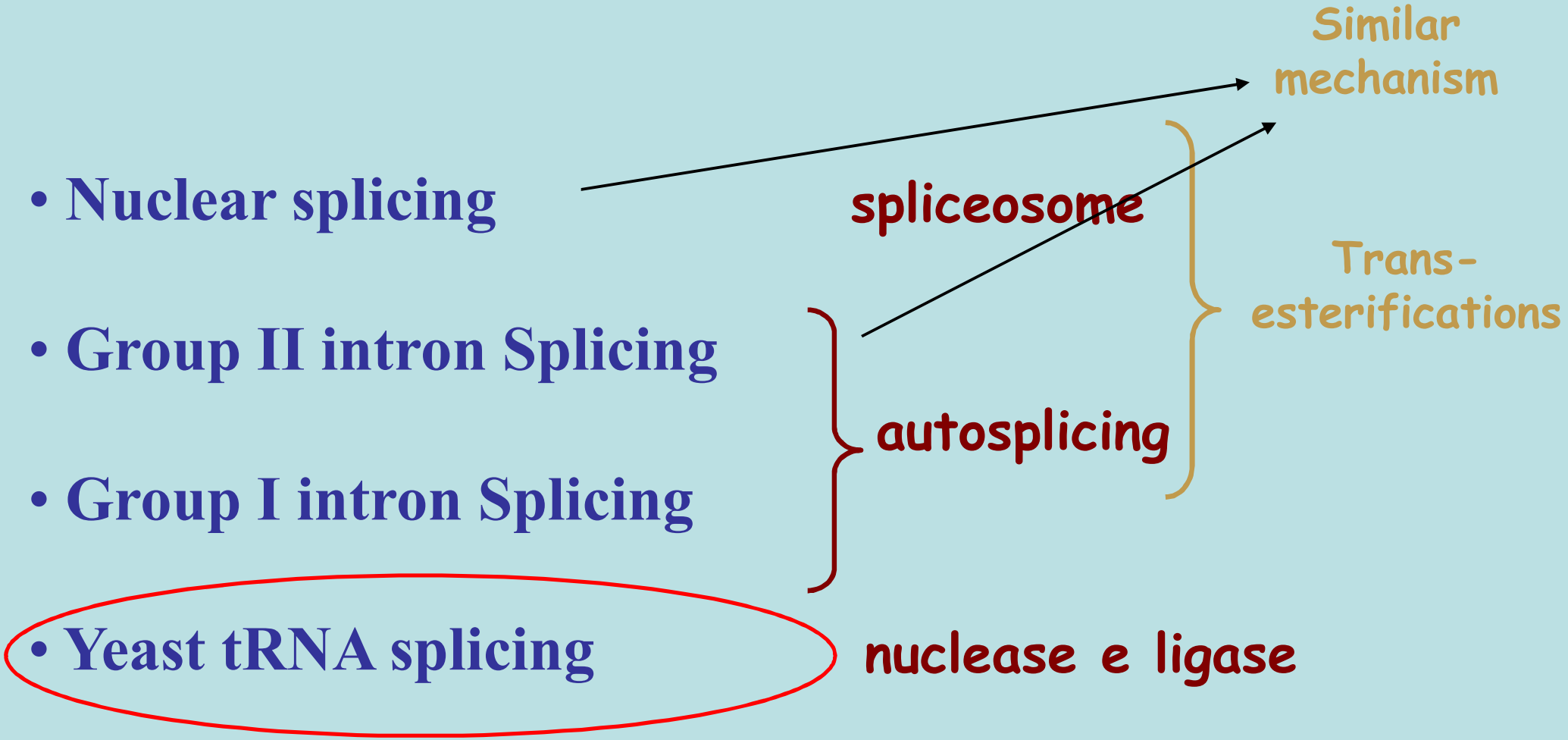
Splicing types



Some 59 of the 272 nuclear tRNA genes in the yeast S. cerevisiae are interrupted.

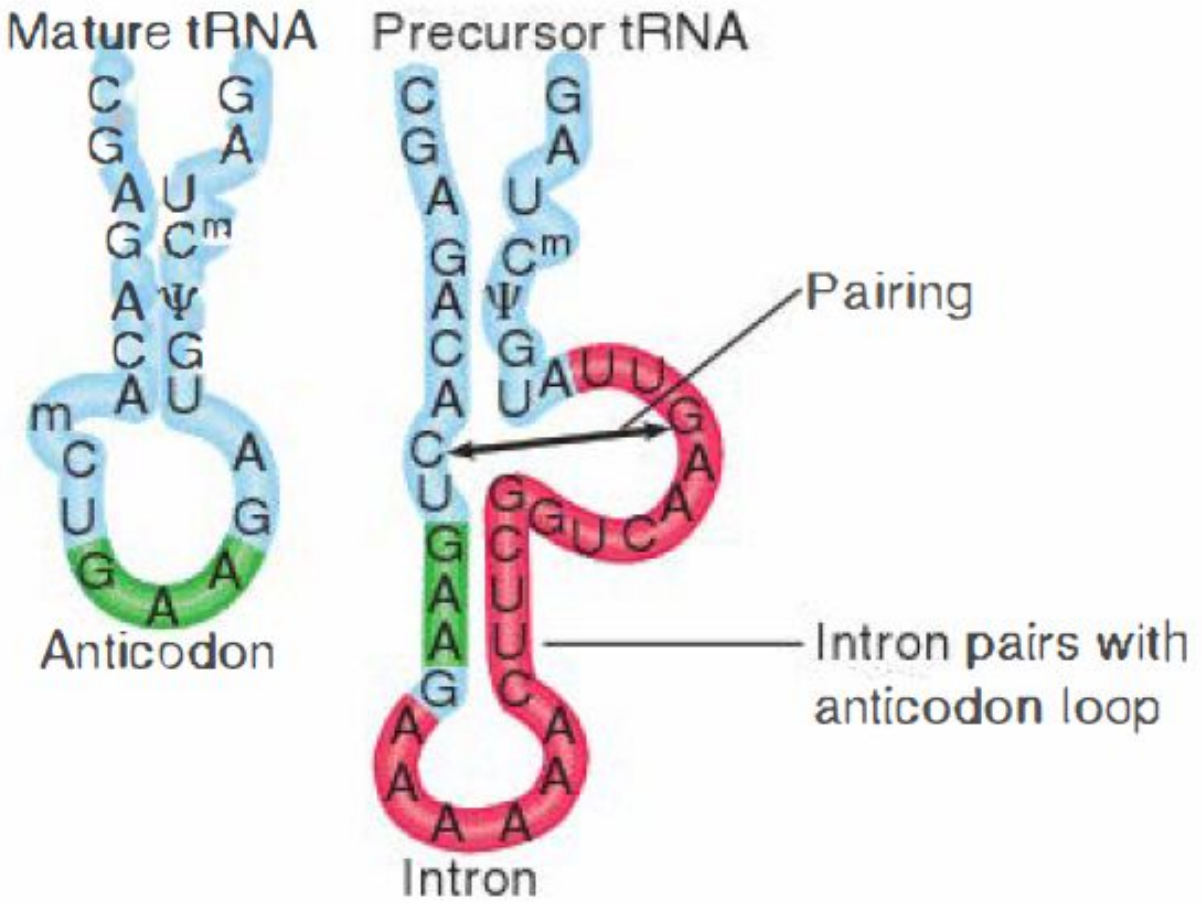


FIGURE 21.34 The intron in yeast $tRNA^{Phe}$ base pairs with the anticodon to change the structure of the anticodon arm. Pairing between an excluded base in the stem and the intron loop in the precursor may be required for splicing.

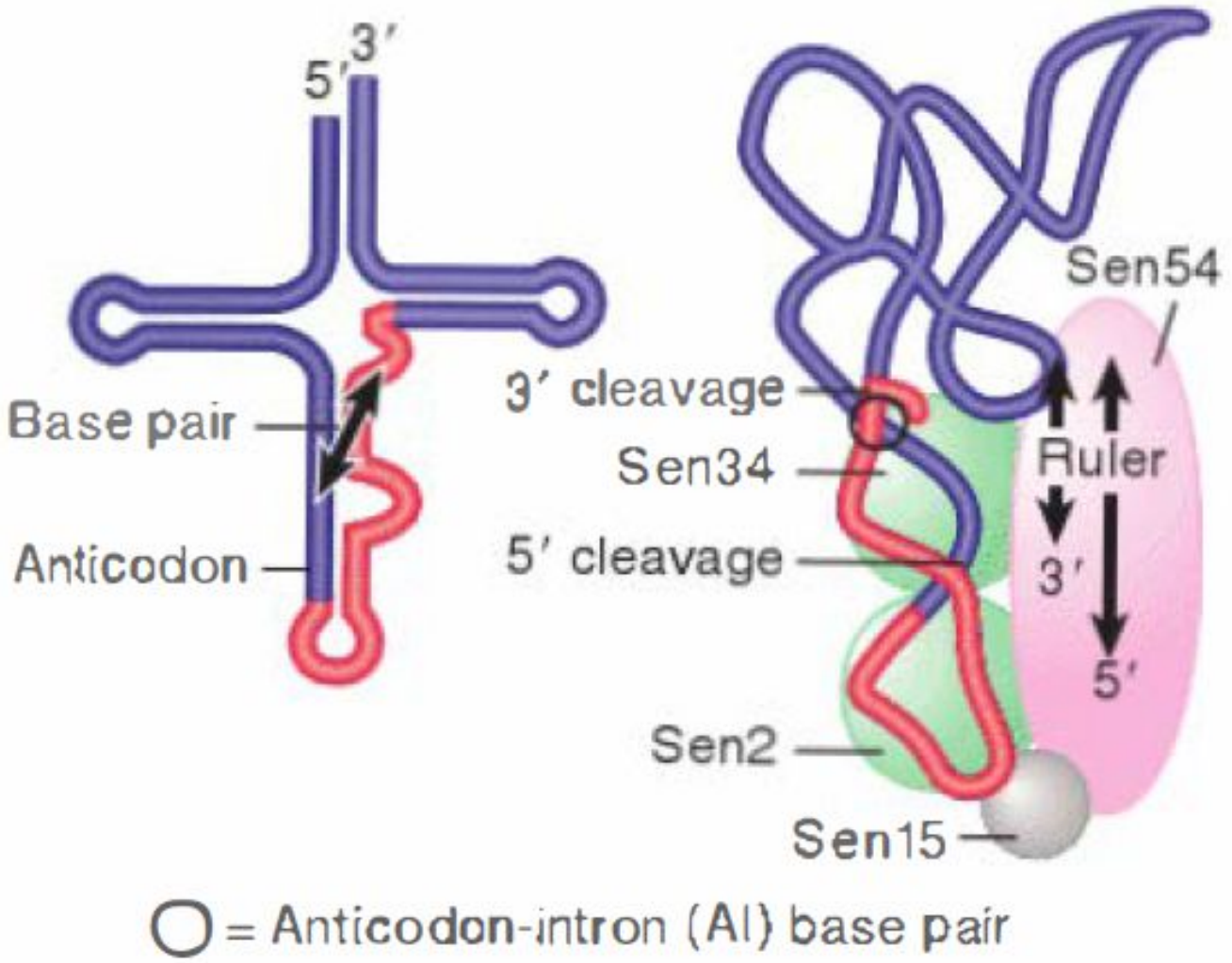


FIGURE 21.36 The 3′ and 5′ cleavages in *S. cerevisiae* pre-tRNA are catalyzed by different subunits of the endonuclease. Another subunit may determine location of the cleavage sites by measuring distance from the mature structure. The AI base pair is also important.

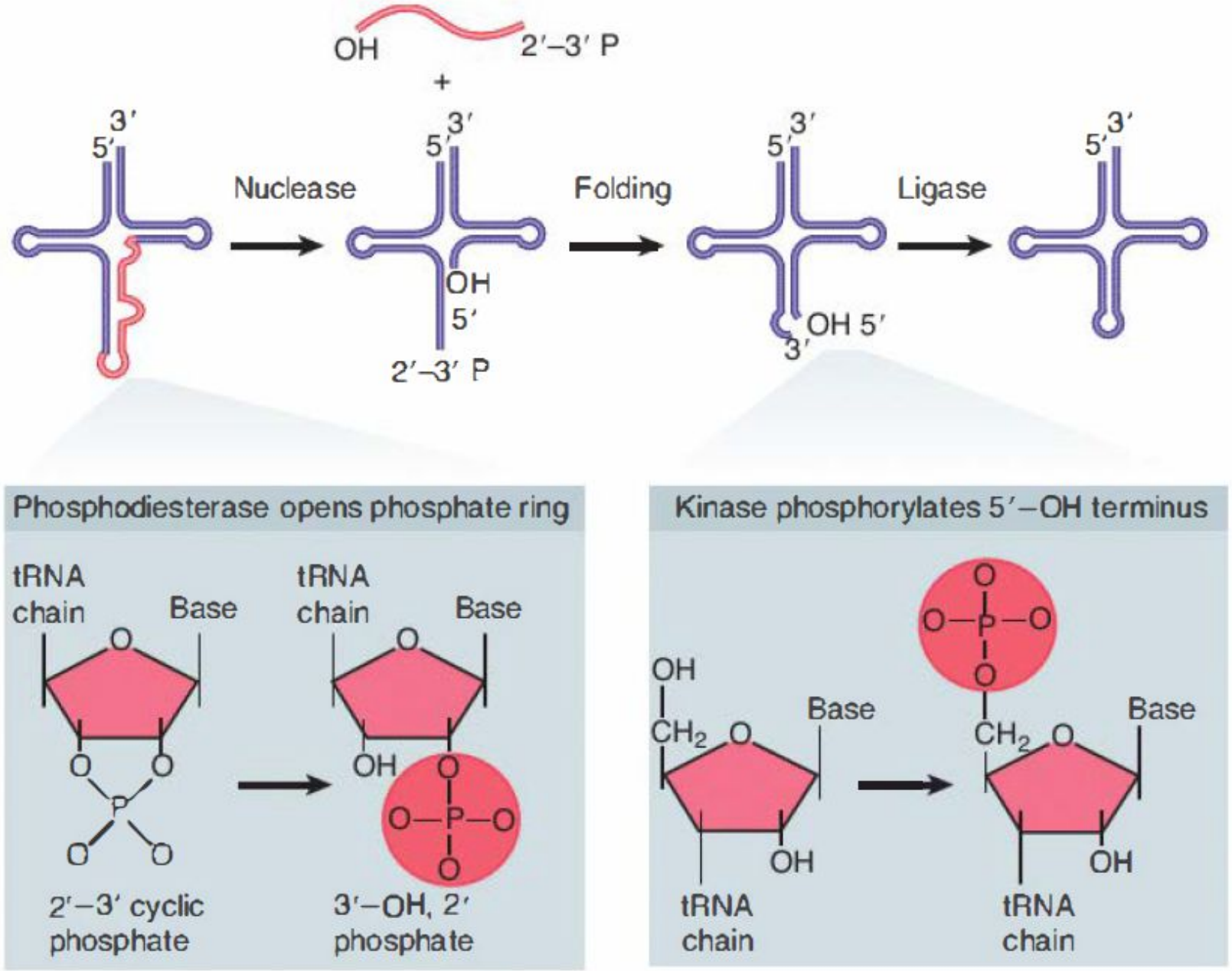
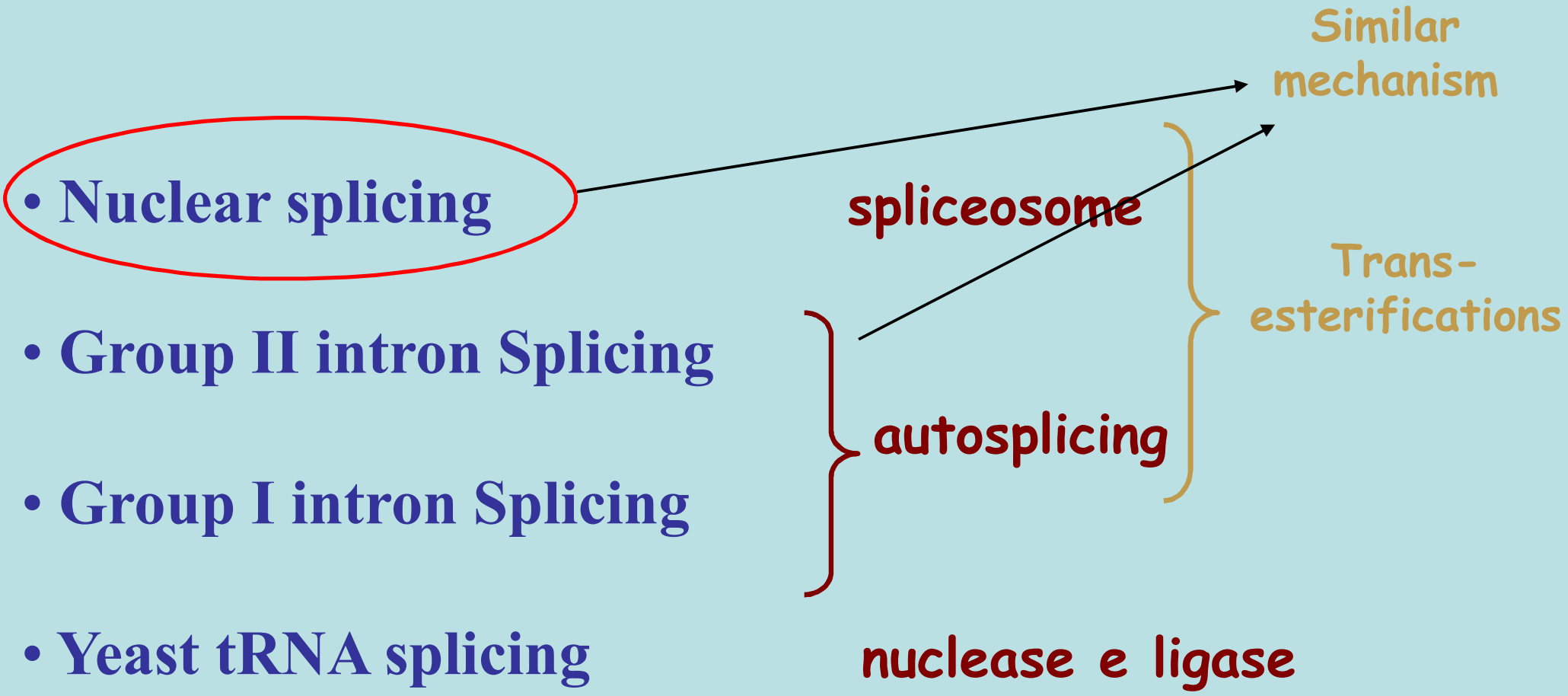


FIGURE 21.38 Splicing of tRNA requires separate nuclease and ligase activities. The exon–intron boundaries are cleaved by the nuclease to generate 2′–3′ cyclic phosphate and 5′–OH termini. The cyclic phosphate is opened to generate 3′–OH and 2′ phosphate groups. The 5′–OH is phosphorylated. After releasing the intron, the tRNA half molecules fold into a tRNA-like structure that now has a 3′–OH, 5′–P break. This is sealed by a ligase.

Splicing types



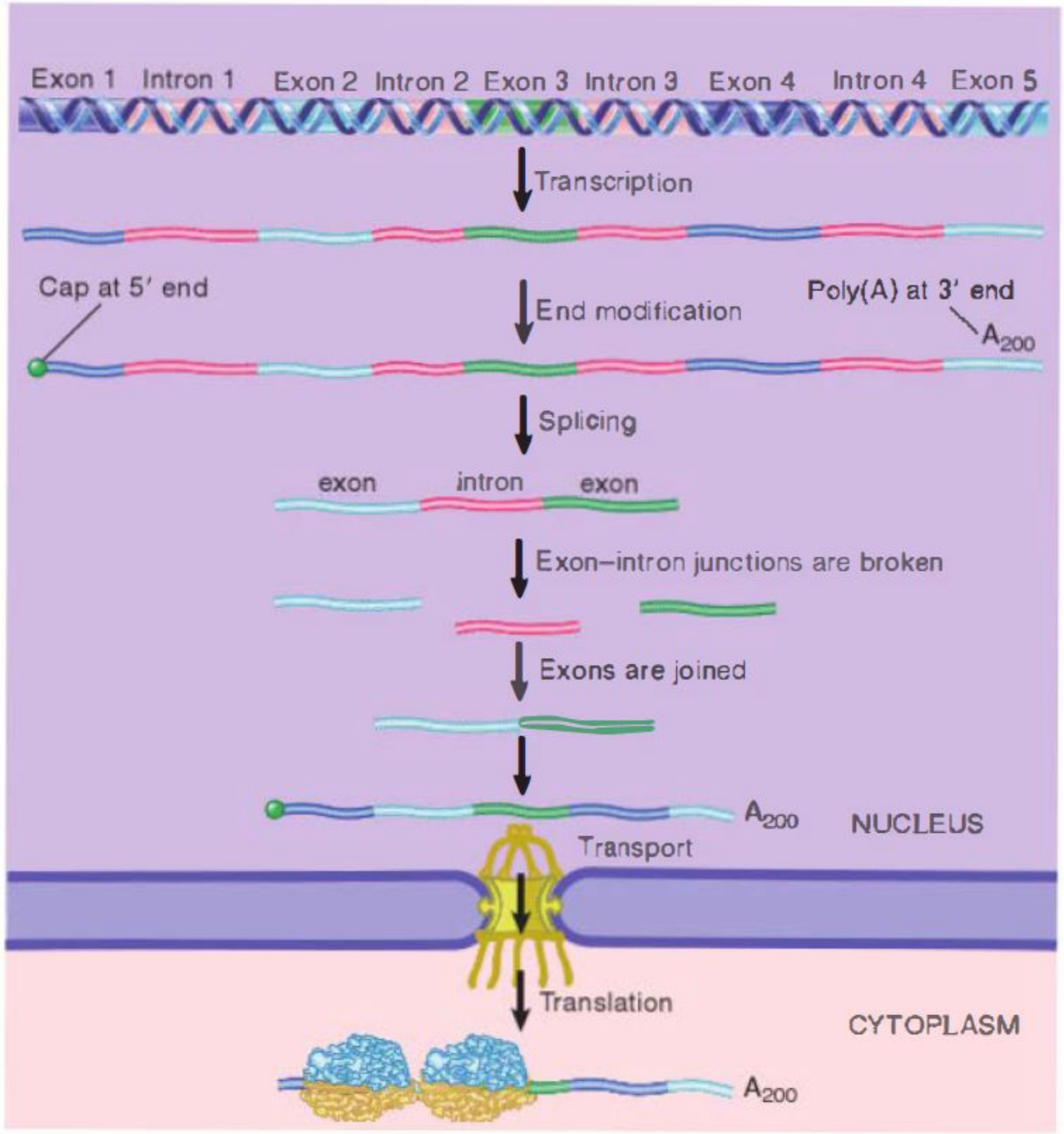


FIGURE 21.1 RNA is modified in the nucleus by additions to the 5′ and 3′ ends and by splicing to remove the introns. The splicing event requires breakage of the exon–intron junctions and joining of the ends of the exons. Mature mRNA is transported through nuclear pores to the cytoplasm, where it is translated.

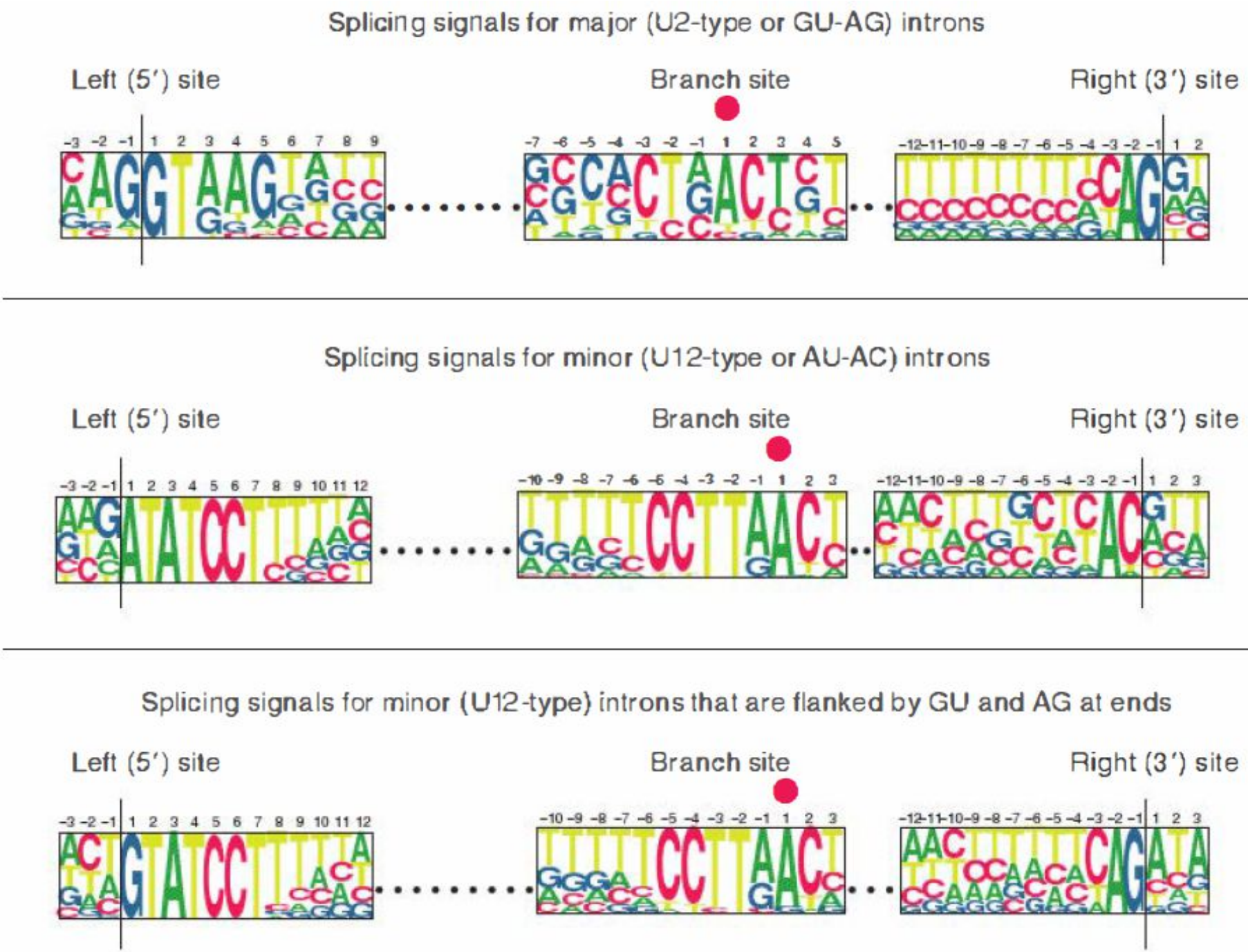


FIGURE 21.3 The ends of nuclear introns are defined by the GU-AG rule (shown here as GT-AG in the DNA sequence of the gene). Minor introns are defined by different consensus sequences at the 5′ splice site, branch site, and 3′ splice site.

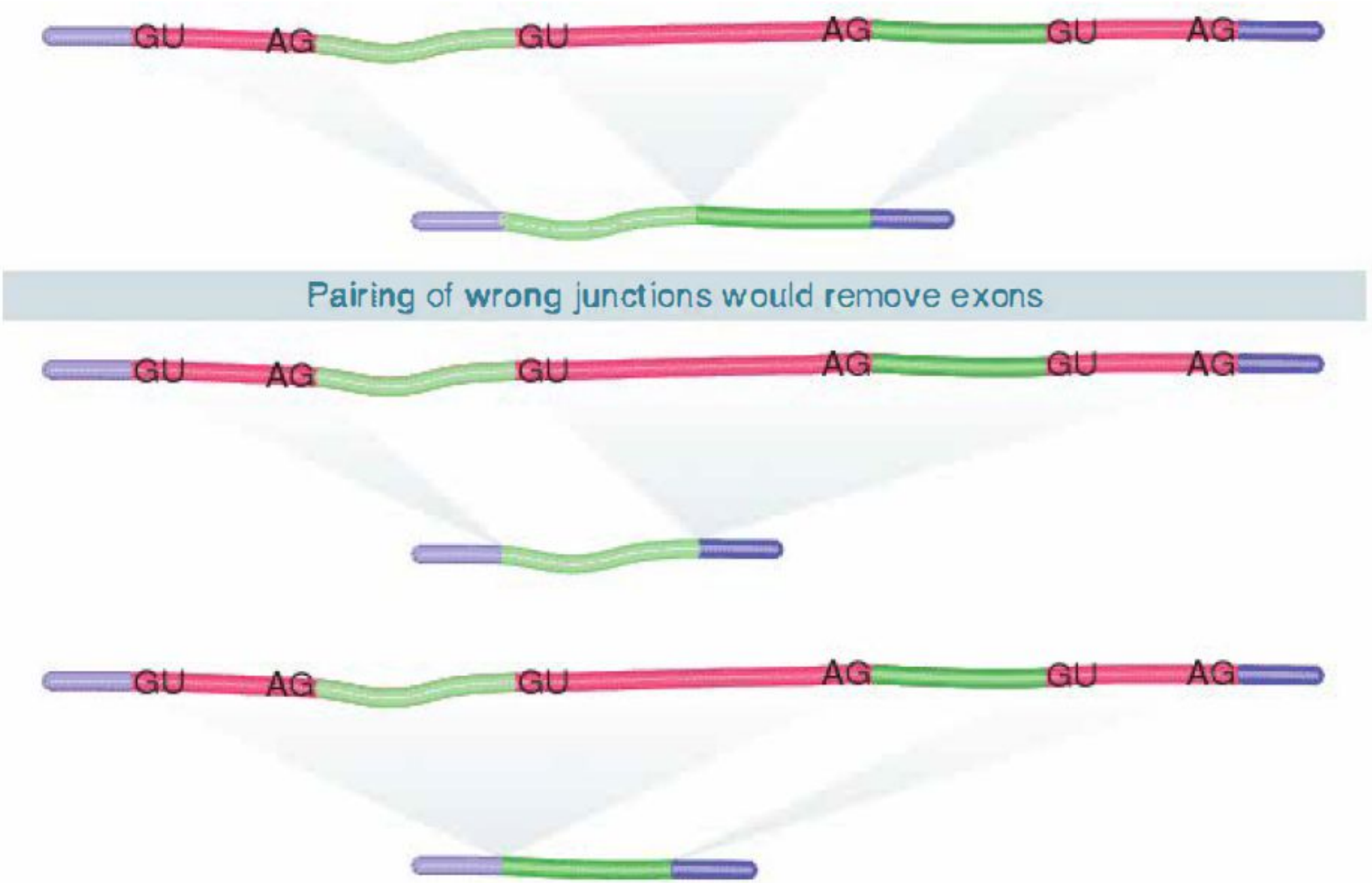
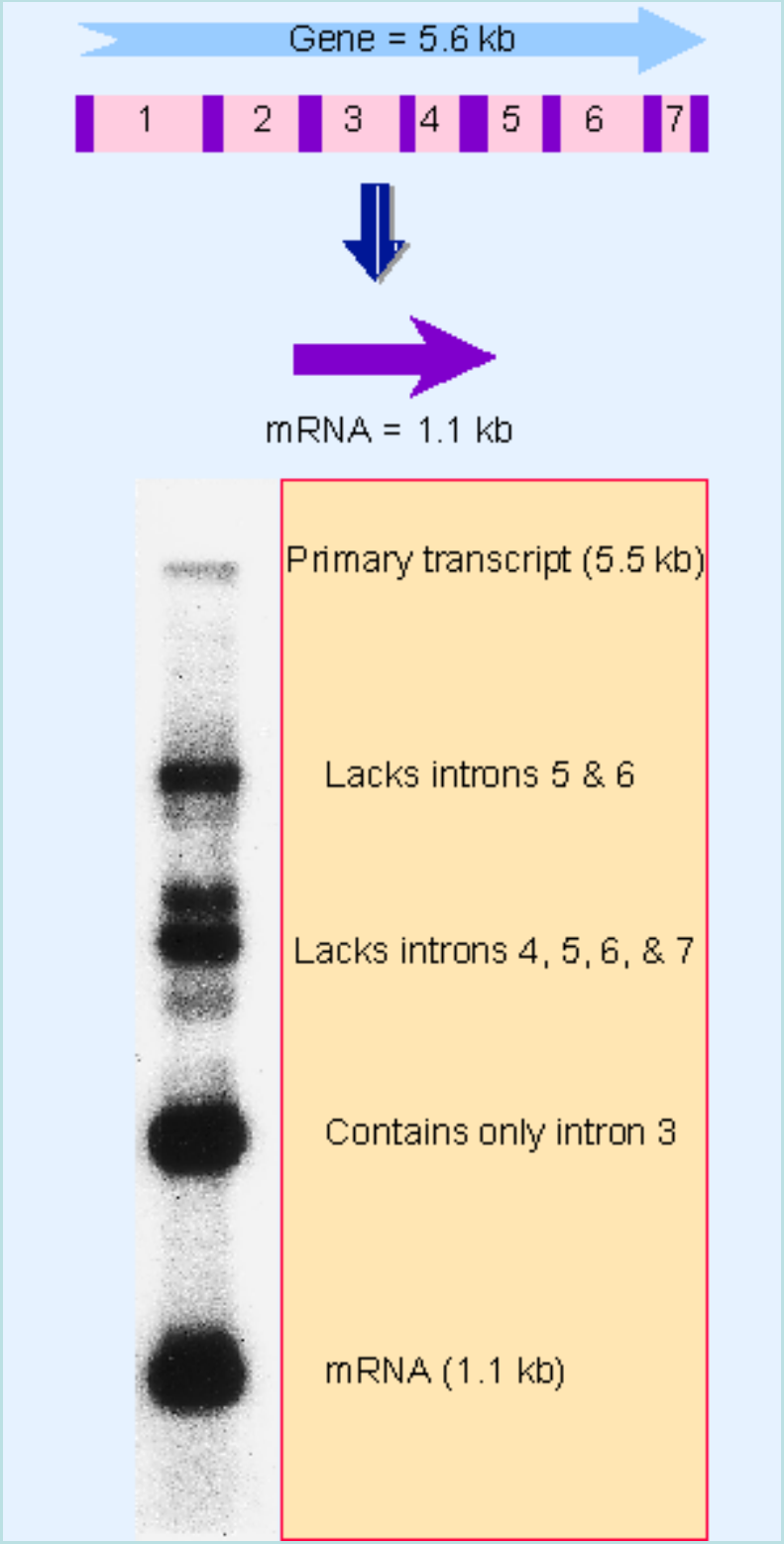


FIGURE 21.4 Splicing junctions are recognized only in the correct pairwise combinations.



Are introns removed in a specific order from a particular RNA? Using RNA blotting, we can identify nuclear RNAs that represent intermediates from which some introns have been removed. There is a discrete series of bands, which suggests that splicing occurs via definite pathways. (If the seven introns were removed in an entirely random order, there would be more than 300 precursors with different combinations of introns, and we should not see discrete bands.)

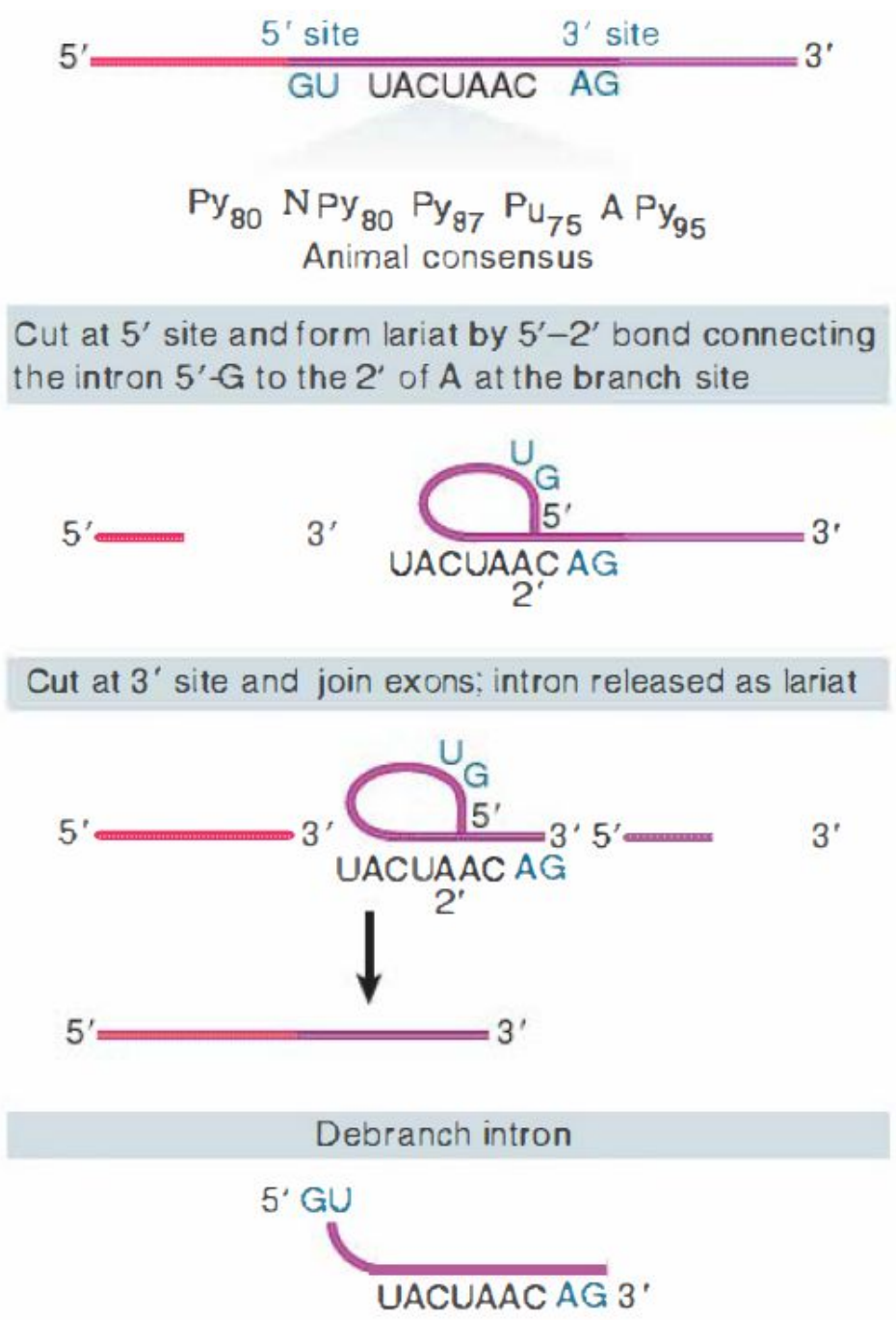


FIGURE 21.5 Splicing occurs in two stages. First the 5′ exon is cleaved off, and then it is joined to the 3′ exon.

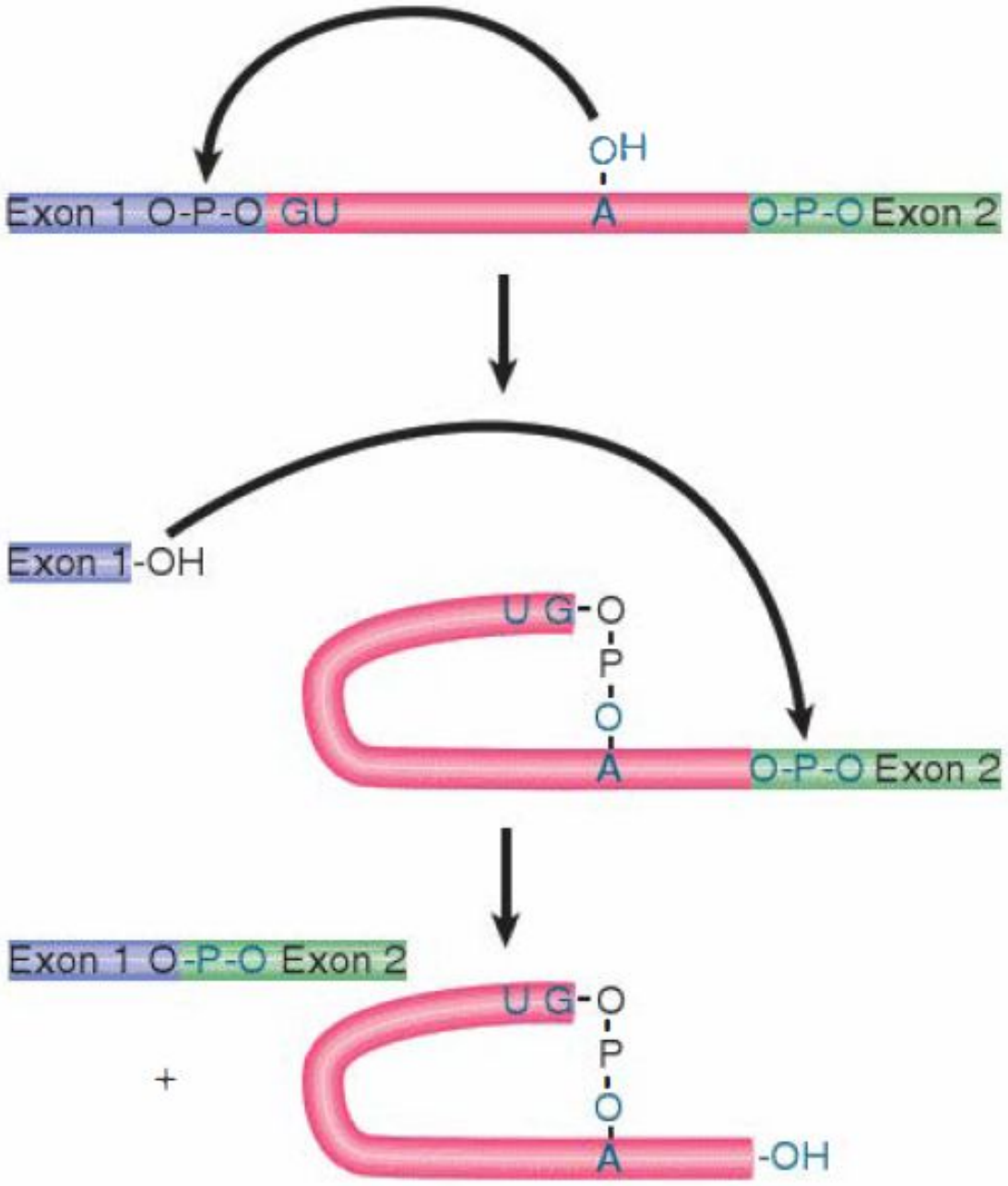


FIGURE 21.6 Nuclear splicing occurs by two transesterification reactions, in which an –OH group attacks a phosphodiester bond.

The spliceosome complex is made by snRNP and other proteins

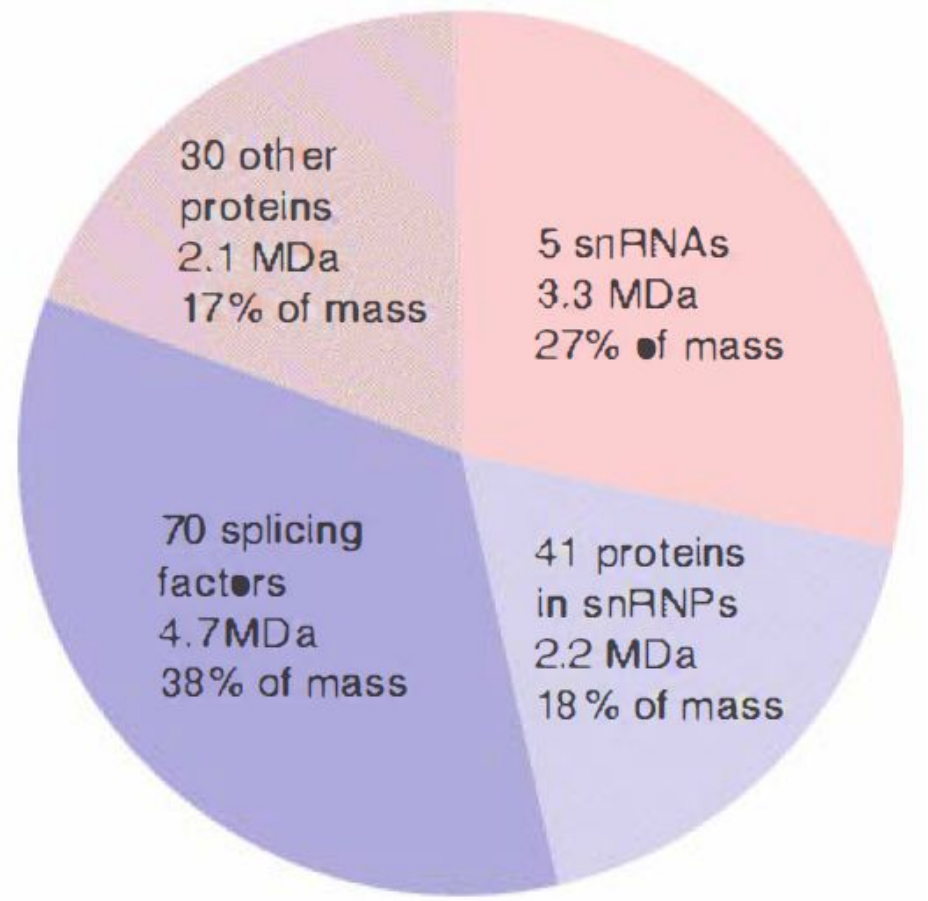


FIGURE 21.7 The spliceosome is ~12 megadaltons (MDa). Five snRNPs account for almost half of the mass. The remaining proteins include known splicing factors as well as proteins that are involved in other stages of gene expression.

50-60S

5 snRNP (proteins + RNA)

snurps

- **U1**
- **U2**
- **U4/U6**
- **U5**

Epitope in common with all snRNP proteins

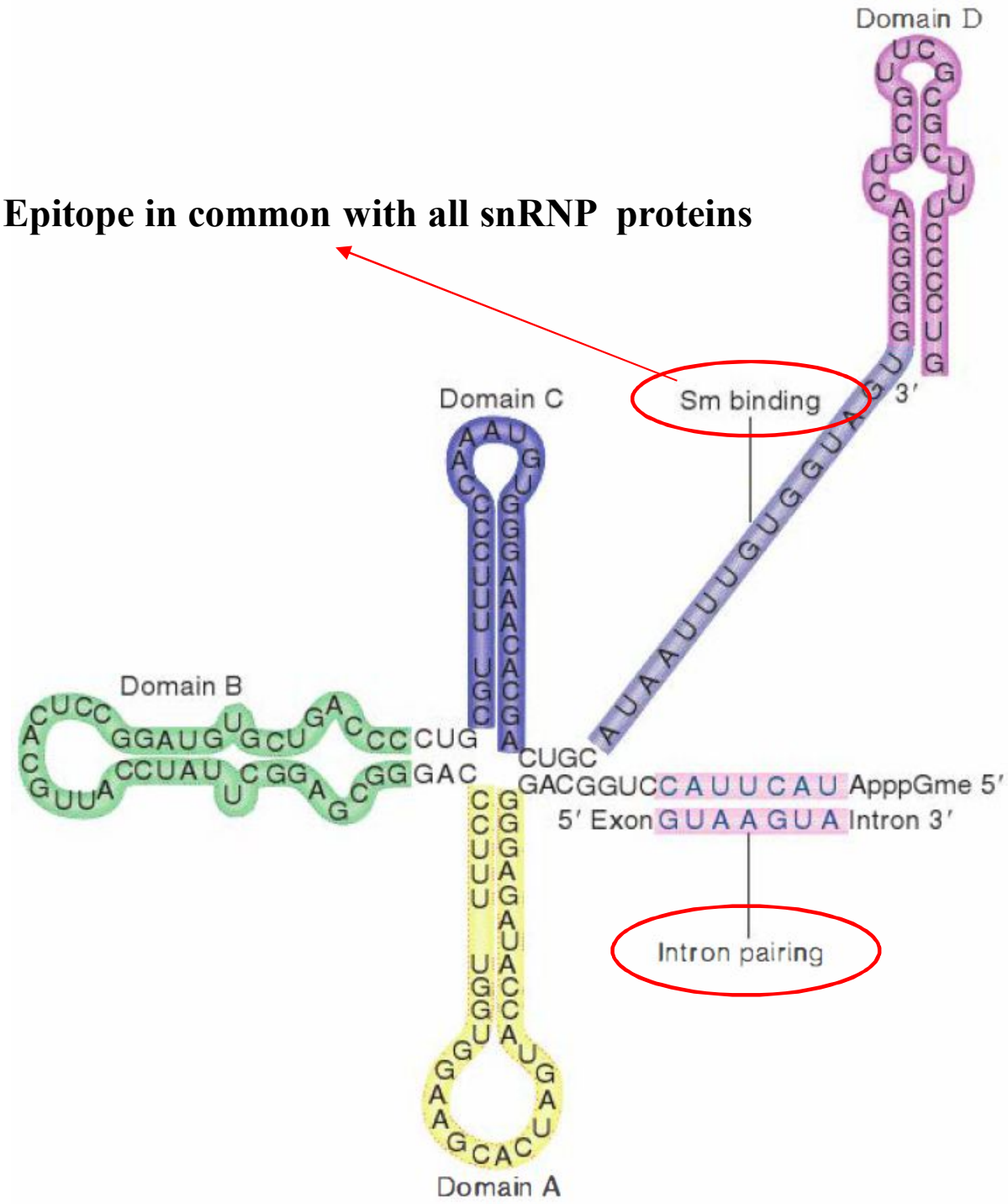
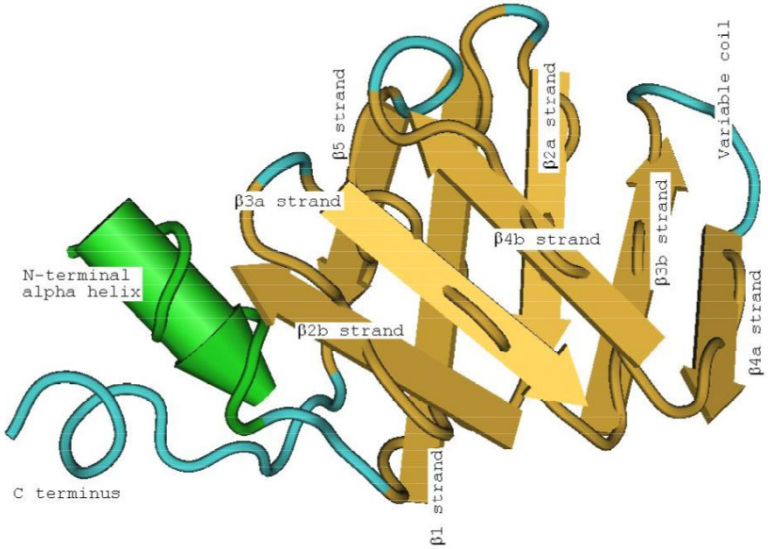


FIGURE 21.8 U1 snRNA has a base-paired structure that creates several domains. The 5′ end remains single stranded and can base pair with the 5′ splice site.

The Sm proteins were first discovered as antigens targeted by so called **Anti-Sm antibodies** in a patient with a form of systemic lupus erythematosus (SLE), a debilitating autoimmune disease. They were named Sm proteins in honor of this patient, Stephanie Smith.



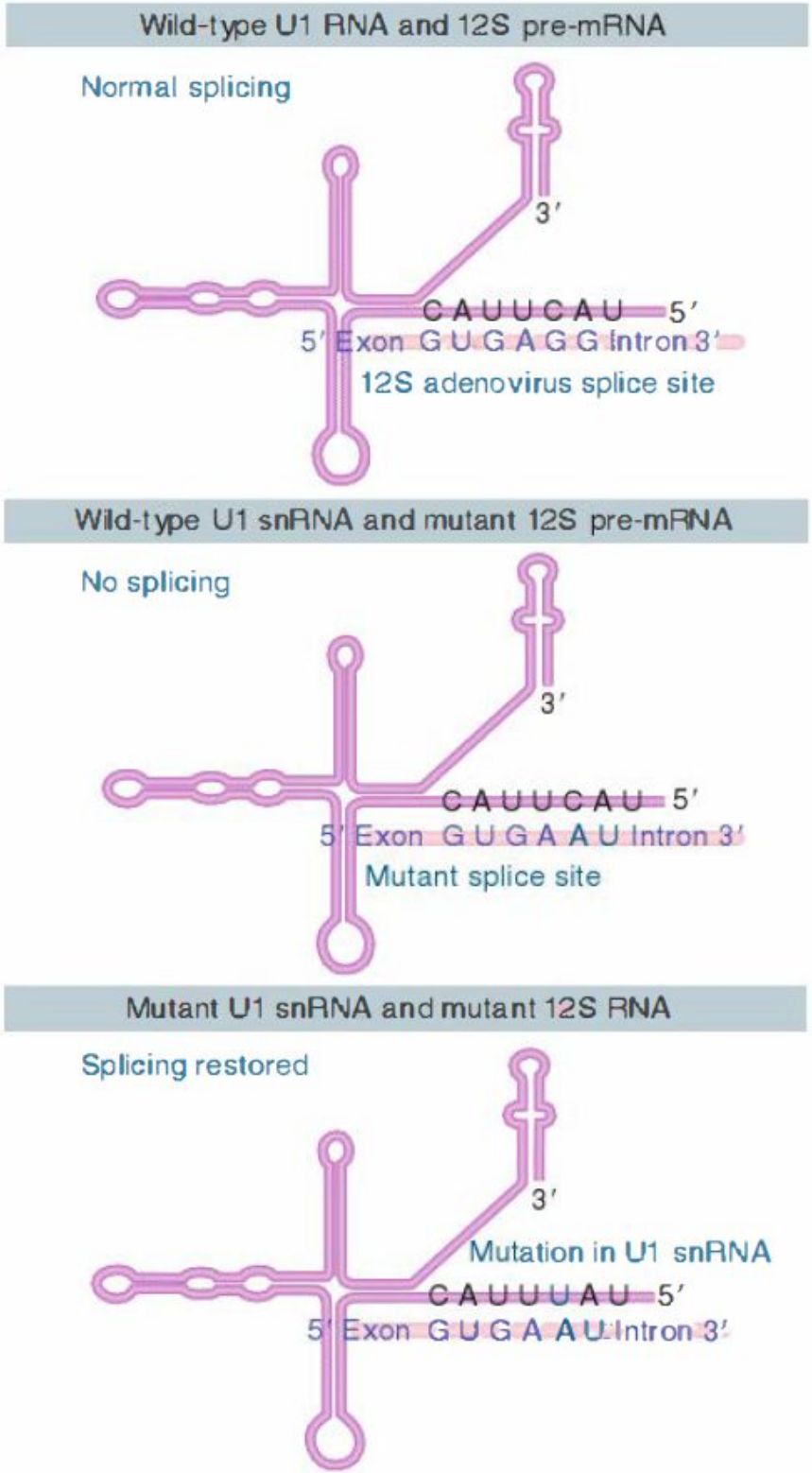


FIGURE 21.9 Mutations that abolish function of the 5′ splice site can be suppressed by compensating mutations in U1 snRNA that restore base pairing.

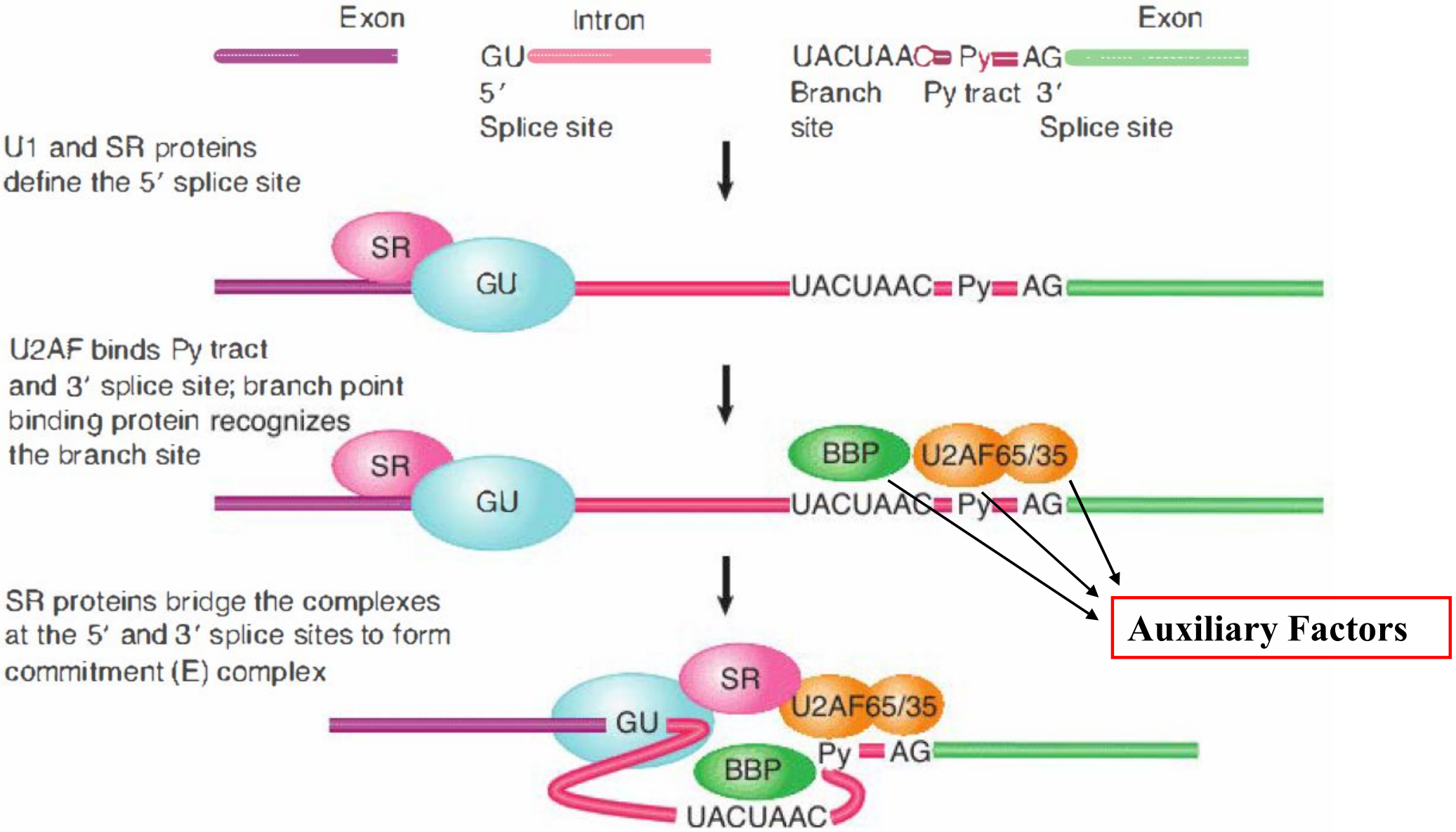


FIGURE 21.10 The commitment (E) complex forms by the successive addition of U1 snRNP to the 5′ splice site, U2AF to the pyrimidine tract/3′ splice site, and the bridging protein SF1/BBP.

U2 snRNA pairs at the branch point at the 3' end of the intron

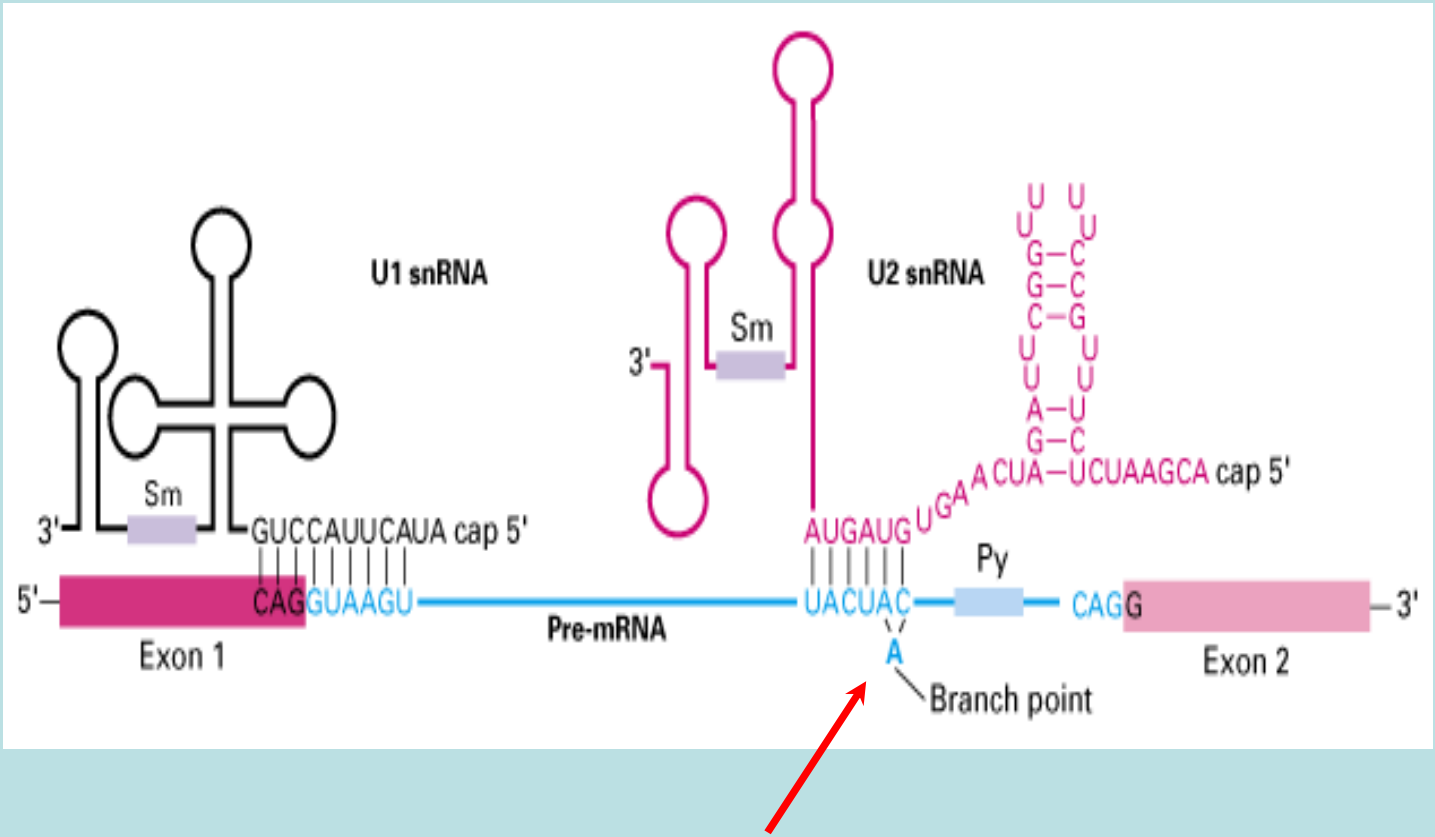
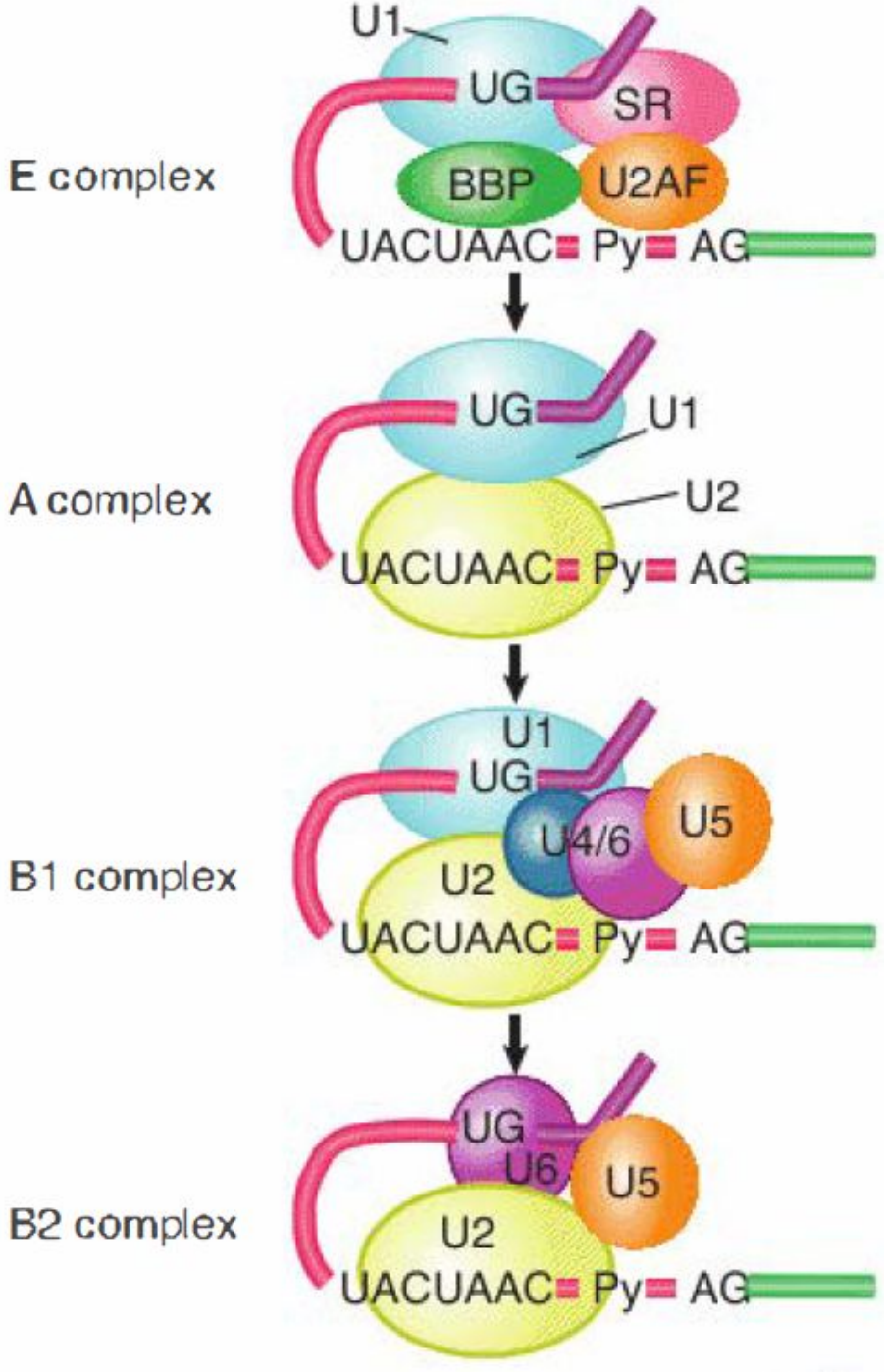


FIGURE 21.12 The splicing reaction proceeds through discrete stages in which spliceosome formation involves the interaction of components that recognize the consensus sequences.



E complex

Formation of commitment complex in which U1 is base paired with the 5′ splice site

A complex

U2 addition to base pair with the branch site in the presence of ATP

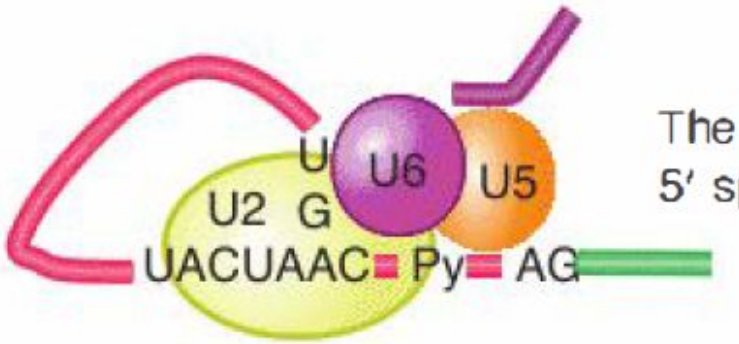
B1 complex

Joining of U4/6 and U5 tri-snRNPs

B2 complex

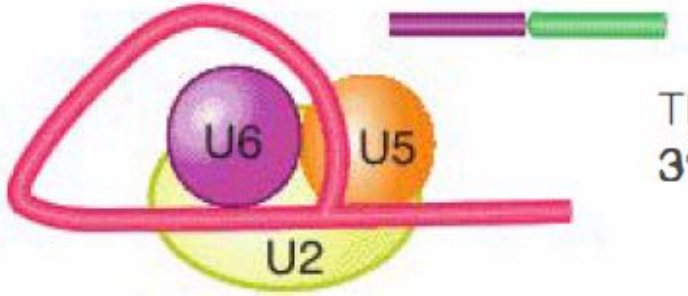
U1 and U4 release
Formation of the catalytic center in which U6 base pairs with the 5′ splice site; U6 also base pairs with U2; U2 remains base paired with the branch site; U5 interacts with both exons through its loop

C1 complex



The first step of transesterification
5′ splice site cleaved, lariat formed

C2 complex



The second step of transesterification
3′ splice site cleaved, exons ligated

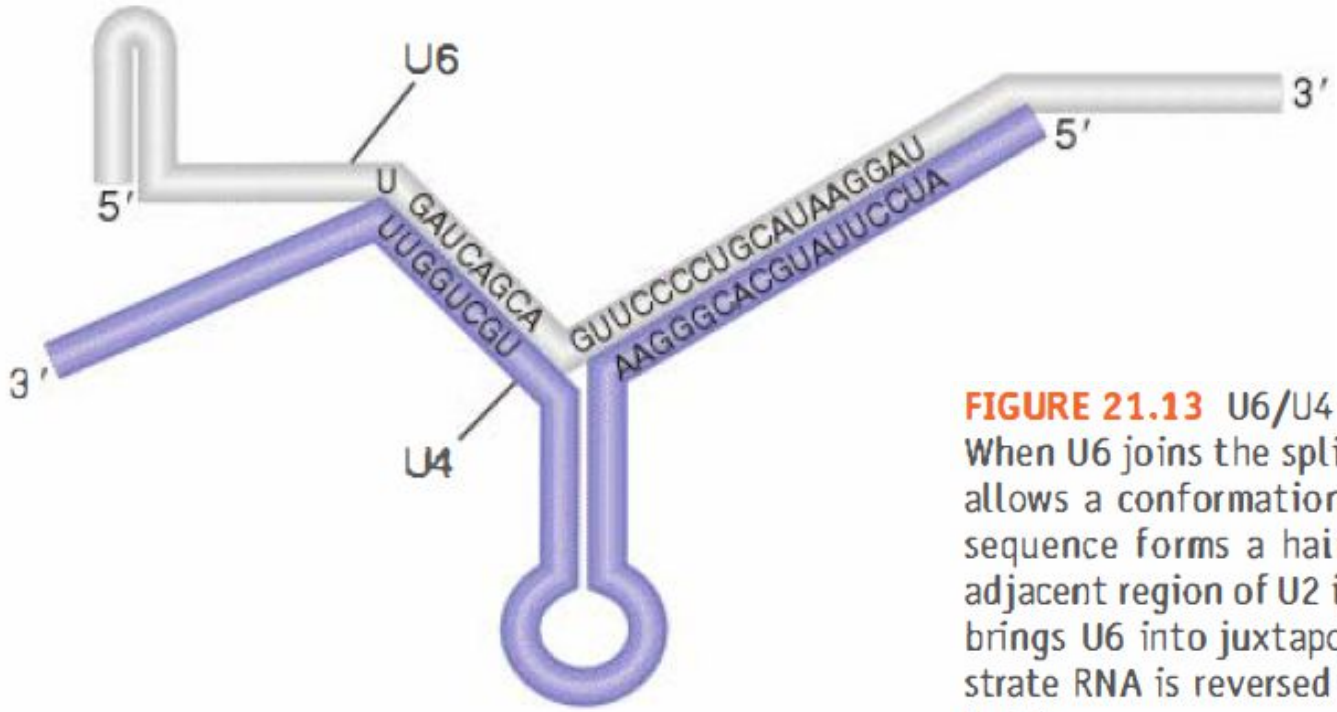
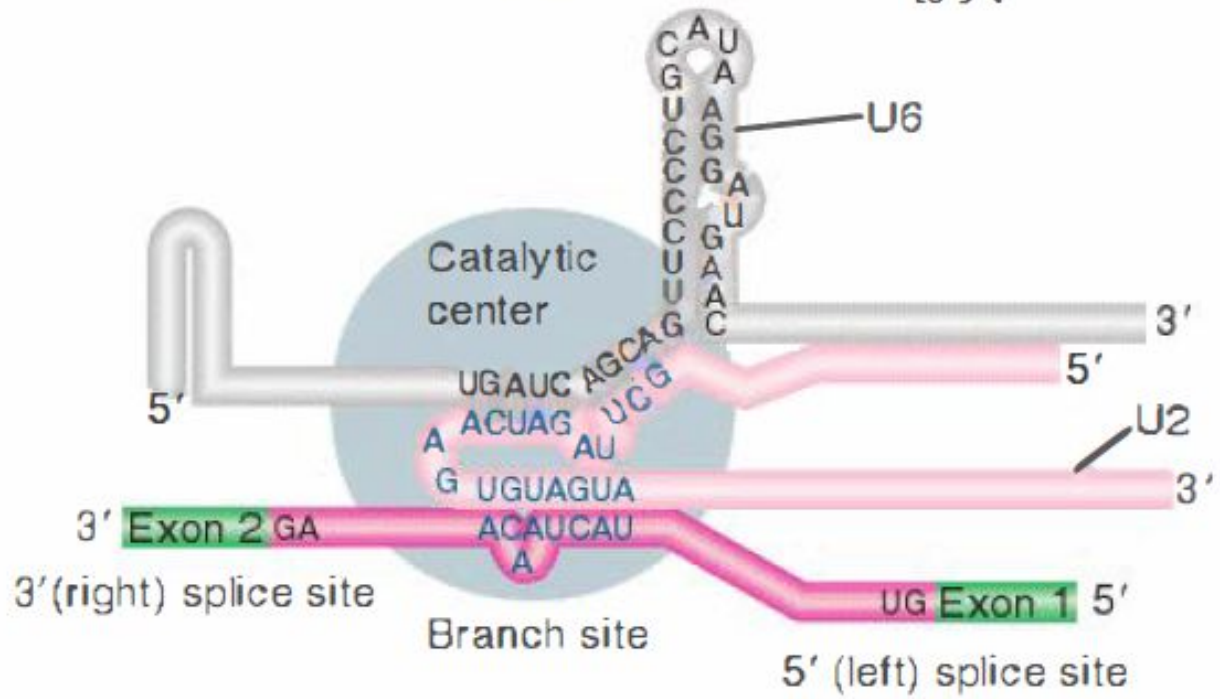


FIGURE 21.13 U6/U4 pairing is incompatible with U6/U2 pairing. When U6 joins the spliceosome it is paired with U4. Release of U4 allows a conformational change in U6; one part of the released sequence forms a hairpin and the other part pairs with U2. An adjacent region of U2 is already paired with the branch site, which brings U6 into juxtaposition with the branch. Note that the substrate RNA is reversed from the usual orientation and is shown 3′ to 5′.



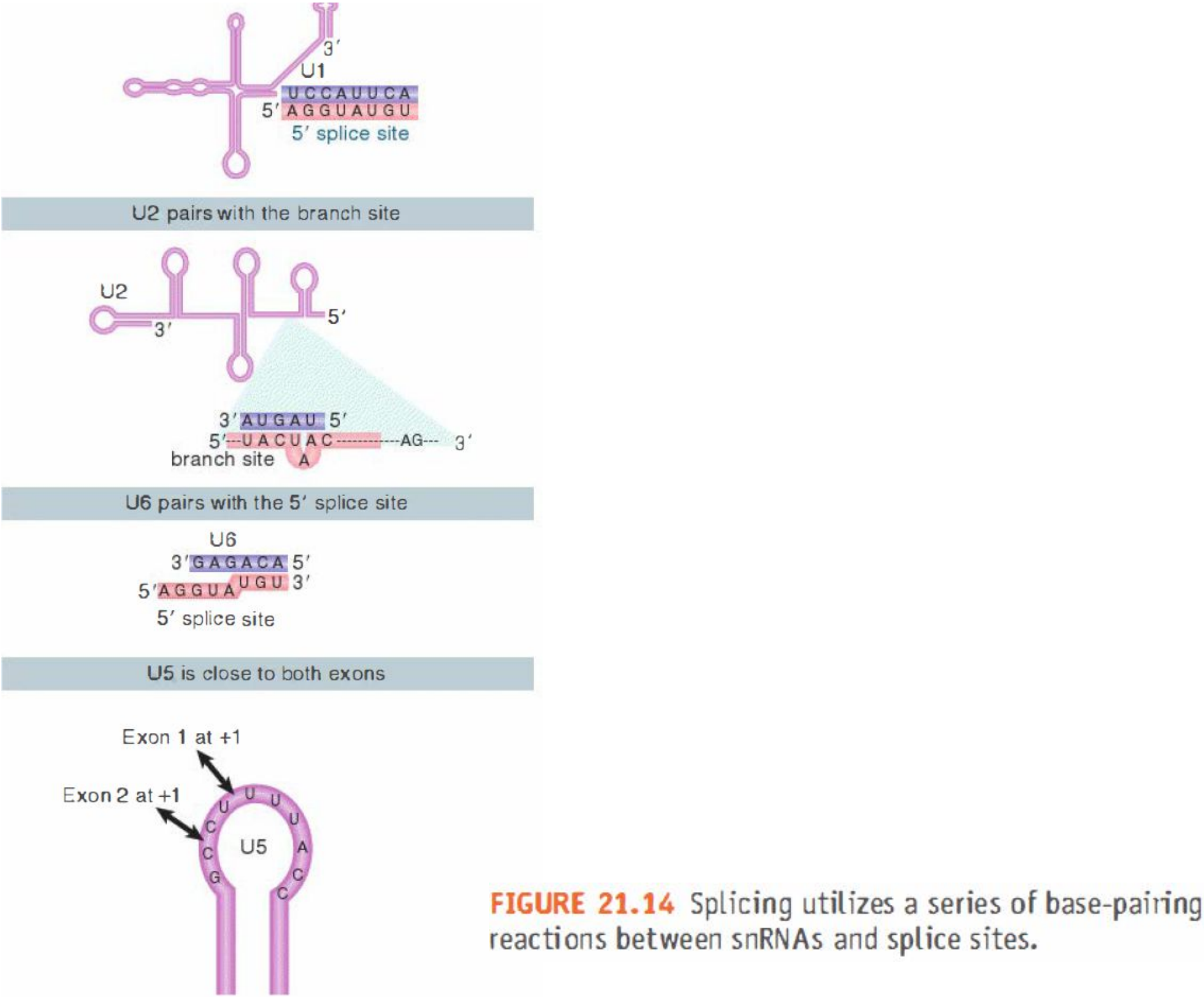
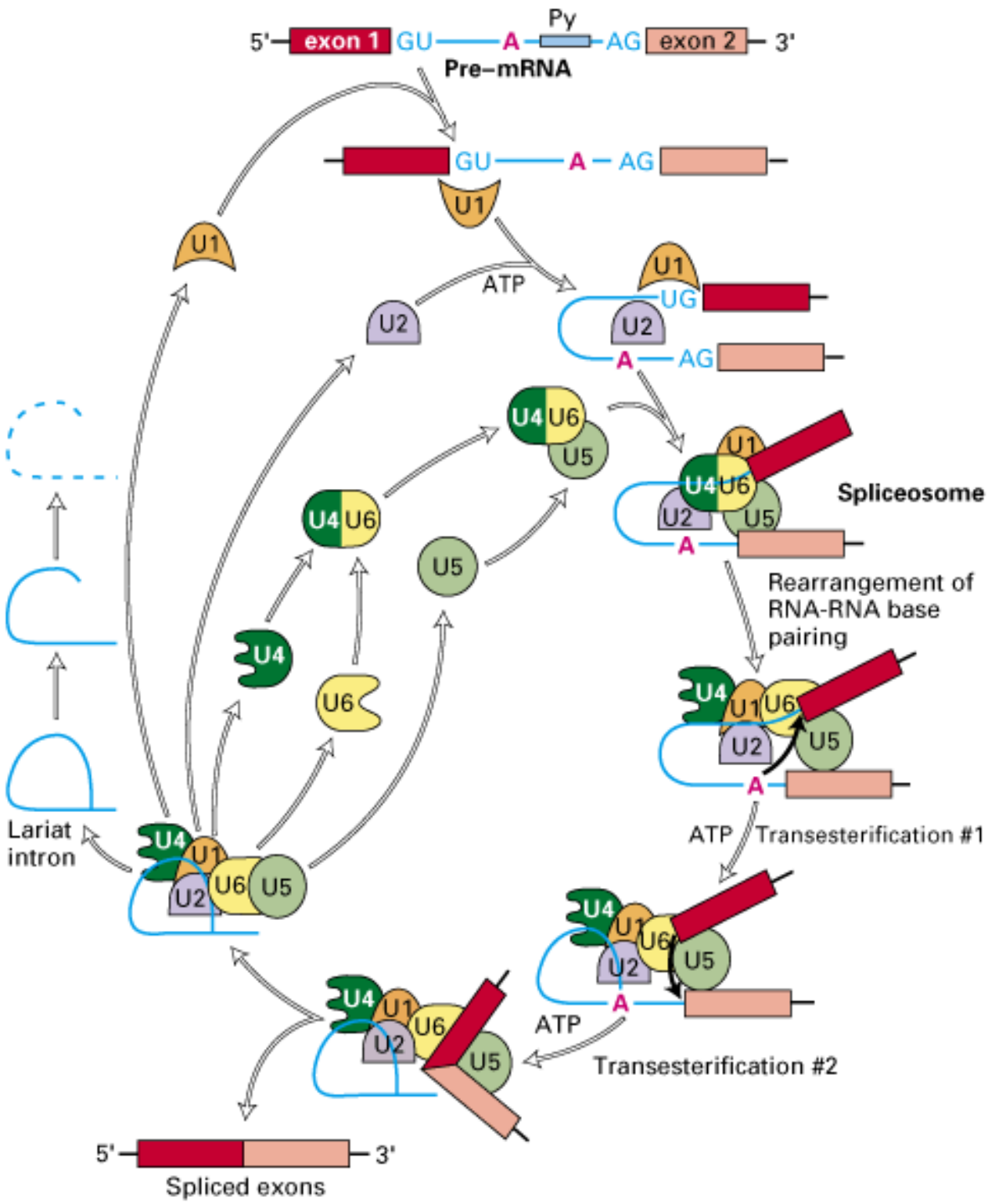


FIGURE 21.14 Splicing utilizes a series of base-pairing reactions between snRNAs and splice sites.


5'
exon 1
GU
A
Py
AG
exon 2
3'
Pre–mRNA
GU
A
AG
U1
U2
ATP
U1
UG
U2
A
AG
U4 U6
U5
U1
U4 U6
U2
U5
A
Spliceosome
Rearrangement of
RNA-RNA base
pairing
U4
U6
U4
U1
U6
U2
U5
A
ATP
Transesterification #1
Lariat
intron
U4
U1
U2
U6
U5
U4
U1
U6
U2
U5
A
ATP
Transesterification #2
U4
U1
U6
U2
U5
5'
3'
Spliced exons

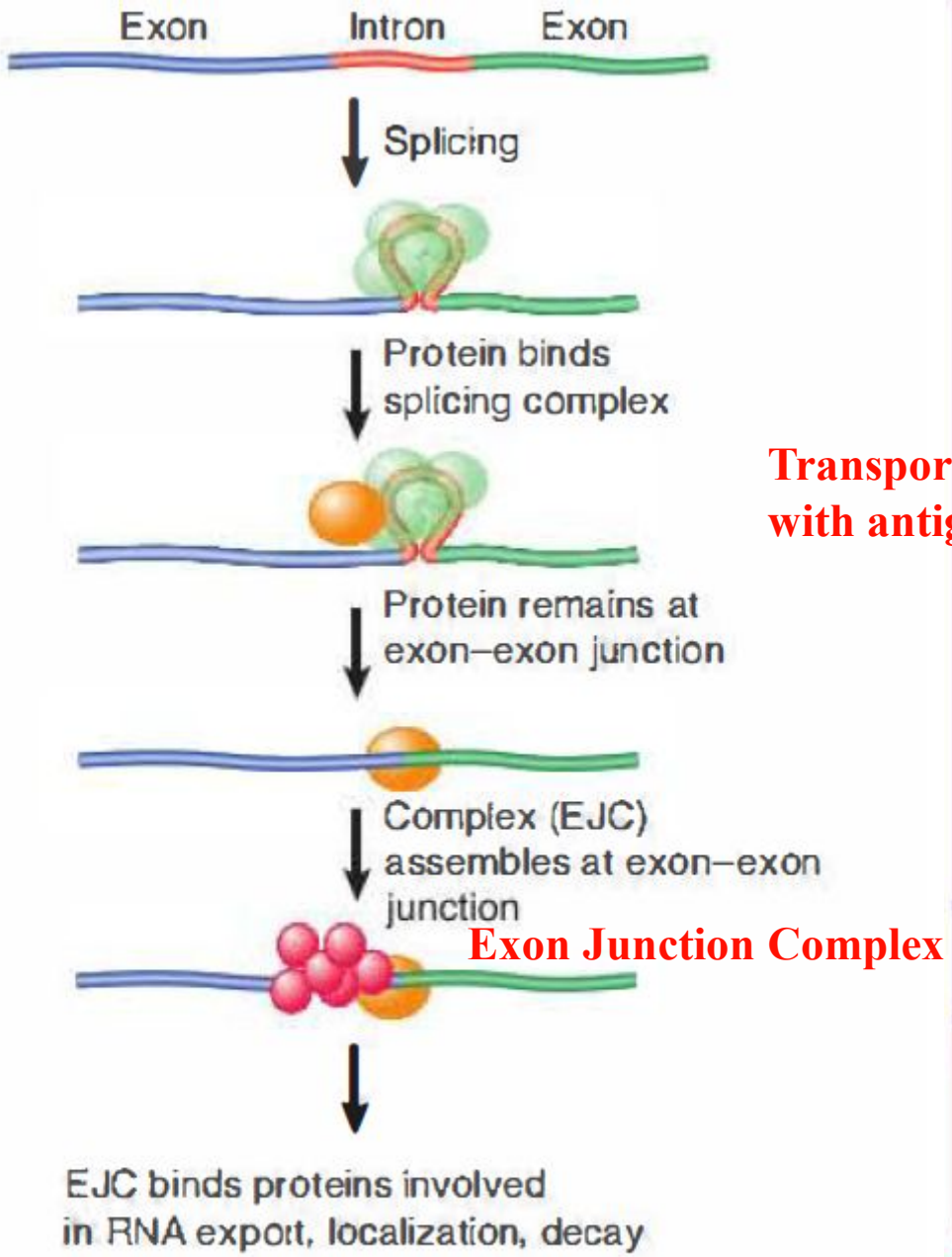


FIGURE 21.18 The exon junction complex (EJC) is deposited near the splice junction as a consequence of the splicing reaction.

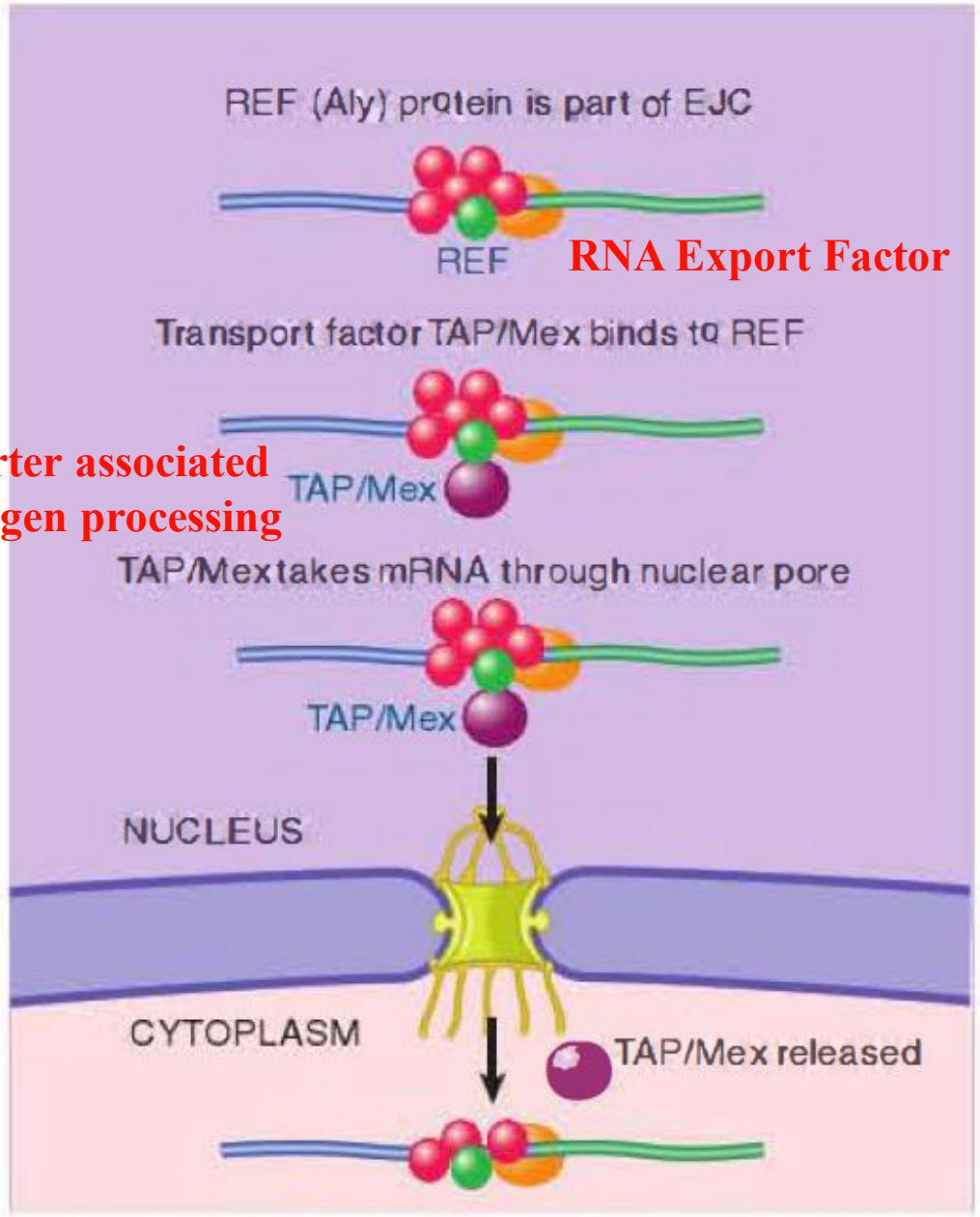


FIGURE 21.19 An REF protein (shown in green) binds to a splicing factor and remains with the spliced RNA product. REF binds to a transport protein (shown in purple) that binds to the nuclear pore.

Alternative splicing

- **Alternative splicing contributes to structural and functional diversity of gene products starting from a single pre-mRNA.**
- **It's controlled by a series of Alternative Splicing Factor (ASF/SF2) selecting the proper splicing junctions in order to obtain the variant you want.**

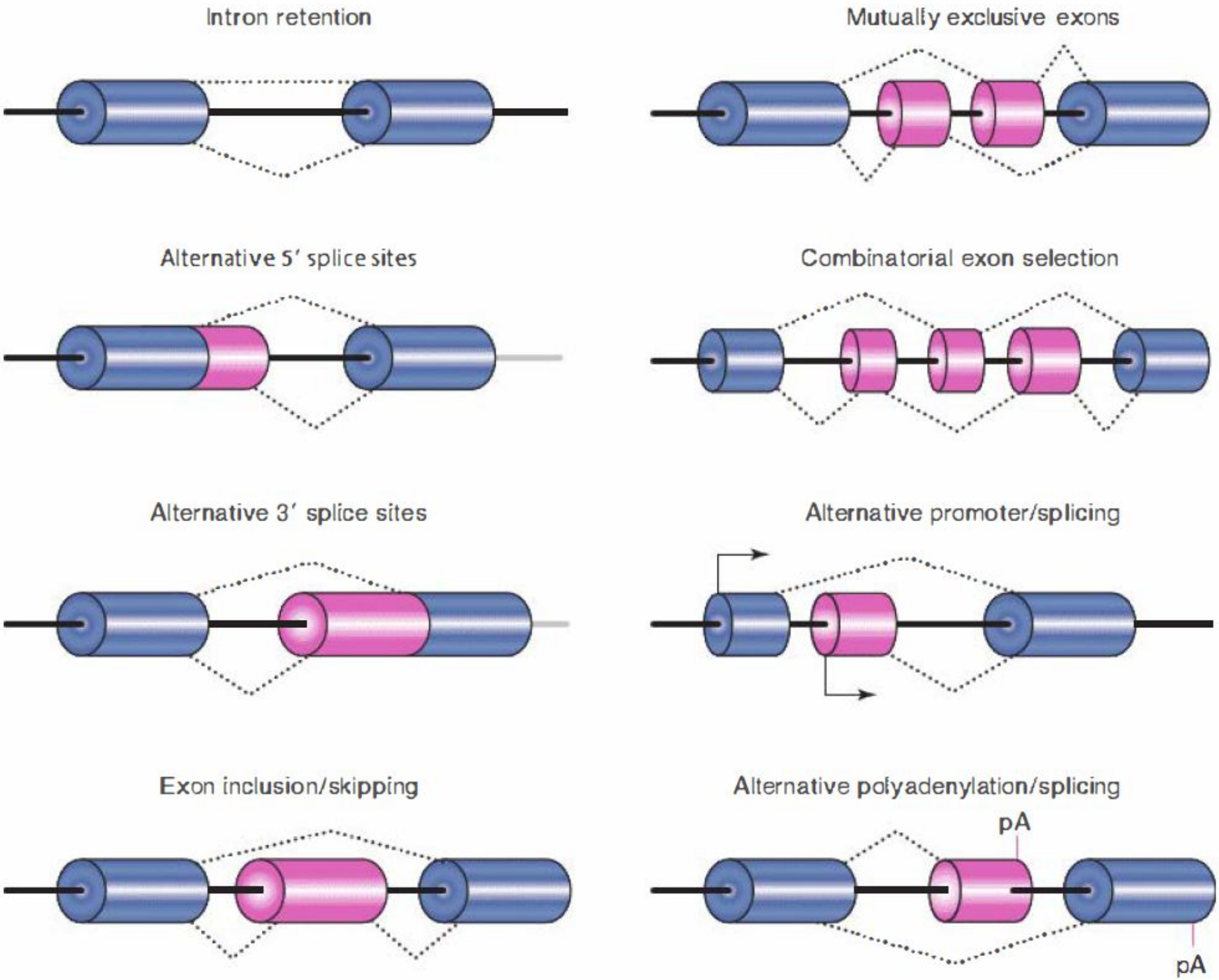


FIGURE 21.21 Different modes of alternative splicing.

Trans-Splicing

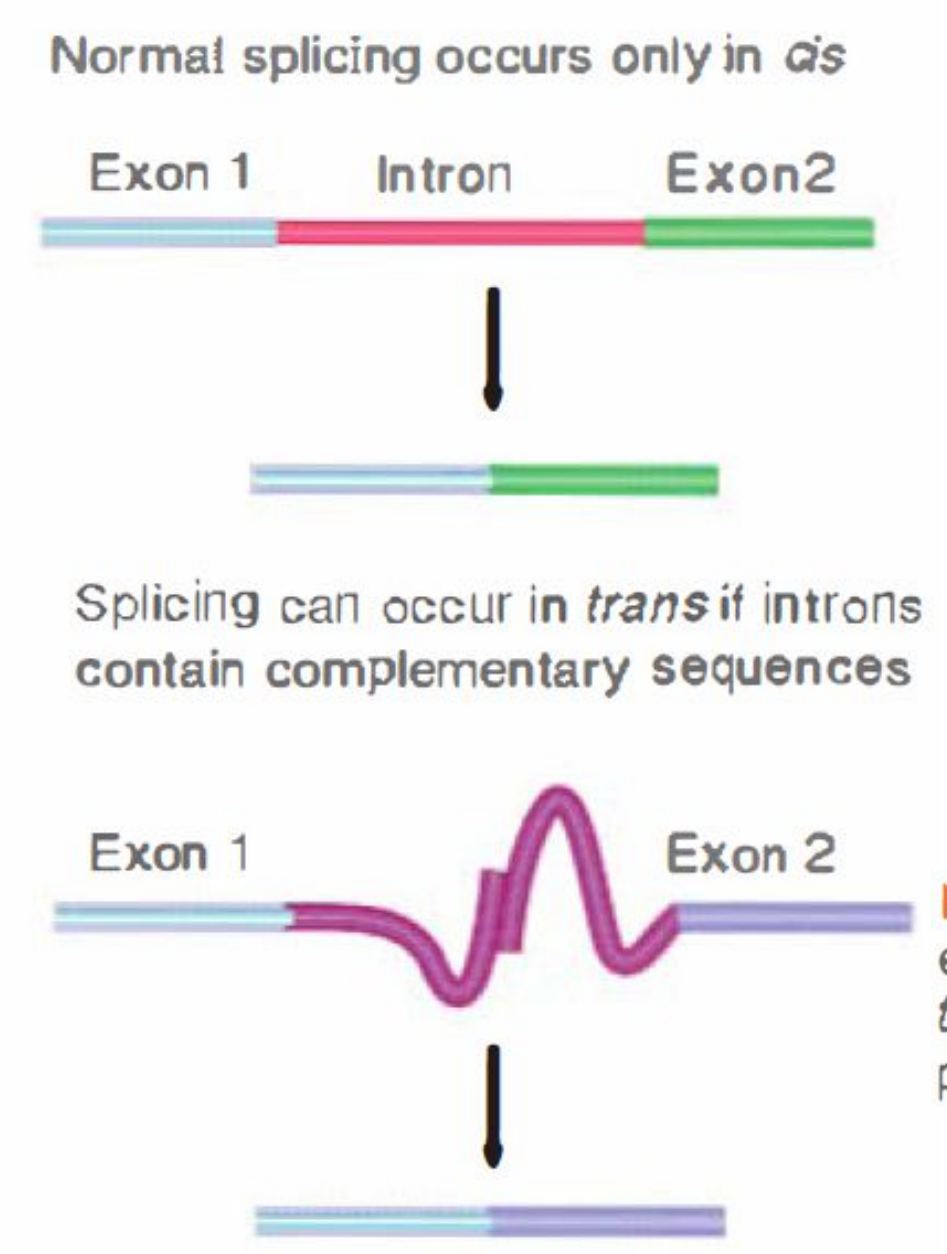


FIGURE 21.26 Splicing usually occurs only in *cis* between exons carried on the same physical RNA molecule, but *trans*-splicing can occur when special constructs that support base pairing between introns are made.

Trans-Splicing → exceedingly rare event *in vivo*

Es. RNA SL (Spliced Leader) in *Tripanosoma* and *worms* → a short sequence (SL RNA) is spliced to the 5 ' ends of many precursor mRNAs.

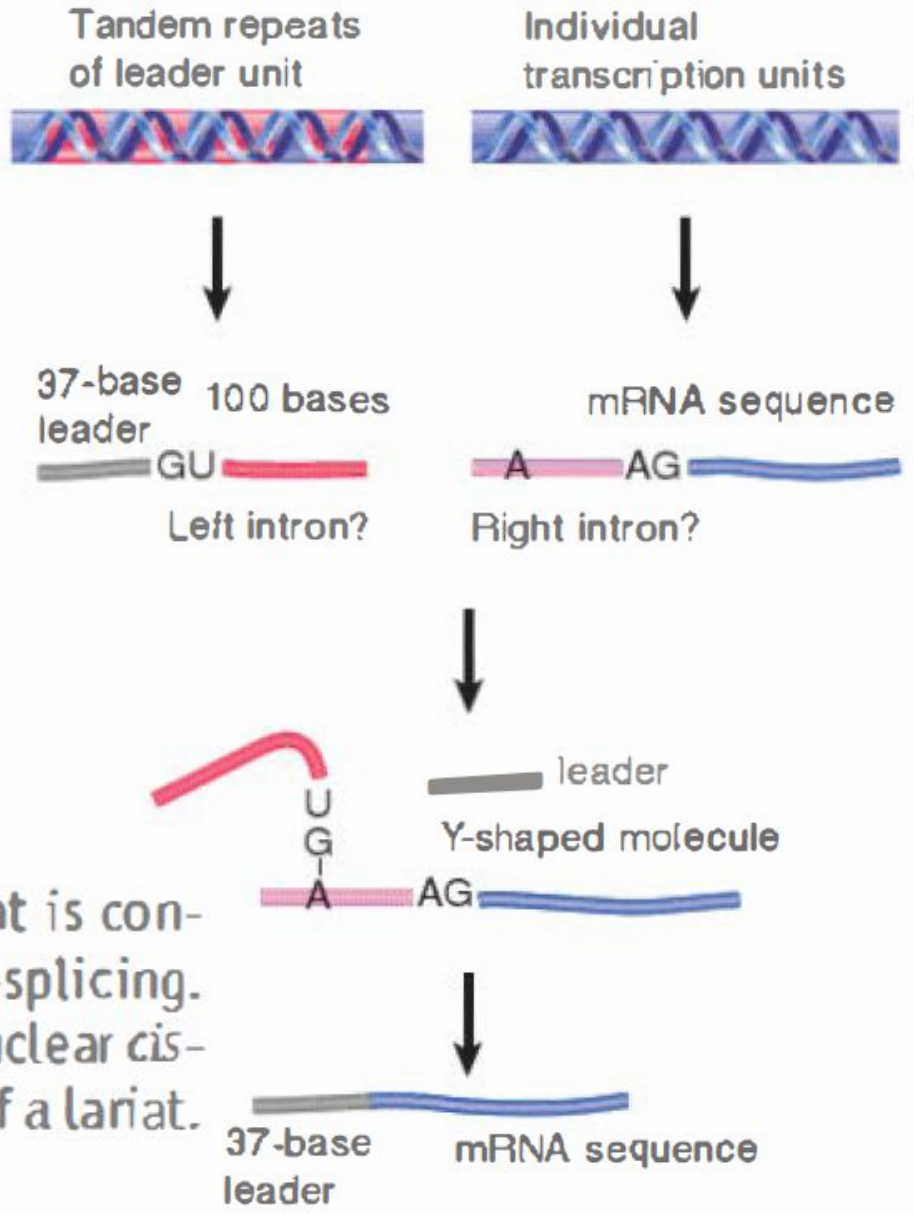


FIGURE 21.27 The SL RNA provides an exon that is connected to the first exon of an mRNA by *trans*-splicing. The reaction involves the same interactions as nuclear *cis*-splicing but generates a Y-shaped RNA instead of a lariat.