MCB Chapter 11

Topic D

Post-Transcriptional Controls

Reading : 404-443

Post-Transcriptional Controls

All processes following transcription initiation

- Elongation - till the end
- Transport to cytoplasm
- Stability of mRNA
- Cellular localization

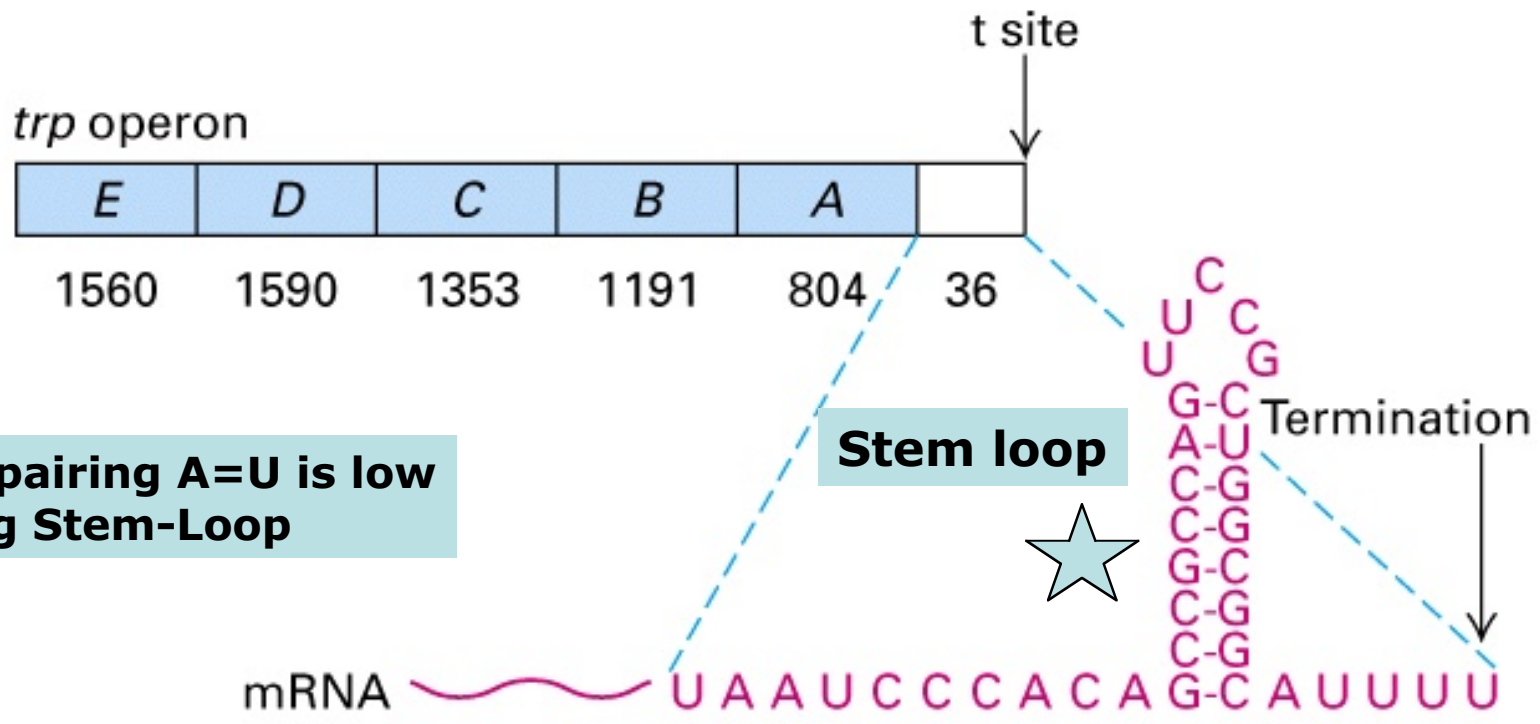
All added to the Regulation of gene expression

Transcription termination

- Several mechanisms in bacteria and eukaryotic cells

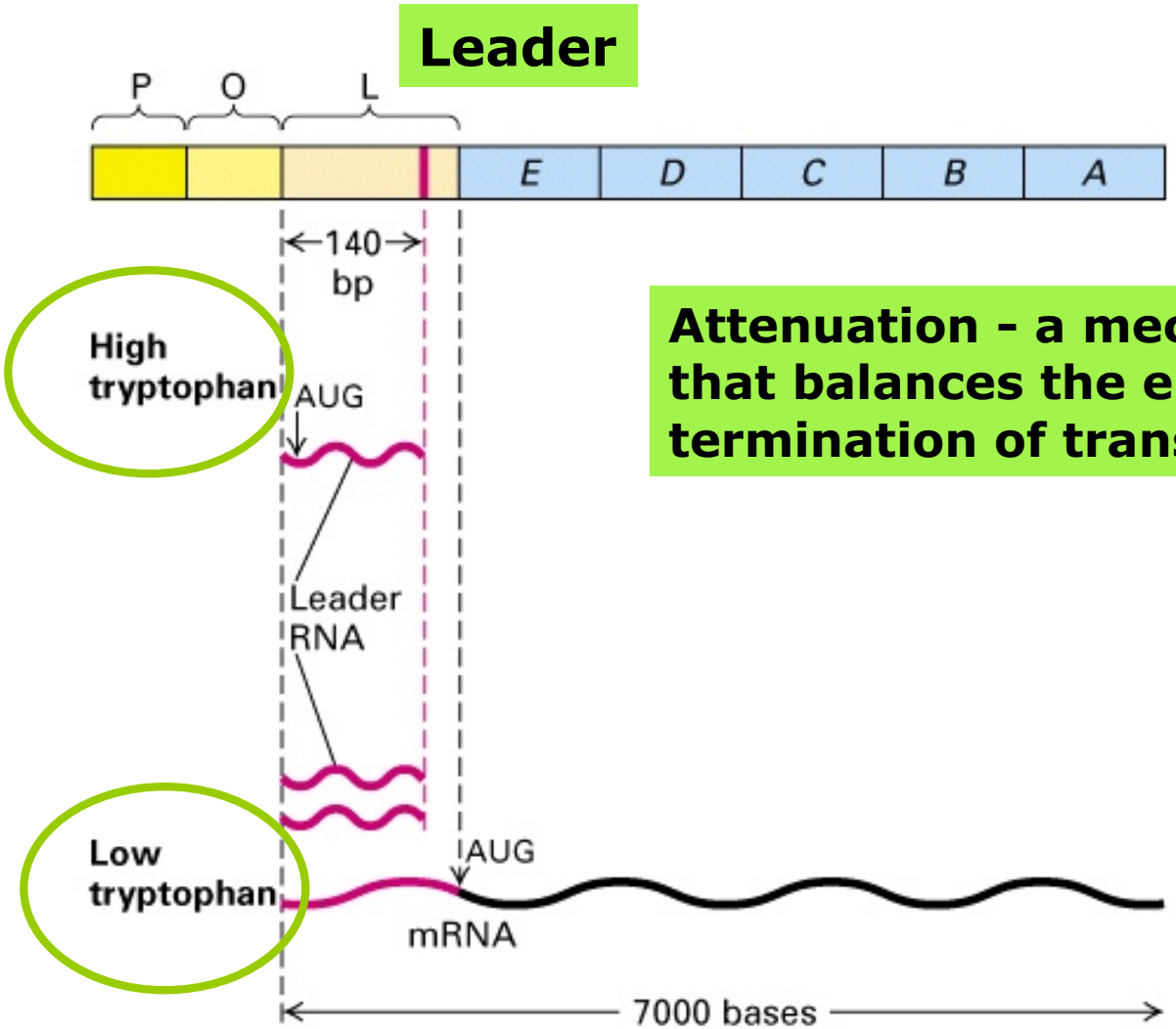
- **In bacteria** - two principle mechanisms involve RNA polymerase
  Requires the termination factor ***Rho***
  Rho independent
- **In eukaryotes**, the mechanisms for terminating transcription differ for each of the 3 types of RNA polymerase
  Pol - I **(pre-rRNA)**
  Pol - II
  Pol - III **(tRNA & 5S-rRNA)**

Rho-independent termination occurs at characteristic sequences in *E. coli* DNA



1. **Base-pairing A=U is low**
2. **Strong Stem-Loop**

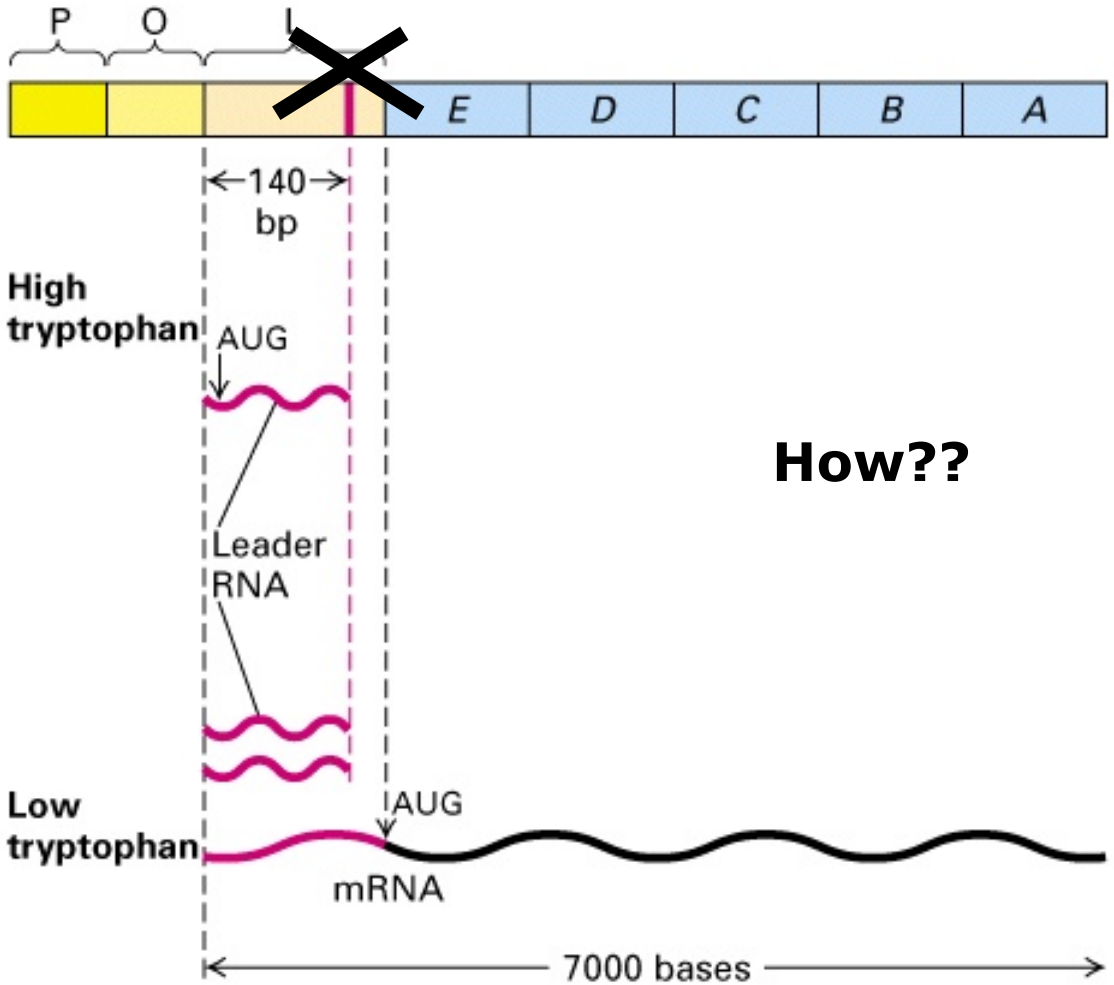
Premature termination by attenuation helps regulate expression of some bacterial operons



Attenuation - a mechanism that balances the elongation/ termination of transcription

Figure 11-2

Mutations in the attenuator -leads to excess of tryptophan biosynthesis transcripts

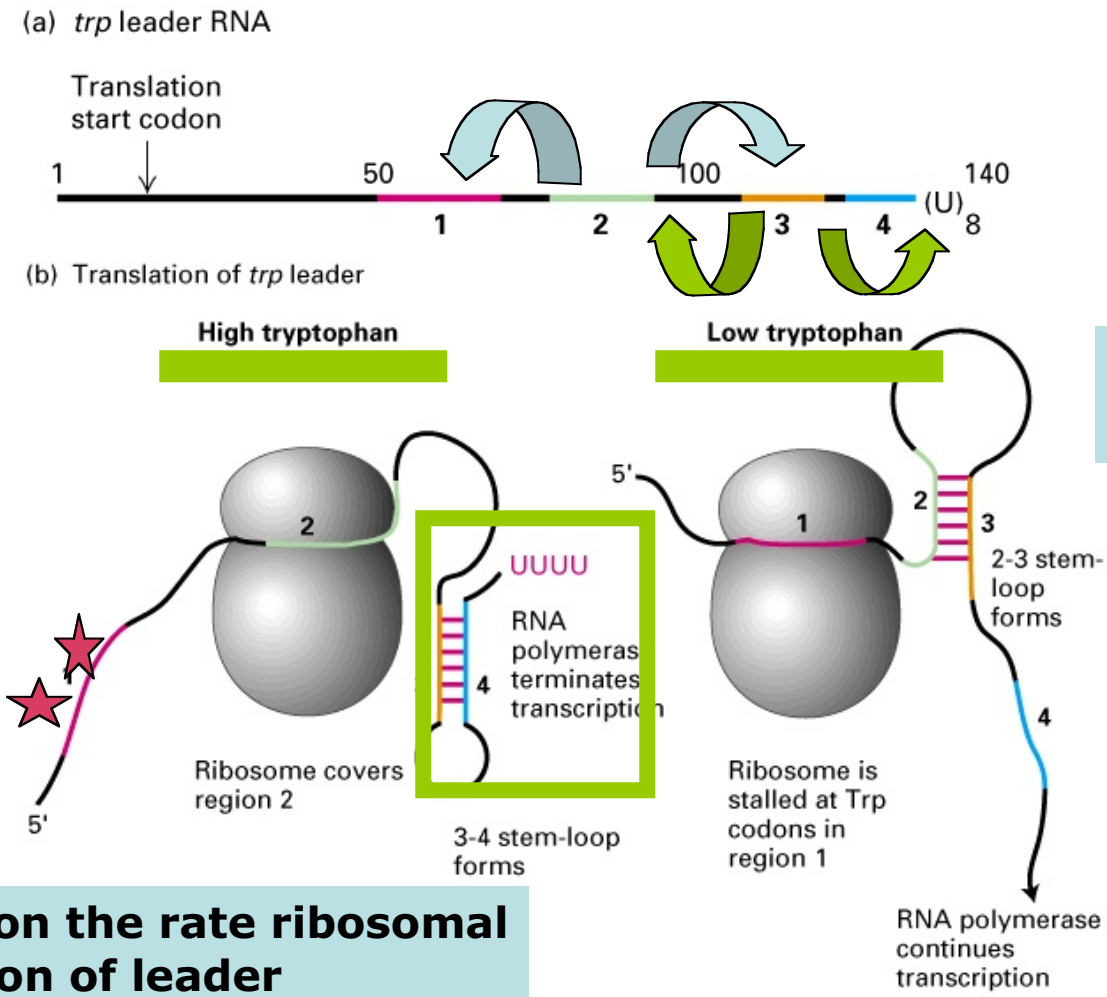


How??

Figure 11-2

Mechanism of attenuation of *trp*-operon transcription - a Rho independent

1. Alternative Base-pairing



2-3 Stem loop

2. Depend on the rate ribosomal translation of leader
3. This depend on $tRNA^{-trp}$

A Rho independent mechanism of attenuation valid for Phe, His, Ile, Leu & Val

- The leader seq includes the relevant aa
- The leader seq is rapidly degraded after translation
- For attenuation in different cases - RNA binding proteins that stabilize base-pairing are essential.

  E. coli bgl operon (for glucose containing polysacharadies)

RNA binding protein- stabilize a non-attenuated stem-loop.
The protein is activated by glucose phosphorylation
Thus, in the presence of glucose - bgl operon is functioning.

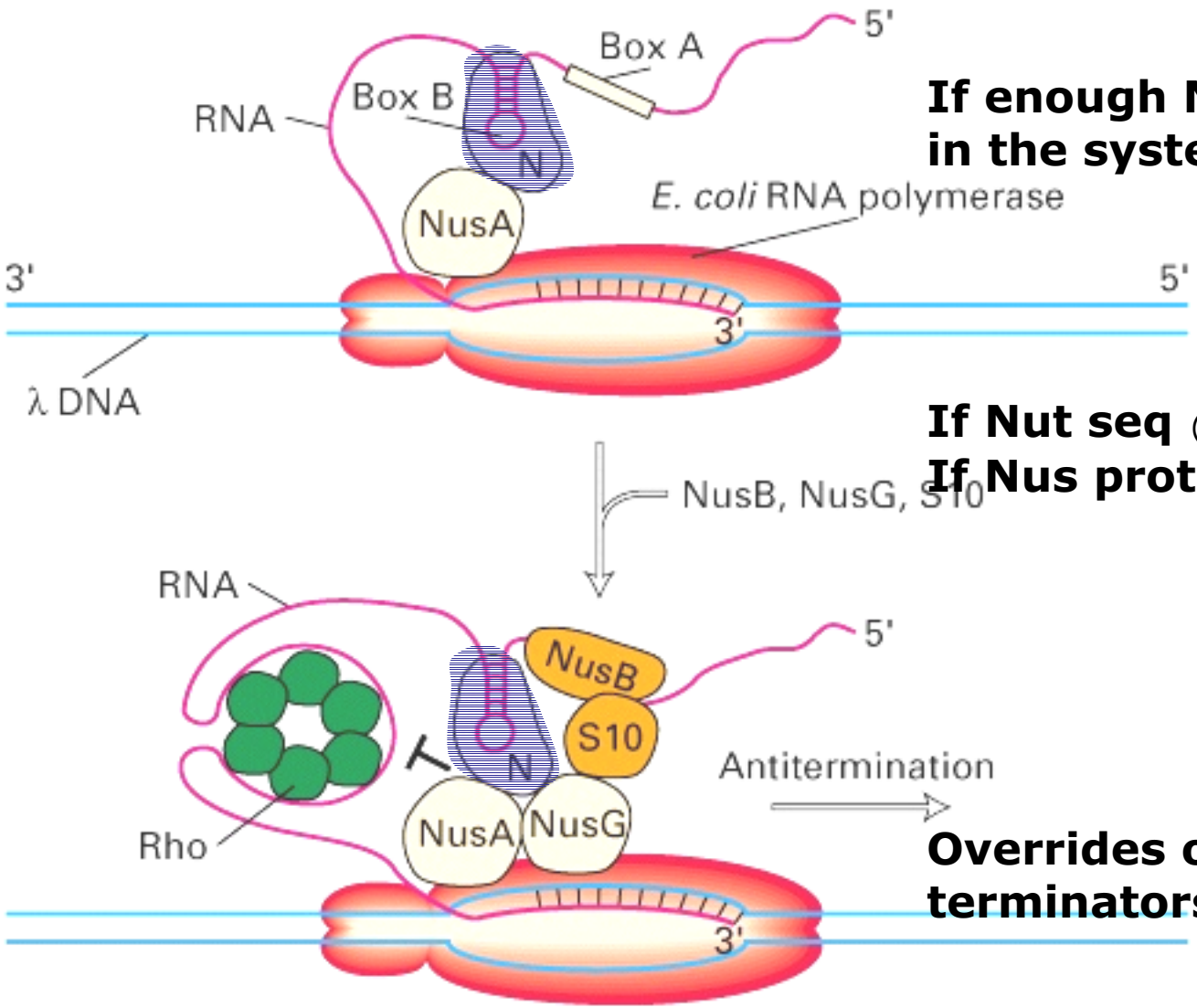
Figure 11-4

Rho-dependent termination sites are present in some λ-phage and *E. coli* genes

Rho was discovered after λ-phage infection, How?

- The Rho factor is a hexameric protein around which a 70- to 80-base segment of the growing RNA transcript wraps
- Rho then moves along the RNA in the 3′ direction until it eventually unwinds the RNA-DNA hybrid at the active site of RNA polymerase
- Whether transcription is terminated or not depends on whether Rho "catches up" to RNA polymerase
- Rho-dependent sites have no clear consensus sequence and Rho-dependent termination operates at relatively few operons

Anti-terminator by λ-phage N + E. coli proteins



If enough N- protein in the system..

If Nut seq (N -utilizatization)
If Nus proteins

Overrides other terminators

Three eukaryotic RNA polymerases employ different termination mechanisms

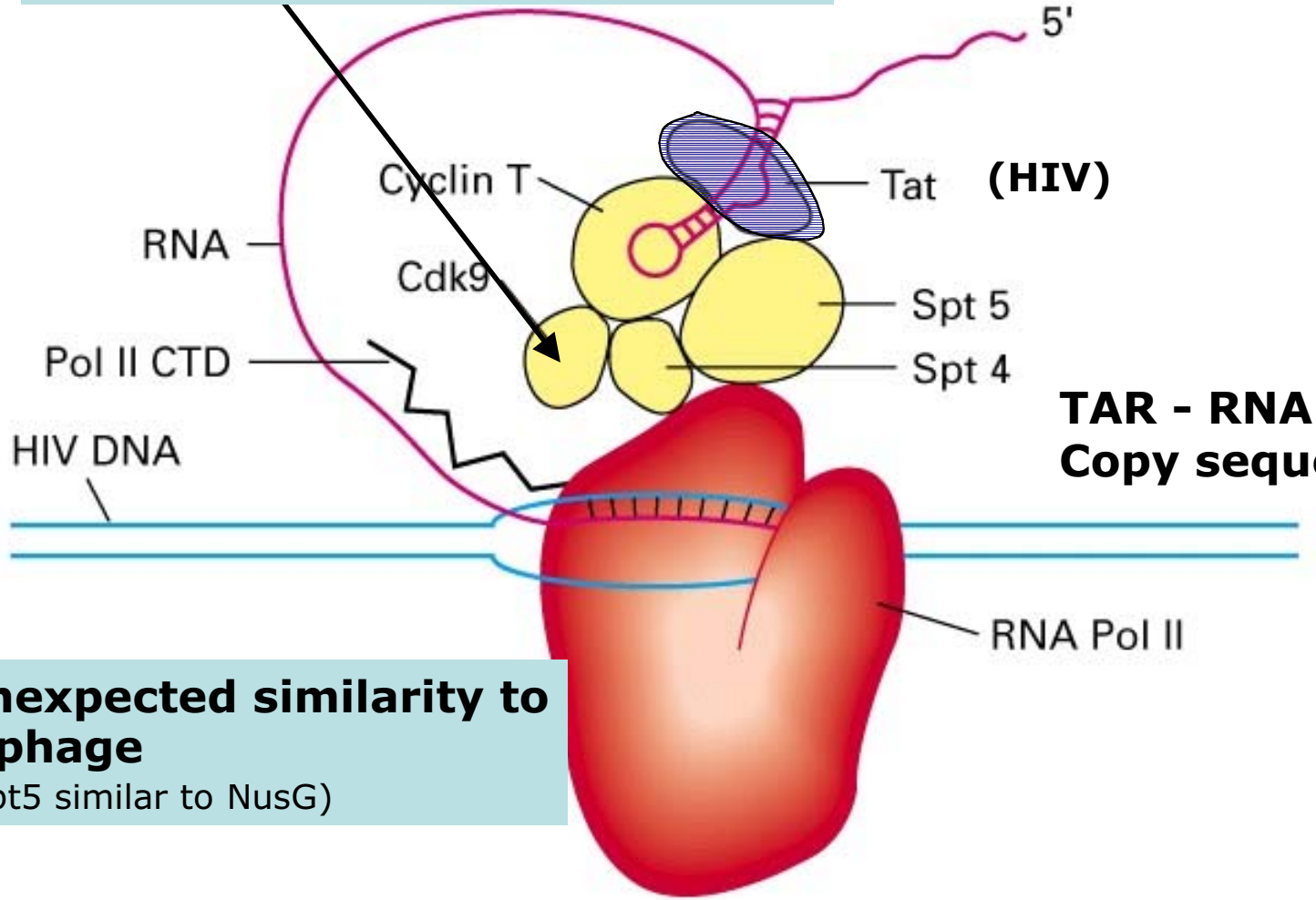
- **RNA polymerase I** is terminated by a mechanism that requires a polymerase-specific termination factor, which binds downstream of the transcription unit (A **DNA**-binding protein not a **RNA** binding as Rho)

- **RNA polymerase II** is terminated in a region 0.5-2 kb beyond the poly(A) addition site, and termination is coupled to the process that cleaves and polyadenylates the 3′ end of a transcript

- **RNA polymerase III** is terminated after polymerizing a series of U residues (no stem-loop is requested)

Transcription of HIV genome is regulated by an anti-termination mechanism

Kinase - the substrate CTD of pol II



TAR - RNA
Copy sequence for Tat

Unexpected similarity to λ-phage
(Spt5 similar to NusG)

Figure 11-6

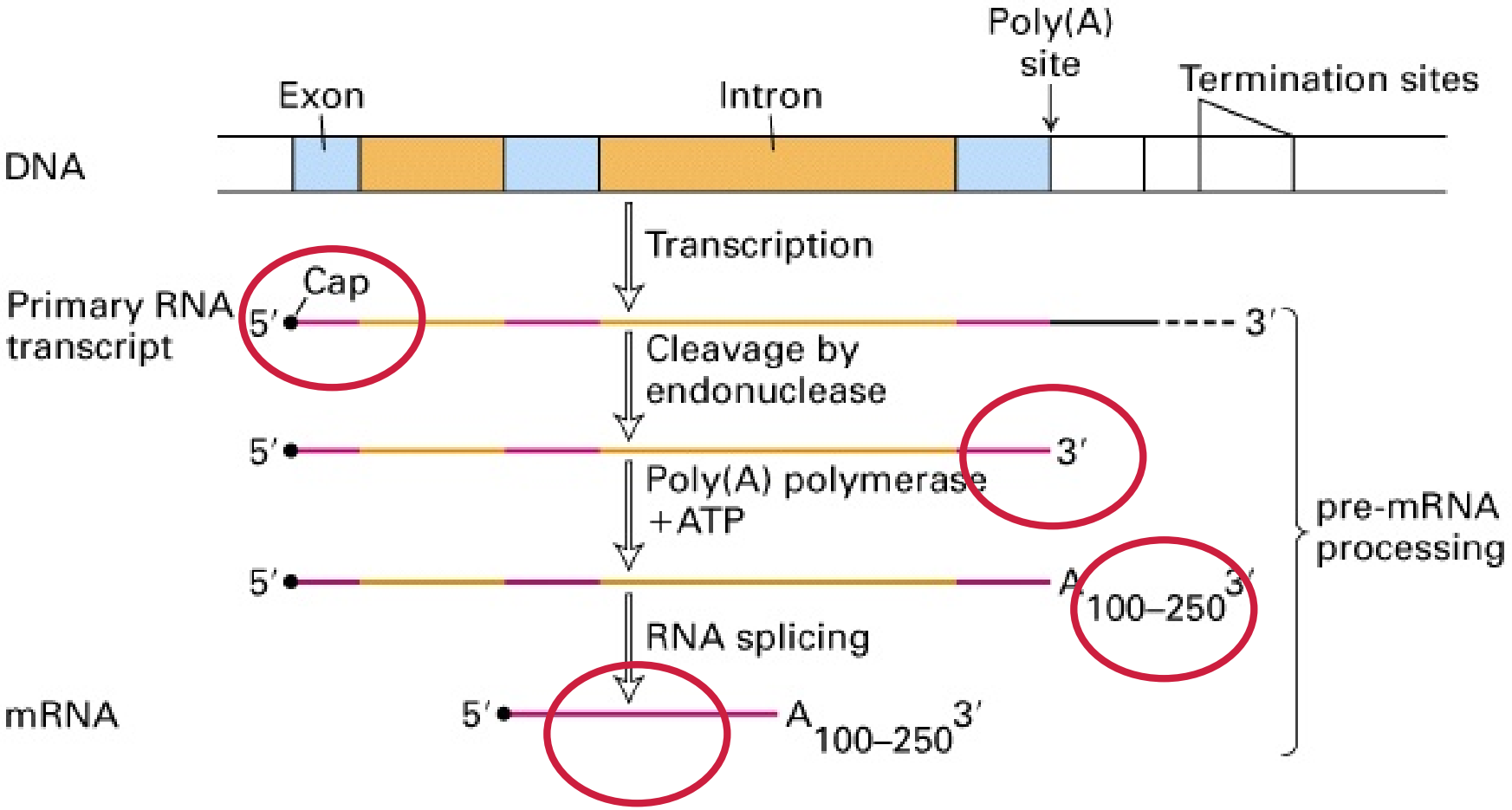
Eukaryotic RNA -pol II transcription termination

HIV example

Drosophila HSP (heat shock)
polII pause but stay attached
HS activates HSTF that relief pol II from pausing

Rapid response! No assembly time is wasted

Processing of eukaryotic mRNA



The 5′-cap is added to nascent RNAs after initiation by RNA polymerase II

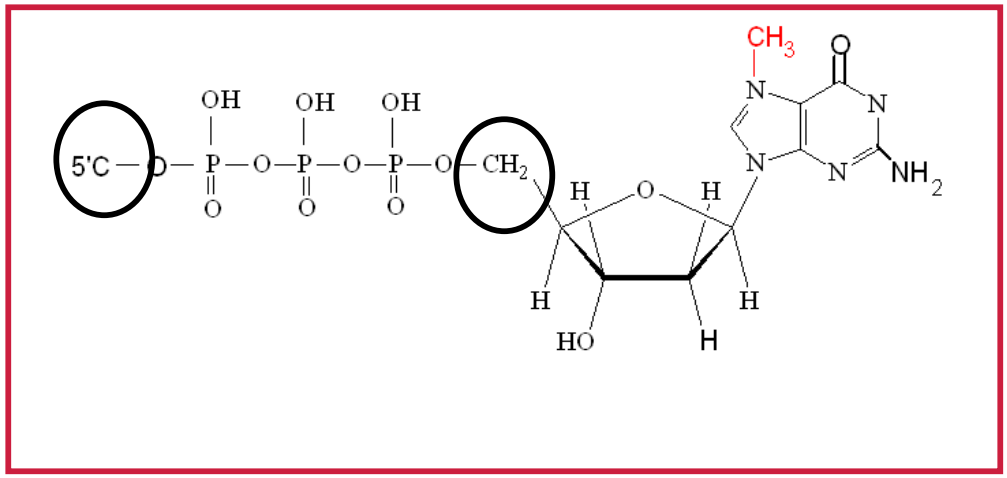
~25 nt

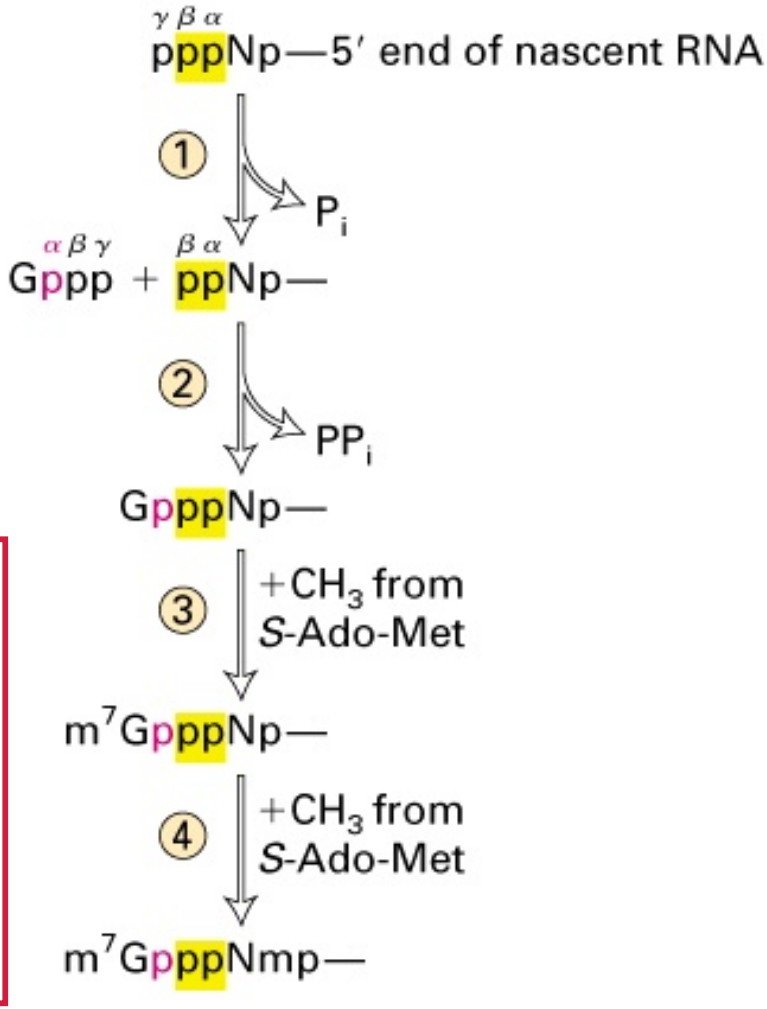
7-methylguanosine

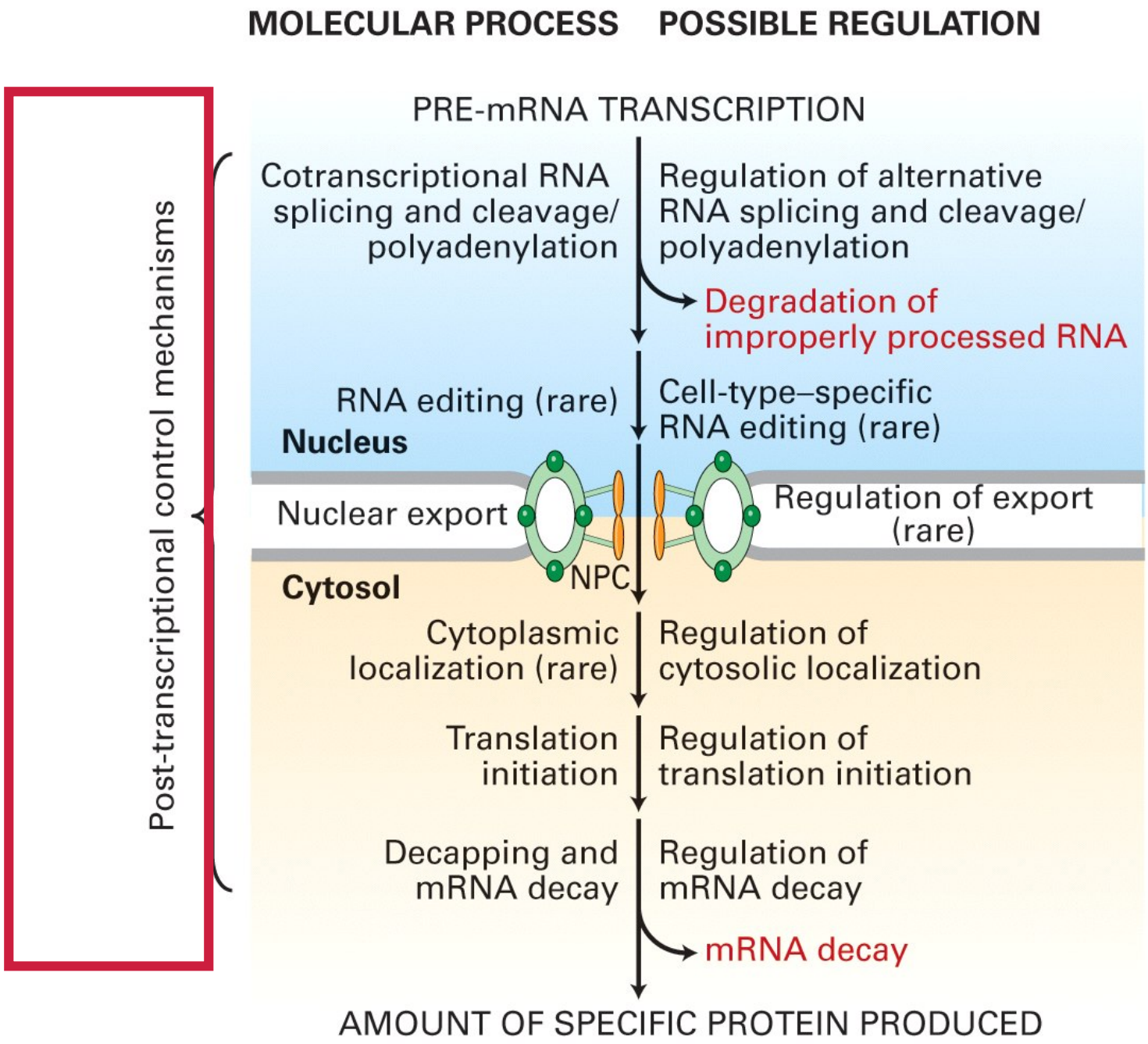
5′-5′ link

Dimeric capping enzyme - associated with CTD of pol II (only)

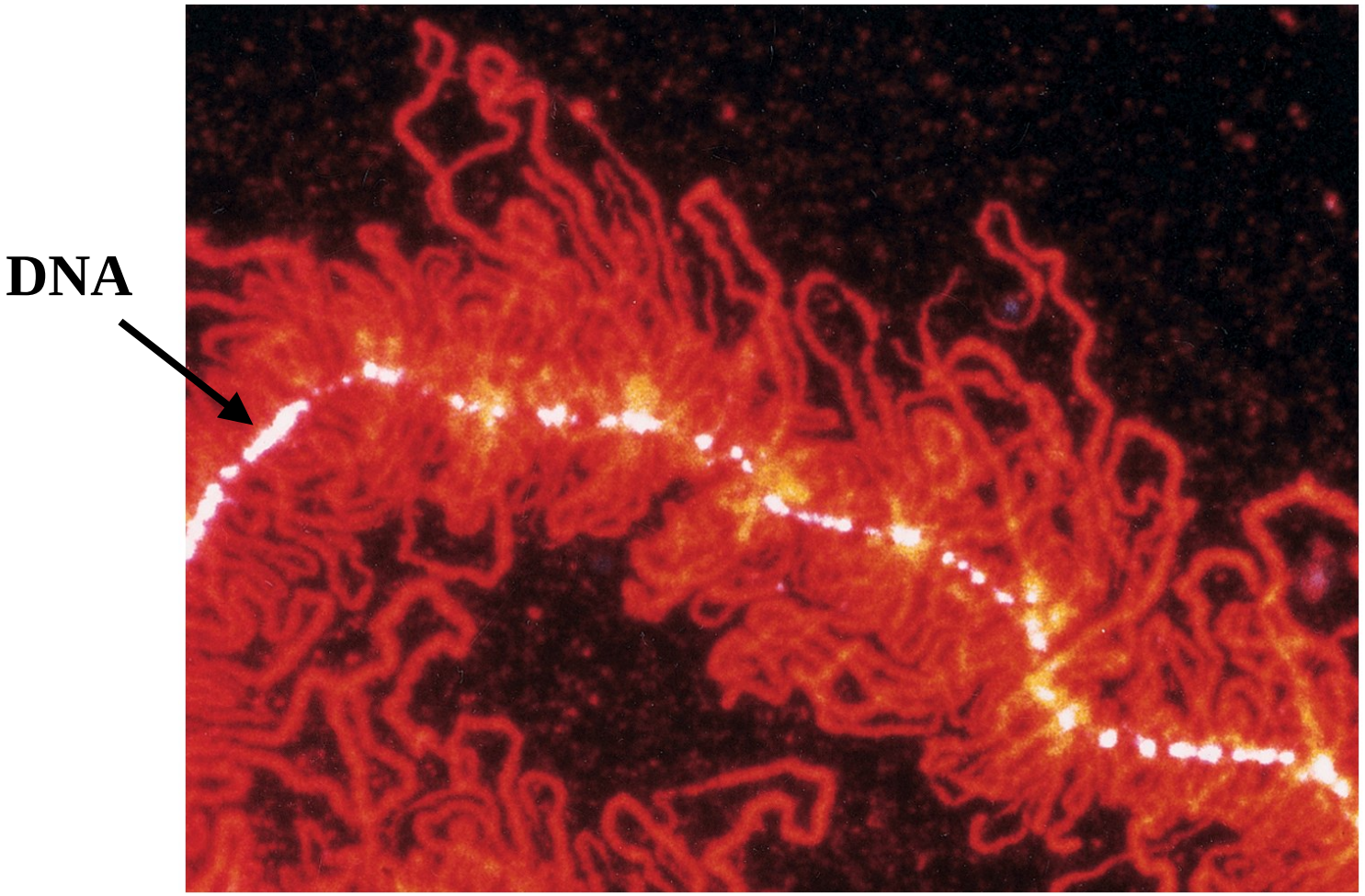
Methylation also on the ribose of the 1st (and) 2nd base






MOLECULAR PROCESS
POSSIBLE REGULATION
Post-transcriptional control mechanisms
PRE-mRNA TRANSCRIPTION
Cotranscriptional RNA splicing and cleavage/ polyadenylation
Regulation of alternative RNA splicing and cleavage/ polyadenylation
Degradation of improperly processed RNA
RNA editing (rare)
Cell-type–specific RNA editing (rare)
Nucleus
Nuclear export
Regulation of export (rare)
NPC
Cytosol
Cytoplasmic localization (rare)
Regulation of cytosolic localization
Translation initiation
Regulation of translation initiation
Decapping and mRNA decay
Regulation of mRNA decay
mRNA decay
AMOUNT OF SPECIFIC PROTEIN PRODUCED

Pre-mRNA are associated with hnRNP proteins



Identifying hnRNP proteins

UV high dose - cross linking
Poly dT column from nuclear extract

Many proteins 30-120,000 daltons

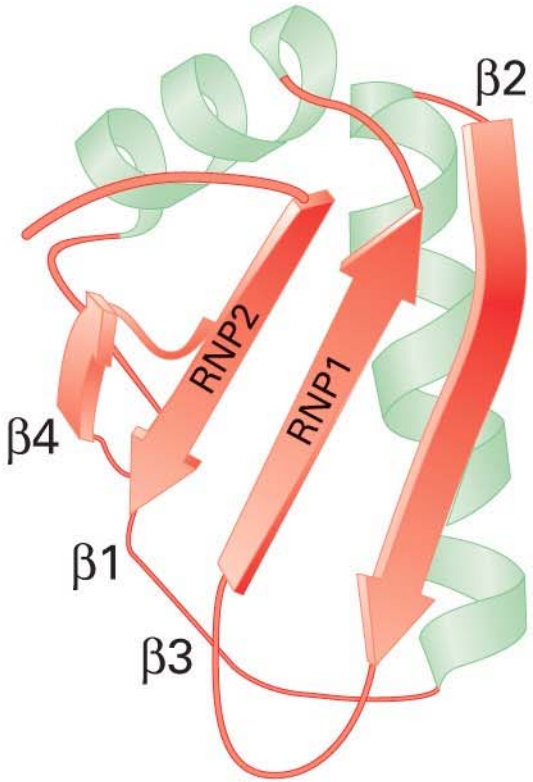
Several proteins are alternatively spliced

Each binds to a 'preferred' site (I.e. 3' of introns)

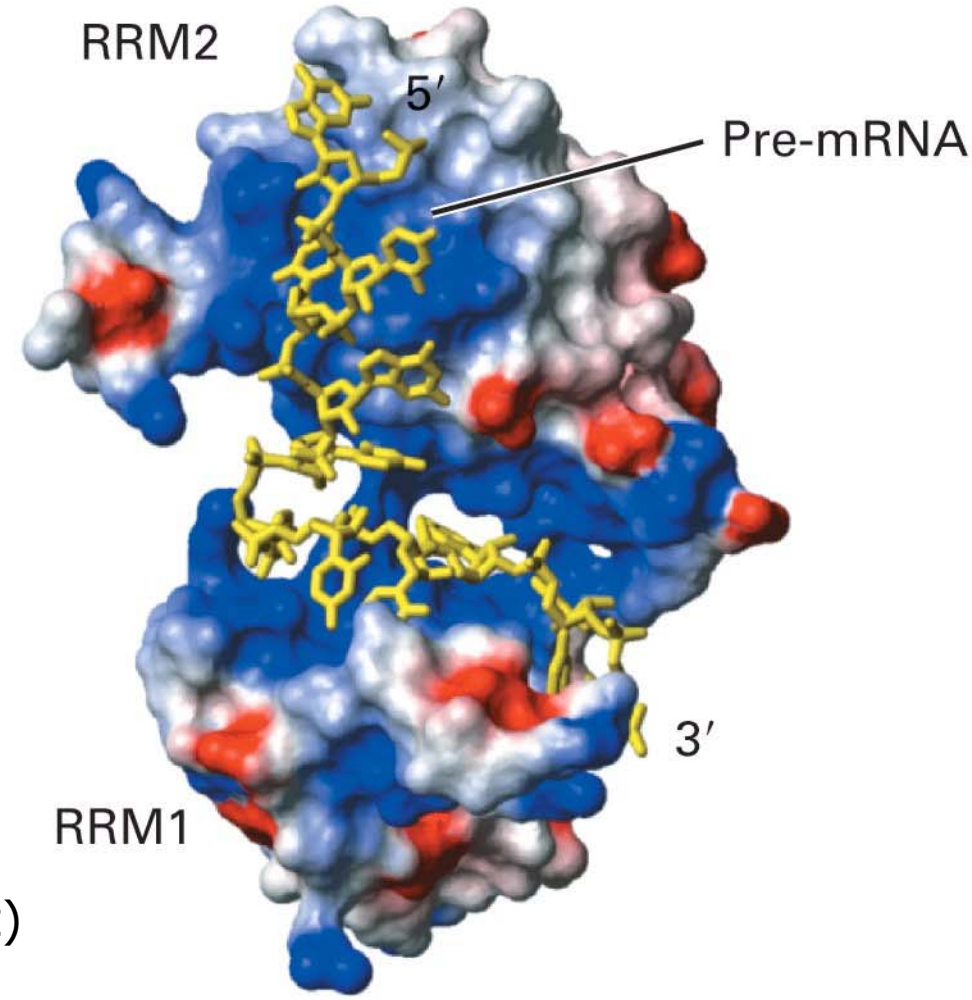
Mostly modular structure

RNP motif (=RBD) - most common

(a) RNA recognition motif (RRM)



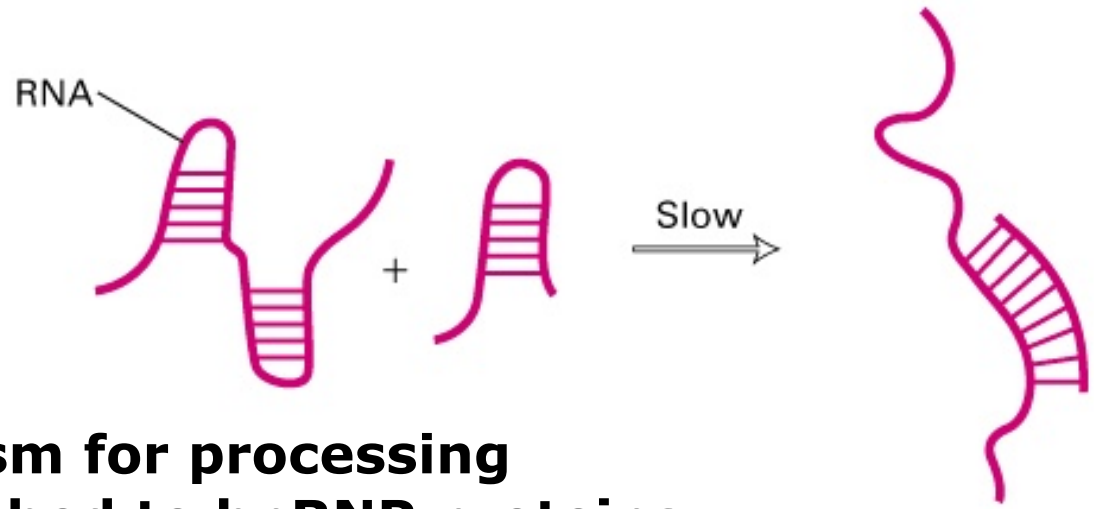
(b) Sex-lethal RRM domains



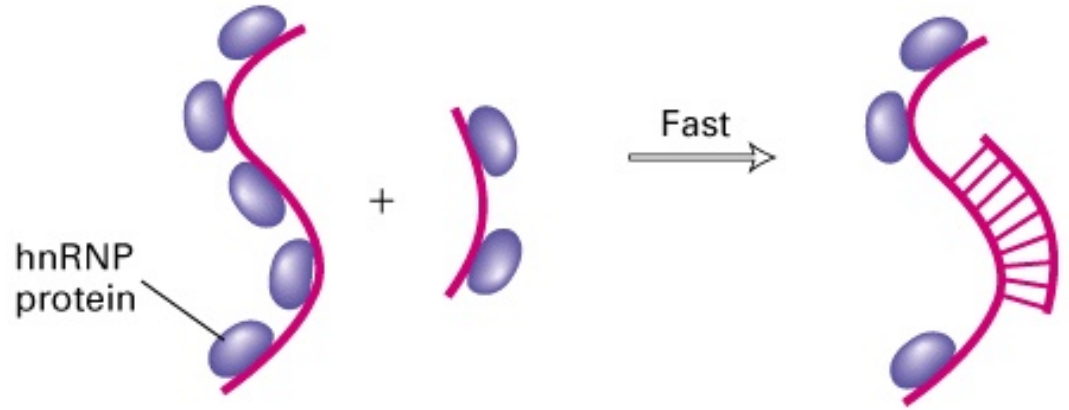
Many Arginine (as for N and Tat)

KH domain (45 aa), in Fragile X- gene (FMR-1)

hnRNP proteins may assist in processing and transport of mRNAs



A 'unified' mechanism for processing
IF hnRNAs are attached to hnRNP proteins



Pre-mRNAs are cleaved at specific 3′ sites and rapidly polyadenylated

All (but histones) have 3′-poly A

The ‘extra’ 3′ transcript very rapidly degraded

What are the signals for endonuclease?

5′- AAUAAA -3′ (10-35 nt upstream)
5′- AUUAAA -3′

If mutated -rapidly degraded

Pre-mRNAs are cleaved at specific 3′ sites and rapidly polyadenylated

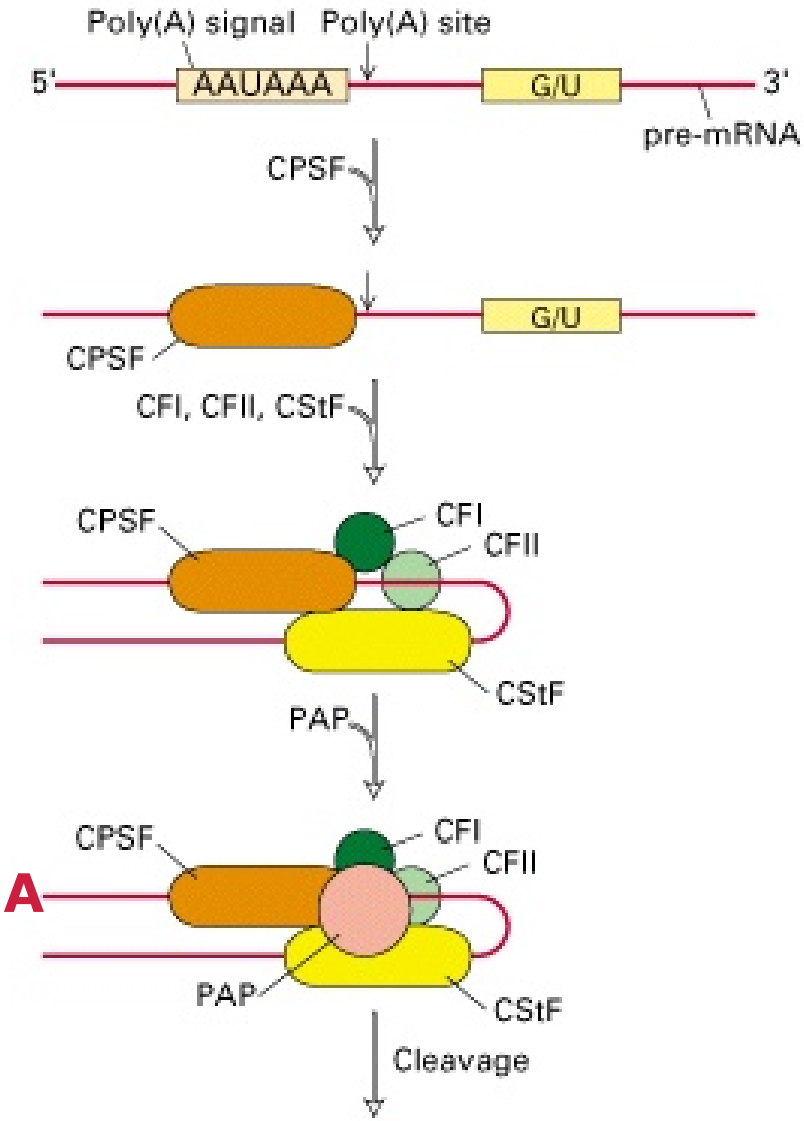
CPSF - Cleavage and Polyadenylation Specificity factor

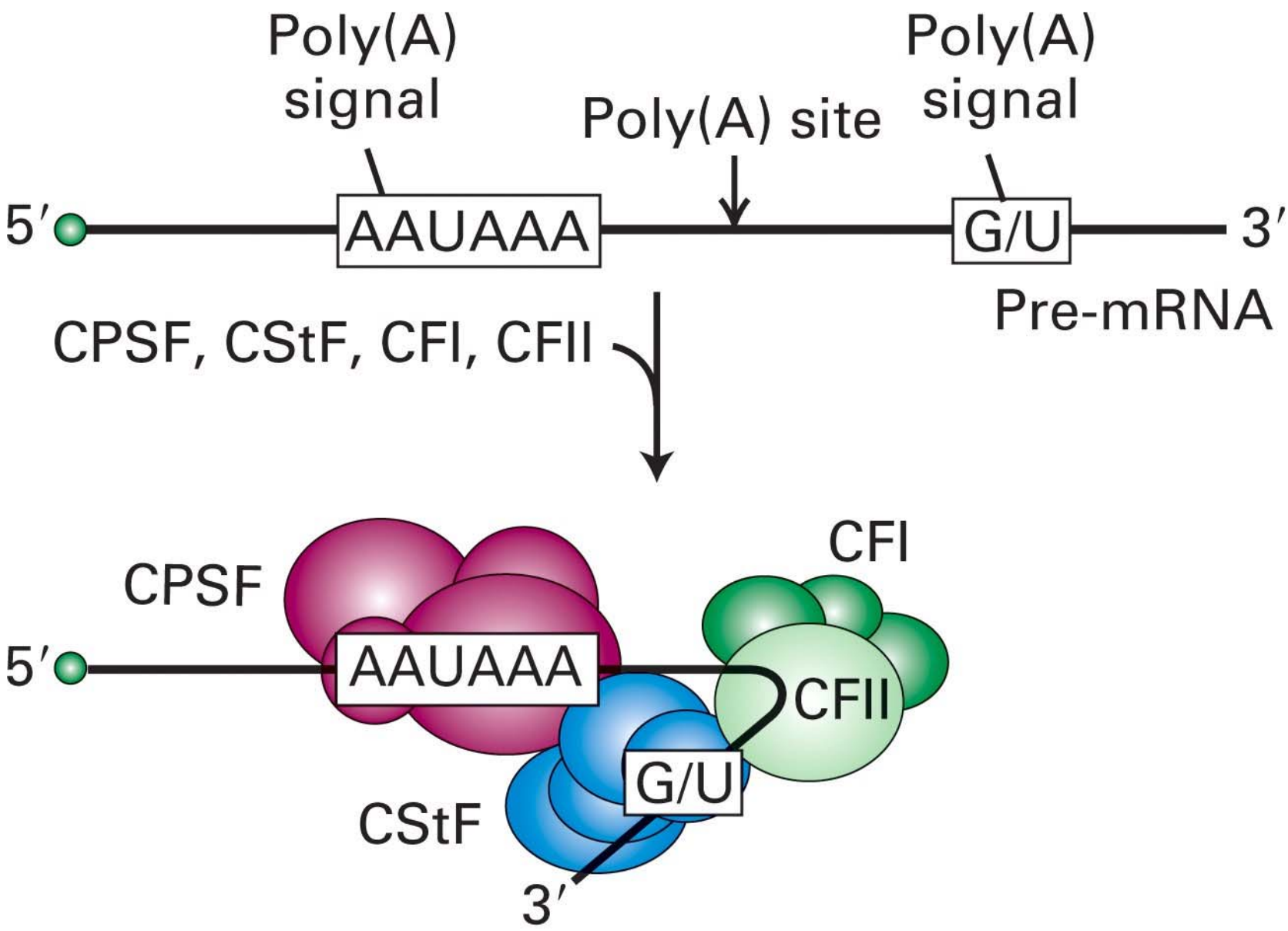
CStF - Cleavage Stimulatory factor

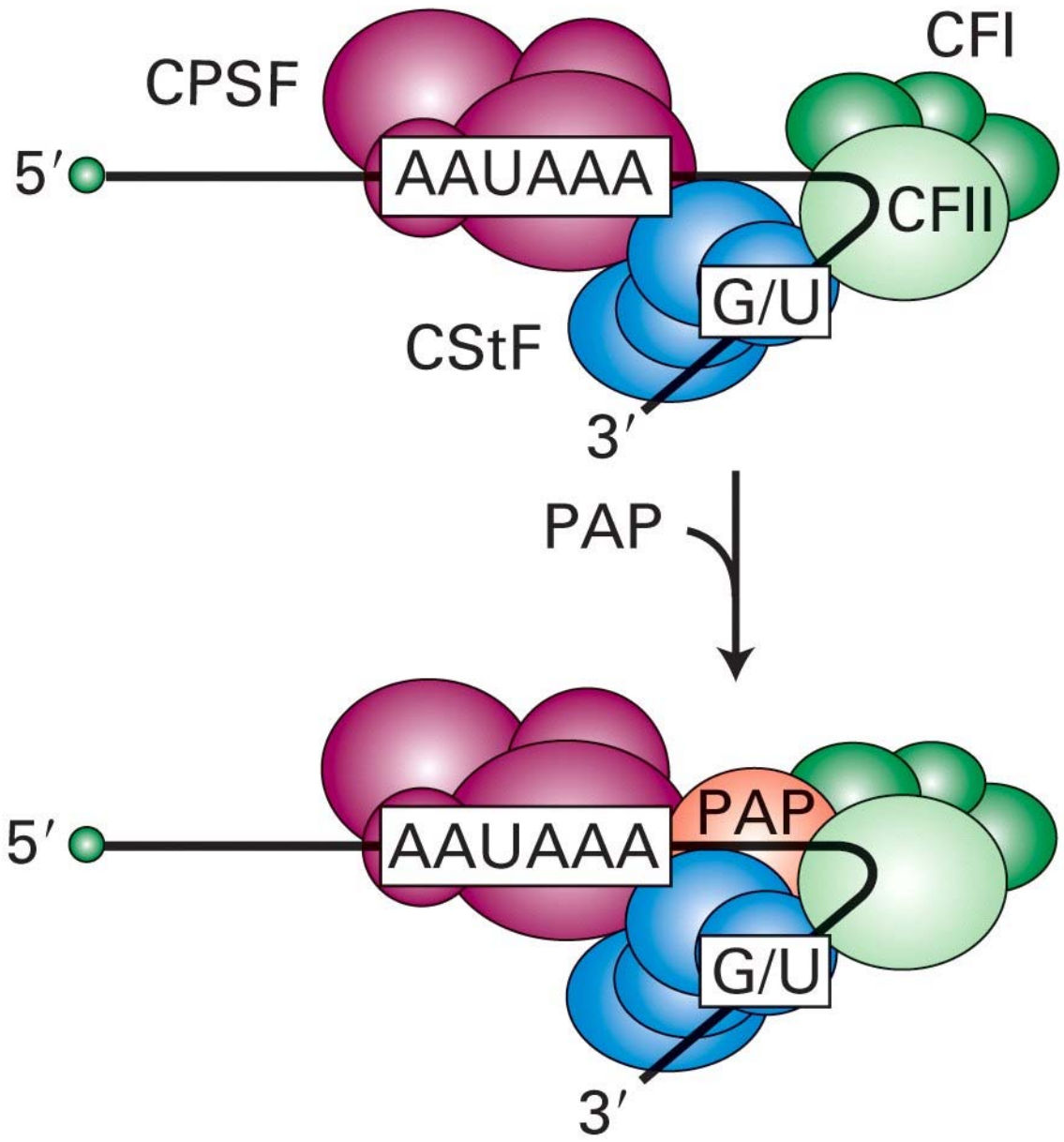
CFI- Cleavage factor I

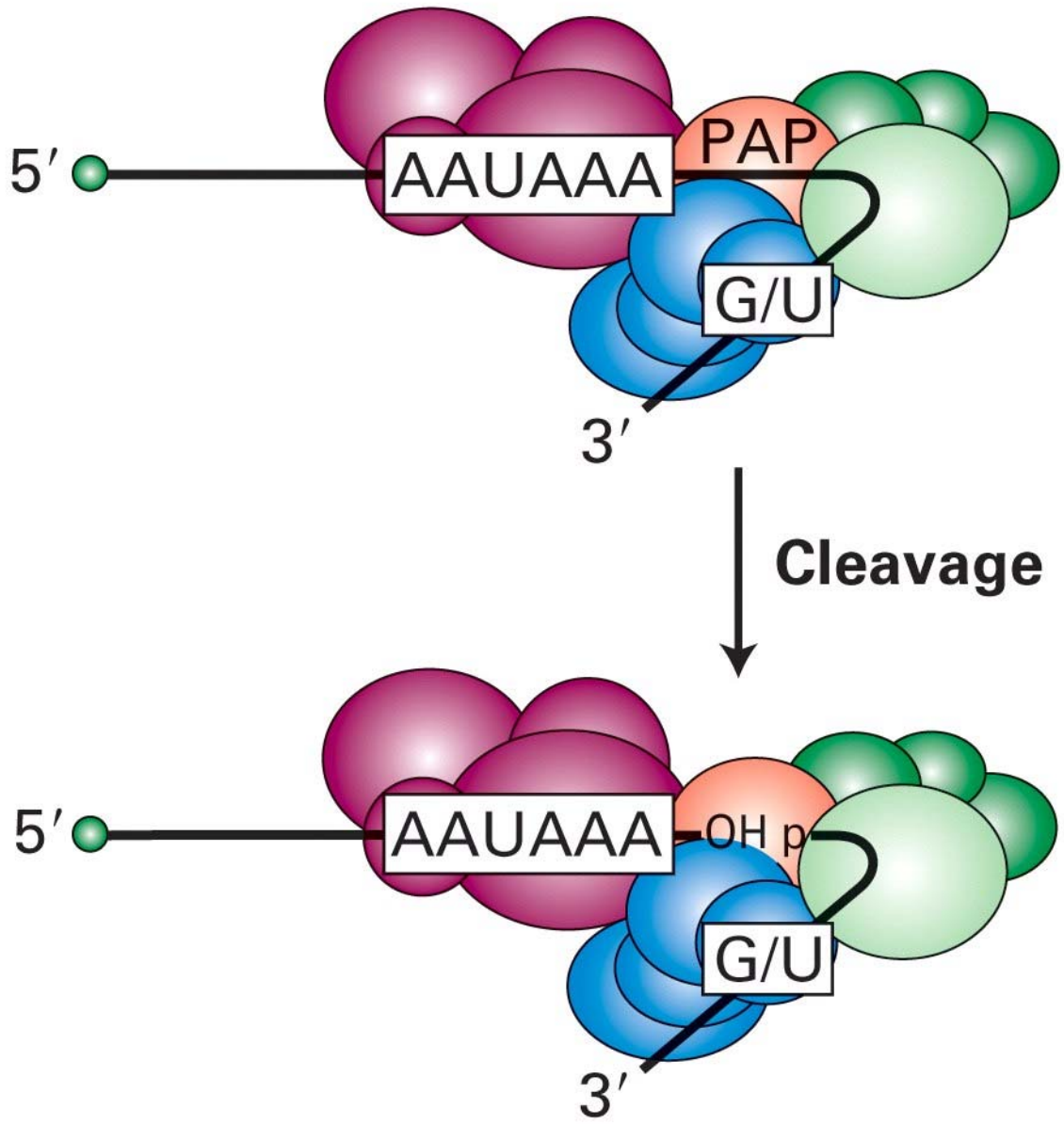
PAP - poly (A) Polymerase

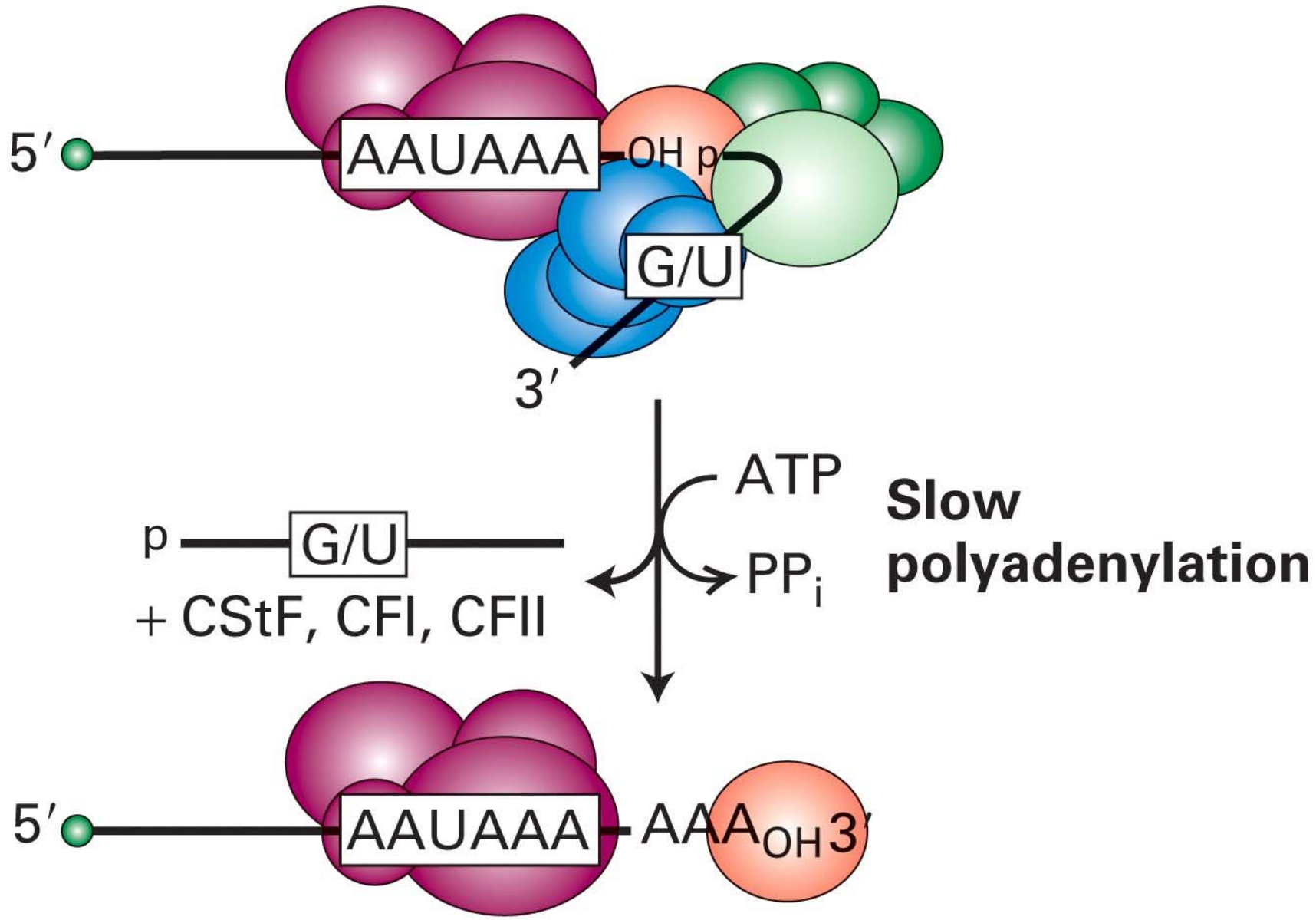
Coupled -cleave+poly A

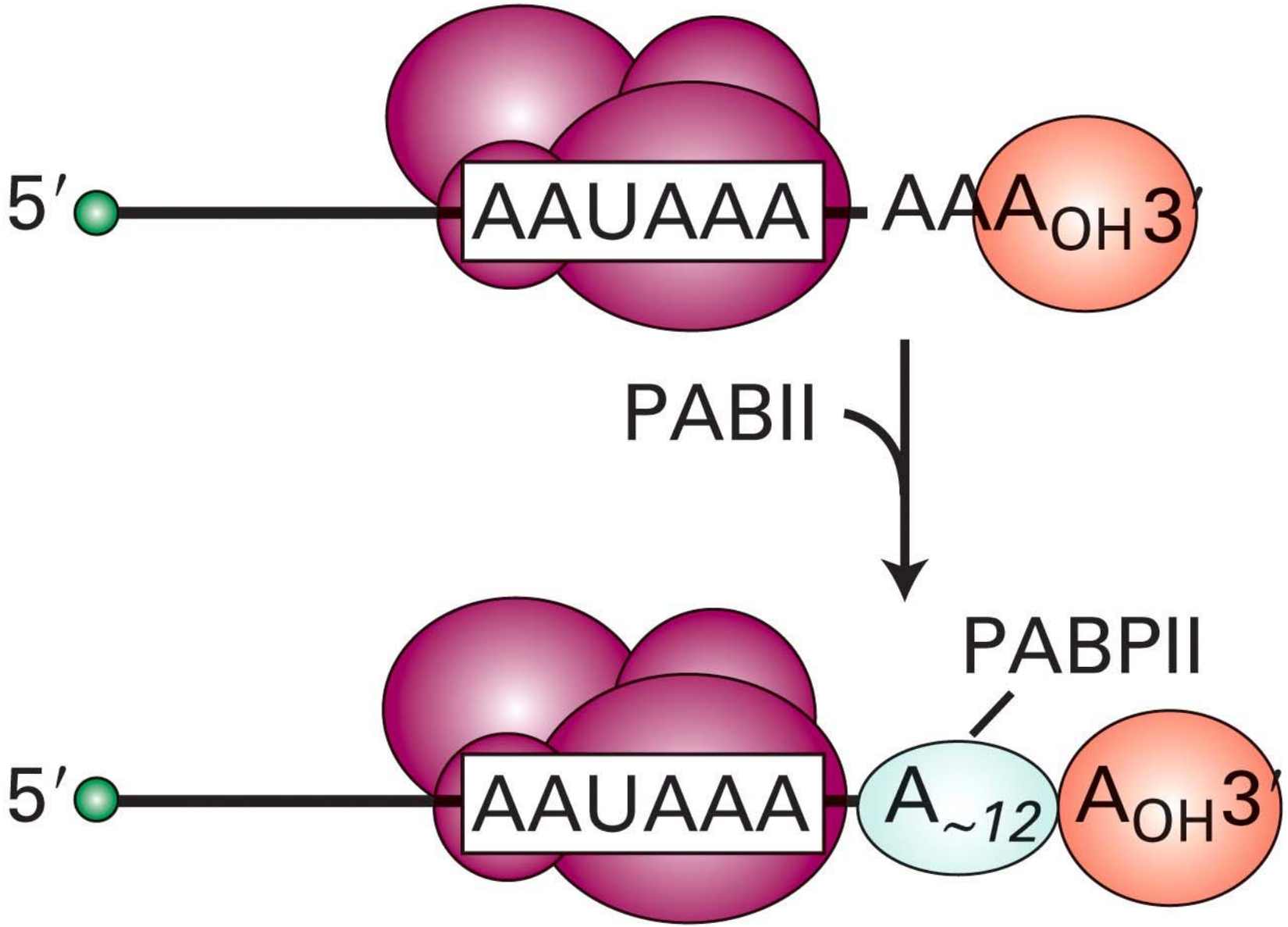


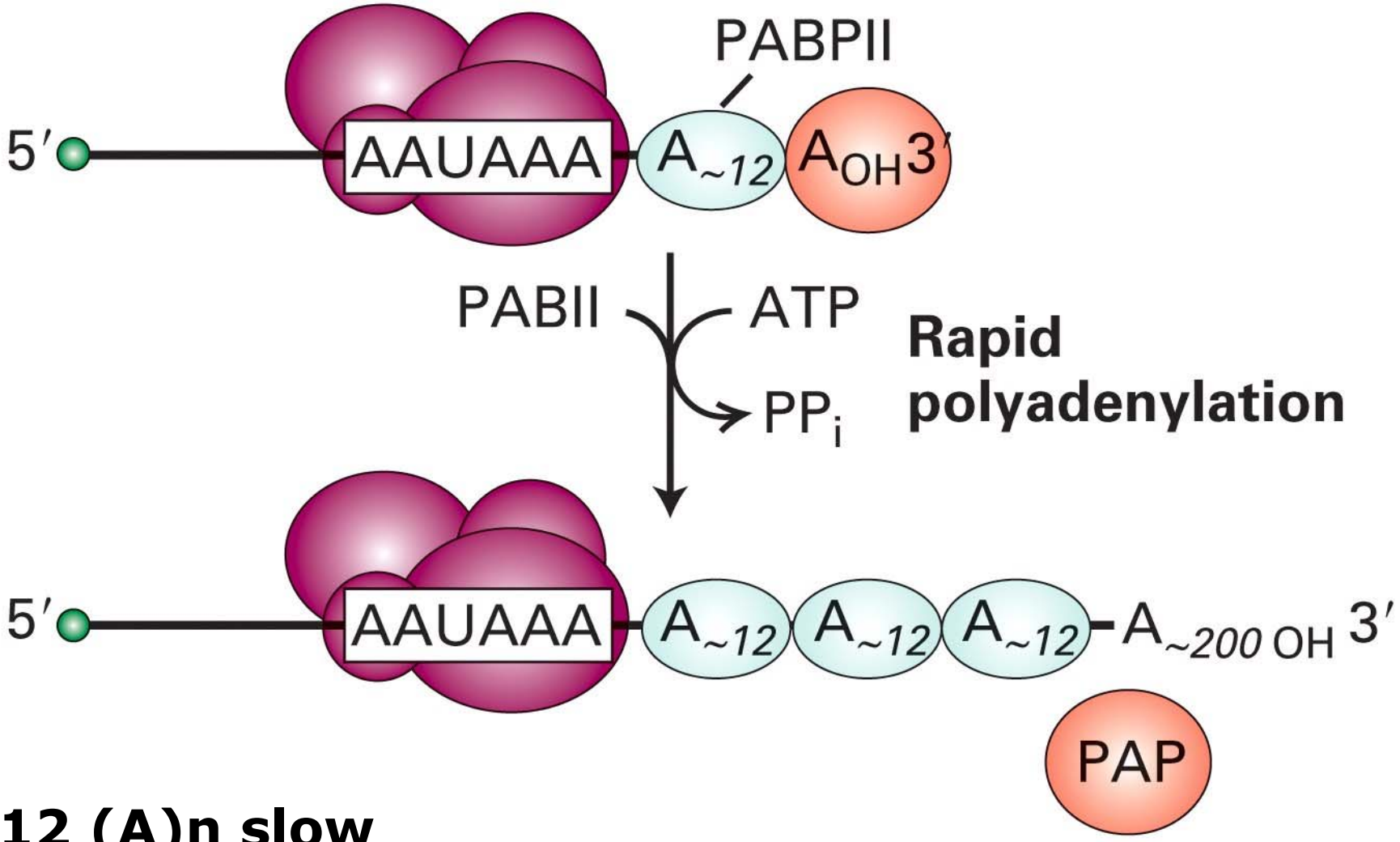

Poly(A)
signal
Poly(A) site
Poly(A)
signal
5′
AAUAAA
G/U
3′
Pre-mRNA
CPSF, CStF, CFI, CFII
CPSF
CFI
5′
AAUAAA
CFII
G/U
CStF
3′


CPSF
CFI
5′
AAUAAA
CFII
G/U
CStF
3′
PAP
5′
AAUAAA
PAP
G/U
3′


5′
AAUAAA
PAP
G/U
3′
Cleavage
5′
AAUAAA
OH p
G/U
3′

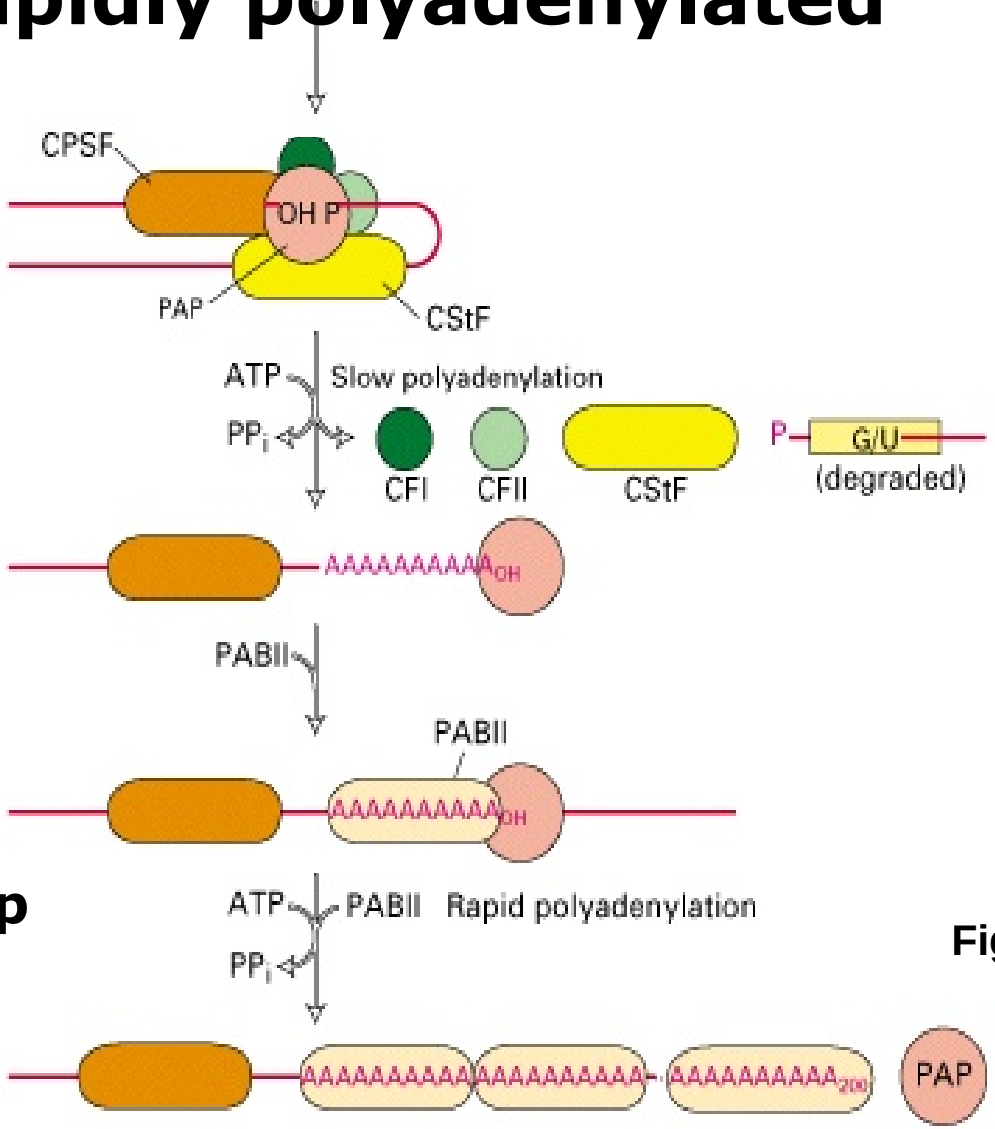

5′
AAUAAA
OH p
G/U
3′
ATP
PPi
Slow
polyadenylation
p
G/U
+ CStF, CFI, CFII
5′
AAUAAA
AAAOH3′


5′
AAUAAA
AAA
OH
3′
PABII
PABPII
5′
AAUAAA
A
~12
A
OH
3′



12 (A)n slow
200-250 (A)n fast

Pre-mRNAs are cleaved at specific 3′ sites and rapidly polyadenylated

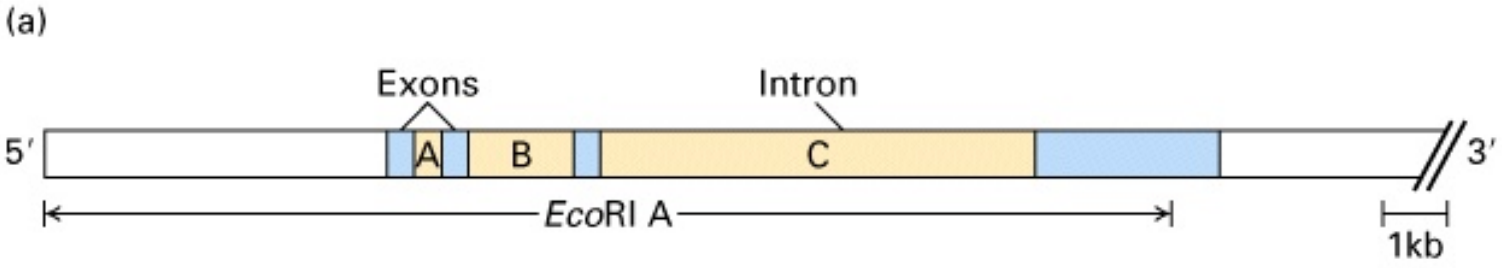


PABII (in nuclei)

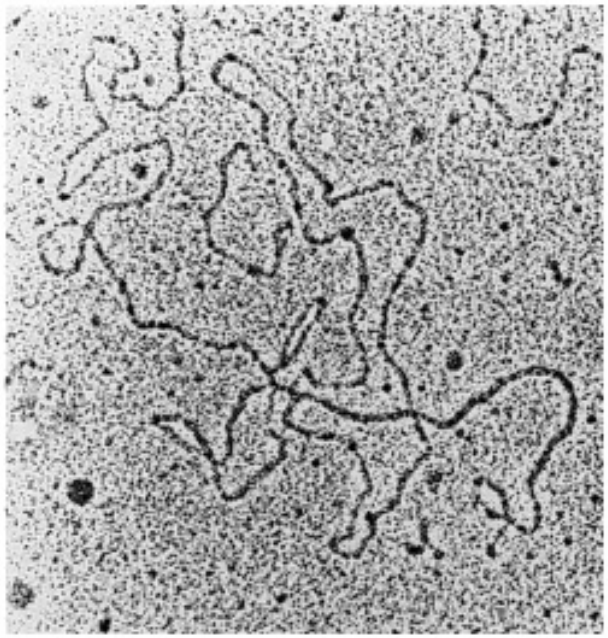
Signal for PAP to Add (A) and to stop

Figure 11-12

During the final step in formation of mature, functional mRNA, introns are removed and exons are spliced together



(b)



Adenovirus- Capsid

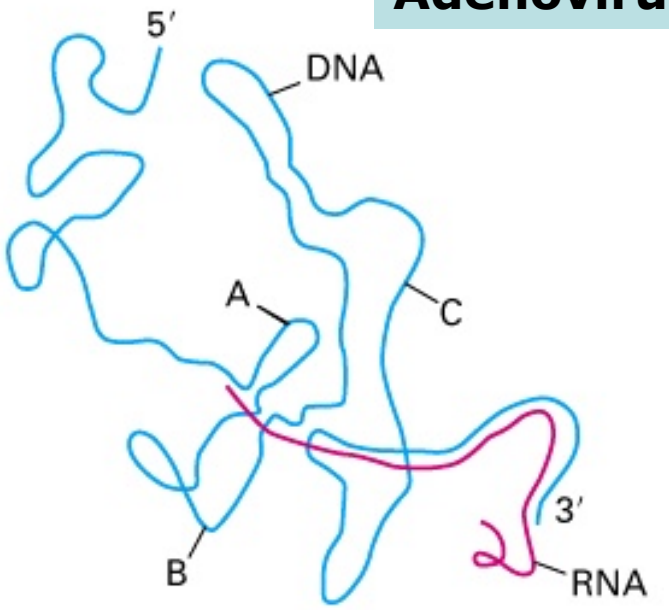
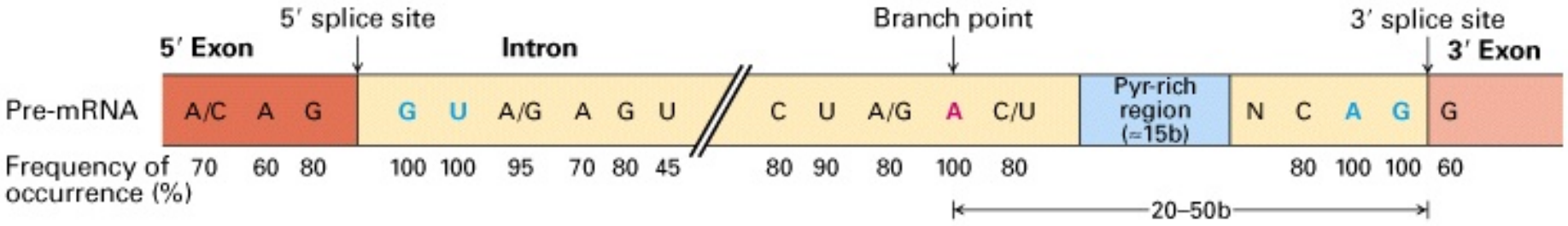


Figure 11-13

Splicing occurs at short, conserved sequences

Consensus sequences around 5′ and 3′ splice sites in vertebrate pre-mRNA



How to determine the borders??
Genomic - cDNA ESTs…

Fusion construct with half introns from genes - perfect product

Splicing mechanism
splicing type I, II, tRNA and mRNA

Primary transcript

Most dramatic processing -mRNA (euk) tRNA (euk+pro)

The introns -1977 Philip Sharp Richard Roberts

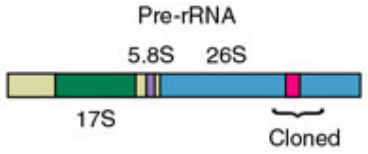
Exons <1000 nt (ave. 100-200 nt)

Intron up to 20,000 and more, some are 60 only

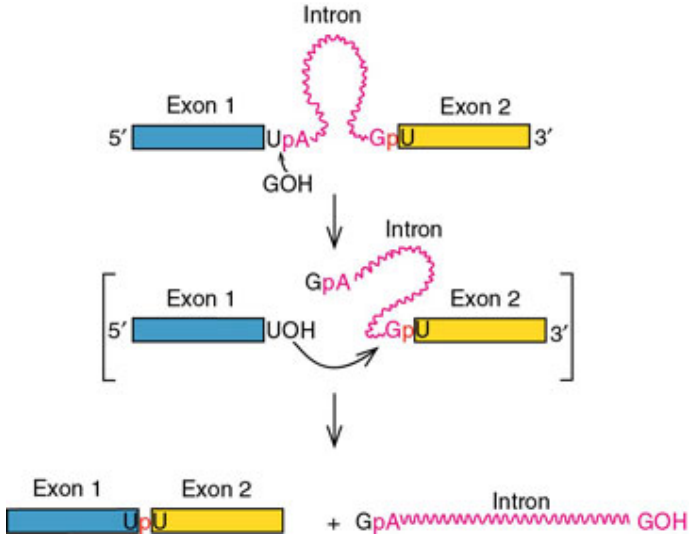
Splicing mechanism
splicing type I, II, tRNA and mRNA

Type I

Type 1 - Pre-rRNA
Mitochondria, chloroplast tRNA, rRNA, mRNA



Use GMP for 'creating OH end'
No energy (ie ATP)
Ligation



Type 2 - mitochondria mRNA -
Fungi, algae, plant

Also rare in bacteria

Self-splicing group I introns were the first examples of catalytic RNA

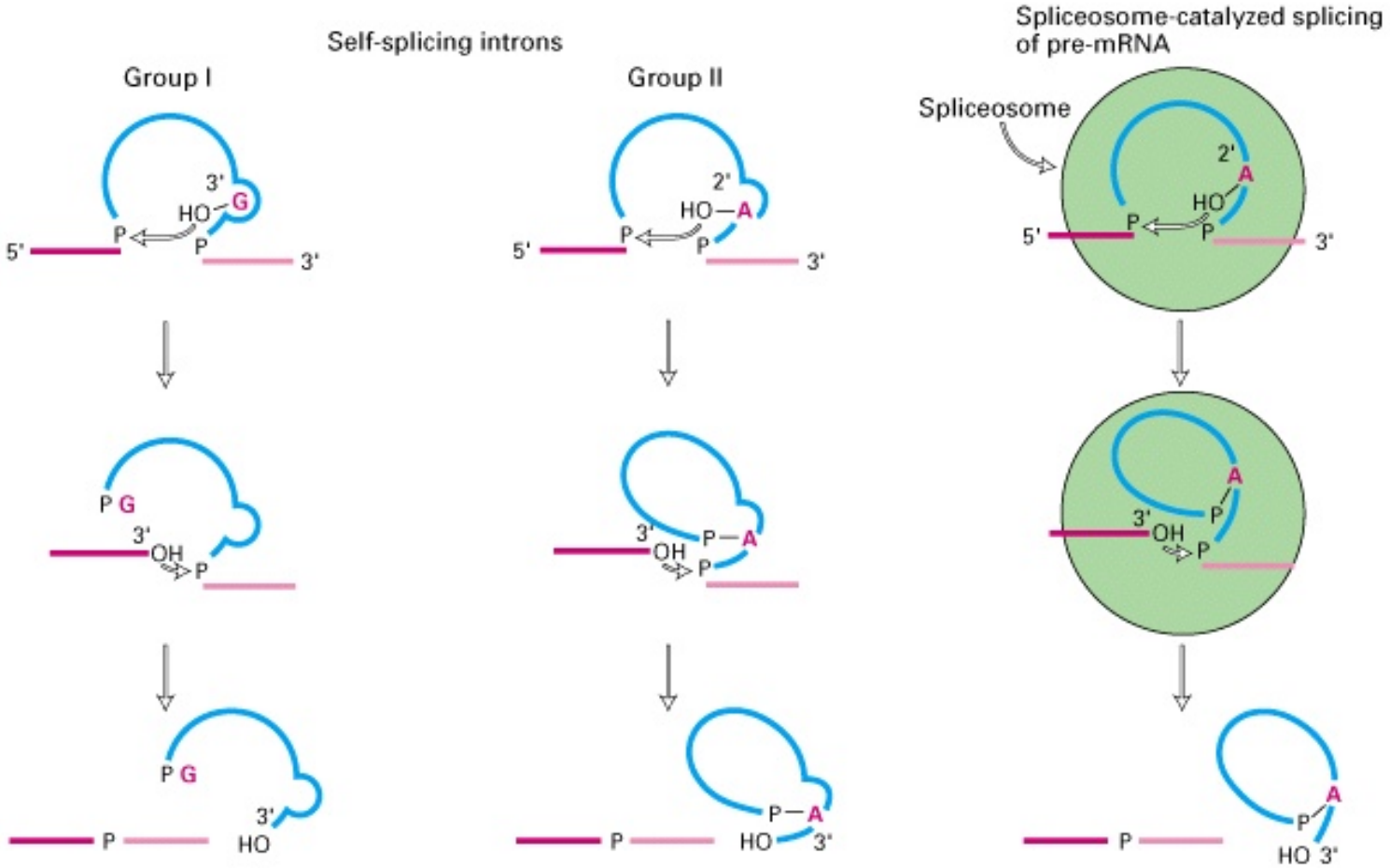


Figure 11-51

A with 3 phosphoester bond

All pre-tRNAs undergo cleavage and base modification

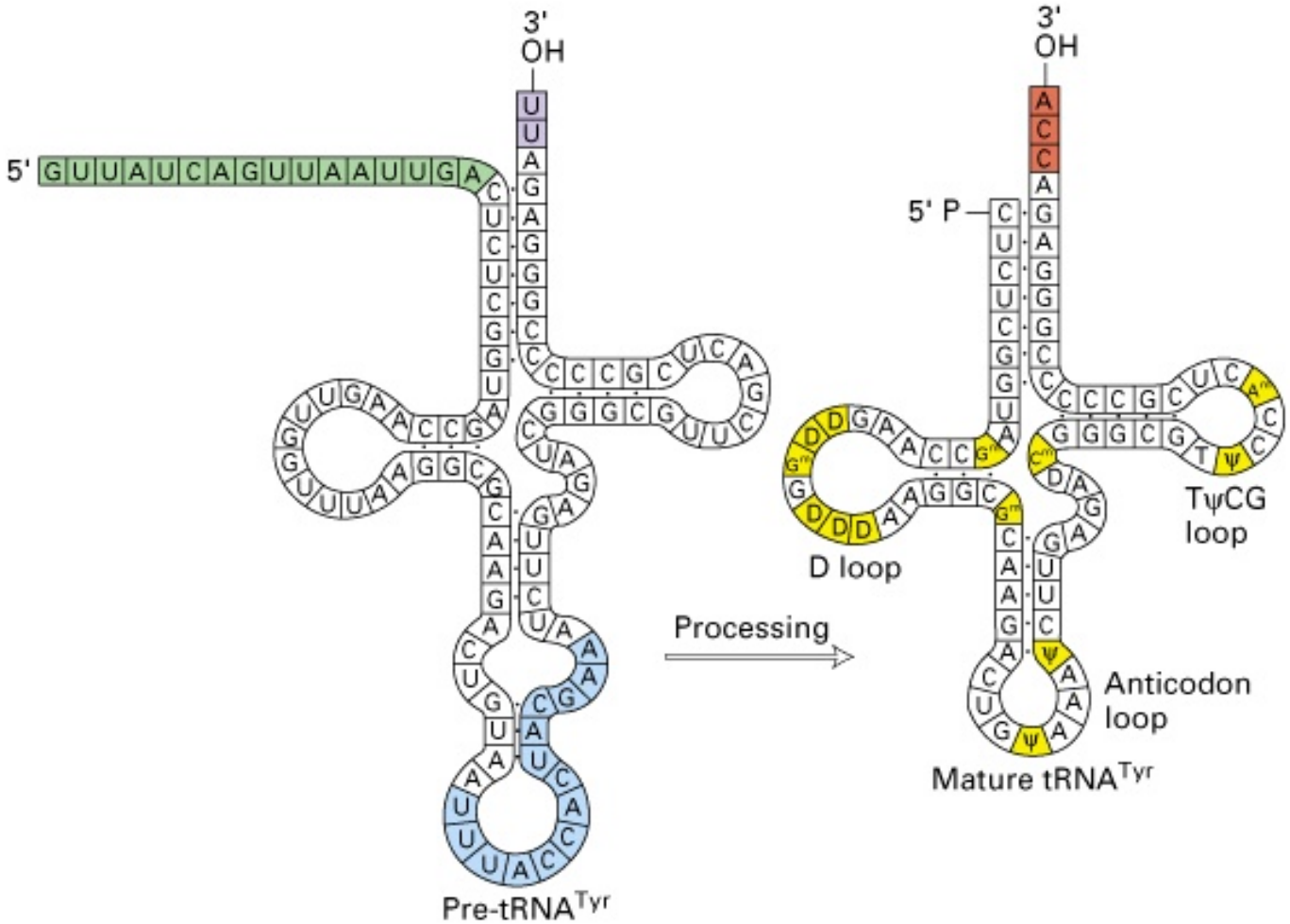
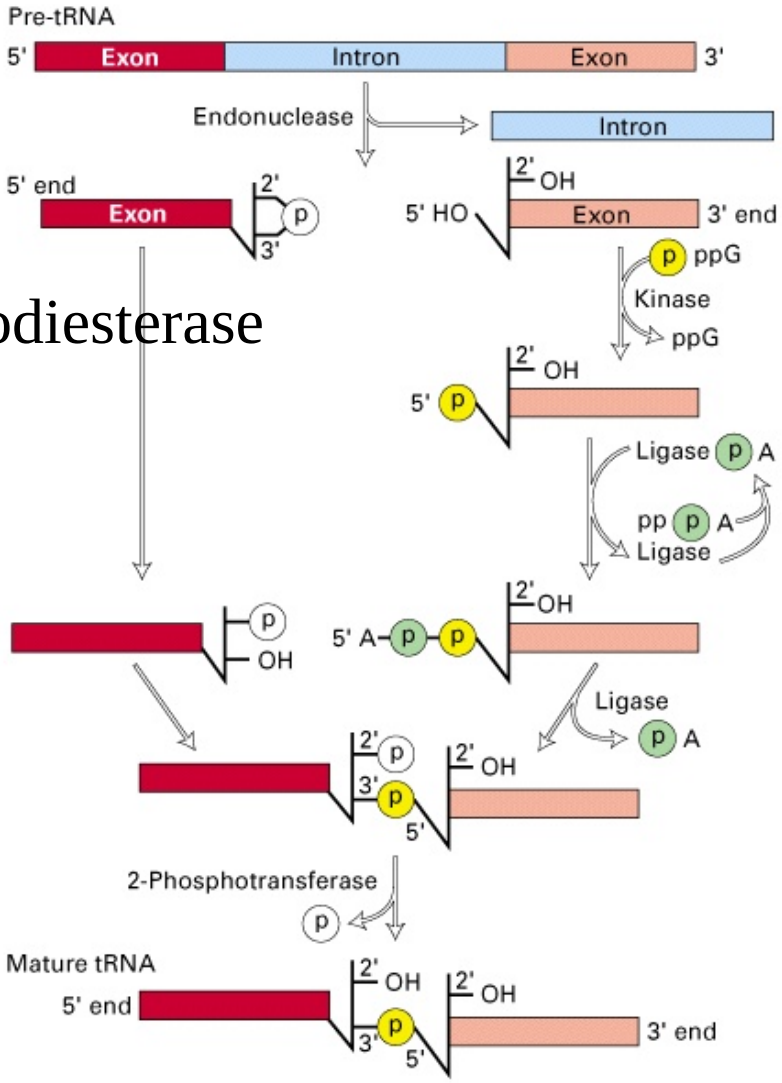


Figure 11-52

Splicing of pre-tRNAs differs from other splicing mechanisms

2-3 cyclic
nucleotide phosphodiesterase



The splicing mechanisms type I, II

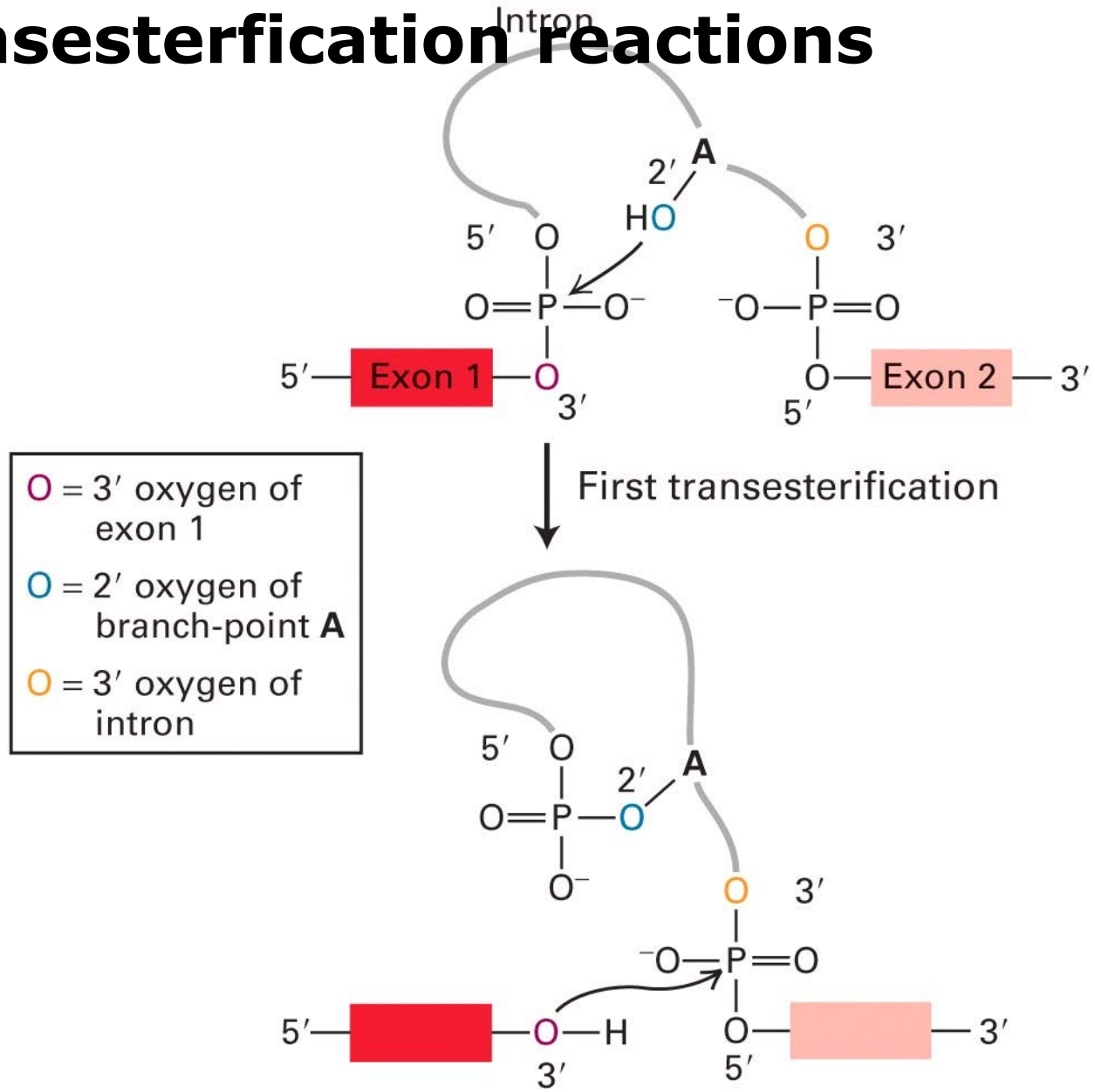
The introns are Self splicing!! T. Cech, 1982

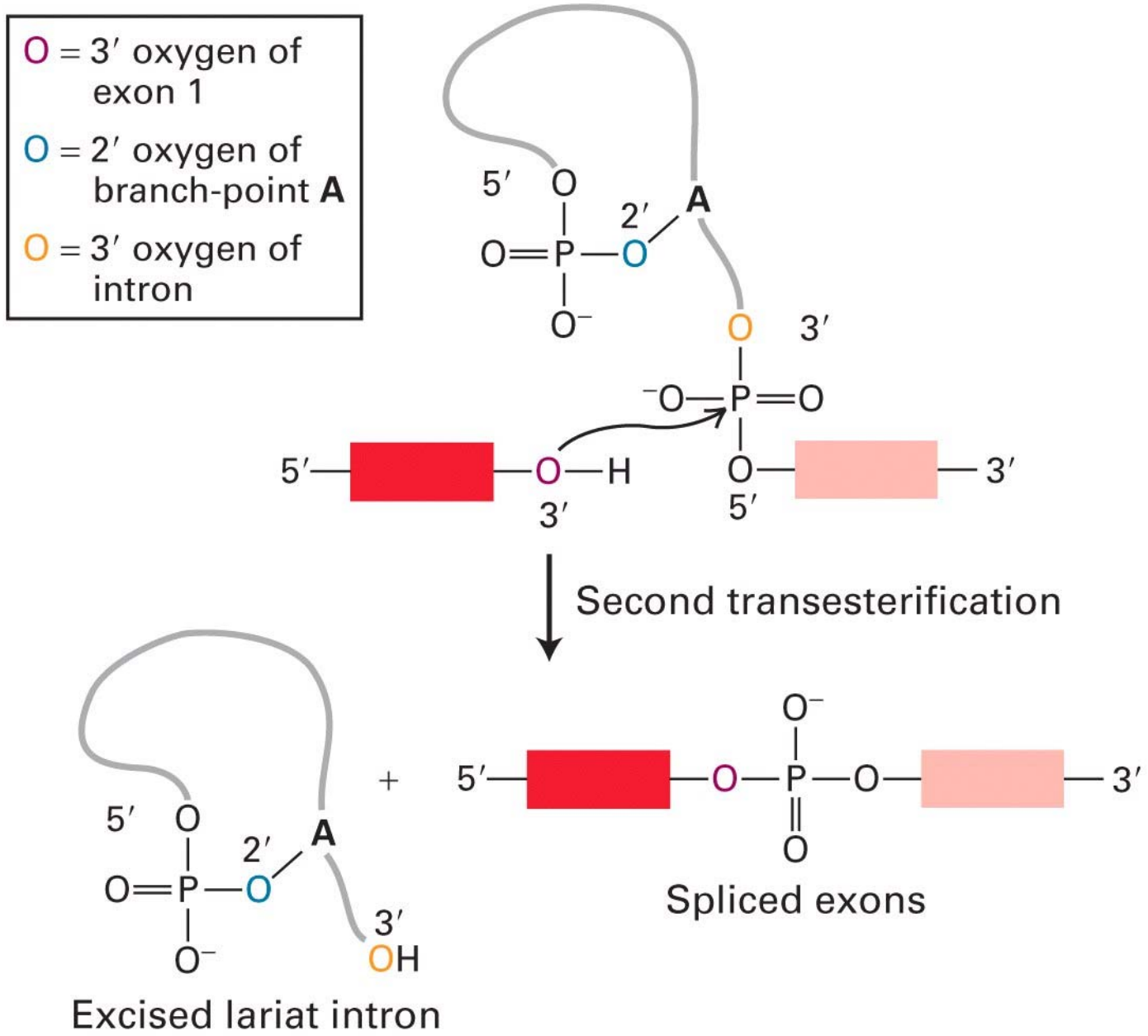
No proteins are involved

Isolated DNA of protozoa - with intron +RNAp (bacteria) resulted in spliced RNA

In mRNA eukaryotes- with the aid of RNA-protein Complex - small nuclear ribonucleoproteins (snRNPs)

Splicing proceeds via two sequential transesterfication reactions




O = 3′ oxygen of exon 1
O = 2′ oxygen of branch-point A
O = 3′ oxygen of intron
5′
O
2′
A
O
P
O
O⁻
O
3′
⁻O
P
O
5′
O
H
3′
O
5′
3′
Second transesterification
5′
O
2′
A
O
P
O
O⁻
3′
OH
+
O⁻
5′
O
P
O
O
3′
Excised lariat intron
Spliced exons

Analysis of RNA products formed in an in vitro splicing reaction

Lariat Structure

Branch point

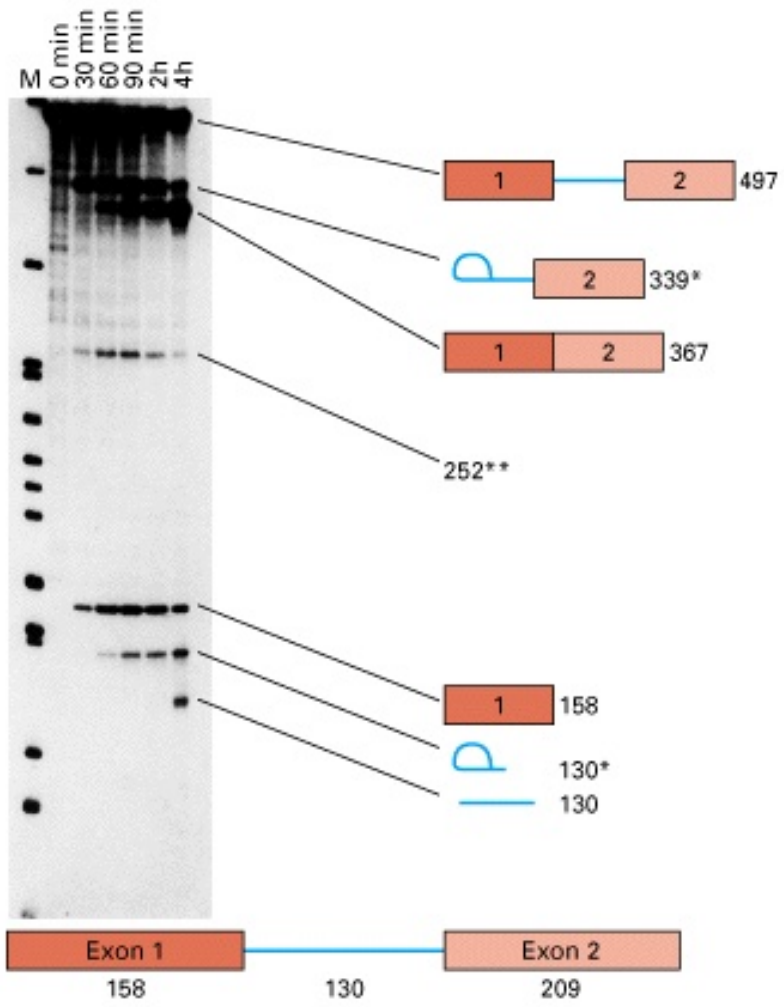
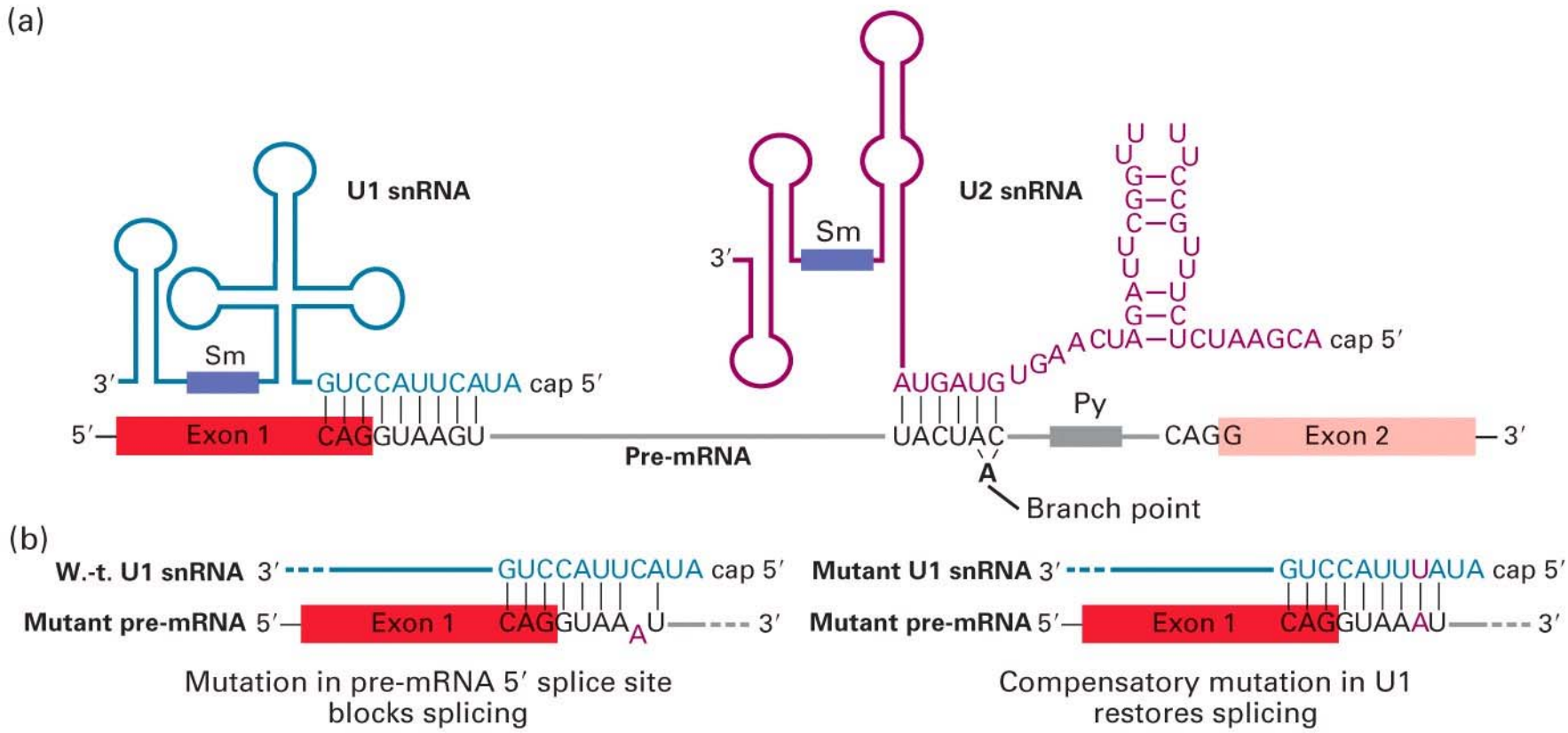
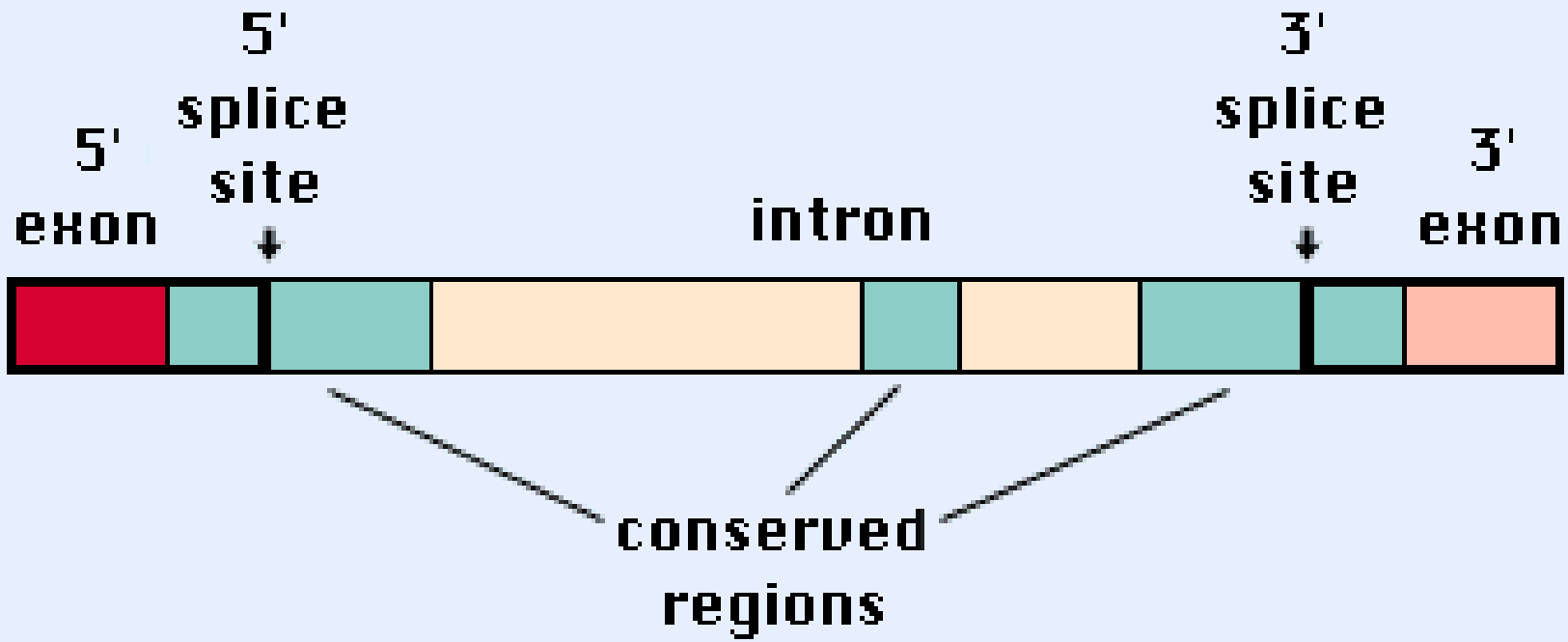


Figure 11-15

Small nuclear RNAs (snRNAs) assist in the splicing reaction




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

The spliceosomal splicing cycle