Zoo-342 Molecular biology
Lecture 9

Eukaryotic transcription

Ribosomes and Ribosomal RNA:

- **Ribosomes** are complexes in the cell where protein **synthesis occurs**.
- They are composed of **protein** and **rRNA** (ribosomal RNA).
- Ribosomes can make up as much as 25% of the mass of the cell.

Measuring rRNA components: the Svedberg Unit:

- Ribosomes and other small molecules and particles are measured in units that describe their rate of sedimentation during density gradient centrifugation in sucrose.
- This technique is developed by Svedberg, which gives information on the size and shape of the particle due to speed of sedimentation.
- This unit sedimentation is named the **Svedberg unit, S.**
- Ribosomes in all organisms are made of two subunits contain one or two rRNA molecule.
- These subunits are 50S for large subunit and 30S for the smaller one.
- The 30S ribosomal subunits are composed of 21 proteins and one of 16S rRNA.

- The 50S subunit is composed of 34 proteins and two of rRNAs, 23S and 5S (Figure 1).
- The rRNAs are functional molecules; they are required for the ribosome to translate a protein.

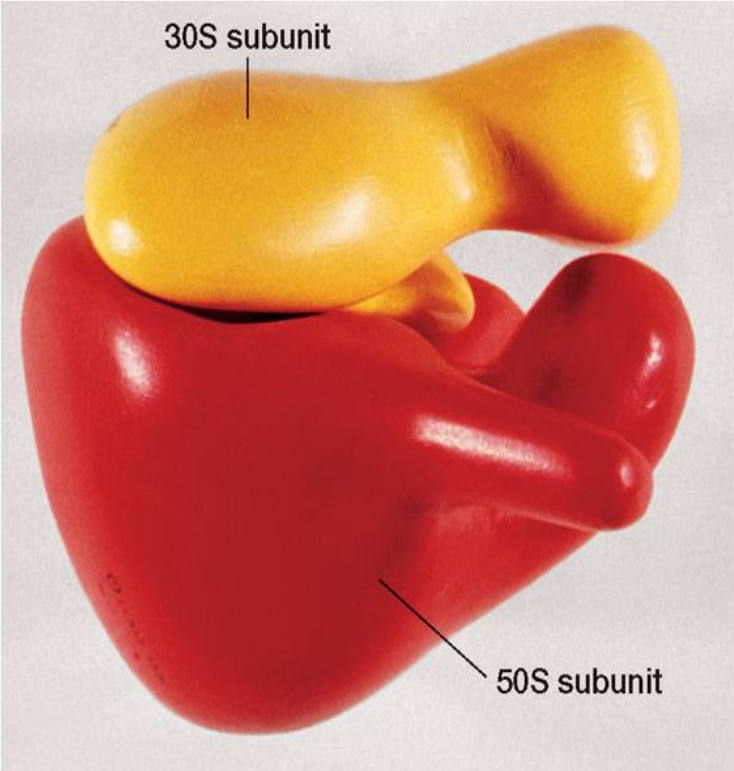

Figure 1: The *E. coli* ribosome

Transfer RNA:

- mRNA molecule carries the information transcribed from the gene (DNA) and binds to a ribosome.
- The transfer RNAs (tRNAs), which are attached to specific individual amino acids, interact with the mRNA in the ribosome.
- The genetic code in the mRNA is read in group of three adjacent nucleotides, called **codons**. The nucleotides of the mRNA codon can pair with the complementary three bases called the **anticodon** on a tRNA (Figure 2).
- Each different tRNA carries a specific amino acid is attached to its proper tRNA by one of a group of enzymes called **aminoacyl-tRNA**.
- The average of tRNA is about 80 nt long. Like rRNAs, the tRNAs are functional molecules and are not translated, which means that they also lack an ORF, translation initiation and termination codons, 5′ and 3′ UTRs.

- The aminoacyl-tRNA attaches the specific amino acid to the 3′-hydroxyl of the tRNA which is composed of the sequence 5′-CCA-3′. Adding of CCA sequence occurs when this enzyme recognizes the anticodon in the tRNA.
- In eukaryotes, a CCA trinucleotide sequence is added at the 3′ end by a nucleotidyl transferase enzyme.

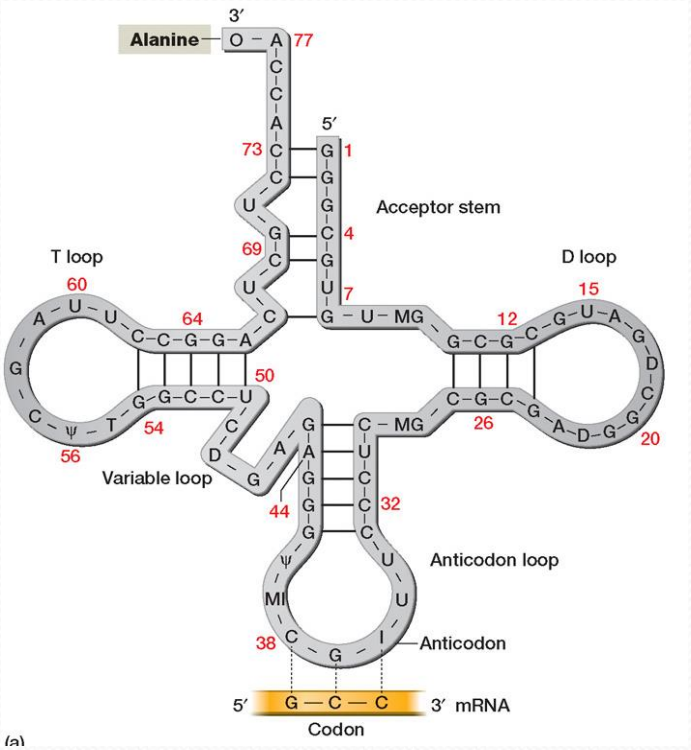

Figure 2: Structure of tRNA

Eukaryotic transcription:

- Many of the general features of transcription are the same in both prokaryotes and eukaryotes.
- Prokaryotes have a **single RNA polymerase** that transcribes **all the genes**, whereas **eukaryotes** have **three** major **RNA polymerase**, each one transcribes just **one gene**.
- The eukaryotes RNA polymerases are **I, II** and **III**.
- **RNA polymerase I** transcribes three largest **rRNA** genes (28S, 18S, and 5.8S rRNAs).
- **RNA polymerase II** transcribed **mRNAs**, (the RNAs that will serve as templates for translation), **snRNA** and **miRNA** genes.

- **RNA polymerase III** transcribes small genes, the 5S rRNA gene and the tRNA genes.
- The bacterial RNA polymerase **recognizes** and **binds** the promoter, but the eukaryotic RNA polymerases do not recognize the promoter sequence in the DNA.
- **Transcription factors** are proteins that bind to different DNA sequences within the promoter to form a complex that regulates RNA polymerase binding, recognize the DNA promoter for RNA polymerase.

Enzyme	Function
Prokaryotic	
RNA polymerase	Transcribes all genes
Primase	RNA primer synthesis during DNA replication
Eukaryotic	
RNA polymerase I	Transcribes large rRNA genes (nucleolar organizer genes)
RNA polymerase II	Transcribes protein-coding genes (mRNAs) and most snRNA and miRNA genes
RNA polymerase III	Transcribes 5S rRNA, U6 snRNA, and all the tRNA genes
Primase	RNA primer synthesis during DNA replication
Mitochondrial RNA polymerase	Transcribes genes in mitochondrial DNA
Chloroplast RNA polymerase	Transcribes genes in chloroplast DNA

- In eukaryotes, **mRNA** is synthesized in the **nucleus**, but **protein** synthesis take place in the **cytoplasm**.
- Transcription of the eukaryotic gene results in **a primary transcript** (pre-mature mRNA) that must be **modified** before it reaches the **mature** state.
- Primary transcript undergoes **three** different modification before transported into the cytoplasm:

A. 5′ Cap: 7-methyl-guanosine is added in the wrong direction 5′⇢ 5′ (Figure 3). This 5′ cap serves four purposes:

1. it protects the mRNA from a 5′⇢ 3′ degradation.
2. Essential for the proper removal of the **first intron** in the pre-mRNA.
3. It is important for the transport of the mRNA out of the nucleus.
4. The ribosome recognizes and binds to the cap.

B. 3′ Poly (A) tail: the poly (A) polymerase adds from 15 to over 250 adenines to the new 3′ end of the RNA.

The poly (A) tail serves three purposes:

A. Essential for the proper removal of the **last intron** in the pre-mRNA.

B. Enhances the ability of the mRNA to be translated.

C. Improves the stability of the mRNA in the cytoplasm.

C. Removes of introns:

- In eukaryotes, some segments of DNA are transcribed into RNA but never appear in the final mature RNA species.
- Theses sequences are called intervening sequences or introns.

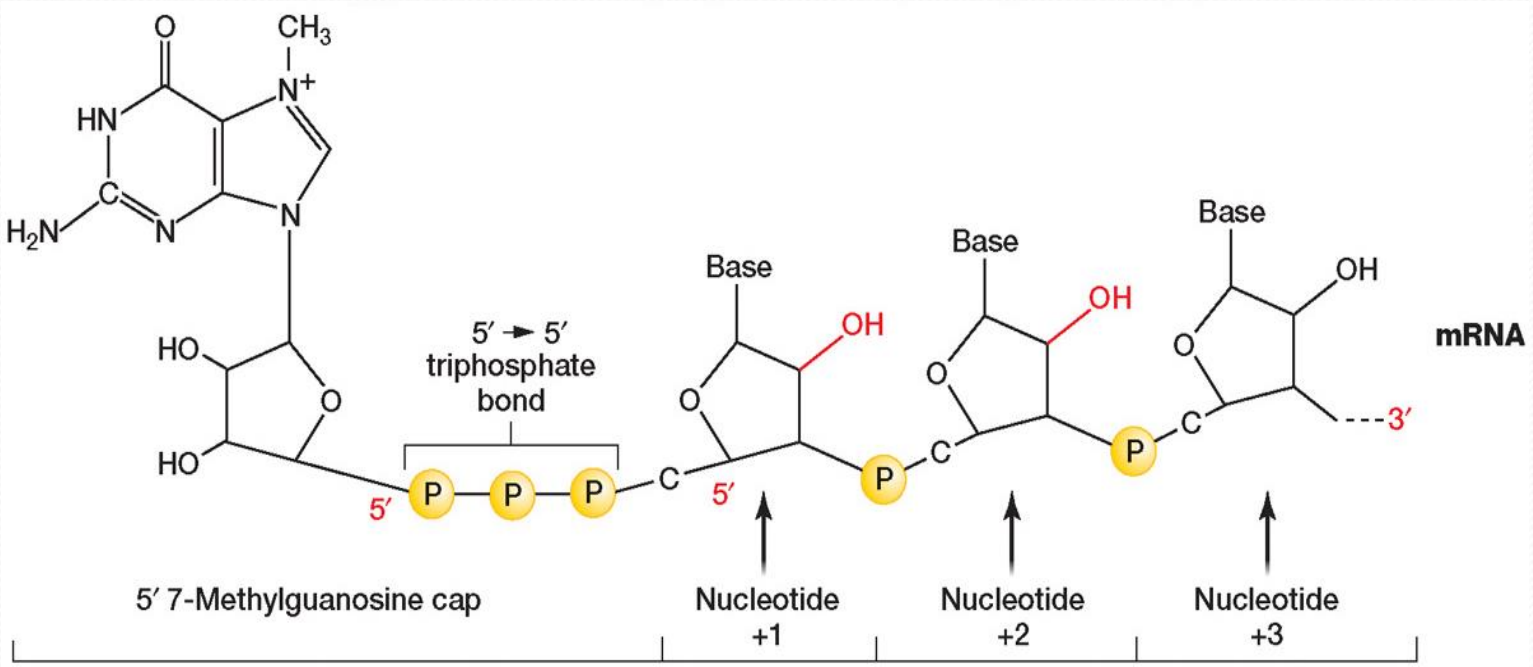

Figure 3: The 5′ cap on mRNA