

## Ch 26.2: RNA processing

Unlike DNA, RNA molecules are highly processed:

End maturation (5' and 3')

Splicing

Modification

Different RNAs, different processing systems

- mRNA: 5'cap, 3'poly A tail, splicing, modifications

- rRNA: extensive cleavage (5' and 3' ends),  
modification

- tRNA: 5' and 3' end maturation, splicing,  
extensive modification

### mRNA processing in eukaryotes

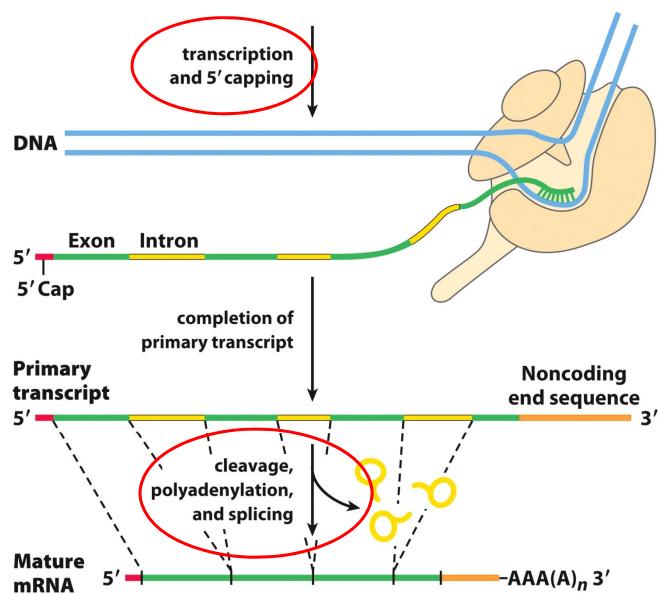


Figure 26-11  
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## 5' end maturation of eukaryotic mRNA: m<sup>7</sup>G cap

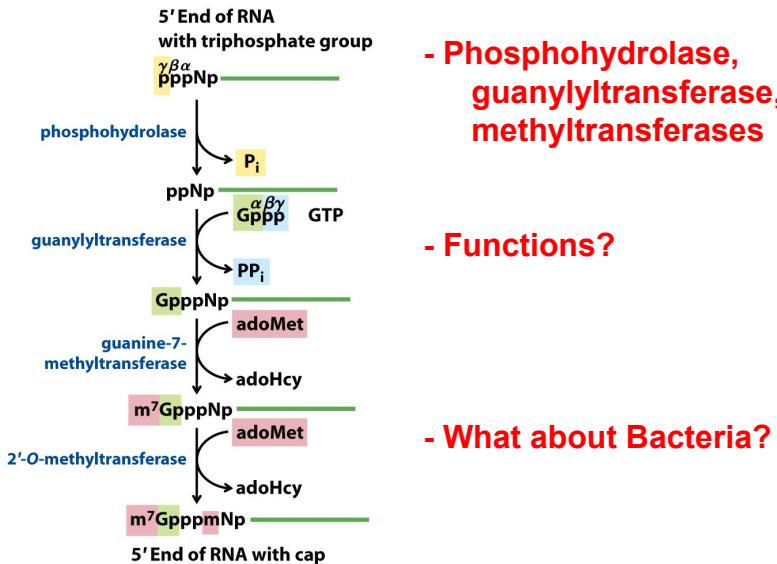


Figure 26-13b  
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## 3' end maturation of eukaryotic mRNA: poly(A) tail

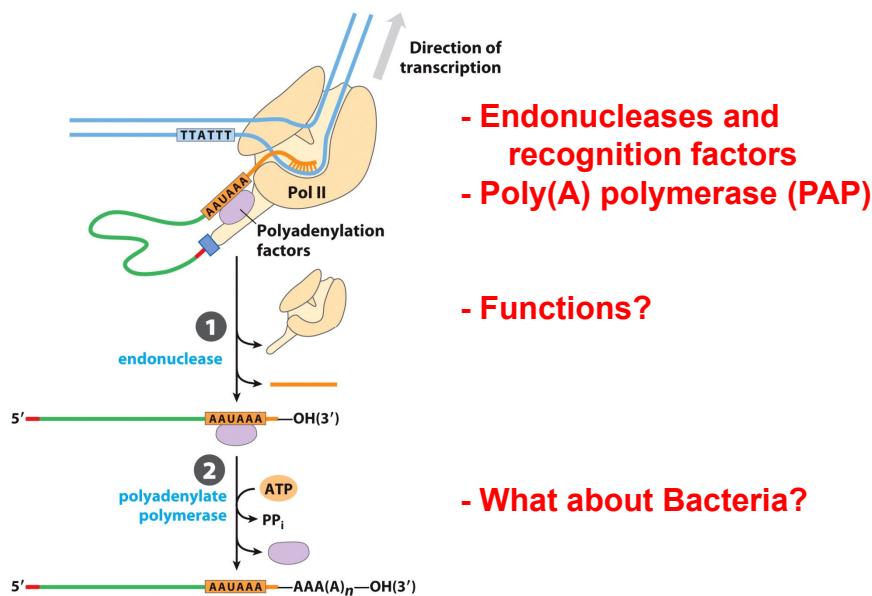
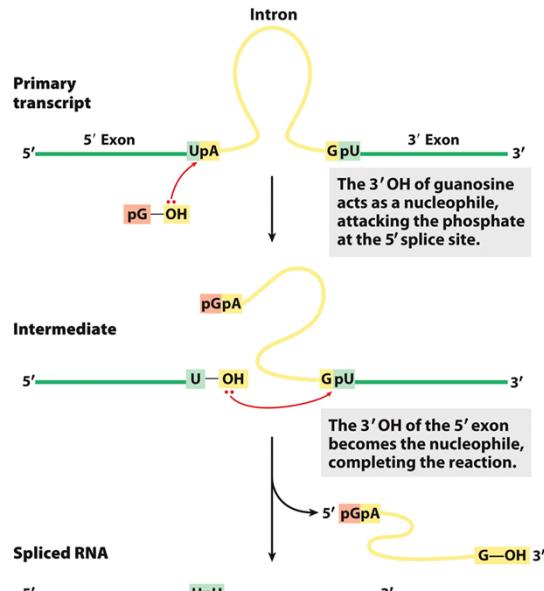


Figure 26-17  
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## Three types of splicing reactions

1. **Group I intron** (rRNA, tRNA, mRNA- all domains)
  - self-splicing (catalytic RNA)
  - guanosine nucleophile
2. **Group II intron** (organellar RNAs, mRNA in bacteria)
  - self-splicing (catalytic RNA)
  - internal A (in intron) nucleophile
3. **Spliceosomal intron** (eukaryotic nuclear mRNAs)
  - snRNP (RNA + protein), but catalytic RNA
  - internal A (in intron) nucleophile

## Mechanism of the Group I intron

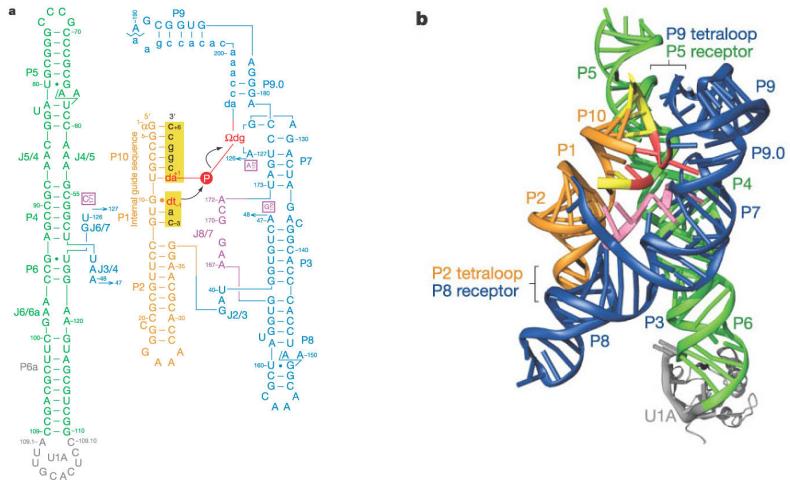


Thomas Cech

Unnumbered 26 p1037  
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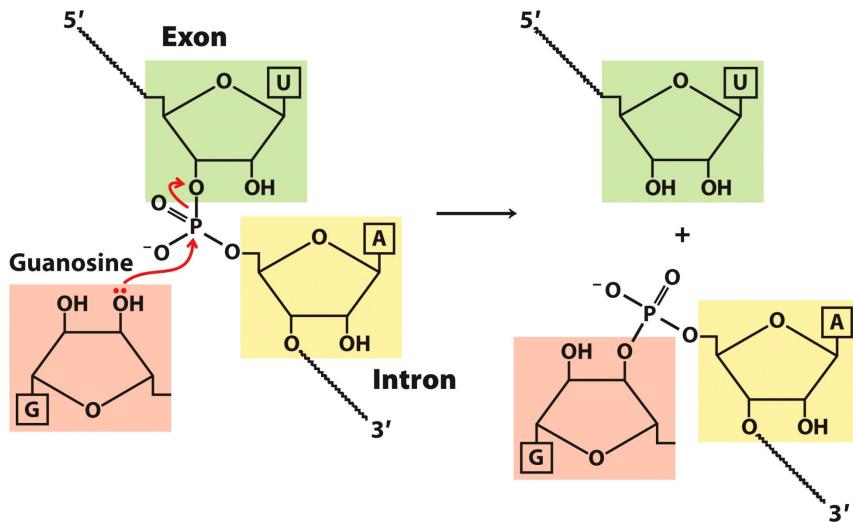
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# Crystal structure of a self-splicing Group I intron



Adams et al., Nature 2004

## RNA splicing: transesterification reactions



**Figure 26-13**  
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## Mechanism of the Group II intron

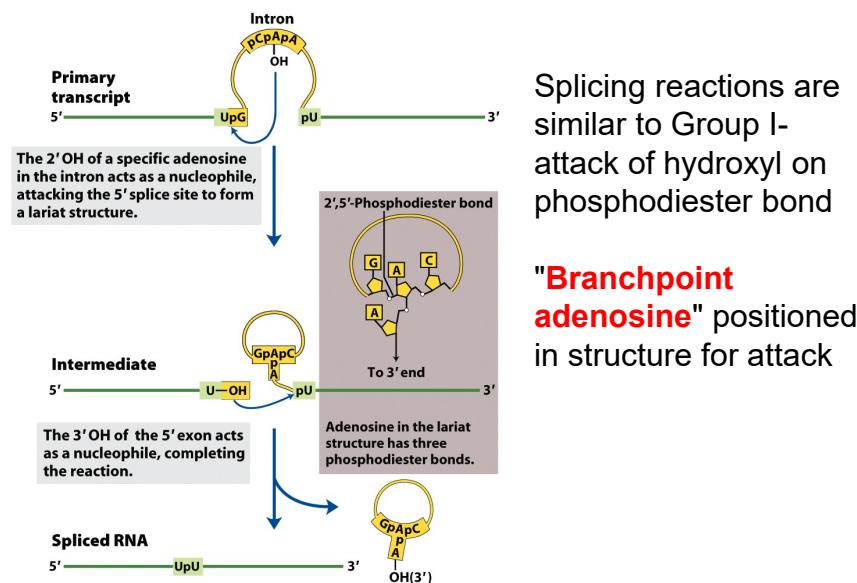
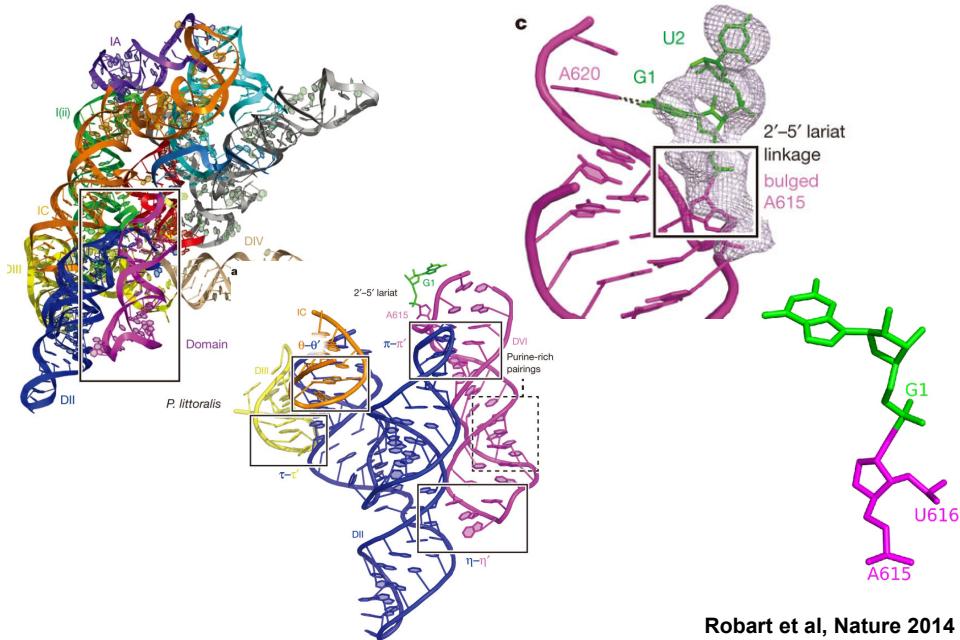


Figure 26-16  
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## Crystal structure of a self-splicing Group II intron



## Three types of splicing reactions

1. **Group I intron** (rRNA, tRNA, mRNA- all domains)
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## Mechanism of the spliceosome

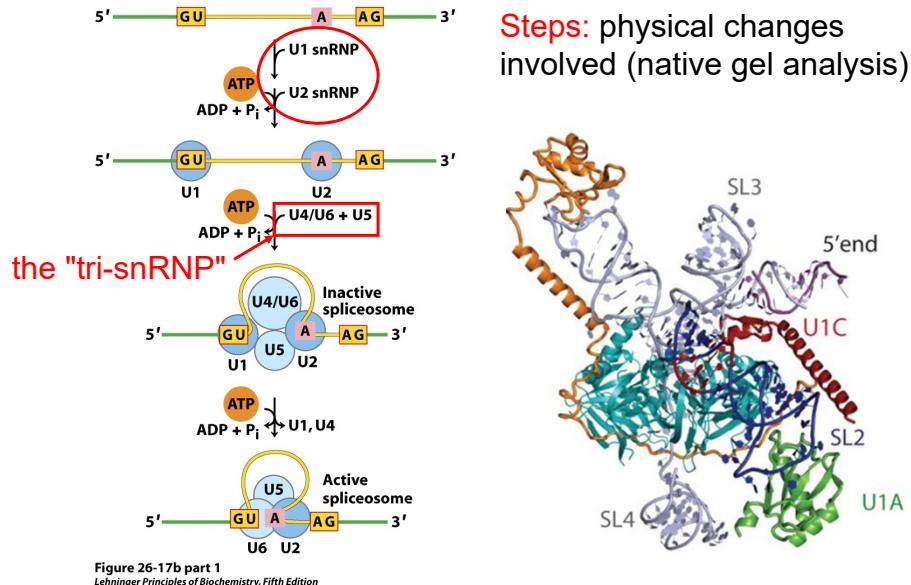


Figure 26-17b part 1  
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## Roles for base-pairing in the spliceosome

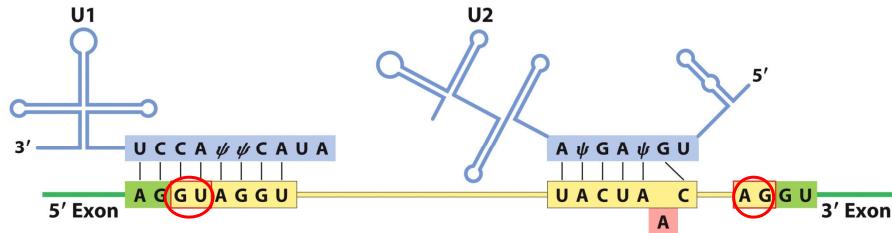


Figure 26-16a  
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Sequences at 5' and 3' splice site and branchpoint are highly conserved

- **GU-AG rule** to define splice sites
- **Role of base pairing** between snRNA:mRNA

## Mechanism of the spliceosome

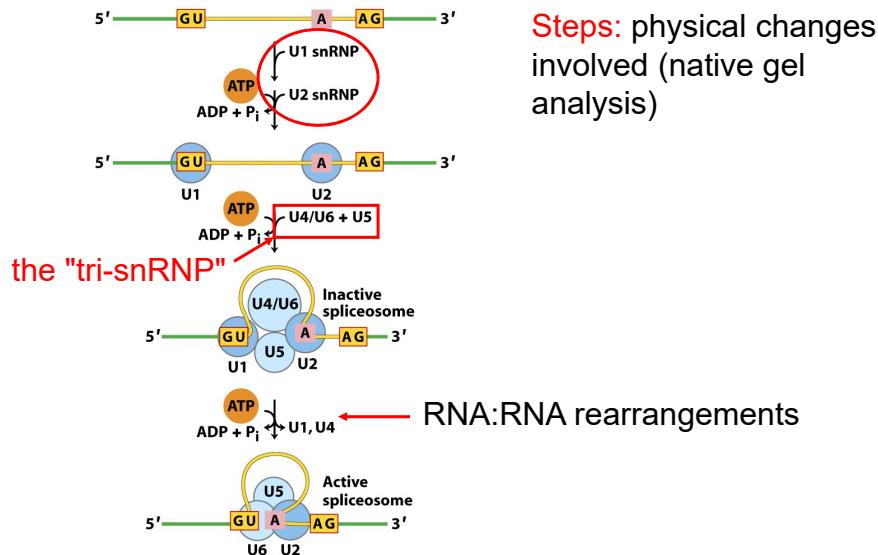
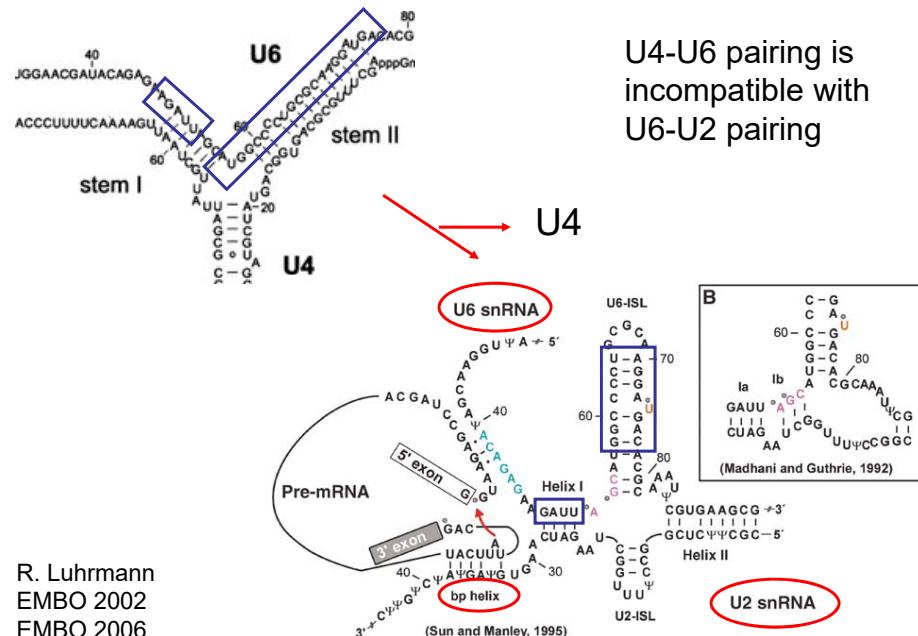


Figure 26-17b part 1  
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## RNA:RNA rearrangements required for catalysis



R. Luhrmann  
EMBO 2002  
EMBO 2006

## Mechanism of the spliceosome

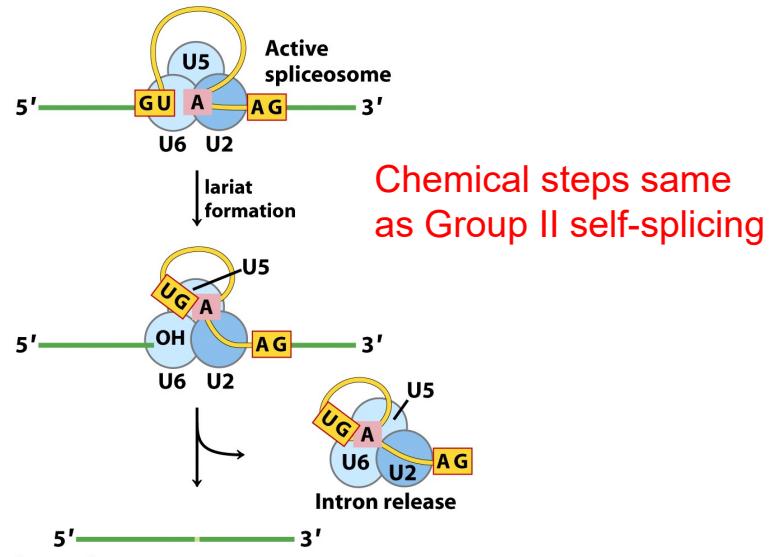
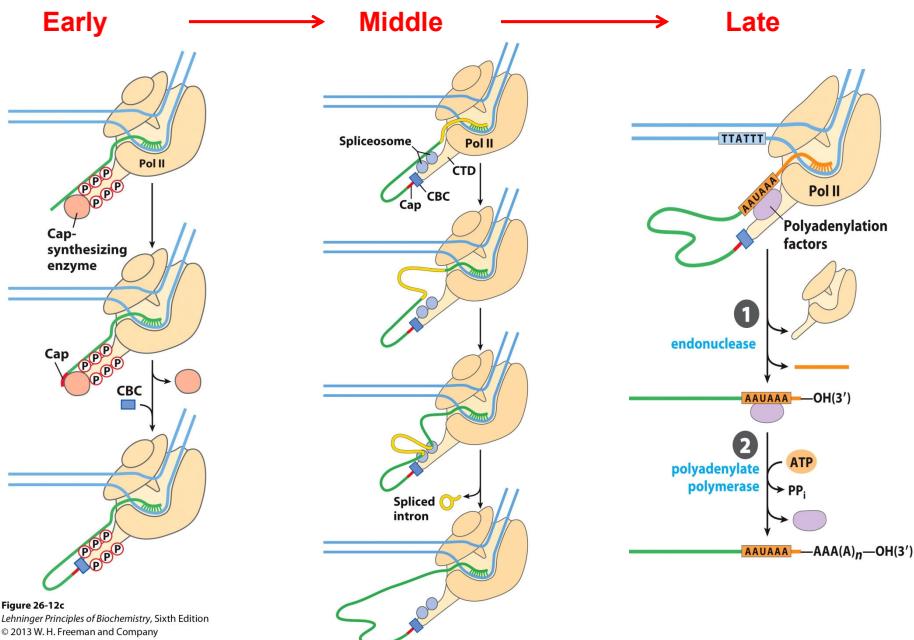


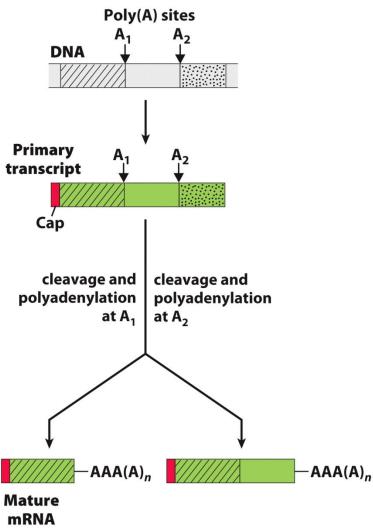
Figure 26-17b part 2  
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## Role of the Pol II CTD in RNA processing

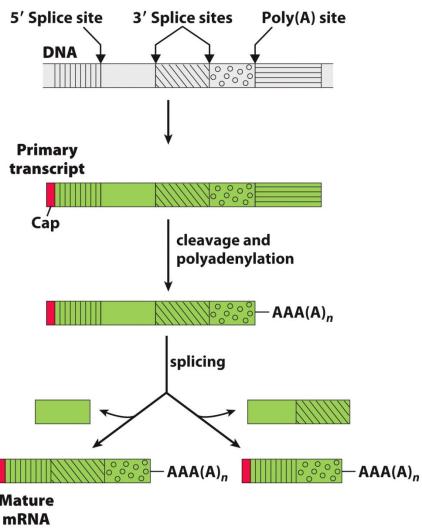


## Alternative processing events generate diversity

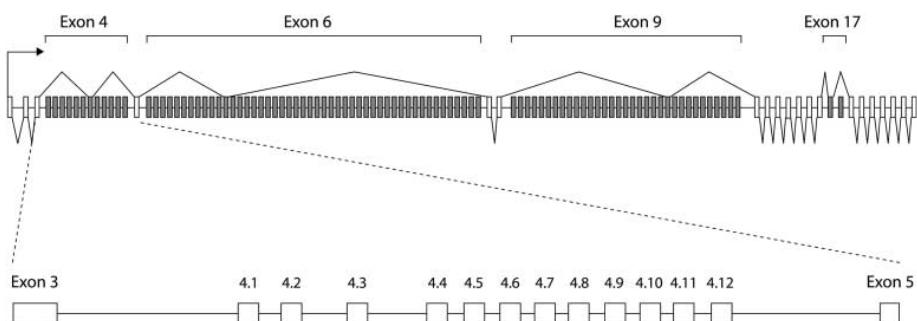
### Alternative polyadenylation



### Alternative splicing



## Drosophila DSCAM: extreme alternative splicing



### DSCAM: Down syndrome cell adhesion molecule

- 115 exons; 20 constitutive, 95 alternatively spliced
- Pick one exon from each of four clusters (4, 6, 9, 17)
- Total of 38,016 different mRNAs possible!!
- Questions: how many are made?  
how is it regulated?

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### rRNA processing from a single large transcript

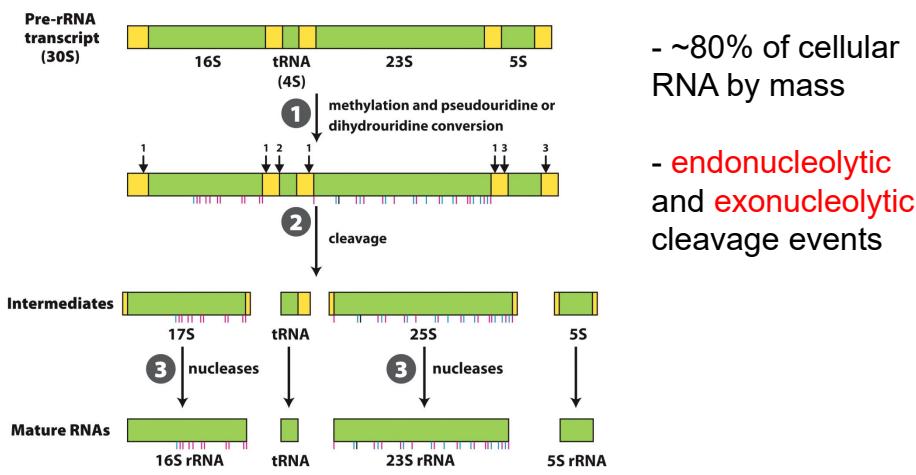


Figure 26-23  
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## rRNA contains modified nucleotides

### Nucleotide modification:

- Base or sugar chemically changed
- rRNA typically contains three modified nucleotides:
  - 2'-Omethyl ribose (2'OMe)
  - pseudouridine ( $\Psi$ )
  - dihydrouridine (D)

### Machinery:

- Bacteria: site-specific protein enzymes
- Eukaryotes: snoRNPs (RNA guides position of mod)

## rRNA contains modified nucleotides

### Box C/D snoRNP: 2'-OMe

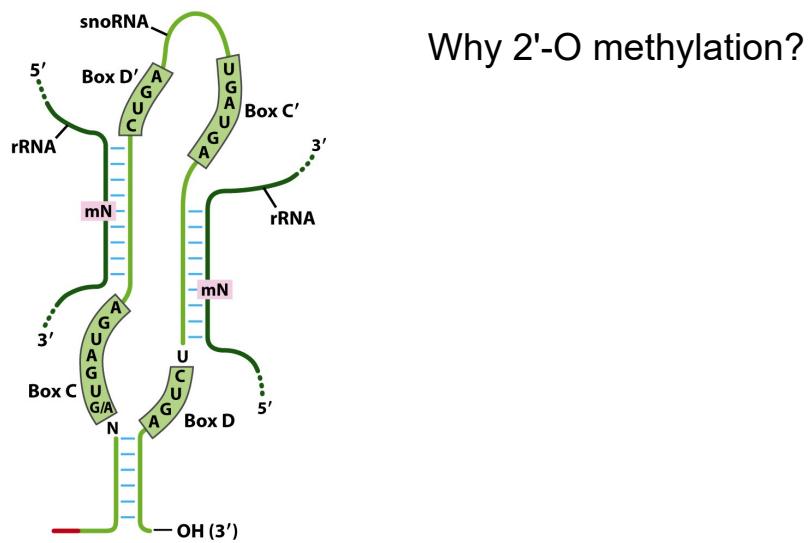
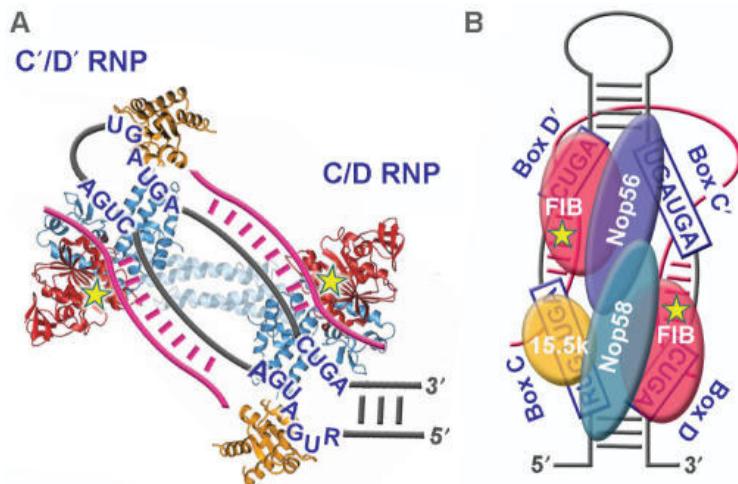


Figure 26-26a  
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## snoRNA guides proteins to specific location in target RNA



Reichow et al, *NAR* 35: 1452 (2007)

## rRNA contains modified nucleotides

### Box H/ACA snoRNP: pseudo-U

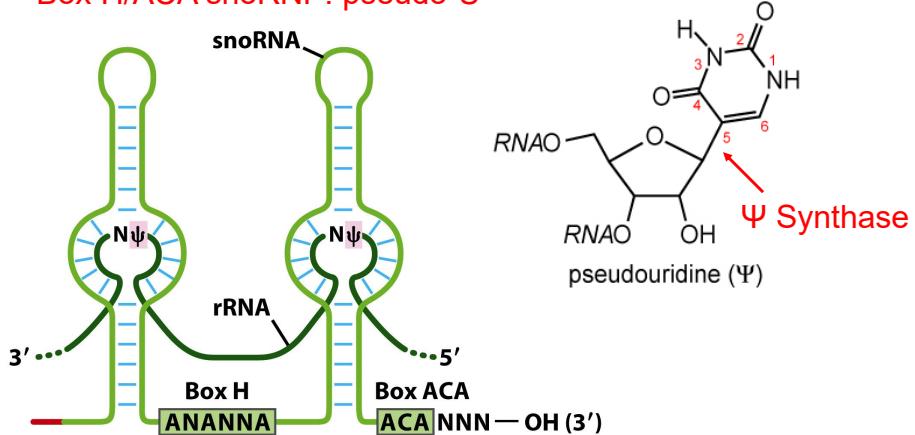


Figure 26-26b  
Lodish et al., Principles of Biochemistry, Fifth Edition  
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Pseudouridine: most common modified nucleotide  
- why have pseudouridine in RNA?

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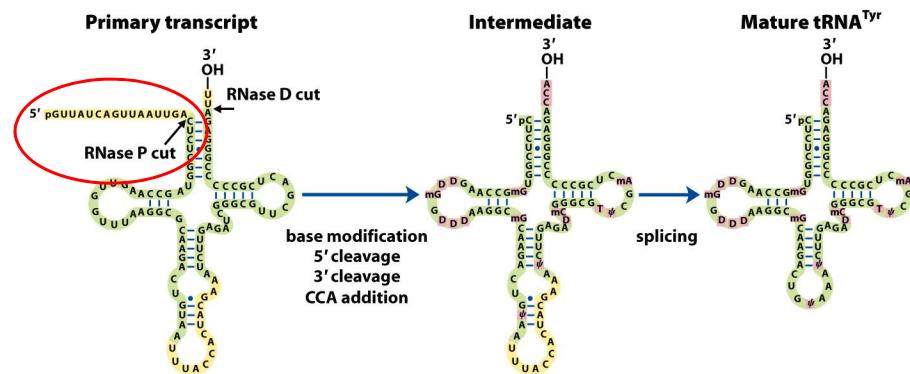
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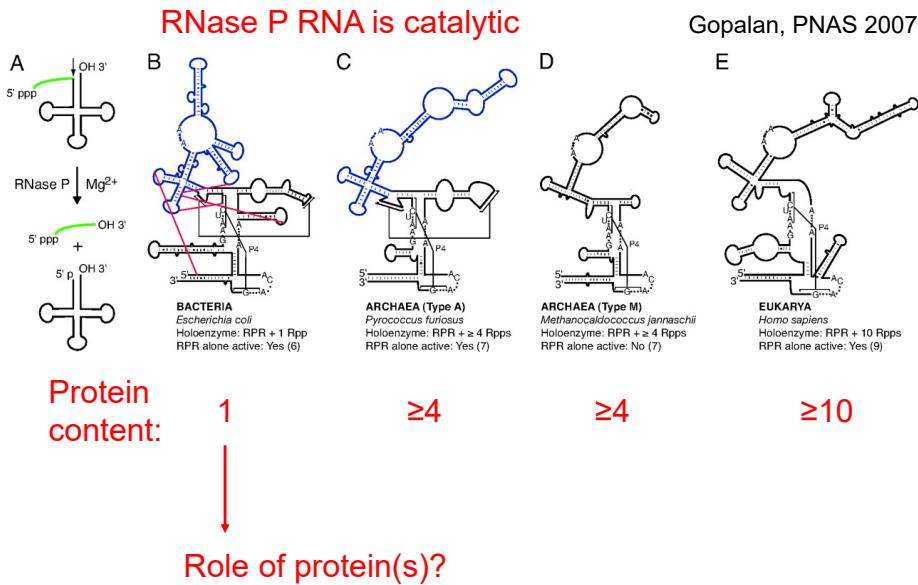
### Multiple steps for tRNA processing in all organisms



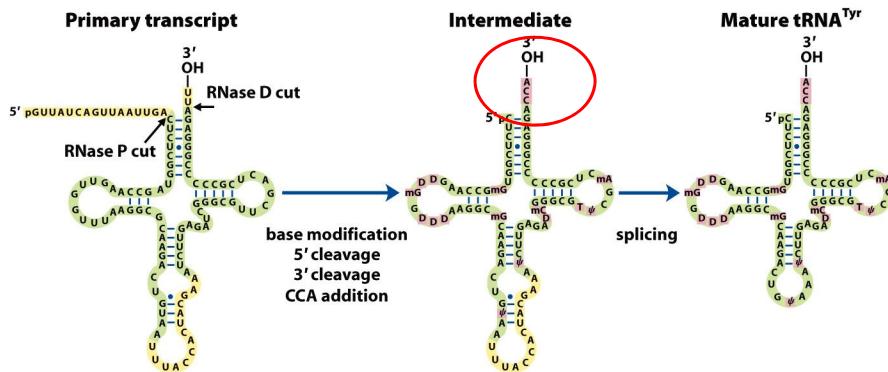
5' end processing:

- RNase P universal: RNA + protein(s)

## RNase P: Diversity in protein composition



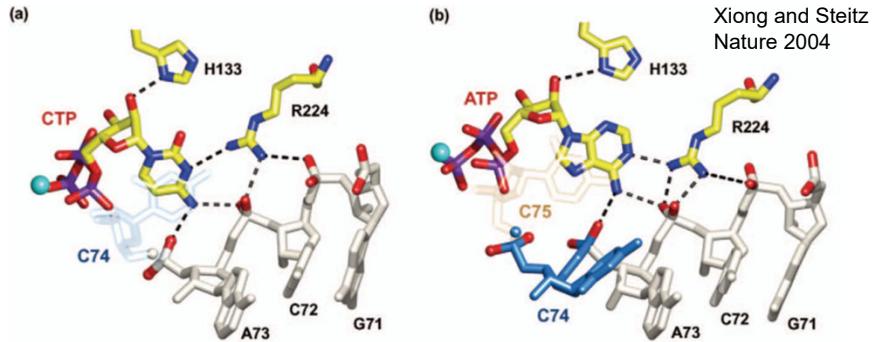
## Multiple steps for tRNA processing in all organisms



CCA addition (if not encoded):

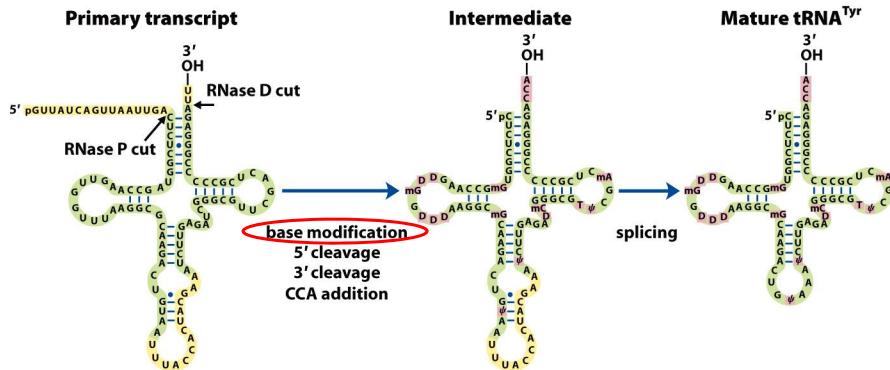
- CCA-adding enzyme is ancient pol β family member
- Template-independent synthesis

## tRNA nucleotidyltransferase (CCA-adding enzyme): non-templated nucleotide addition



How does CCA enzyme know identity and order of NTPs to add?

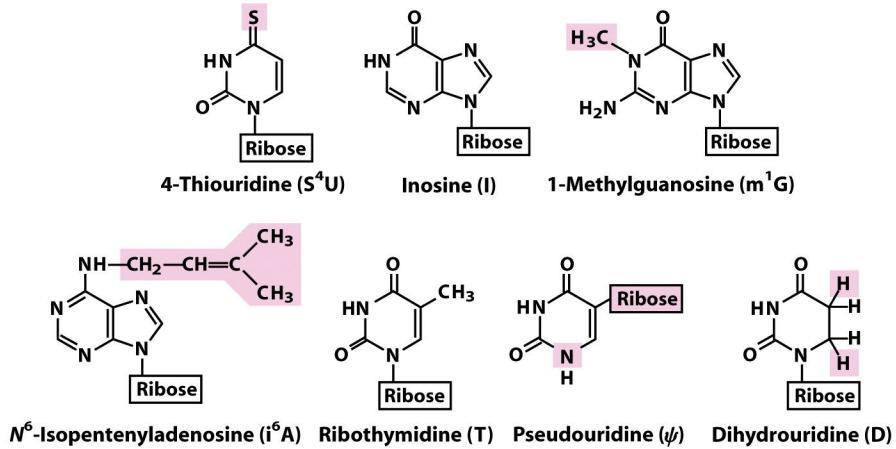
## Multiple steps for tRNA processing in all organisms



### tRNA modification:

- every tRNA species has different set of modifications
- catalyzed by individual modification enzymes (proteins)

## Some modified nucleotides in tRNA



- tRNA nucleotide modifications catalyzed by **protein enzymes**
  - Function?

## Base modifications found in *S. cerevisiae* tRNA

