Transcription termination in eukaryotes

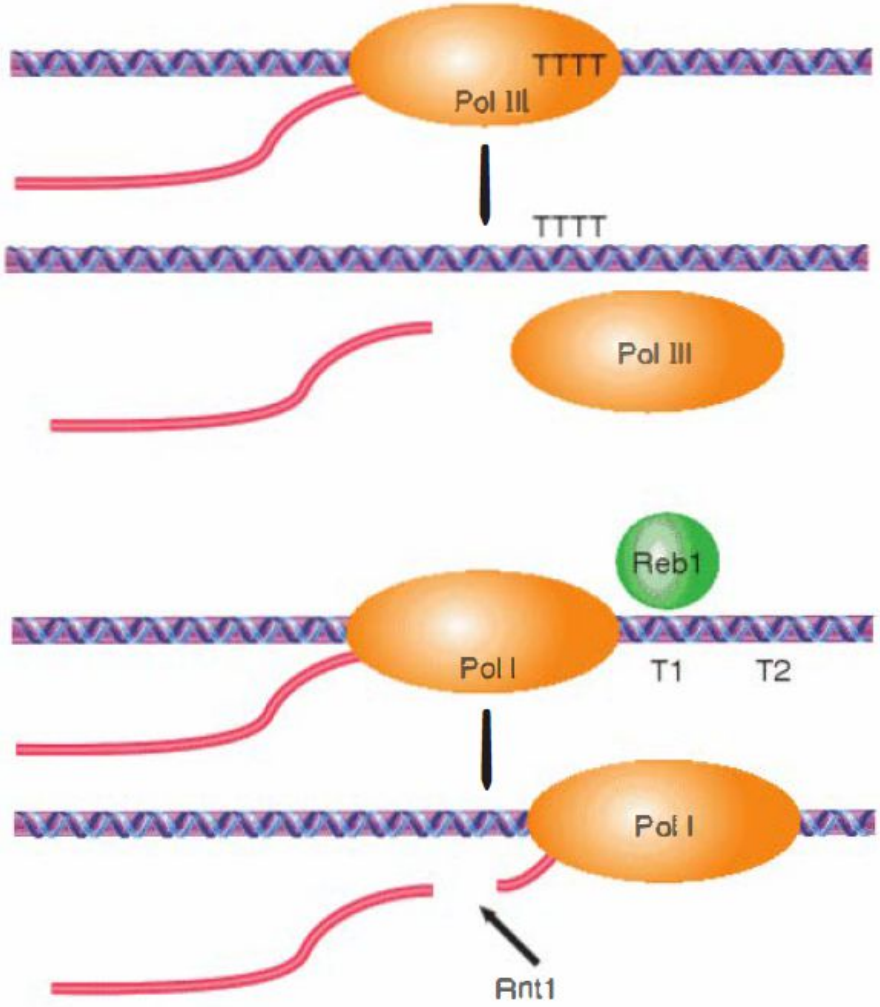


FIGURE 21.31 Transcription by Pol III and Pol I uses specific terminators to end transcription.

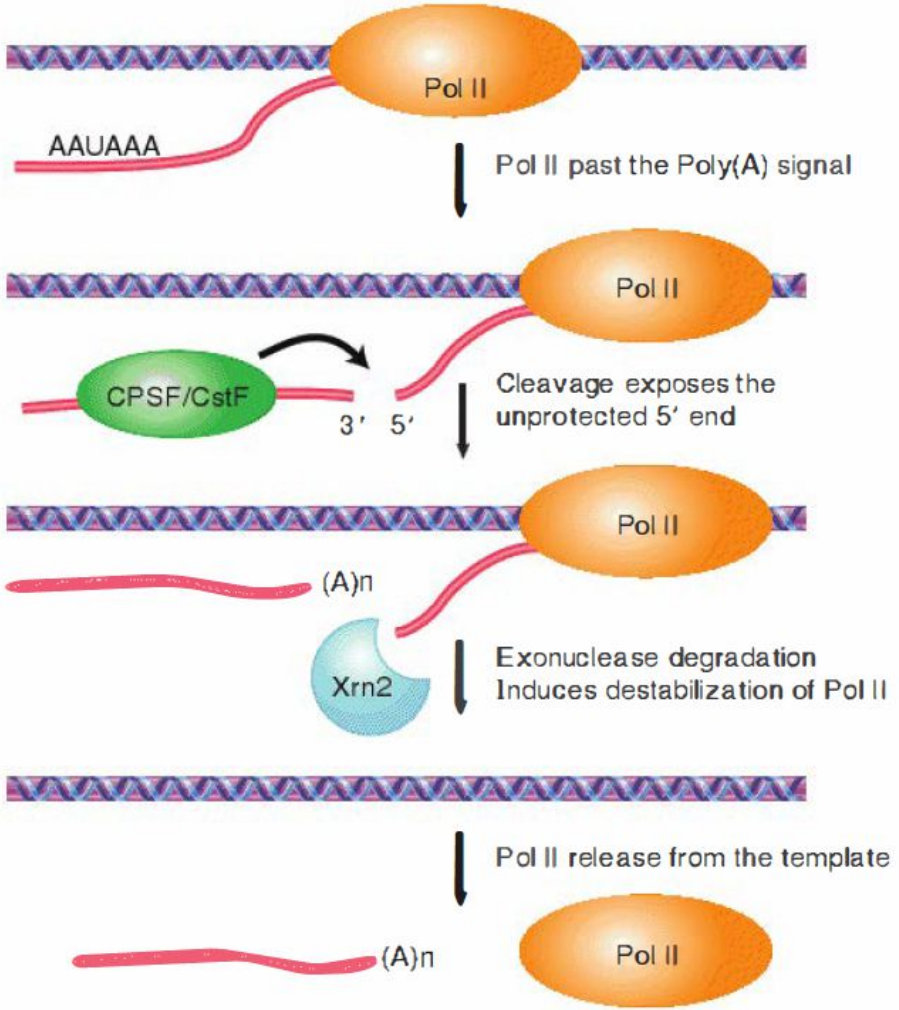


FIGURE 21.32 3' end formation of Pol II transcripts facilitates transcriptional termination.

PolI e III

PolII

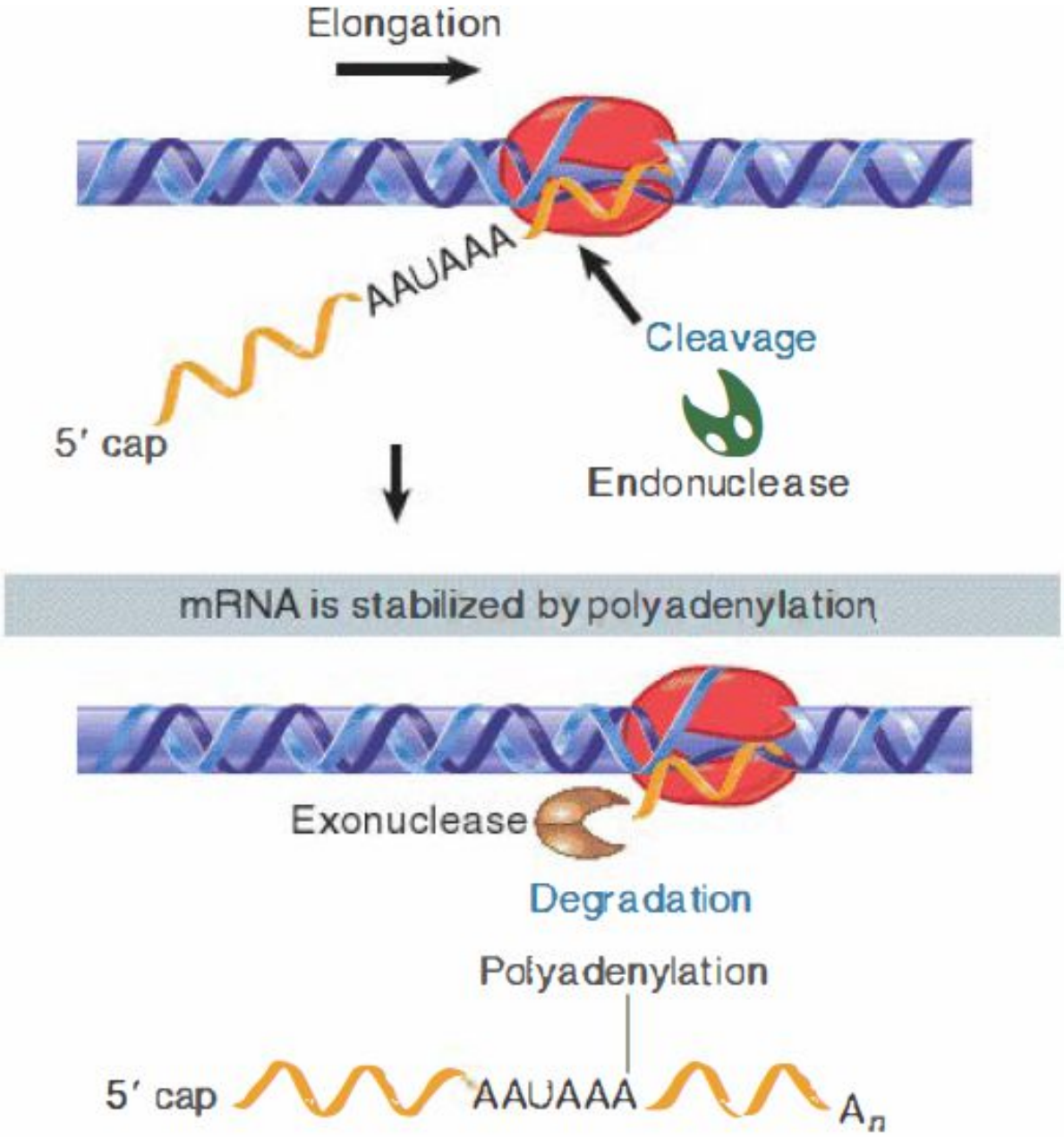
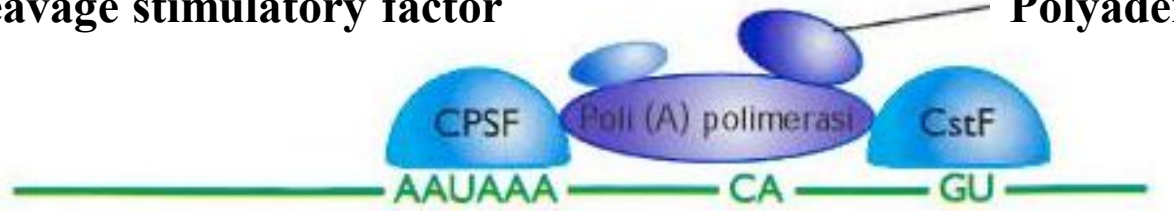


FIGURE 21.29 The sequence AAUAAA is necessary for cleavage to generate a 3′ end for polyadenylation.

Polyadenylation of eukaryotic mRNA

Polyadenylation complex assembly

CPSF → Cleavage polyadenylation stimulating factor
CstF → Cleavage stimulatory factor



Polyadenylation

EdiSES

CPSF recognizes the seq. *AAUAAA*

CstF binds GU, stimulates CPSF

CFI, CFII endonuclease

PAP Poly(A) Polymerase

PABP Poly(A) Binding Protein

Limits the addiction of A from the polymerase at about 200 bp.

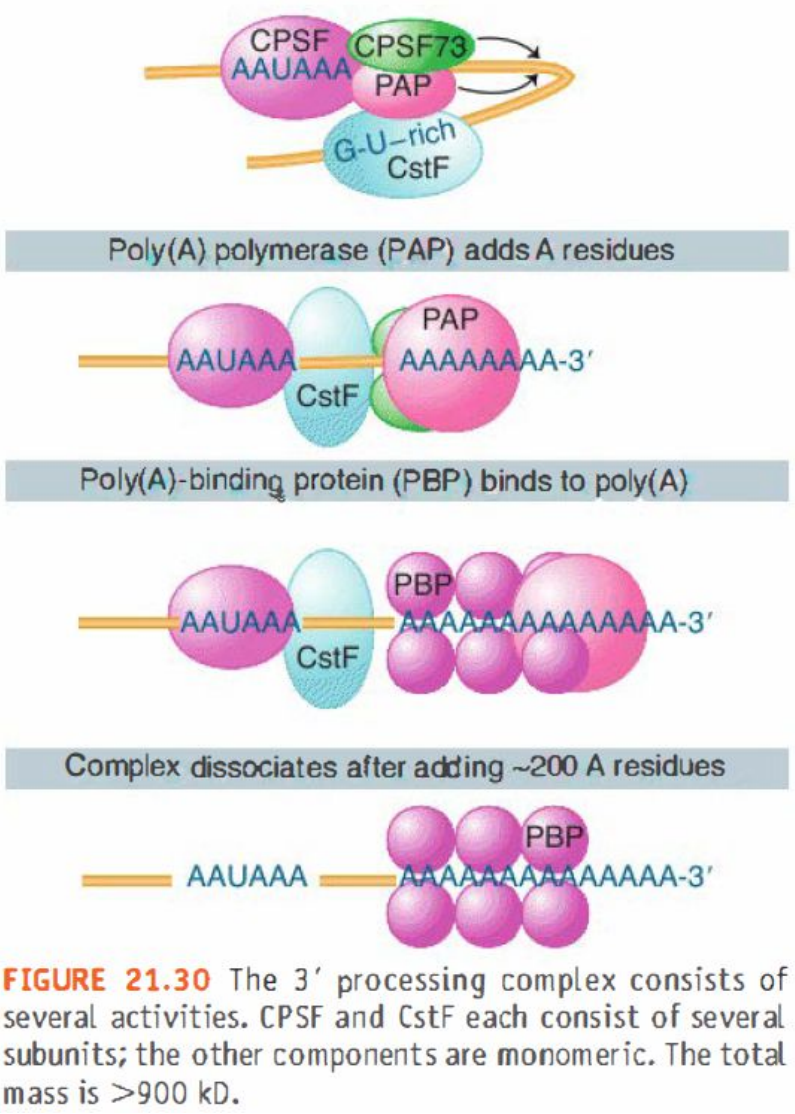


FIGURE 21.30 The 3′ processing complex consists of several activities. CPSF and CstF each consist of several subunits; the other components are monomeric. The total mass is >900 kD.

Maturation (processing) of RNA

Ribosomal RNA

About 80 % of the total RNA in growing cells is made up by ribosomal RNA.

It is synthetized starting from a precursor (pre-rRNA) which undergoes a series of changes up to the final mature form.

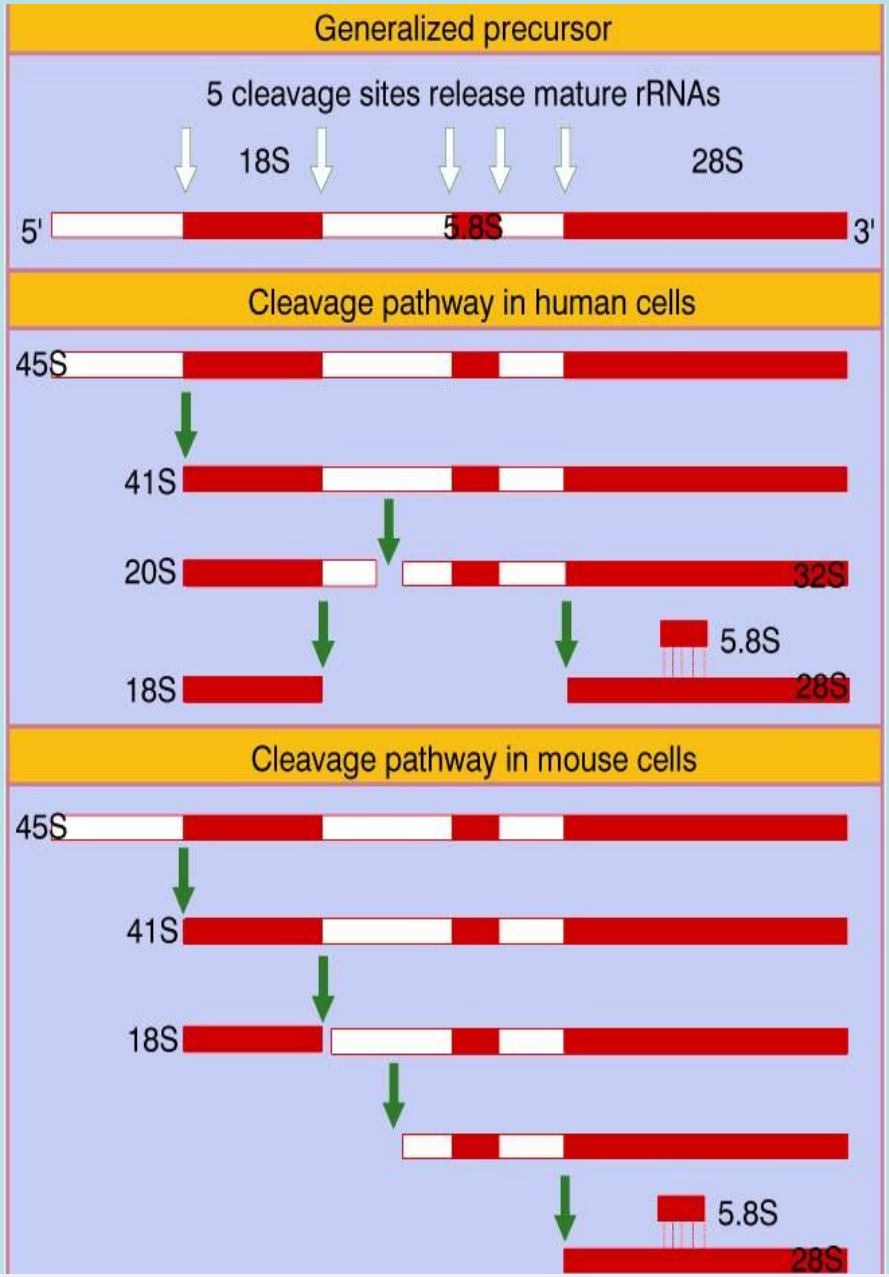


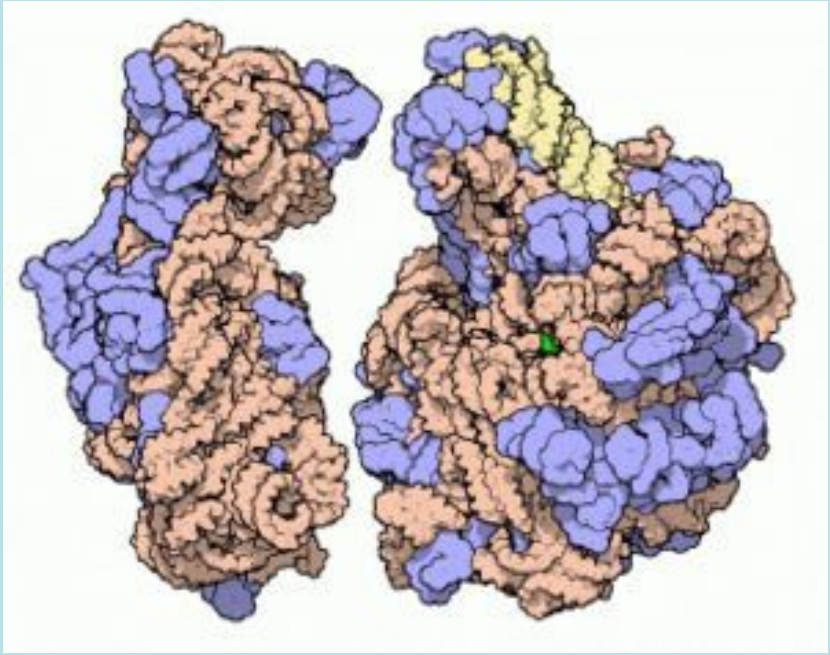
Figure 10.50 Mature rRNAs are released from the 45S mammalian precursor by cleavages within each spacer region.

Light subunit

- **18S**

Heavy subunit

- **28S + 5.8S**
- **5S** (from e different trancription unit)



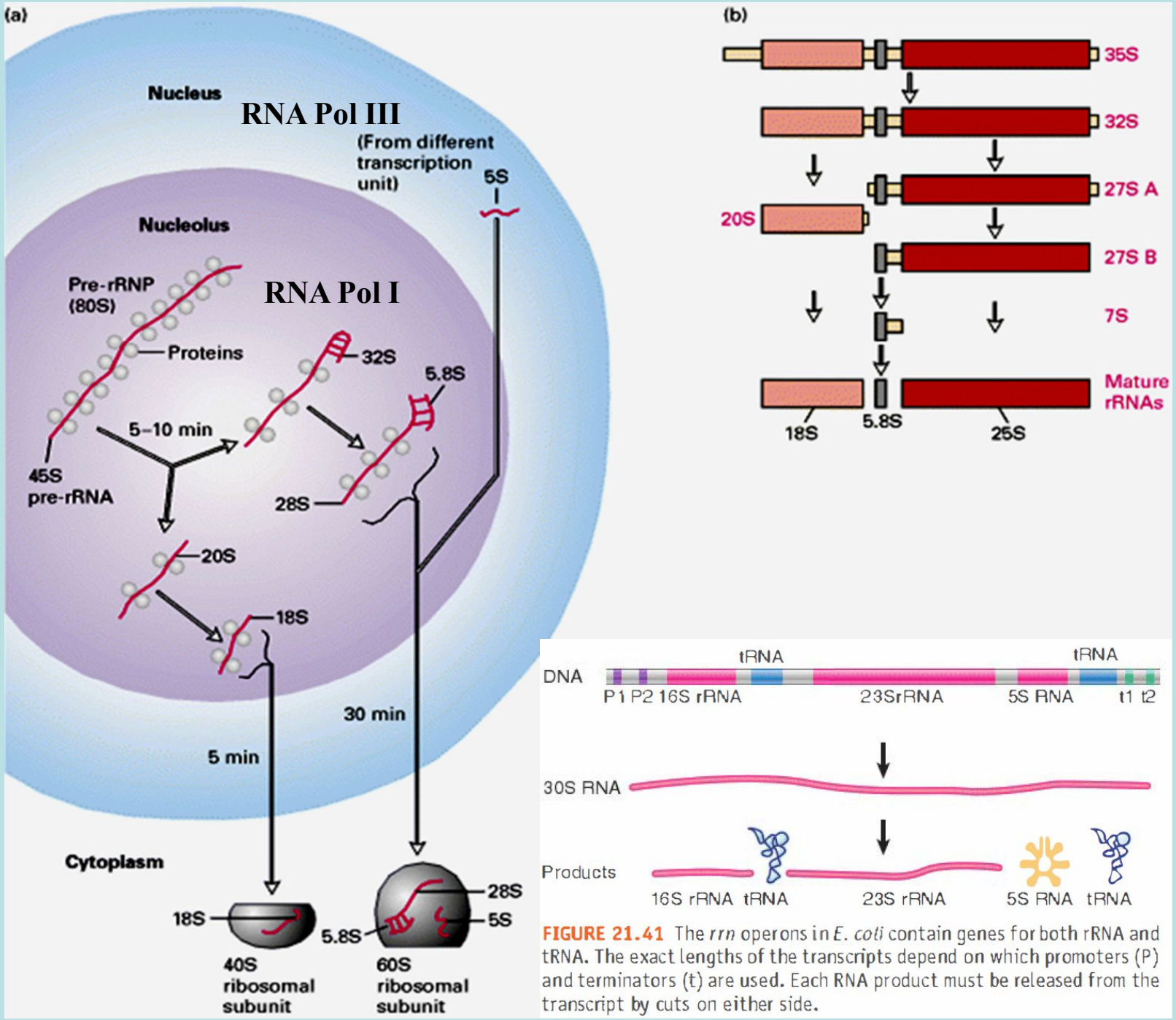


FIGURE 21.41 The *rrn* operons in *E. coli* contain genes for both rRNA and tRNA. The exact lengths of the transcripts depend on which promoters (P) and terminators (t) are used. Each RNA product must be released from the transcript by cuts on either side.

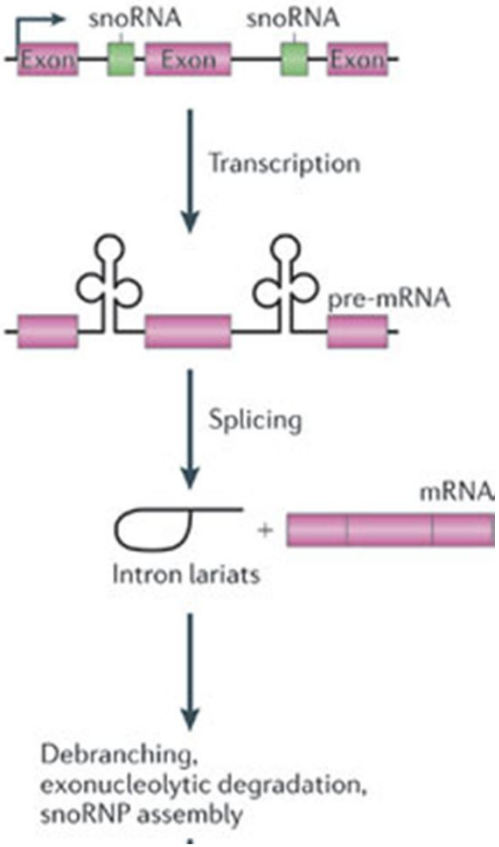
rRNA modifications

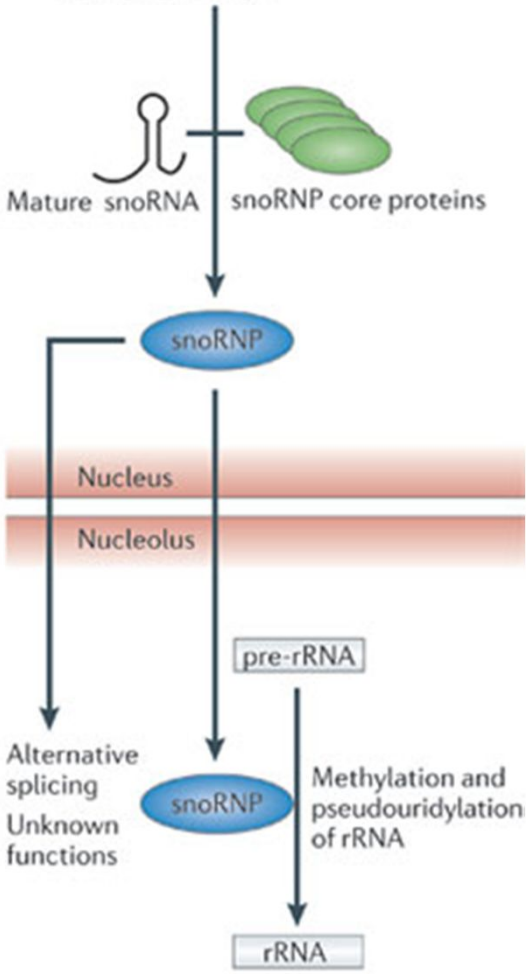
- Methylation
- Pseudouridylation

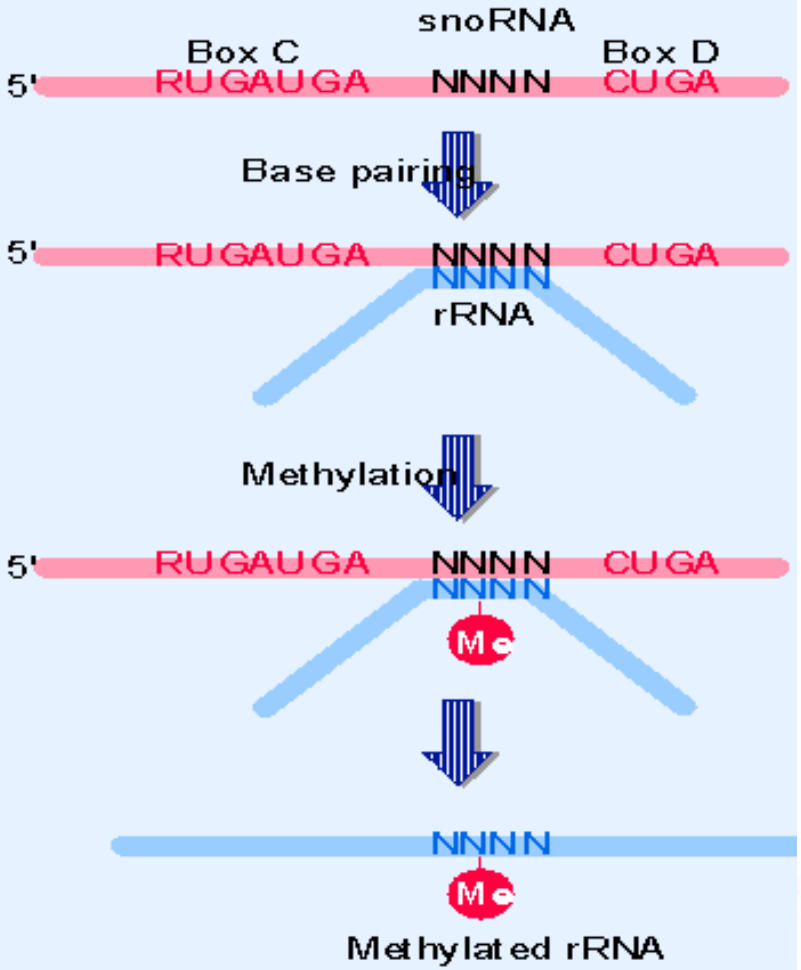
snoRNA

- Small nucleolar RNA
- Forming the snoRNP (ribonucleoprotein particles)
- Needed for maturation of the rRNA

- Box C/D → methylation
- Box H/ACA → pseudouridylation


snoRNA
snoRNA
Exon
Exon
Exon
Transcription
pre-mRNA
Splicing
mRNA
+
Intron lariats
Debranching,
exonucleolytic degradation,
snoRNP assembly


Mature snoRNA
snoRNP core proteins
snoRNP
Nucleus
Nucleolus
pre-rRNA
Alternative
splicing
Unknown
functions
snoRNP
Methylation and
pseudouridylation
of rRNA
rRNA



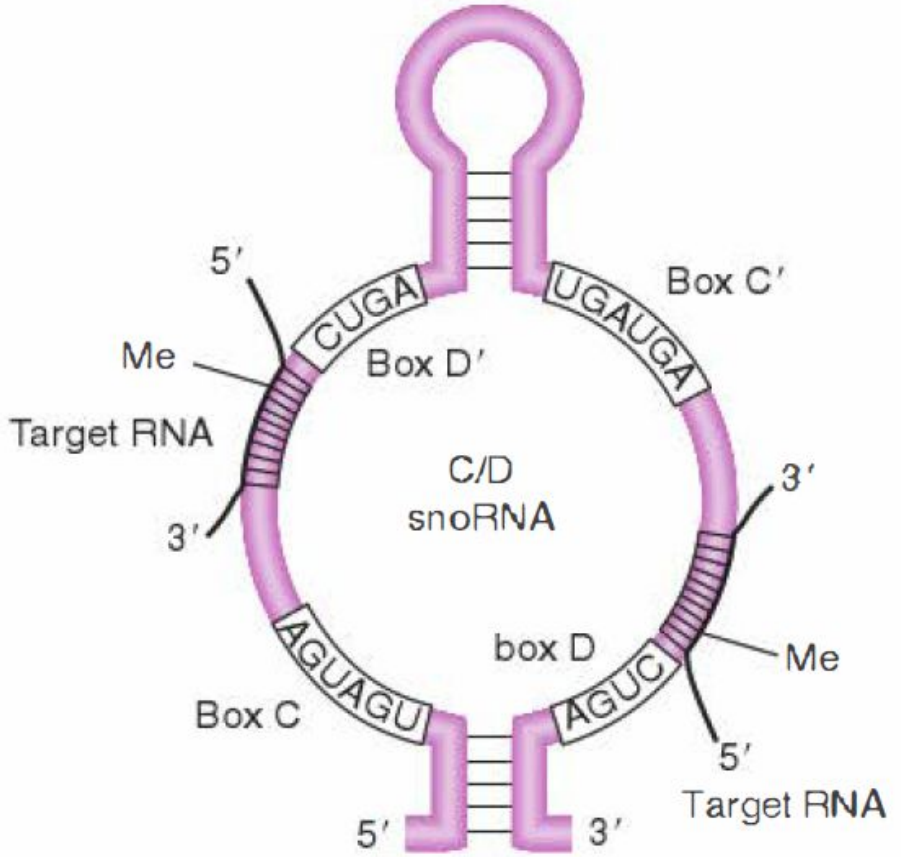


FIGURE 21.42 A snoRNA base pairs with a region of rRNA that is to be methylated.

A snoRNA base pairs with a region of rRNA that is to be methylated.

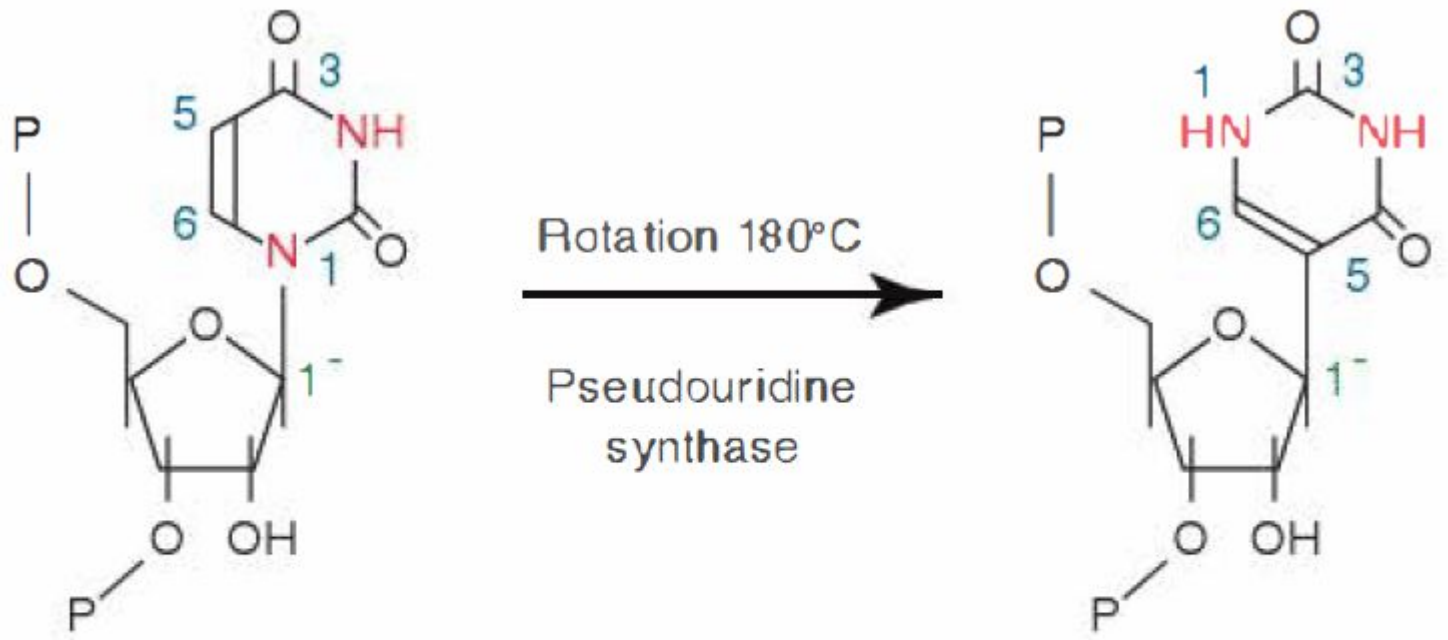


FIGURE 21.43 Uridine is converted to pseudouridine by replacing the N1-sugar bond with a C5-sugar bond and rotating the base relative to the sugar..

Conversion U→ Ψ in the non-paired regions

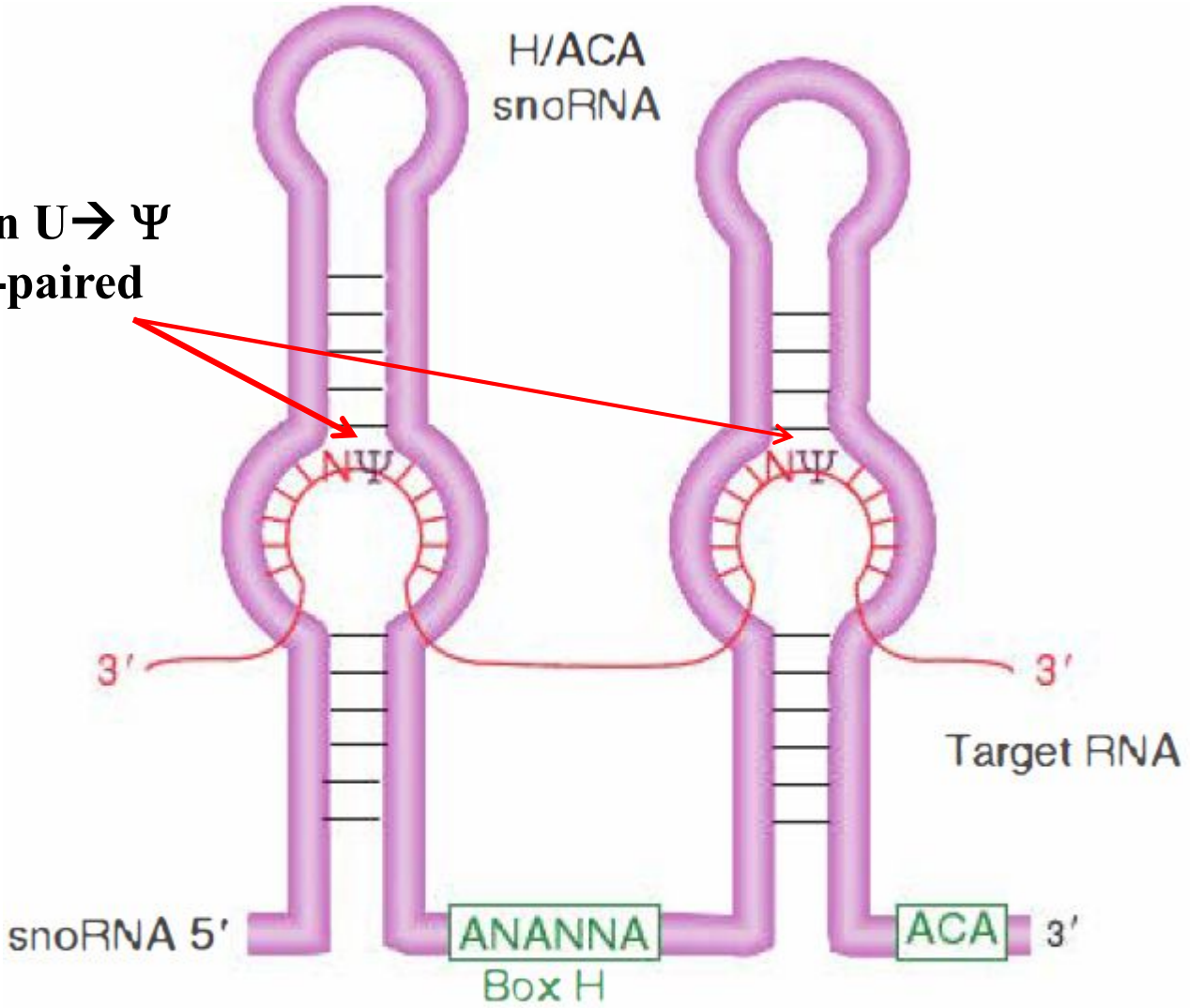


FIGURE 21.44 H/ACA snoRNAs have two short, conserved sequences and two hairpin structures, each of which has regions in the stem that are complementary to rRNA. Pseudouridine is formed by converting an unpaired uridine within the complementary region of the rRNA.

mRNA

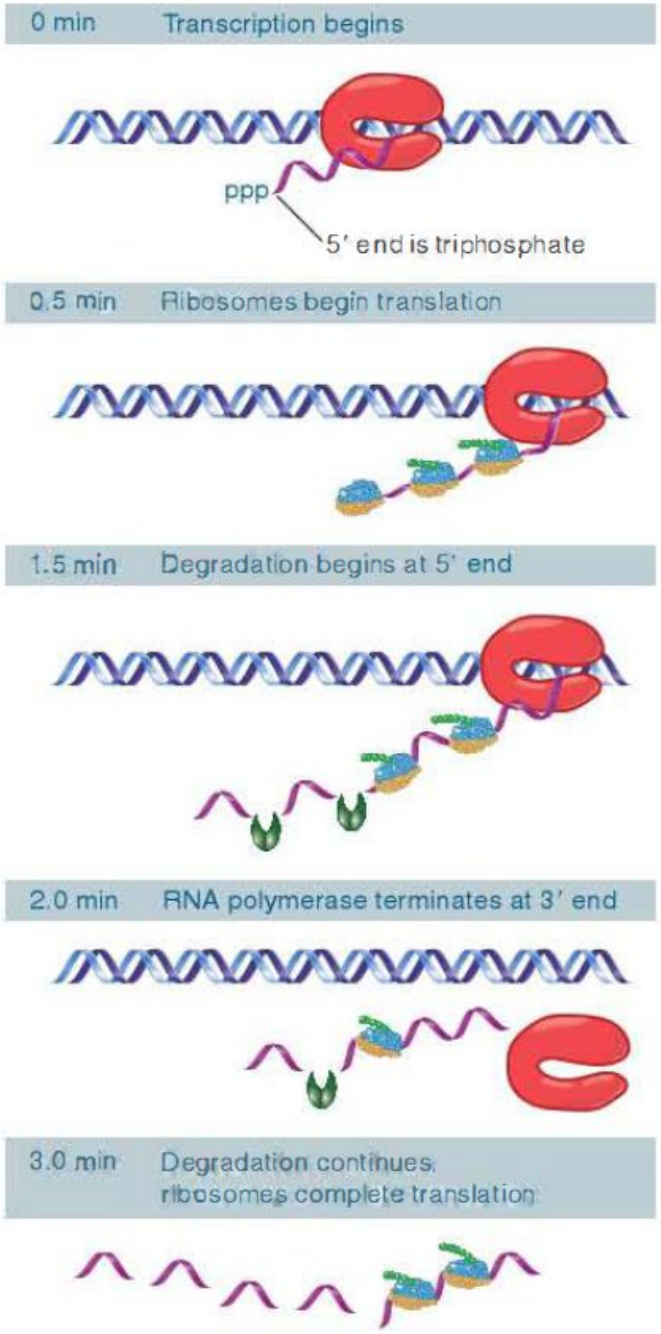
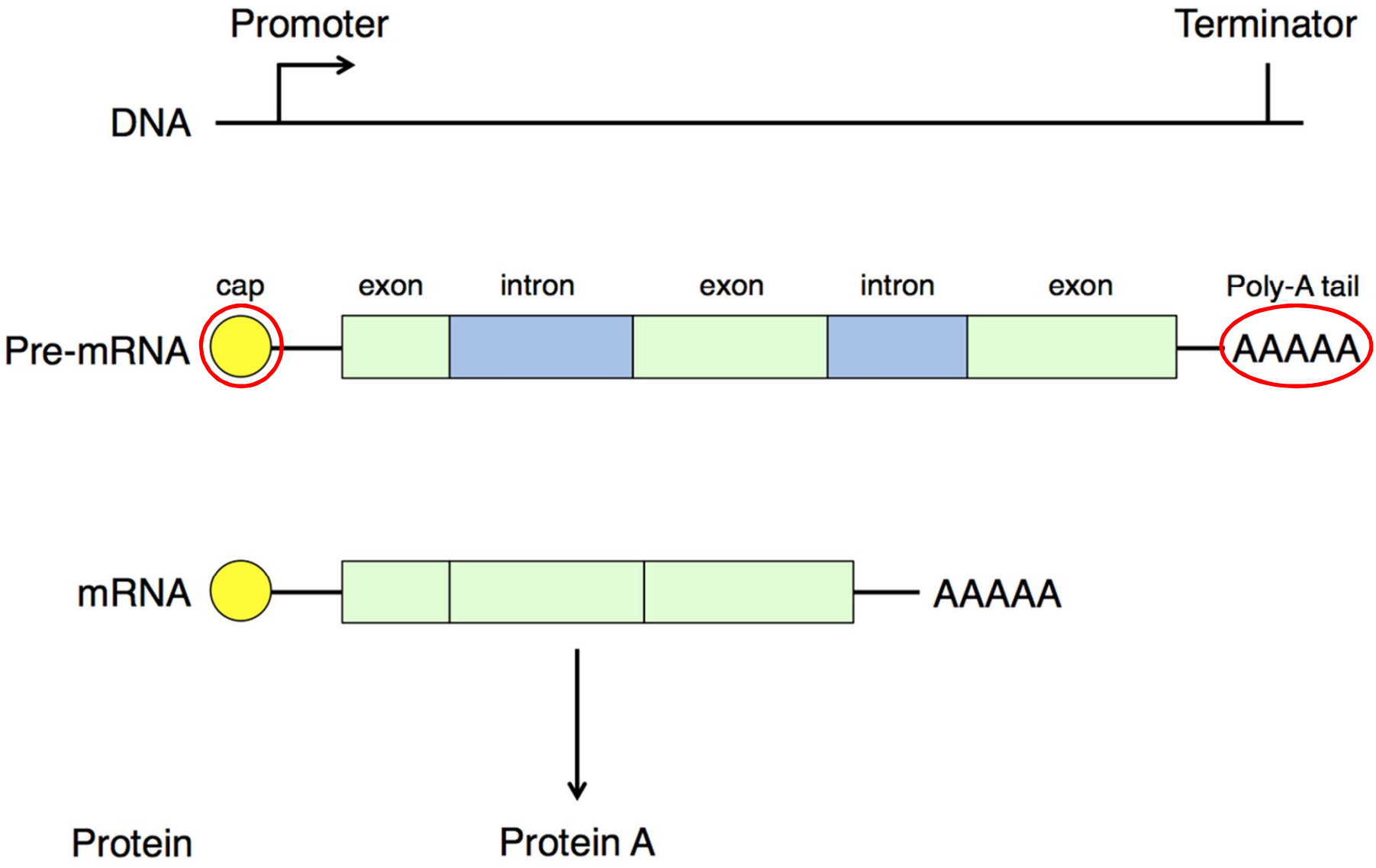
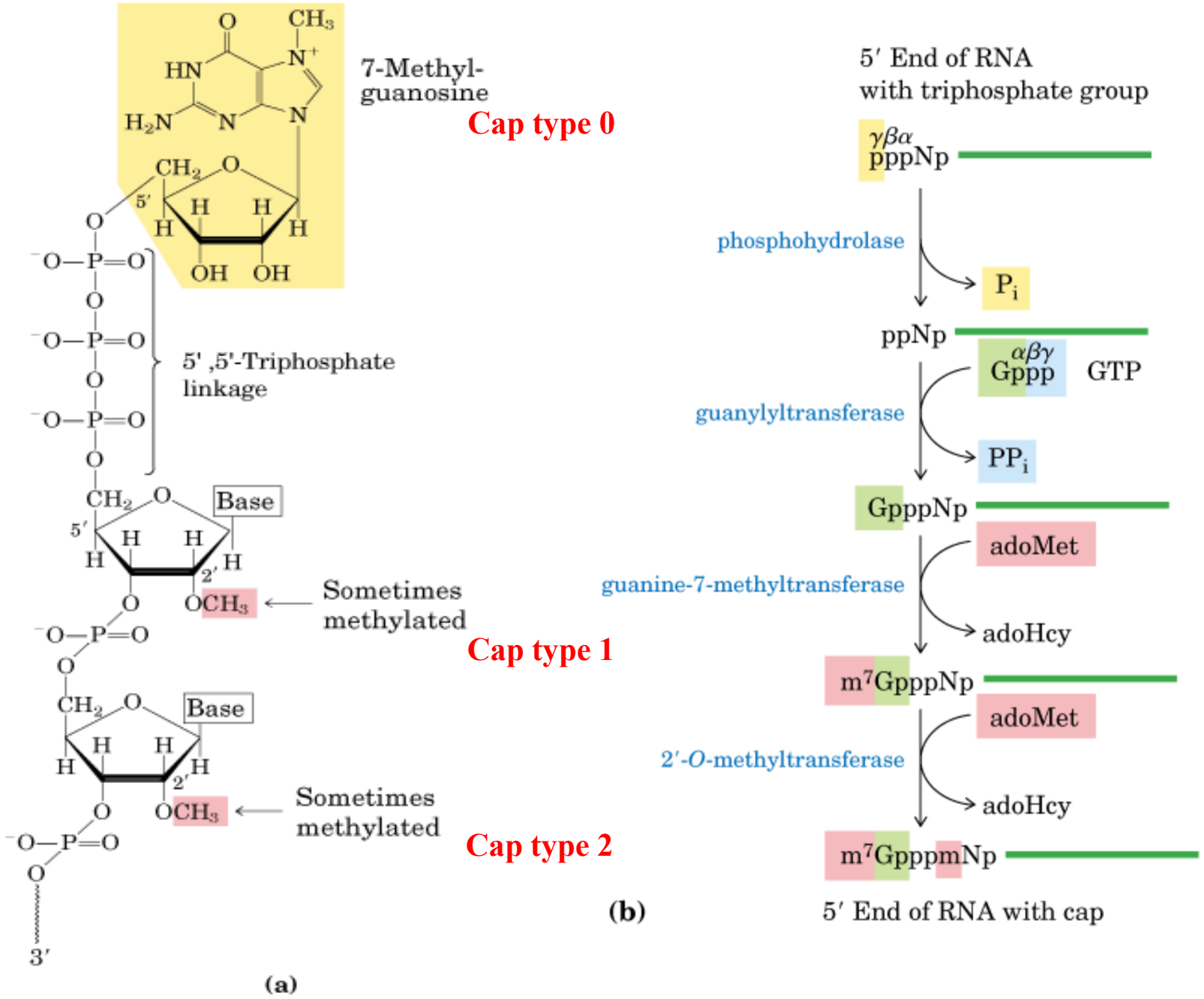


FIGURE 24.53 mRNA is transcribed, translated, and degraded simultaneously in bacteria.


Promoter
Terminator
DNA
cap
exon
intron
exon
intron
exon
Poly-A tail
Pre-mRNA
AAAAA
mRNA
AAAAA
Protein
Protein A


7-Methyl-guanosine
Cap type 0
5′ ,5′-Triphosphate linkage
Base
Sometimes methylated
Cap type 1
Base
Sometimes methylated
Cap type 2
3′
(a)
5′ End of RNA with triphosphate group
γβα pppNp
phosphohydrolase
Pi
ppNp
αβγ Gppp
GTP
guanylyltransferase
PPi
GpppNp
adoMet
guanine-7-methyltransferase
adoHcy
m7GpppNp
adoMet
2′-O-methyltransferase
adoHcy
m7GpppmNp
(b)
5′ End of RNA with cap

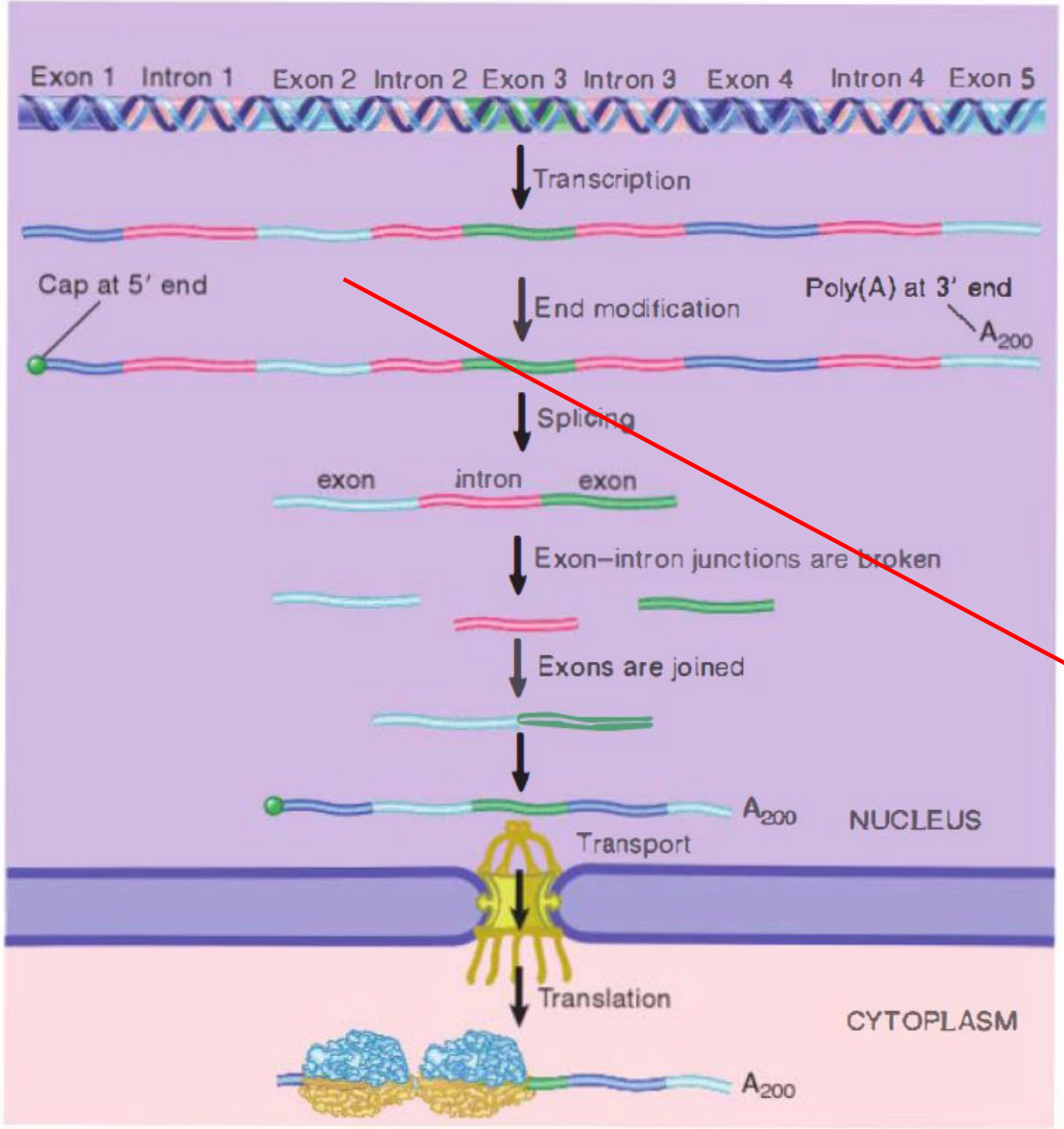
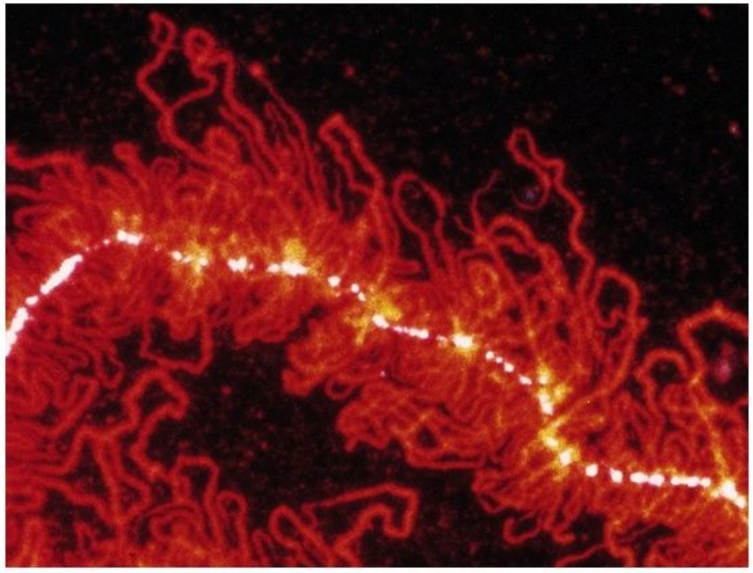
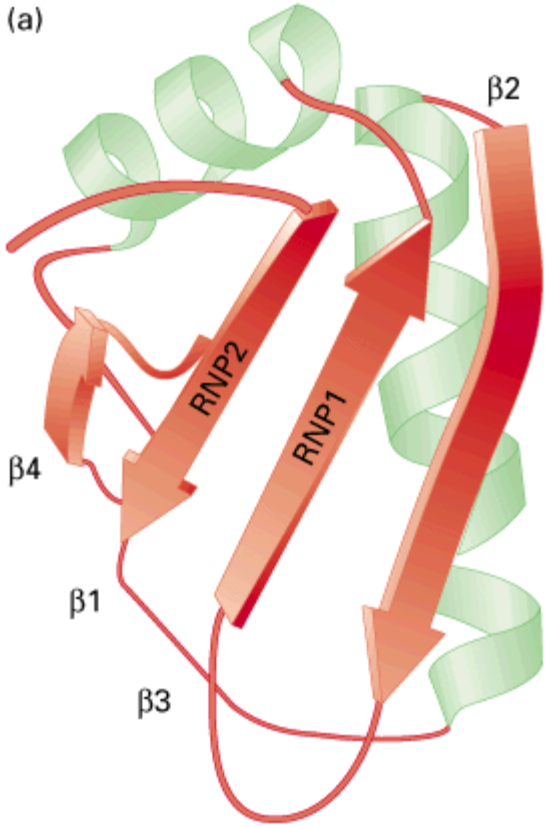


FIGURE 21.1 RNA is modified in the nucleus by additions to the 5′ and 3′ ends and by splicing to remove the introns. The splicing event requires breakage of the exon–intron junctions and joining of the ends of the exons. Mature mRNA is transported through nuclear pores to the cytoplasm, where it is translated.

Before processing, capped RNAs exist as RNA-protein complexes that are associated with heterogeneous ribonucleoprotein particles (hnRNPs) that consist of heterogeneous nuclear RNAs (hnRNAs) and hnRNP proteins. These can be visualized by staining fixed transcriptionally active chromatin with antibodies against hnRNP proteins (red) and dyes for DNA (white): The structure is known as a "lampbrush" chromosome

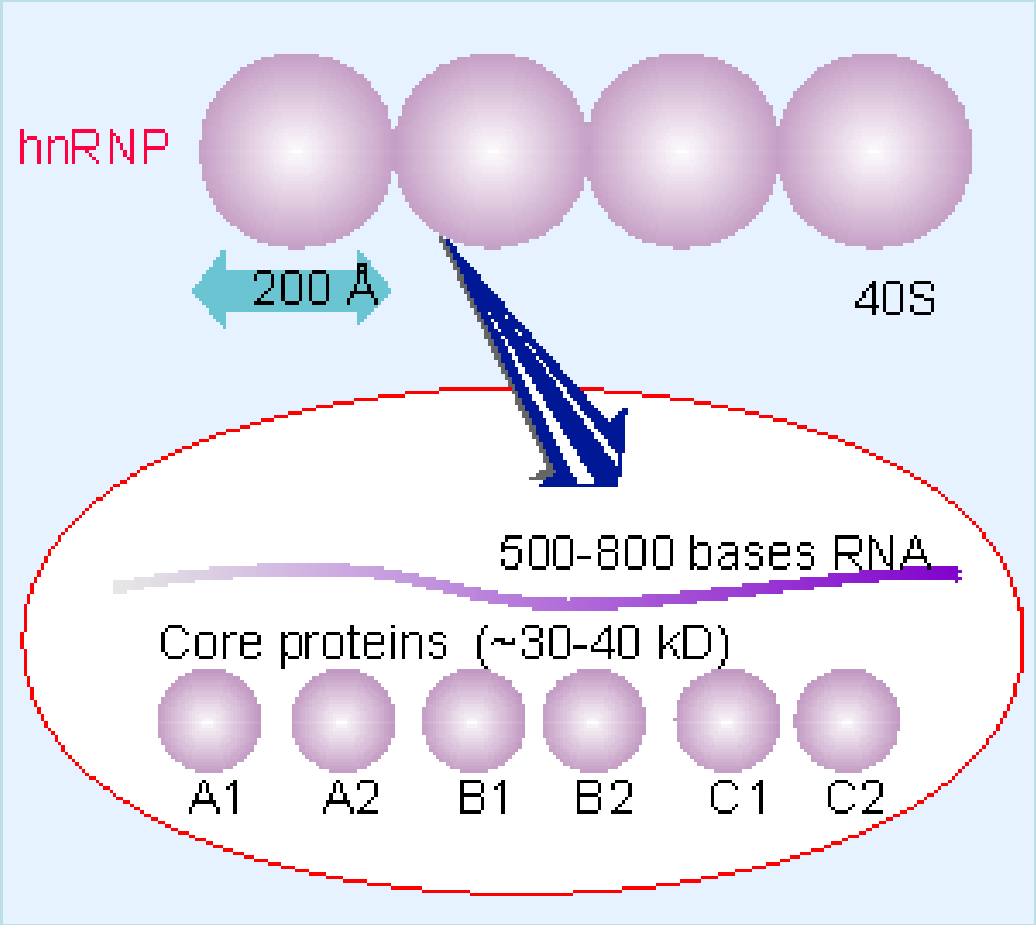


In the nucleus, nascent pre-mRNA associates with hnRNP (heterogeneous nuclear ribonucleoparticles) proteins.



RNP motif domain

It prevents the formation of short secondary structures dependent on base-pairing of complementary regions, making the pre-mRNA accessible for interactions with other macromolecules



hnRNA exists as a ribonucleoprotein particle organized as a series of beads.

Prokaryotic mRNA degradation occurs during the process of translation.

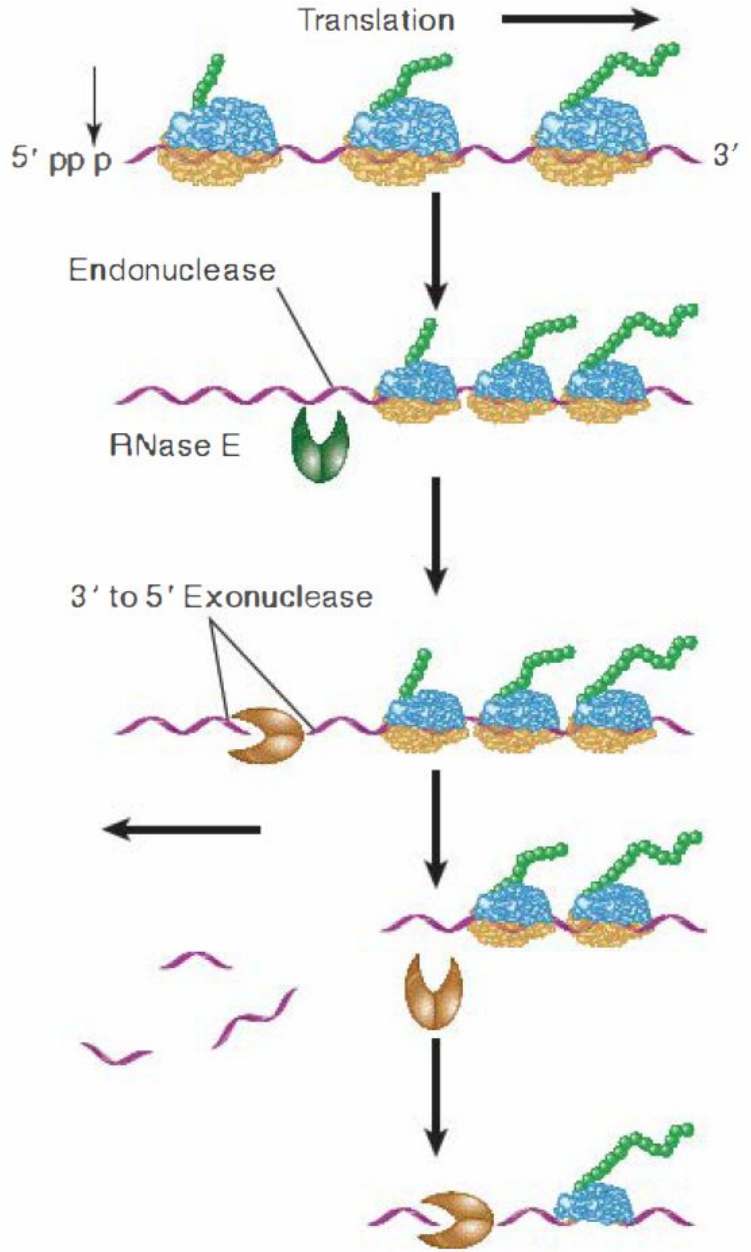


FIGURE 22.5 Degradation of bacterial mRNAs. Bacterial mRNA degradation is initiated by cleavage of the triphosphate 5′ terminus to yield a monophosphate. mRNAs are then degraded in a two-step cycle: an endonucleolytic cleavage, followed by 3′ to 5′ exonuclease digestion of the released fragment. The endonucleolytic cleavages occur in a 5′ to 3′ direction on the mRNA, following the passage of the last ribosome.

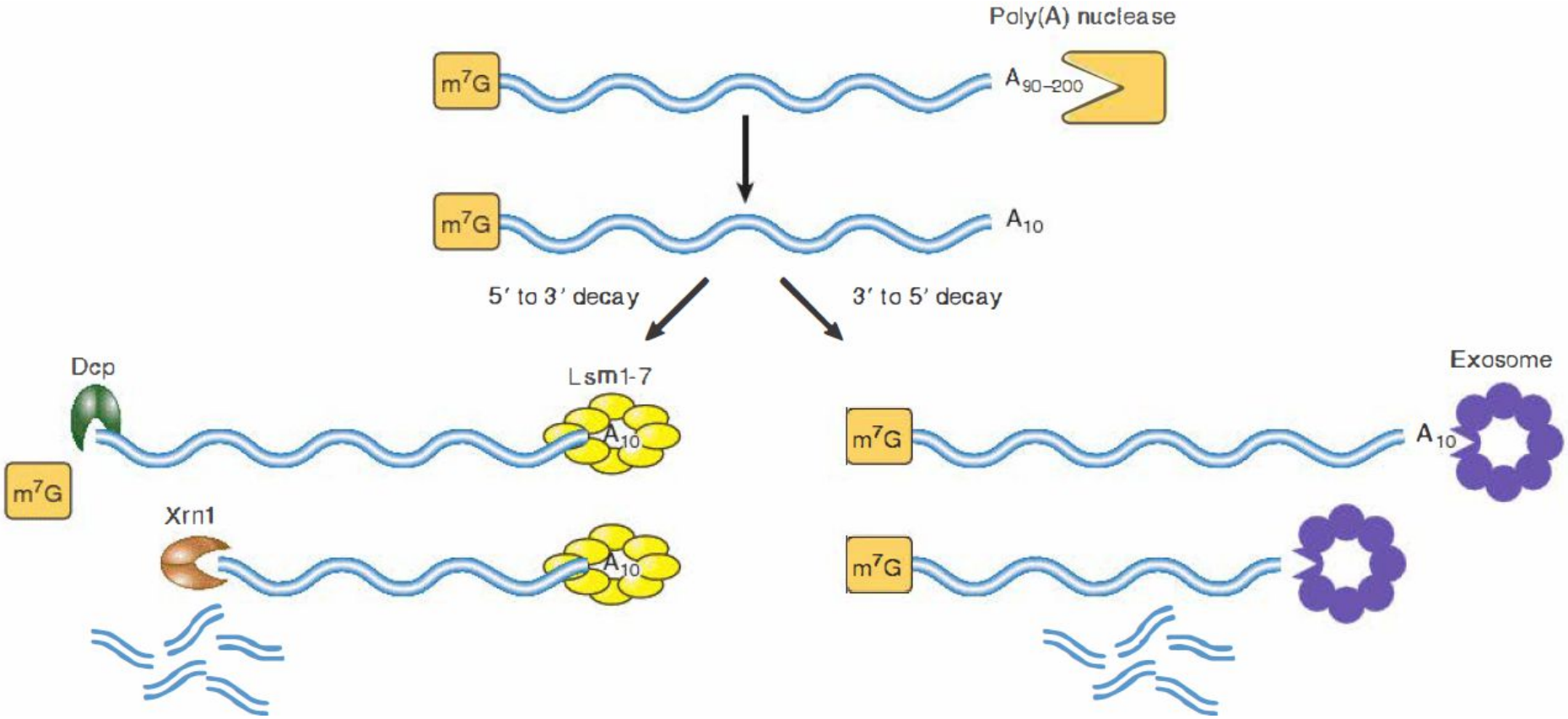


FIGURE 22.6 The major deadenylation-dependent decay pathways in eukaryotes. Two pathways are initiated by deadenylation. In both, poly(A) is shortened by a poly(A) nuclease until it reaches a length of about 10 Å. Then an mRNA may be degraded by the 5' to 3' pathway or by the 3' to 5' pathway. The 5' to 3' pathway involves decapping by Dcp and digestion by the Xrn1 exonuclease. The 3' to 5' pathway involves digestion by the exosome complex.

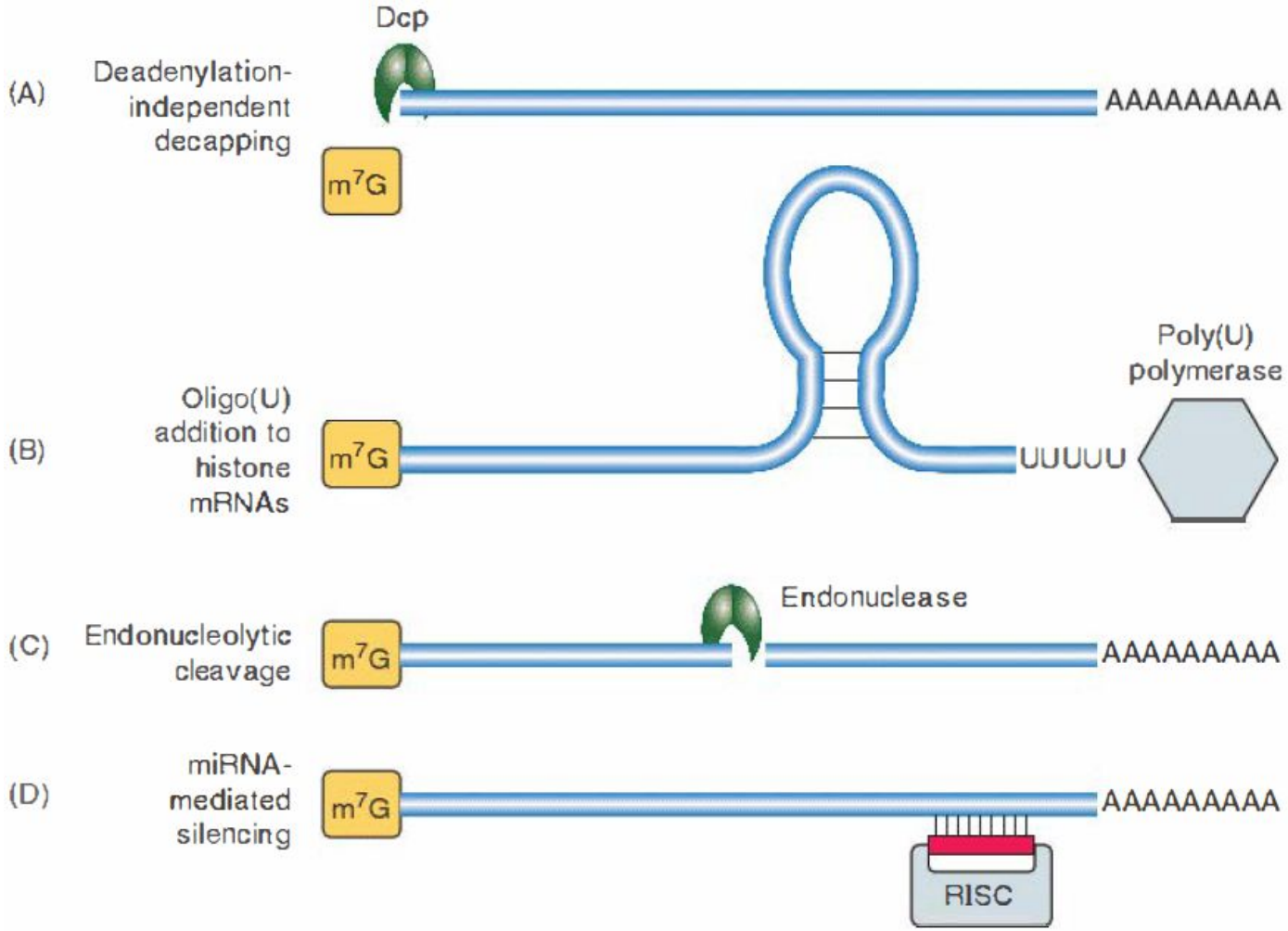


FIGURE 22.8 Other decay pathways in eukaryotic cells. The initiating event for each pathway is illustrated. (A) Some mRNAs may be decapped before deadenylation occurs. (B) Histone mRNAs receive a short poly(U) tail to become a decay substrate. (C) Degradation of some mRNAs can be initiated by a sequence-specific endonucleolytic cut. (D) Some mRNAs can be targeted for degradation or translational silencing by complementary guide miRNAs.

Pathway	Initiating event	Secondary step(s)	Substrates
Deadenylation-dependent 5′ to 3′ digestion	Deadenylation to oligo(A)	Oligo(A) binding by Lsm complex Decapping 5′ to 3′ exonuclease digestion by XRN1	Probably most polyadenylated mRNAs
Deadenylation-dependent 3′ to 5′ digestion	Deadenylation to oligo(A)	3′ to 5′ exonuclease digestion by exosome	Probably most polyadenylated mRNAs
Deadenylation-independent decapping	Decapping	5′ to 3′ exonuclease digestion	Few specific mRNAs
Endonucleolytic pathway	Endonuclease cleavage	5′ to 3′ and 3′ to 5′ exonuclease digestion	Few specific mRNAs
Histone mRNA pathway	Oligouridylation	Oligo(U) binding by Lsm complex Decapping and 5′ to 3′ exonuclease digestion by XRN1 3′ to 5′ digestion by exosome	Histone mRNAs in mammals
miRNA pathway	Base pairing with miRNA in RISC	Endonucleolytic cleavage or translational repression	Many mRNAs (extent unknown)

FIGURE 22.9 Table summarizing key elements of mRNA decay pathways in eukaryotic cells.

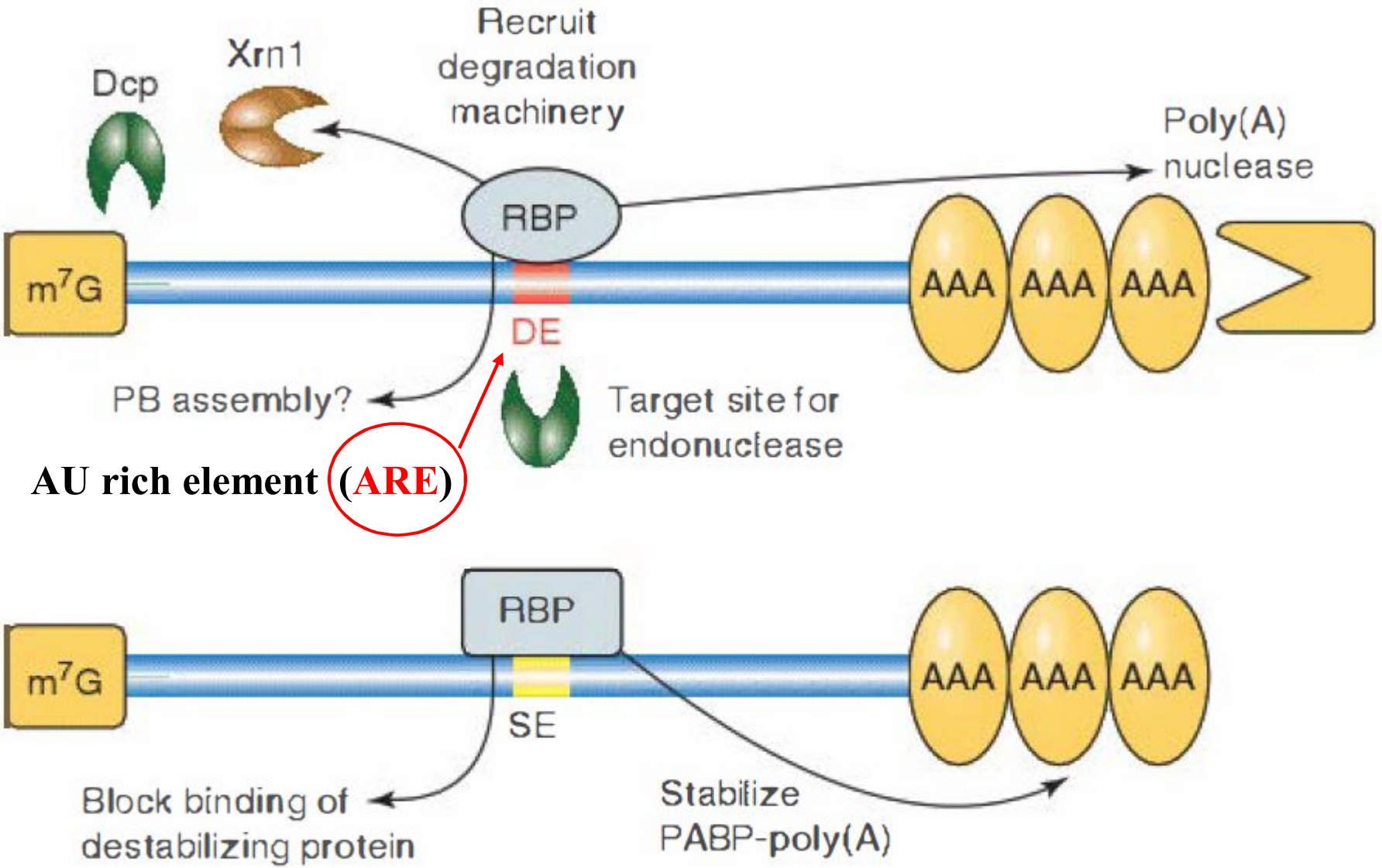


FIGURE 22.10 Mechanisms by which destabilizing elements (DEs) and stabilizing elements (SEs) function. Effects of DEs and SEs on mRNA stability are mediated primarily through the proteins that bind to them. One exception is a DE that acts as an endonuclease target site.

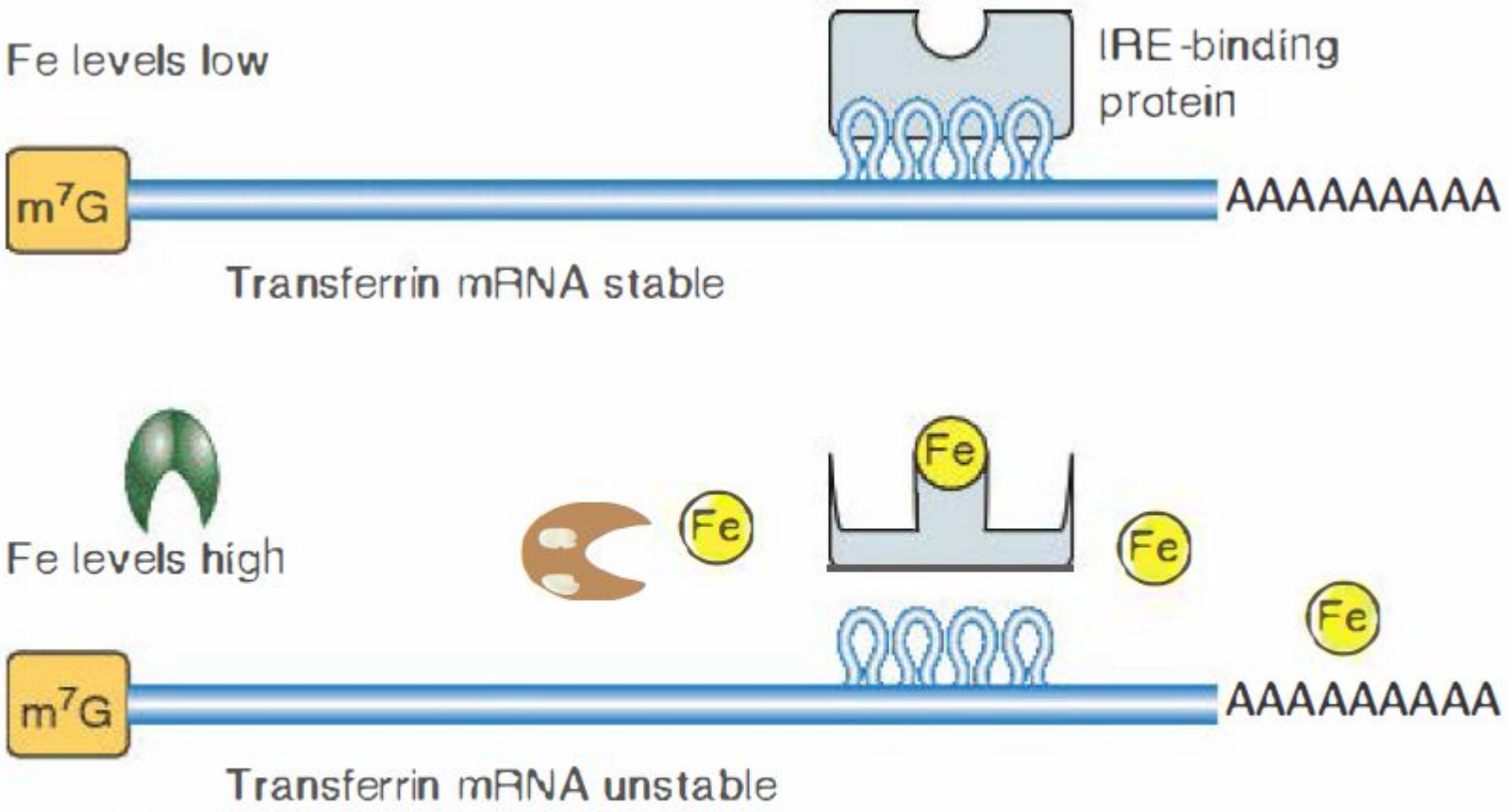


FIGURE 22.11 Regulation of transferring mRNA stability by iron (Fe) levels. The IRE in the 3′ UTR is the binding site for a protein that stabilizes the mRNA. The IRE-binding protein is sensitive to iron levels in the cell, binding to the IRE only when iron is low.

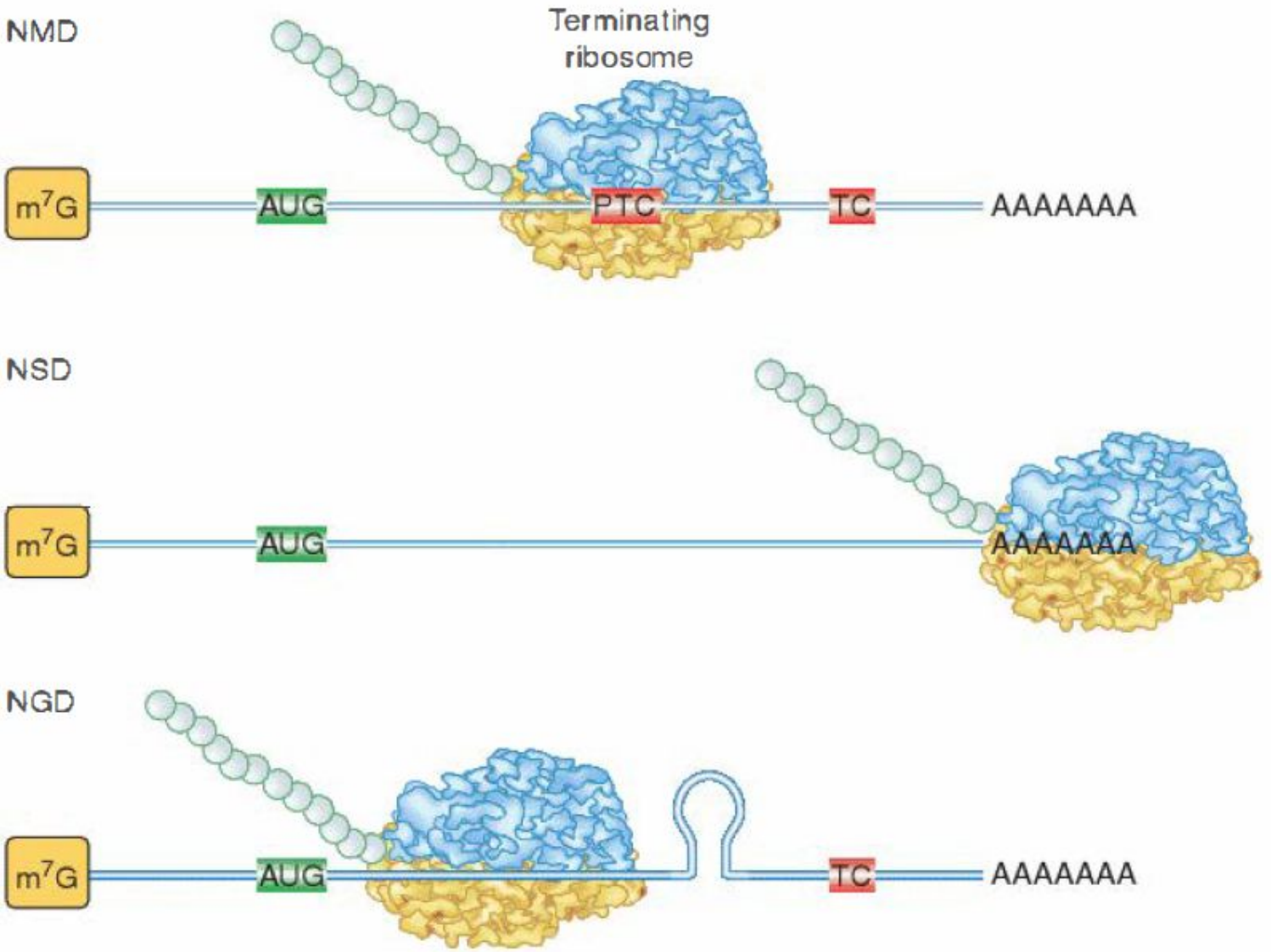


FIGURE 22.13 Substrates for cytoplasmic surveillance systems. Nonsense-mediated decay (NMD) degrades mRNAs with a premature termination codon (PTC) positioned ahead of its normal termination codon (TC). Nonstop decay (NSD) degrades mRNAs lacking an in-frame termination codon. No-go decay (NGD) degrades mRNAs having a ribosome stalled in the coding region.

Regulatory RNA

- Noncoding antisense RNAs can be used to regulate gene expression (RNA interference).

- A regulator RNA can function by forming a duplex region with a target RNA that may block initiation of translation, cause termination of transcription, or create a target for an endonuclease → RNA degradation.

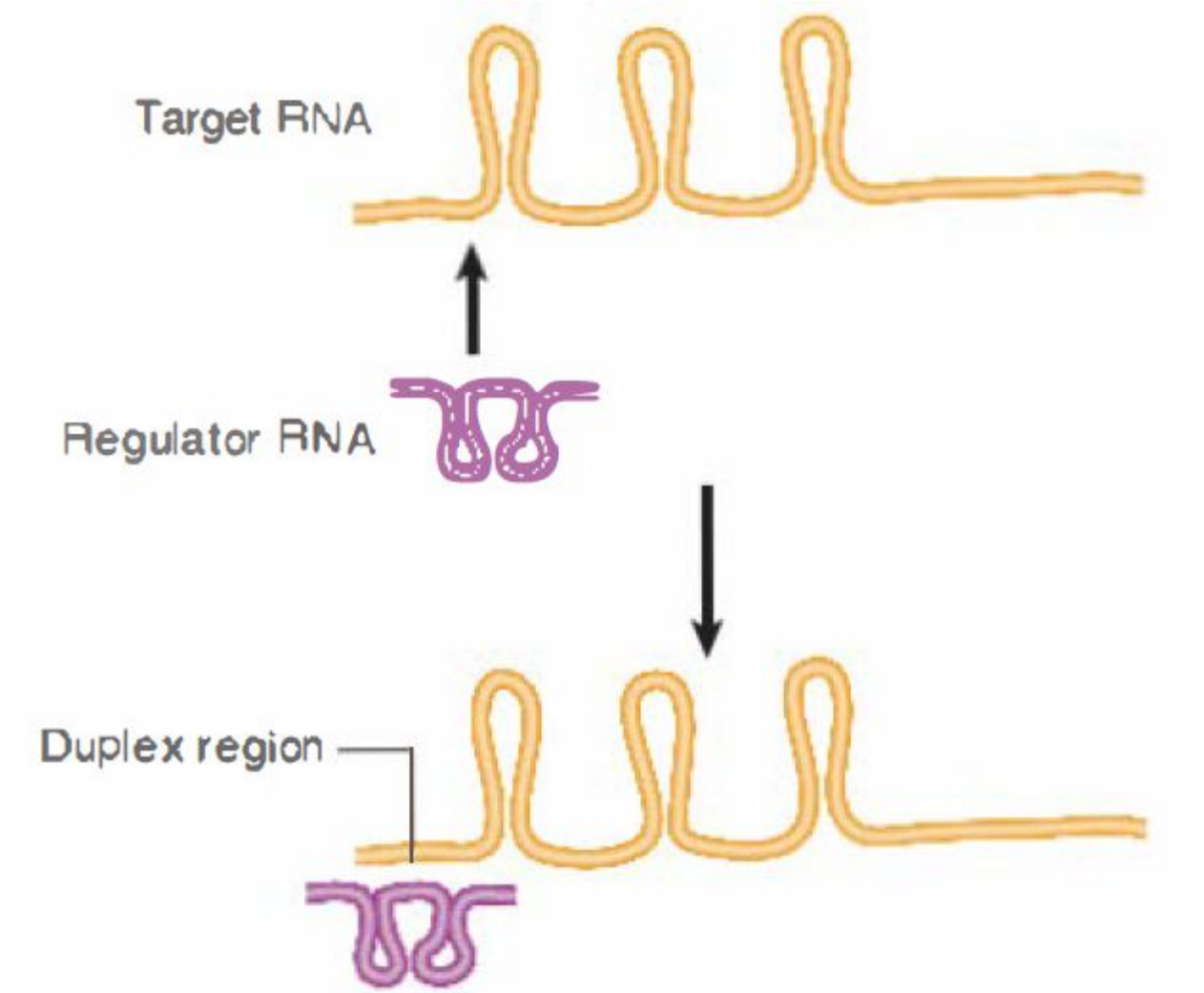
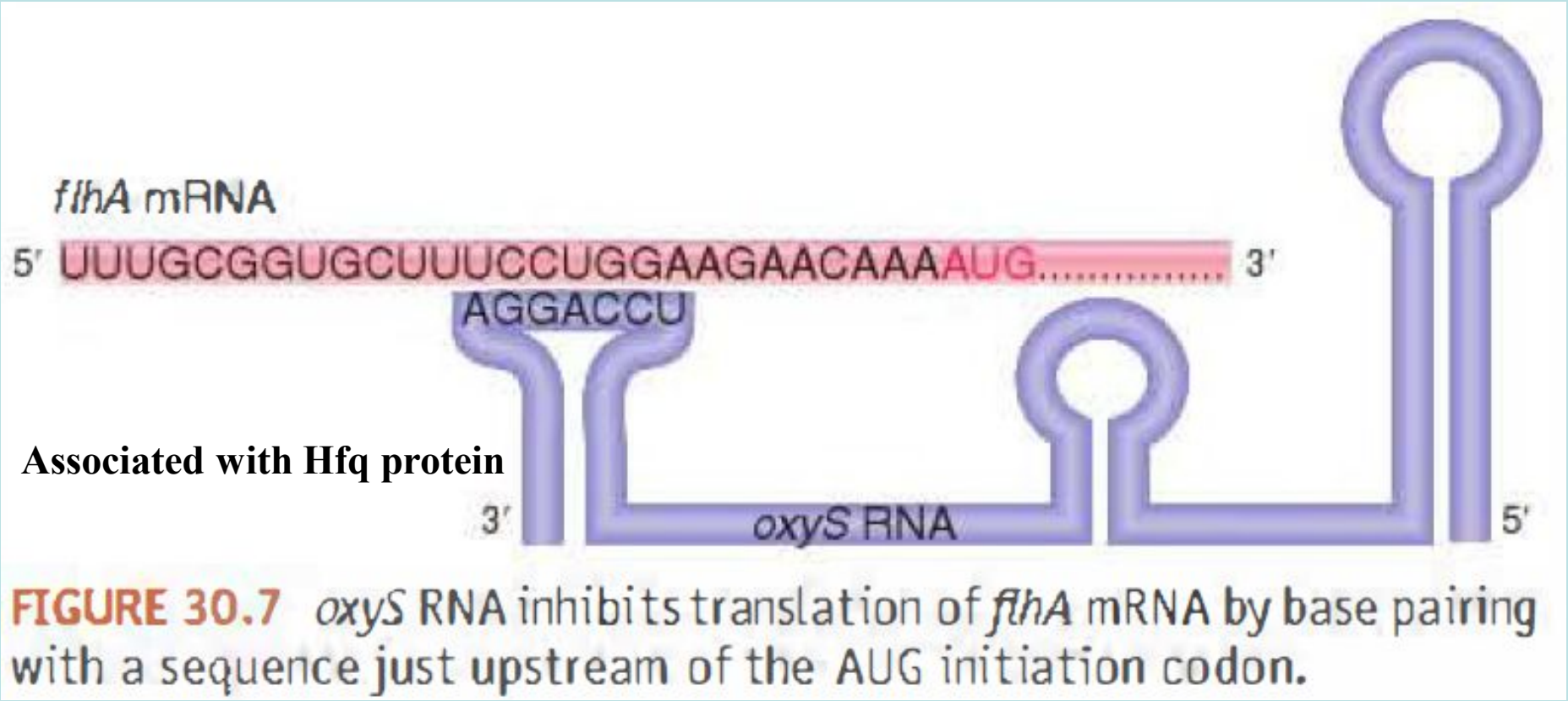


FIGURE 30.1 A regulator RNA is a small RNA with a single-stranded region that can pair with a single-stranded region in a target RNA.

An example of a bacterial regulator RNAs
sRNA (short RNA)



Associated with Hfq protein

FIGURE 30.7 *oxyS* RNA inhibits translation of *flhA* mRNA by base pairing with a sequence just upstream of the AUG initiation codon.

"OxyS RNA is a small <u>non-coding RNA</u> which is induced in response to oxidative stress in *Escherichia coli*. This RNA acts as a global regulator to activate or repress the expression of as many as 40 genes, by an <u>antisense</u> mechanism, including the fhlA-encoded transcriptional activator and the rpoS-encoded sigma(s) subunit of RNA polymerase "

RNA interference

RNAi

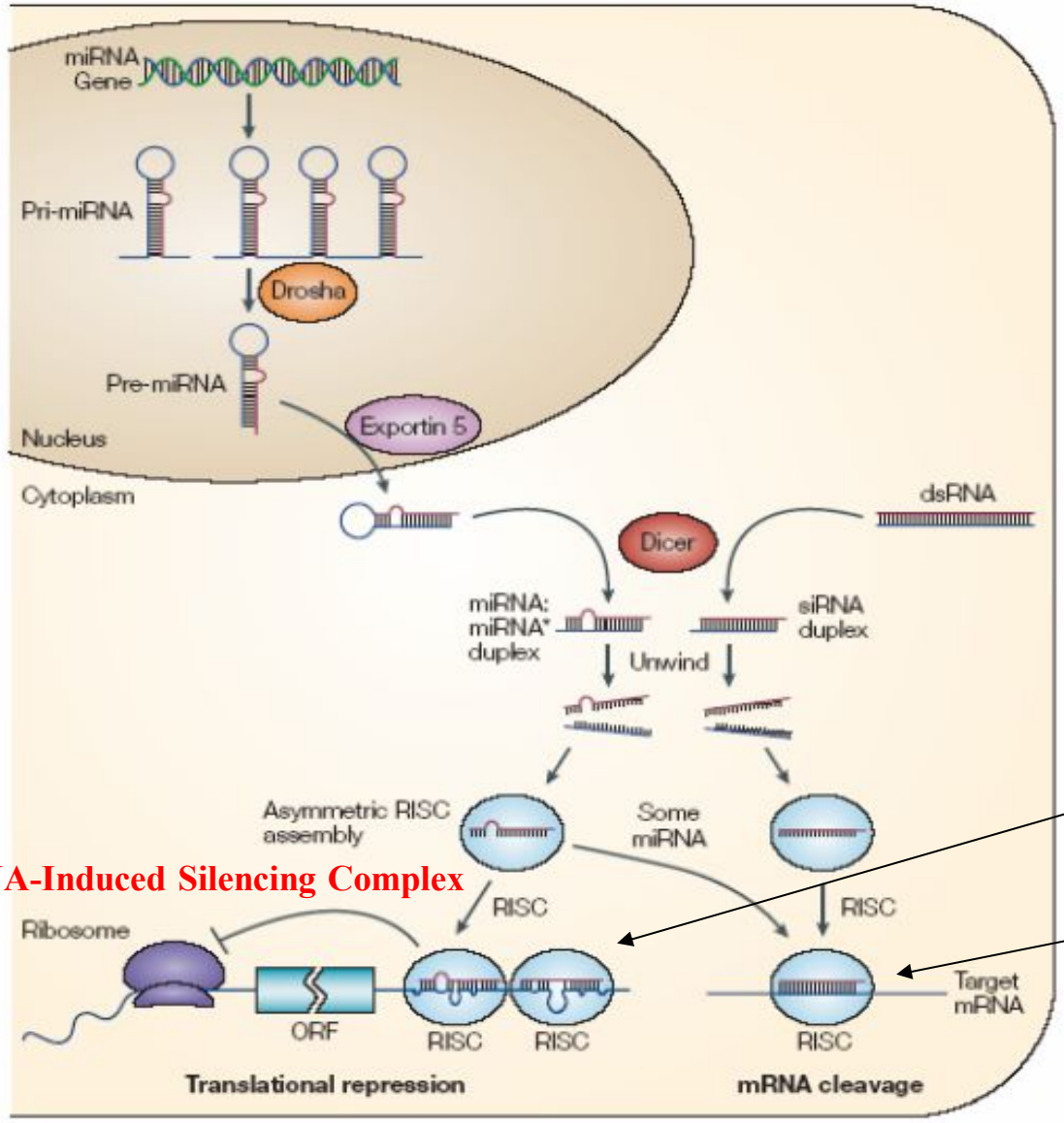
- RNAi is a powerful, conserved biological process through which the small, double-stranded RNAs specifically silence the expression of homologous genes, largely through degradation of their cognate mRNA.
- **its** responsible for post-transcriptional gene silencing of the gene from which it was derived
- Endogenous cellular mechanisms
- Effecter molecules for functional genomics
- Great potential as therapeutic agents for treatment of human disease

Advantage of RNAi

- Downregulation of gene expression simplifies "knockout" analysis.
- Easier than use of antisense oligonucleotides. siRNA more effective and sensitive at lower concentration.
- Cost effective
- High Specifity

  middle region 9-14 are most sensitive
- With siRNA, the researcher can simultaneously perform experiments in any cell type of interest
- Can be labelled
- Ease of transfection by use of vector

RNA interference



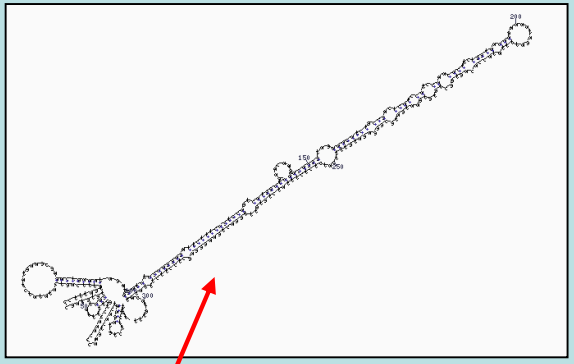
RNA-Induced Silencing Complex

miRNA → microRNA
dsRNA → double strand RNA
siRNA → small interfering RNA

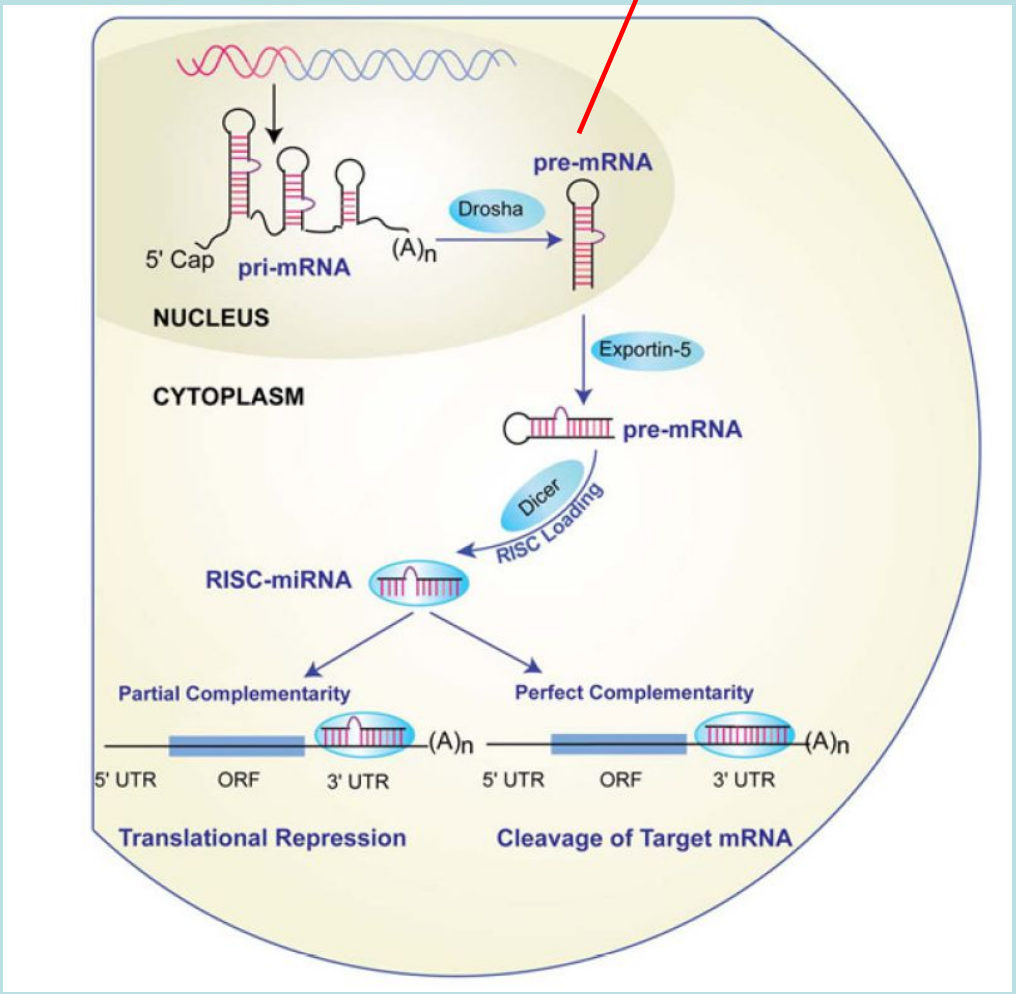
Imperfect pairing

Perfect base pairing

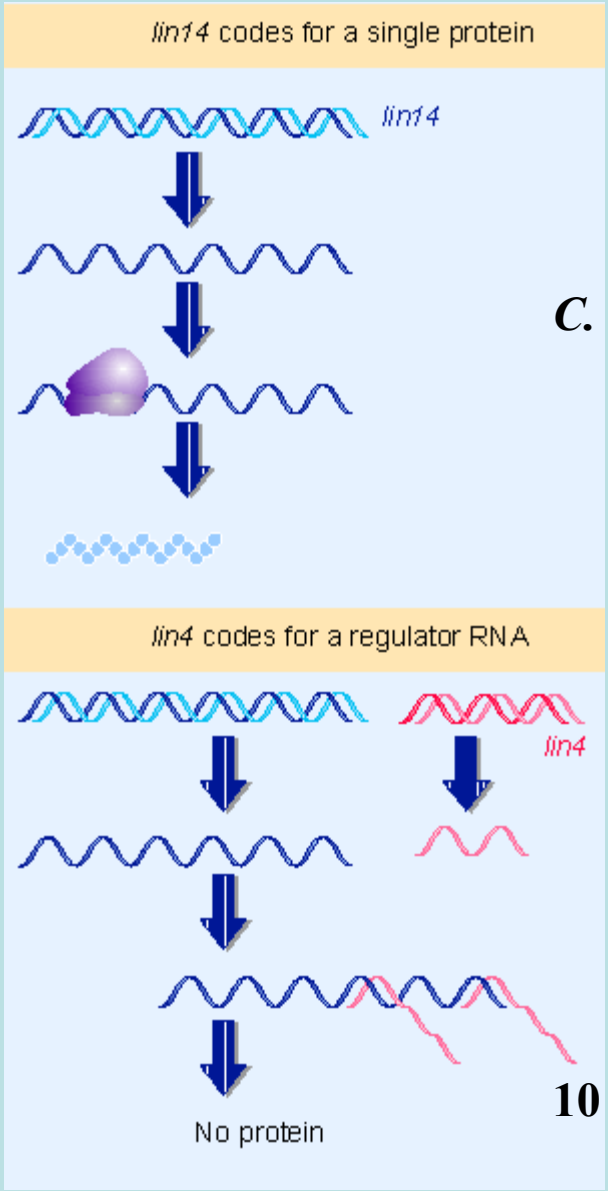
MicroRNAs



- Eukaryotic genomes (animals and plants) code for many short (-22 bases) RNA molecules called microRNAs.
- MicroRNAs regulate gene expression by base pairing with complementary sequences in target mRNAs.



MicroRNAs



C. elegans

10 bases in 7 repetitions at the 3' UTR

dsRNA

- **Viral origin**
- **Hexogen (artificially inserted in cells)**

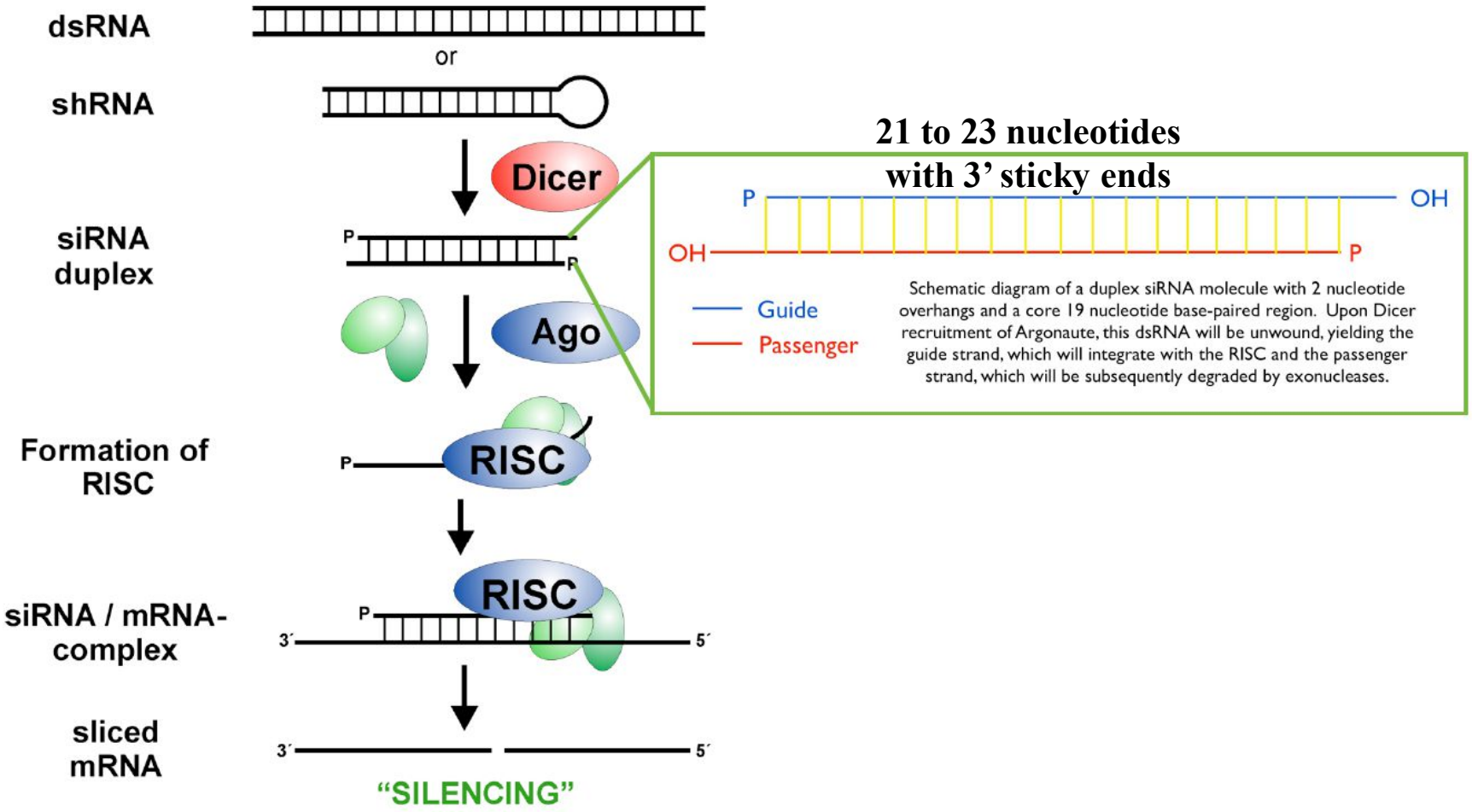
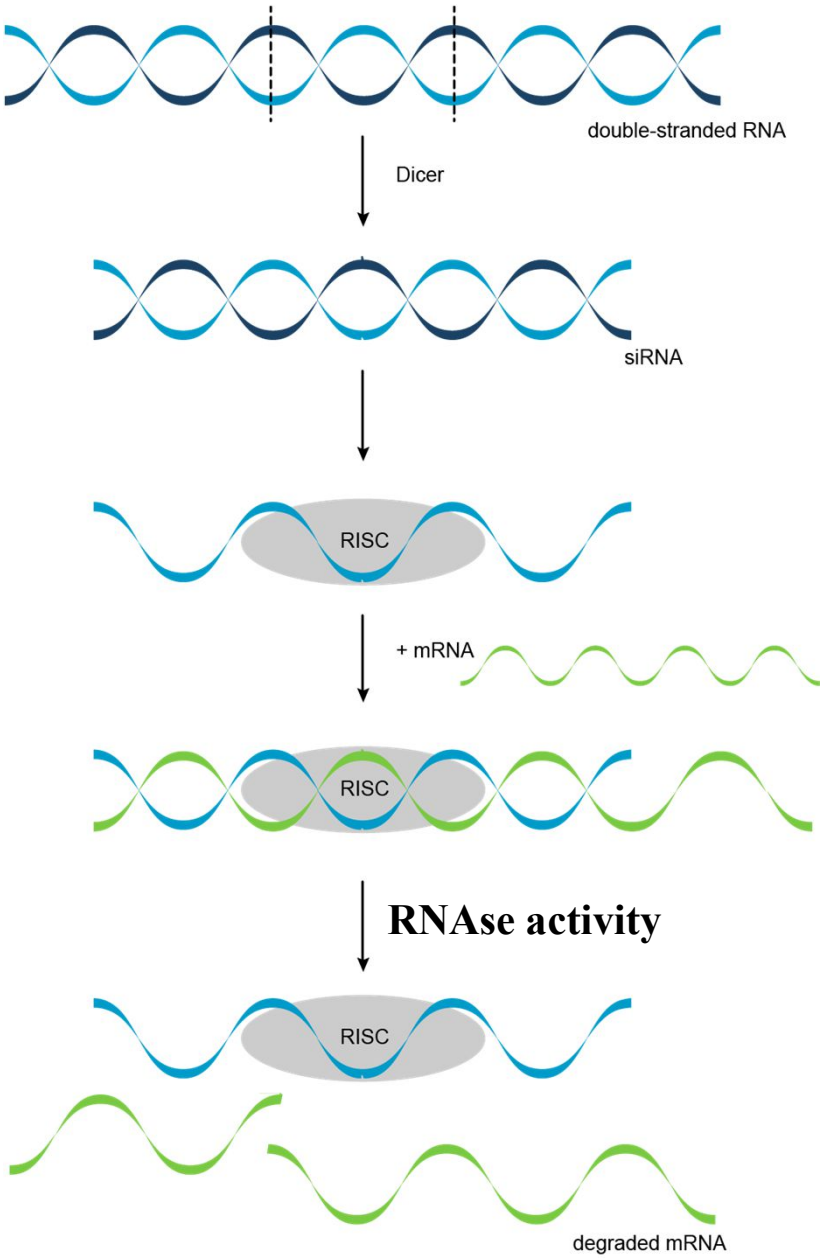


Diagram illustrating the major steps in siRNA biogenesis and subsequent siRNA-mediated gene silencing.

RNAi is mediated by siRNAs



RNAi applications

- Genome-wide RNAi screening
  - Done in *C. elegans*
    - 19 757 protein coding genes (predicted)
    - 16 757 inactivated using RNAi
  - New standard for systematic genome wide functional studies
- RNAi as a solution for mammalian genetics
- Defense against Infection by viruses
- Potential therapeutic use
- Prevents viral infection
- Inhibits the expression of viral antigens
- Suppresses the transcription of viral genome
- Blocks viral replication
- Silences viral accessory genes
- Hinders the assembly of viral particles & Displays roles in virus-host interactions