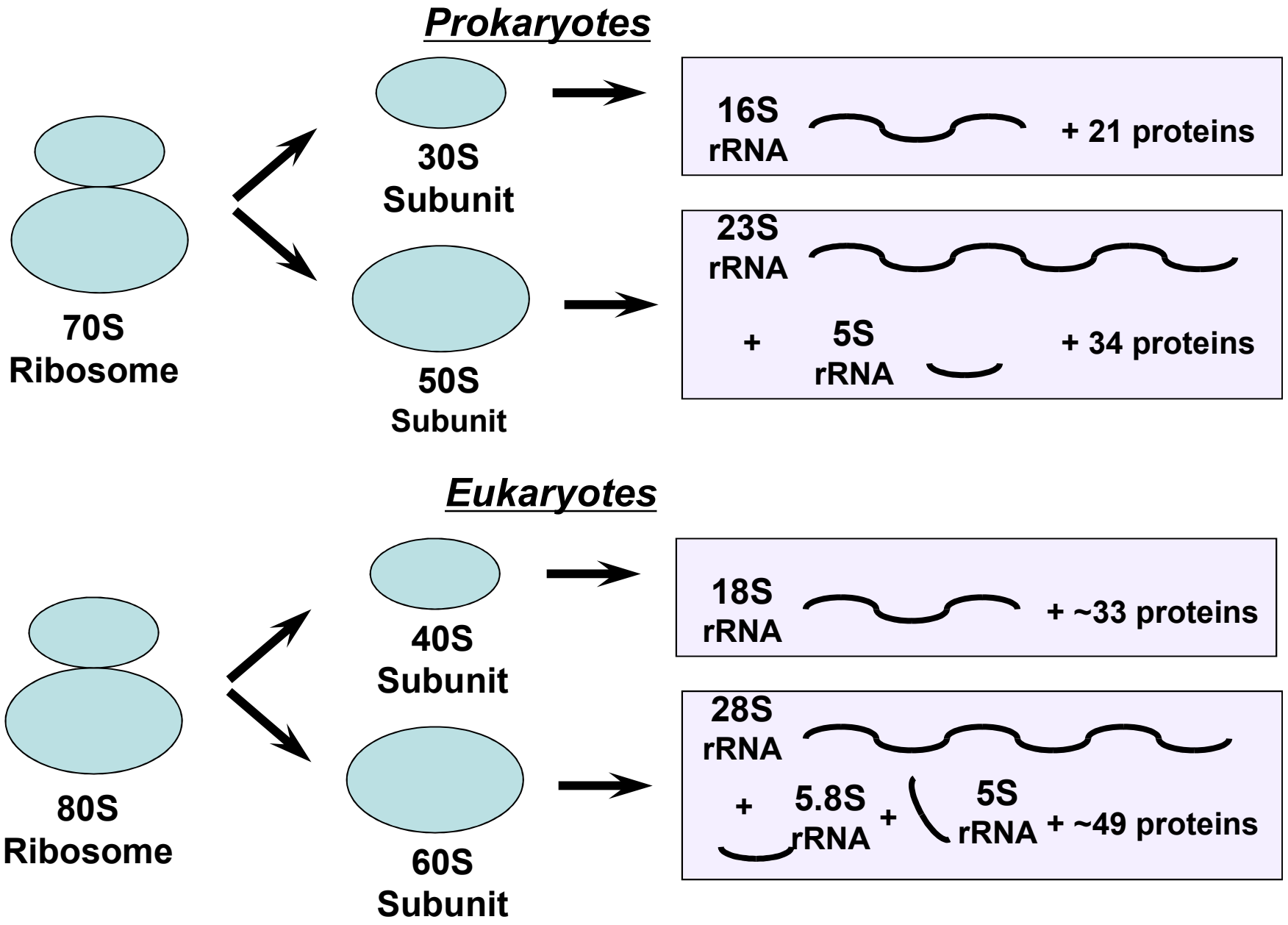
- They are located in the cytoplasm. In eukaryotes, they are free or associated with the nuclear membrane or the membrane of the endoplasmic reticulum.

- Three of the ribosomal RNAs are transcribed in the fibrillar areas of the nucleolus by RNA polymerase I in the form of a 35S precursor of approximately 14,000 nucleotides. The sequence of this precursor is hydrolyzed into 5.8S, 18S, and 28S ribosomal RNA.

- The 4th 5S ribosomal RNA (encoded by a different gene) is transcribed by RNA polymerase III in the nucleoplasm.

- Ribosomal RNAs associated with proteins are imported from the nucleolus and form the two types of subunits (small and large) of ribosomes. Ribosomes are therefore colossal ribonucleoprotein complexes (300,000 atoms).

Composition of Ribosomes



Translation in prokaryotes

a) Initiation of translation

The initiation actors:

- mRNA
- Ribosome
- Nformylméthionine tRNA (Initiator tRNA)
- initiation factors (**IF**):

IF3 (30S small subunit recognition on **Shine Dalgarno** sequence),

IF2 (fixation of the 1st tRNA),

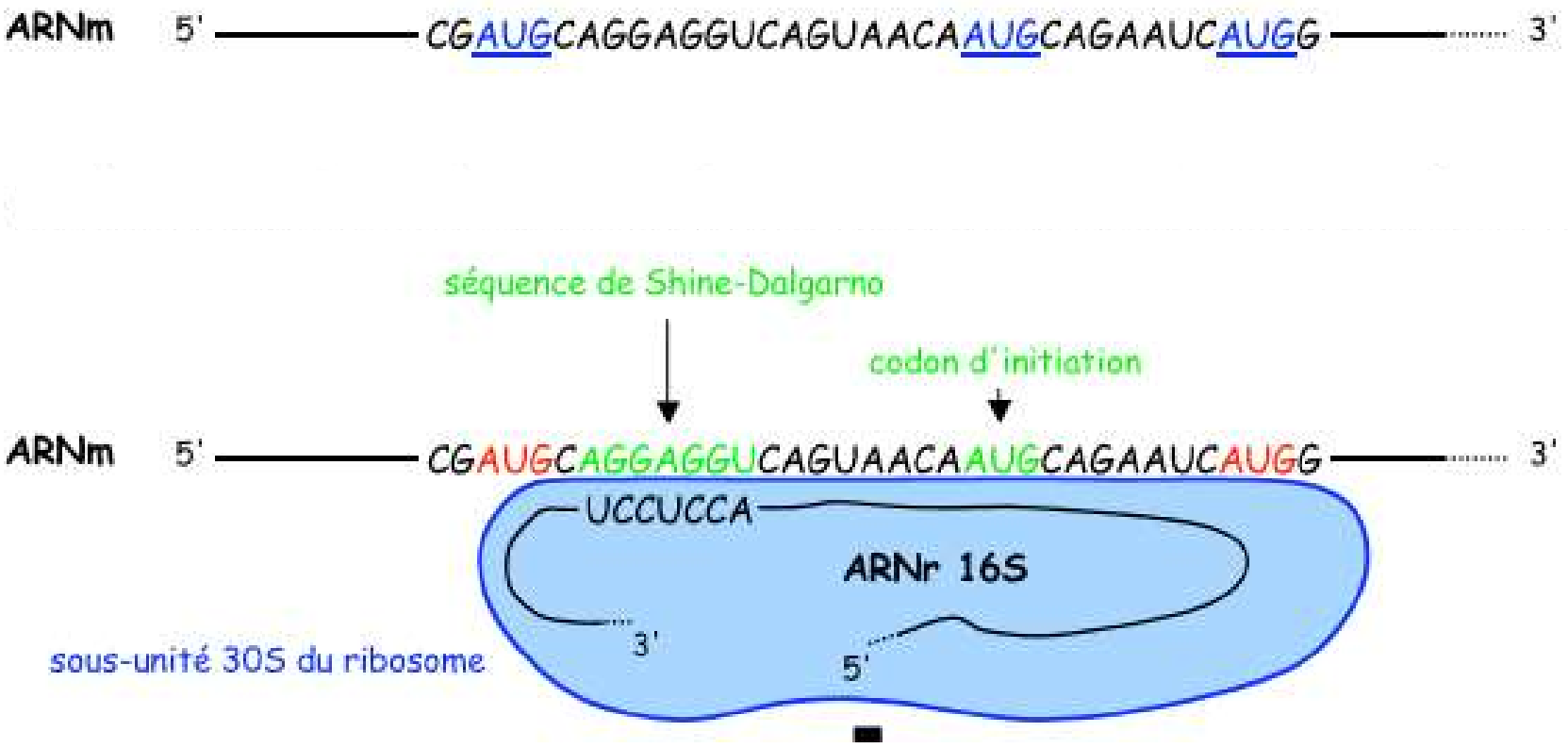
IF1 (50S large subunit attachment)

Architecture of the initiation sequences :

- approximately 15 nucleotides long
- complementary to Shine Dalgarno sequence (included in 16S rRNA of small 30S ribosomal subunit)
- rich in purine with conserved sequence:

AGGAGG

- upstream (5' side) of the START codon (AUG)

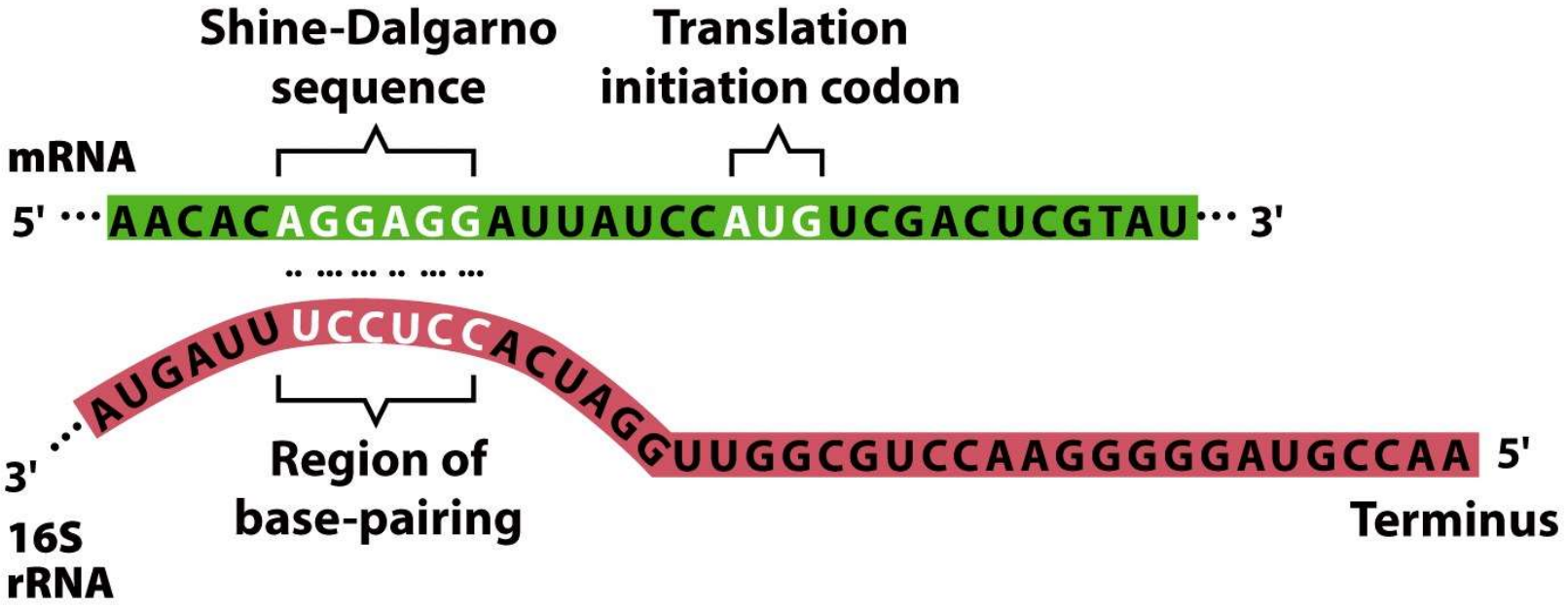


Architecture of the initiation sequences :

- approximately 15 nucleotides long
- complementary to Shine Dalgarno sequence (included in 16S rRNA of small 30S ribosomal subunit)
- rich in purine with conserved sequence:

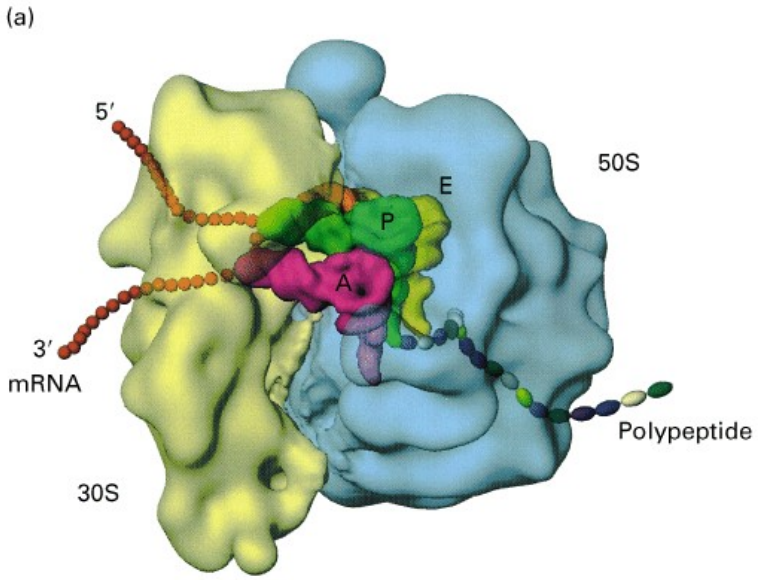
AGGAGG

- upstream (5' side) of the START codon (AUG)



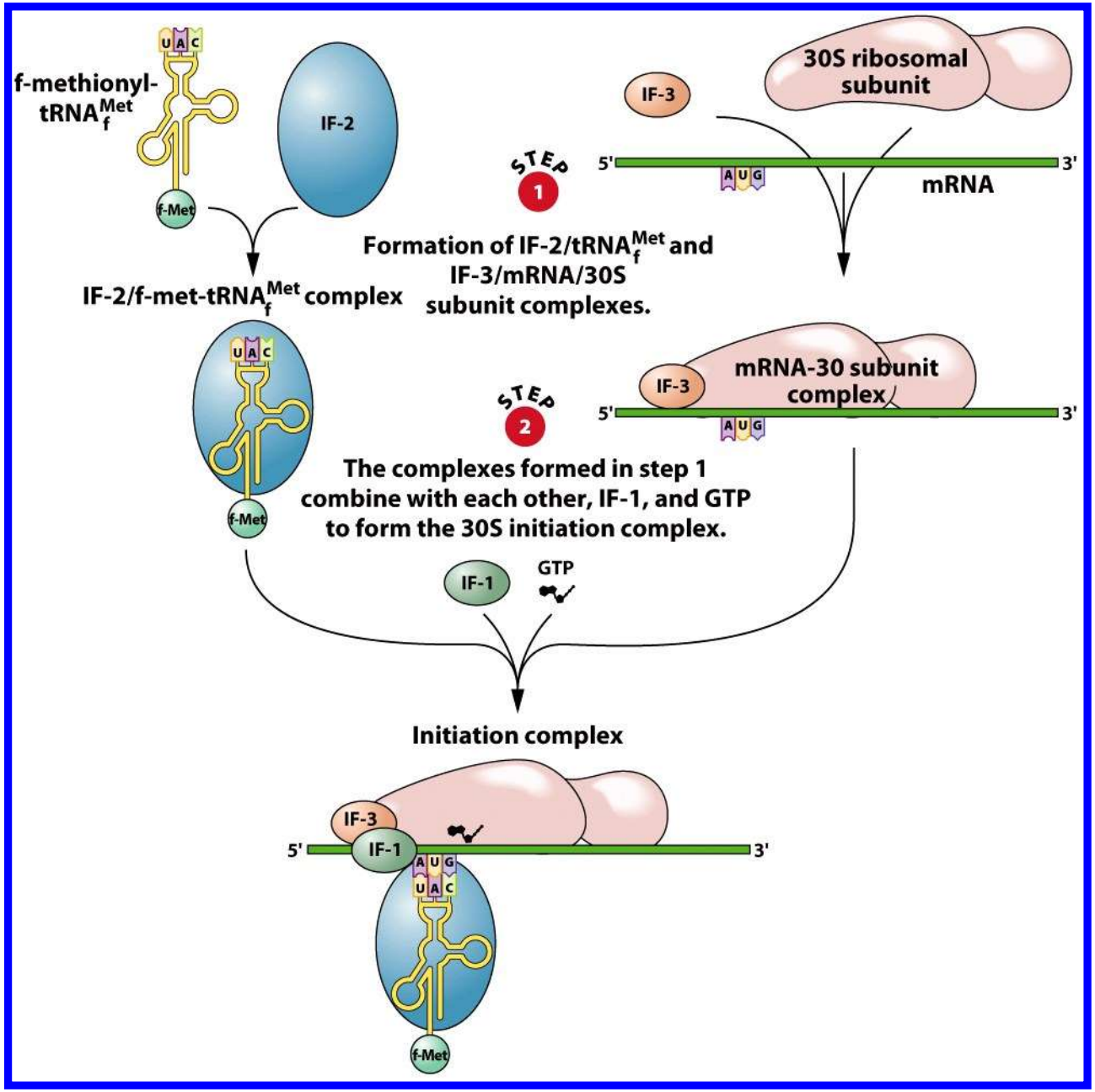
Binding of the 1st amino acyl tRNA to the small subunit

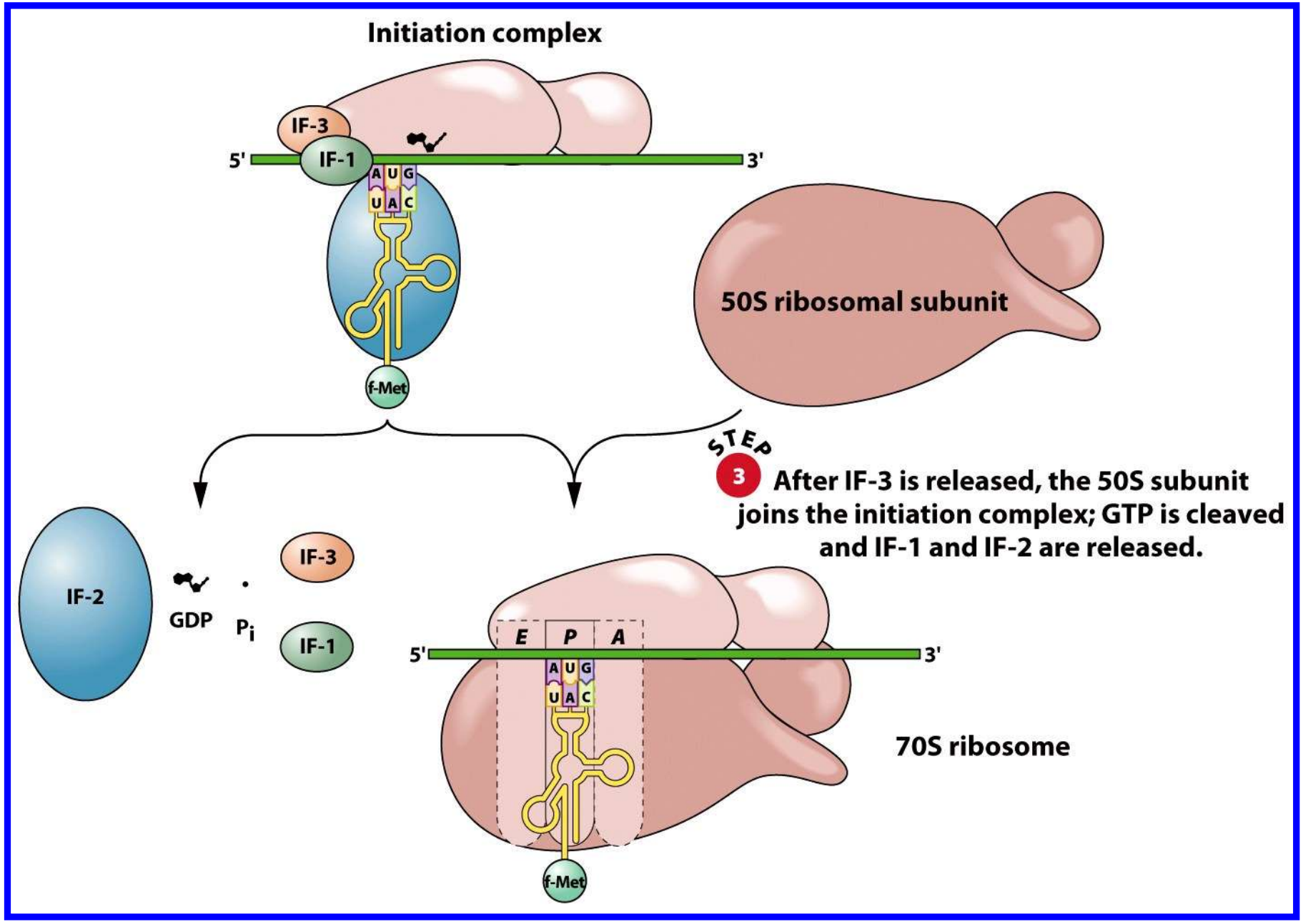
- Nformyl methionine tRNA
- Fixation on P site ("peptidyl")
- Intervention of IF2 which hydrolyzes GTP



Fixation of the large subunit

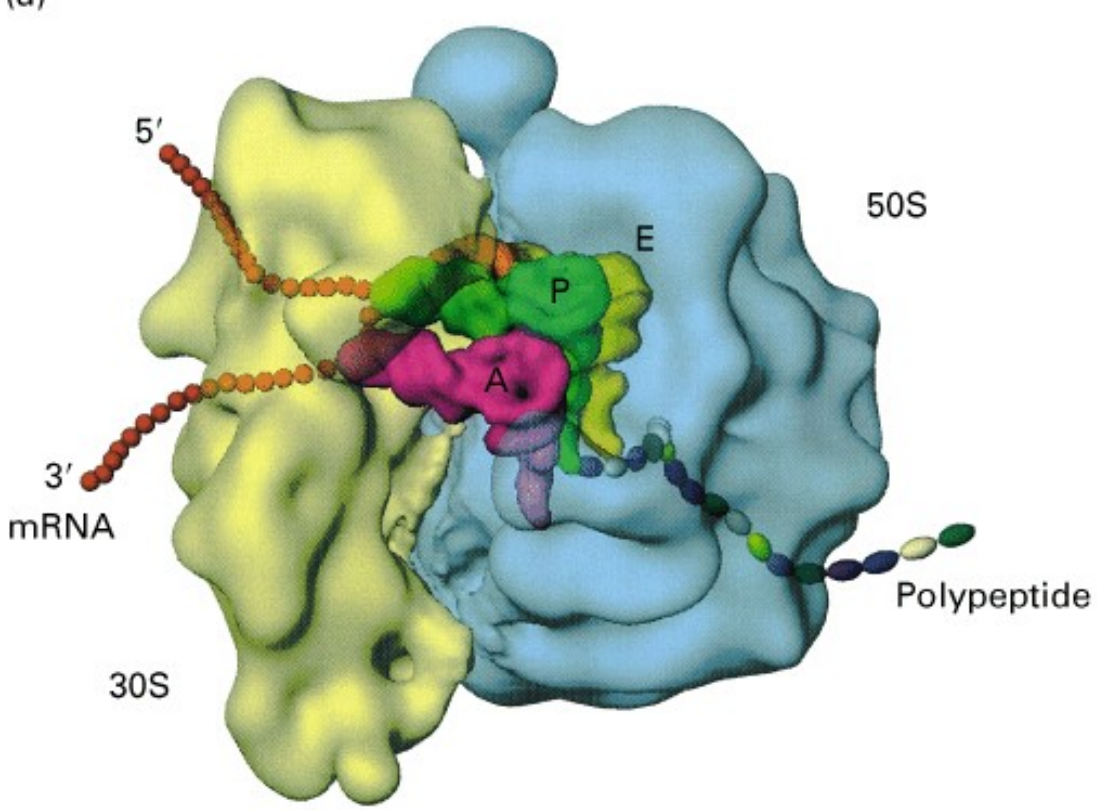
- Intervention of IF1
- Attachment of the second aa-tRNA to the A site
- The ribozyme catalytic site of the large subunit forms a peptide bond (without energy consumption)
- IF release (consumes one GTP)


f-methionyl-tRNA$_f^{Met}$
U A C
f-Met
IF-2
IF-2/f-met-tRNA$_f^{Met}$ complex
30S ribosomal subunit
IF-3
STEP 1
5'
A U G
3'
mRNA
Formation of IF-2/tRNA$_f^{Met}$ and IF-3/mRNA/30S subunit complexes.
STEP 2
mRNA-30 subunit complex
The complexes formed in step 1 combine with each other, IF-1, and GTP to form the 30S initiation complex.
IF-1
GTP
Initiation complex

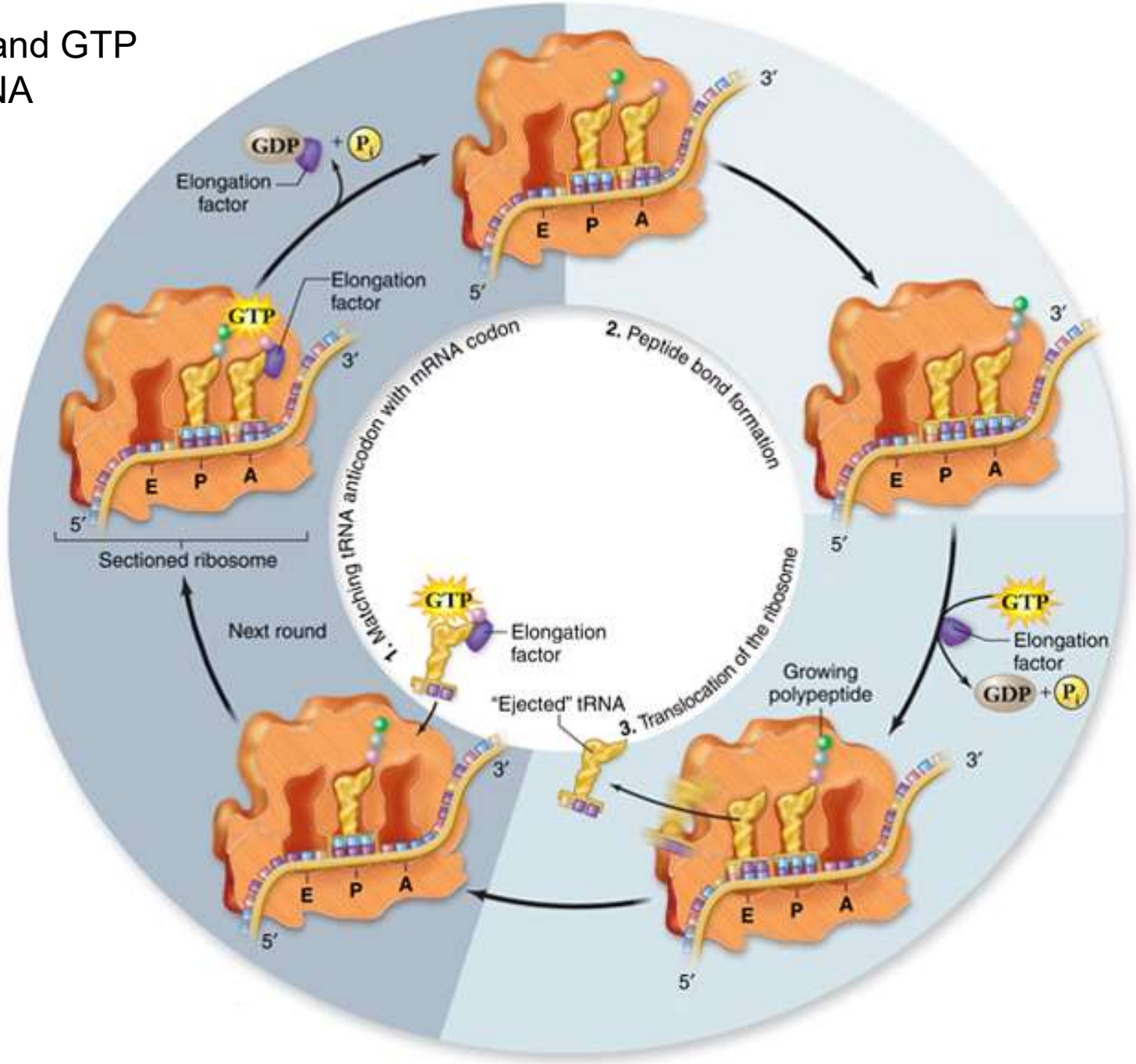

Initiation complex
IF-3
IF-1
5'
3'
A U G
U A C
f-Met
50S ribosomal subunit
STEP 3
After IF-3 is released, the 50S subunit joins the initiation complex; GTP is cleaved and IF-1 and IF-2 are released.
IF-2
GDP
Pi
IF-3
IF-1
E P A
5'
3'
A U G
U A C
f-Met
70S ribosome

b) Elongation

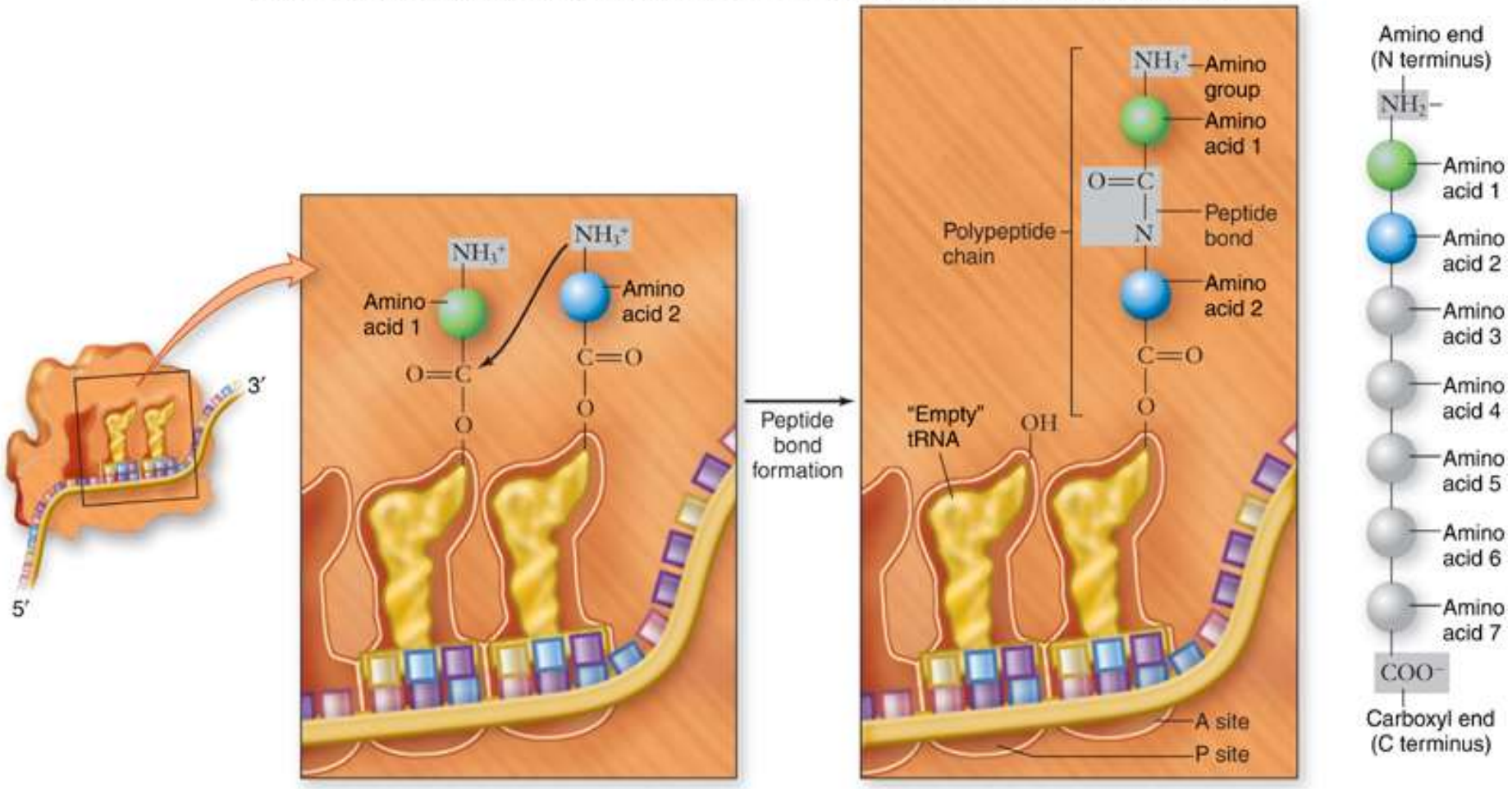
- Sens 5' → 3'
-3 sites ribosome
    - **Site A** (amino)
    - **Site P** (peptide)
    - Site E (exit)



Elongation factors and GTP are required for tRNA recruitment and translocation



b) Elongation
Peptide bonds



b) Elongation

- **Site A** (amino) : Arrival of aminoacyl tRNA interacting with elongation factors (EF-Tu) + GTP for codon/anticocon pairing
- **Site P** (peptide) : synthesis of the peptide bond by action of a peptide transferase of the large subunit (50S) (without energy consumption)

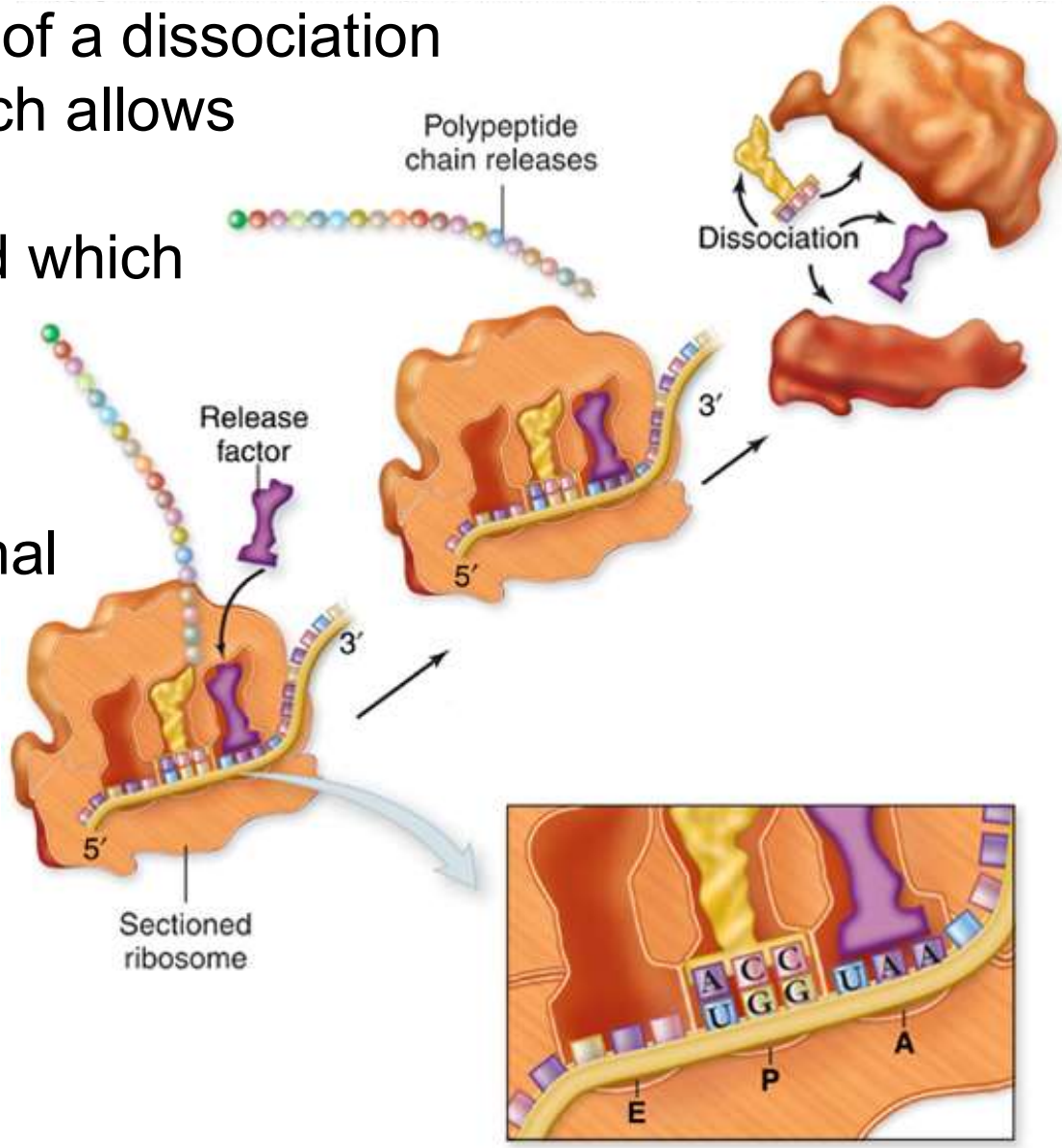
- Translocation :
  - ntervention of a translocase factor(EFG)
  - consommes 1 GTP
  - Progression of 3 nucléotides

-Elongation speed: 20 aa/sec (not very fast but several ribosomes at the same time)

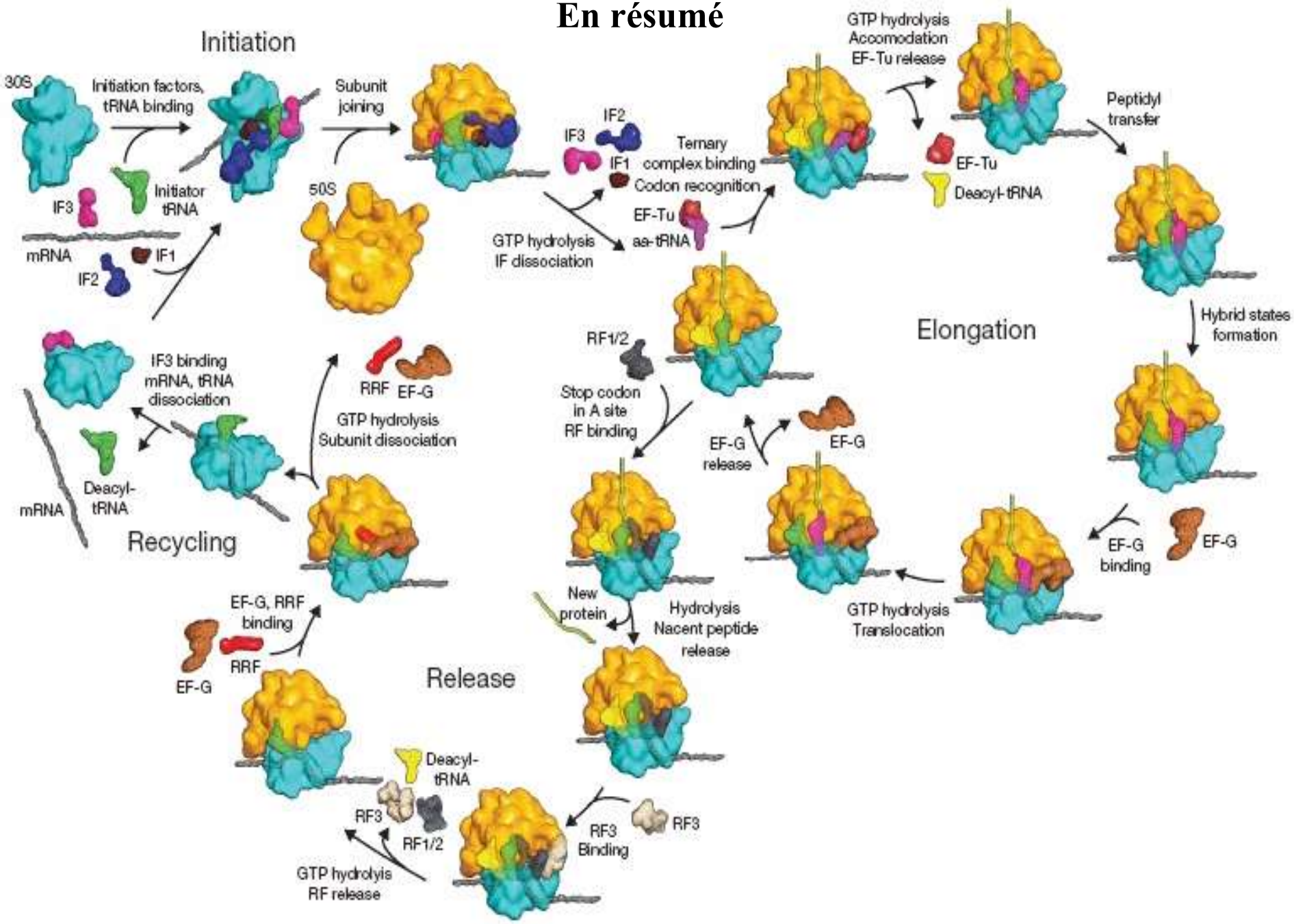
c) Termination

- Arrival of the STOP codon at site A
- allows the establishment of a dissociation factor **(release factor)** which allows the hydrolysis of the COOH-tRNA + 1 GTP bond which releases the newly synthesized polypeptide.

-separation of the 2 ribosomal subunits + 1 GTP



En résumé



From: Schmeing, T. Martin, V. Ramakrishnan. 29 October 2009. What recent ribosome structures have revealed about