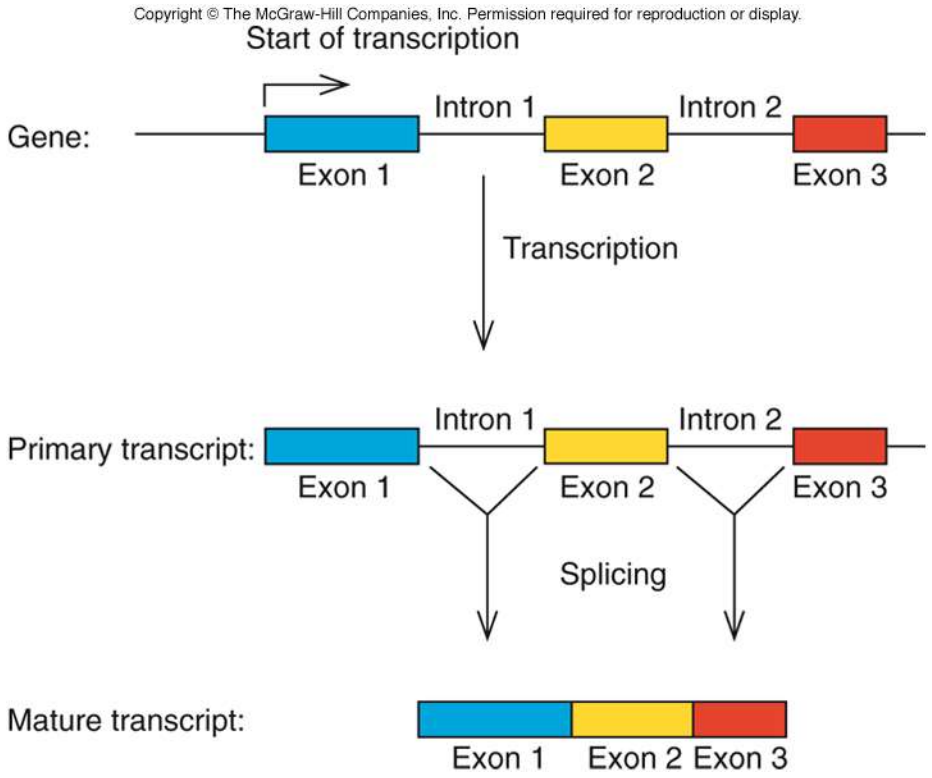
**SOS in Biochemistry, Jiwaji University, Gwalior**
**M.Sc. II Semester (2019-20)**
**Paper BCH 201: Fundamentals of Molecular Biology (Unit IV)**

# Self Splicing

## (pre-rRNA Splicing)

# 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)

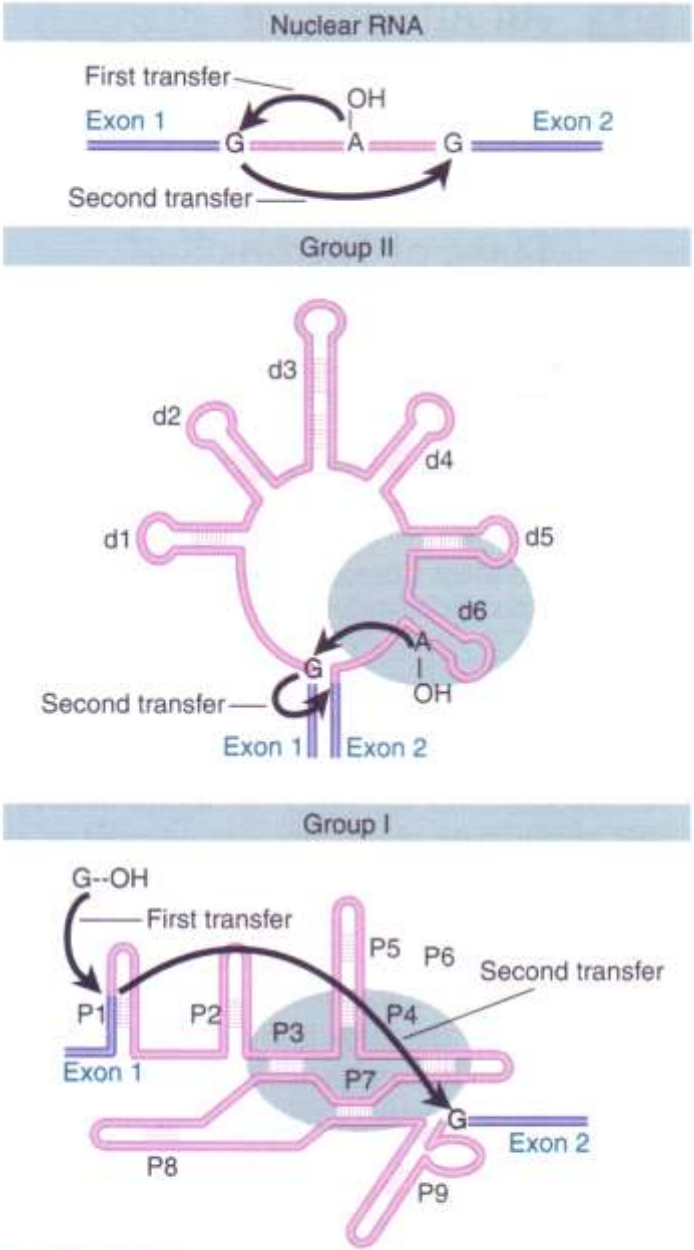



**FIGURE 21.15** Three classes of splicing reactions proceed by two transesterifications. First, a free OH group attacks the exon 1–intron junction. Second, the OH created at the end of exon 1 attacks the intron–exon 2 junction.

# Self Splicing

# NOBEL PRIZE – 1989

## Chemistry

**For the discovery of**

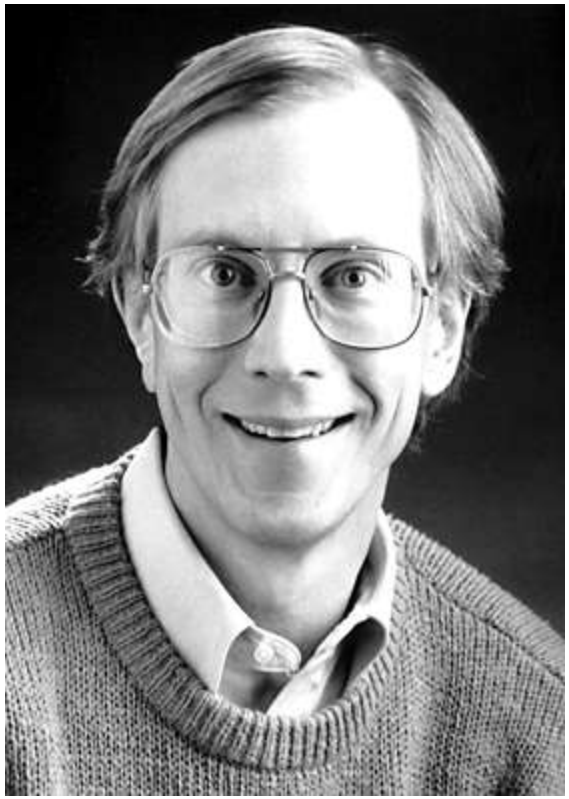
# Catalytic Properties of RNA

**The Nobel Prize was shared by two American Scientists:**

1. **Prof. Sidney Altman, Yale University, New Haven, CT, USA (1/2)**
2. **Thomas R. Cech, University of Colorado, Boulder, CO, USA (1/2)**

## Sidney Altman

**Born:** 7 May 1939, Montreal, Canada
**Affiliation at the time of the award:** Yale University, New Haven, CT, USA
**Field:** Biochemistry
**Prize share:** 1/2



## Thomas R. Cech

**Born:** 8 December 1947, Chicago, IL, USA
**Affiliation at the time of the award:** University of Colorado, Boulder, CO, USA
**Field:** Biochemistry
**Prize share:** 1/2

# Work: Catalytic Properties of RNA

# Self-Splicing RNAs

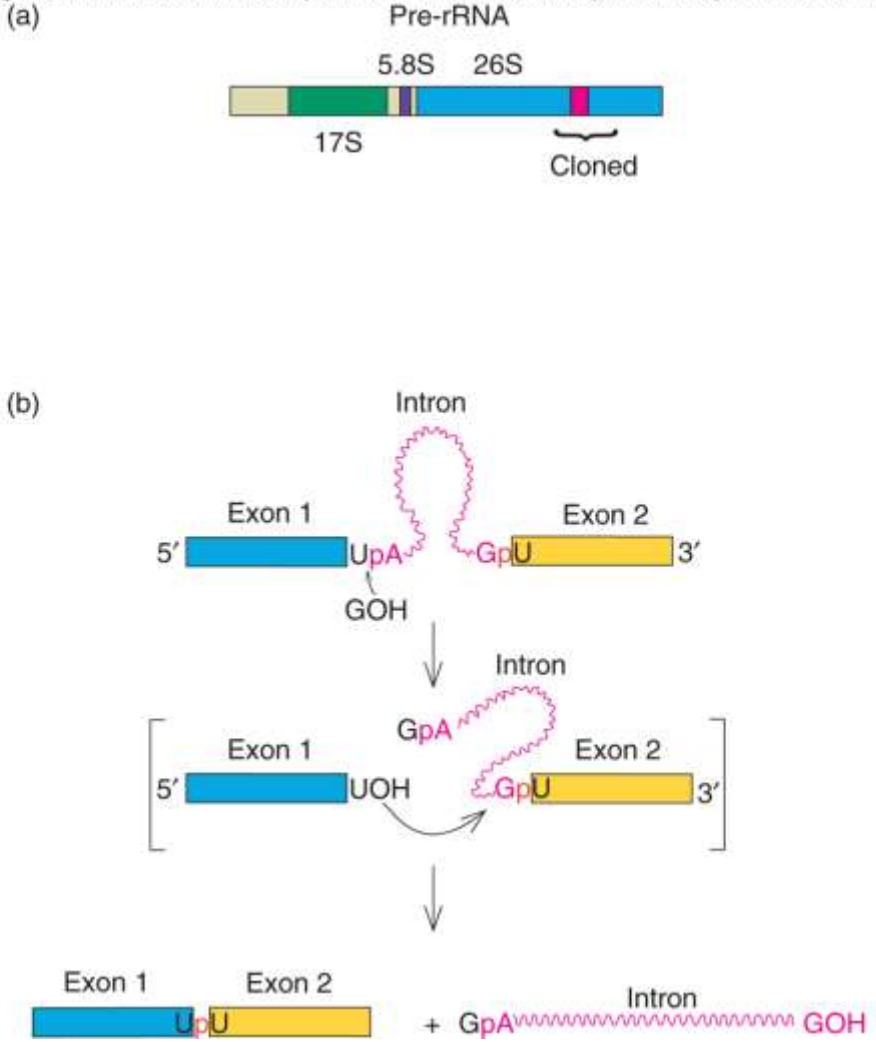
- **Some RNAs could splice themselves without aid from a spliceosome or any other protein**
- ***Tetrahymena* 26S rRNA gene has an intron, splices itself in vitro**
  - **Group I introns are a group of self-splicing RNAs**
  - **Another group, Group II introns also have some self-splicing members**

# Group I Introns

- Group I introns can be removed in vitro with no help from protein
- Reaction begins with attack by a guanine nucleotide on the 5'-splice site
  - Adds G to the 5'-end of the intron
  - Releases the first exon
- Second step, first exon attacks the 3'-splice site
  - Ligates 2 exons together
  - Releases the linear intron
- Intron cyclizes twice, losing nucleotides each time, then linearizes a last time

# Group I Intron: *Tetrahymena* 26S rRNA precursor

Fig. 14.43 



# TRANSESTERIFICATION REACTION

(Transfer of phosphoester)

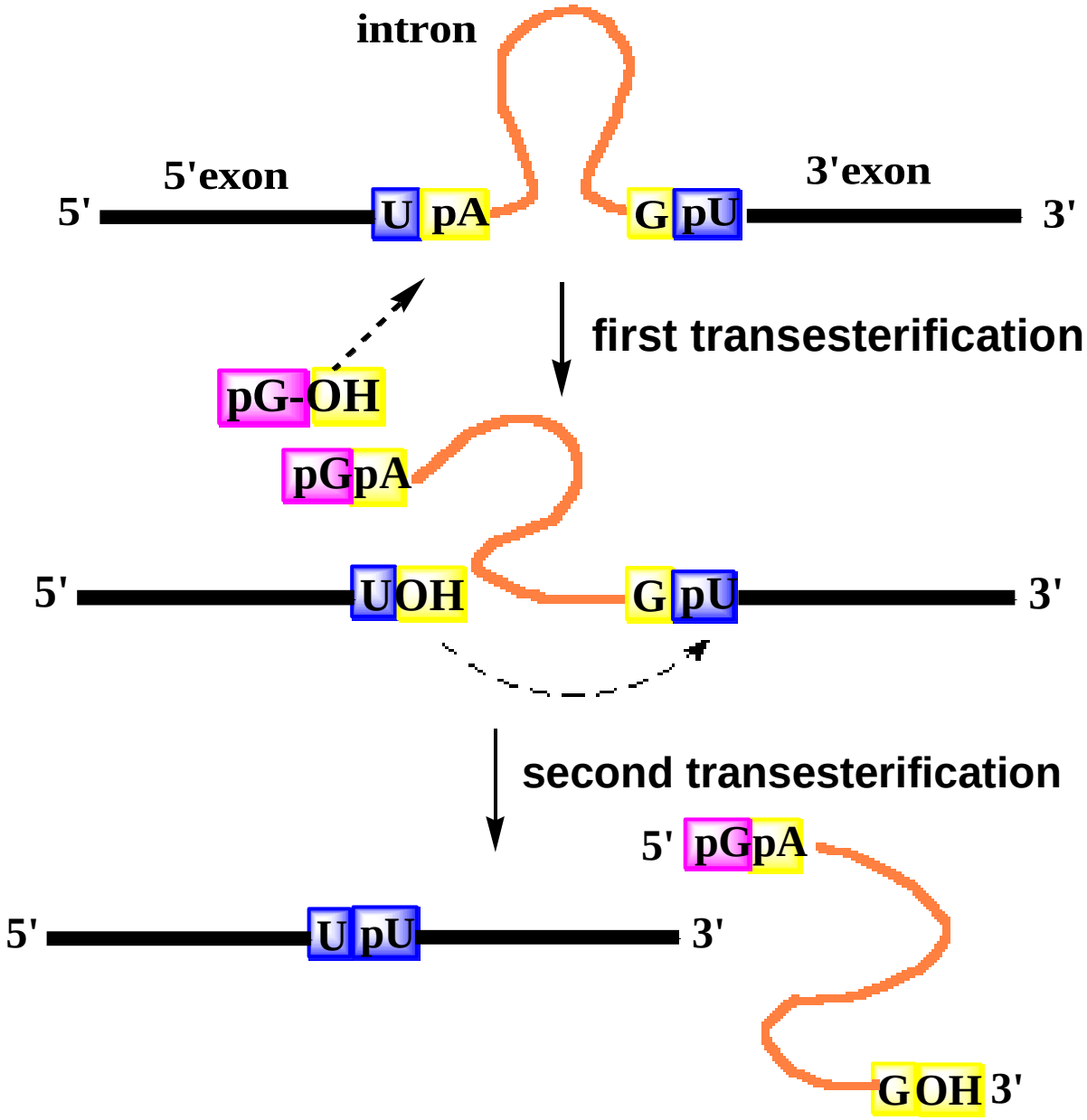
The esters are of phosphate rather than carbon & some of R groups are very long eg –

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

R″ = IVS + 3′ exon

R‴ = Guanosine or GTP

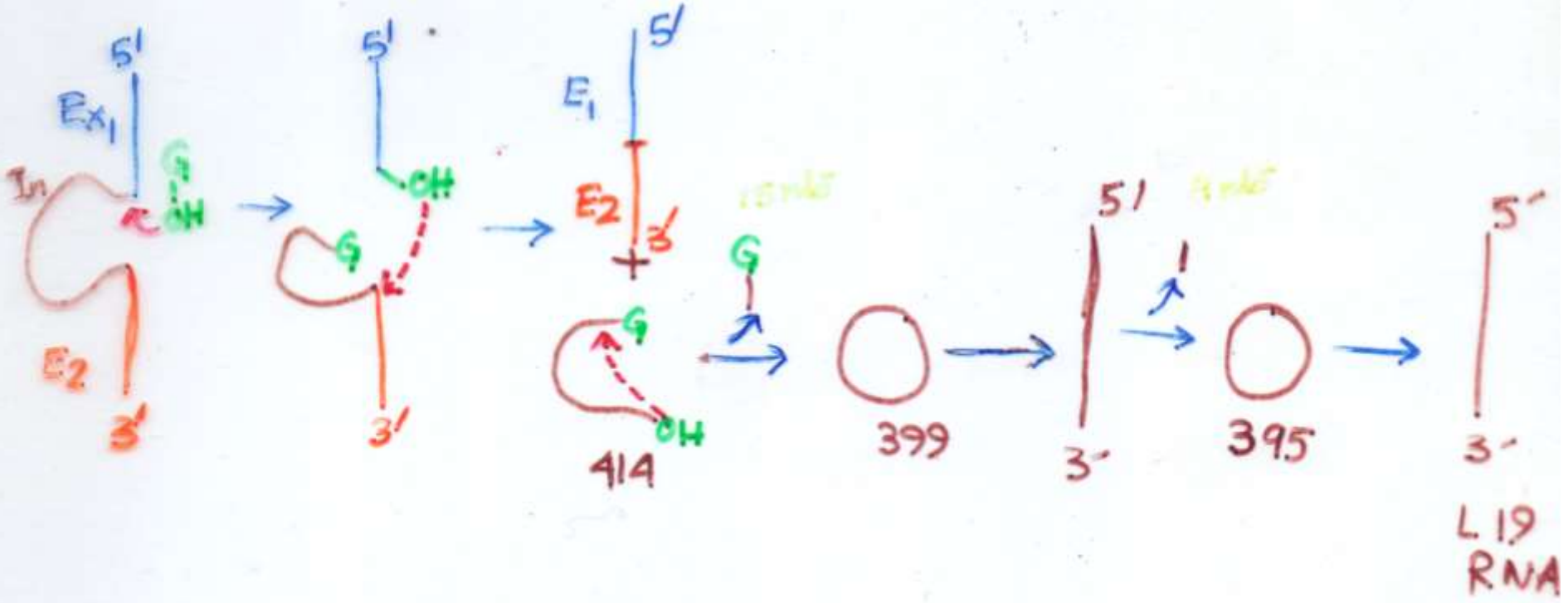
# Twice Transesterification

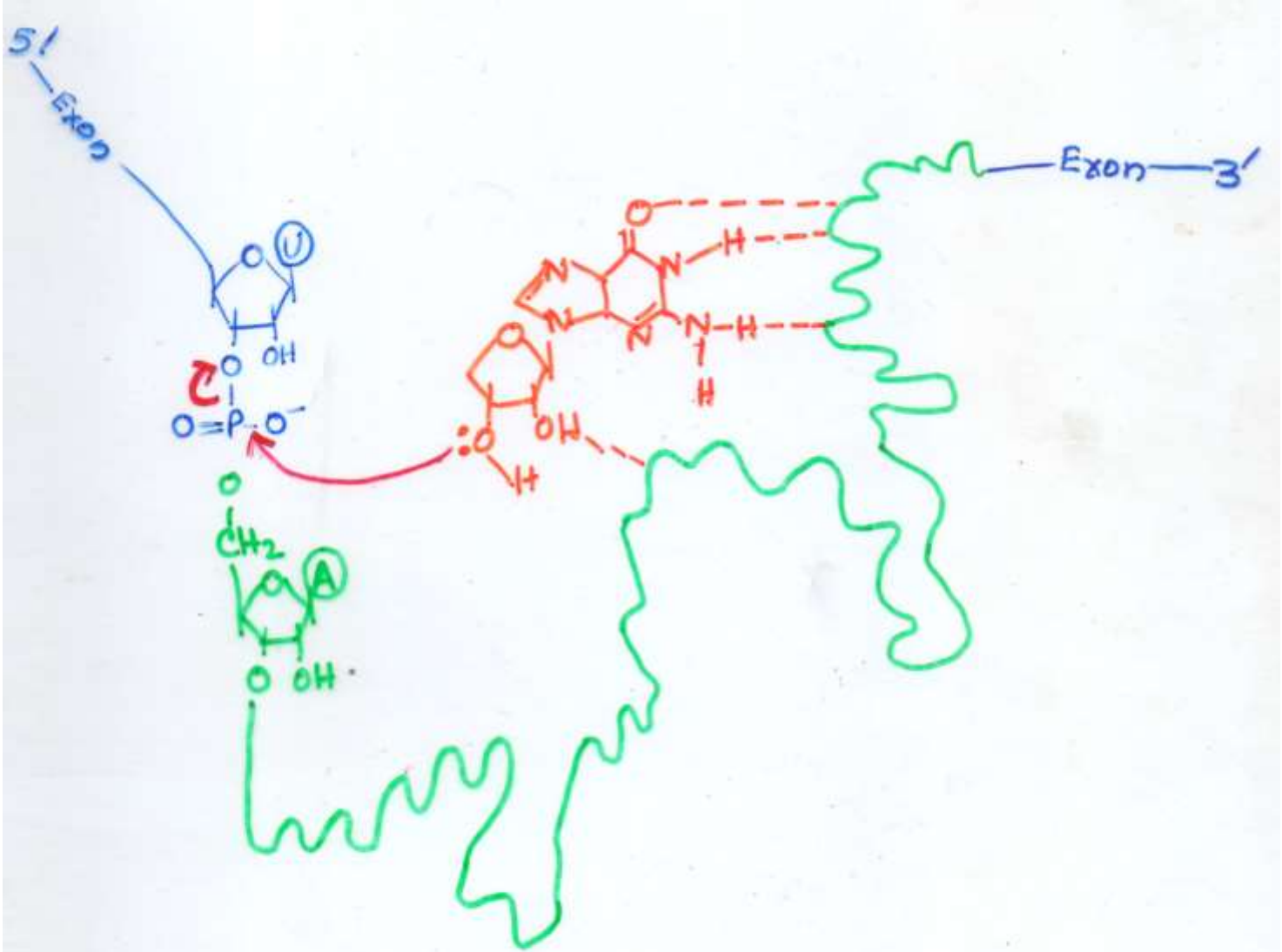


# Group II Introns

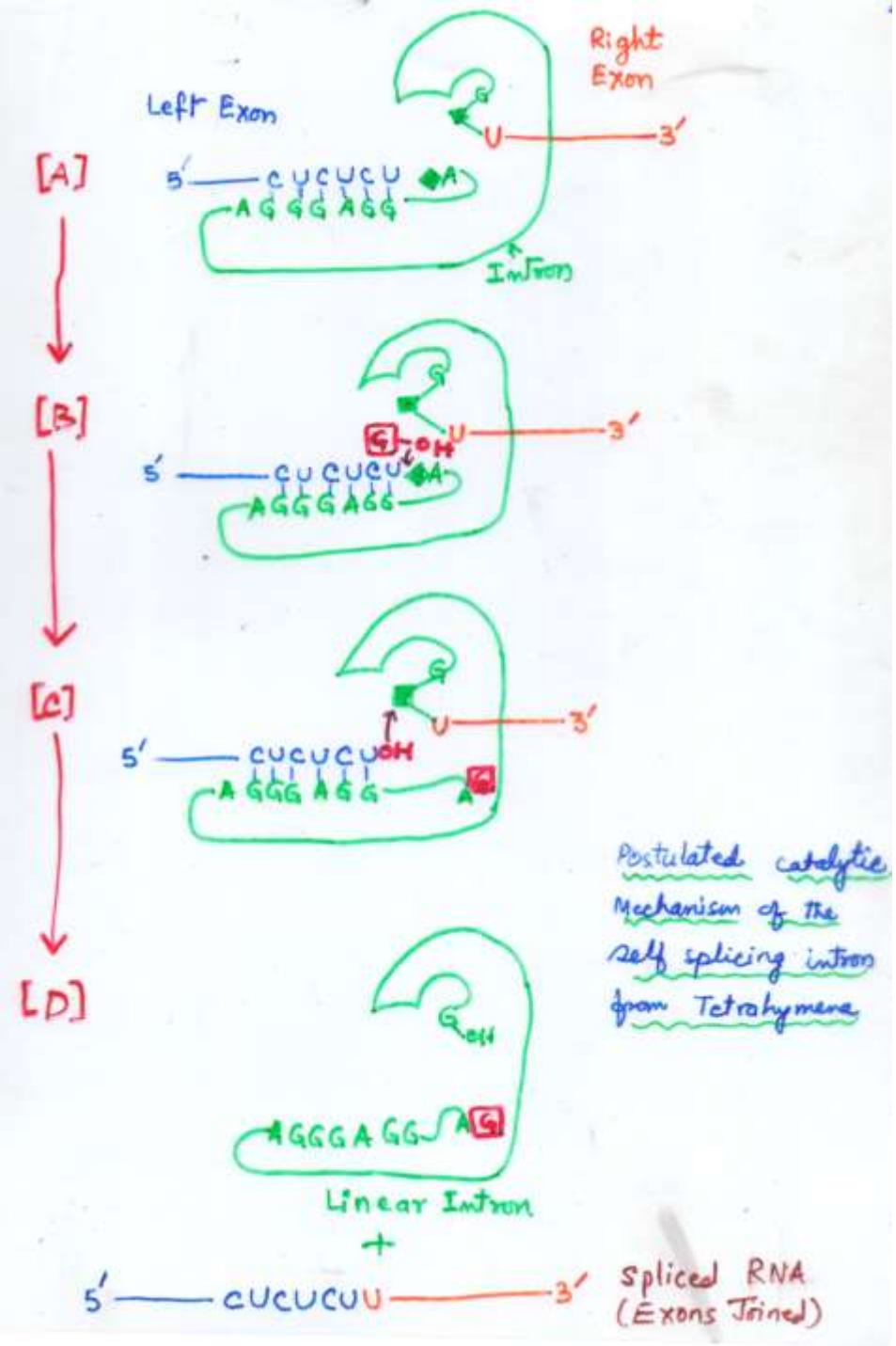
- **RNAs containing group II introns self-splice by a pathway using an A-branched lariat intermediate, like spliceosome lariats**
- **Secondary structures of the splicing complexes involving spliceosomal systems and group II introns are very similar**

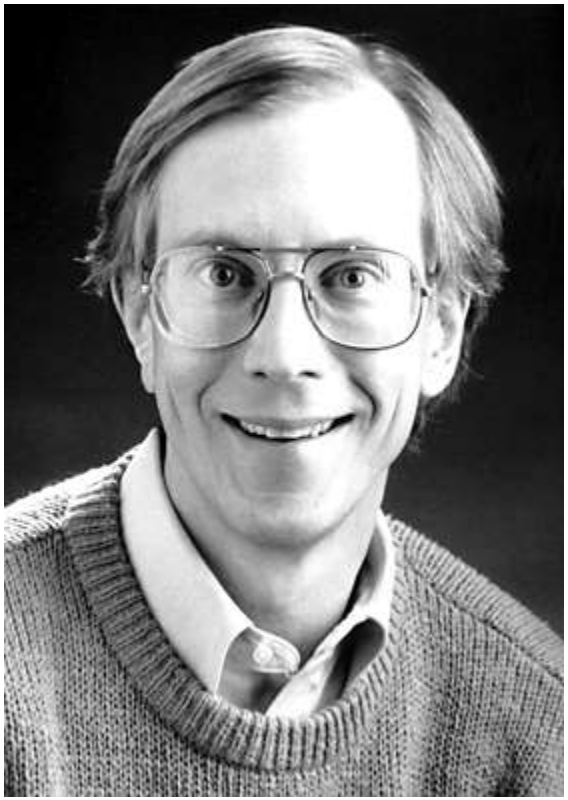
# SELF SPLICING OF A RIBOSOMAL RNA PRECURSOR FROM TETRAHYMENA






PROPOSED MODEL OF BINDING OF GUANOSINE AT THE ACTIVE SITE OF RNA ENZYME

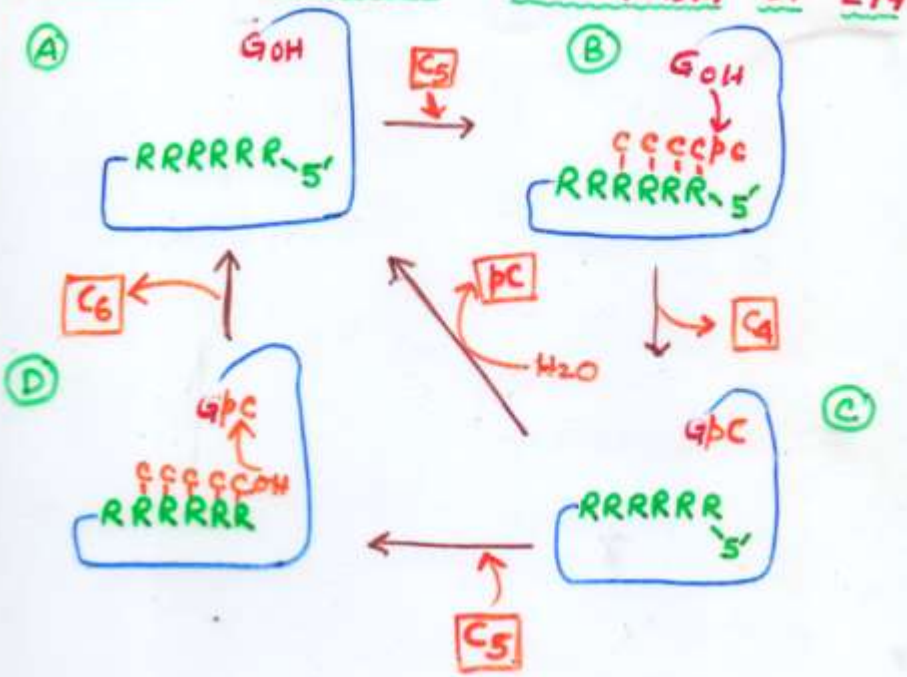

Right Exon
Left Exon
[A]
5′ CUCUCU A
AGGGAGG
Intron
3′
[B]
G
OH
U
3′
5′ CUCUCU A
AGGGAGG
[C]
G
U
3′
5′ CUCUCU OH
AGGGAGG A
[D]
G OH
AGGGAGG A
Linear Intron
+
5′ CUCUCUU 3′
Spliced RNA
(Exons Joined)
Postulated catalytic Mechanism of the self splicing intron from Tetrahymena



# Thomas R. Cech

**Born:** 8 December 1947, Chicago, IL, USA
**Affiliation at the time of the award:** University of Colorado, Boulder, CO, USA
**Field:** Biochemistry
**Prize share:** 1/2

## PROPOSED CATALYTIC MECHANISM OF L19 RNA




(A) Enzyme alone

(B) $C_5$ is hydrogen bonded to the enzyme and the terminal pc is linked covalently to the terminal G of the enzyme. The remaining $C_4$ moeily is free to dissociate from the enzyme

(C) Hydrolysis of enzyme restores the enzyme to its original state

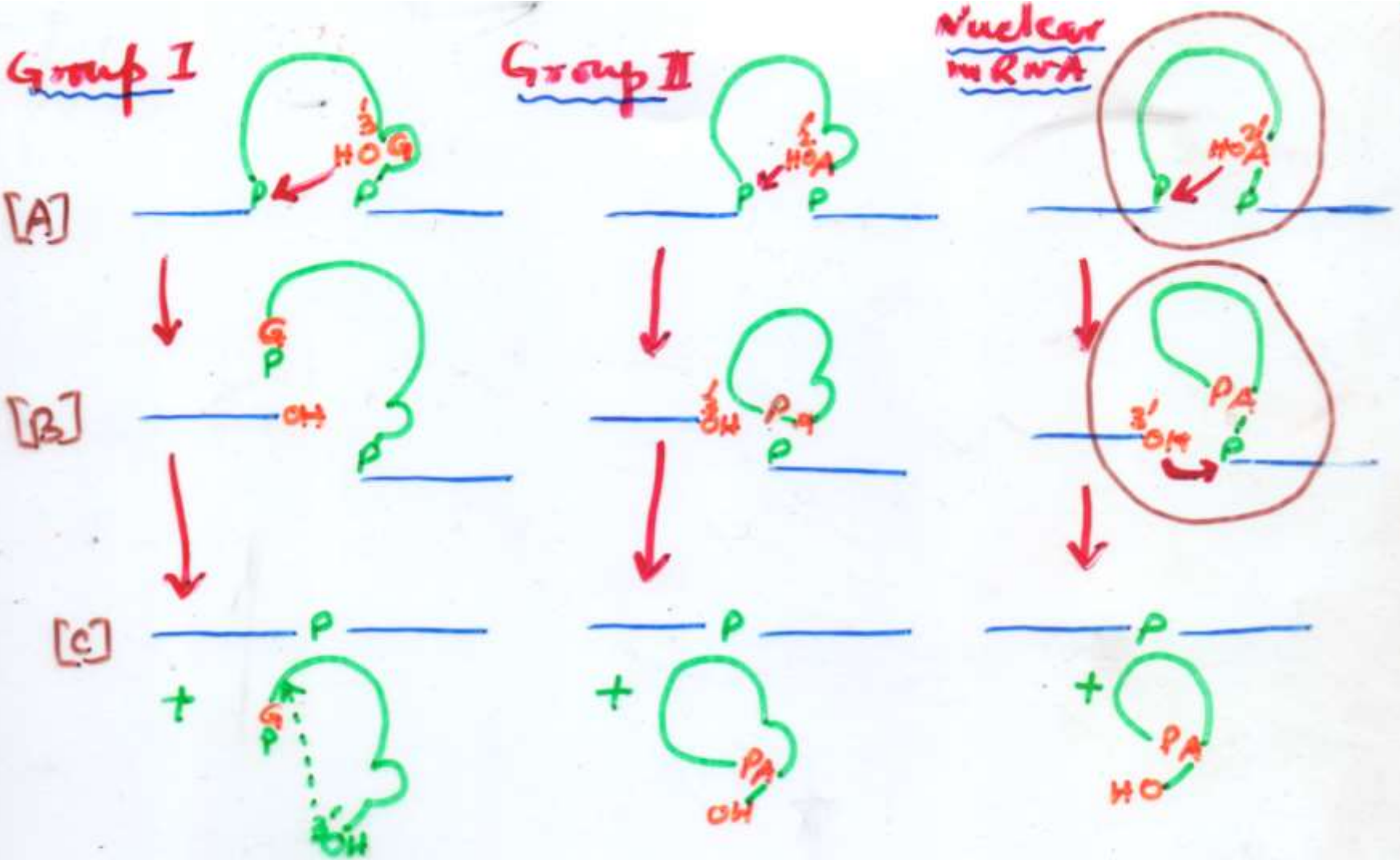
(D) Alternatively, the covalently attached pc can be attacked by a second $C_5$ molecule to form $C_6$.

**This discovery suggest that RNAs at an early stage of evolution could have replicated itself without the participation of proteins.**

# Phillip A. Sharp

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

- Philip Sharp has proposed that spliceosome catalyzed splicing of mRNA precursors evolved from RNA catalyzed self splicing.
- Gp II splicing may well be an intermediate between Group I splicing and that occuring in the nuclei of higher eukaryotes.
- A major step in this transition was the transfer of catalytic power from the intron itself to other molecules.
- The formation of spliceosomes gave introns a new freedom because they were no longer constrained to provide the catalytic centre for splicing.
- Moreover, external catalysis can be more easily regulated.




Comparision of self-splicing and Spliceosome catalyzed splicing