Post-transcriptional Modifications

Post-transcriptional Processing of Primary Transcripts in Prokaryotes & Eukaryotes

Post-transcriptional Processing

Involves phosphodiester bond cleavage and loss of certain nucleotides

Precursor Transcripts

In Prokaryotes

Extra bases

In Eukaryotes

Introns

Processing involves phosphodiester bond cleavage and loss of certain nucleotides

Processing in Prokaryotes

Processing involves different Ribonucleases

Processing involves two types of enzymes that can cleave phosphodiester bonds in RNA→

1. **Endoribonucleases** – **cleave at internal sites in the RNA, resulting in two smaller RNA**

2. **Exonucleases** – **sequentially remove single nucleotides from one end of the RNA**

Processing Ribonucleases of *E. coli*

PROCESSING

Enzymes	Types	Products	Specificity
1. RNase III	Endo	3'-OH 5'-PO4	specific long ds RNA
2. RNase D	3'→5' exo	5' NMPs	Non-specific but stops at CCA
3. RNase E	Endo	–	specific
4. RNase F	Endo	–	specifically cuts 3' to tRNA like structure
5. RNase P	Endo	3'-OH 5'-PO4	Specifically cuts 5' to tRNA like structure
6. RNase M16	Endo	–	specifically cuts pre 16S to 16S rRNA
7. RNase M23	Endo	–	specific, cuts pre 23S to 23S rRNA
8. RNase M5	Endo	–	specific, cuts pre 5S to 5S rRNA

Processing Ribonucleases of *E. coli* *(contd….)*

DEGRADATION

Enzymes	Types	Products	Specificity
1. RNase I	Endo	3'-PO4 oligos	Non-specific
2. RNase II	3'→5' exo	5'-NMP	Non-specific
3. Polynucleoside phosphorylase	3'→5' exo	5'-NDP	Non-specific
4. RNase H	Endo	–	Non-specific digest RNA only RNA-DNA duplex
5. RNase A	Endo	Py-3'-PO4	specific cuts 3' to Py
6. RNase T1	Endo	G-3'-PO4	specific cuts 3' to G
7. S1 nuclease	Endo	5' NMP	Non-specific cuts ss DNA/ss RNA
8. Bovine spleen phosphodiesterase	5'→3' exo	3' NMP	Non-specific
9. Snake venom phosphodiesterase	3'→5' exo	3' NMP	Non specific

pre-tRNA Processing

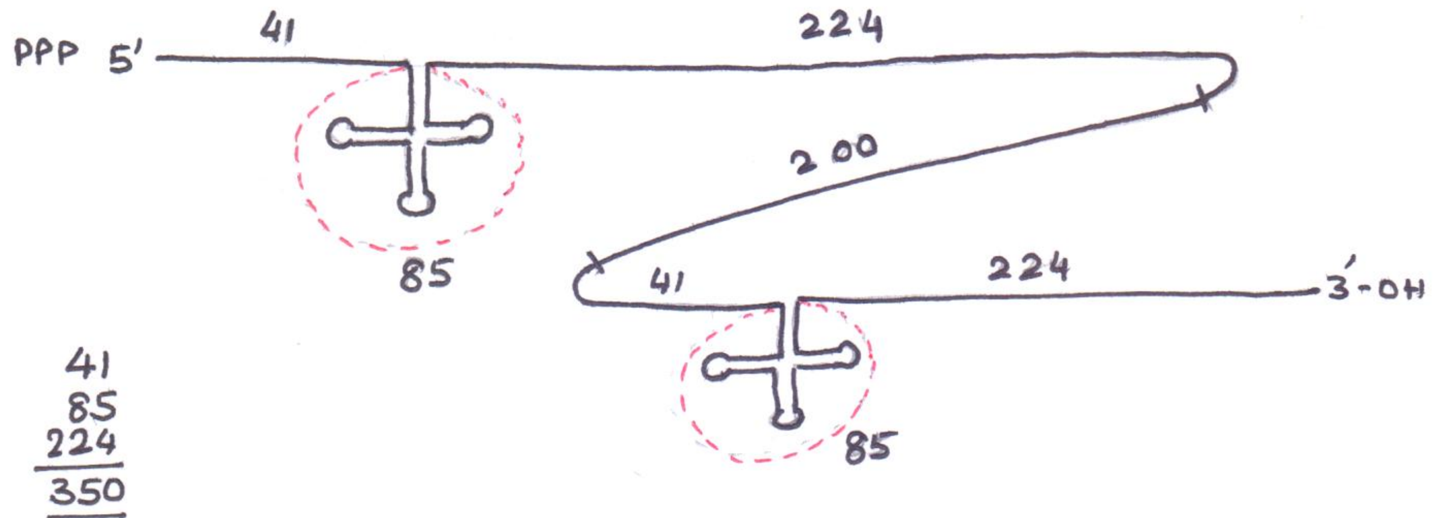
(Prokaryotes)

Processing of pre-tRNA $_1^{tyr}$ transcript in *E. coli*

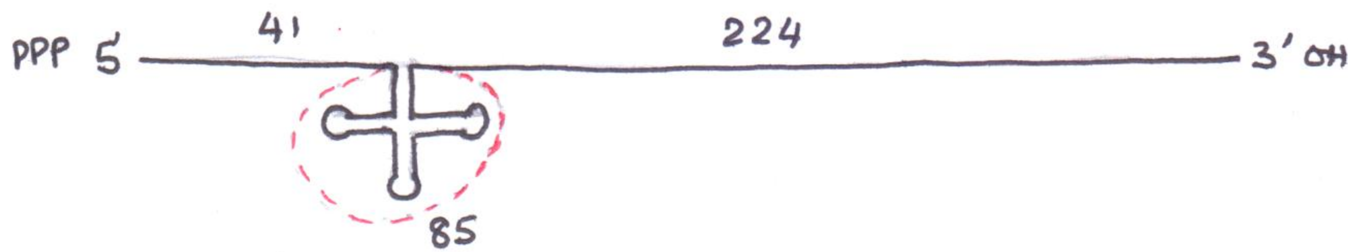
Pre-tRNA $_1^{tyr}$ Transcript in *E. coli*

- In *E. coli*, there are two copies of the pre-tRNA $_1^{tyr}$ gene, i.e., two identical adjuscent copies of the DNA from which this tRNA is transcribed.
- Each gene consists of 350 nucleotide pairs separated by a spacer of 200 nucleotide pairs.

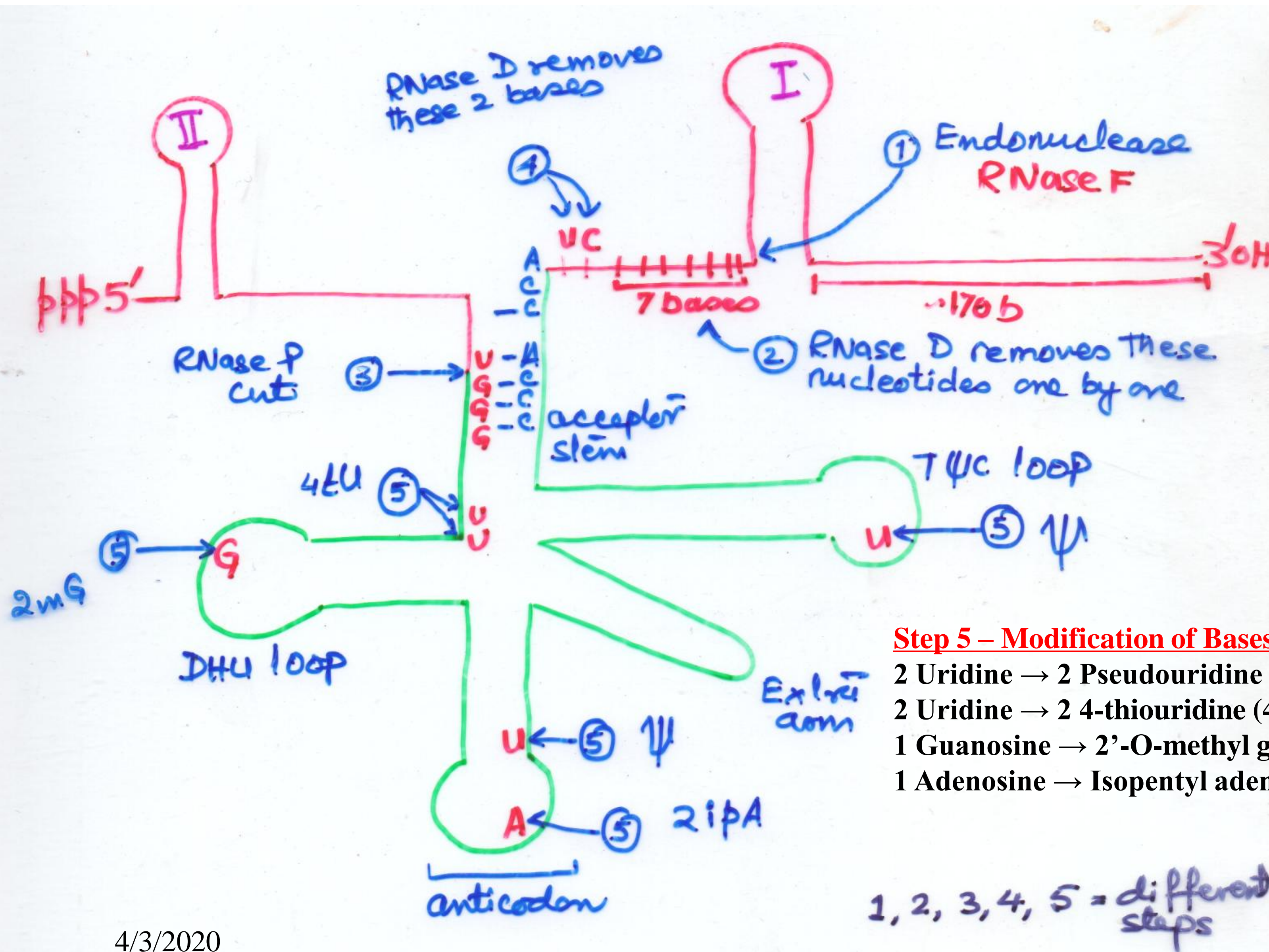
A – The complete transcript with two adjuscent identical tRNA segments and spacer region



B – A single genetic unit



Stages in the processing of pre-tRNA^{tyr} in *E. coli*



Step 5 – Modification of Bases

2 Uridine → 2 Pseudouridine (ψ)
2 Uridine → 2 4-thiouridine (4tU)
1 Guanosine → 2’-O-methyl guanosine (2mG)
1 Adenosine → Isopentyl adenosine (2ipA)

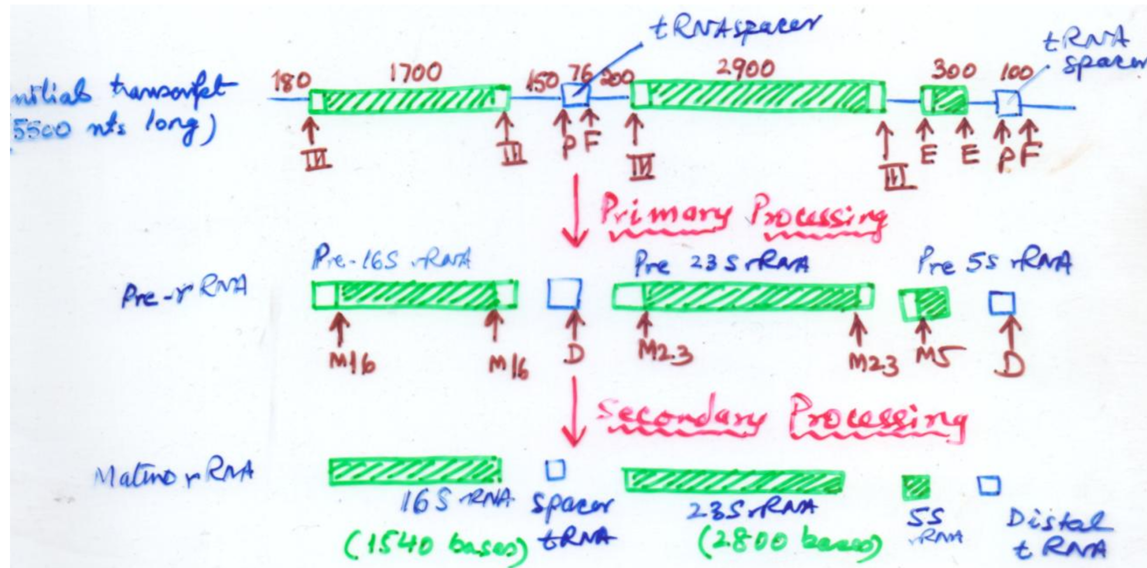
Points to be remembered………

- **All tRNA molecules are terminated by CCA-3'-OH. The precursor contain this sequence, so the terminus is generated at the appropriate cut.**
- **However, precursor of some tRNA molecules lack a terminal CCA.**
- **To such molecules a CCA end is added by the enzyme tRNA nucleotidyl transferase.**
- **Multiple copies of a particular tRNA molecule are commonly found in a single transcription unit.**
- **e.g., 4 copies of $tRNA^{Leu}$ → in one precursor molecule**
- **The occurance of different tRNA in the same precursor is also frequent.**
- **e.g., one $tRNA^{Ser}$ & one $tRNA^{Thr}$ are present in a single transcription unit in *E. coli*.**

pre-rRNA Processing

(Prokaryotes)

Stages in the processing of rRNA in *E. coli*



Steps:

1) Primary Processing events include :-
   1. endonucleolytic actio[n] by RNase III to produ[ce] pre 16S & pre 23S rRNA
   2. then the action of specific ribonucleases to produce tRNA & pre 5S rRNA

2) Secondary Processing events include :- endonucleolytic action of M16, M23 & M5 to generate 16S, 23S & 5S rRNA respectively.

3) Extra bases on the 3' end of the tRNAs are removed by exonuclease- RNase D

Processing in Eukaryotes

Splicing Reaction

(Eukaryotes)

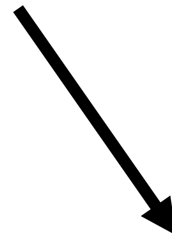
pre-tRNA Splicing

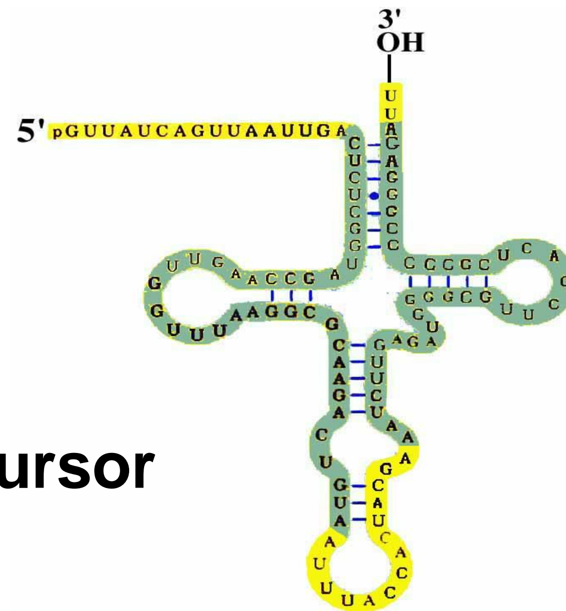
(Eukaryotes)

Precursor transcription

DNA

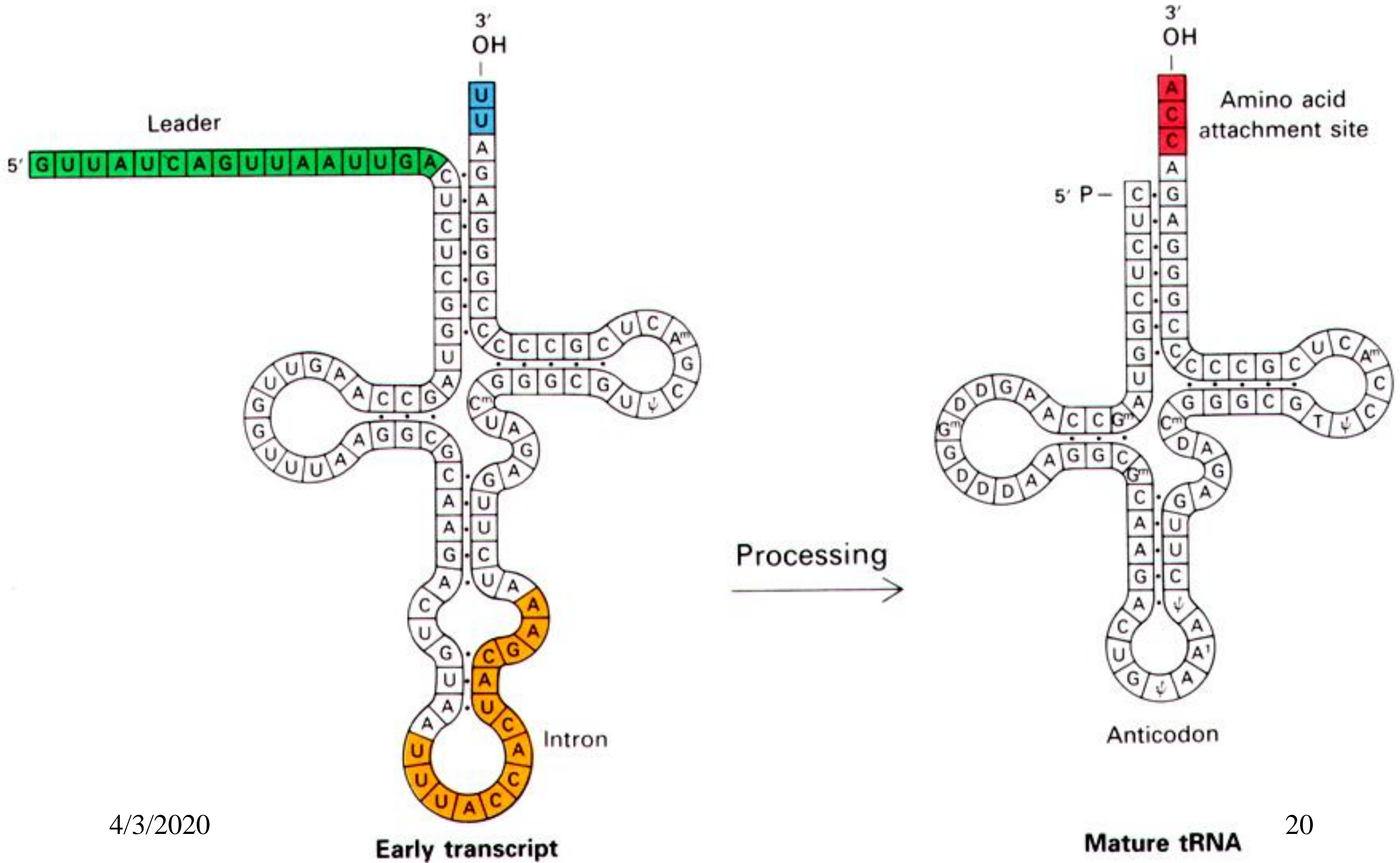
RNA-pol III



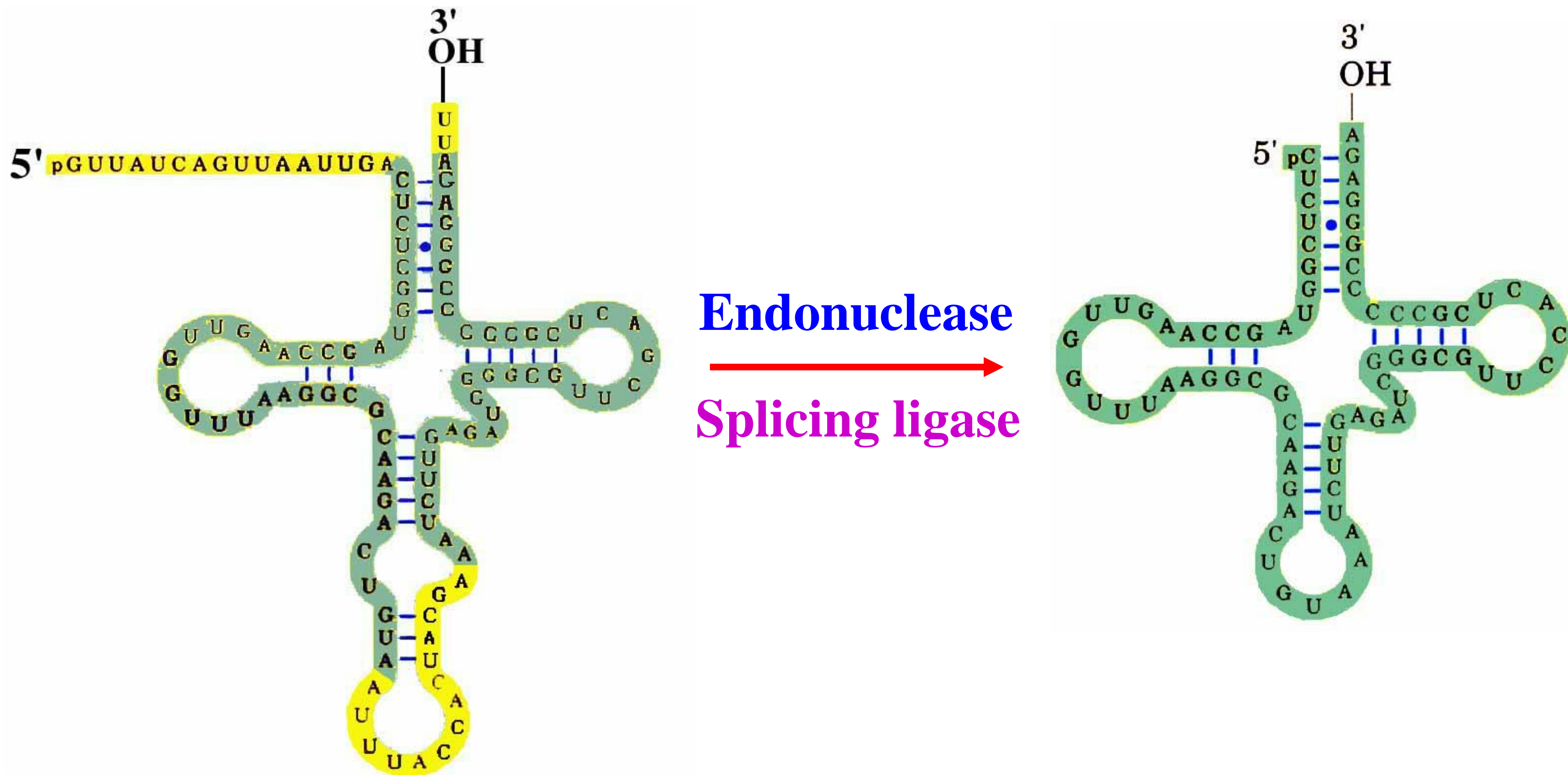


tRNA precursor

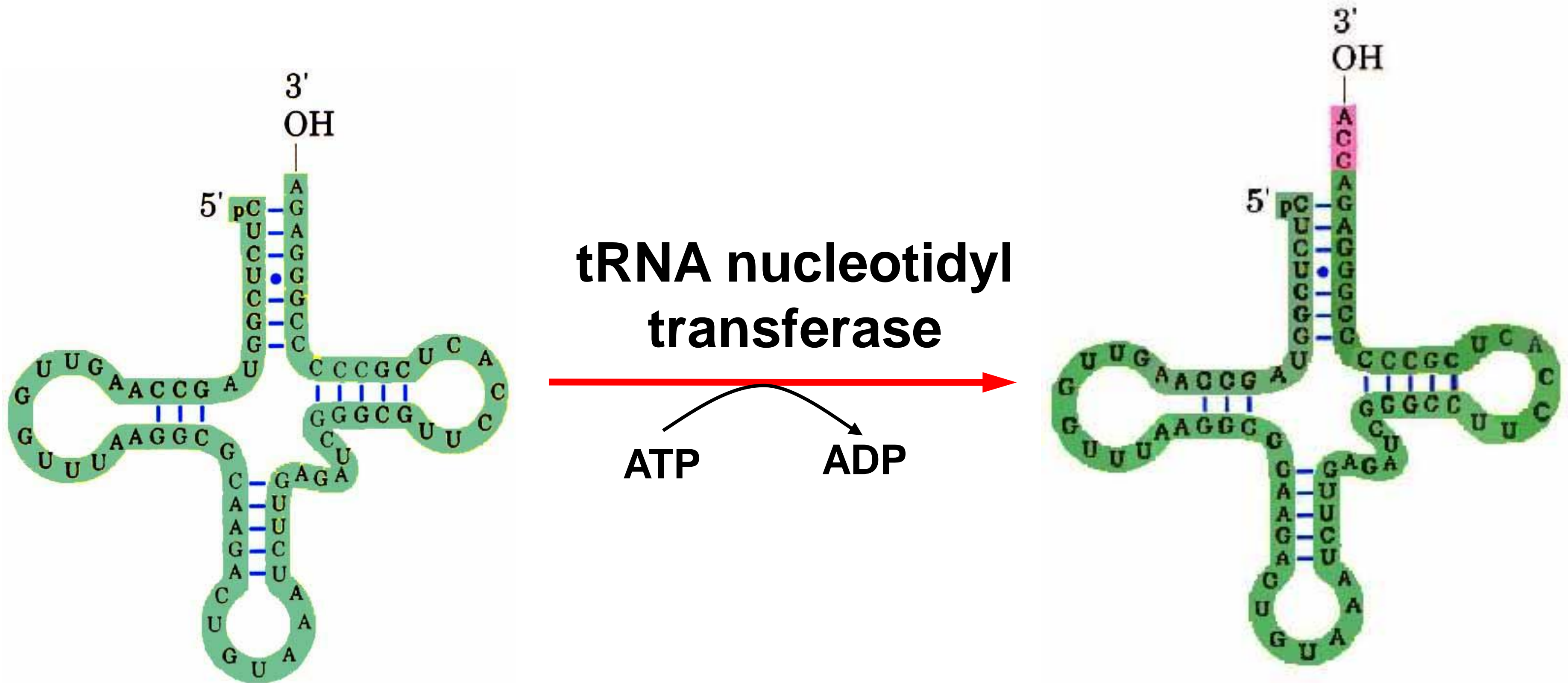
Splicing of tRNA



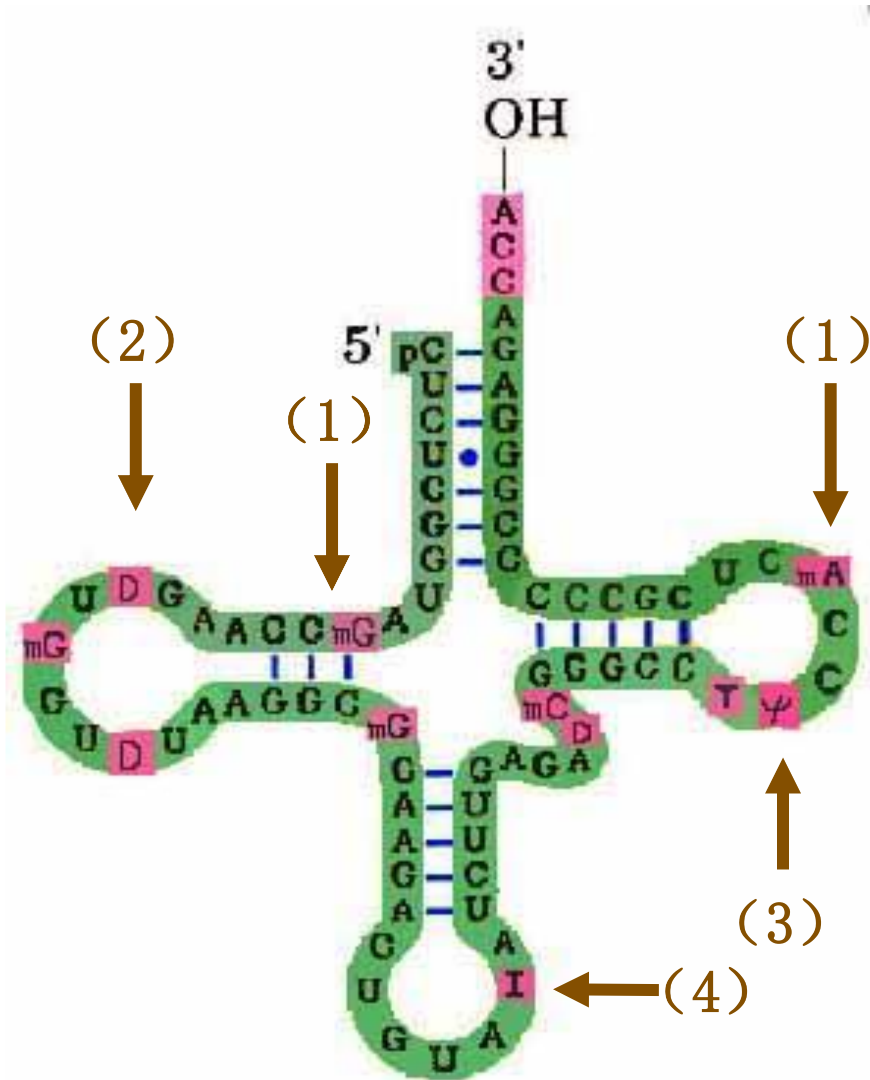
Cleavage



Addition of CCA-OH



Base modification



1. **Methylation**
   A→mA, G→mG
2. **Reduction**
   U→DHU
3. **Transversion**
   U→ψ
4. **Deamination**
   A→I

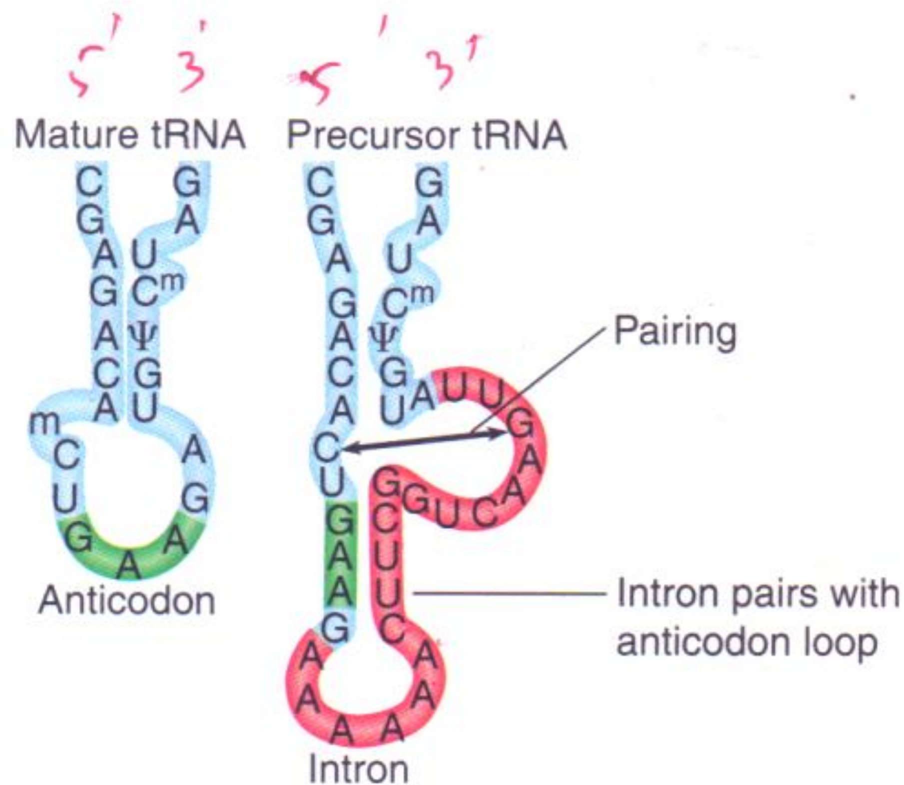


FIGURE 21.34 The intron in yeast tRNA^{Phe} base pairs with the anticodon to change the structure of the anticodon arm. Pairing between an excluded base in the stem and the intron loop in the precursor may be required for splicing.

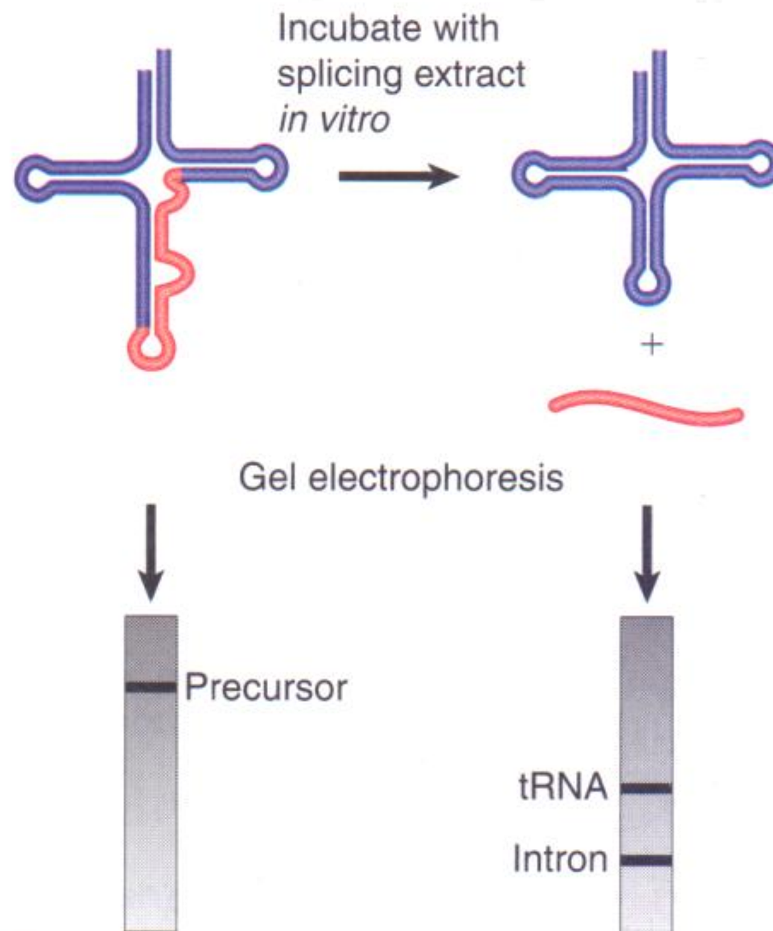


FIGURE 21.35 Splicing of yeast tRNA *in vitro* can be followed by assaying the RNA precursor and products by gel electrophoresis.

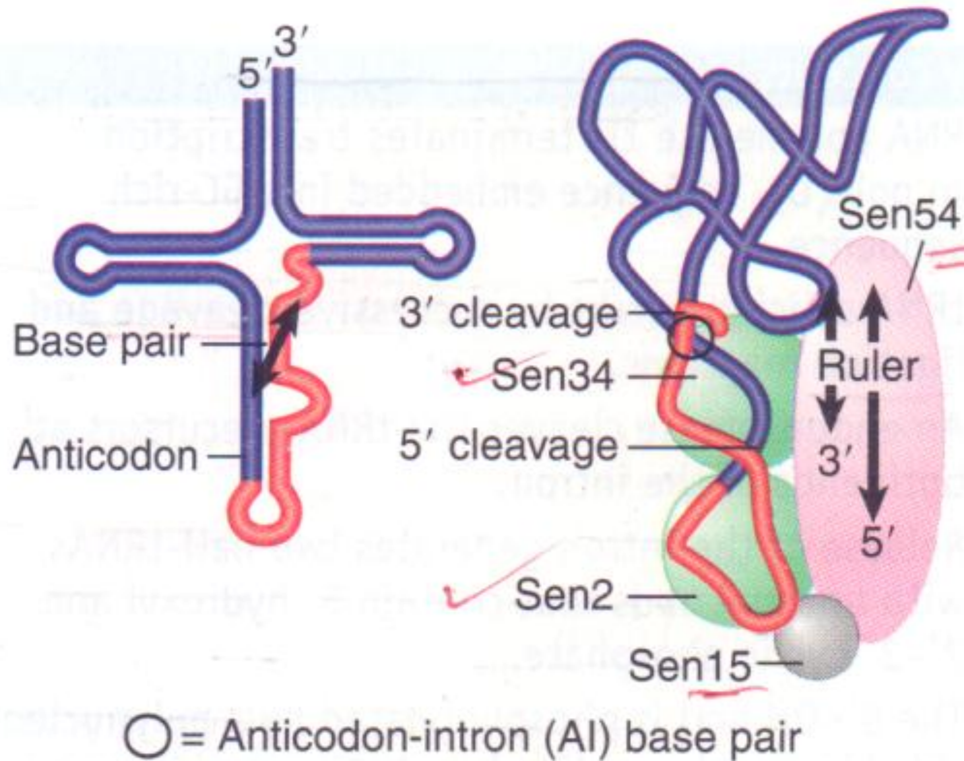


FIGURE 21.36 The 3′ and 5′ cleavages in *S. cerevisiae* pre-tRNA are catalyzed by different subunits of the endonuclease. Another subunit may determine location of the cleavage sites by measuring distance from the mature structure. The AI base pair is also important.

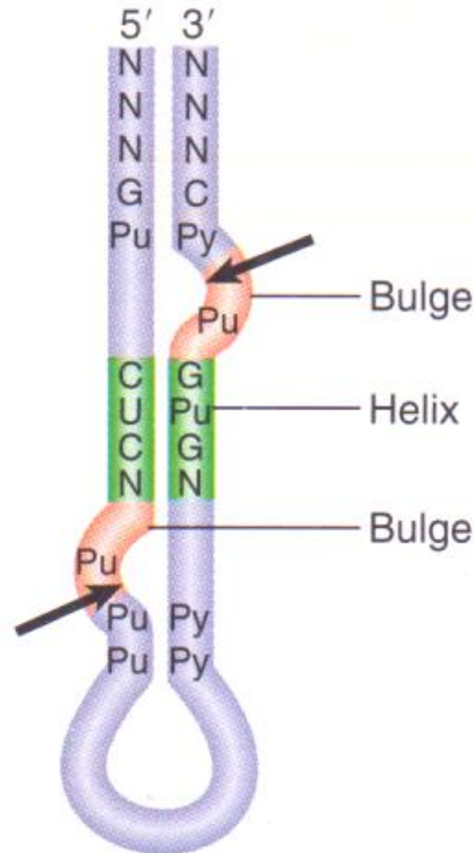
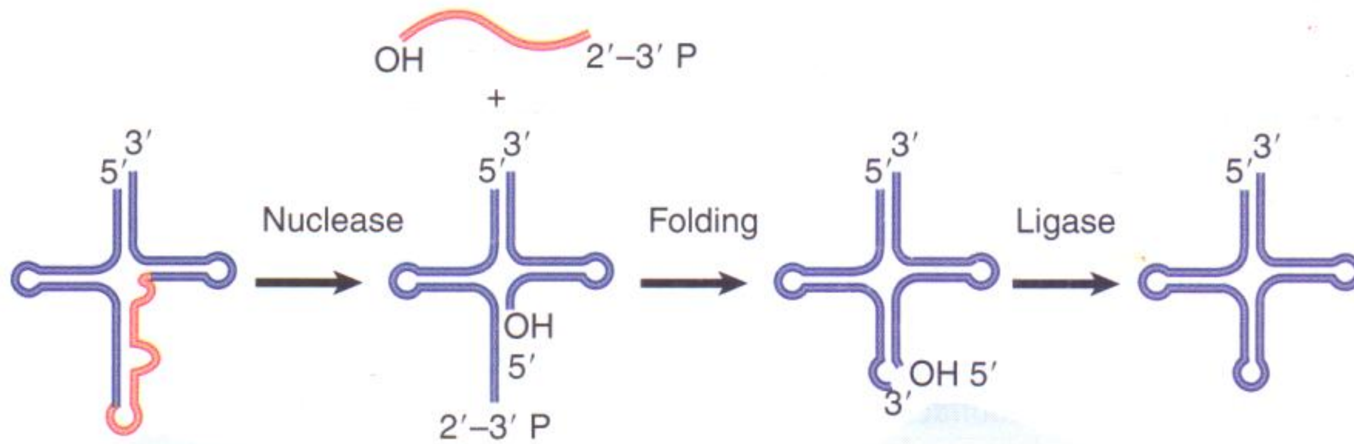
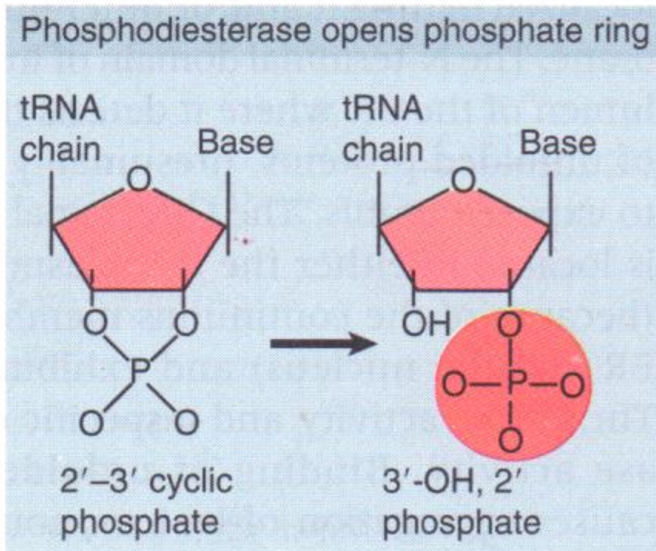


FIGURE 21.37 Archaeal tRNA splicing endonuclease cleaves each strand at a bulge in a bulge-helix-bulge motif.





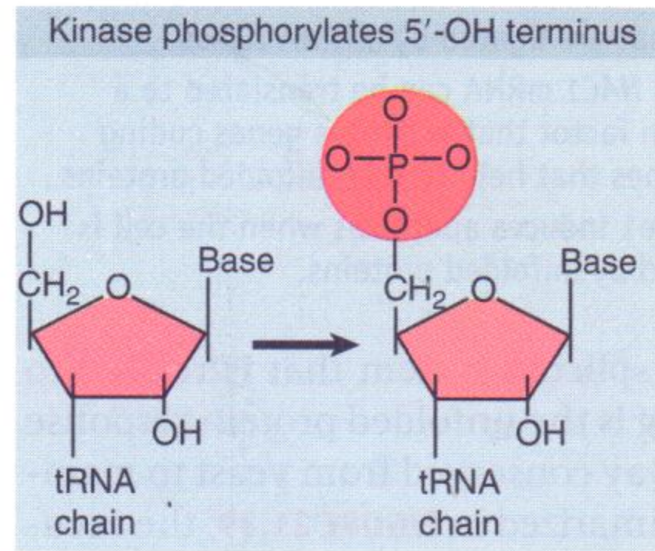
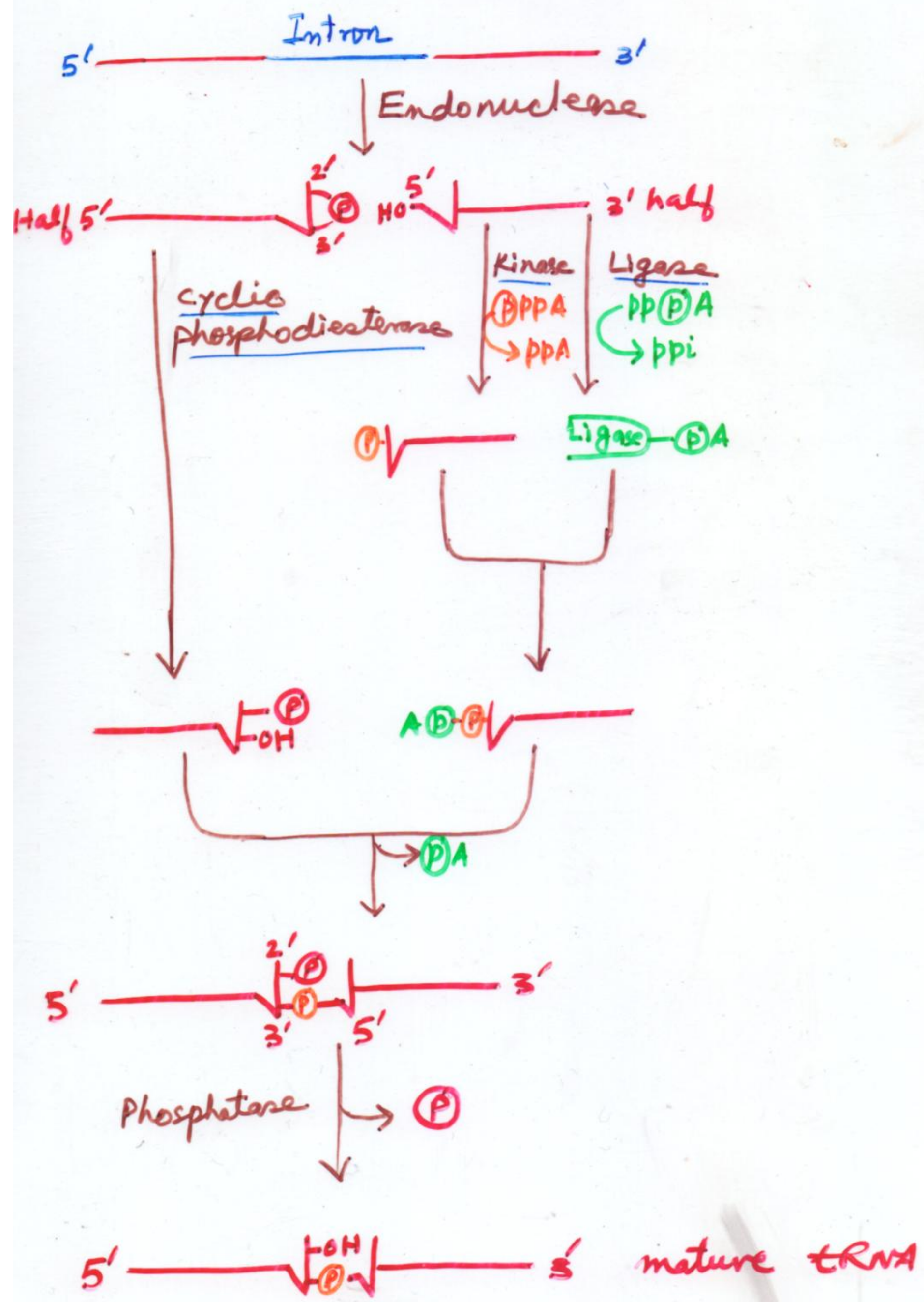


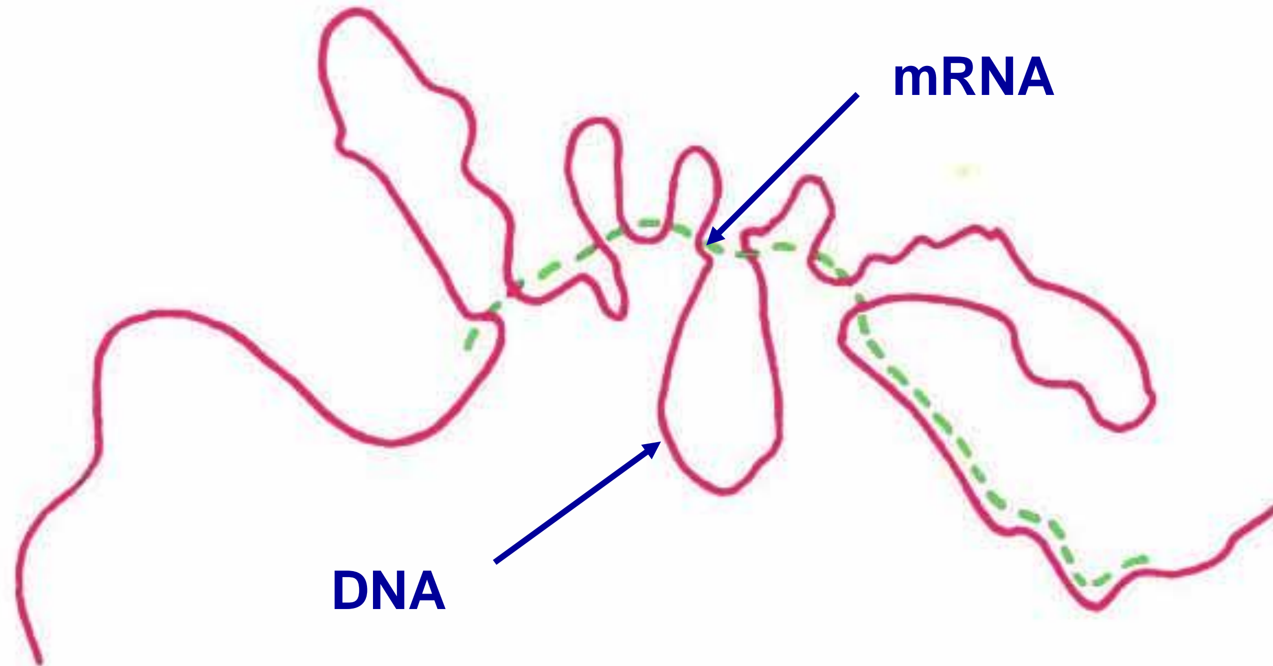
FIGURE 21.38 Splicing of tRNA requires separate nuclease and ligase activities. The exon–intron boundaries are cleaved by the nuclease to generate 2′ to 3′ cyclic phosphate and 5′ OH termini. The cyclic phosphate is opened to generate 3′-OH and 2′ phosphate groups. The 5′-OH is phosphorylated. After 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 of Yeast tRNA
Intron
5′
3′
Endonuclease
2′
5′
HO
3′
Half 5′
3′ half
Kinase
Ligase
PPPA
PPA
PPPA
PPi
cyclic phosphodiesterase
P
Ligase
PA
P
OH
APP
PA
2′
P
P
5′
3′
3′
5′
Phosphatase
P
OH
P
5′
3′
mature tRNA

pre-mRNA Splicing

(Eukaryotes)

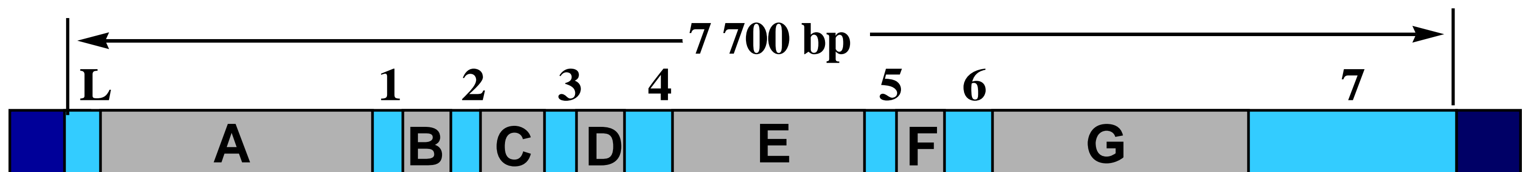
c. mRNA splicing



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 to G are non-coding regions

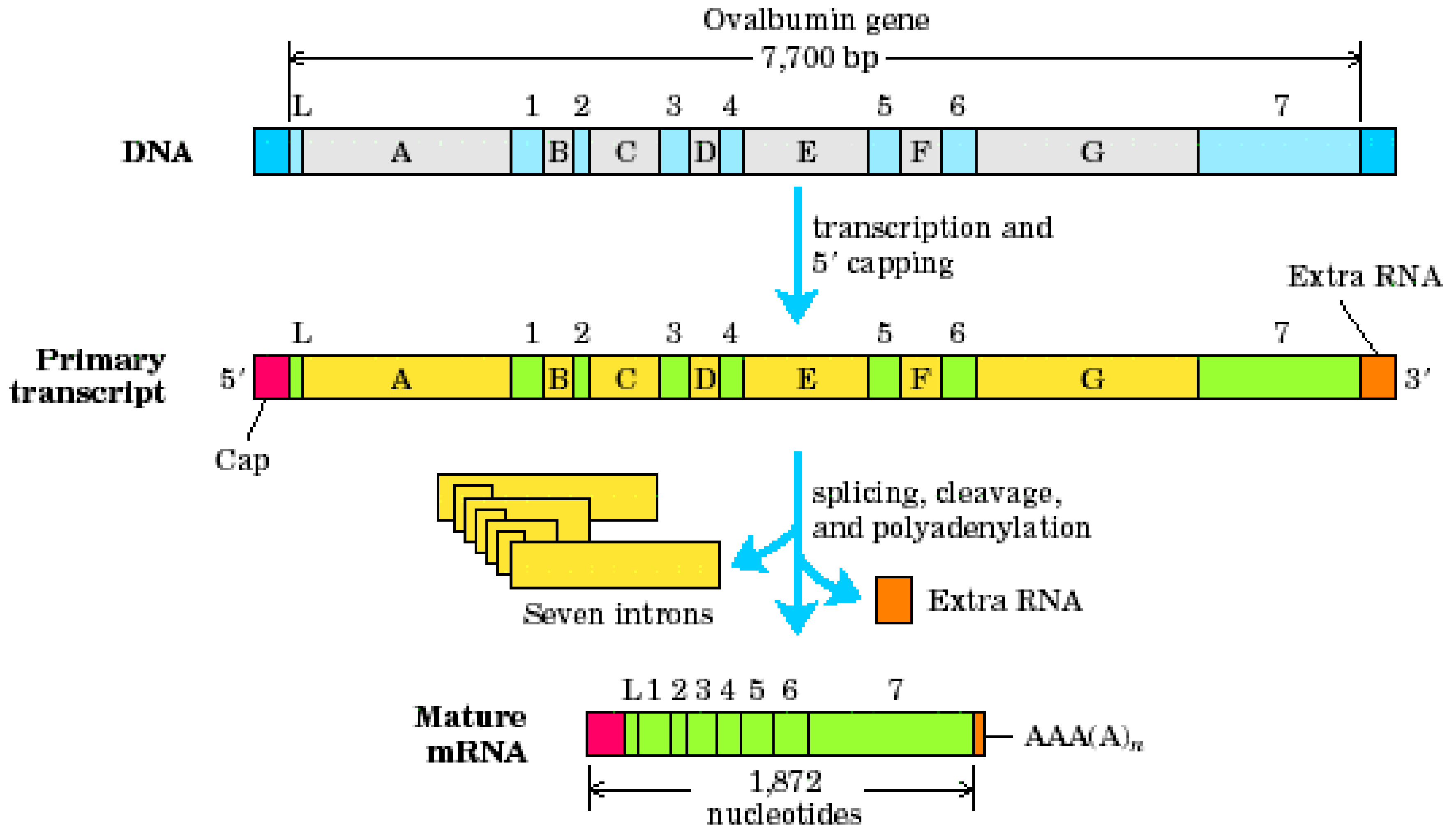
1 to 7 are coding regions

Exon and intron

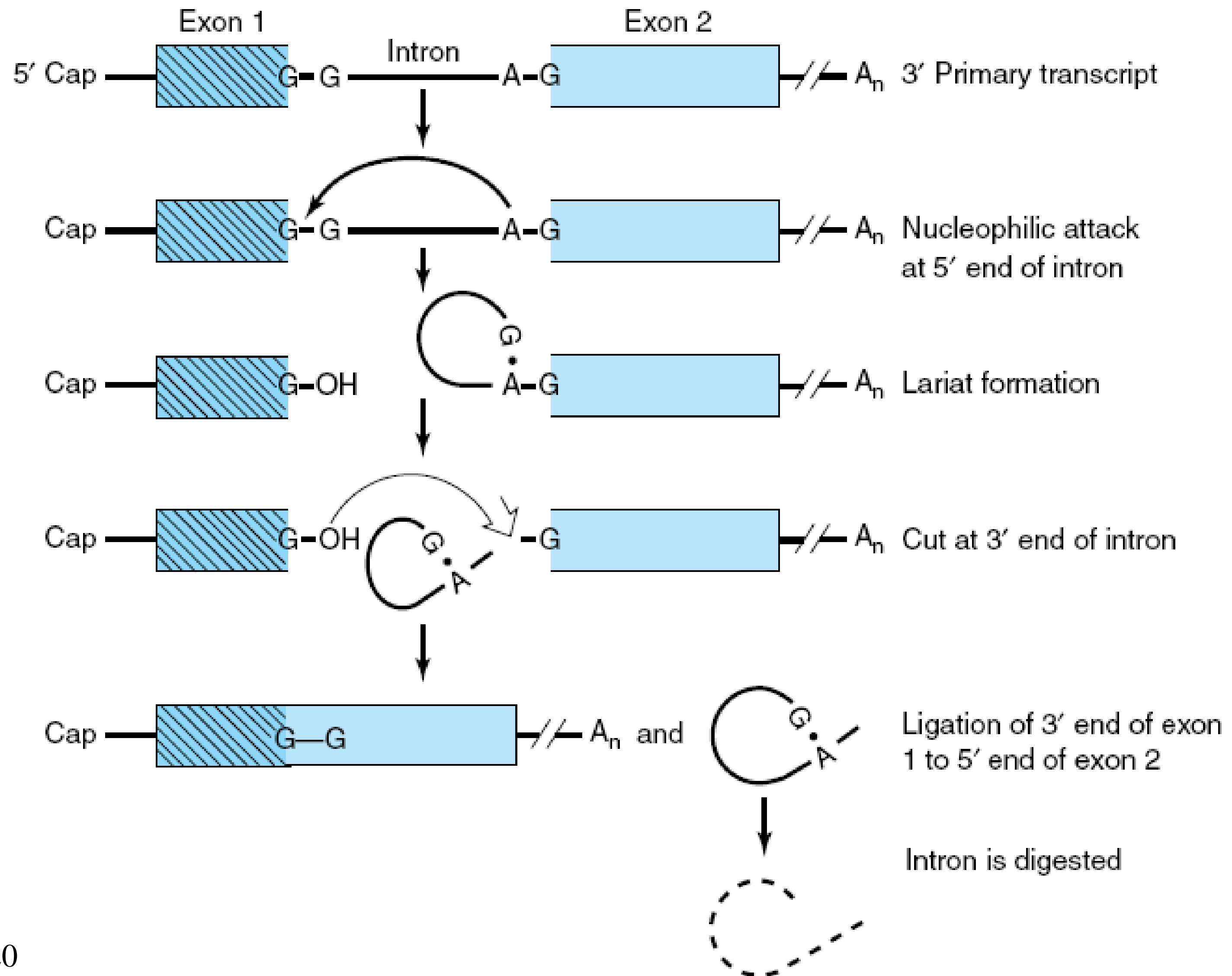
Exons are the coding sequences that appear on split genes and primary transcripts, and will be expressed to matured mRNA.

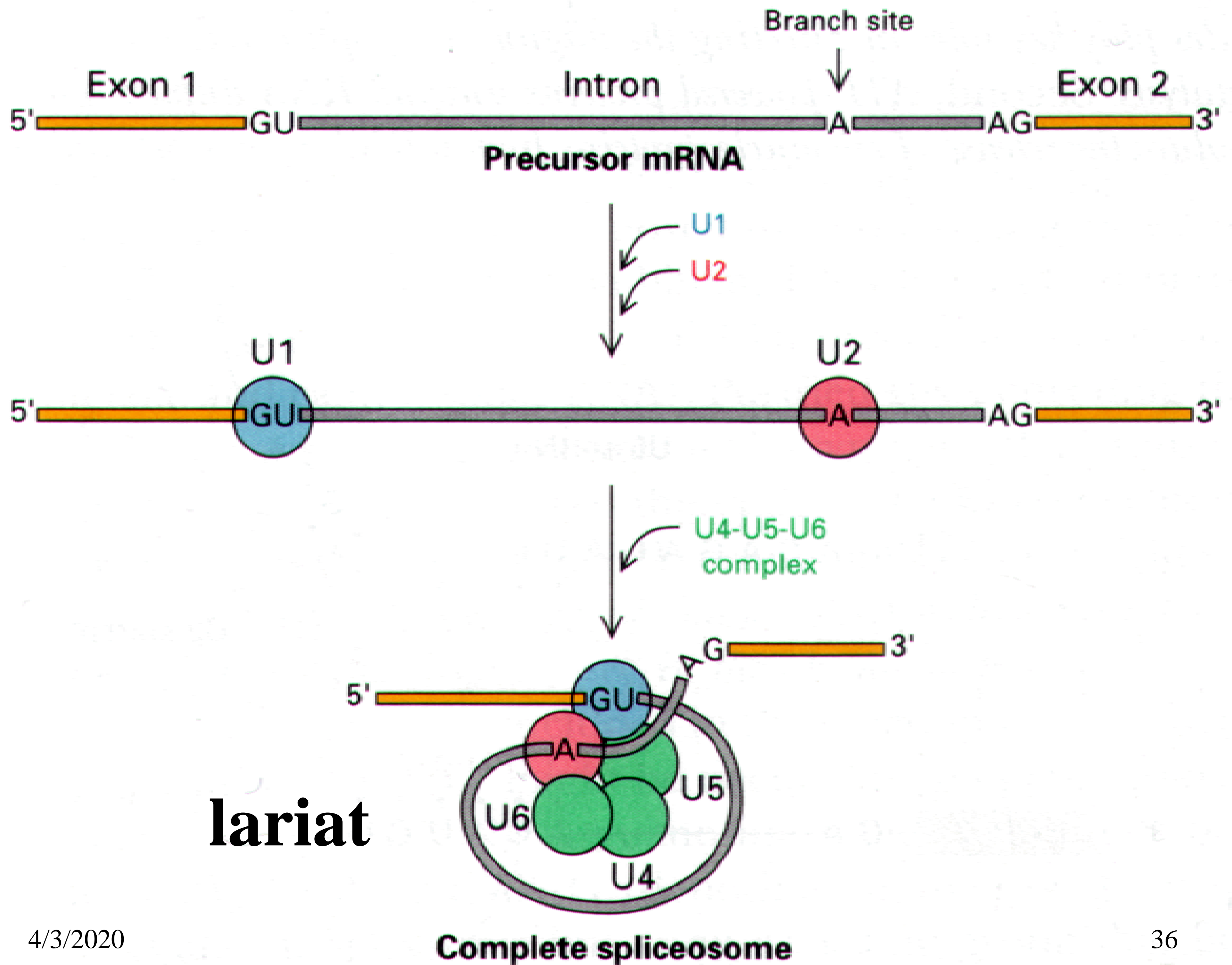
Introns are the non-coding sequences that are transcripted into primary mRNAs, and will be cleaved out in the later splicing process.

mRNA splicing

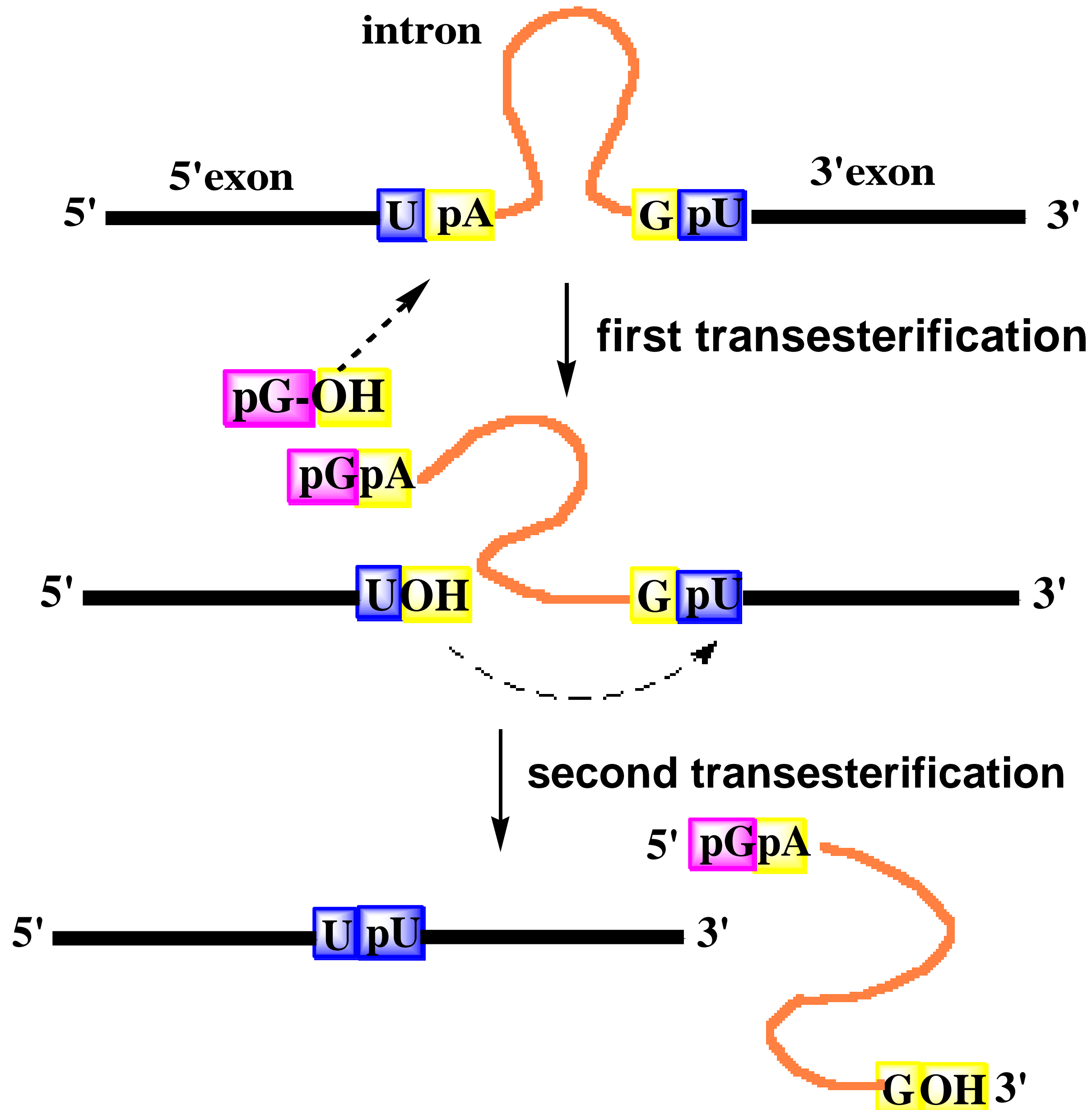


Splicing mechanism




Branch site
Exon 1
Intron
Exon 2
5'
GU
A
AG
3'
Precursor mRNA
U1
U2
U1
U2
5'
GU
A
AG
3'
U4-U5-U6
complex
AG
3'
5'
GU
A
U5
U6
U4
lariat
Complete spliceosome

Twice transesterification

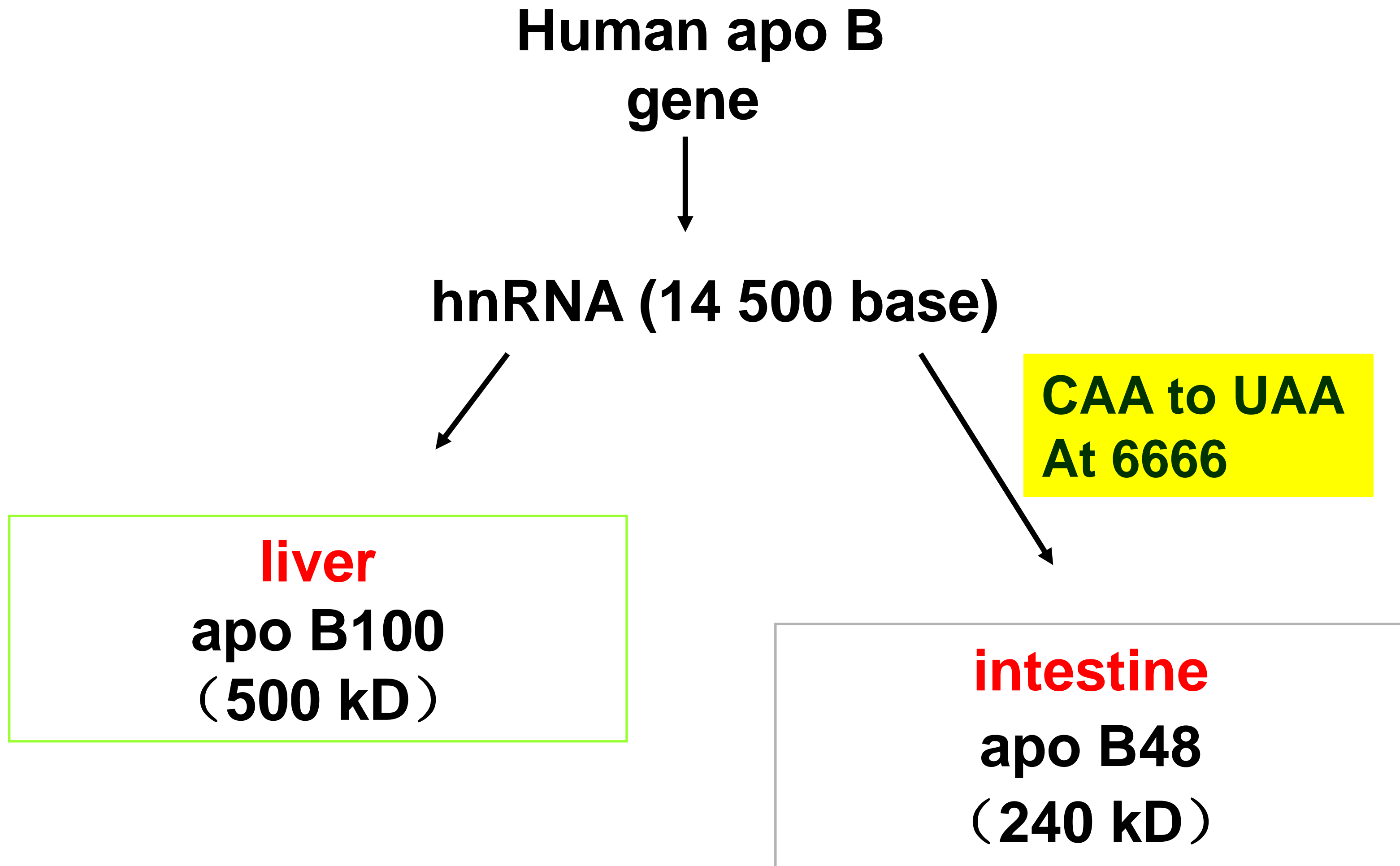


mRNA Editing

mRNA Editing

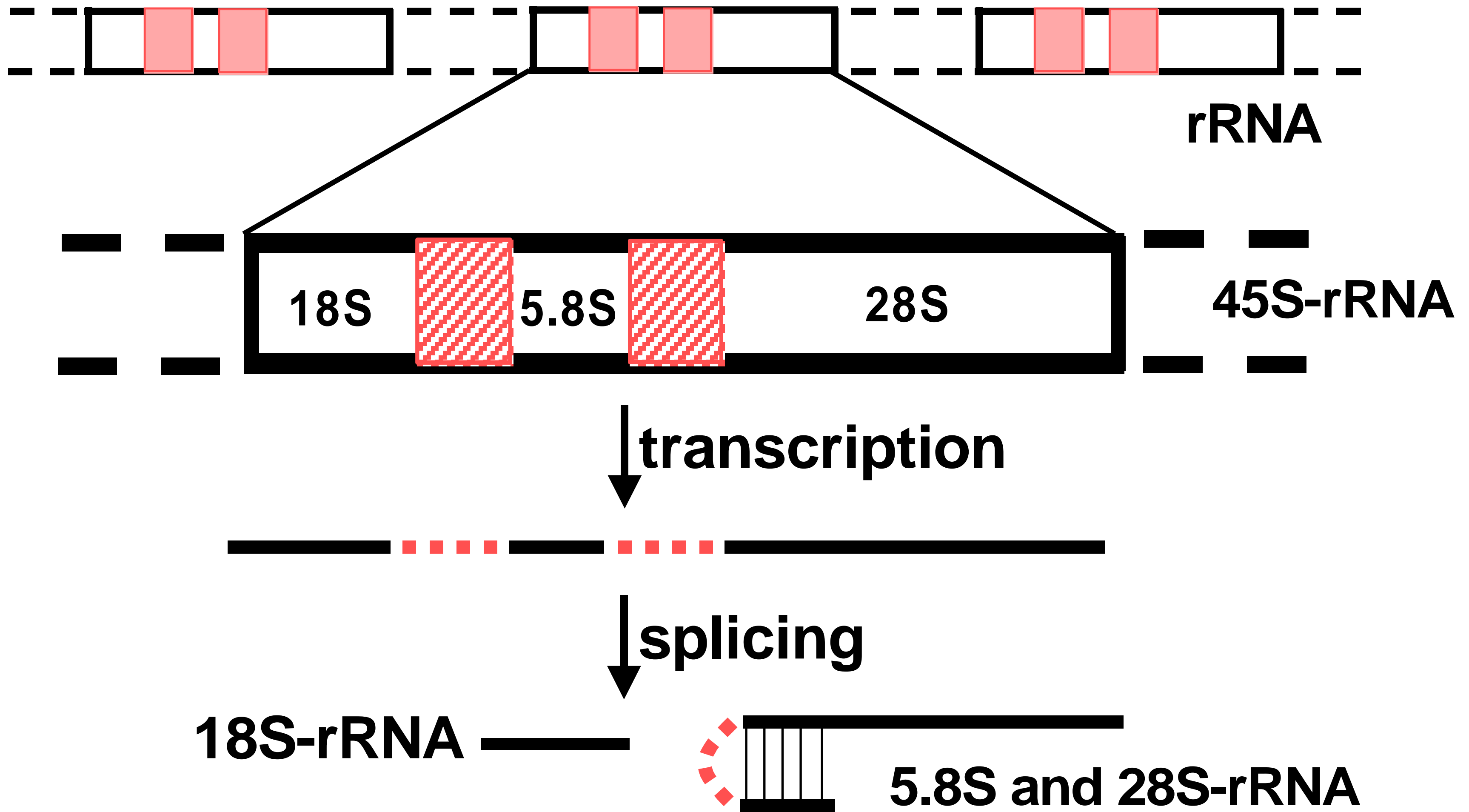
- **Taking place at the transcription level**
- **One gene responsible for more than one proteins**
- **Significance: gene sequences, after post-transcriptional modification, can be multiple purpose differentiation.**

Different pathway of apo B



Modification of rRNA

- **45S transcript in nucleus is the precursor of 3 kinds of rRNAs.**
- **The matured rRNA will be assembled with ribosomal proteins to form ribosomes that are exported to cytosolic space.**

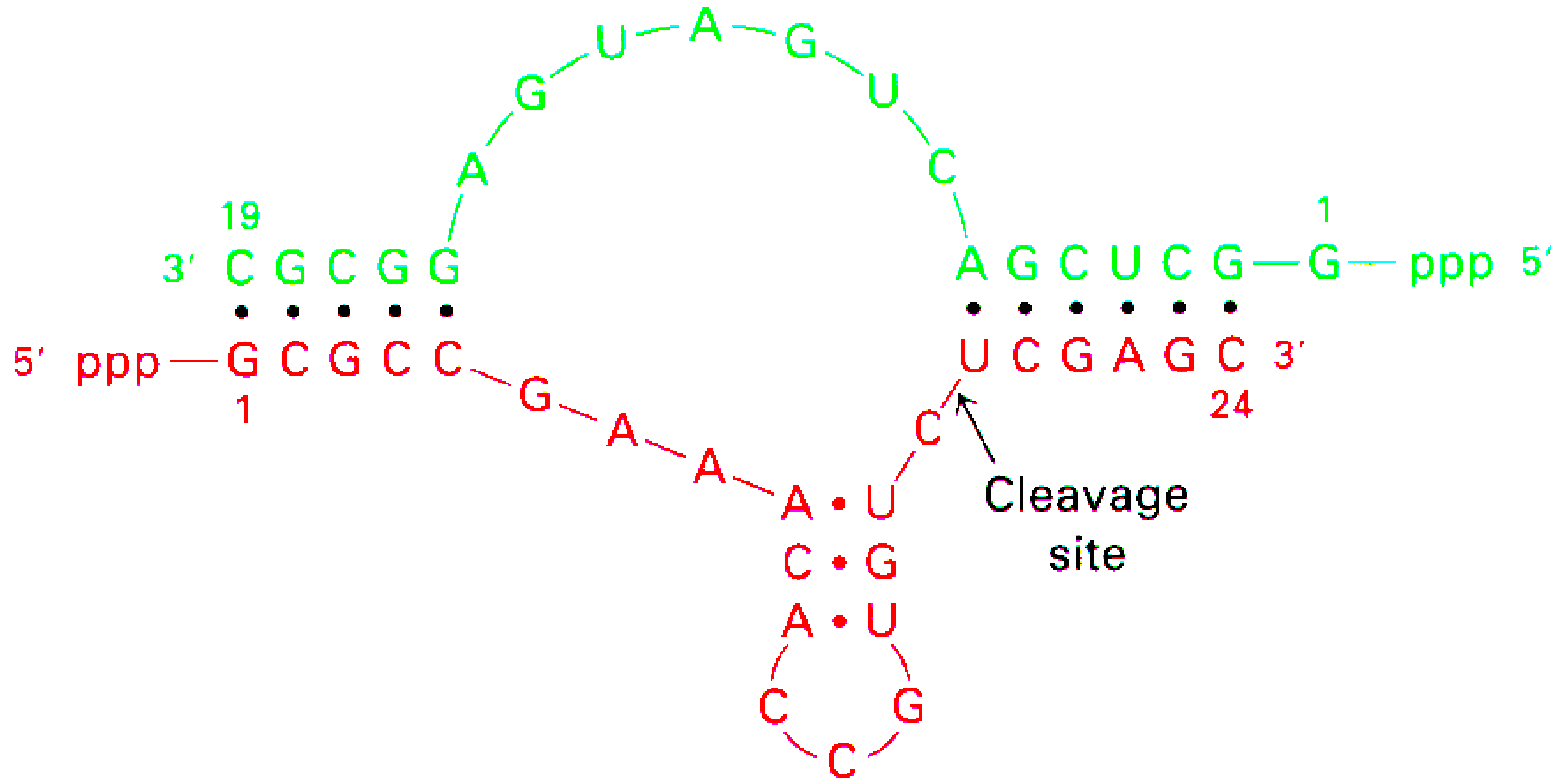

rRNA
18S
5.8S
28S
45S-rRNA
transcription
splicing
18S-rRNA
5.8S and 28S-rRNA

Ribozyme

- The rRNA precursor of tetrahymena has the activity of self-splicing (1982).
- The catalytic RNA is called ribozyme.
- Self-splicing happened often for intron I and intron II.

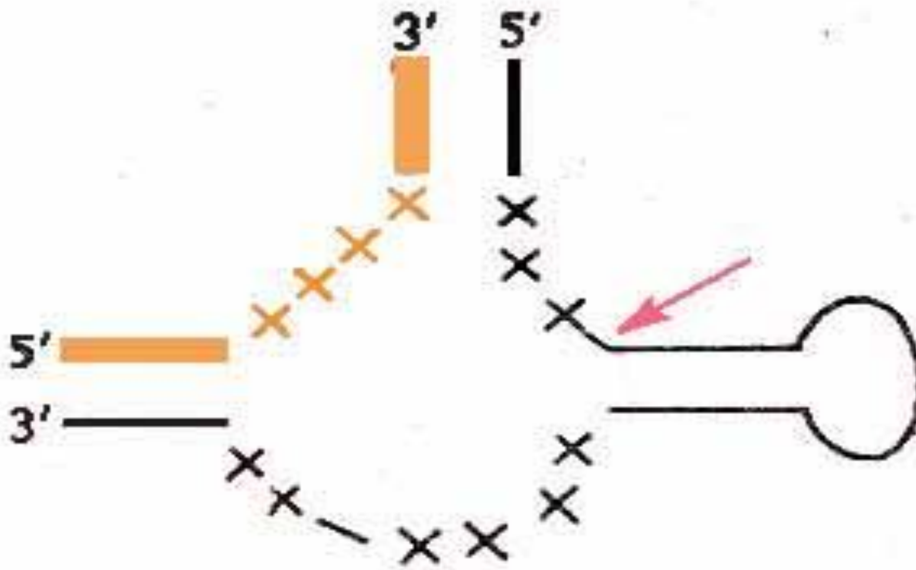
- **Both the catalytic domain and the substrate locate on the same molecule, and form a hammer-head structure.**
- **At least 13 nucleotides are conserved.**

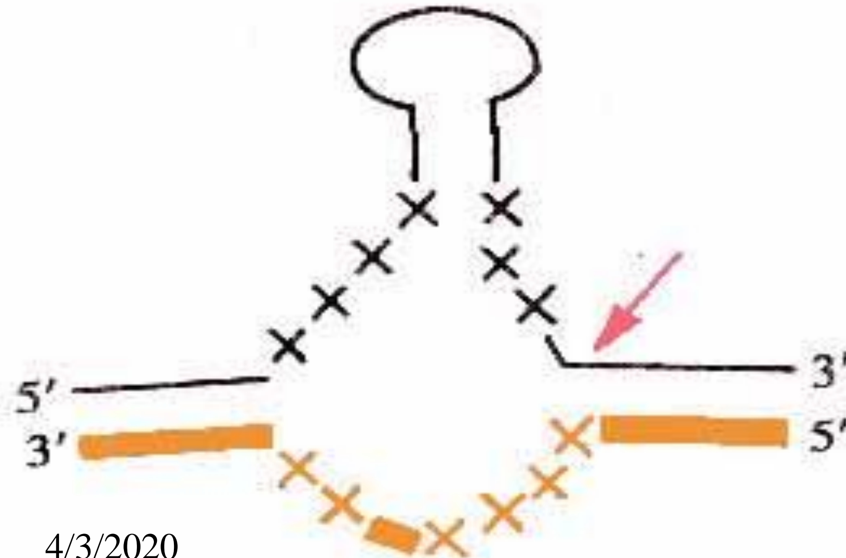
Hammer-head



Significance of ribozyme

- **Be a supplement to the central dogma**
- **Redefine the enzymology**
- **Provide a new insights for the origin of life**
- **Be useful in designing the artificial ribozymes as the therapeutical agents**





Artificial ribozyme

- **Thick lines: artificial ribozyme**
- **Thin lines: natural ribozyme**
- **X: consensus sequence**
- **Arrow: cleavage point**