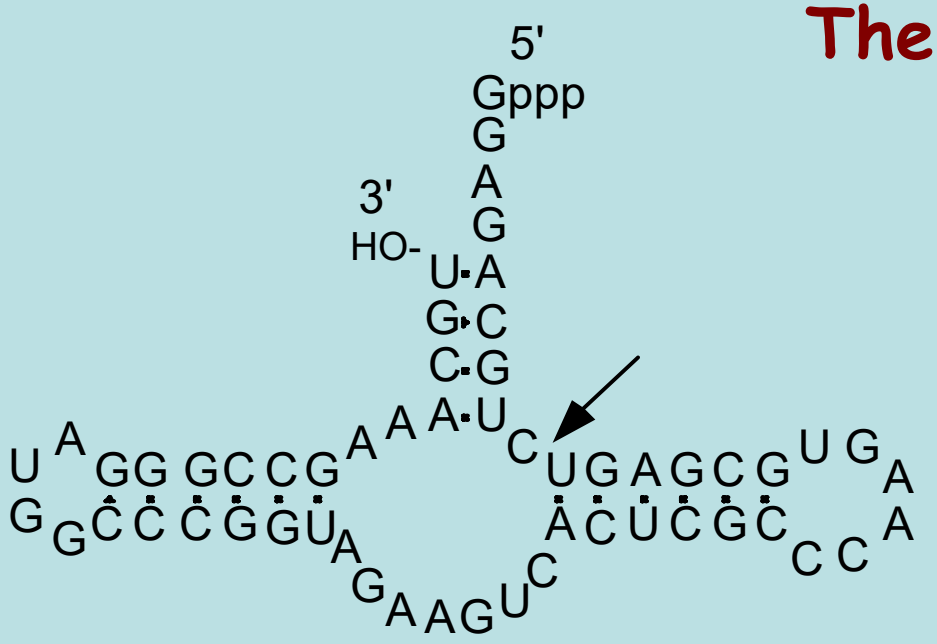
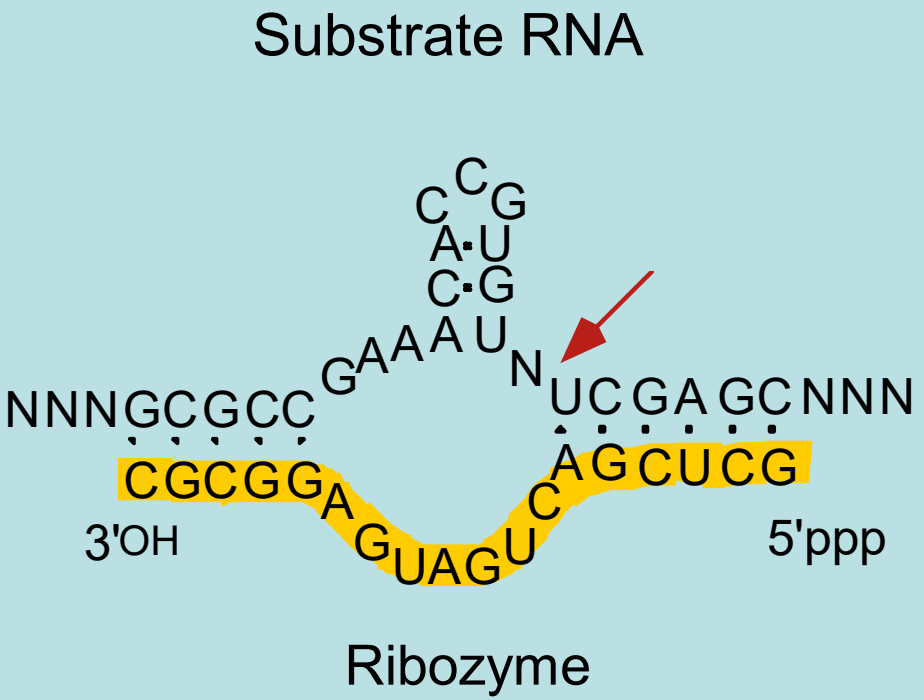
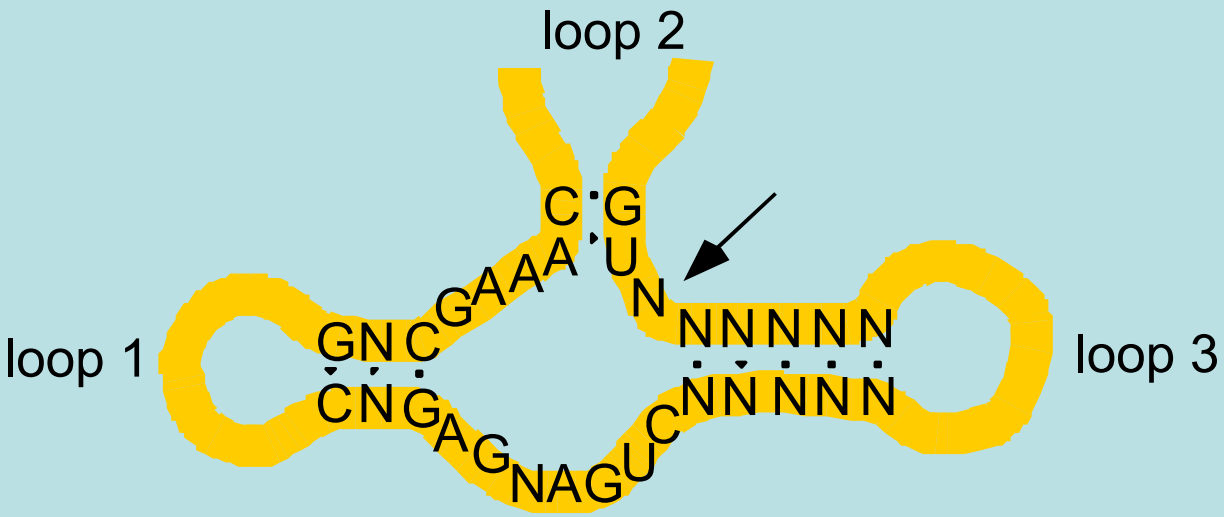
Catalytic RNA (ribozyme)

- RNAse P
- Viroids and virusoids
- Grup I and II introns

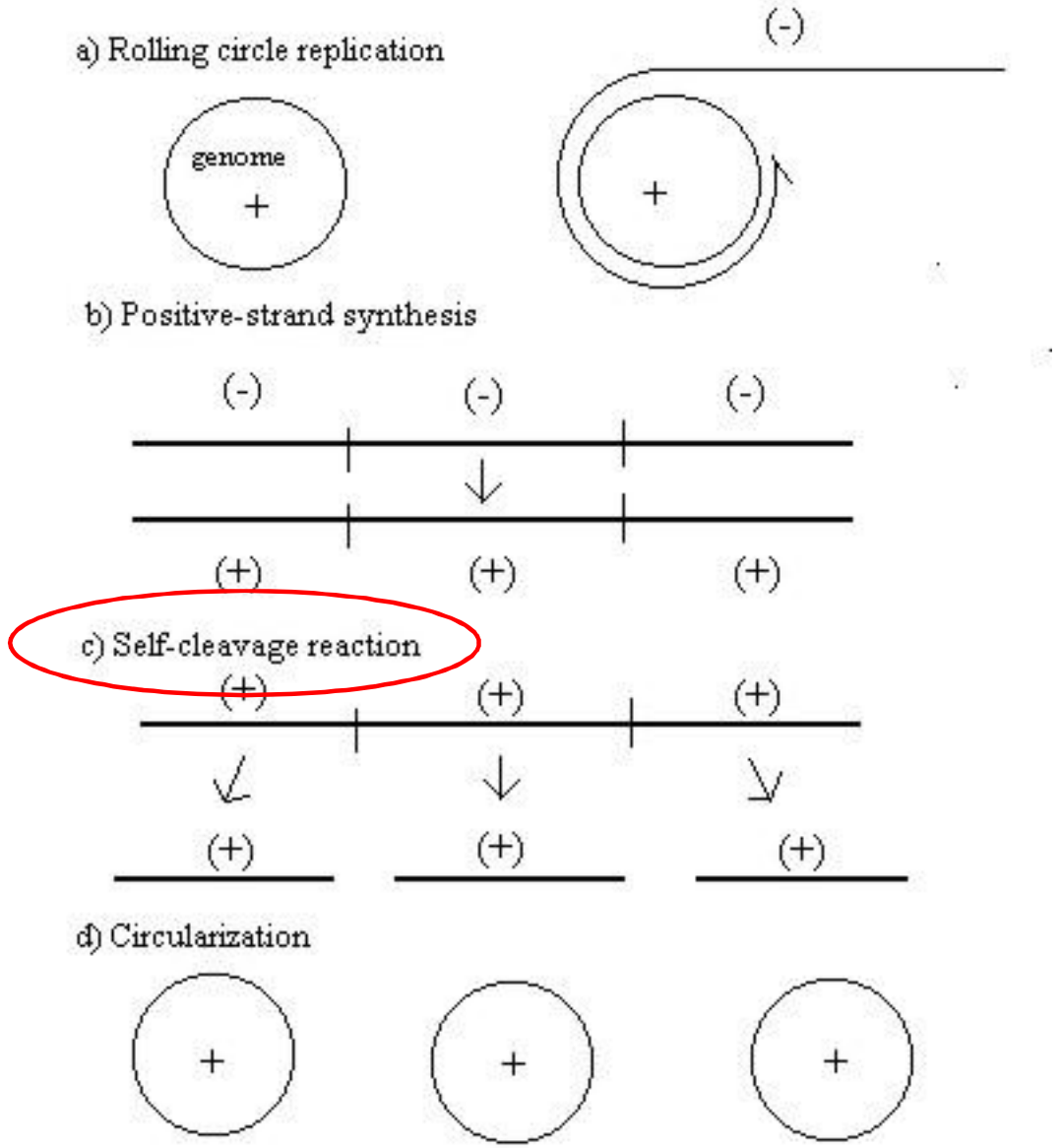
The hammerhead structure



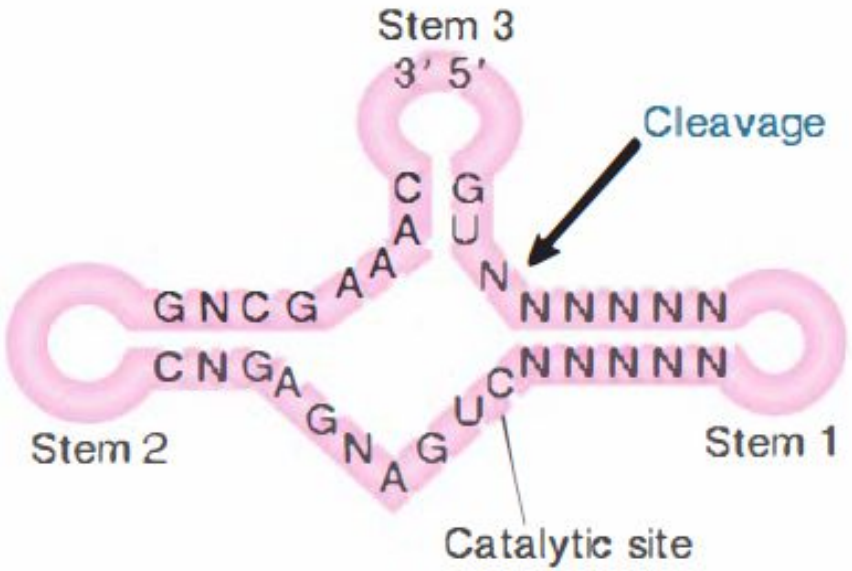




Viroids and virusoids



Consensus hammerheads have three stem loops and conserved bases



Hammerheads can be created by interaction between two complementary RNA molecules

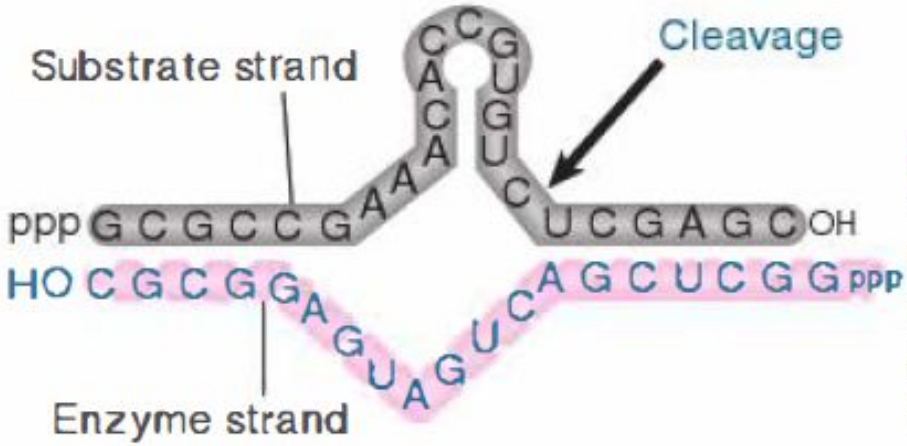
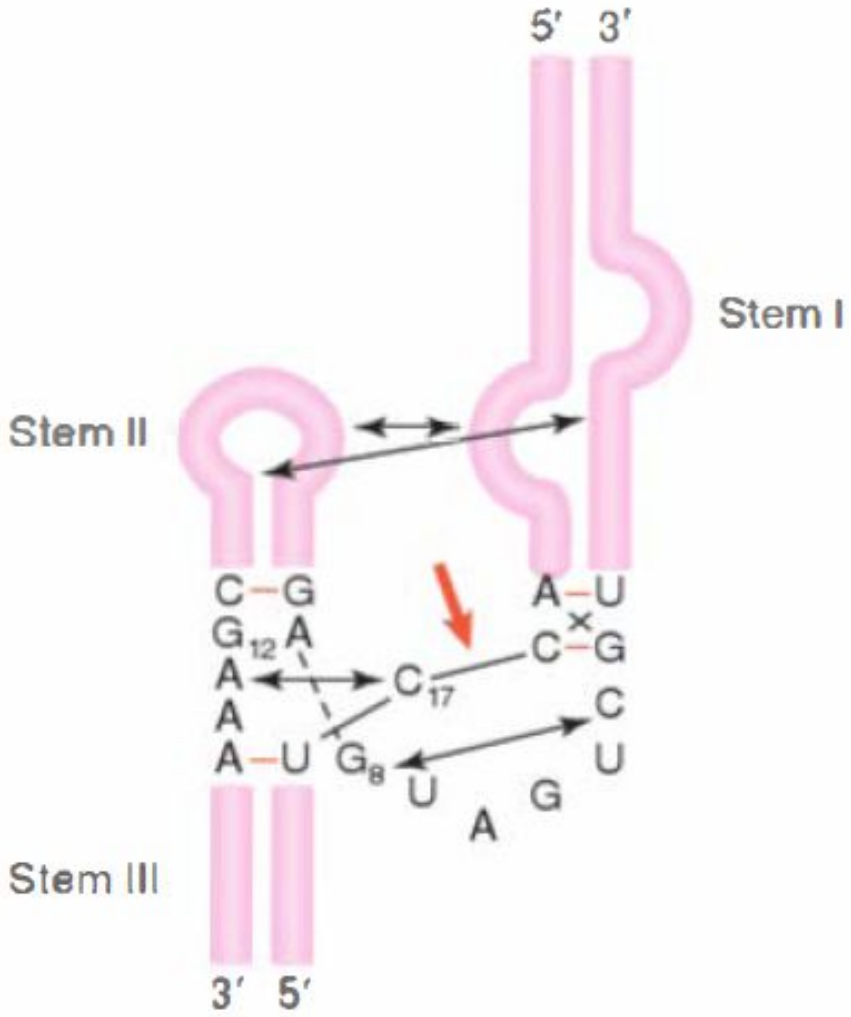


FIGURE 23.14 Self-cleavage sites of viroids and virusoids have a consensus sequence and form a **hammerhead** secondary structure by intramolecular pairing. Hammerheads can also be generated by pairing between a substrate strand and an "enzyme" strand. The three loop regions at the end of the stems are optional.



2’-3’ cyclic phosphodiester

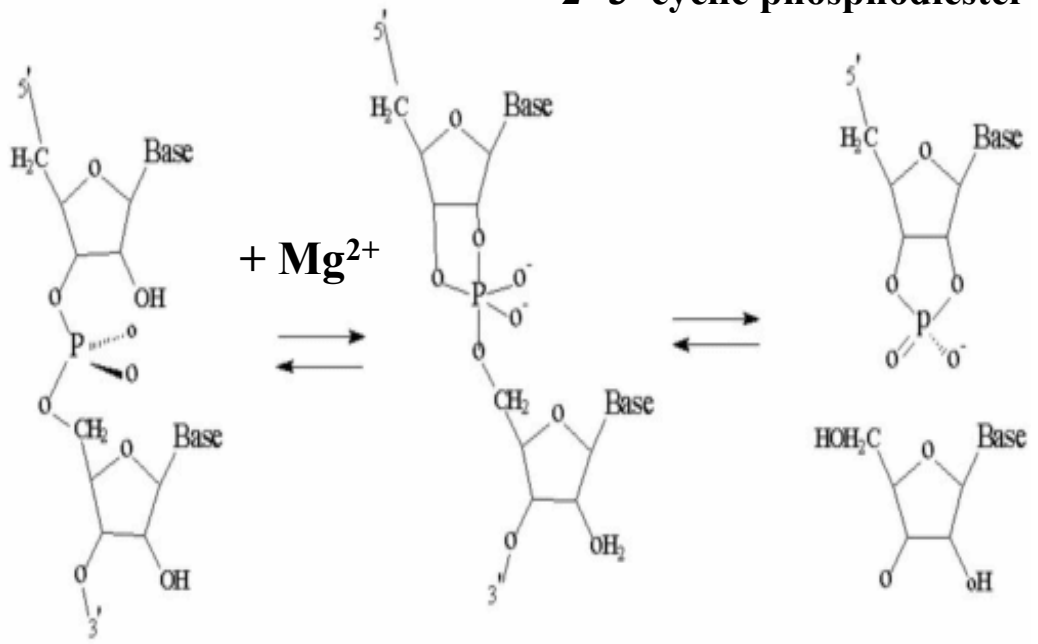


FIGURE 23.15 The hammerhead ribozyme structure is held in an active tertiary conformation by interactions between stem loops, indicated by arrows. The site of cleavage is marked with a red arrow. Adapted from M. Martick and W. G. Scott, *Cell* (126): 309–320.

Enzyme	Substrate	K_m (mM)	k_{cat} (min^{-1})	k_{cat}/K_m (mM^{-1} min^{-1})
19-base virusoid	24-base RNA	0.0006	0.5	8.3×10^2
L-19 intron	CCCCCC	0.04	1.7	4.2×10^1
RNase P RNA	pre-tRNA	0.00003	0.4	1.3×10^4
RNase P complete	pre-tRNA	0.00003	29	9.7×10^5
RNase T1	GpA	0.05	5700	1.1×10^5
β-galactosidase	lactose	4.0	12,500	3.2×10^3

FIGURE 23.9 Reactions catalyzed by RNA have the same features as those catalyzed by proteins, although the rate is slower. The K_m gives the concentration of substrate required for half-maximum velocity; this is an inverse measure of the affinity of the enzyme for substrate. The k_{cat} gives the number of substrate molecules transformed in unit time by a single catalytic site.

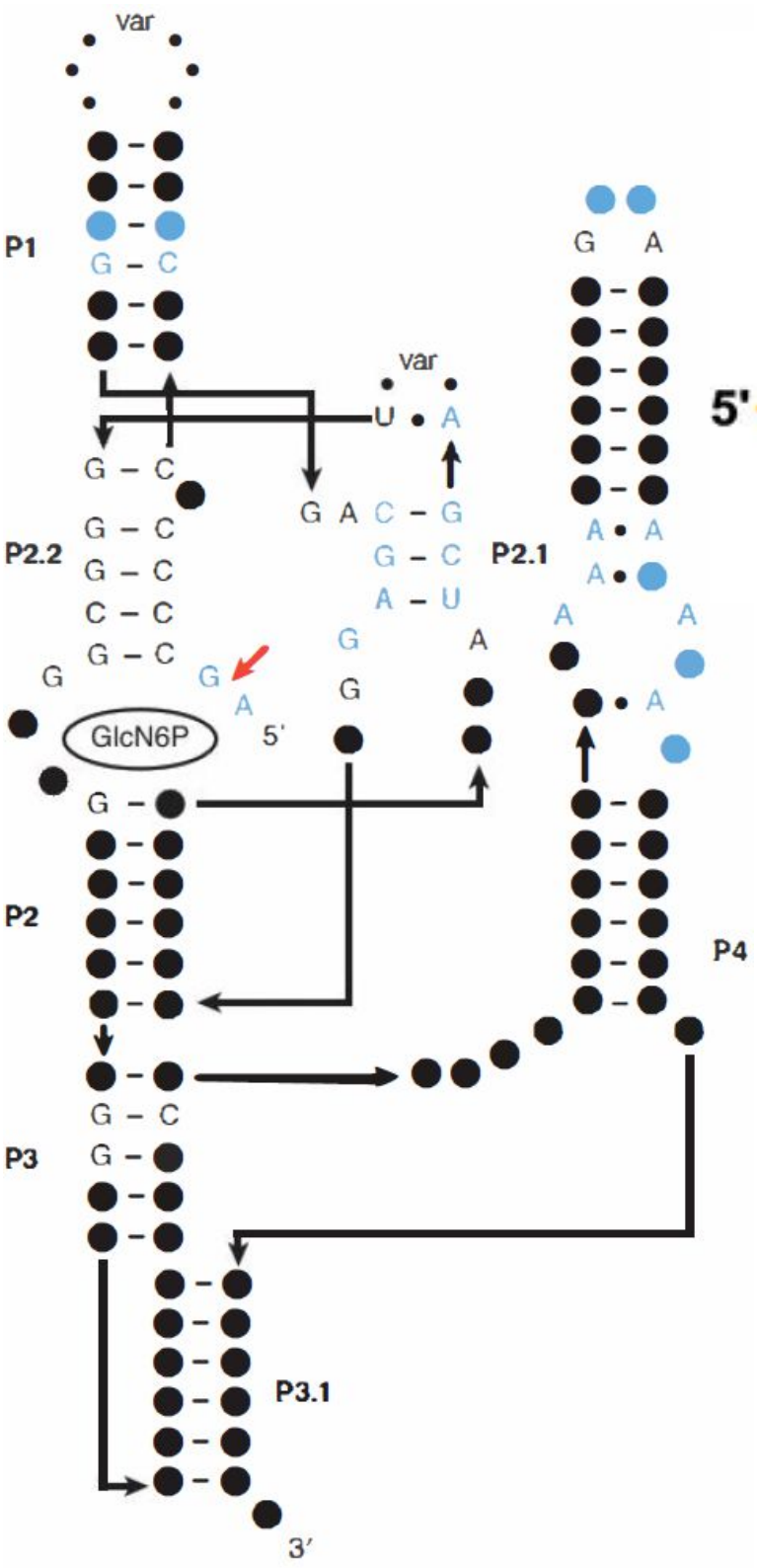
Riboswitch

"A **riboswitch** is a part of an mRNA molecule that can directly bind a small target molecule and whose binding of the target affects the gene's activity. Thus, an mRNA that contains a riboswitch is directly involved in regulating its own activity, depending on the presence or absence of its target molecule. "

Most known riboswitches occur in eubacteria, but functional riboswitches of one type (the TPP (binds thiamin pyrophosphate) riboswitch have been discovered in plants and certain fungi

Riboswitches are conceptually divided into two parts: an aptamer and an expression platform. The aptamer directly binds the small molecule, and the expression platform undergoes structural changes in response to the changes in the aptamer. The expression platform is what regulates gene expression.
Expression platforms typically turn off gene expression in response to the small molecule, but some turn it on. Expression platforms include:

- The formation of rho-independent transcription termination hairpins
- Folding in such a way as to sequester the ribosome-binding site, thereby blocking translation.
- Self-cleavage (i.e. the riboswitch contains a **ribozyme** that cleaves itself in the presence of sufficient concentrations of its metabolite).
- Folding in such a way as to affect the splicing of the pre-mRNA.



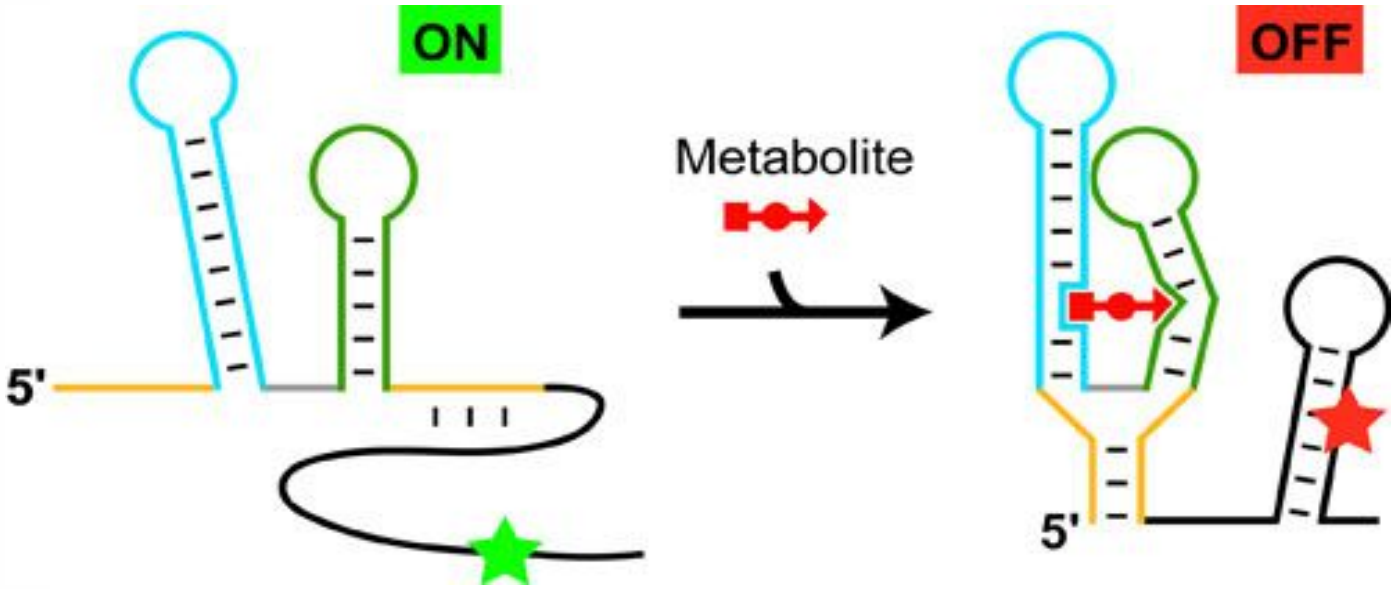
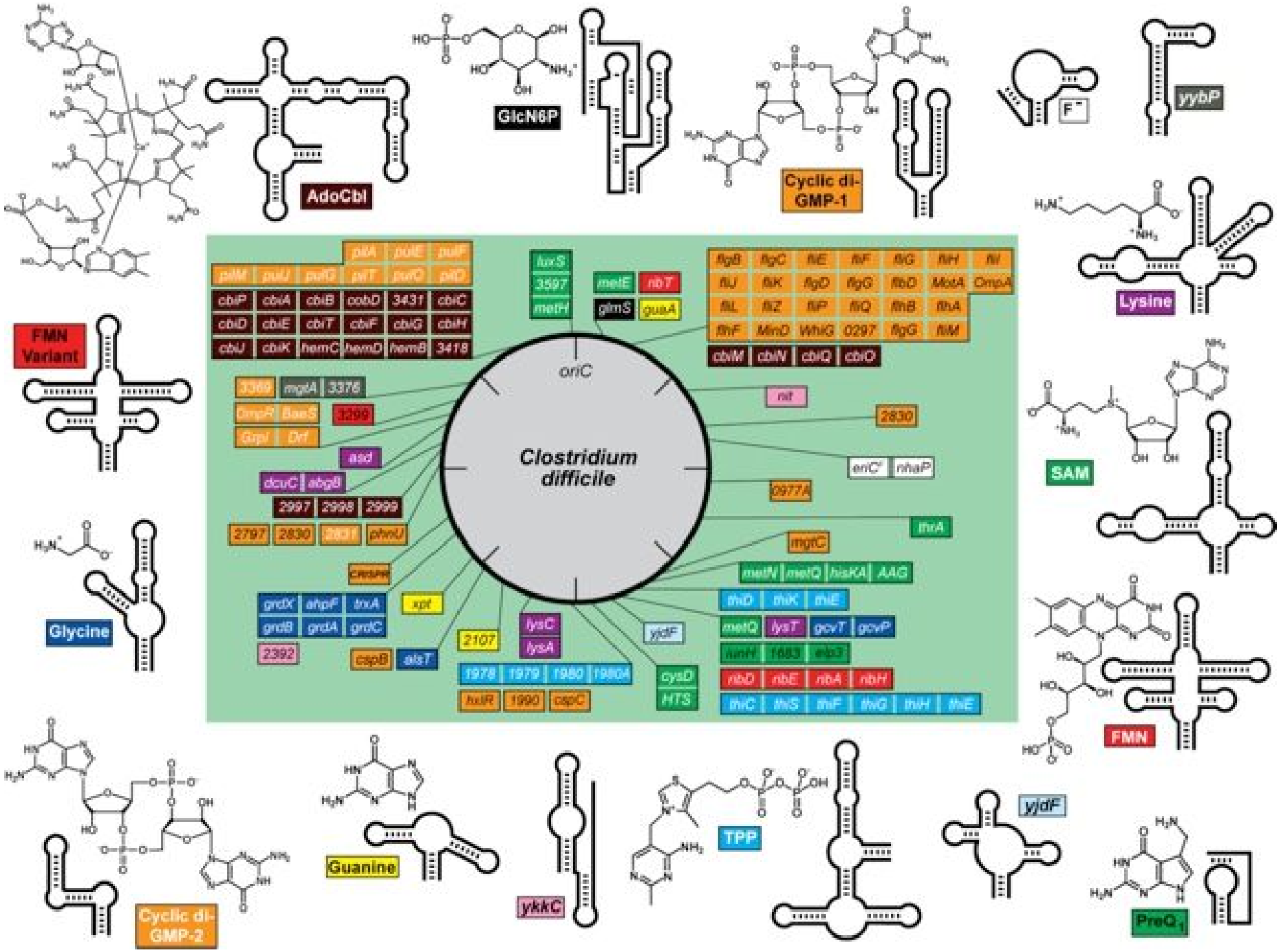
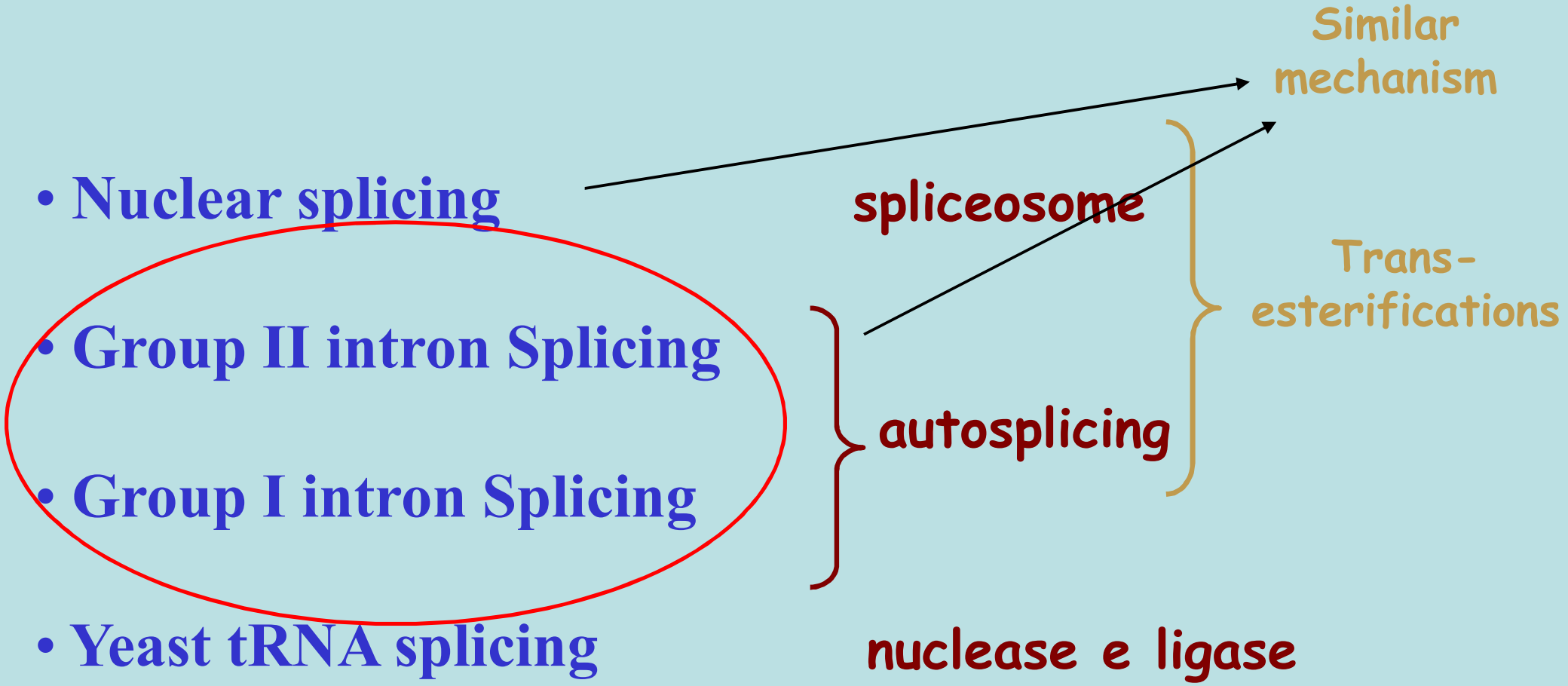


FIGURE 23.10 A ribozyme is contained within the 5′ untranslated region of the mRNA encoding the enzyme that produces glucosamine-6-phosphate (GlcN6P). When GlcN6P binds to the ribozyme, it cleaves off the 5′ end of the mRNA, thereby inactivating it and preventing further production of the enzyme. Regions important for maintaining the active tertiary structure are shown in blue, while the cleavage site is indicated by a red arrow. Reprinted from *Curr. Opin. Struct. Biol.*, vol. 17, T. E. Edwards, D. J. Klein, and A. R. Ferré-D' Amaré, Riboswitches: small-molecule recognition ..., pp. 273–279. Copyright 2007, with permission from Elsevier [http://www.sciencedirect.com/science/journal/0959440X].


AdoCbl
GlcN6P
Cyclic di-GMP-1
F⁻
yybP
Lysine
FMN Variant
SAM
Glycine
FMN
Cyclic di-GMP-2
Guanine
ykkC
TPP
yjdF
PreQ1
Clostridium difficile
oriC
pilA pilE pilF
pilM pilJ pilQ pilT pilC pilD
cbiP cbiA cbiB cobD 3431 cbiC
cbiD cbiE cbiT cbiF cbiG cbiH
cbiJ cbiK hemC hemD hemB 3418
luxS 3597 metH
metE nbT
glmS guaA
flgB flgC fliE fliF flgG fliH fliI
fliJ fliK flgD flgG flbD MotA OmpA
fliL fliZ fliP fliQ flhB flhA
flhF MinD WhiG 0297 flgG fliM
cbiM cbiN cbiQ cbiO
3389 mgtA 3376
OmpR BaeS 3299
GspI Dnf
asd
dcuC atoB
2997 2998 2999
2797 2830 2831 phnU
CRISPR
grdX ahpF trxA
grdB grdA grdC
2892
xpt
cspB alsT
2107
lysC lysA
1978 1979 1980 19804
fusR 1990 cspC
ydjF
cysD HTS
nit
2830
oriC' nhaP
0977A
thrA
mgtC
metN metQ hisKA AAG
thiD thiK thiE
metQ lysT gcvT gcvP
tunH 1683 ebg3
nbD nbE nbA nbH
thiC thiS thiF thiG thiH thiE

Splicing types



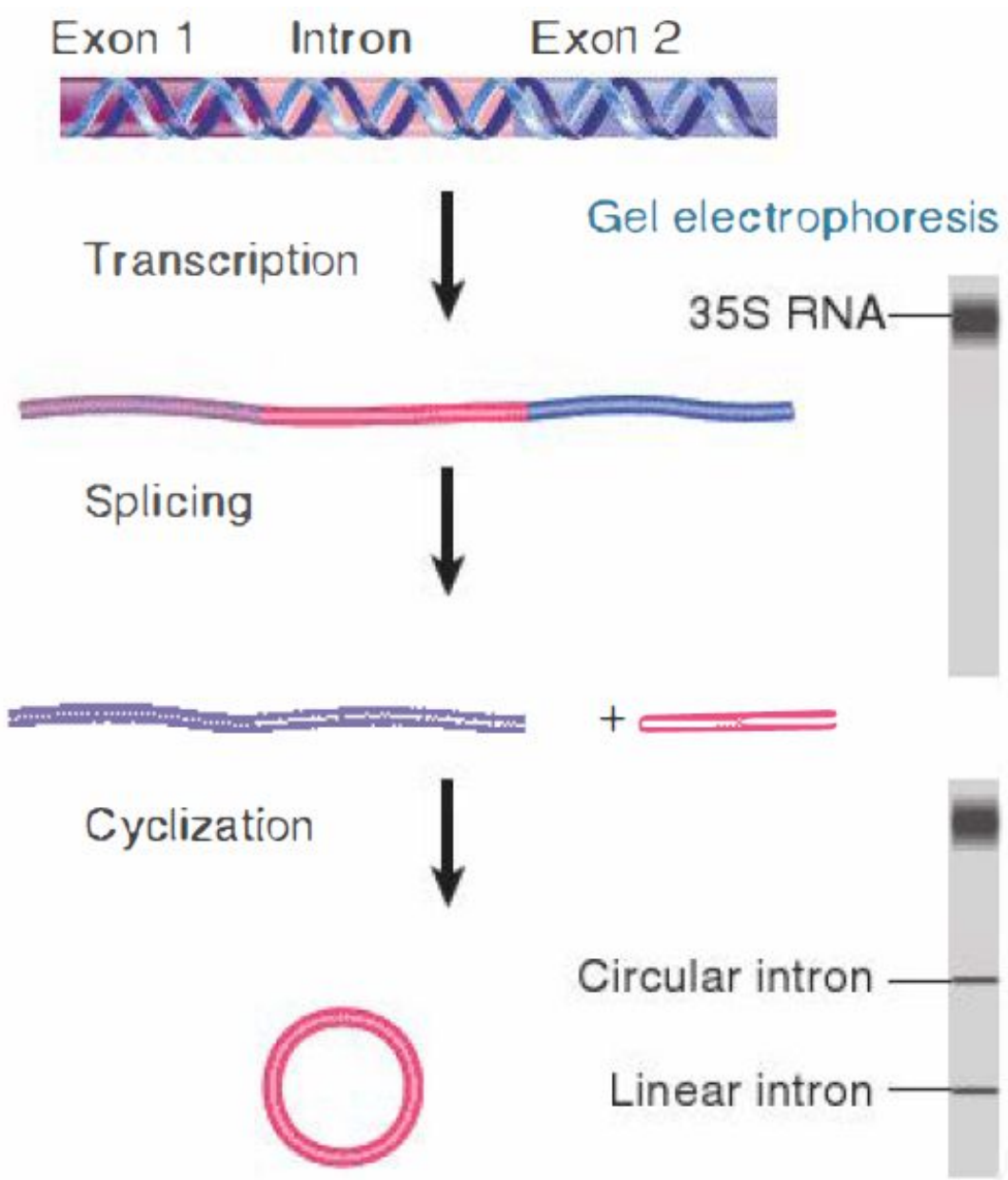


FIGURE 23.1 Splicing of the *Tetrahymena* 35S rRNA precursor can be followed by gel electrophoresis. The removal of the intron is revealed by the appearance of a rapidly moving small band. When the intron becomes circular, it electrophoreses more slowly, as seen by a higher band.

Type I Introns

- rRNA genes in simple eukaryotes
- Mitochondrial genes
- Some bacterial genes and T4 phage genes

Type II Introns

- Organelles genes (mitochondria and chloroplast)

Type I introns splicing

Presence of mono and divalent cation and G-OH

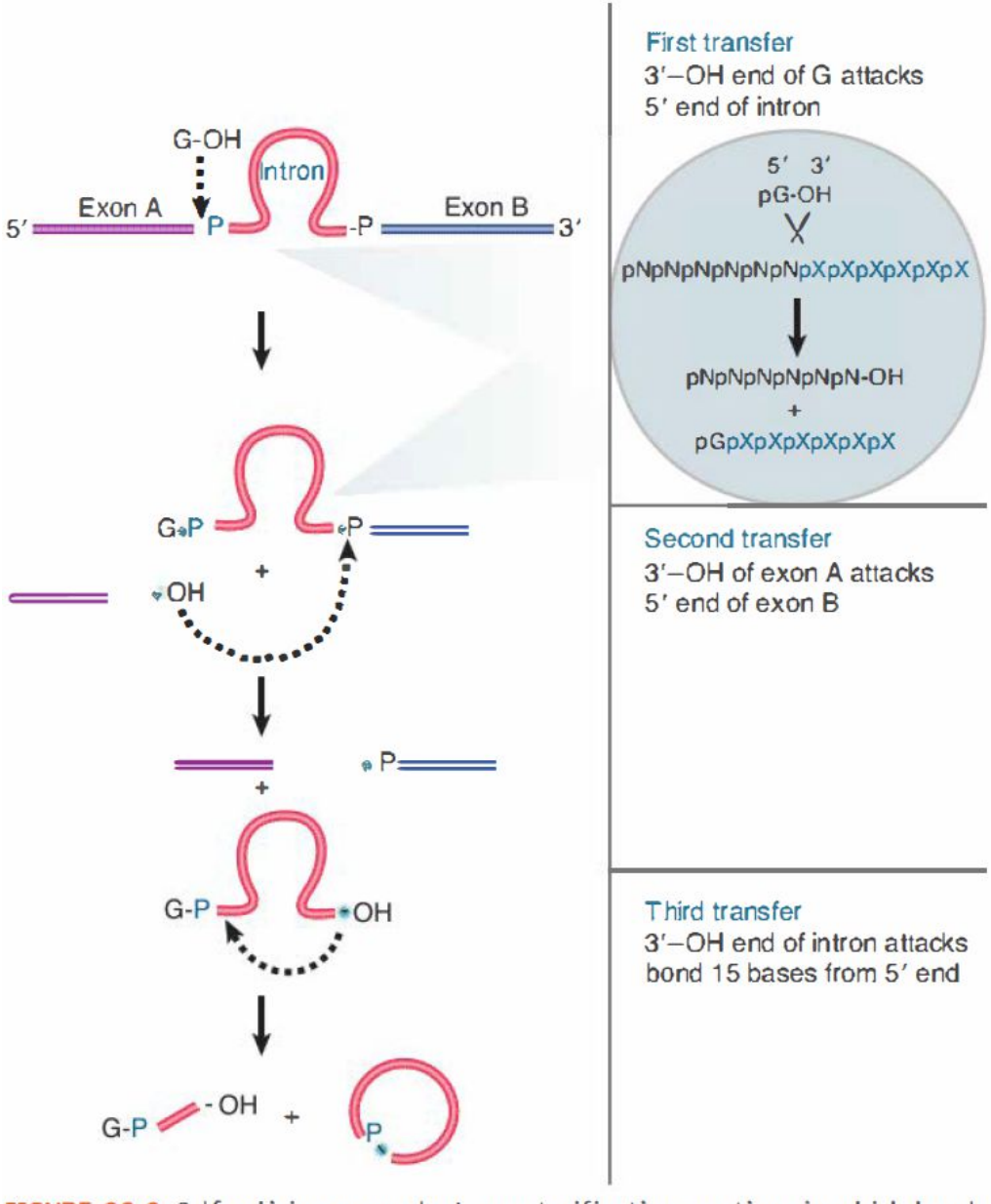


FIGURE 23.2 Self-splicing occurs by transesterification reactions in which bonds are exchanged directly. The bonds that have been generated at each stage are indicated by the blue circles.

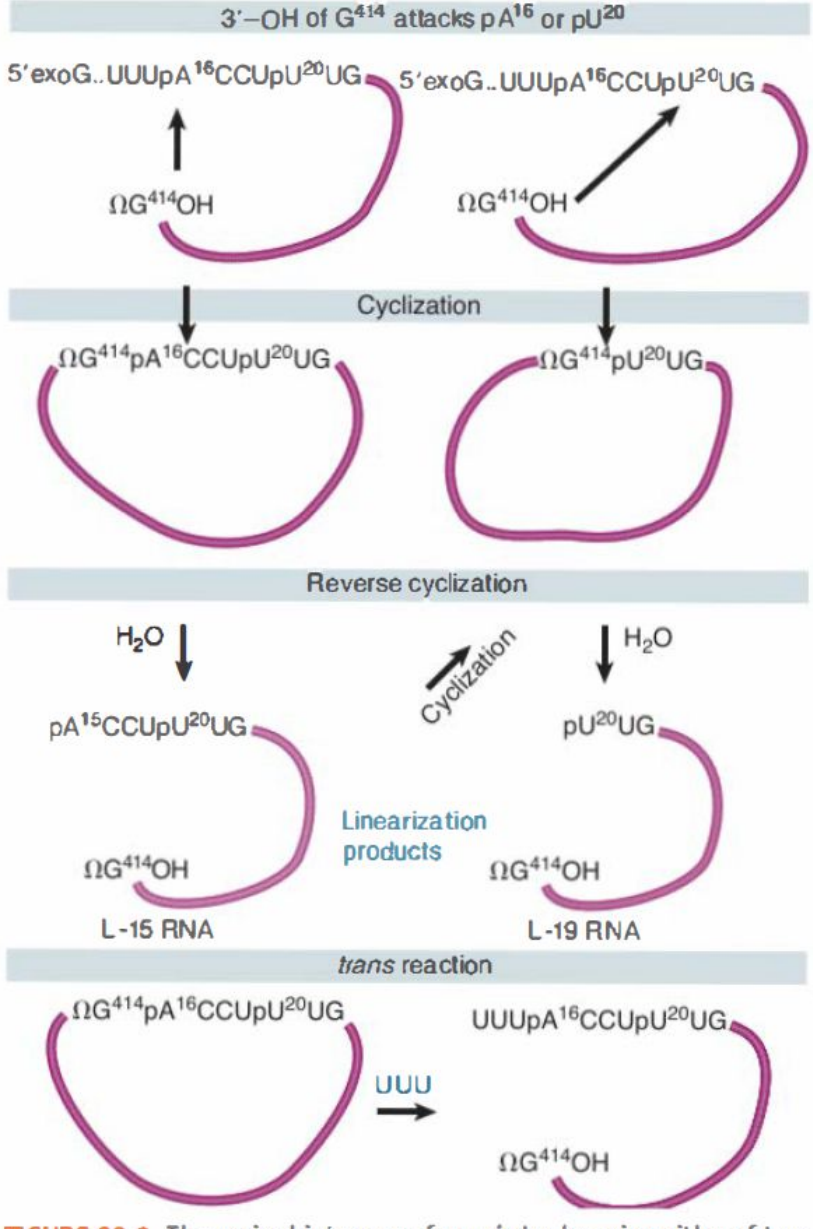
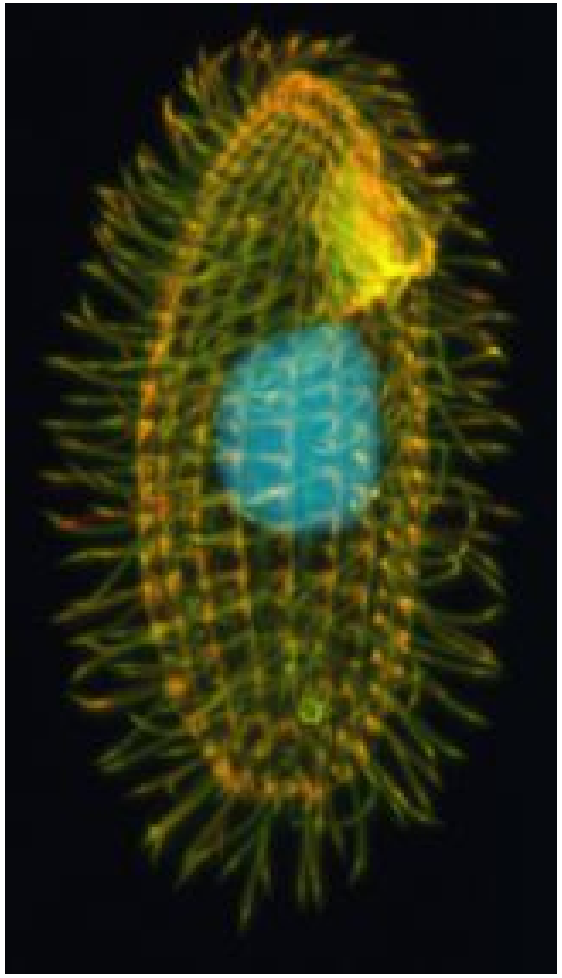
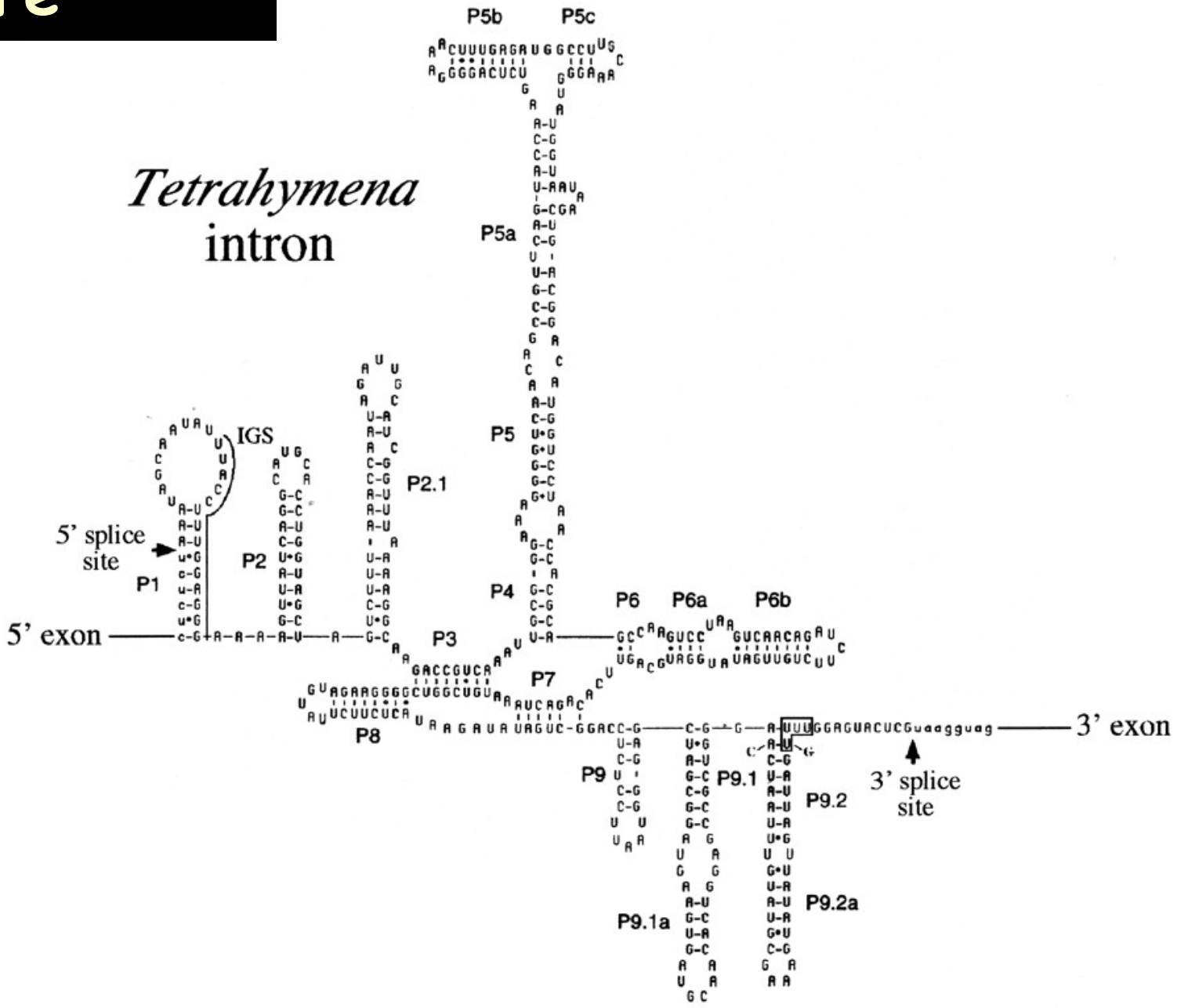


FIGURE 23.3 The excised intron can form circles by using either of two internal sites for reaction with the 5′ end and can reopen the circles by reaction with water or oligonucleotides.

Tetrahymena rRNA intron structure





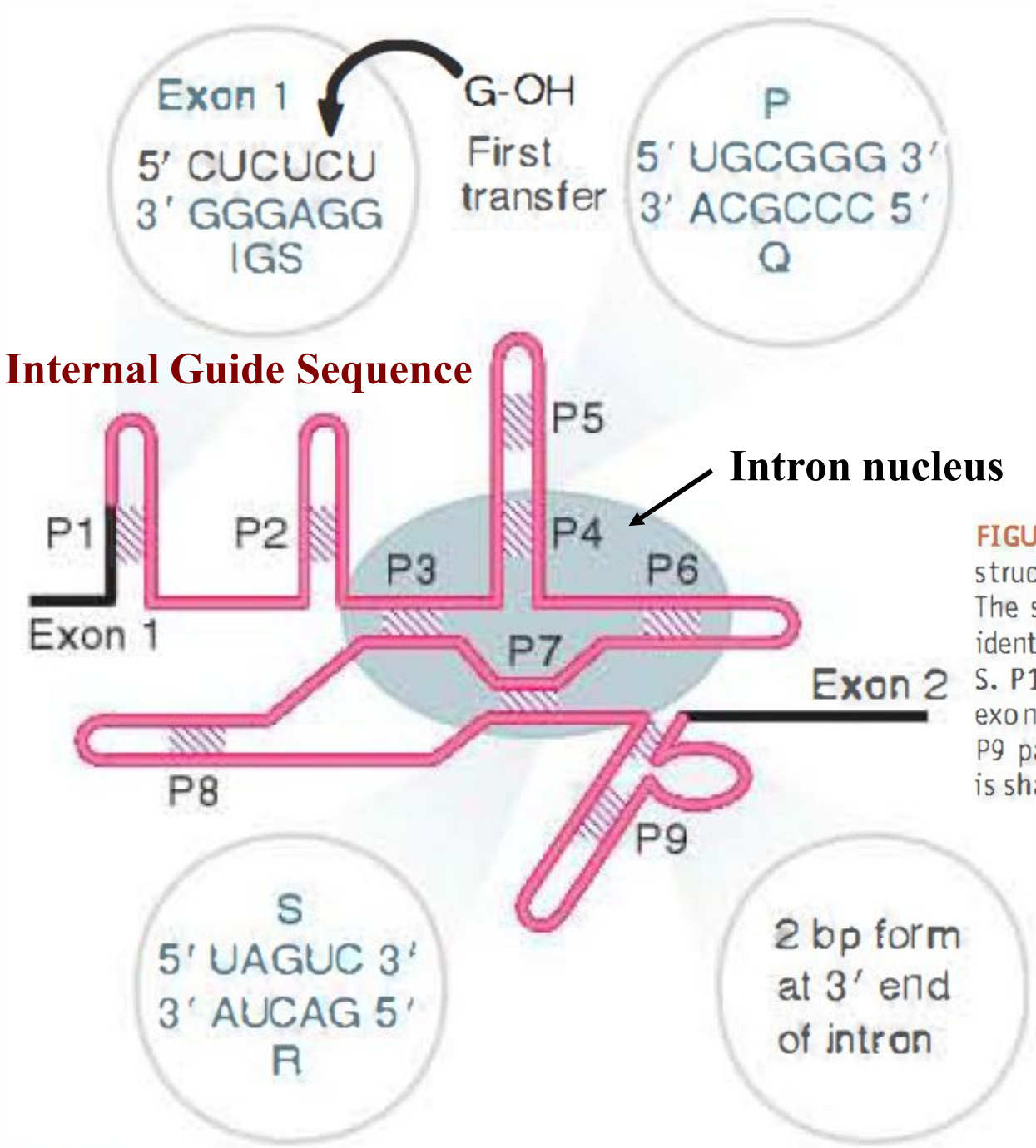


FIGURE 23.4 Group I introns have a common secondary structure that is formed by nine base-paired regions. The sequences of regions P4 and P7 are conserved and identify the individual sequence elements P, Q, R, and S. P1 is created by pairing between the end of the left exon and the IGS of the intron; a region between P7 and P9 pairs with the 3′ end of the intron. The intron core is shaded in gray.

Type I introns conserved elements

Introns	P	Q	R (9L)	S (2)
Mitochondrial type I	UGCUGG	UCAGCAG	GACUA	UAGUC
rDNA Tetrahymena	UGCGGG	CCACGCA	GACUA	UAGUC
Thymidylate synt. T4	ACGG G	CCCGU	GACUA	UAGUC

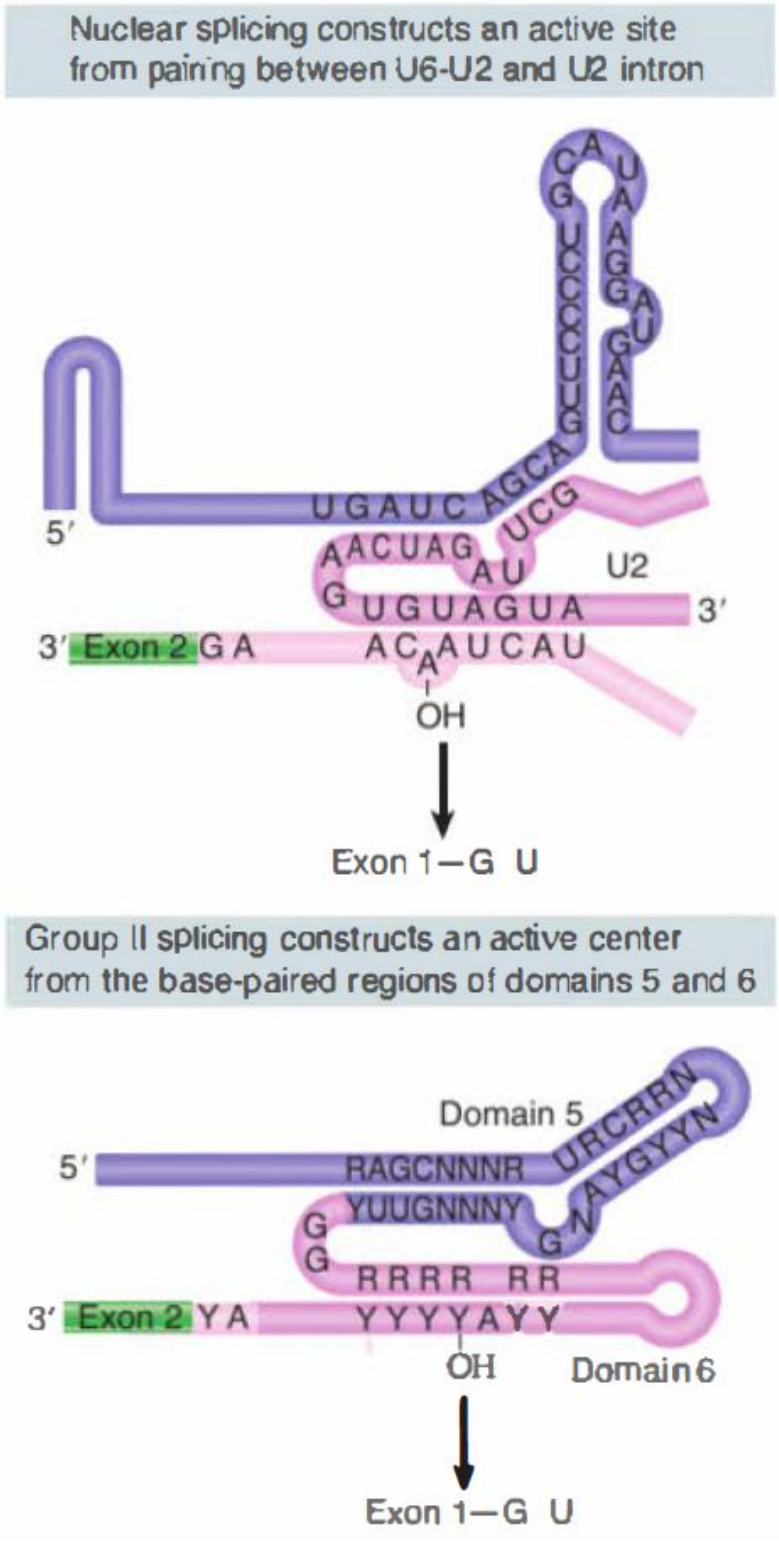


FIGURE 21.16 Nuclear splicing and group II splicing involve the formation of similar secondary structures. The sequences are more specific in nuclear splicing; group II splicing uses positions that may be occupied by either purine (R) or pyrimidine (Y).

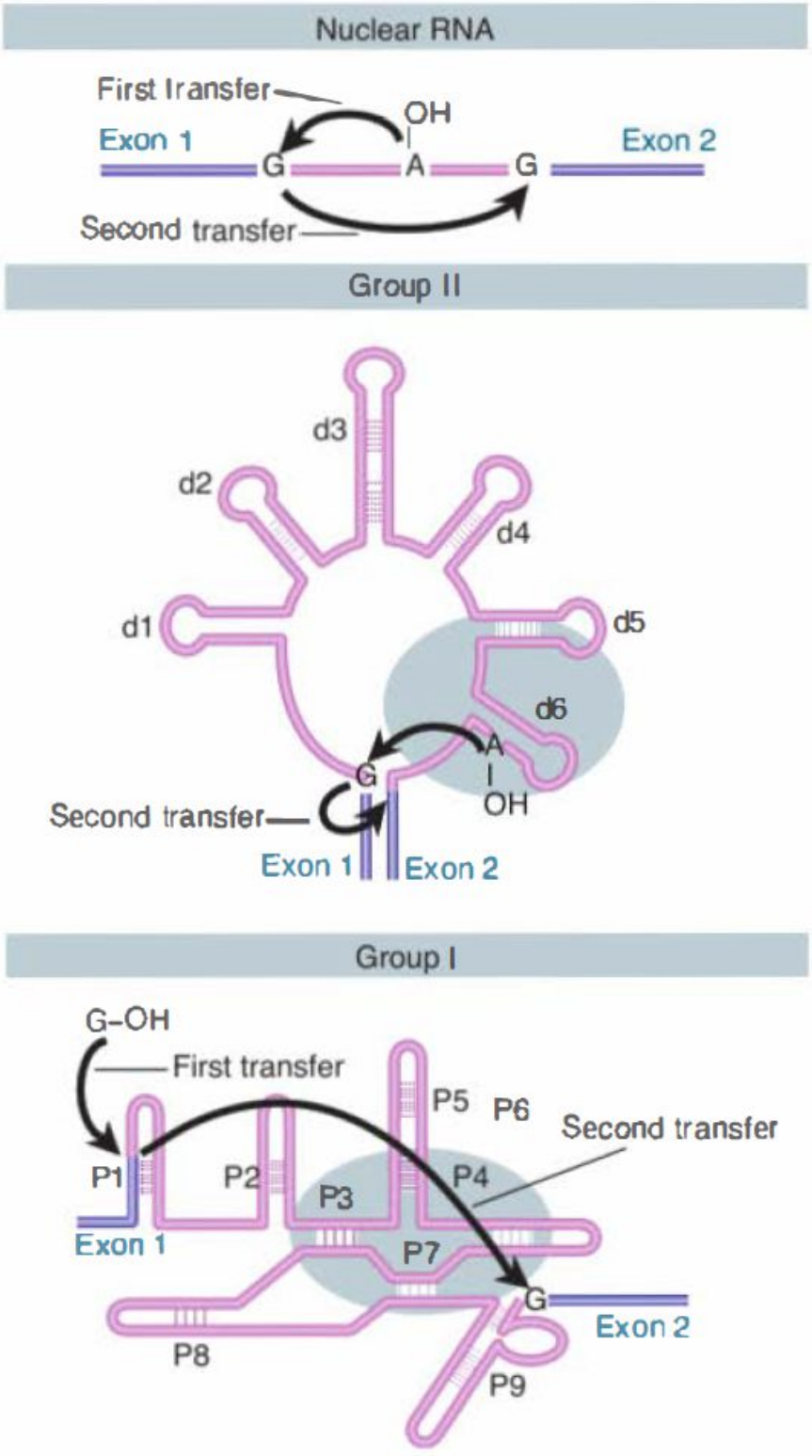


FIGURE 21.15 Three classes of splicing reactions proceed by two transesterifications. First, a free –OH group attacks the exon 1–intron junction. Second, the –OH created at the end of exon 1 attacks the intron–exon 2 junction.

RNA Editing

Individual bases post-transcriptional modification of mRNA

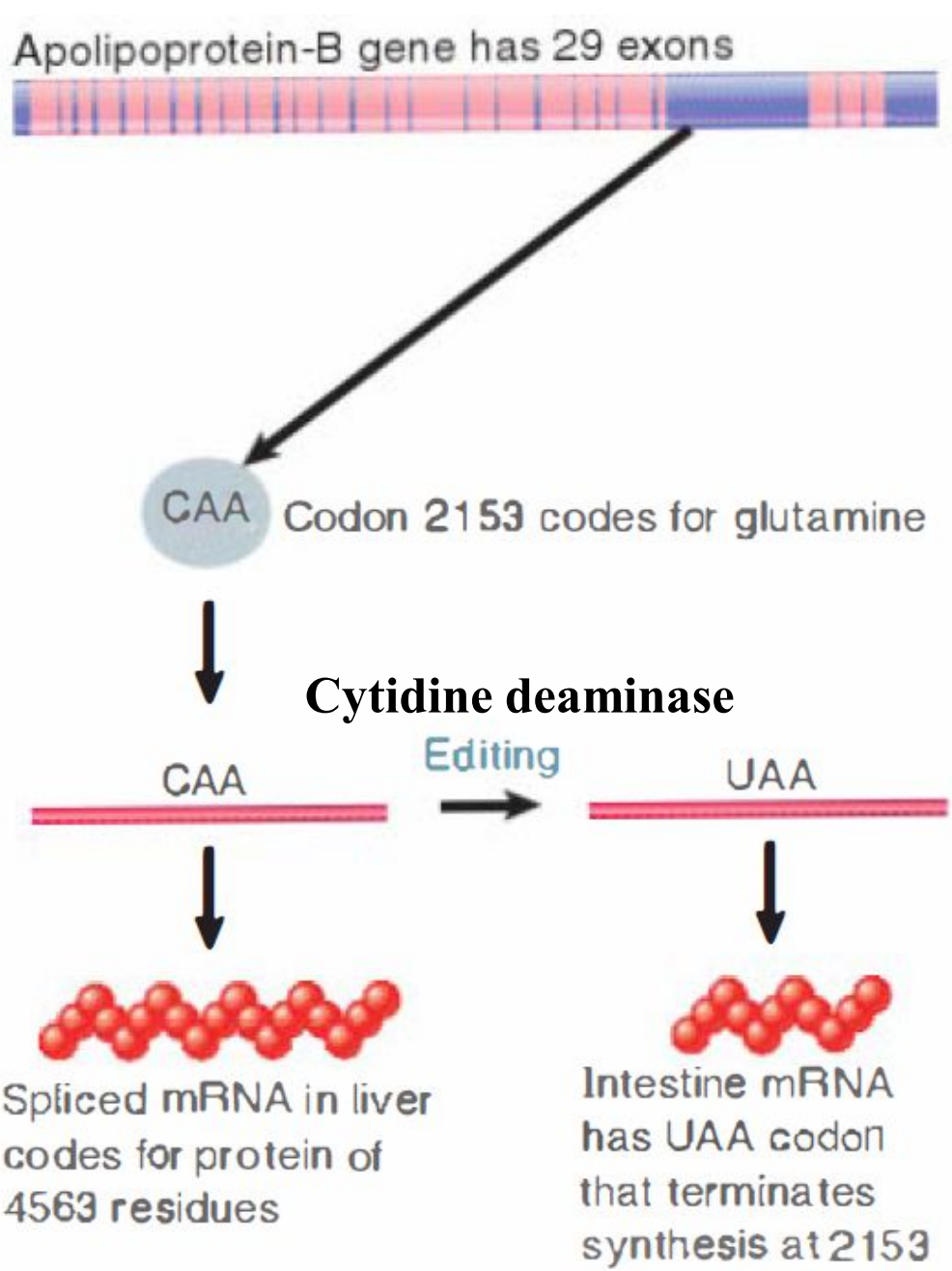


FIGURE 23.16 The sequence of the apo-B gene is the same in the intestine and liver, but the sequence of the mRNA is modified by a base change that creates a termination codon in the intestine.

The transformation of an adenine into a inosine allows a more specific pairing in a duplex RNA region (glutamate receptor in rat brain)

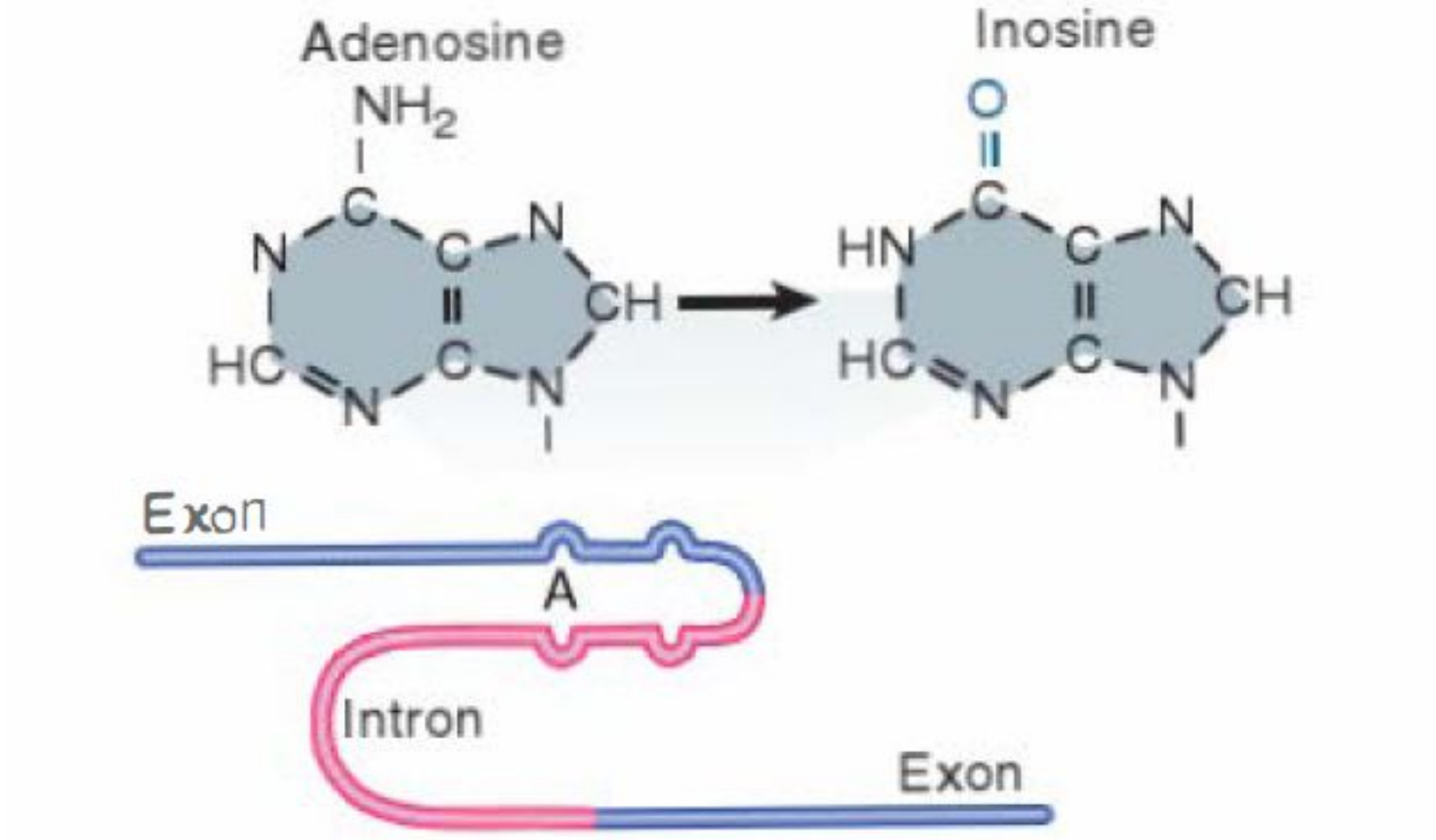


FIGURE 23.17 Editing of mRNA for the glutamate receptor, GluR-B, occurs when a deaminase acts on an adenine in an imperfectly paired RNA duplex region.

Mitochondrial coxII gene (Cytochrome Oxidase) in *Trypanosoma*

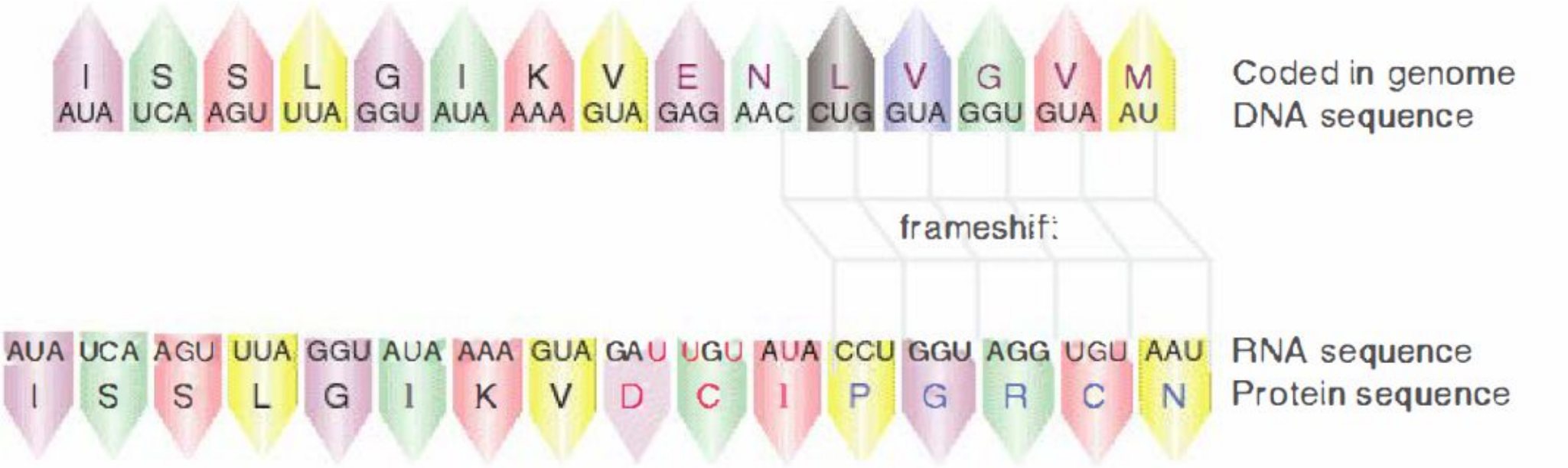


FIGURE 23.18 The mRNA for the trypanosome *coxII* gene has a frameshift relative to the DNA; the correct reading frame observed in the protein is created by the insertion of four uridines (shown in red).

Mature mRNA (with the right ORF) is obtained with the insertion of 4 U compared to the sequence of the gene.

Mitochondrial coxIII gene (Cytochrome Oxidase) in *Trypanosoma brucei*

FIGURE 23.19 Part of the mRNA sequence of *T. brucei coxIII* shows many uridines that are not encoded in the DNA (shown in red) or that are removed from the RNA (shown as Ts in blue boxes).

U added

T removed

Cytochrome b in *Leishmania*

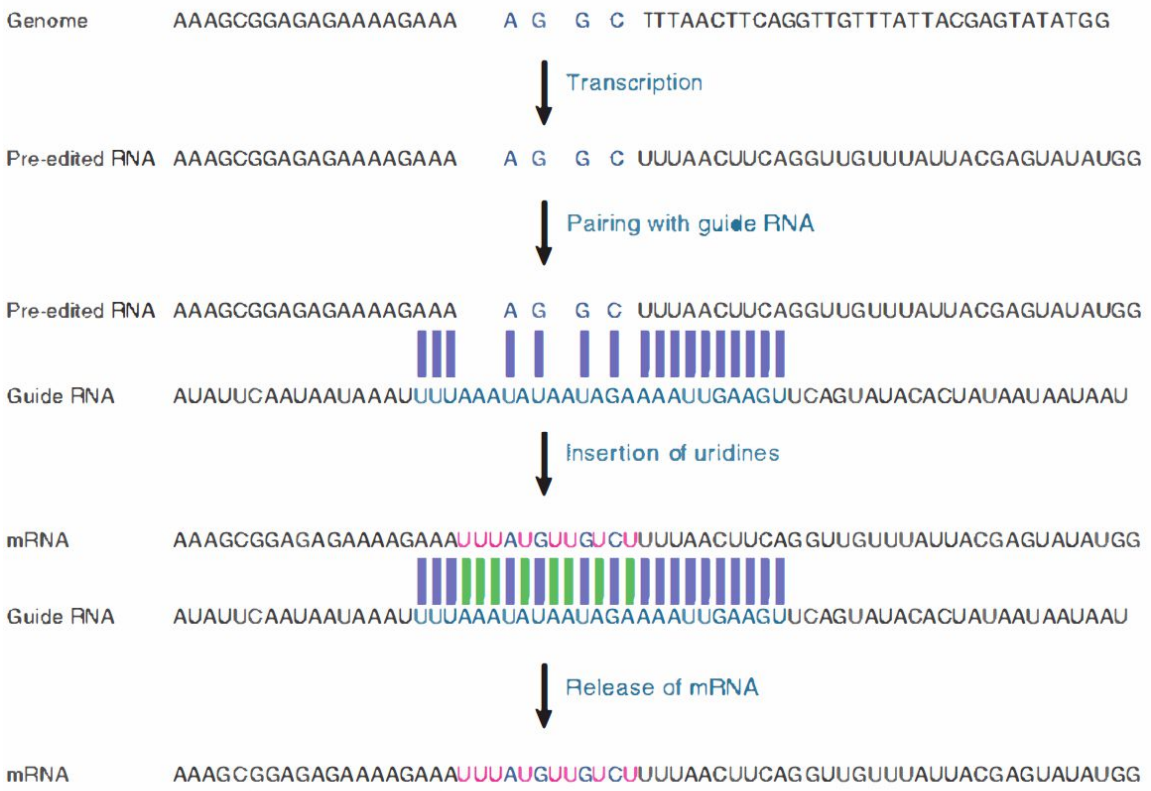


FIGURE 23.20 Pre-edited RNA base pairs with a guide RNA on both sides of the region to be edited. The guide RNA provides a template for the insertion of uridines. The mRNA produced by the insertions is complementary to the guide RNA.

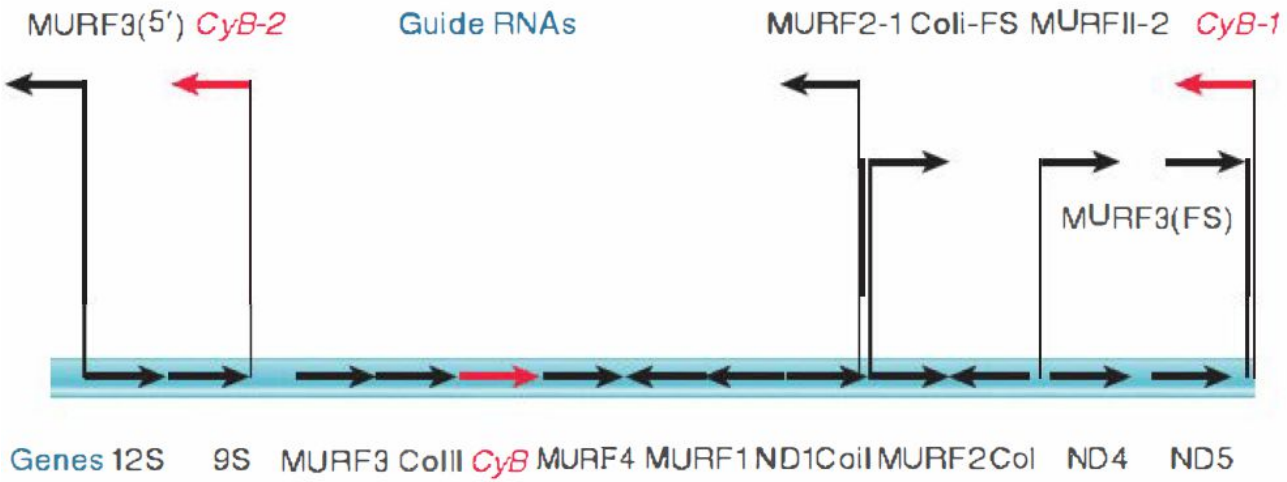


FIGURE 23.21 The *Leishmania* genome contains genes encoding pre-edited RNAs interspersed with units that encode the guide RNAs required to generate the correct mRNA sequences. Some genes have multiple guide RNAs. *CyB* is the gene for pre-edited cytochrome b, and *CyB-1* and *CyB-2* are genes for the guide RNAs involved in its editing.

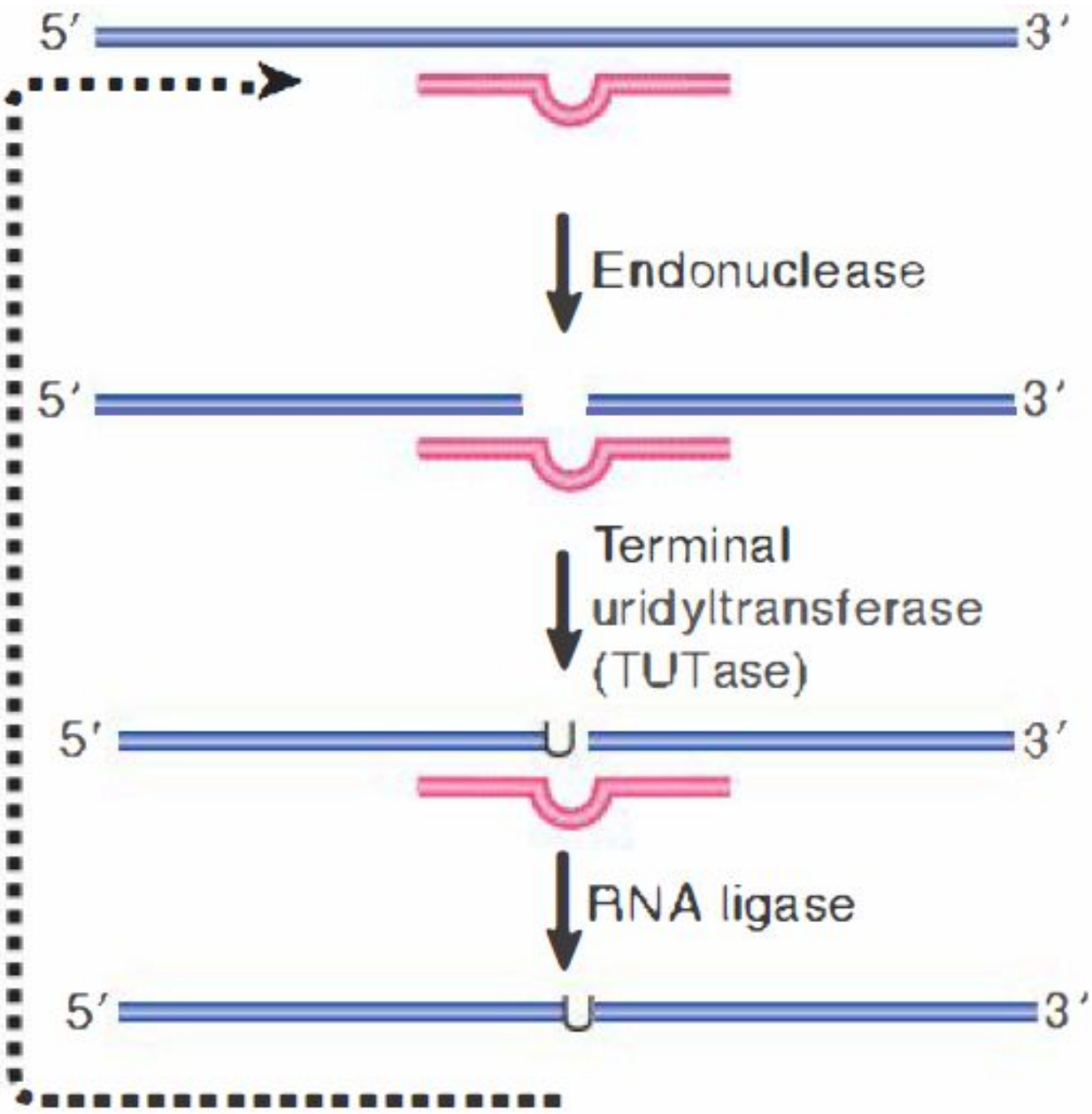
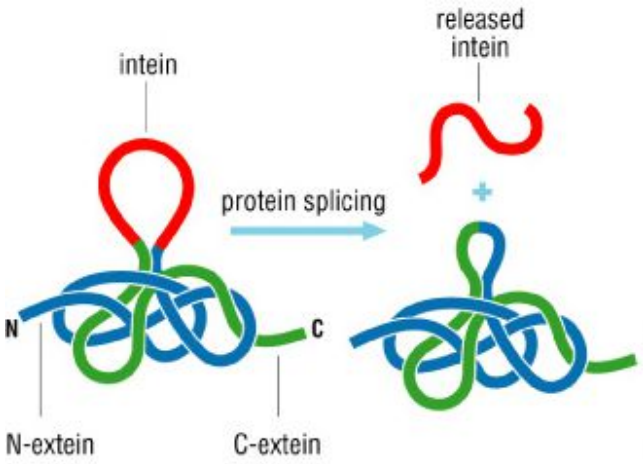
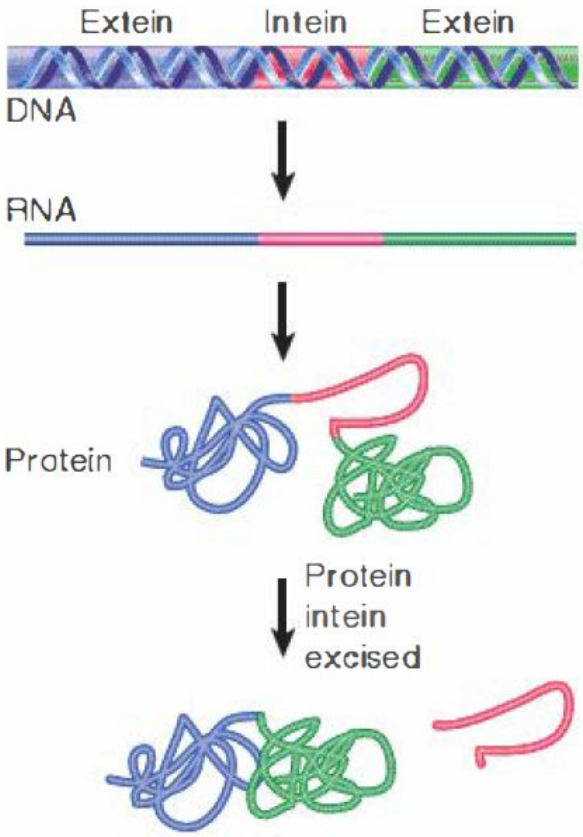


FIGURE 23.22 Addition or deletion of U residues occurs by cleavage of the RNA, removal or addition of the U, and ligation of the ends. The reactions are catalyzed by a complex of enzymes under the direction of guide RNA.

Protein Splicing





Cys o Ser

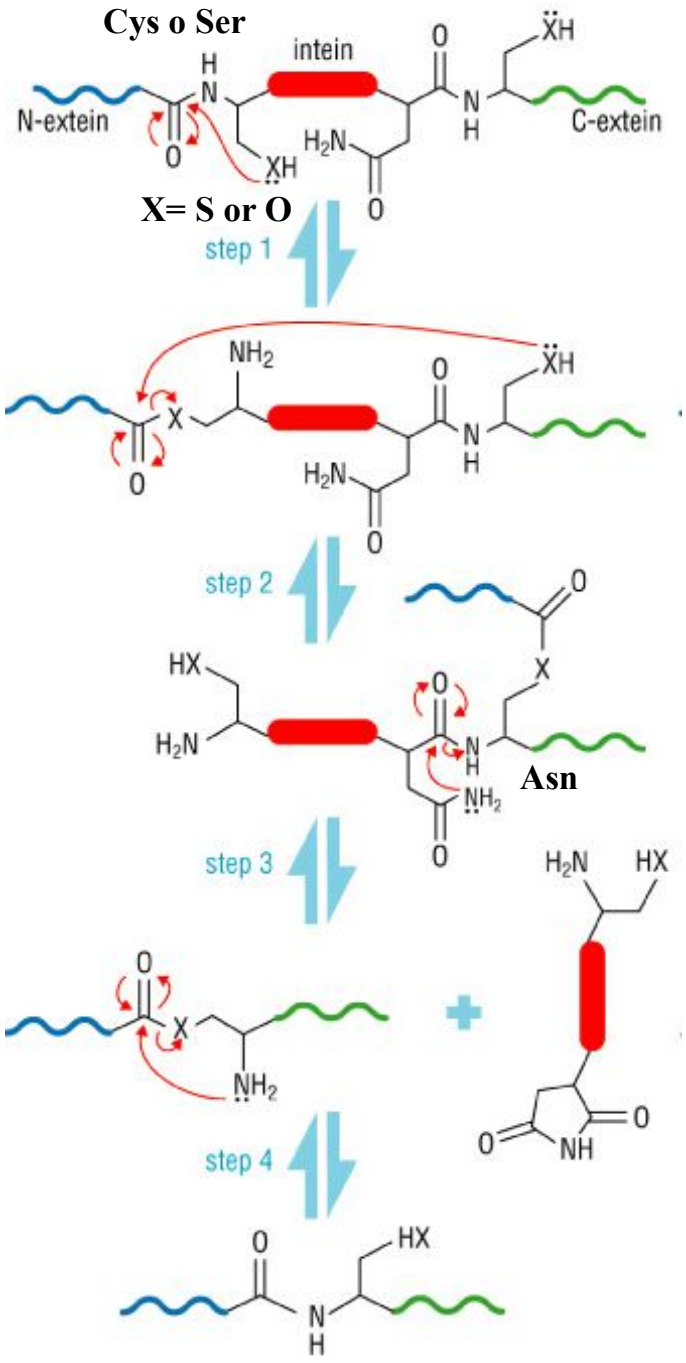


FIGURE 23.23 In protein splicing, the exteins are connected by removing the intein from the protein.