- **Processing of mRNA**
- **Synthesis & Processing of ribosomal RNA (rRNA)**
- **Synthesis & Processing of tRNA**

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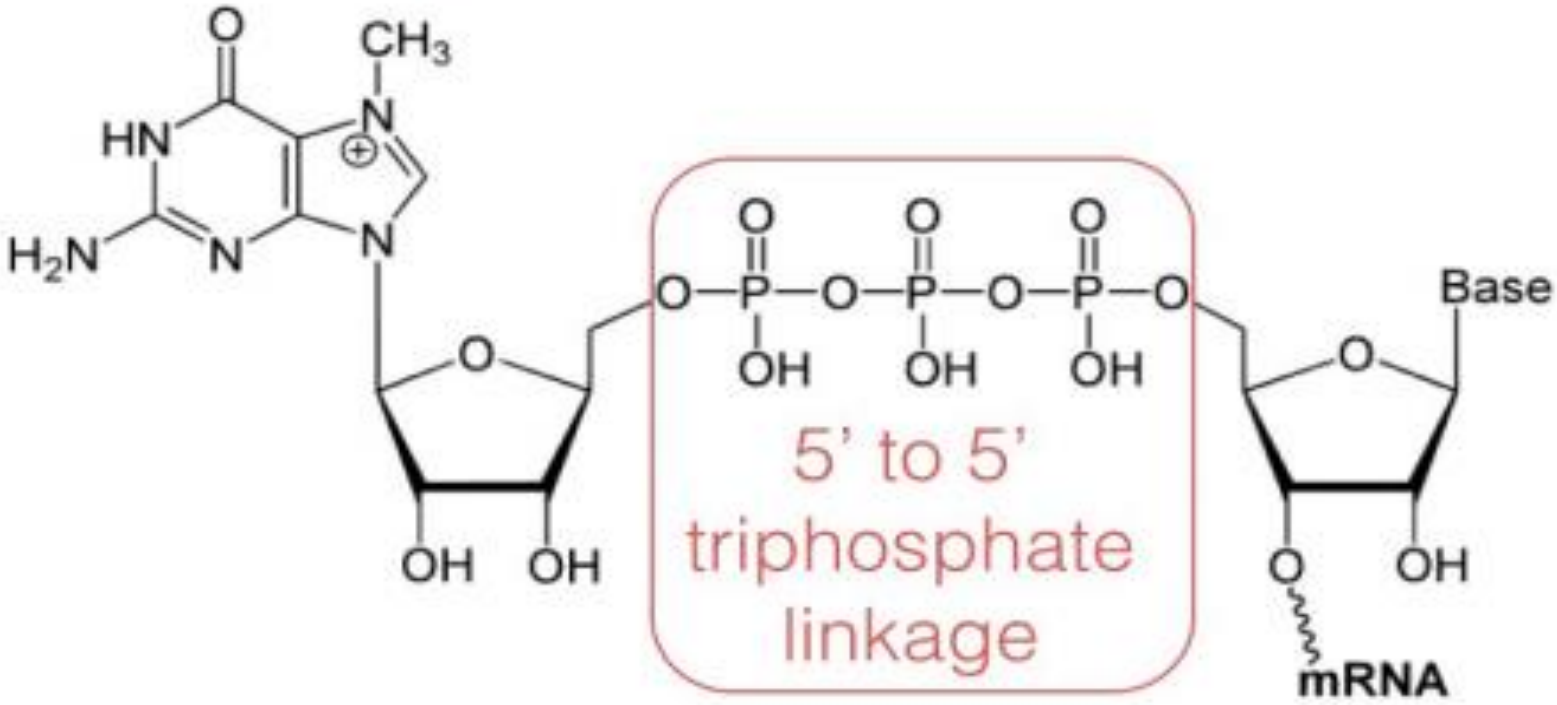
Processing of mRNA
(Post transcription modifications)

A. 5\-Capping :

- The RNA is capped as soon as it emerges from the RNA-exit channel of polymerase. This happens when the transcription cycle has progressed only as far as the transition from the initiation to elongation phases.
- The cap is a **7- methylguanosine triphosphate** attached to the 5\– terminal end of the mRNA **(which terminates at a triphosphate group).**
- One of the terminal phosphate groups is removed by **RNA triphosphatase**, leaving a bisphosphate group

- GTP is added to the terminal bisphosphate by mRNA **guanylyltransferase**, losing a pyrophosphate from the GTP substrate in the process. This results in the **unusual 5\to 5\ triphosphate linkage.**

7-meG cap



5' end of mRNA

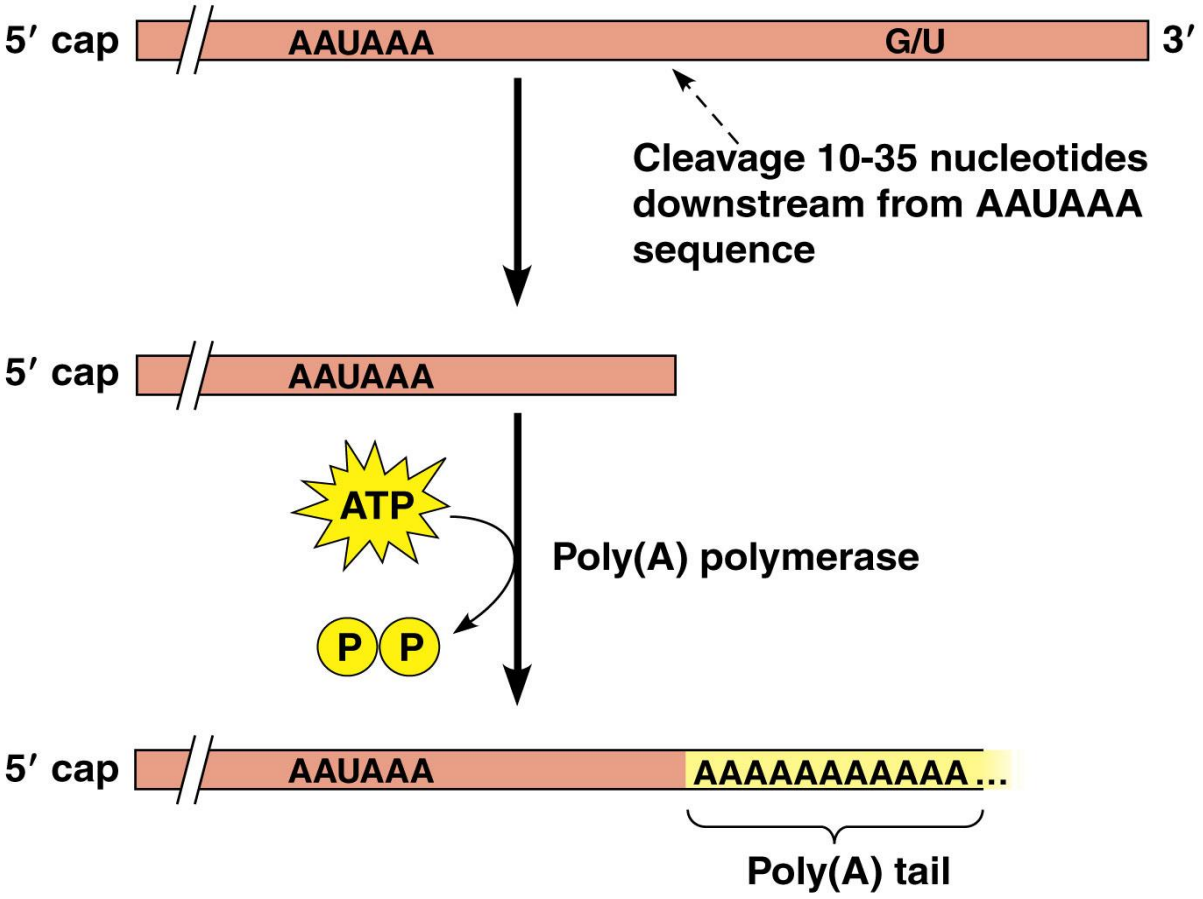
- **Methylation** of this terminal guanine is catalyzed by **guanine-7-methyltransferase**.
- S adenosylmethionine, SAM, (active methionine) is the source of methyl group. **Methylation of N-7 of guanine of the GTP cap occurs in the nucleus.**
- In the cytoplasm, methylation may occur at 2\ OH of ribose of some nucleotides, and at N-6 of adenine of some nucleotides **(secondary methylations)**

❖Importance of capping:

- It protects the 5\ end of the mRNA from **5\ exonuclease** enzyme.
- It helps its recognition by the ribosome.
- It helps the initiation of protein synthesis.
- Eukaryotic mRNA lacking the cap are not efficiently translated.
- Helps transport of mRNA to the cytoplasm.

B. Addition of poly(A) tail:

- ✓ The final RNA processing event, polyadenylation of the 3\ end of the mRNA, is intimately linked with the termination of transcription
- ✓ It is the addition of poly- A tail at the 3\ end of mRNA (100-200 A bases).
- ✓ This poly–A tail is not transcribed from DNA but added after transcription by the enzyme polyadenylate polymerase using ATP as a substrate.
- ✓ This occurs after the mRNA is cleaved 15-20 nucleotides downstream from the AAUAAA recognition sequence.
- ✓ The poly–A tail immediately binds several copies of a poly (A) binding proteins that protect mRNA against 3\ exonuclease.



❖Importance of poly-A tail:

- It stabilizes the mRNA & protects it from exonucleases enzymes. The length of poly (A) tail determines the half life time of mRNA.
- Increases the efficiency of translation.
- It facilitate their exit from the nucleus .After the mRNA enters the cytosol, the poly-A tail is gradually shortened.

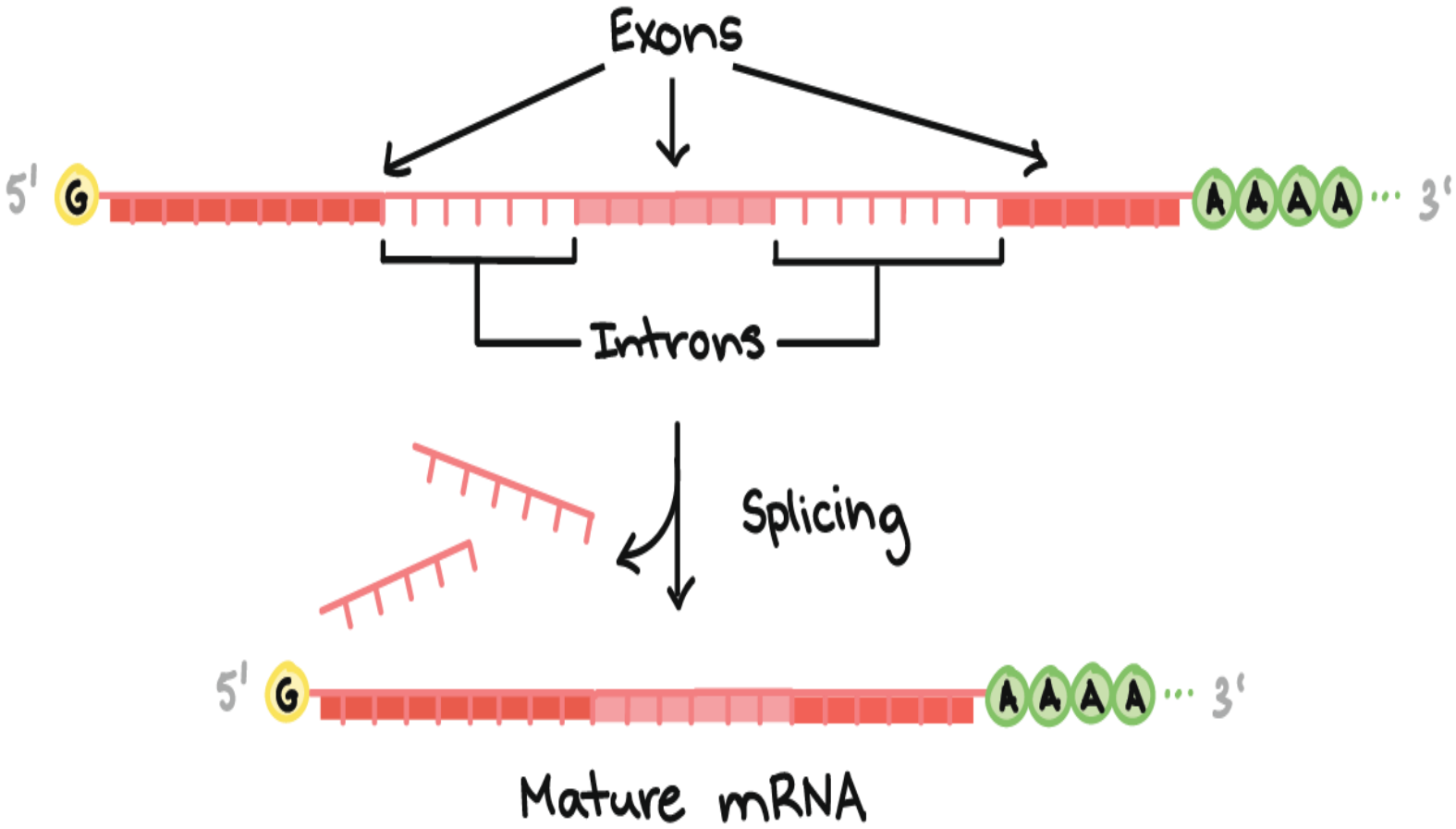
C. Removal of introns and splicing of exons :

- It means excision of introns and joining the ends of exons to **leave only the functional mRNA molecule.**

- This process occurs in the nucleus by the help of **the small nuclear ribonucleoproteins** (**snRNP, or snurps**) which are composed of small nuclear RNA (snRNA) and proteins.

- Snurps acting on mRNA are called **spliceosomes** . This is an example of catalytic RNAs or RNA enzymes, which are termed **ribozymes**.

- The sequence of bases at the exon-intron junction determines the site of splicing.


Exons
5'
G
A A A A
3'
Introns
Splicing
5'
G
A A A A
3'
Mature mRNA

- One type of β thalassemia appears to result from nucleotide change at the exon-intron junction leading to failure to remove introns, reducing the synthesis of the β globin chain.

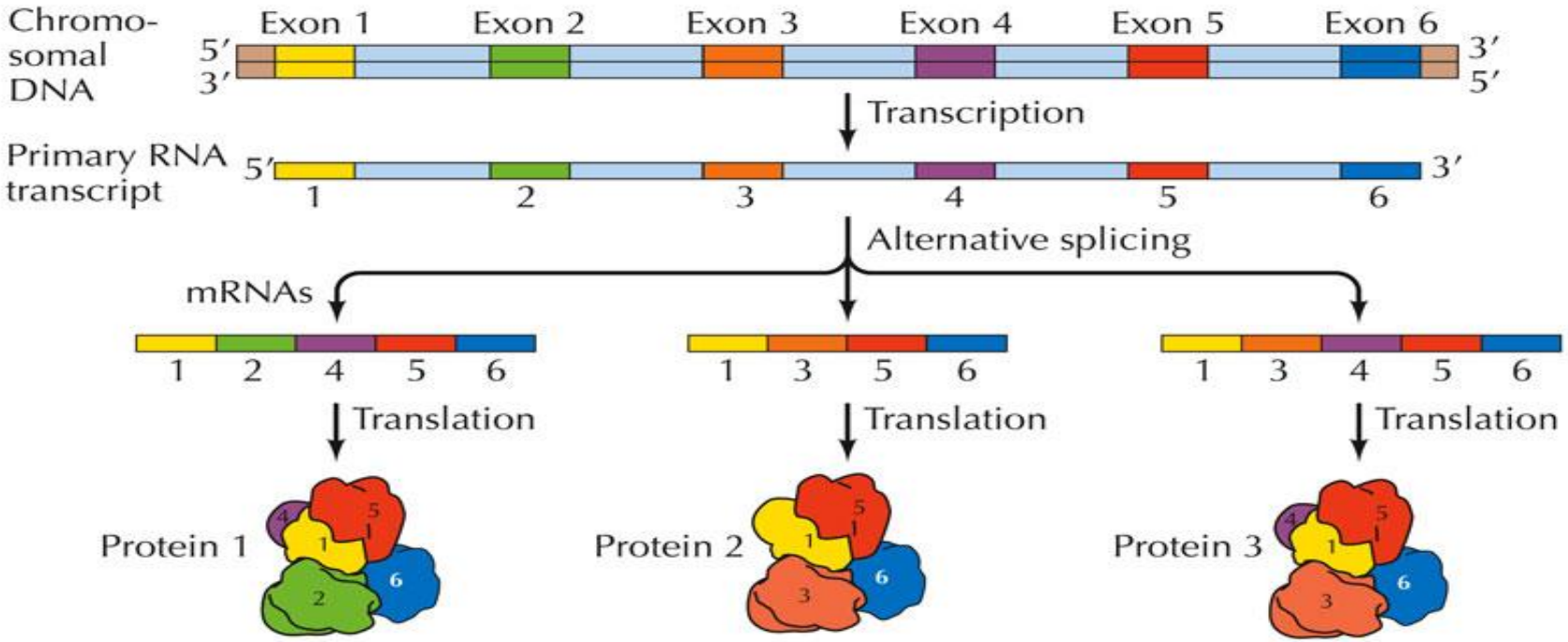
- Patients with systemic lupus erythematosis (SLE) produce antibodies against snRNP.

- Histone mRNAs (replication-dependent histones that are expressed during the S-phase of the cell cycle) do not contain introns.

- **Two advantages are suggested for having protein- coding genes organized as exons & introns:**

1- **Alternative splicing** may lead to the formation of different types or new types of mRNA molecules or proteins.

2- Also this will **decreases the possibility of effective mutations** ,(that result in protein abnormalities or disease), if it occurs at the regions of introns.



THE CELL, Fourth Edition, **Figure 5.5**

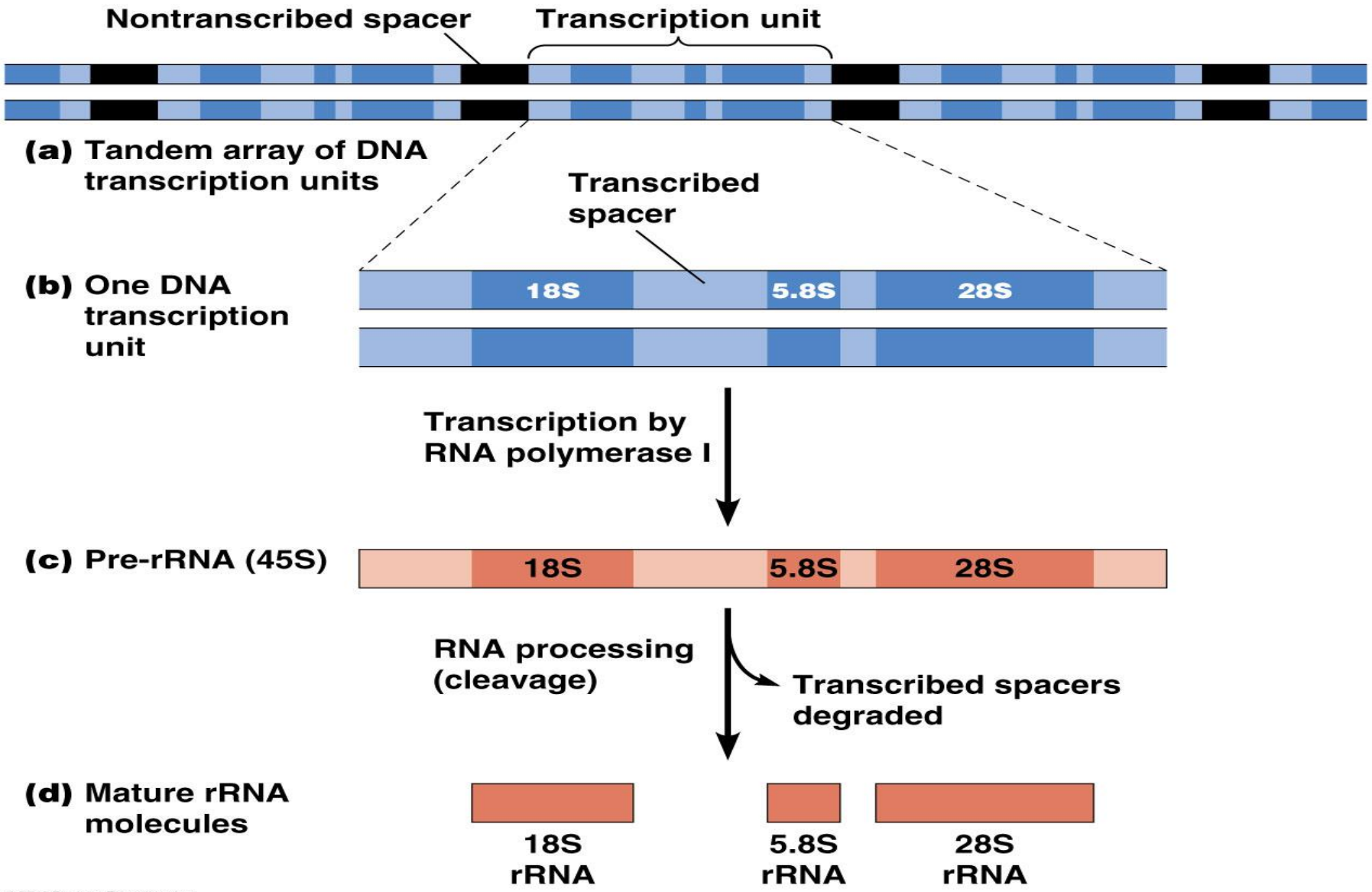
- **Alternative splicing**, or **differential splicing**, is a regulated process during gene expression that **results in a single gene coding for multiple proteins.**
- In this process, particular exons of a gene may be included within or excluded from the final, processed messenger RNA (mRNA) produced from that gene.
- Notably, alternative splicing allows the human genome to direct the synthesis of many more proteins than would be expected from its 20,000 protein-coding genes.

Synthesis & Processing of ribosomal RNA (rRNA)

- The primary transcripts of the mammalian rRNA include a 45S rRNA (pre-rRNA) & a 5S rRNA.
- The 45S rRNA is synthesized by RNA polymerase I then undergoes RNA processing in the nucleus which cleaves the precursor to release the mature 18S, 5.8S, 28S rRNA

- The 45S genes for 18S, 5.8S and 28S rRNA are typically **clustered together and tandemly repeated** (one copy each of 18S, 5.8S and 28S occur, followed by untranscribed spacer DNA, then another set occur and so on).
- **5S RNA gene** is transcribed by **RNA polymerase III**
- Hundreds of copies of these genes are present in every cell. This large number of genes is required to synthesize sufficient copies of each type of rRNA to form the 10^7 ribosomes required for each cell replication.

Unlike pre-rRNA genes, 5S-rRNA genes are transcribed by RNA polymerase III in the nucleoplasm outside of the nucleolus. **Without further processing**, 5S RNA diffuses to the nucleolus, where it assembles with the 28S and 5.8S rRNAs and proteins into large ribosomal subunits. When assembly of ribosomal subunits in the nucleolus is complete, they are transported through nuclear pore complexes to the cytoplasm, where they appear first as free subunits.



Synthesis & Processing of tRNA

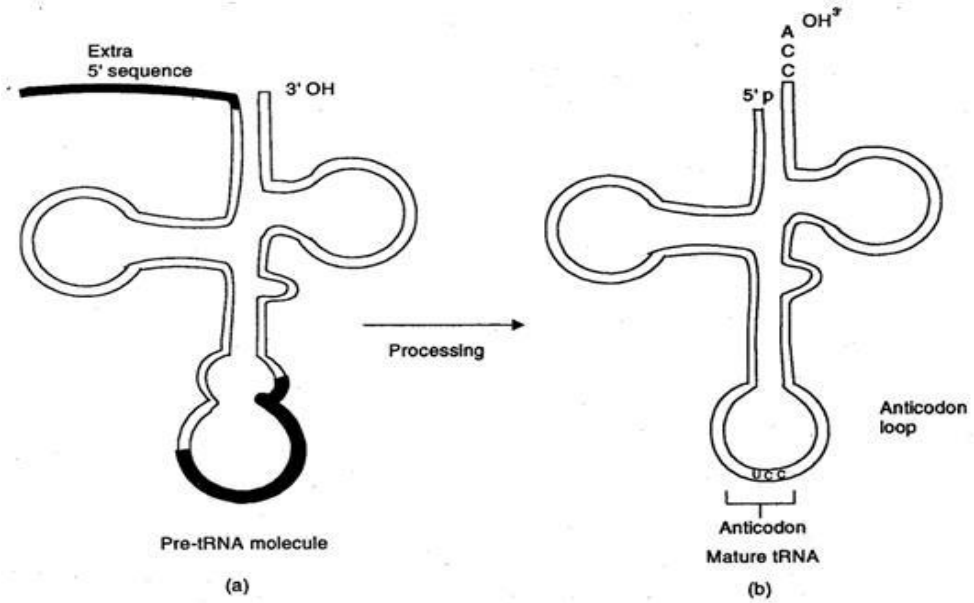
- Eukaryotic tRNA genes are all transcribed by **RNA polymerase III.**
- The primary transcript (pre-tRNA molecules) requires up to 4 different types of RNA processing steps as follows:

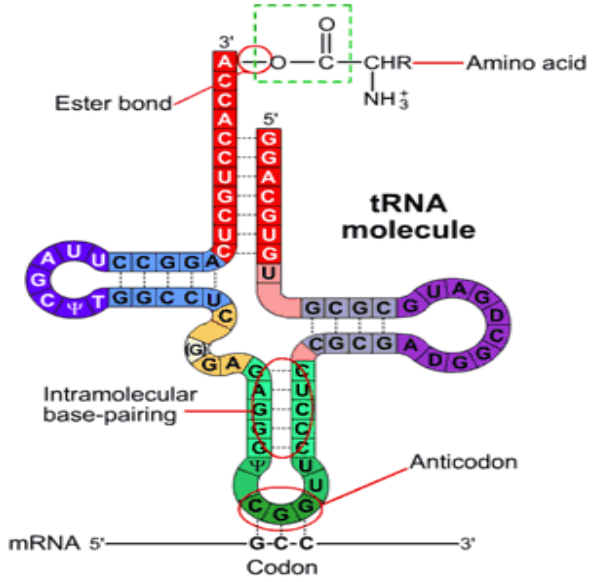
1- Addition of the CCA sequence at the 3\ end by the nucleotidyl transferase.

2- Excision of the nucleotide extension at the 5\end.

3- Excision of introns present in the anticodon loop.

4-Modification of some bases by methylation of uracil into thymine or reduction of uracil into dihydrouracil and formation of pseudouracil


Extra
5' sequence
3' OH
Processing
OH
A
C
C
5' p
Anticodon
loop
U C C
Anticodon
Pre-tRNA molecule
Mature tRNA
(a)
(b)


3'
O
C
CHR
Amino acid
NH3+
Ester bond
5'
tRNA
molecule
Intramolecular
base-pairing
Anticodon
mRNA 5'
3'
G-C-C
Codon