Processing of rRNA and tRNA

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Topic - Processing of rRNA and tRNA

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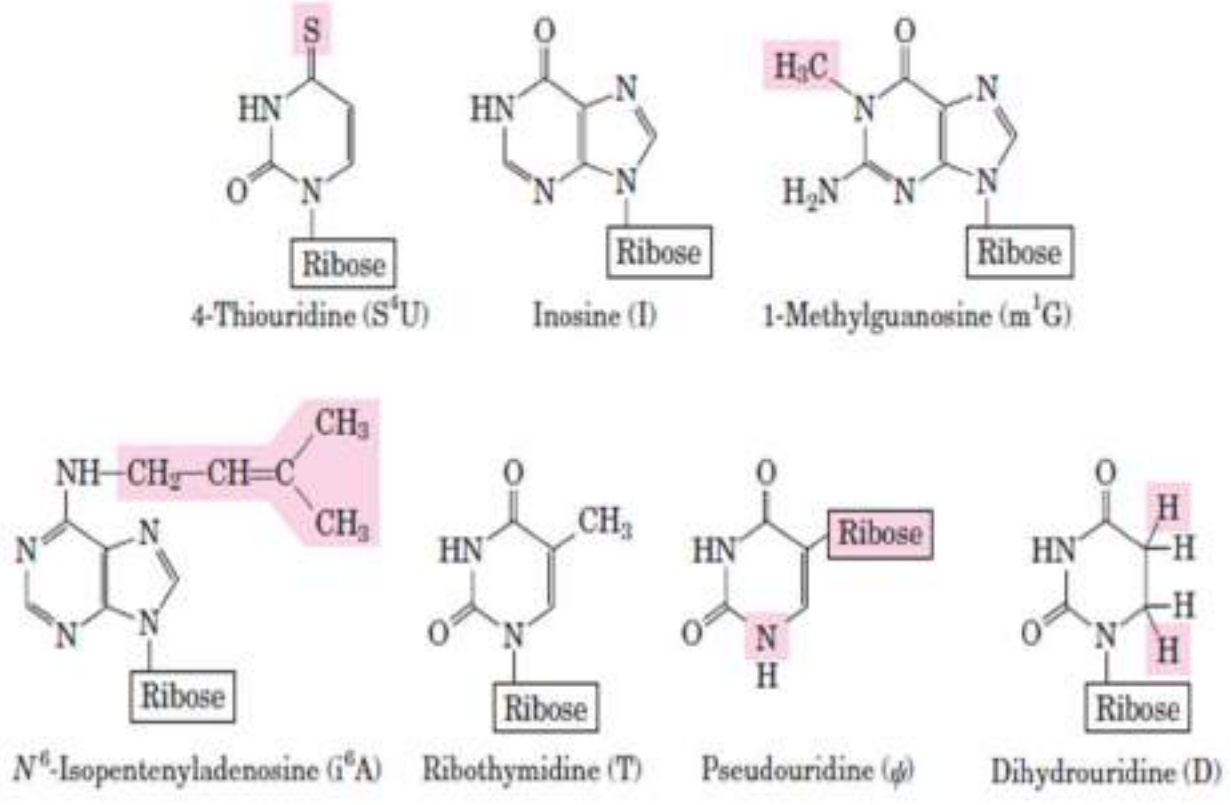
Introduction

- Posttranscriptional processing is not limited to mRNA.
- Ribosomal RNAs of bacterial, archaeal, and eukaryotic cells are made from longer precursors called **preribosomal RNAs**, or pre-rRNAs.
- Transfer RNAs are similarly derived from longer precursors.
- These RNAs may also contain a variety of modified nucleosides; some examples are shown below.



Source- Lehninger Principles of Biochemistry; Nelson and Cox, 6th edition

Figure- Some modified bases of rRNAs and tRNAs, produced in posttranscriptional reactions. The standard symbols are shown in parentheses. Note the unusual ribose attachment point in pseudouridine. This is just a small sampling of the 96 modified nucleosides known to occur in different RNA species, with 81 different types known in tRNAs and 30 observed to date in rRNAs (approx data). For current detail see RNA database.

rRNA Processing

Ribosomal RNAs In bacteria, 16S, 23S, and 5S rRNAs (and some tRNAs, although most tRNAs are encoded from other places) arise from a single 30S RNA precursor of about 6,500 nucleotides. RNA at both ends of the 30S precursor and segments between the rRNAs are removed during processing.

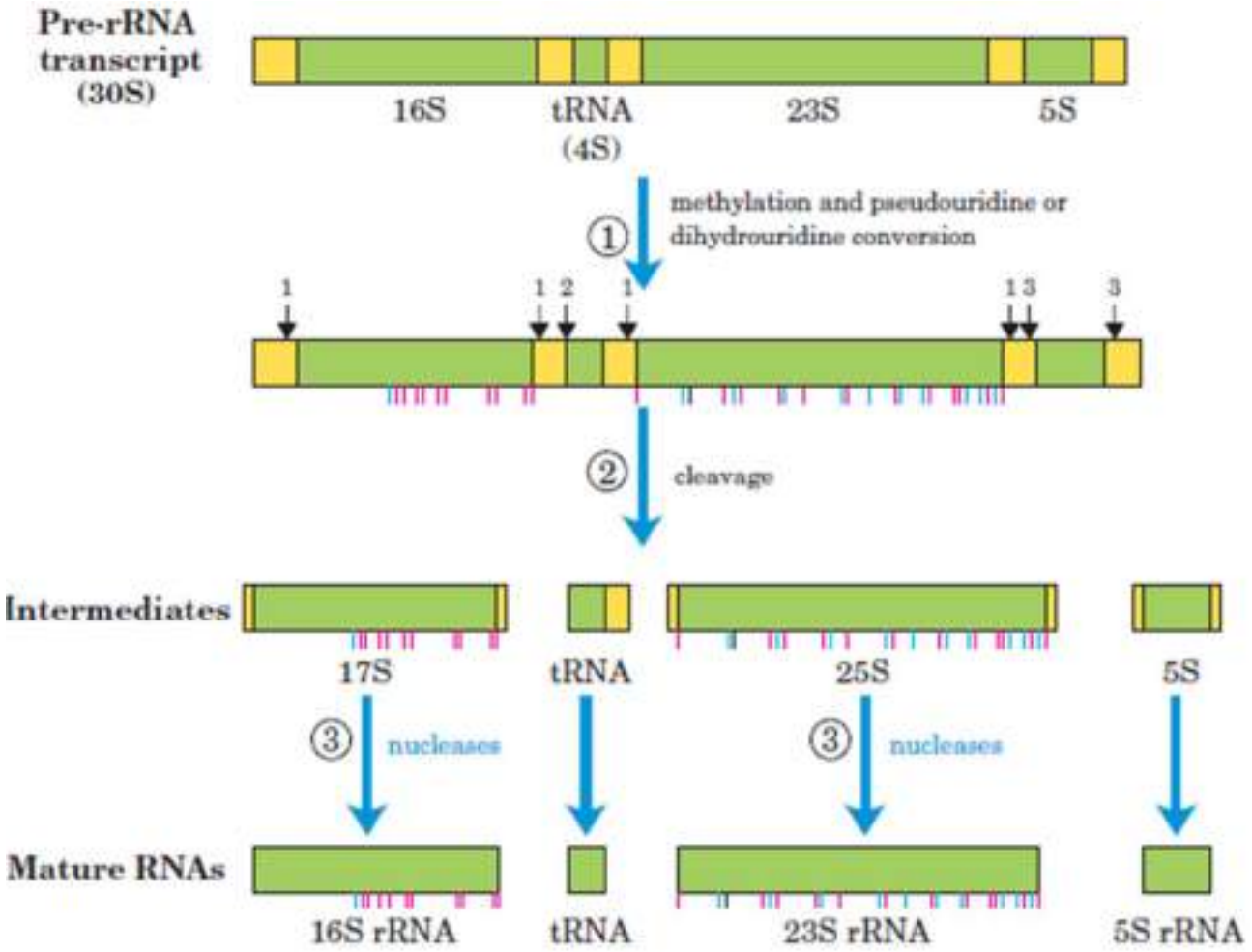


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Figure- Processing of pre-rRNA transcripts in bacteria.

1- Before cleavage, the 30S RNA precursor is methylated at specific bases (red tick marks), and some uridine residues are converted to pseudouridine (blue tick marks) or dihydrouridine (black tick mark) residues. The methylation reactions are of multiple types, some occurring on bases and some on 2'-hydroxyl groups.

2- Cleavage liberates precursors of rRNAs and tRNA(s). Cleavage at the points labeled 1, 2, and 3 is carried out by the enzymes RNase III, RNase P, and RNase E, respectively. **RNase P is a ribozyme**.

3- The final 16S, 23S, and 5S rRNA products result from the action of a variety of specific nucleases. The seven copies of the gene for pre-rRNA in the *E. coli* chromosome differ in the number, location, and identity of tRNAs included in the primary transcript. Some copies of the gene have additional tRNA gene segments between the 16S and 23S rRNA segments and at the far 3' end of the primary transcript.

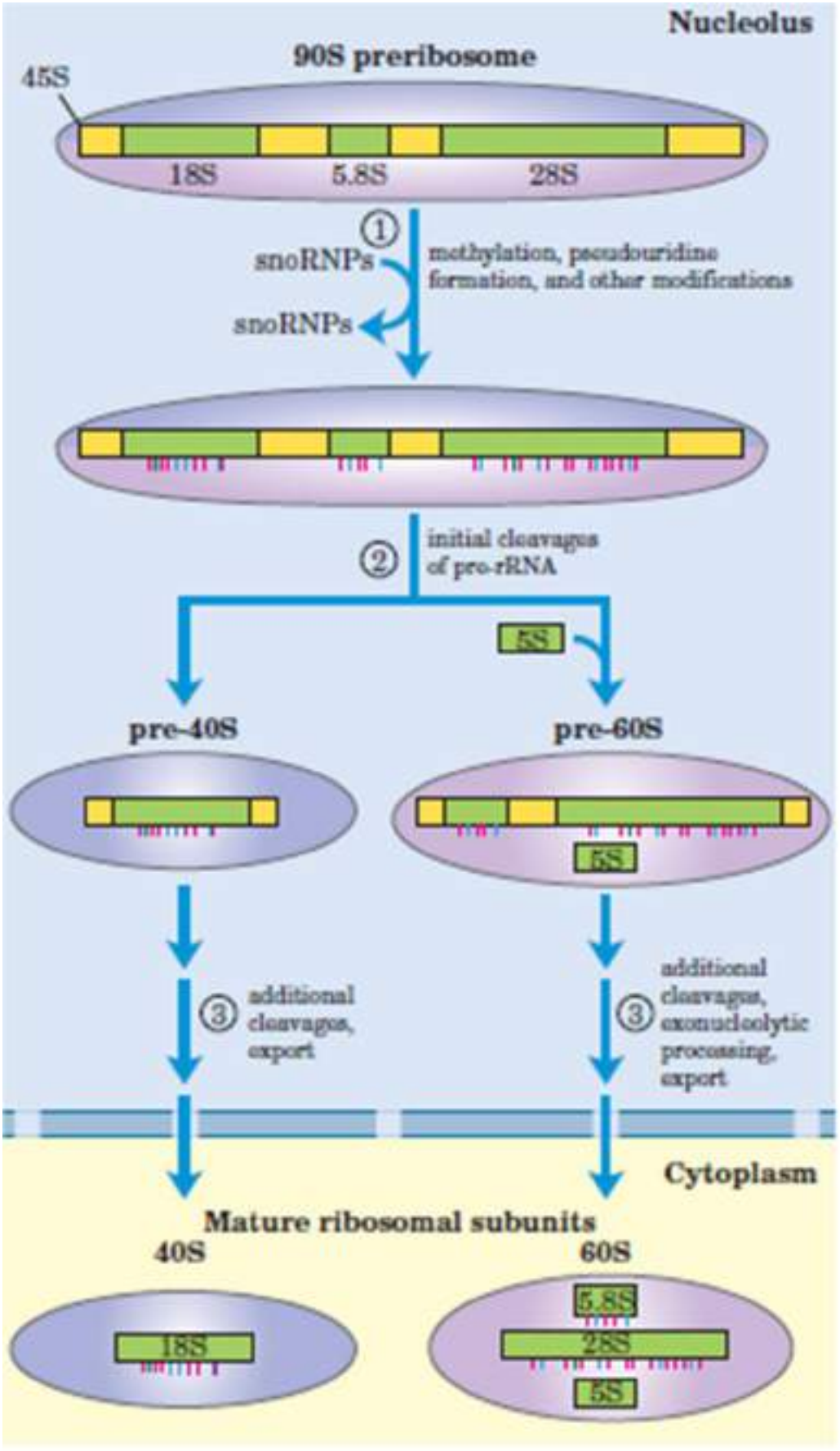
The 16S and 23S rRNAs contain modified nucleosides. In *E. coli*, the 11 modifications in the 16S rRNA include a pseudouridine and 10 nucleosides methylated on the base or the 2-hydroxyl group, or both.

The 23S rRNA has 10 pseudouridines, 1 dihydrouridine, and 12 methylated nucleosides. In bacteria, each modification is generally catalyzed by a distinct enzyme.

Methylation reactions use *S*-adenosylmethionine as cofactor. No cofactor is required for pseudouridine formation.

- The genome of *E. coli* encodes seven pre-rRNA molecules. All of these genes have essentially identical rRNA-coding regions, but they differ in the segments between these regions.
- The segment between the 16S and 23S rRNA genes generally encodes one or two tRNAs, with different tRNAs produced from differen pre-rRNA transcripts.
- Coding sequences for tRNAs are also found on the 3' side of the 5S rRNA in some precursor transcripts.

The situation in eukaryotes is more complicated. A 45S pre-rRNA transcript is synthesized by RNA polymerase I and processed in the nucleolus to form the 18S, 28S, and 5.8S rRNAs characteristic of eukaryotic ribosomes. There is a tight coupling between rRNA transcription, rRNA maturation, and ribosome assembly in the nucleolus. Each complex includes the ribonucleases that cleave the rRNA precursor, the enzymes that modify particular bases, large numbers of small nucleolar RNAs, or **snoRNAs**, that guide nucleoside modification and some cleavage reactions, and ribosomal proteins.



Source- Lehninger Principles of Biochemistry; Nelson and Cox, 6th edition

Figure- Processing of pre-rRNA transcripts in vertebrates.

During transcription, the 45S primary transcript is incorporated into a nucleolar 90S preribosomal complex, in which rRNA processing and ribosome assembly are tightly coupled.

1- The 45S precursor is methylated at more than 100 of its 14,000 nucleotides, either on the bases or on the 2'-OH groups, some uridines are converted to pseudouridine, and a few other modifications occur.

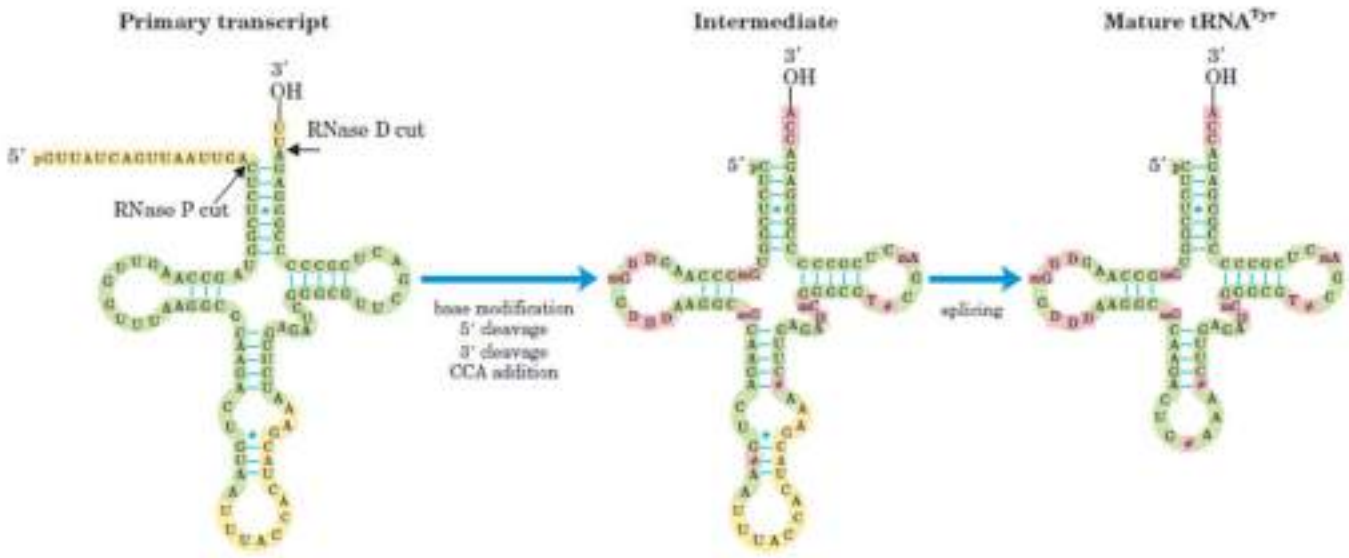
2- A series of enzymatic cleavages of the 45S precursor produces the 18S, 5.8S, and 28S rRNAs, and the ribosomal subunits gradually take shape with the assembling ribosomal proteins. The cleavage reactions and all of the modifications require small nucleolar RNAs (snoRNAs) found in protein complexes (snoRNPs) in the nucleolus that are reminiscent of spliceosomes. The 5S rRNA is produced separately.

- The most common nucleoside modifications in eukaryotic rRNAs are, again, conversion of uridine to pseudouridine and adoMet-dependent nucleoside methylation (often at 2’-hydroxyl groups).
- These reactions rely on snoRNA-protein complexes, or **snoRNPs**, each consisting of a snoRNA and four or five proteins, which include the enzyme that carries out the modification.
- There are two classes of snoRNPs, both defined by key conserved sequence elements referred to as lettered boxes. The box H/ACA snoRNPs are involved in pseudouridylylation, and box C/D snoRNPs function in 2’-*O*-methylations.
- Unlike the situation in bacteria, the same enzyme may participate in modifications at many sites, guided by the snoRNAs.
- The snRNAs and snoRNAs not only facilitate RNA processing reactions but are themselves synthesized as larger precursors, and then processed.

tRNA processing

- **Transfer RNAs** Most cells have 40 to 50 distinct tRNAs, and eukaryotic cells have multiple copies of many of the tRNA genes. Transfer RNAs are derived from longer RNA precursors by enzymatic removal of nucleotides from the 5’ and 3’ ends.
- In eukaryotes, introns are present in a few tRNA transcripts and must be excised. Where two or more different tRNAs are contained in a single primary transcript, they are separated by enzymatic cleavage. The endonuclease RNase P, found in all organisms, removes RNA at the 5’ end of tRNAs.
- This enzyme contains both protein and RNA. The RNA component is essential for activity, and in bacterial cells it can carry out its processing function with precision even without the protein component. RNase P is therefore another example of a catalytic RNA, as described in more detail below. The 3’ end of tRNAs is processed by one or more nucleases, including the exonuclease RNase D.

- Transfer RNA precursors may undergo further posttranscriptional processing. The 3-terminal trinucleotide CCA(3’) to which an amino acid is attachedduring protein synthesis is absent from some bacterial and all eukaryotic tRNA precursors and is added during processing.
- This addition is carried out by tRNA nucleotidyltransferase, an unusual enzyme that binds the three ribonucleoside triphosphate precursors in separate active sites and catalyzes formation of the phosphodiester bonds to produce the CCA(3’) sequence. The creation of this defined sequence of nucleotides is there fore not dependent on a DNA or RNA template—the template is the binding site of the enzyme.
- The final type of tRNA processing is the modification of some bases by methylation, deamination, or reduction. In the case of pseudouridine, the base (uracil) is removed and reattached to the sugar through C-5’. Some of these modified bases occur at characteristic positions in all tRNAs.



Source- Lehninger Principles of Biochemistry; Nelson and Cox, 6th edition

Figure- Processing of tRNAs in bacteria and eukaryotes. The yeast tRNATyr (the tRNA specific for tyrosine binding, is used to illustrate the important steps.

The nucleotide sequences shown in yellow are removed from the primary transcript. The ends are processed first, the 5' end before the 3' end. CCA is then added to the 3' end, a necessary step in processing eukaryotic tRNAs and those bacterial tRNAs that lack this sequence in the primary transcript.

While the ends are being processed, specific bases in the rest of the transcript are modified. For the eukaryotic tRNA shown here, the final step is splicing of the 14 nucleotide intron. Introns are found in some eukaryotic tRNAs but not in bacterial tRNAs.

SUGGESTED BOOK AND REFERENCES

- LEHNINGER PRINCIPLES OF BIOCHEMISTRY; NELSON AND COX, 6TH EDITION