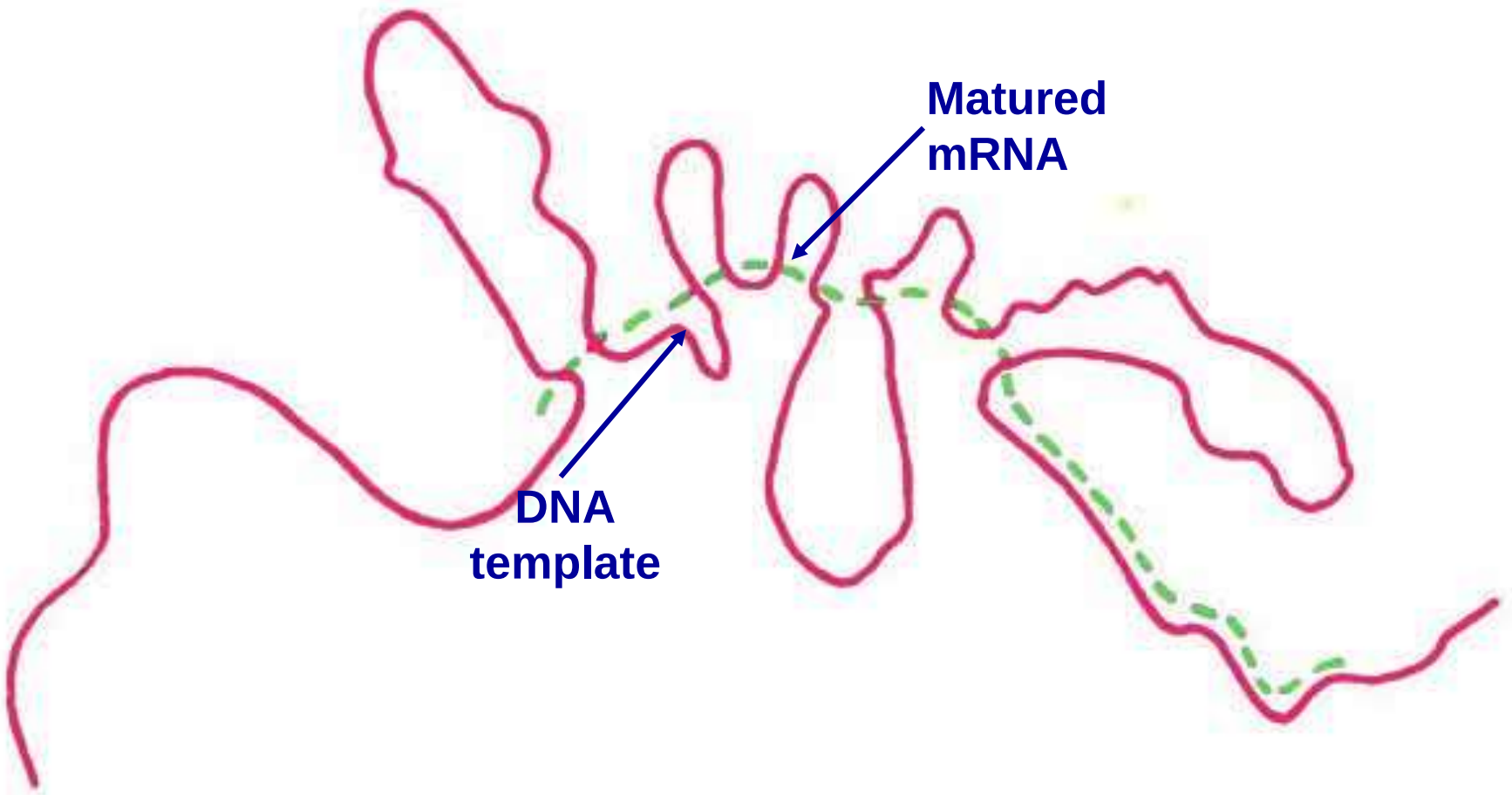
SOS in Biochemistry, Jiwaji University, Gwalior
M.Sc. II Semester (2019-20)
Paper BCH 201: Fundamentals of Molecular Biology (Unit IV)

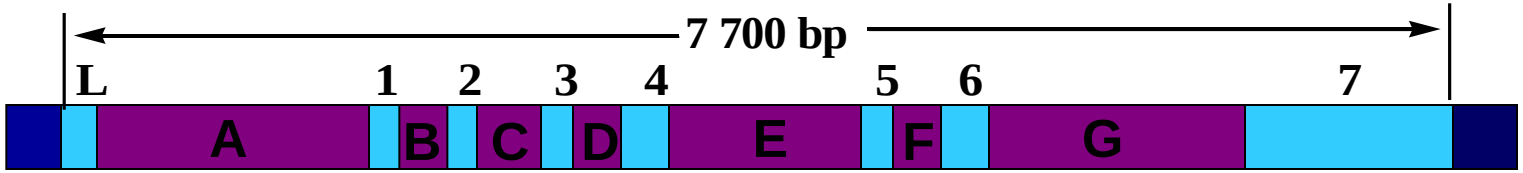
Nuclear pre-mRNA Splicing

The matured mRNAs are much shorter than the DNA templates.



Split Gene

The structural genes are composed of coding and non-coding regions that are alternatively separated.



A~G no-coding region 1~7 coding region

NOBEL PRIZE – 1993

PHYSIOLOGY OR MEDICINE

For the discovery of

Split Genes

The Nobel Prize was shared by two American Scientists:

1. **Richard J. Roberts, New England Biolabs, Beverly, MA, USA (1/2)**
2. **Phillip A. Sharp, Massachusetts Institute of Technology (MIT), Center for Cancer Research, Cambridge, MA, USA (1/2)**

Richard J. Roberts

Born: 6 September 1943, Derby, United Kingdom
Field: Genetics, Molecular Biology
Prize share: 1/2

Phillip A. Sharp

Born: 6 June 1944, Falmouth, KY, USA
Field: Genetics, Molecular Biology
Prize share: 1/2

Work: Split Genes

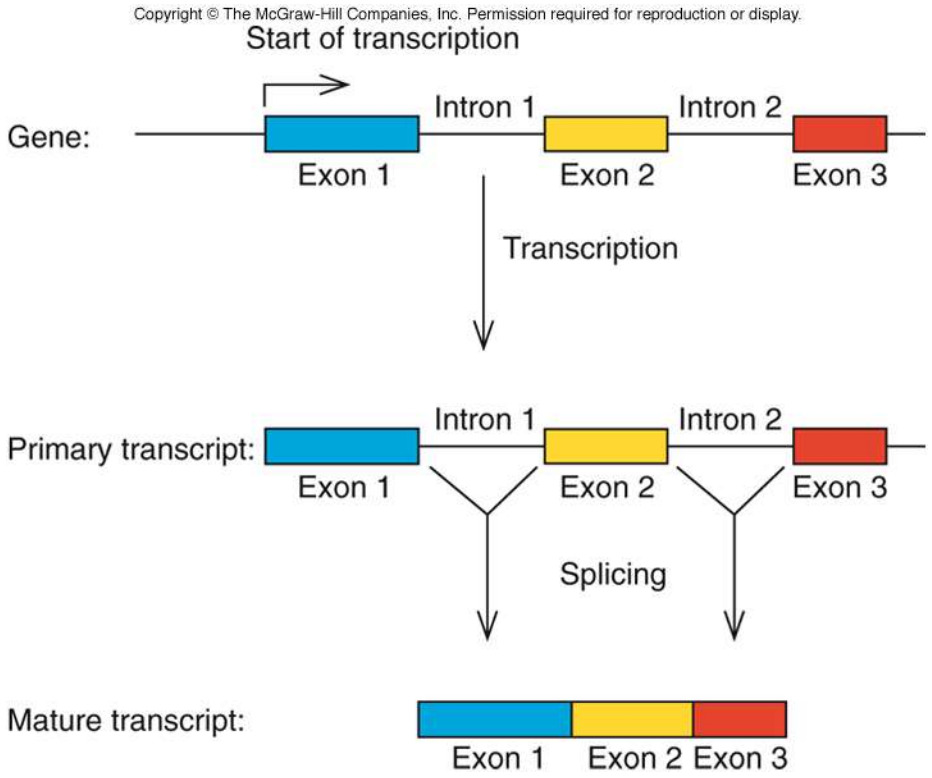
Exon and Intron

Exons are the coding sequences that appear on split genes and primary transcripts, and will be expressed to matured mRNA.

Introns are the non-coding sequences that are transcripted into primary mRNAs, and will be cleaved out in the later splicing process.

Splicing Outline

- Introns are transcribed along with exons in the primary transcript
- Introns are removed as the exons are spliced together



Types of Introns

Intron Type	Where Found
GU–AG introns	Eukaryotic nuclear pre-mRNA
AU–AC introns	Eukaryotic nuclear pre-mRNA
Group I	Eukaryotic nuclear pre-mRNA, organelle RNAs, a few bacterial RNAs
Group II	Organelle RNAs, a few prokaryotic RNAs
Group III	Organelle RNAs
Twintrons (composites of two and/or more group II or III introns)	Organelle RNAs
Pre-tRNA introns	Eukaryotic nuclear pre-tRNAs
Archaeal introns	Various RNAs

TYPES OF INTRONS

Introns in all genes can be divided into three general classes:
(Except nuclear tRNA coding genes)

1. Nuclear pre-mRNA Introns
(With GU-----AG dinucleotide at 5' & 3' ends and a branch site near the 3' end)

2. Group I Introns
(Found in organelles & bacteria)
(Also found in nucleus of lower eukaryotes)

3. Group II Introns
(Found in organelles & bacteria)

...Gp I introns are more common than Gp II introns and both possess auto splicing / self splicing property

...Self splicing introns are classified according to their internal organization
(Each can be folded into a typical type of secondary structure)

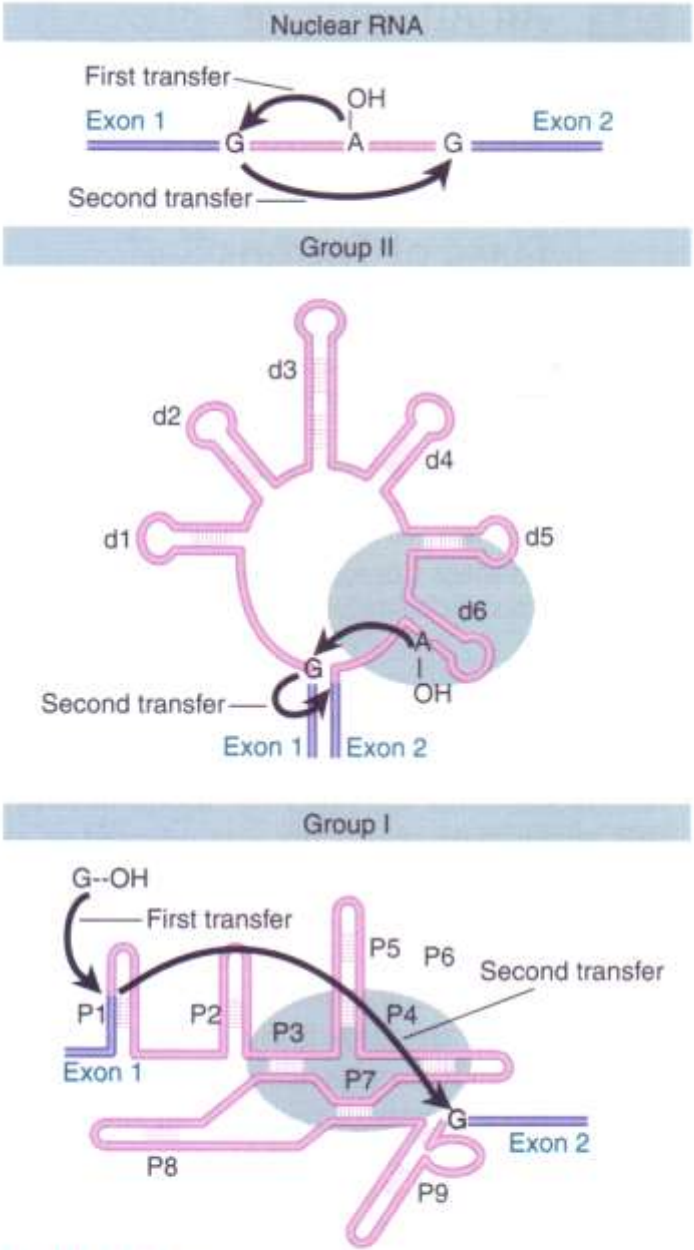
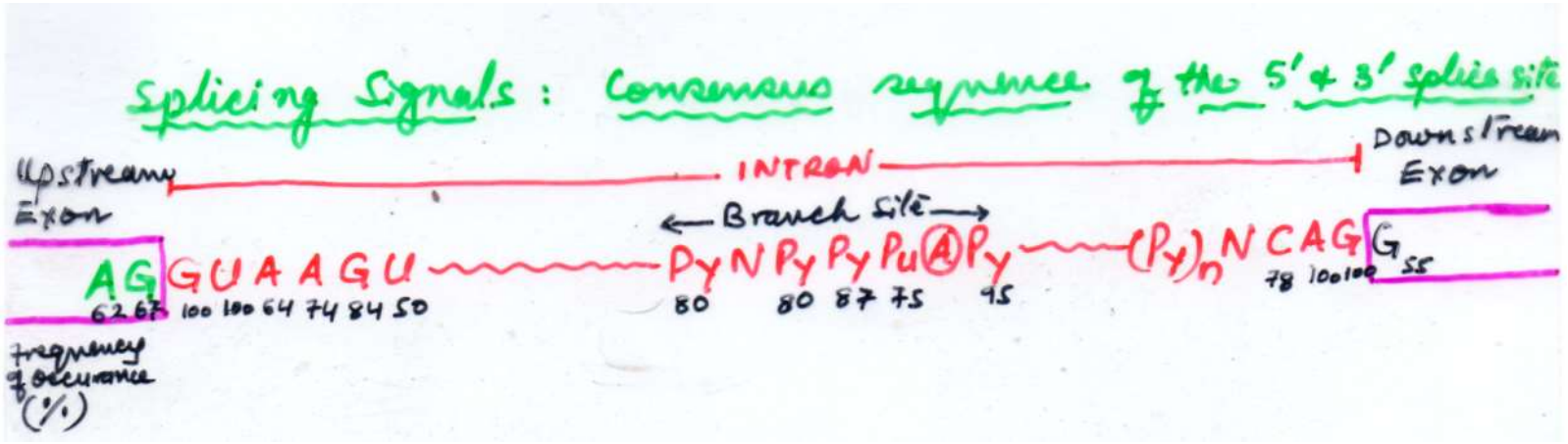
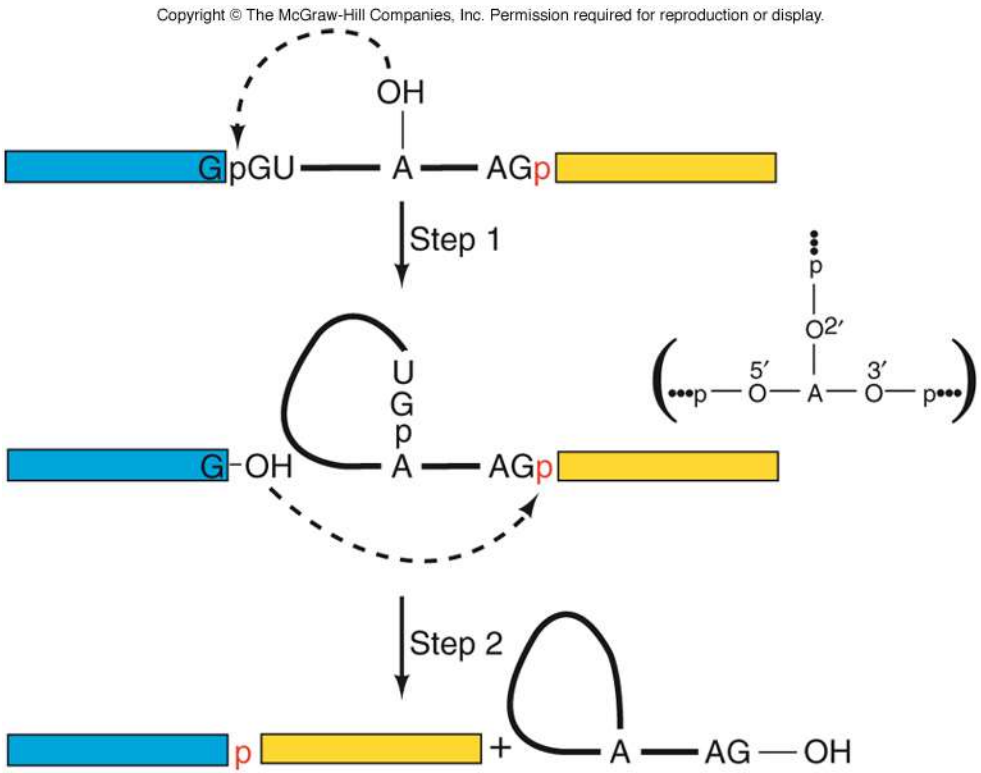


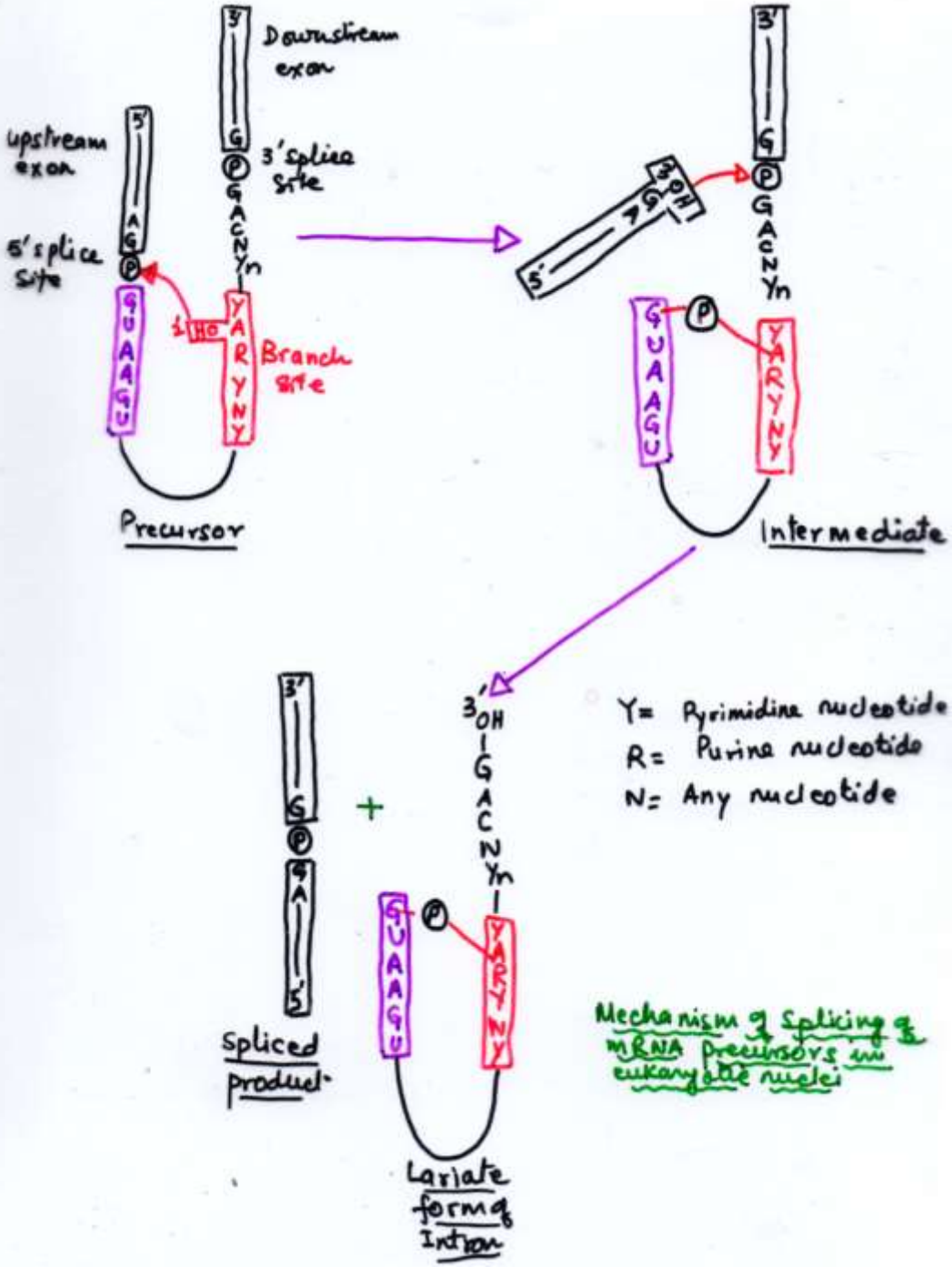
FIGURE 21.15 Three classes of splicing reactions proceed by two transesterifications. First, a free OH group attacks the exon 1–intron junction. Second, the OH created at the end of exon 1 attacks the intron–exon 2 junction.


Splicing Signals : Consensus sequence of the 5' & 3' splice site
Upstream Exon
INTRON
Downstream Exon
← Branch site →
AG GUAAGU ~~~ PyNPyPyPuAPy ~~~ (Py)nNCAGG
62 67 100 100 64 74 84 50
80 80 87 75 95
78 100 100 55
frequency of occurance (%)

Simplified Mechanism of Splicing

- 2'-OH group of A within intron attackes the phosphodiester bond linking the first exon to the intron
- A lariat is formed due to the GU at the 5' end of the intron forming a phosphodiester bond with the branch point A
- The free 3'OH on exon 1 attacks the phosphodiester bond between the intron and exon 2
- The exons are then linked




Downstream exon
3' splice site
Upstream exon
5' splice site
Branch site
Precursor
Intermediate
Spliced product
Lariat forming Intron
Y= Pyrimidine nucleotide
R= Purine nucleotide
N= Any nucleotide
Mechanism of Splicing of mRNA precursors in eukaryotic nuclei

Commitment Complex

Pre-spliceosome

Spliceosome

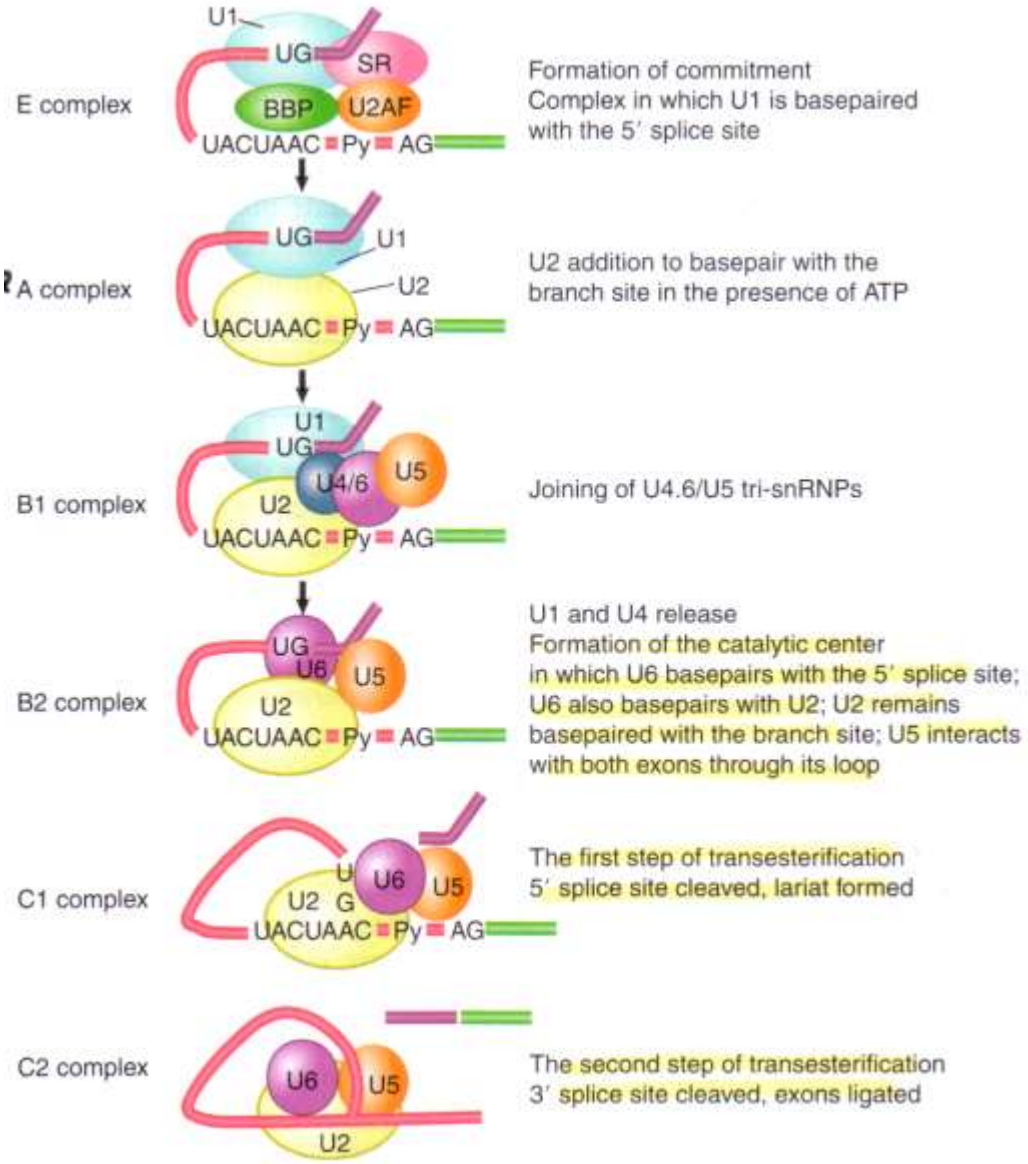


FIGURE 21.12 The splicing reaction proceeds through discrete stages in which spliceosome formation involves the interaction of components that recognize the consensus sequences.

Commitment, Splice Site Selection and Alternative Splicing

- **snRNPs do not have enough specificity and affinity to bind exclusively and tightly at exon-intron boundaries**
- **Additional splicing factors are needed to help snRNPs bind**
- **Some splicing factors are needed to bridge across introns and exons and so define these RNA elements**

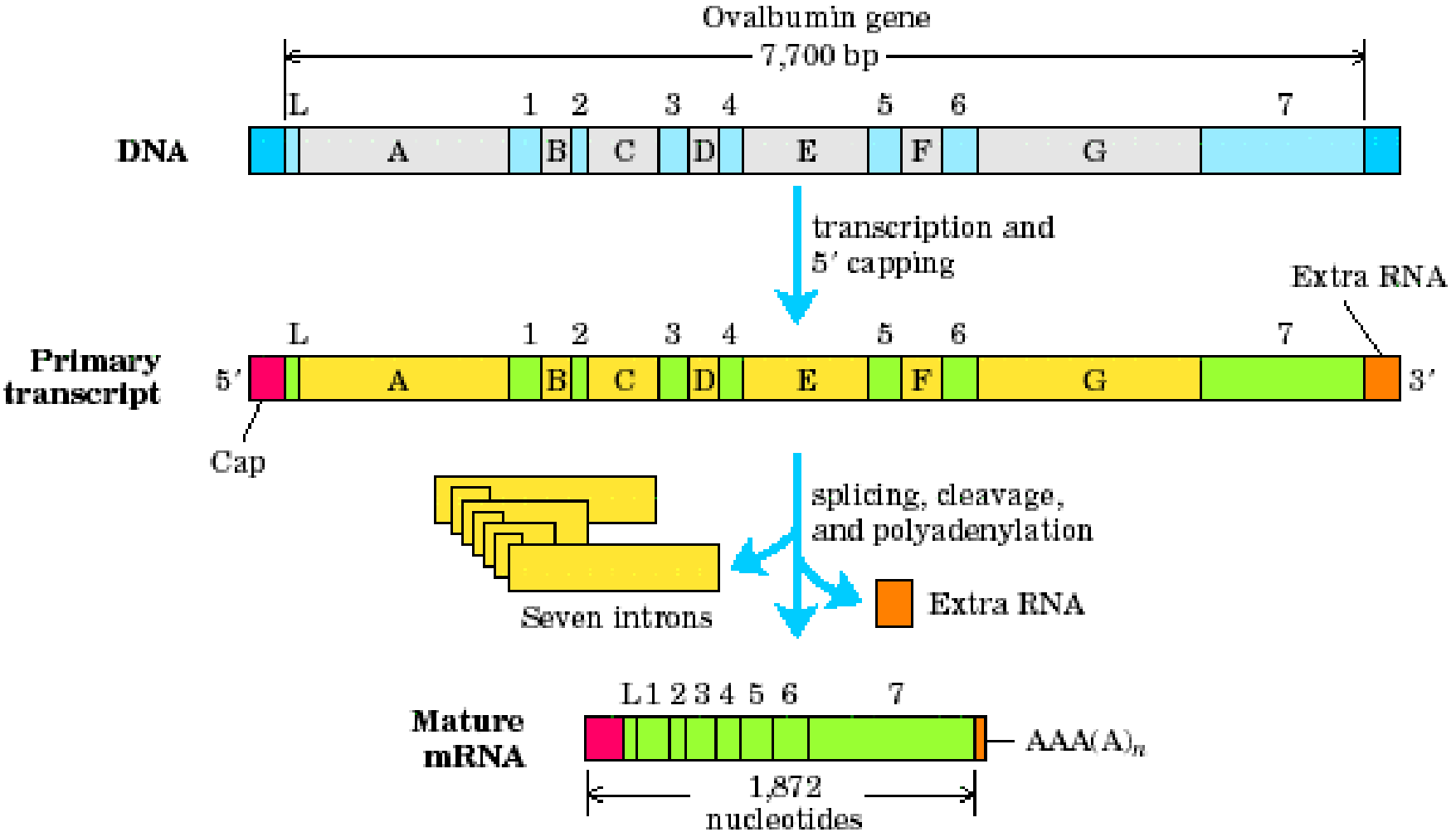
Exon and Intron Definition

- **The spliceosome can recognize either exons or introns in the splicing commitment process, presumably by assembling splicing factors to bridge across exons or introns**
- **If exons are recognized it is exon definition**
- **If introns are recognized it is intron definition**
- **Splicing in a given organism typically uses either exon definition or intron definition**

Number of Introns per primary transcript:

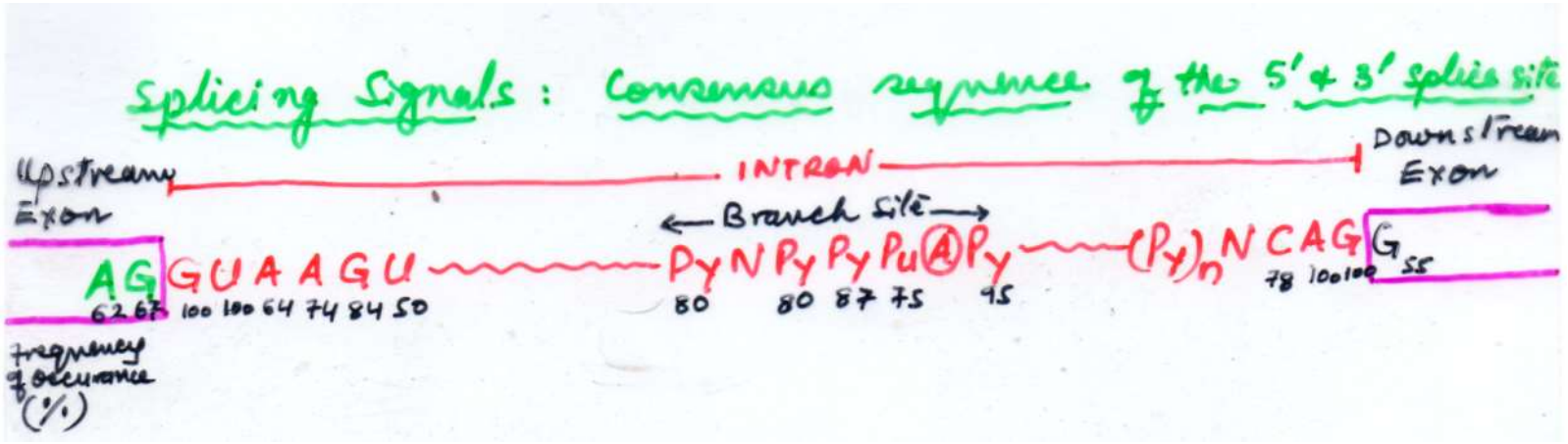
Gene	# of Introns
Histone & Interferon	0
α-Globin	2
Ig L-Chain	2
Ig H-Chain	4
Yeast mt. cytochrome b	6
Ovomucoid	6
Ovalbumin	7
Ovotransferrin	16
Conalbumin	17
α Collagen	52

mRNA Splicing



Base sequence of intron-exon junctions:

	Gene Region	Exon ▼	INTRON	▼ Exon
1.	Ovalbumin (Intron-2)	UAAG	GUGAGC ~~~~~~~~	UUACAG GUUG
2.	Ovalbumin (Intron-3)	UCAG	GUACAC ~~~~~~~~	AUUCAG UCUG
3.	β-Globin (Intron-1)	GCAG	GUUGGU ~~~~~~~~	CCUUAG GCUG
4.	β-Globin (Intron-2)	CAGG	GUGAGU ~~~~~~~~	CCACAG UCUC
5.	Ig λ, Intron,	UCAG	GUCAGC ~~~~~~~~	UUGCAG GGGC
6.	SV40 Early T antigen	UAAG	GUAAAU ~~~~~~~~	UUUUAG AUUC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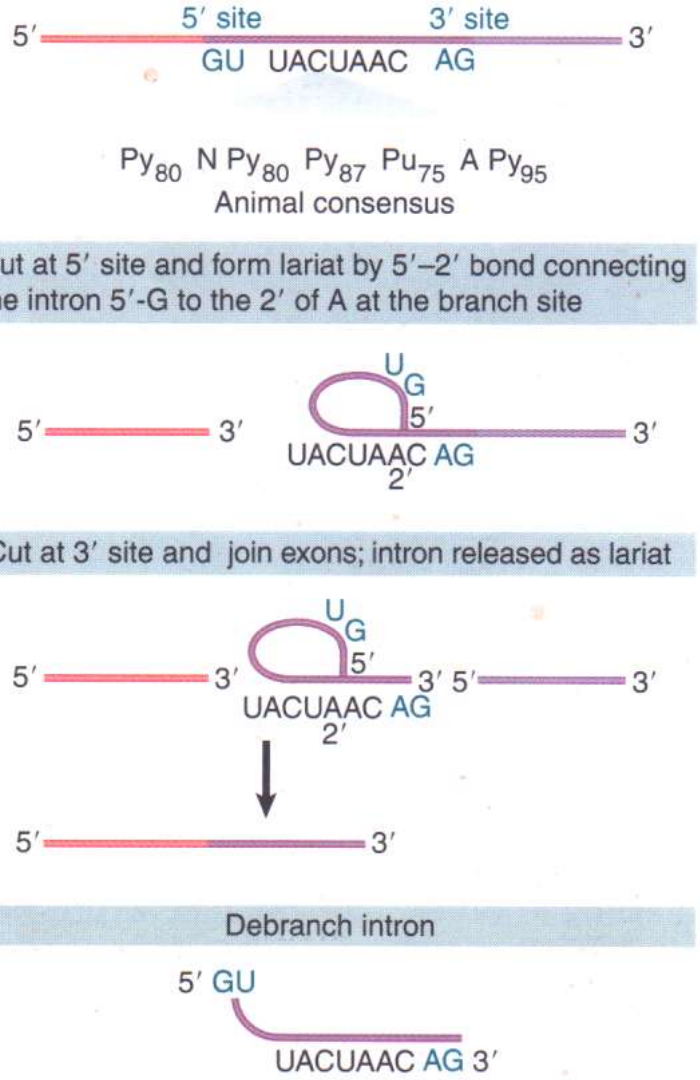


FIGURE 21.5 Splicing occurs in two stages. First the 5′ exon is cleaved off, and then it is joined to the 3′ exon.

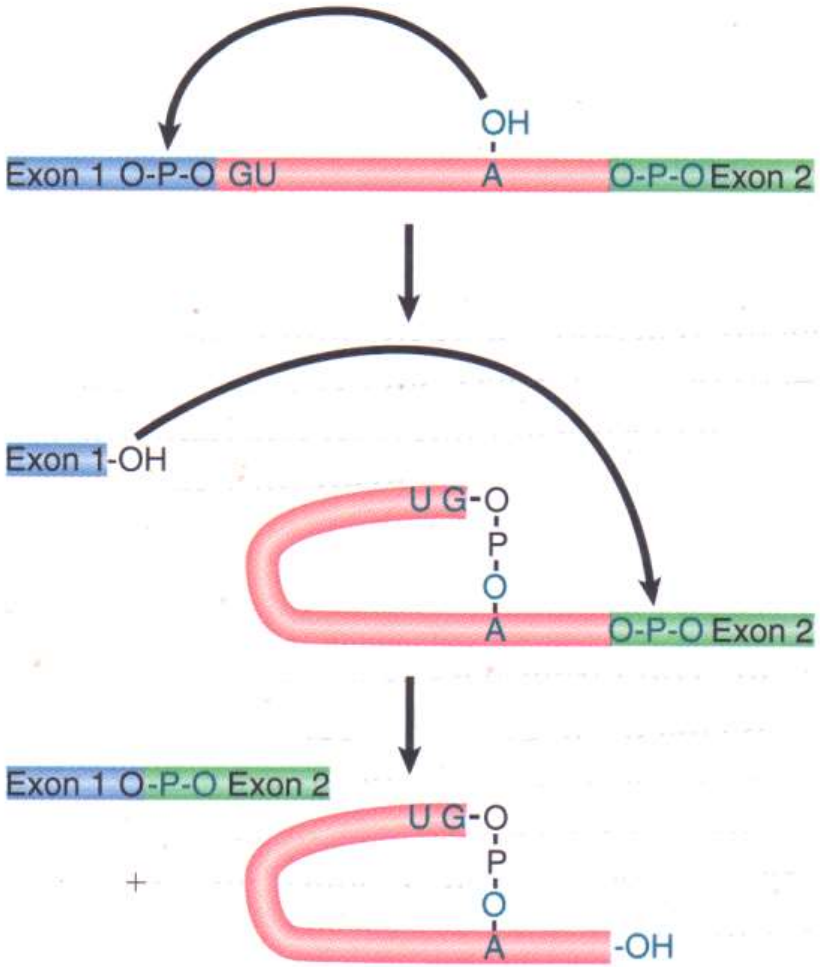


FIGURE 21.6 Nuclear splicing occurs by two transesterification reactions in which an OH group attacks a phosphodiester bond.

TRANSESTERIFICATION REACTION

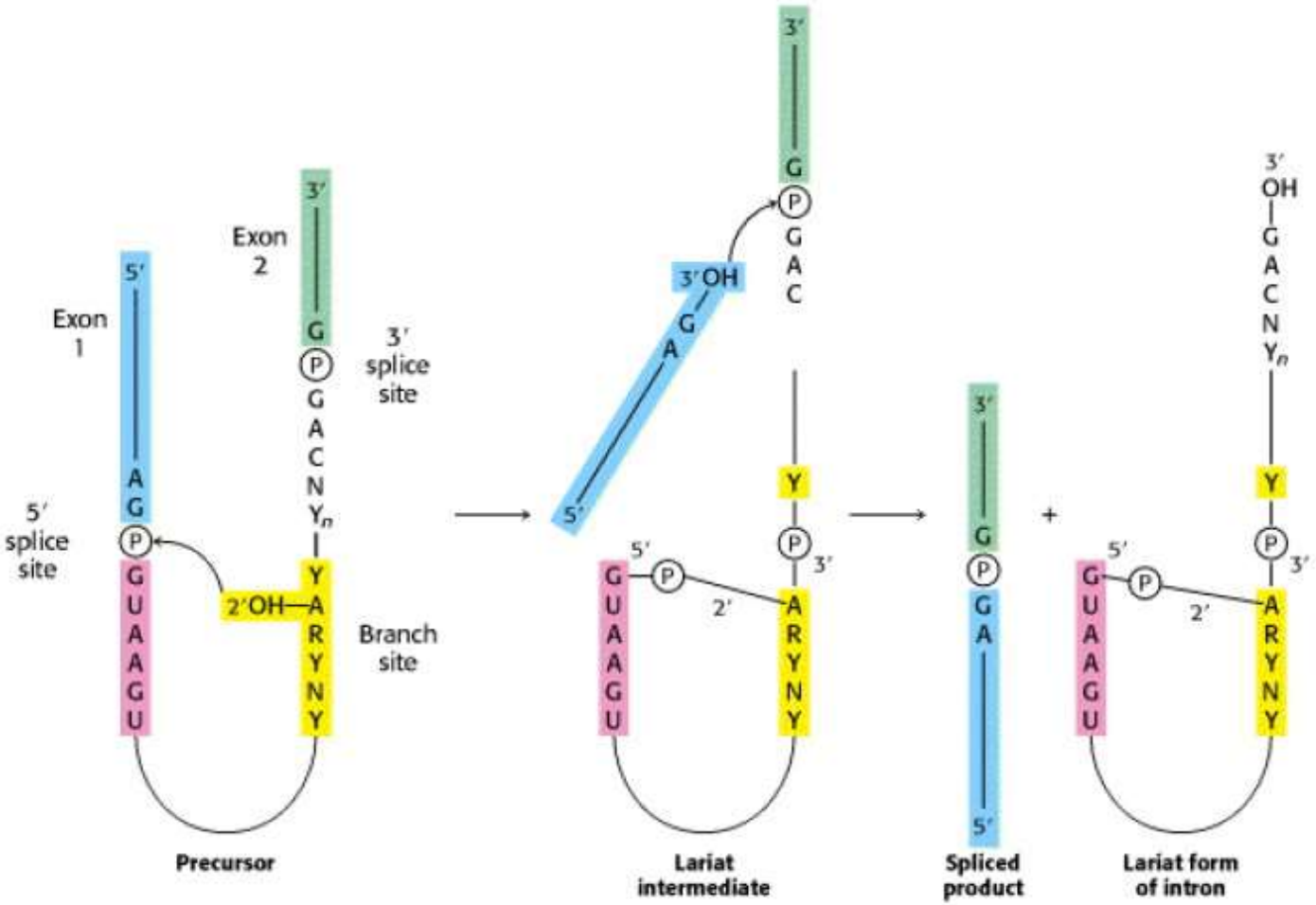
(Transfer of phosphoester)

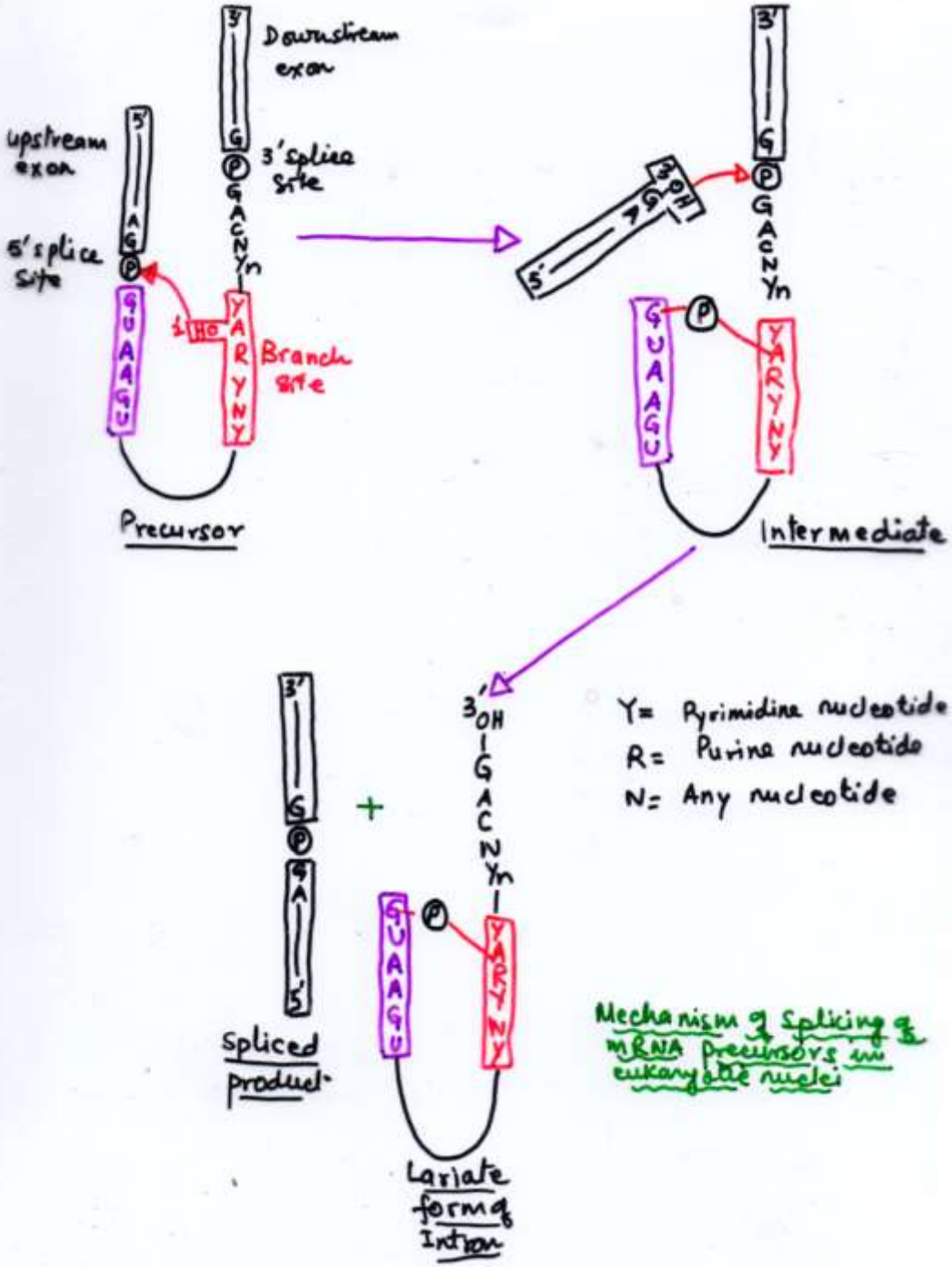
The esters are of phosphate rather than carbon & some of R groups are very long eg –

R′ = 5′ exon, contains ~5,000 nto & has a molecular wt. exceeding 1 million daltons

R″ = IVS + 3′ exon

R‴ = Guanosine or GTP


Exon 1
Exon 2
5′ splice site
3′ splice site
Branch site
2′OH
Precursor
3′OH
Lariat intermediate
Spliced product
Lariat form of intron


Downstream exon
3' splice site
Upstream exon
5' splice site
Branch site
Precursor
Intermediate
Spliced product
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Y= Pyrimidine nucleotide
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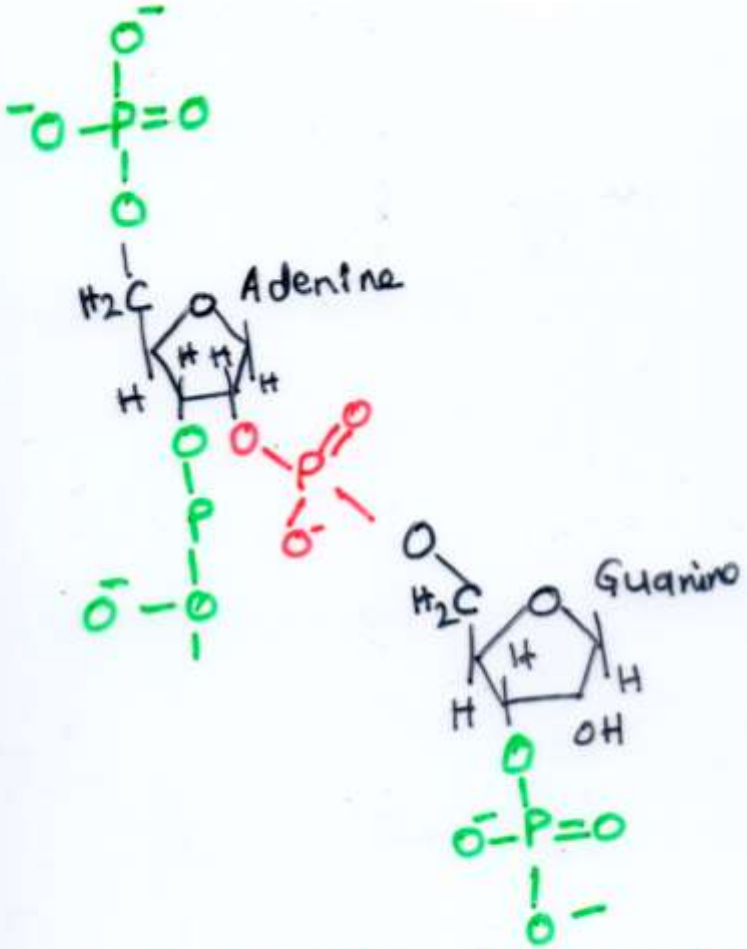
N= Any nucleotide
Mechanism of Splicing of mRNA precursors in eukaryotic nuclei



Structure of the Branch Point in the lariate intermediate formed during splicing

Role of Branch Site

The role of branch site is to identify the nearest 3' splice site as the target for connection to 5' splice site.

Spliceosome Assembly Pathway

snRNPs are required for Splicing

- **The 5’ & 3’ splice sites and the branch site are recognized by components of the splicing apparatus. That assemble to form large complex.**
- **The splicing apparatus contains both proteins and RNAs (in addition to the pre-mRNAs.**
- **Five snRNAs are involved in splicing reaction e.g., U1, U2, U5, U4 & U6.**
- **In their natural state, these snRNA exist as ribonucleoprotein particles called snRNPs (U1 snRNP, U2 snRNP, U5 snRNP, U4 snRNP & U6 snRNP)**
- **SnRNPs associated with splicing signals brings these signals together before any reaction occur.**
- **These complex assembles sequentially on the pre-mRNA.**
- **Splicing occurs only after all the components have assembled.**

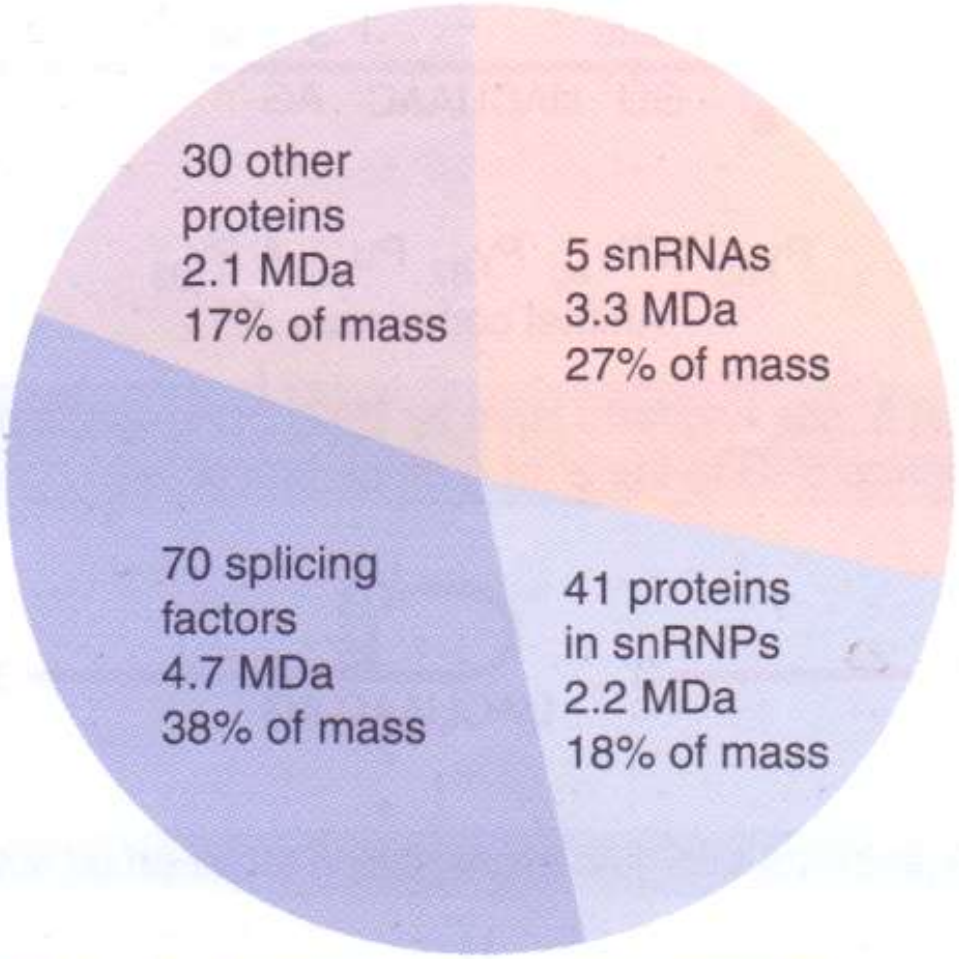


FIGURE 21.7 The spliceosome is ~12 MDa. Five snRNPs account for almost half of the mass. The remaining proteins include known splicing factors, as well as proteins that are involved in other stages of gene expression.

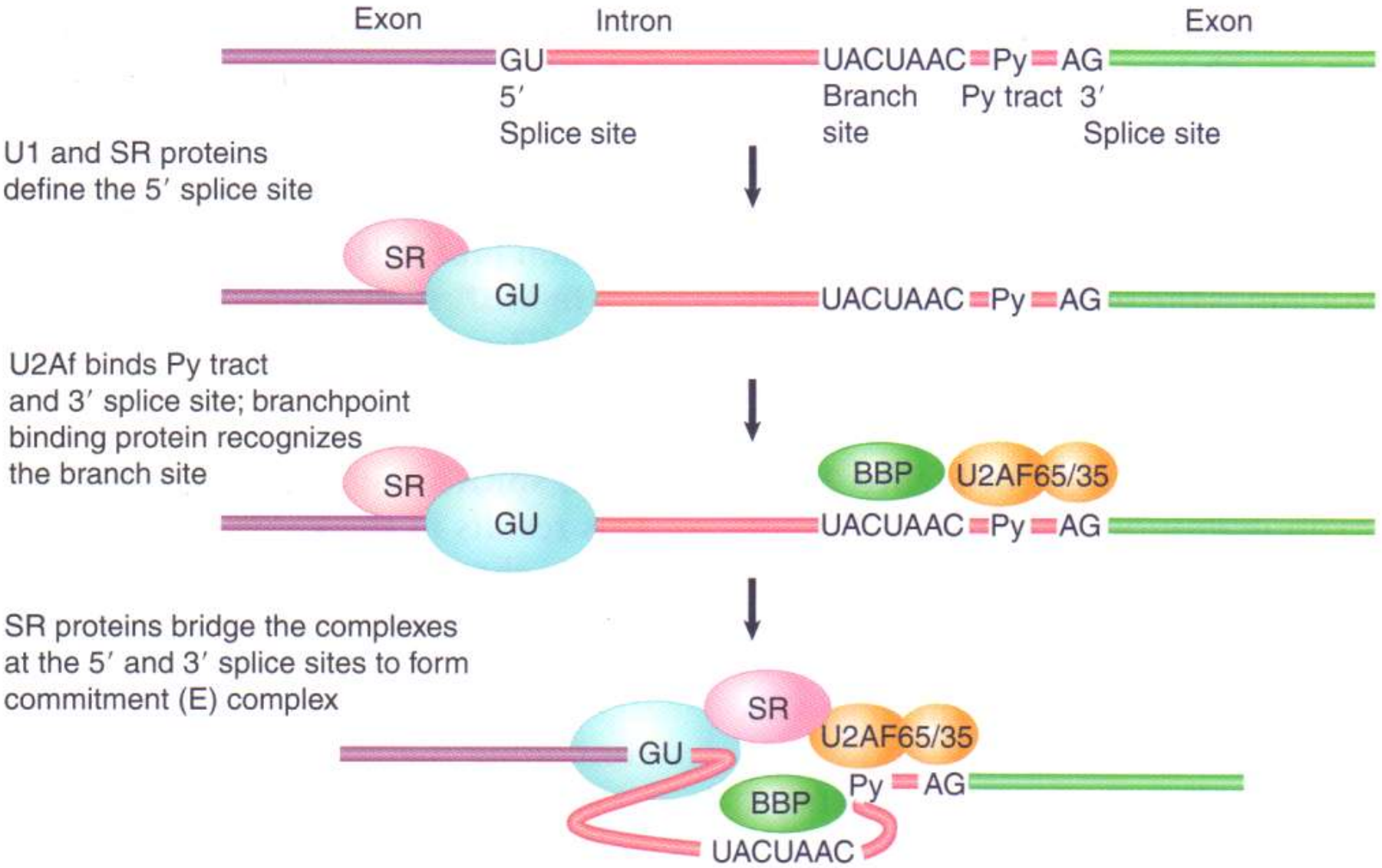


FIGURE 21.10 The commitment (E) complex forms by the successive addition of U1 snRNP to the 5′ splice site, U2AF to the pyrimidine tract/3′ splice site, and the bridging protein SF1/BBP.

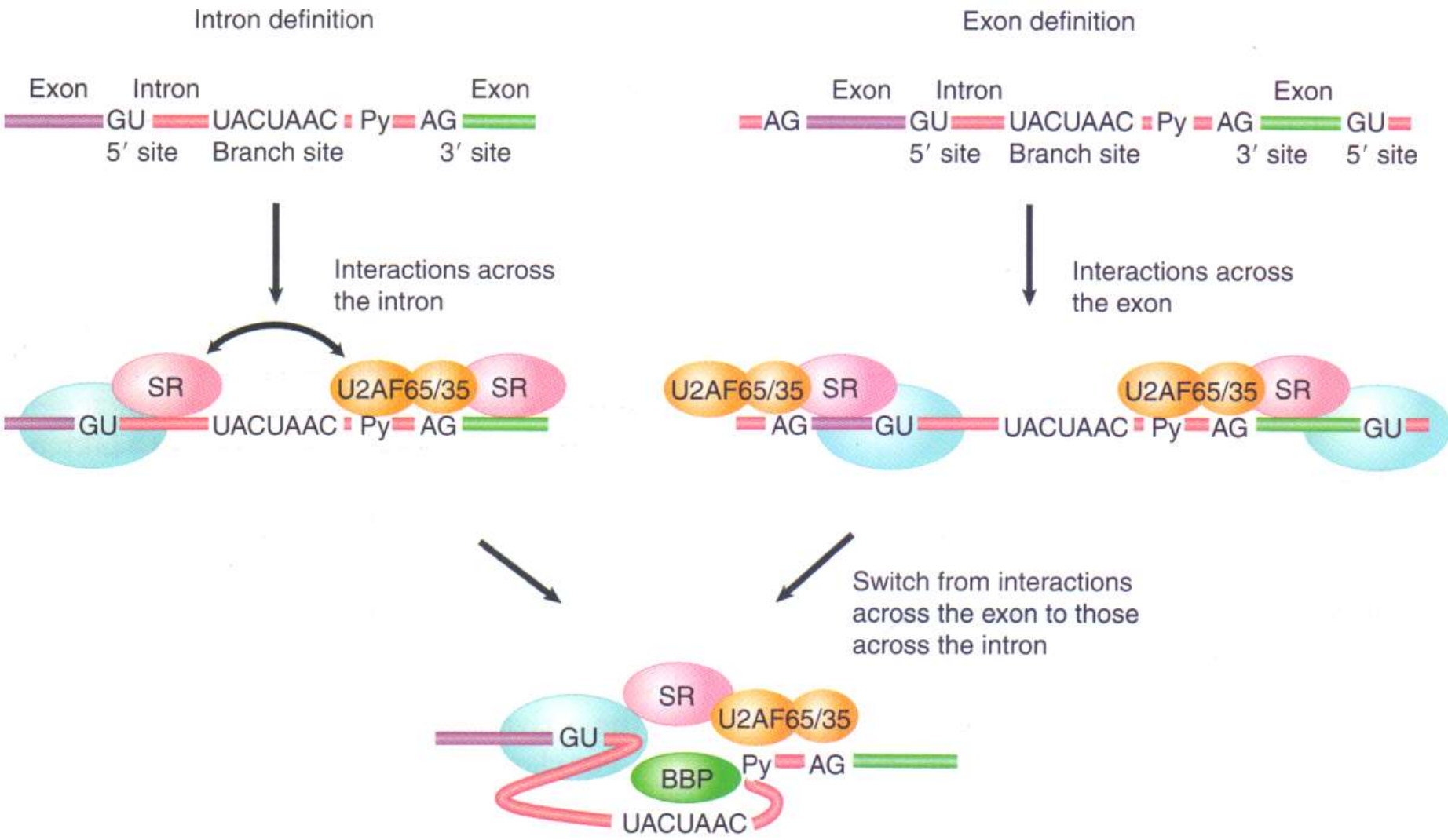
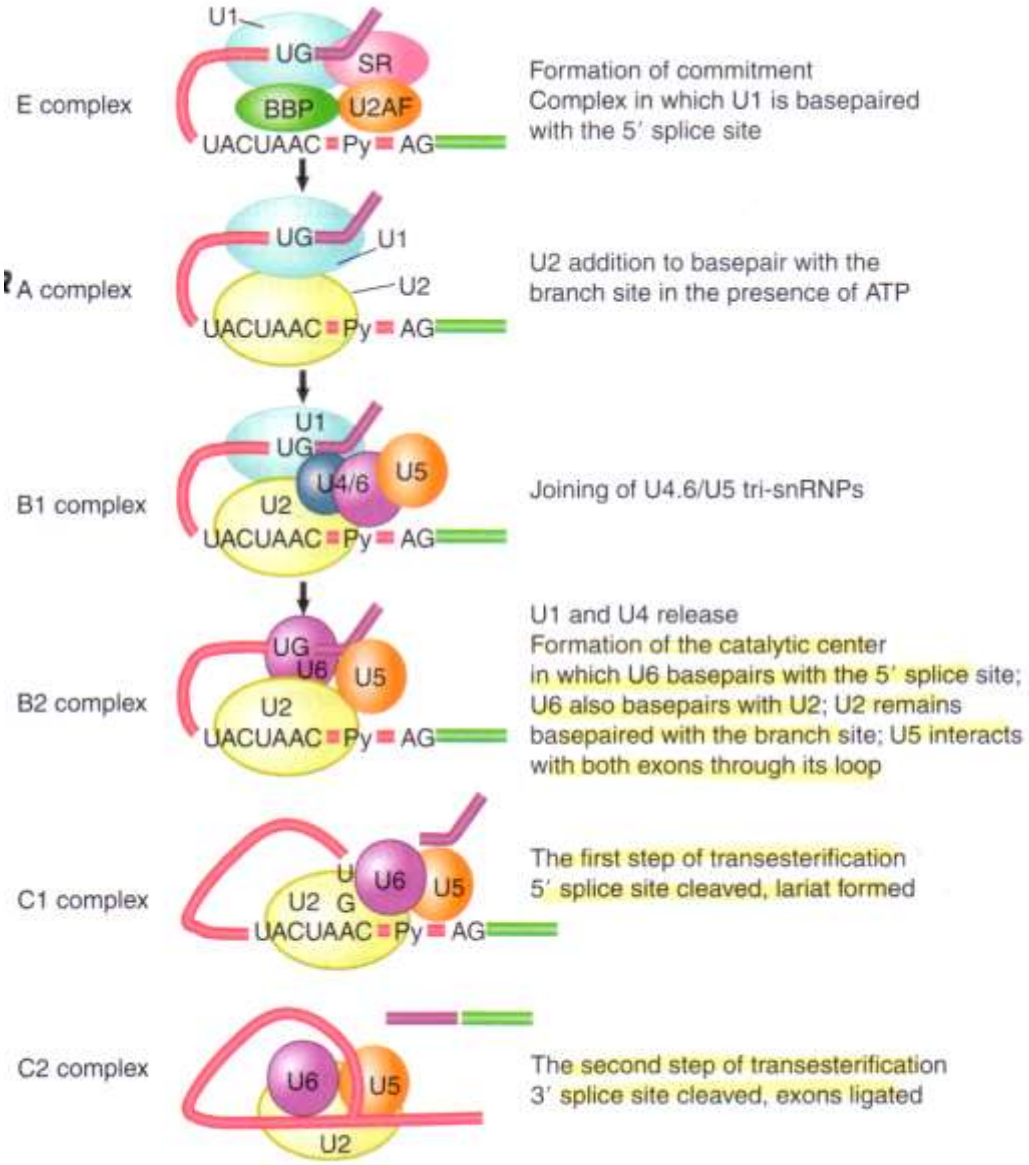


FIGURE 21.11 There are two routes for initial recognition of 5′ and 3′ splice sites by either intron definition or exon definition.

Commitment Complex

Pre-spliceosome

Spliceosome



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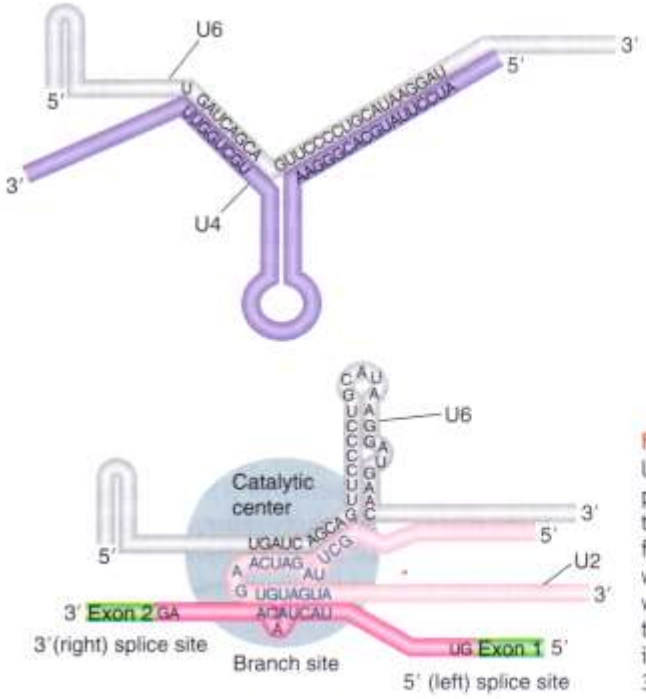

FIGURE 21.13 U6-U4 pairing is incompatible with U6-U2 pairing. When U6 joins the spliceosome it is paired with U4. Release of U4 allows a conformational change in U6; one part of the released sequence forms a hairpin (gray), and the other part (pink) pairs with U2. An adjacent region of U2 is already 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′.

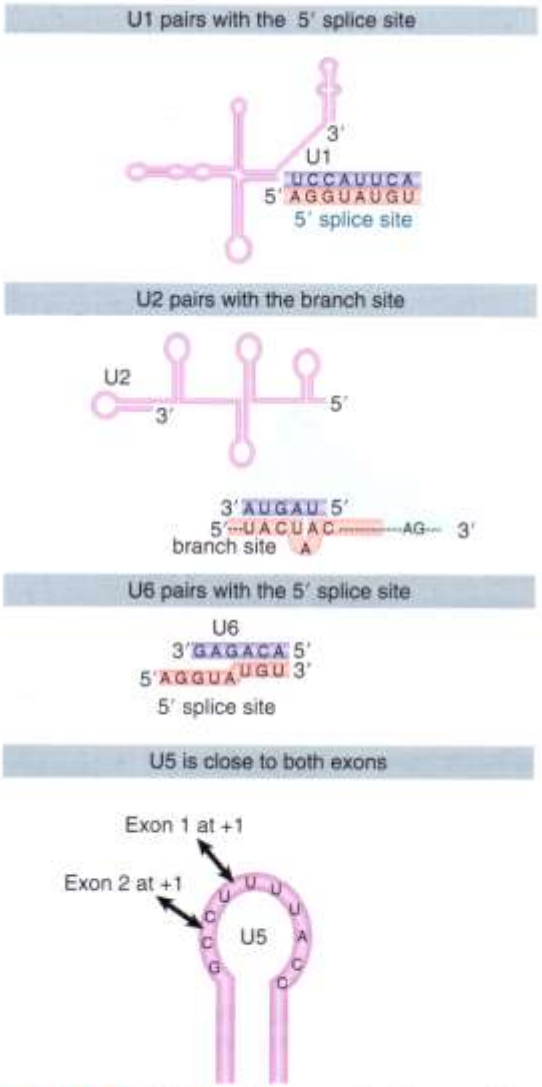

FIGURE 21.14 Splicing utilizes a series of base pairing reactions between snRNAs and splice sites.