

## RNA processing #1

Making ends of RNA

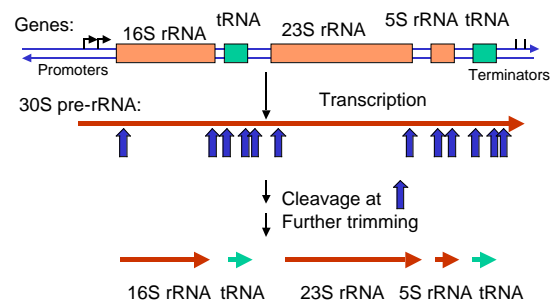
## Types of RNA processing

- A) Cutting and trimming to generate ends:
  - rRNA, tRNA and mRNA
- B) Covalent modification:
  - Add a cap and a polyA tail to mRNA
  - Add a methyl group to 2'-OH of ribose in mRNA and rRNA
  - Extensive changes of bases in tRNA
- C) Splicing
  - pre-rRNA, pre-mRNA, pre-tRNA by different mechanisms.

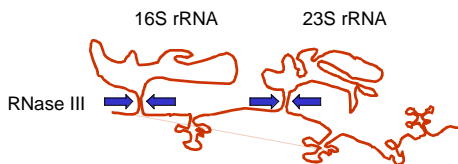
## Cutting and Trimming RNA

- Can use **endonucleases** to cut at specific sites **within** a longer precursor RNA
- Can use **exonucleases** to trim back from the new **ends** to make the mature product
- This general process is seen in **prokaryotes** and **eukaryotes** for all types of RNA

## Excision of mature rRNA and tRNA from pre-rRNA in *E. coli*



## RNase III cuts in stems of stem-loops

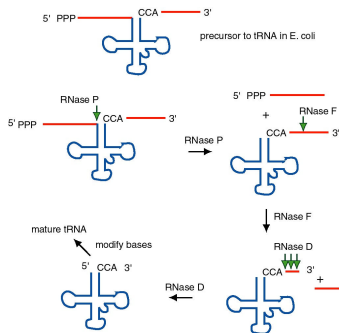


No apparent primary sequence specificity - perhaps RNase III recognizes a particular stem structure.

## Endo- and exonucleases to generate ends of tRNA

- Endonuclease **RNase P** cleaves to generate the 5' end.
- Endonuclease **RNase F** cleaves 3 nucleotides past the mature 3' end.
- Exonuclease **RNase D** trims 3' to 5', leaving the mature 3' end.
- See Figure 3.3.3

### Cleavage of pre-tRNA in *E. coli*



### CCA at 3' end of tRNAs

- Virtually all tRNAs end in the sequence CCA.
- Amino acids are added to the CCA end during “charging” of tRNAs for translation.
- In most **prokaryotic** tRNA genes, the CCA is **encoded in the DNA**.
- For most eukaryotic tRNAs, the **CCA is added after transcription**, in a reaction catalyzed by tRNA nucleotidyl transferase.

### Where is the catalytic activity in RNase P?

RNase P is composed of a 375 nucleotide RNA and a 20 kDa protein.

The protein component will NOT catalyze cleavage on its own.

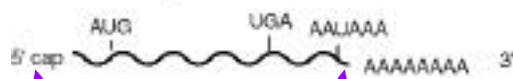
The RNA WILL catalyze cleavage by itself !!!!  
The protein component aids in the reaction but is **not** required for catalysis.  
Thus **RNA** can be an **enzyme**.

Enzymes composed of RNA are called **ribozymes**.

### Covalent modification of RNA

### 5' and 3' ends of eukaryotic mRNA

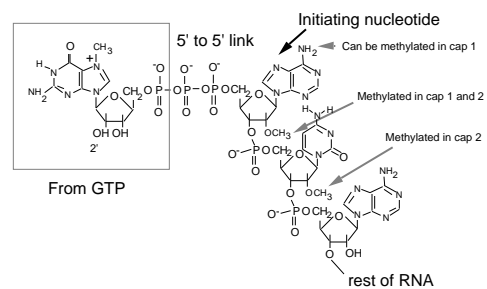
Structure of eukaryotic mRNAs



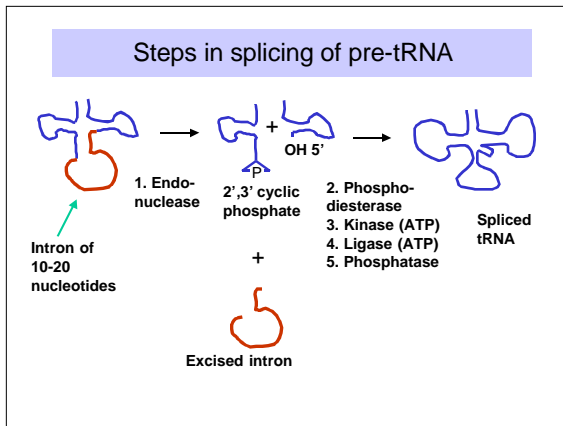
Add a GMP.  
Methylate it and  
1st few nucleotides

Cut the pre-mRNA  
and add A's

### 5' cap structure







- Splicing of Group I and II introns**
- Introns in fungal mitochondria, plastids, Tetrahymena pre-rRNA
  - Group I
    - **Self-splicing**
    - Initiate splicing with a **G** nucleotide
    - Uses a phosphoester transfer mechanism
    - Does **not** require ATP hydrolysis.
  - Group II
    - **self-splicing**
    - Initiate splicing with an **internal A**
    - Uses a phosphoester transfer mechanism
    - Does **not** require ATP hydrolysis

- Splicing of pre-mRNA**
- The introns begin and end with almost invariant sequences: 5' GU...AG 3'
  - Use ATP to assemble a large spliceosome
  - Mechanism is similar to that of the Group II fungal introns:
    - Initiate splicing with an internal A
    - Uses a phosphoester transfer mechanism for splicing