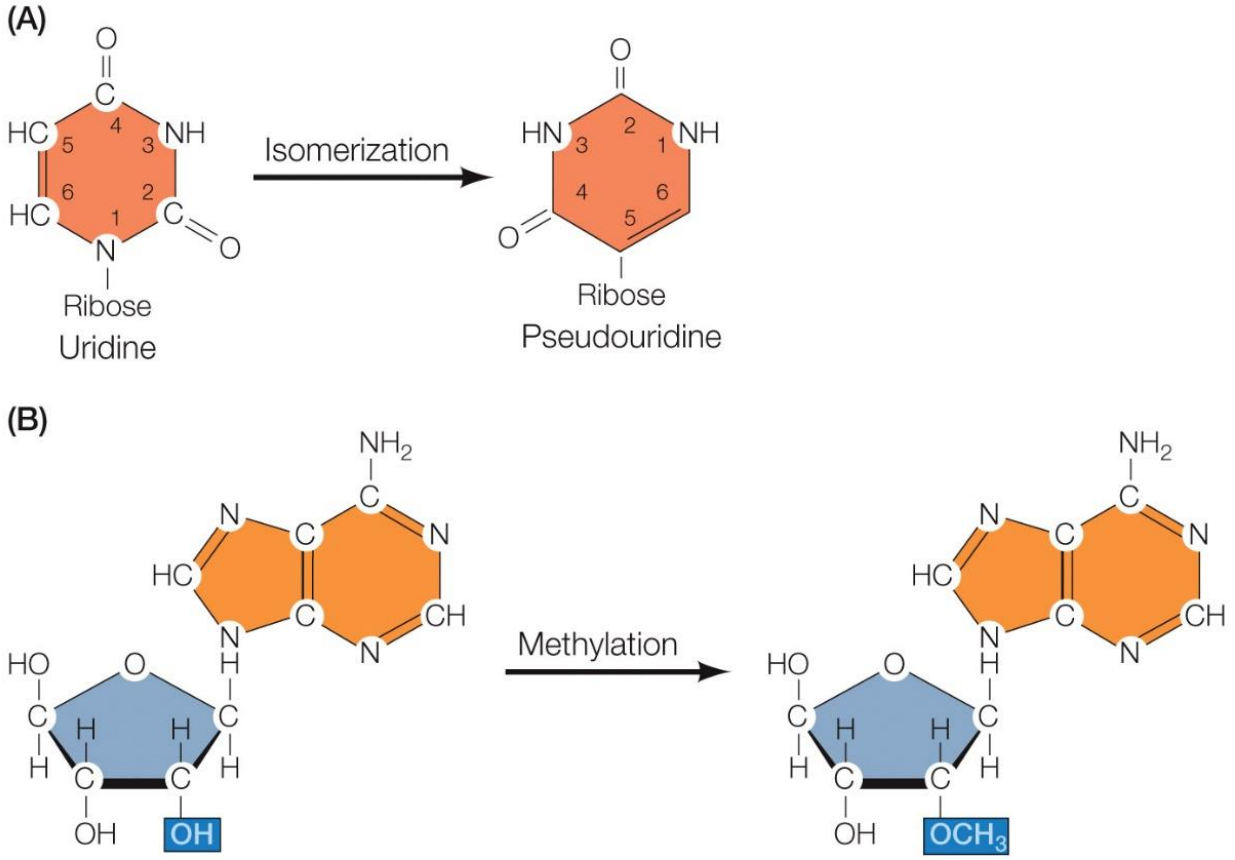
Lecture 10 : RNA Processing and turnover

Learning objectives

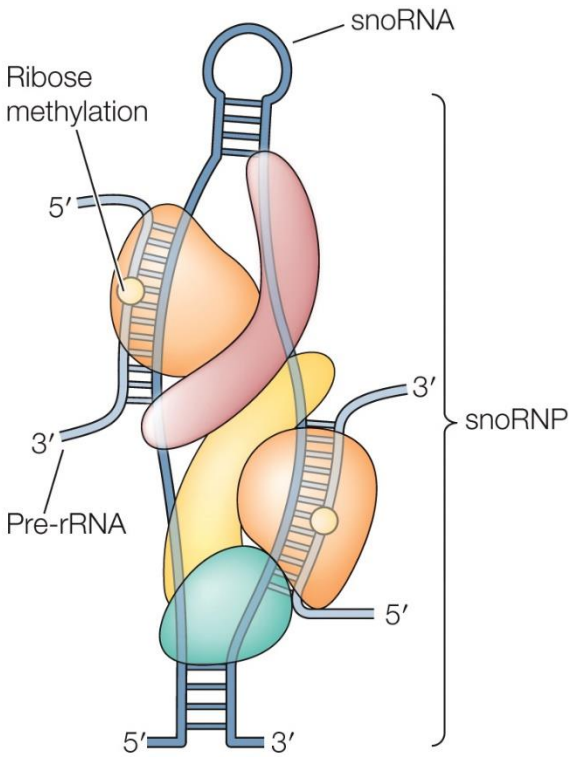
- Summarize the events involved in processing rRNA and tRNAs.
- Diagram mRNA processing
- Describe the roles of snRNAs in mRNA splicing.
- Illustrate patterns of alternative splicing
- Describe RNA editing
- Explain how mRNA degradation can be regulated by the environment

Processing of ribosomal RNAs

1. Cleavage of pre-rRNA into 5.8S, 18S, and 28S rRNAs in eukaryotes
2. Modification: formation of pseudouridine and methylation of ribose residues



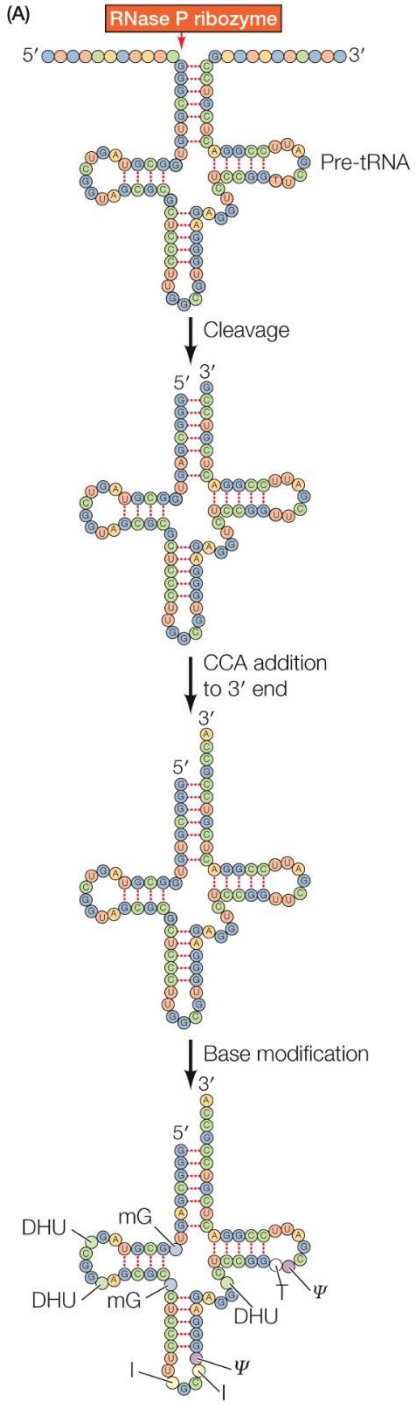
Role of snoRNPs in pre-rRNA processing

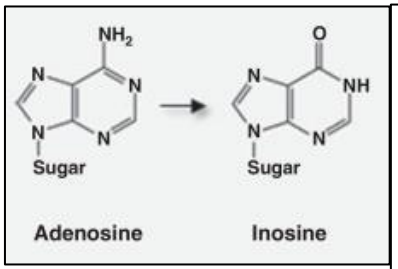


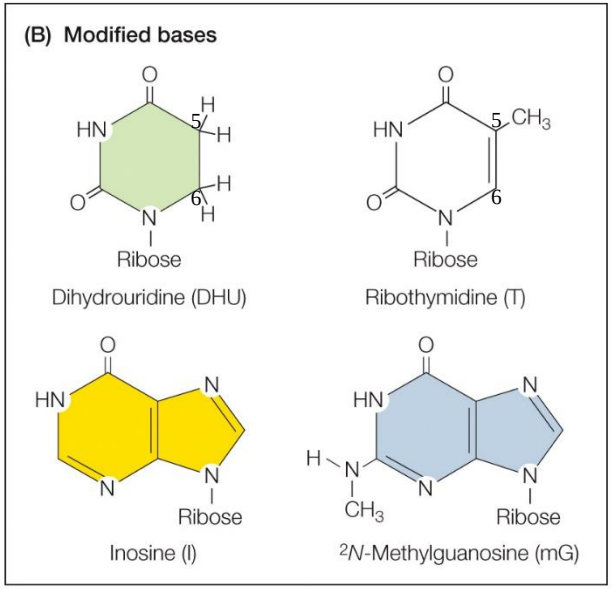
- rRNA modifications are mediated by small ribonucleoprotein particles called **snoRNPs** in the nucleolus
- snoRNPs contain snoRNAs complexed with 8~10 proteins.
- The snoRNAs contain short sequences complementary to the sites of pre-rRNA modification.
- Base pairing between snoRNAs and pre-rRNAs targets the modifying enzymes responsible for methylation or pseudouridylation to the appropriate sites.

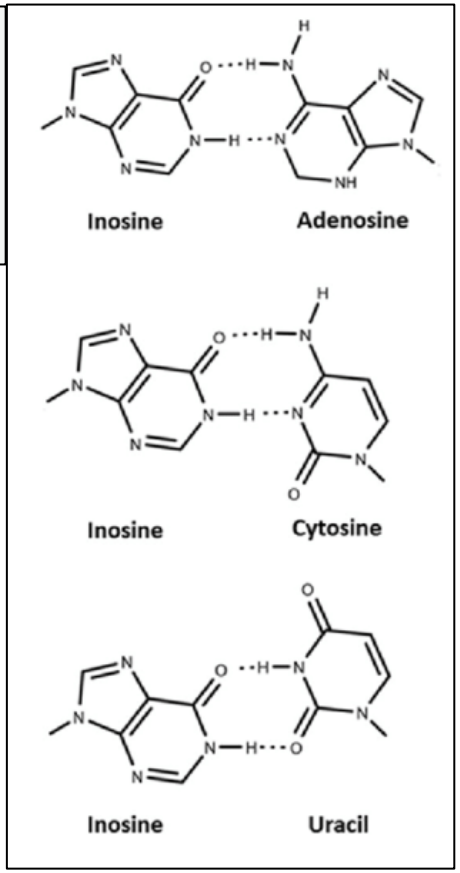
Processing of transfer RNAs

- Cleavage from pre-tRNAs: Cleavage at the 5′ end by the RNase P ribozyme; cleavage at the 3′ end by a conventional protein RNase.
- Addition of a CCA terminus to the 3′ end
- Base modification: dihydrouridine (DHU), ribothymidine (T), inosine (I), methylguanosine (mG), and pseudouridine (ψ).









Processing of mRNA in eukaryotes

Eukaryotic RNAs are transcribed and processed simultaneously in the nucleus

Transcription과 translation이

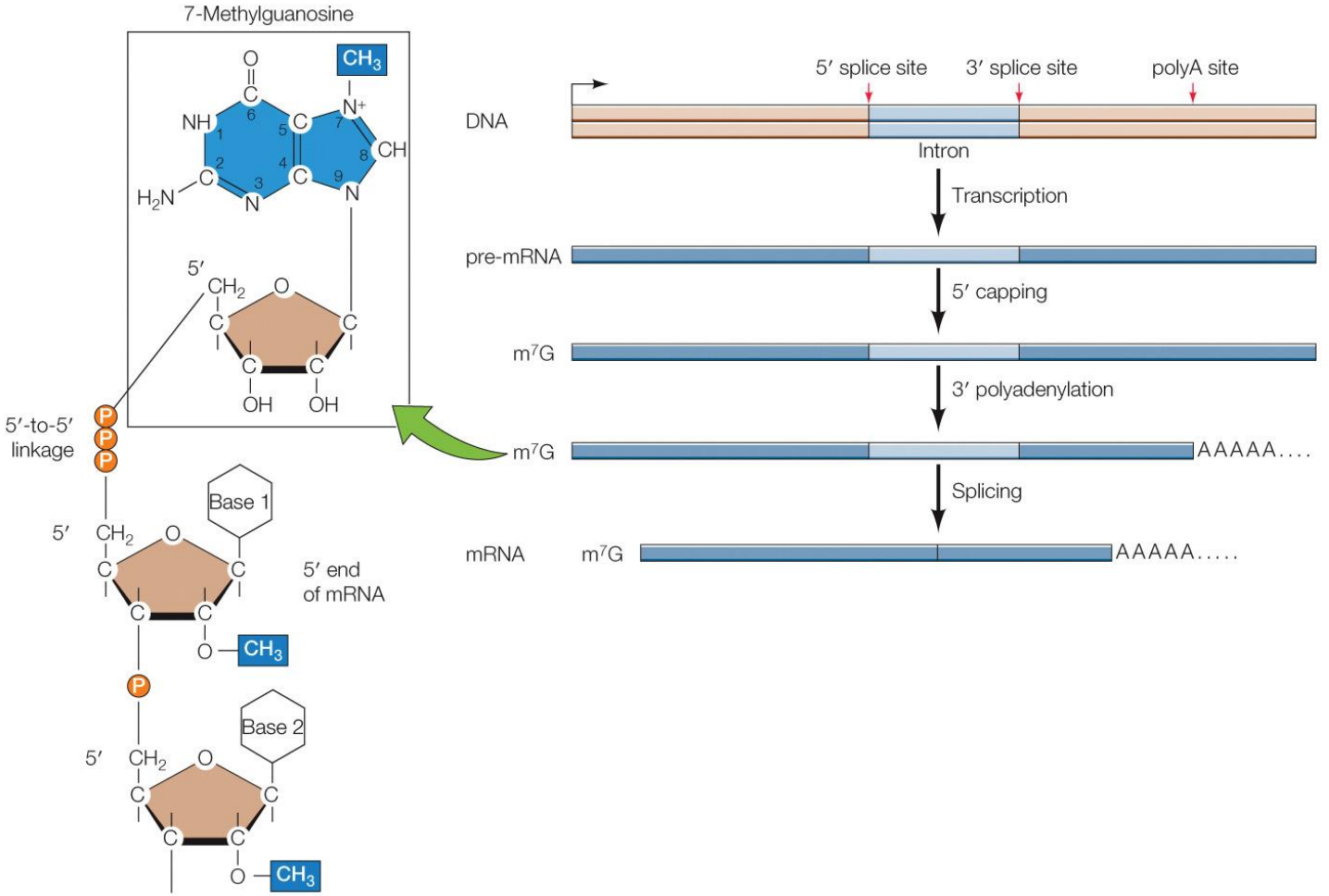
- Prokaryotes에서는 연계되어 일어남; coupled transcription-translation
- Eukaryotes에서는 시간적, 공간적으로 구분됨; 핵막의 존재
- Eukaryotes에서는 유전자에 intron이 존재

→ **RNA processing** for transport, stability, and maturation of RNA

1. RNA capping at 5'end
2. Polyadenylation at 3' end
3. Splicing

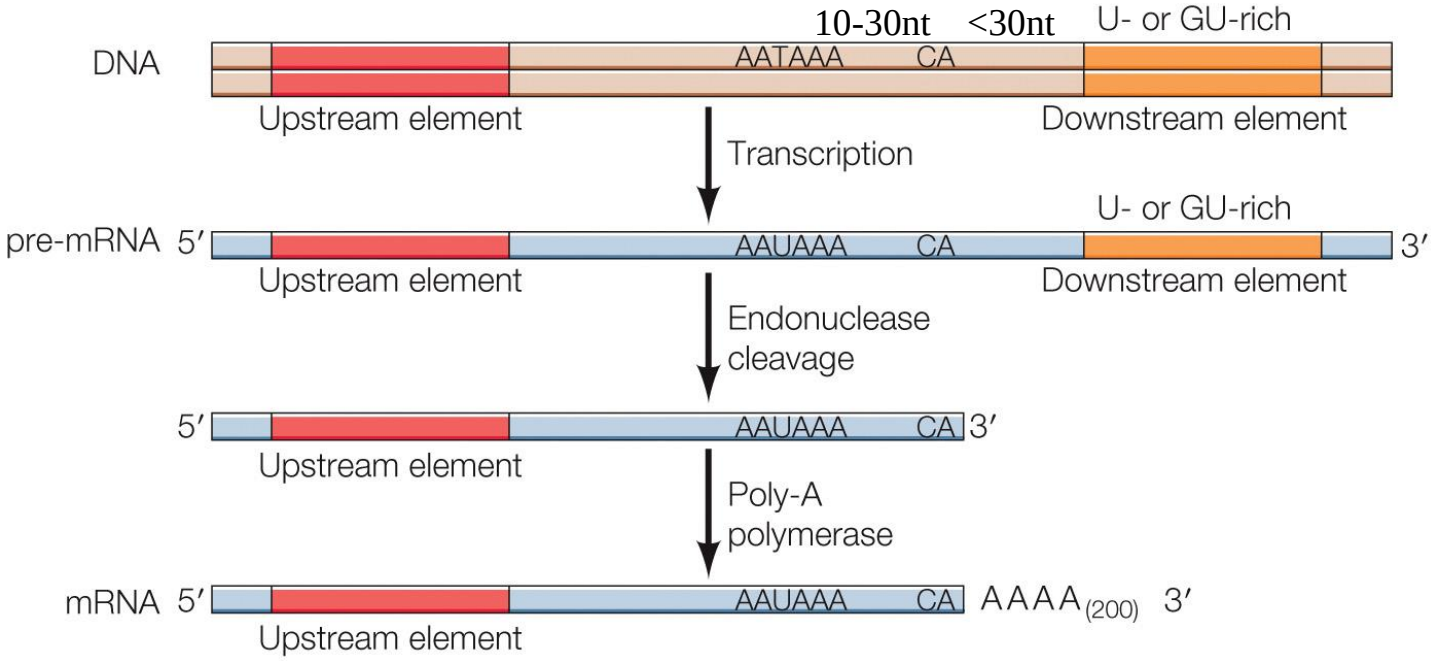
Processing of Eukaryotic Messenger RNAs

1. 5’ capping: 5’-to-5’ GTP addition & methylation → RNA stability & ribosome binding
2. 3’ polyadenylation: cleavage and polyadenylation → RNA export, RNA stability, and translation efficiency
3. RNA splicing: intron removal & RNA export

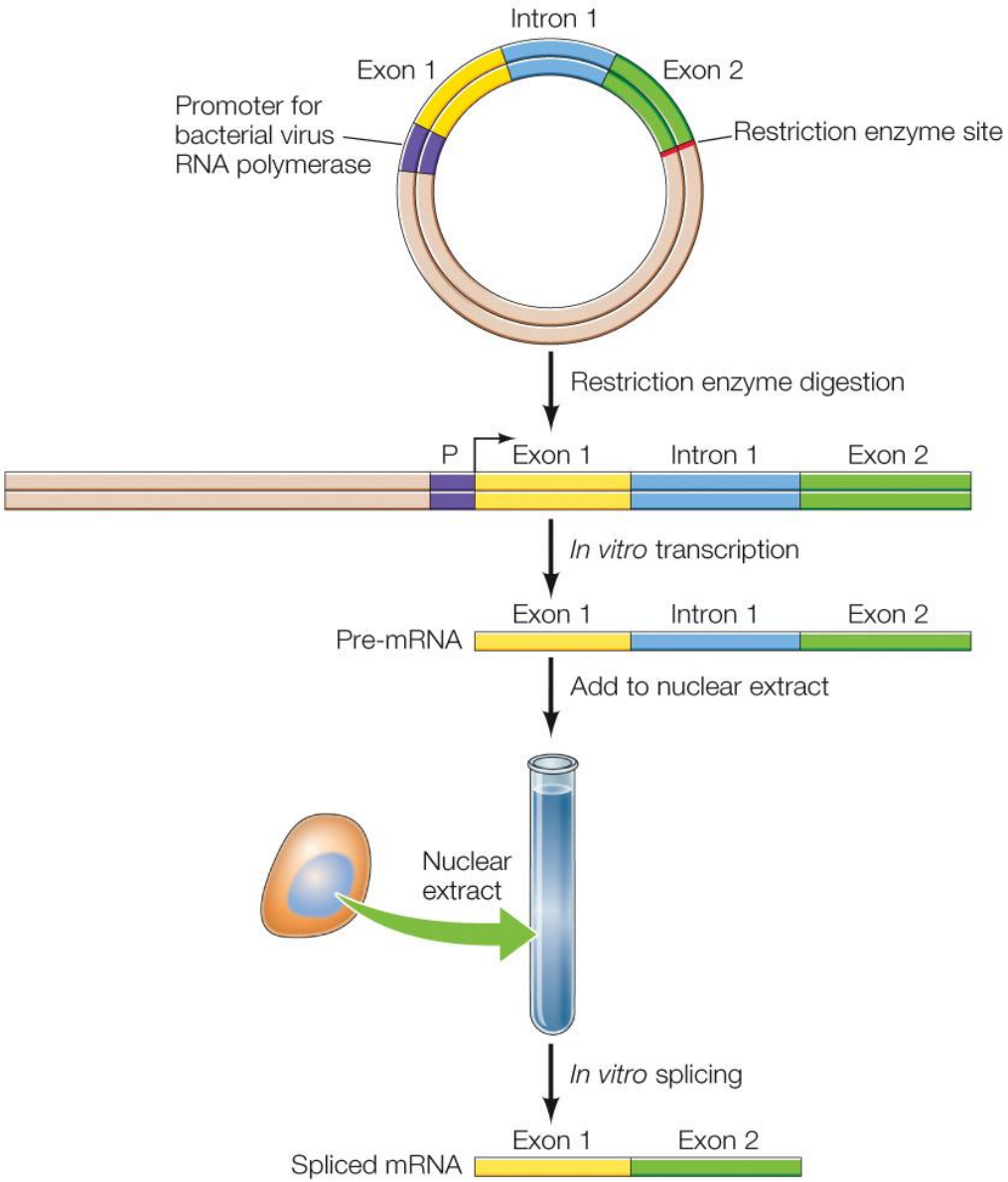


Formation of the 3’ Ends of Eukaryotic mRNAs

- Polyadenylation signals in mammalian cells consist of the hexanucleotide AAUAAA in addition to upstream and downstream (U- or GU-rich) elements.
- An endonuclease cleaves the pre-mRNA 10–30 nucleotides downstream of the AAUAAA, usually at a CA sequence.
- Poly-A polymerase then adds a poly-A tail consisting of about 200 adenines (A) to the 3′ end of the RNA.

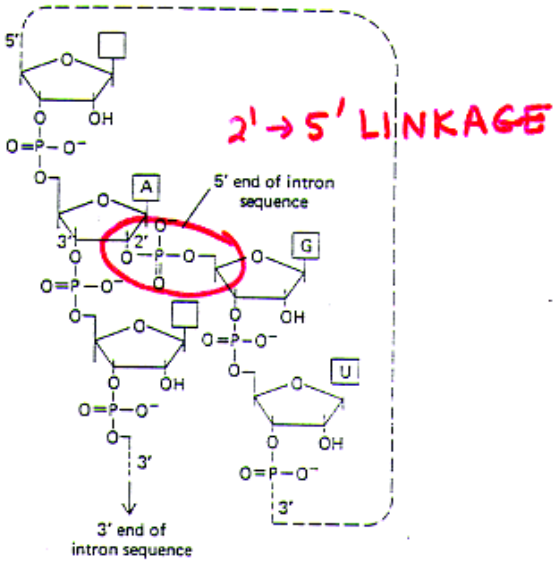


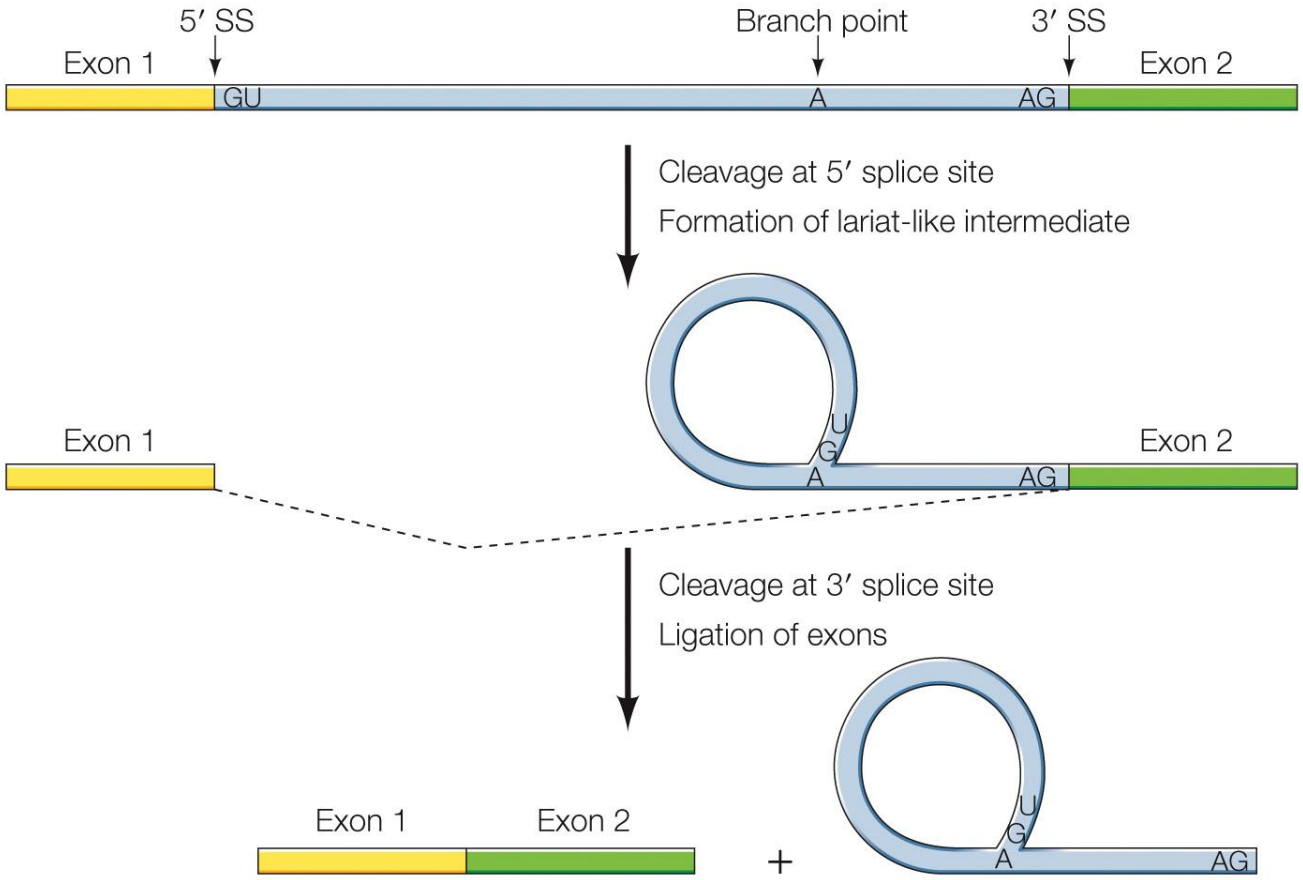
In vitro splicing



Pre-mRNA splicing proceeds in two steps

1. Cleavage at 5’ splice site and formation of lariat-like intermediate (5’-2’)
2. Simultaneous cleavage at the 3’ splice site and ligation of the two exons



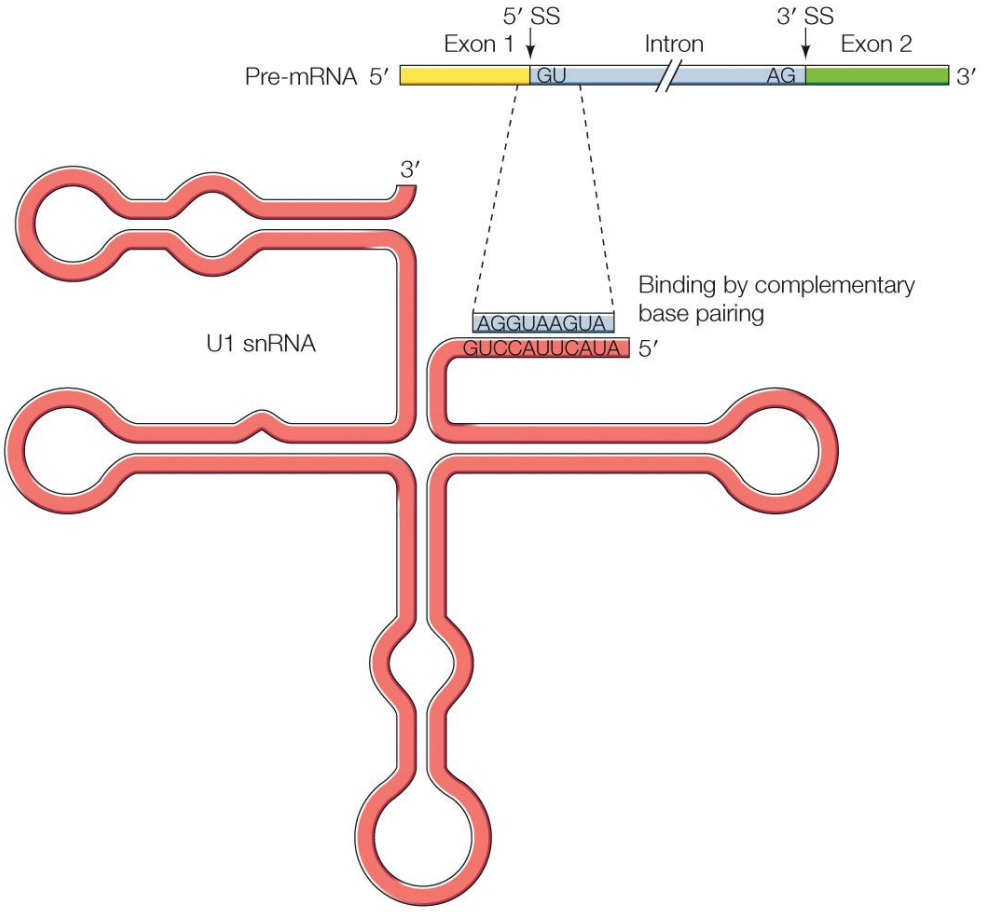


Splicesome

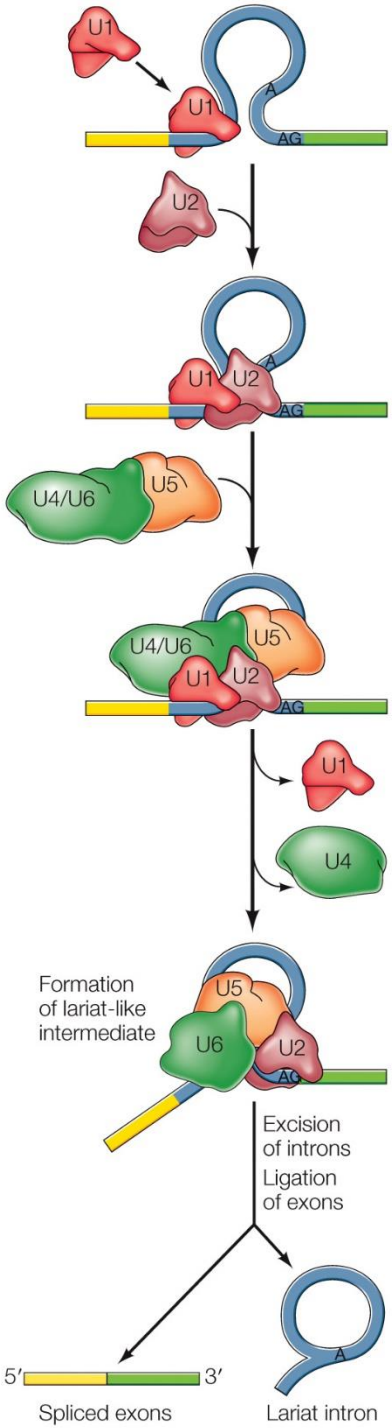
; small nuclear ribonucleoprotein complexes (snRNPs)

• 5 Small nuclear RNAs (snRNAs): U1, U2, U4, U5, U6

• 6~10 proteins



Binding of U1 snRNA to the 5′ Splice Site



Self-Splicing

Intron acts as a ribozyme to direct its own excision from the pre-mRNA molecule

- Group I—cleavage at 5′ SS mediated by a guanosine cofactor. The 3′ end of the free exon then reacts with the 3′ SS to excise the intron
- Group II—cleavage of 5′ SS results from attack by an adenosine nucleotide in the intron, resulting in a lariat-like intermediate

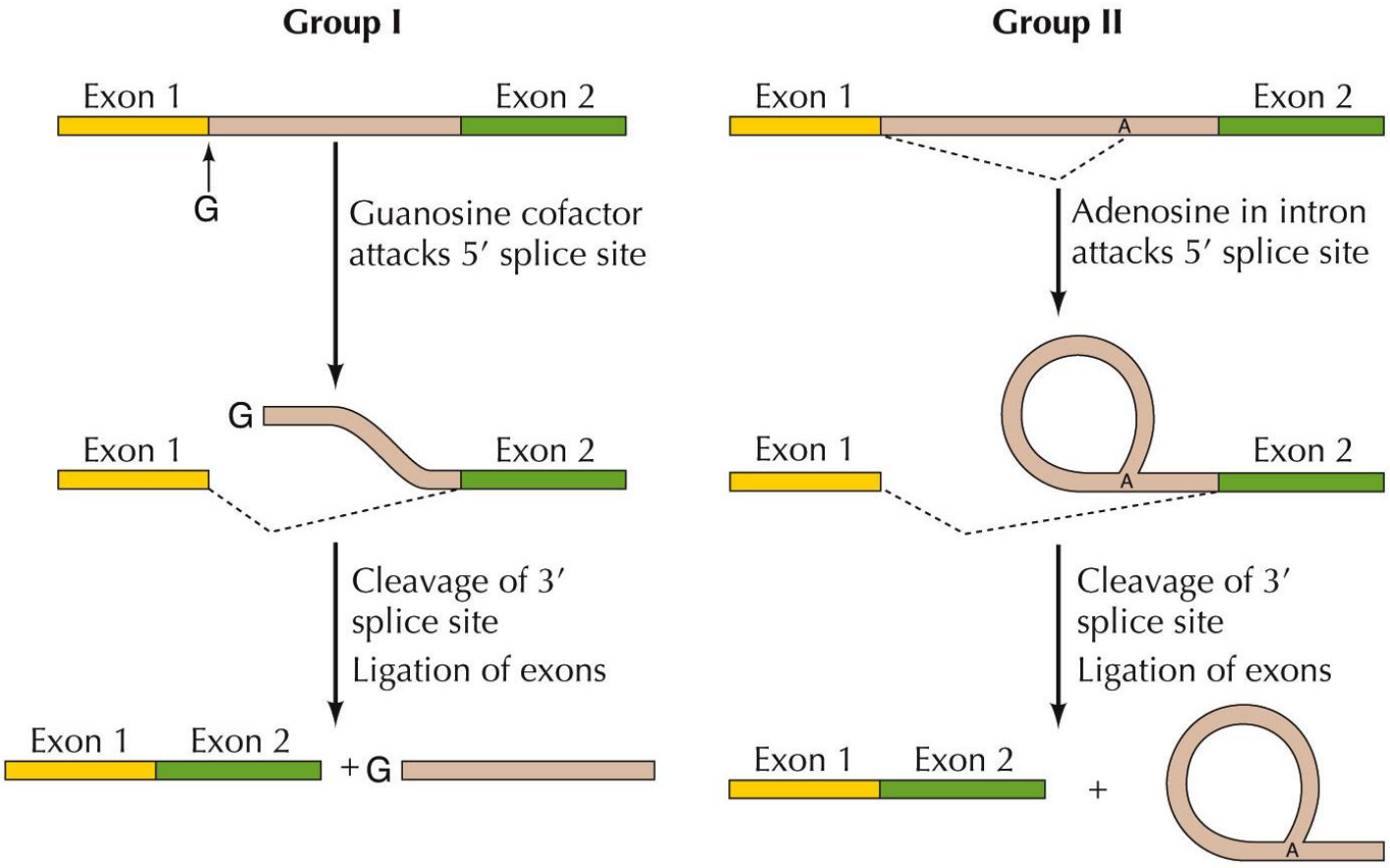
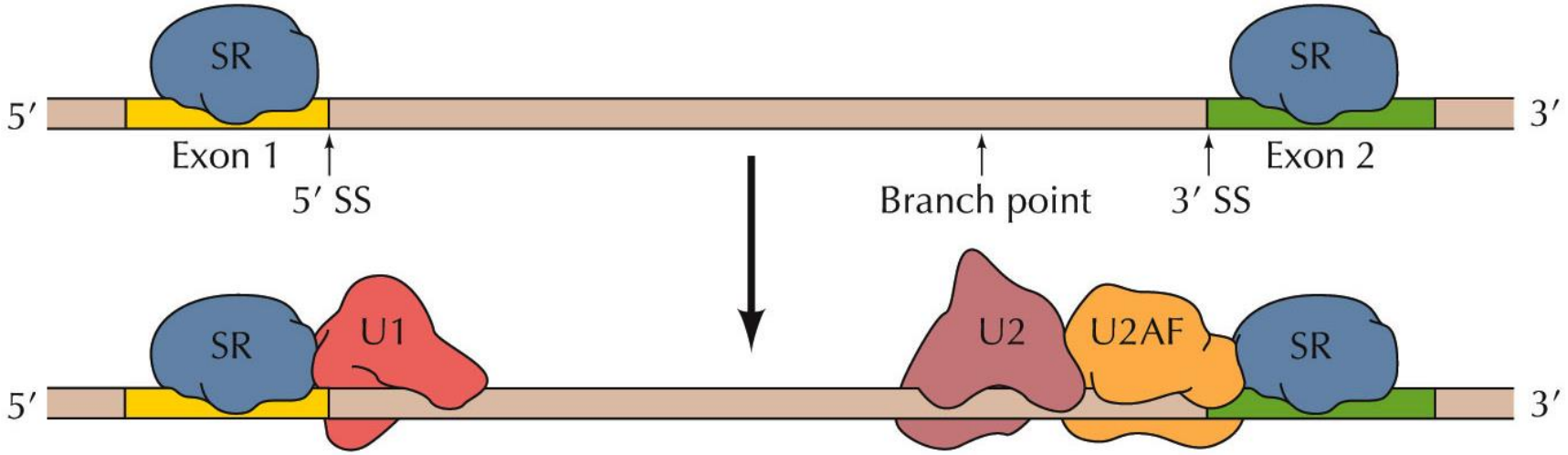


Figure 7.52 Role of splicing factors in spliceosome assembly

Splicing factors; snRNP 성분은 아니지만 spliceosome assembly에 중요한 역할 수행

- Exon의 특정 RNA 서열을 인식하여 결합하고 단백질간 상호작용을 통하여 U1 & U2 snRNPs를 pre-mRNA의 적절한 위치에 결합하도록 유도
- RNA Pol II의 phosphorylated CTD와 결합하여 transcription과 splicing을 연계시킴

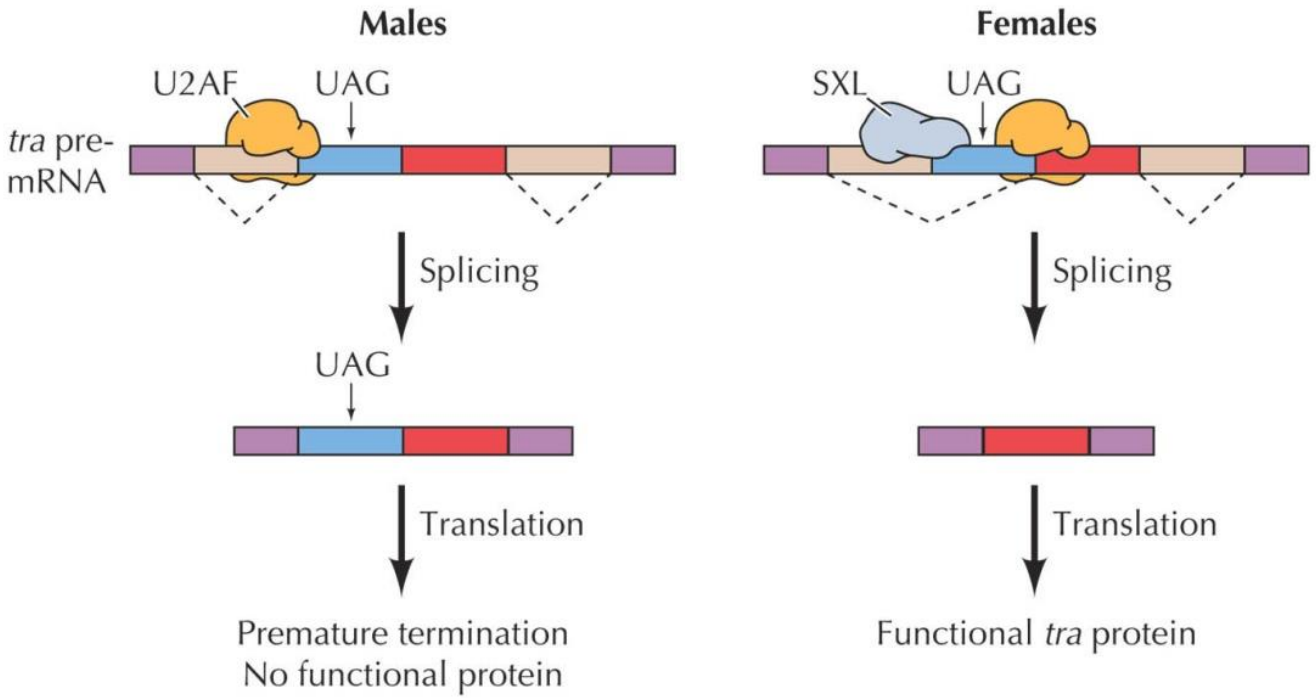
→ splicing이 순서대로 일어나도록 함



Alternative splicing (선택적 스프라이싱)

Different mRNAs are produced from the same gene by different combinations of 5’ and 3’ splice sites

→ tissue-specific and developmental regulation of gene expression



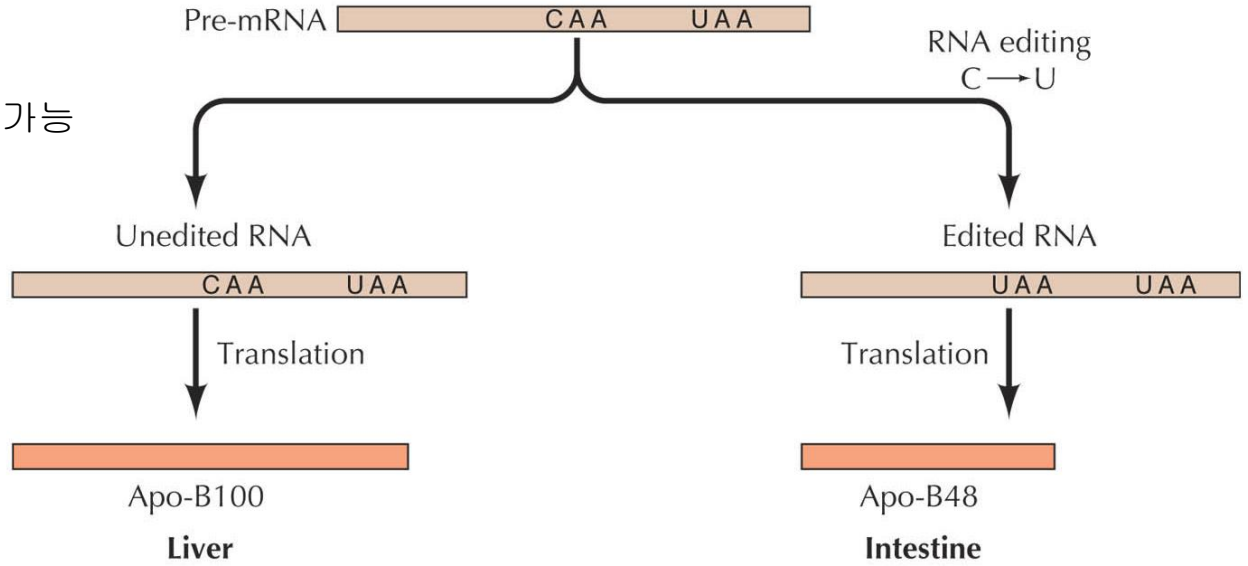
Alternative Splicing in *Drosophila* Sex Determination

RNA editing (RNA 편집)

RNA processing events (other than splicing) that alter the protein-coding sequences of some mRNA

- Addition or removal of U
- Deamination of C to U
- Deamination of A to I

  * I는 U, C, A와 base-pairing 가능



Editing of Apolipoprotein B mRNA

RNA degradation

- **Non-sense-mediated mRNA decay (quality-control system):** degradation of mRNA that lack complete open-reading frames
- **Differential stability of mRNAs in cytoplasm**: half-lives from less than 30 minutes to approx. 20 hours

-- Unstable mRNAs often contain specific AU-rich sequences near their 3' ends that appear to signal rapid degradation by promoting degradation

-- The stability of some mRNAs can be regulated in response to extracellular signals