STRUCTURE AND FUNCTION OF MESSENGER RNA (mRNA)

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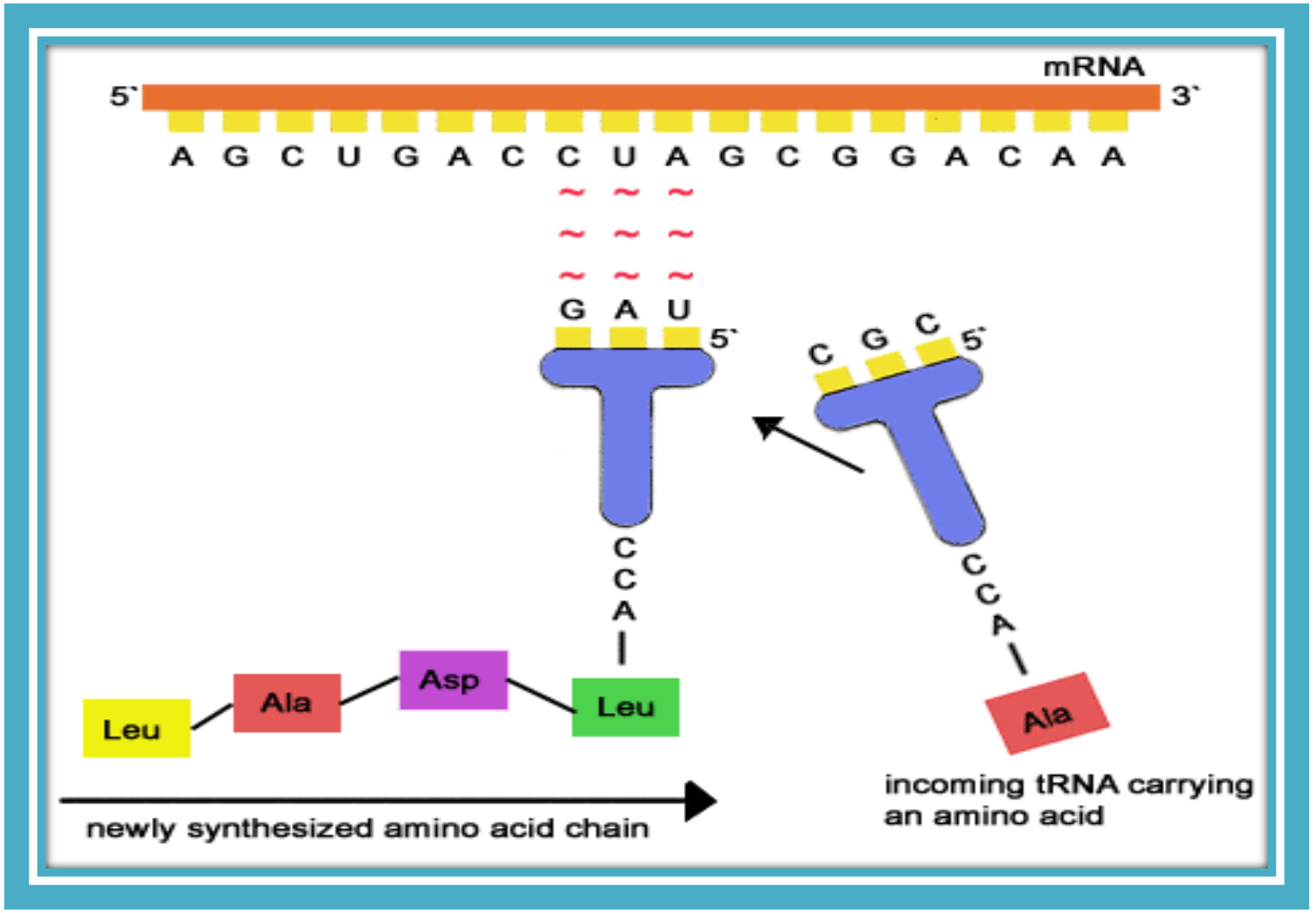
MESENGER RNA (mRNA)

- Messenger RNA is a single stranded RNA.
- The mRNA carries genetic information from DNA to Ribosome present in cytosol, where it is used as a template for protein synthesis.
- Messenger RNA is furnished with genetic code (Message) as sequence of codons .
- Each codon consists of triplet of bases .
- Genetic code is Universal, Comma less, non overlapping , degenerate and Non ambiguous.
- The message of mRNA is read consecutively in 5'→3' direction. Each Amino Acid is recognised by specific codon.
- mRNA comprises only about 5% of the total RNA in the cell but is most heterogenous type of RNA in cell varying in coding region.



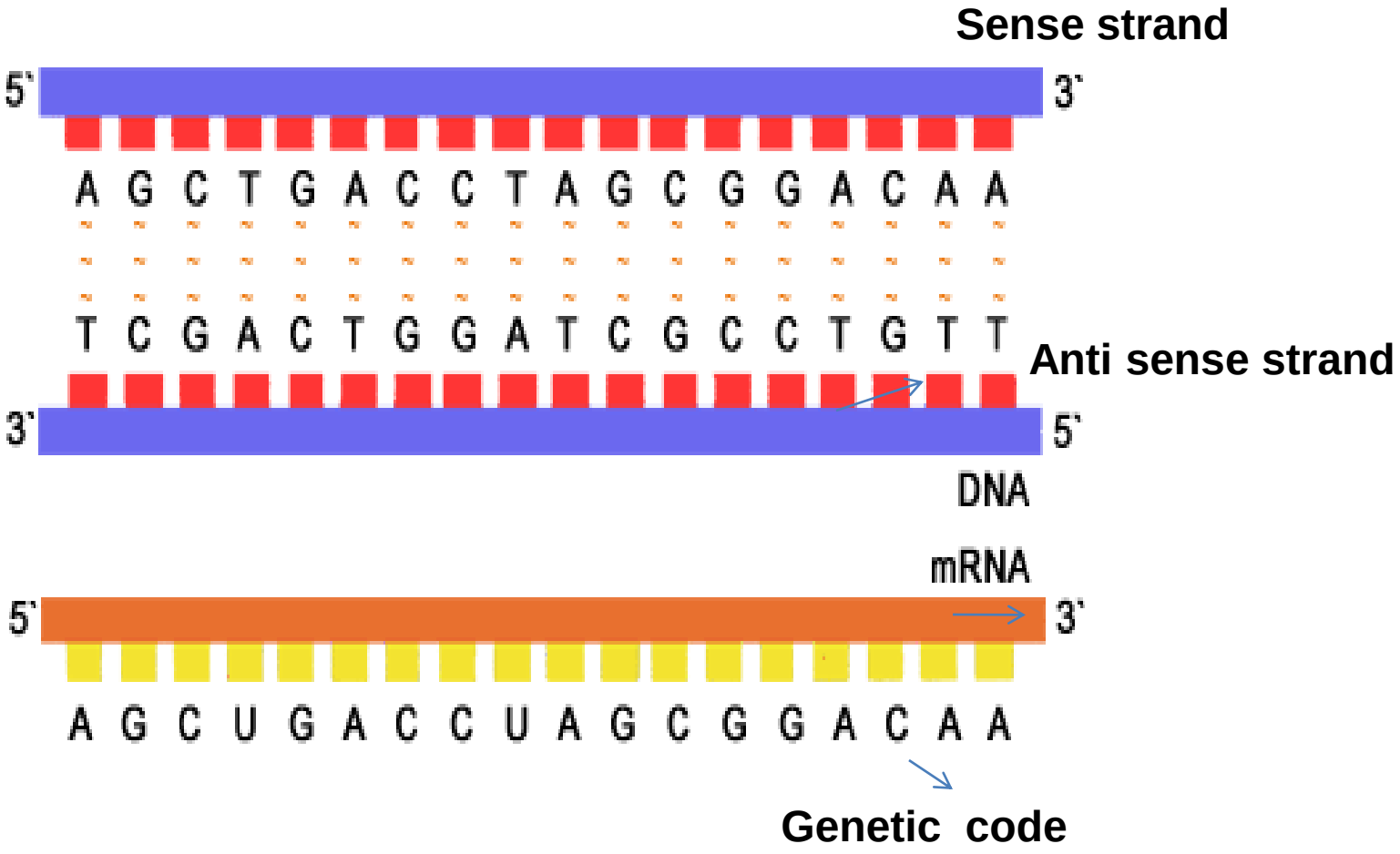
MESSENGER RNA FURNISHED WITH GENETIC CODE

Only a small amount of DNA is used for coding mRNA

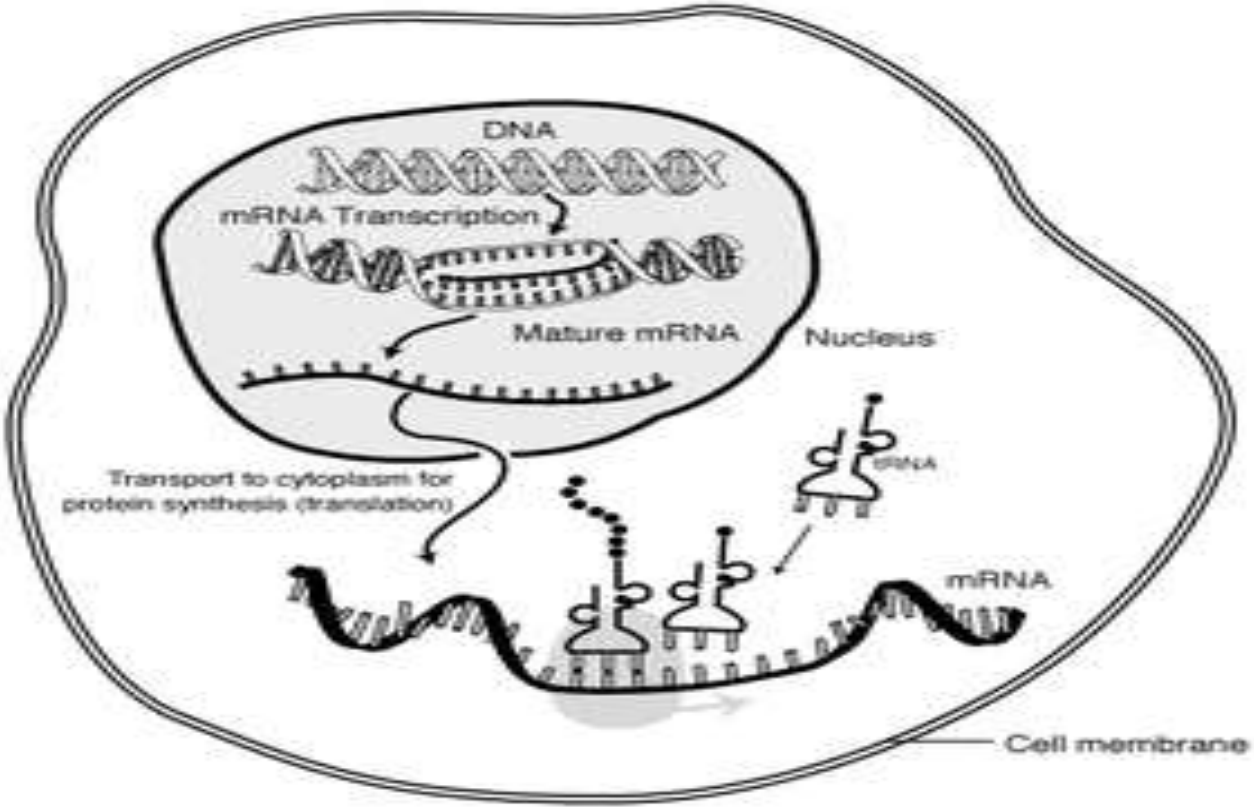
mRNA as other RNAs is synthesized by transcription using DNA template .

In prokaryotes there is only one RNA Polymerase enzyme for transcription of all the 3 RNAs as rRNA, mRNA and tRNA.

But in Eukaryotes there are three RNA Polymerase enzymes as RP I, RP II & RP III. RPII transcribes mRNA. The DNA double helix opens locally by RP and Transcription factors. During transcription only a segment of one strand is used as template (Antisense strand) . The RNA produced has base sequence similar to other strand known as sense strand



mRNA SYNTHESIS BY TRANSCRIPTION



Transcription & Translation

PROKARYOTIC MESSENGER RNA

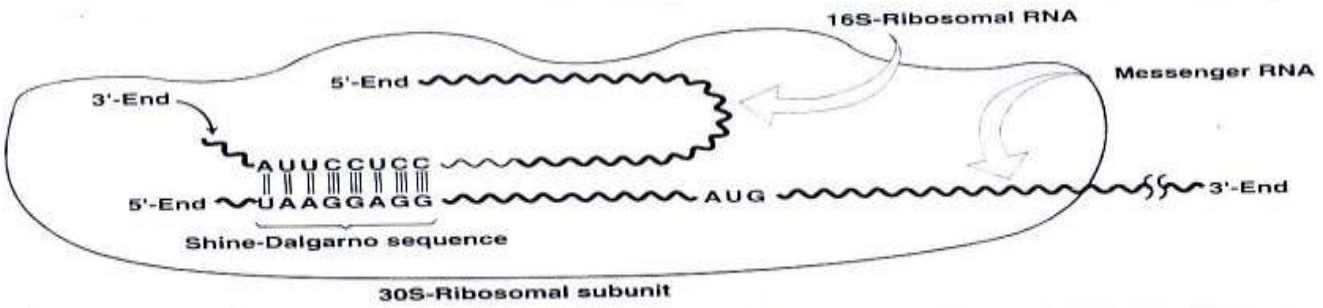
mRNA is short lived between several seconds to few minutes in bacteria but longer lived in eukaryotes possibly because it has to move out of nucleus to the site of protein synthesis the ribosome present in the cytoplasm

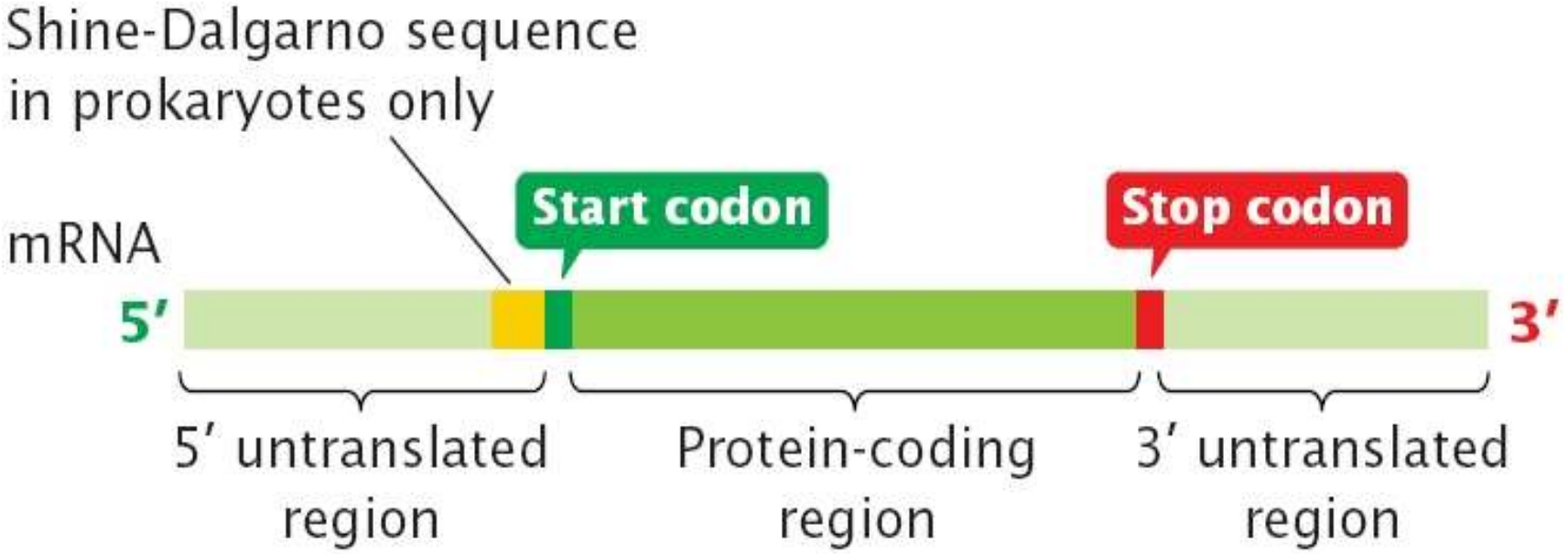
In Prokaryotic organisms ribosome can attach to mRNA, while it is being transcribed. In this situation translation begins at the 5' end of mRNA while the 3' end is still attached to DNA

In prokaryotes transcription and translation are coupled. Prokaryotic mRNA undergoes very little processing after transcription.

Prokaryotic mRNA has at its 5'end has a Shine Dalgarno Sequence which is rich in purine nucleotides and helps binding of mRNA to 30 S subunit of Ribosome by establishing H bonds with pyrimidine nucleotide sequence present on the 3' end of single mRNA (16 S) of smaller sub unit.

Features of Prokaryotic mRNA





General features of Eukaryotic mRNA are as follows

• A cap of ATP/GTP is added at the 5' end after transcription.G or A get methylated for it. Cap facilitates binding of mRNA with ribosome and provide stability

• Cap is followed by Non coding (UTR) region I ,which is used for binding of mRNA with smaller sub unit through its single rRNA.

• Initiator codon AUG follows UTR I which directs to introduce Methionine at first position.

• It is followed by coding region (1200-1500 nucleotides) and can code for polymerisation of 400 to 500 AAs

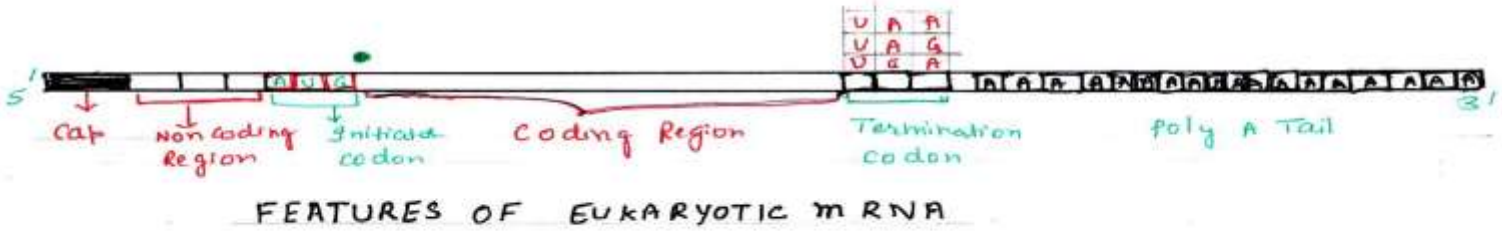
• Towards 3’ end one of the three termination codon provides signal for termination of Polypeptide chain.

• On the 3’end A poly Adenine (50-150-200) tail is added after transcription which also provides stability to mRNA

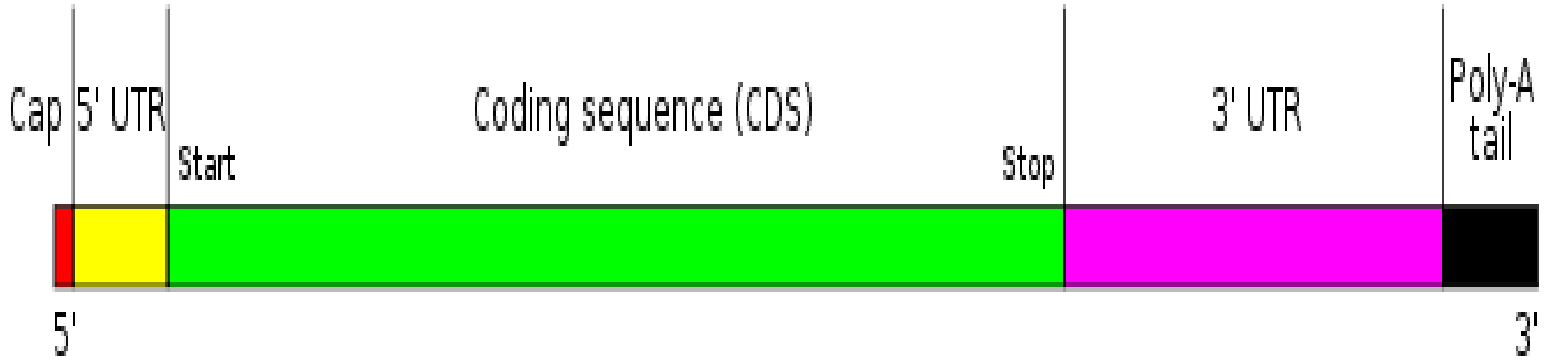
• Precursor Eukaryotic mRNA possess both coding (Exons) and Non coding (Introns) regions and require some processing to give mature functional form.

• In case where introns are present , by spilcing introns are removed and exons in sequence are ligated to form continuous stretch of coding region.

Features of Eukaryotic mRNA



The structure of a typical human protein coding mRNA including the untranslated regions (UTRs)



PROCESSING OF MESSENGER RNA IN EUKARYOTES

Eukaryotic mRNA is synthesized using a part of one strand of DNA as template by the RNA Polymerase II enzyme .

The primary transcript is known as heterogenous nuclear RNA (hnRNA)

The primary transcript before being translocated to the cytoplasm undergoes some modifications in the nucleus to give mature or functional mRNA

These modifications usually include

- **5' Capping**
- **Addition of a poly –A tail at 3' end**
- **Removal of Introns**

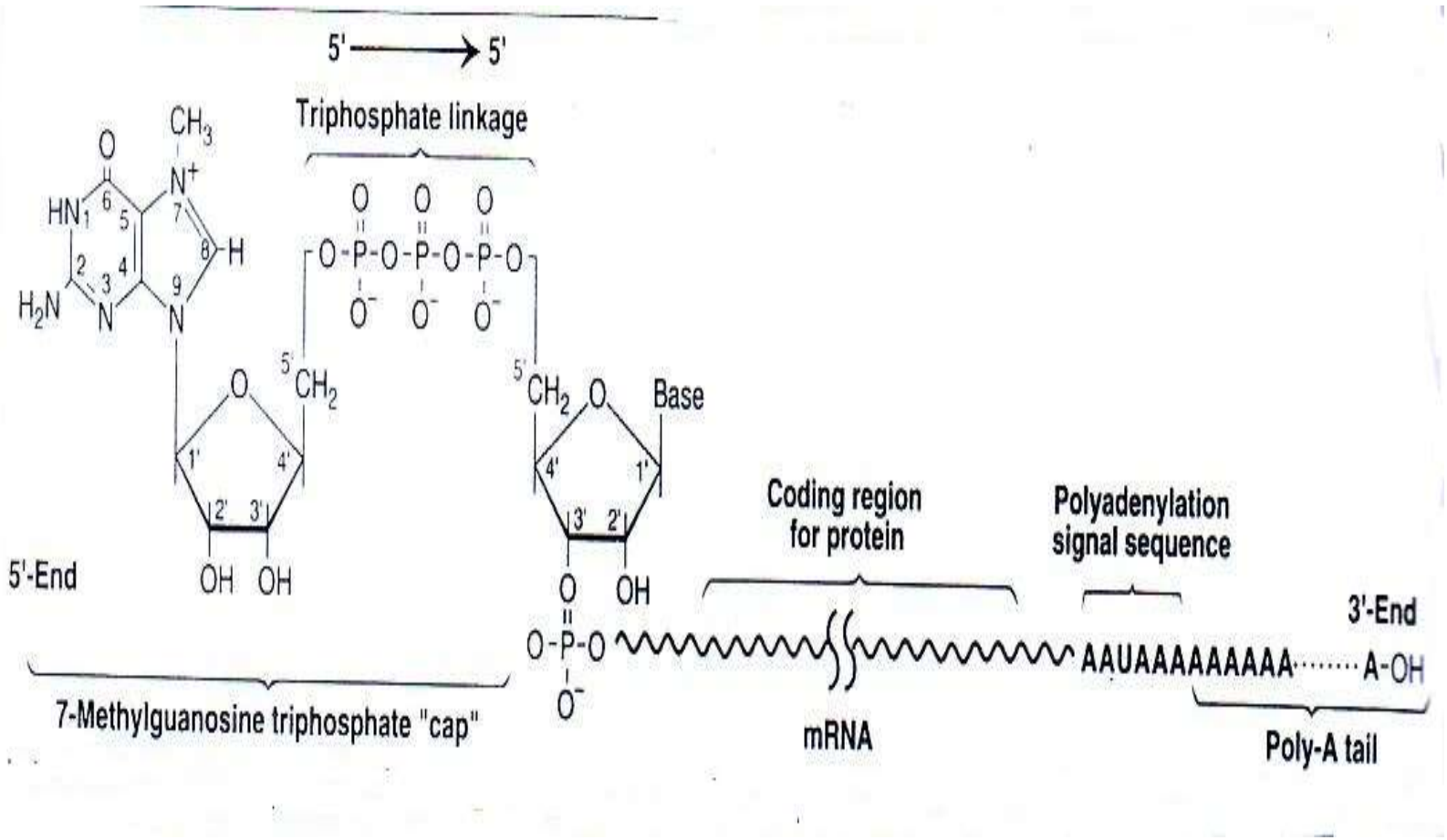
5’ Capping is the first processing step for hnRNA

The cap is a 7-methlyl-guanosine attached backwards to the 5’ terminal end of mRNA forming unusual 5’-5’ triphophate linkage catalyzed by nuclear enzyme guanylyltransferase.

Methylation of this terminal guanine occurs in the cytosol by guanine 7-methyltransferase.

Source of methyl group is S-adenosylmethionine.

The addition of 5’ cap permits initiation of translation and also provides stability to mRNA



Post Transcriptional modification of mRNA showing 7-methylguanosine cap and poly-A tail

Addition of Poly –A Tail

Most eukaryotic mRNAs have a chain of 40 to 200 Adenine nucleotides attached to the 3'end .

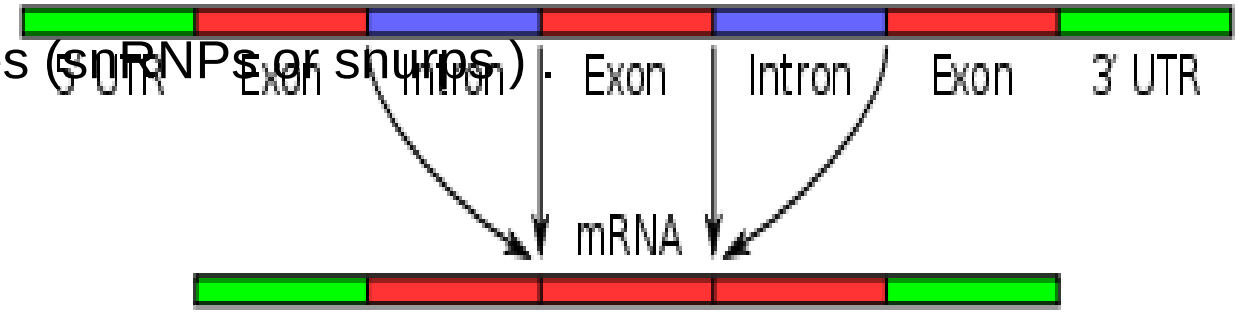
This Poly –A tail is not transcribed from the DNA , but is rather added after transcription by nuclear enzyme polyadenylate polymerase.

The Poly A tail also provides stability and facilitate the exit of mRNA from the nucleus.

After the mRNA enters the cytosol , the poly A tail is gradually shortened

Removal of Introns

Maturation of eukaryotic mRNA usually involves the removal of RNA sequences which do not code for proteins (Introns or Intervening sequences) from the primary transcript . The reamaining coding sequences (Exons) are spliced together to form mature mRNA with continuous coding stretch. The removal of Introns and splicing of exons is helped by small nuclear RNAs (snRNAs) in association with Proteins . These complexes are known as small nuclear ribonucleo protein particles (snRNPs or shurps) .



Simple illustration of an unspliced mRNA precursor, with two introns and three exons (top). After the introns have been removed via splicing, the mature mRNA sequence is ready for translation (bottom)

Mechanism of Exon splicing and removal of Introns by SnRNAP or Snrups

The term Intron (Non coding sequence) was introduced in 1978 by American Biochemist Walter Gilbert. Phillip Allen sharp and Richard J Roberts got the Nobel Prize in Physiology of 1993 for discovery of Introns.

Introns are spliced from primary transcript and exons are joined or ligated to form mature functional RNA

Splicing requires specialized RNAprotein complexes containing a class of eukaryotic RNAs called small nuclear RNA proteins (SnRNAPs) as U1,U2, U4,U5 & U6 are involved in splicing reactions. They are found in abundance in the nuclei of many eukaryotes.

The RNAs contain 106 (U6) to 189(U2) nucleotides and are complexed with proteins to form Snrups

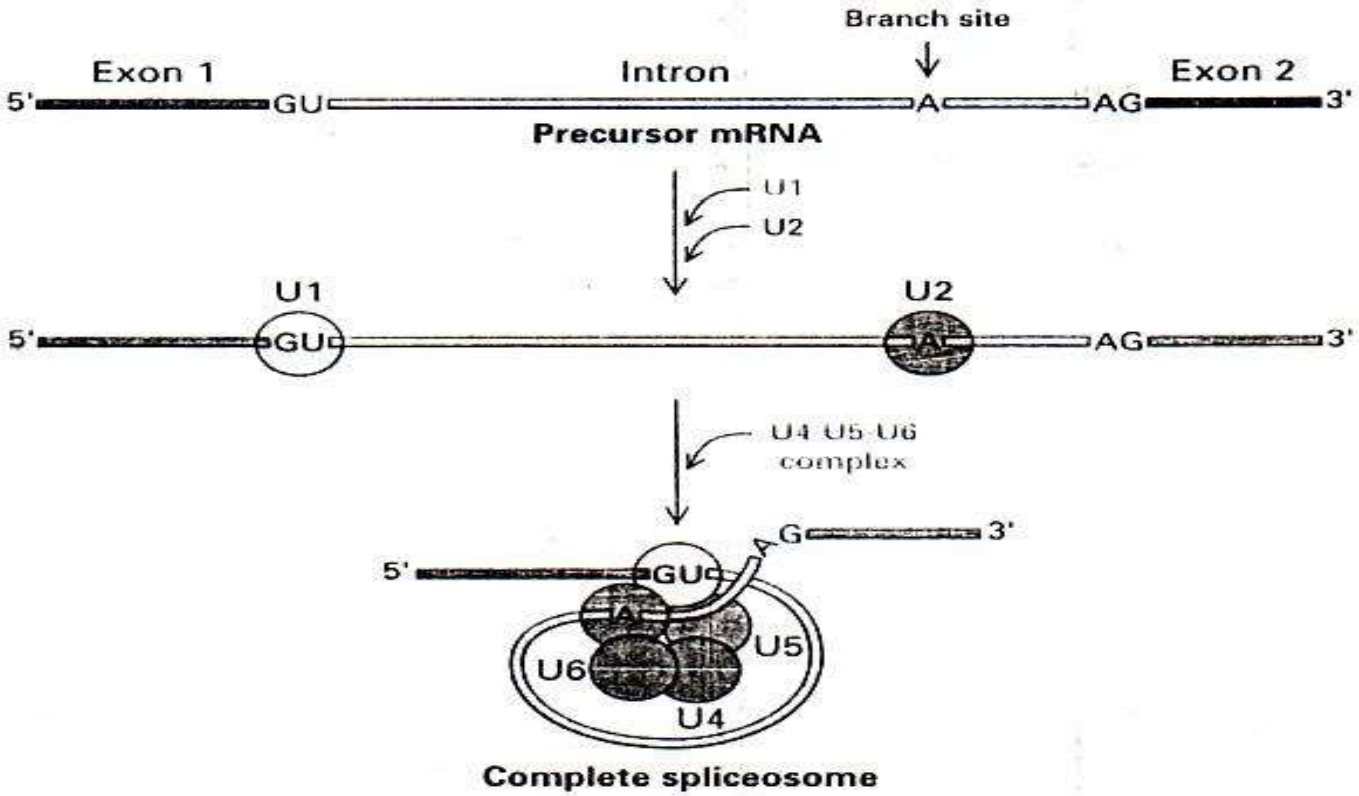


Figure – Pathway of spliceosome assembly U1 binds to 5'splice site and U2 to the branch site. U4,U5 & U6 then join to form complete spliceosome.

Spliceosomes are large (60S) dynamic assemblies of SnRNPs and precursor mRNA

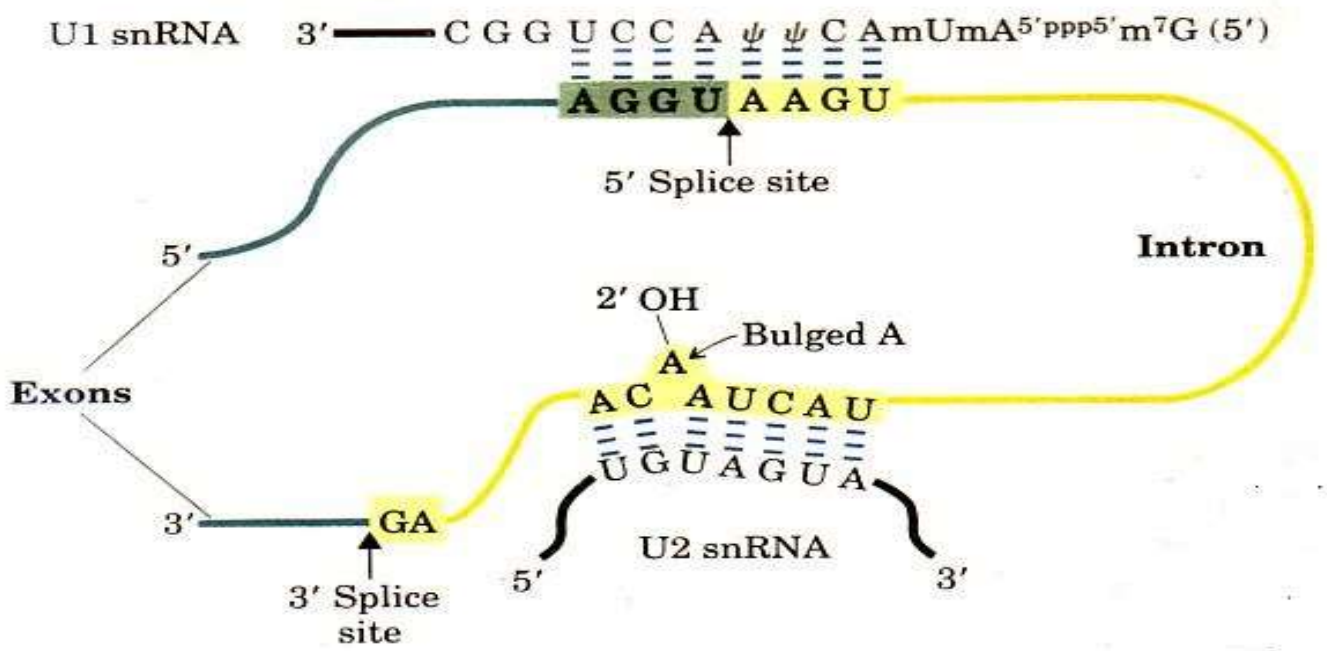
Splicing begins with the recognition of the 5'splice site by U1 SnRNP,which contains a complementary sequence to this splice site. Binding of U1 snRNP to an mRNA precursor protects a 15 nucleotide region at the 5'splice site from digestion.

U2SnRNP then binds the branch site in the intron. The consequent association of U1 and U2 brings together the 5' and 3' ends of the intron which enables U1 also to pair with the 3' splice site.This complex of U1 and U2 and the mRNA precursor is joined by a preassembled U4-U5-U6 complex to form a complete spliceosome.

Each intron starts with GU and ends with AG

They are recognized by components of splicing apparatus consisting of Sn RNPs(U1,U2,U4U5,U6).

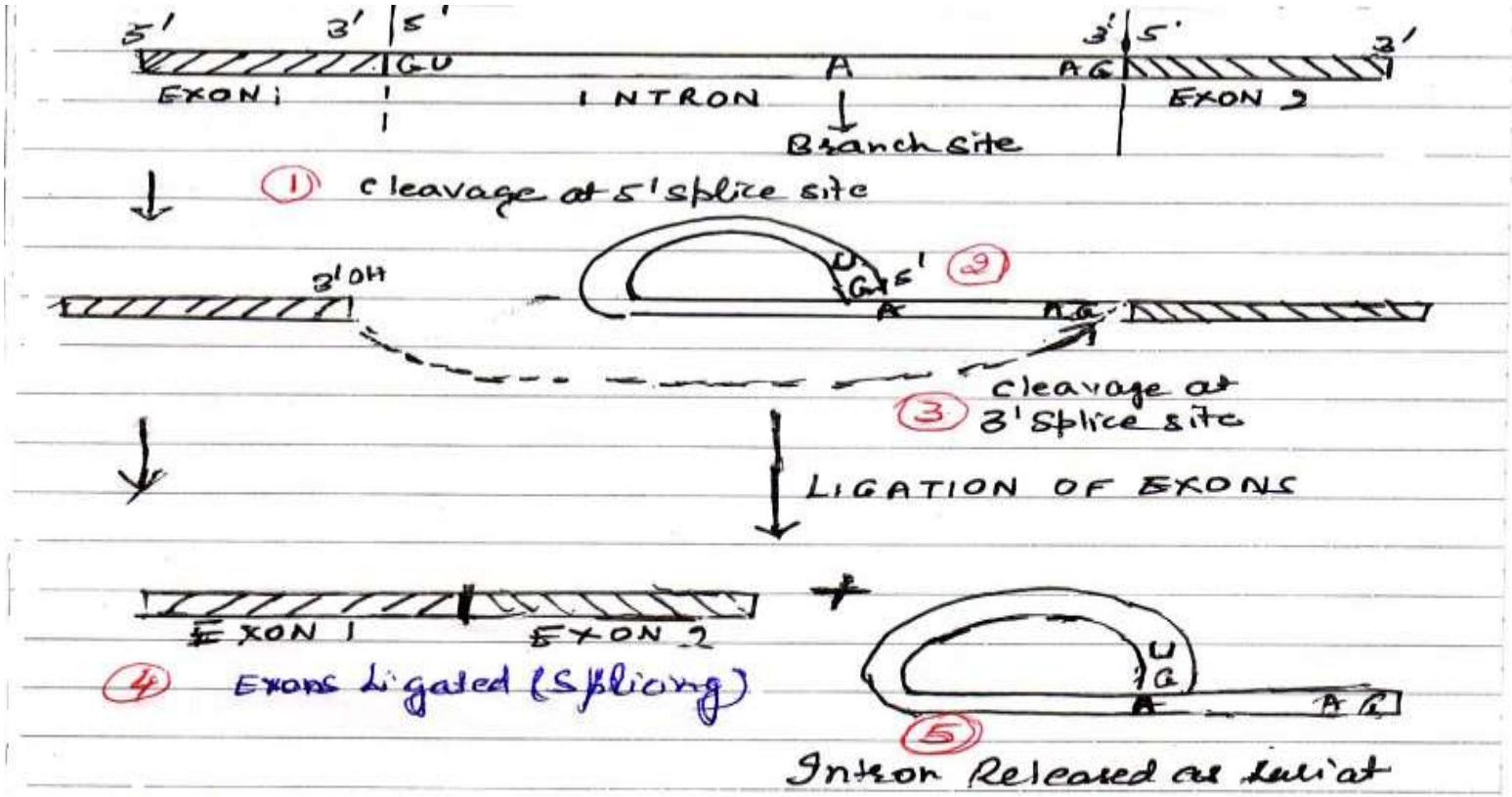
A complex called spliceosome is formed between 5'end GU and 3'end AG of intron. Energy is obtained from ATP. Intron is removed by breaking Phospho Diester Bonds (PDBs) on its both sides. The adjacent exons are



U1 has a sequence complementary near 5'splice site of nuclear mRNA intron and U1 SnRNP binds to this region of primary transcript.

Addition of U2,U4,U5 &U6 lead to formation of complex spliceosome within which actual splicing reaction occurs.

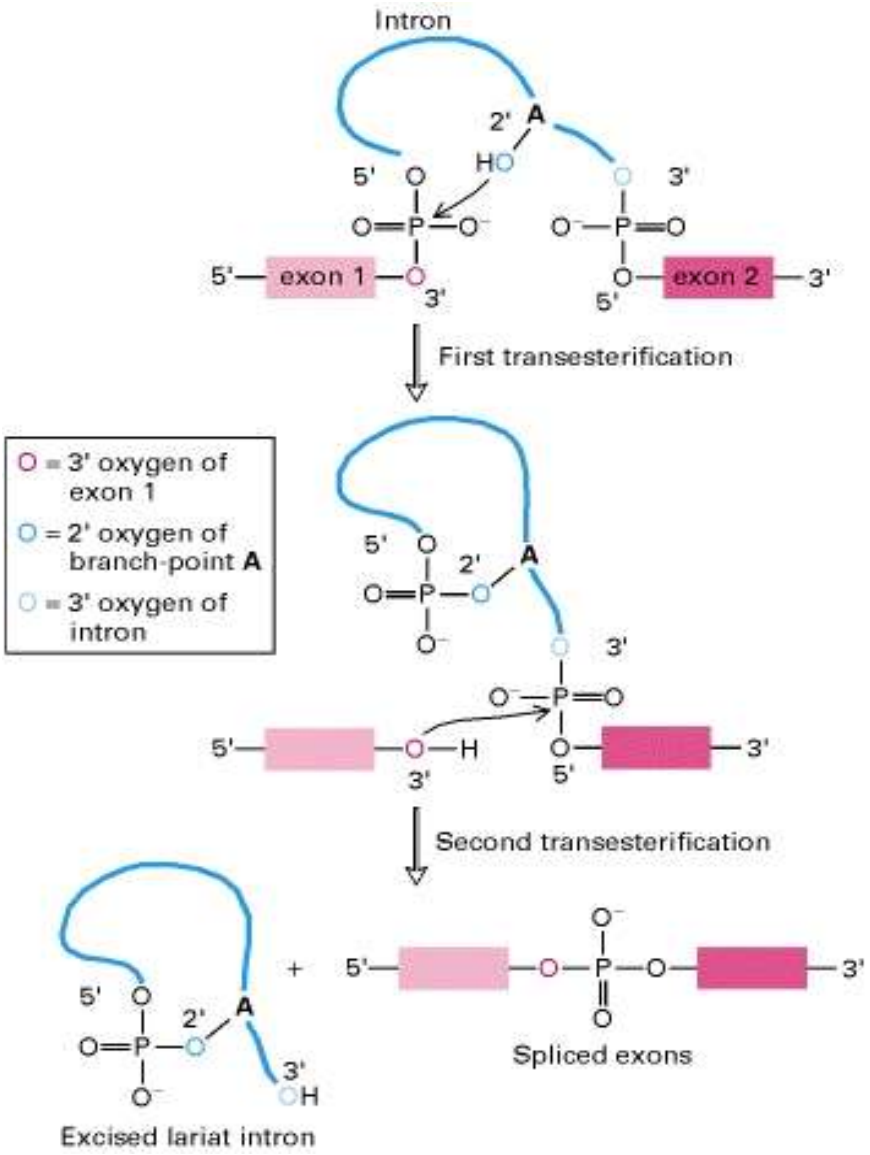
ATP is required for assembly of spliceosome but not for splicing reaction



Spliing of mRNA (Pre) —

Step 1 - cleavage at 5' splice site, 5' end of intron joins branch site, forms lariate (Bending)

Step - 2 - The free 3'OH of exon I (Nucleophile) attacks 3' Splice site of Intron Exons joined, Intron Released

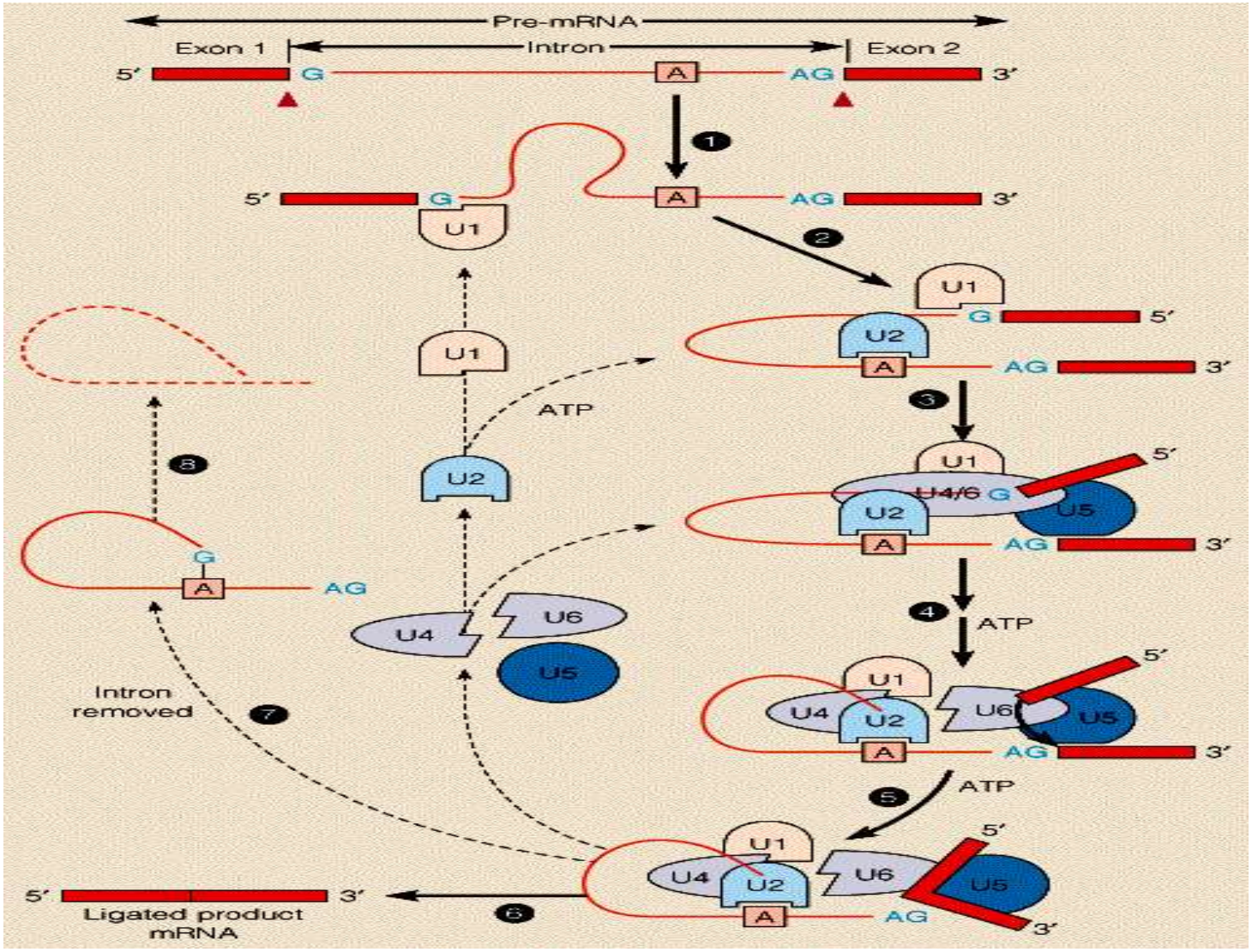


Splicing of exons in pre-mRNA occurs via two transesterification reactions

In the first reaction, the ester bond between the 5'phosphorous of the intron and the 3'oxygen(OH) of exon 1 is exchanged for an ester bond with 2'OH of the branch site A residue.

In second reaction, the ester bond between the 5'phosphorous of exon 2 and the 3'oxygen (OH) of the intron is exchanged for an ester bond with the 3'oxygen of exon 1, releasing the intron as a lariat structure and joining the two exons. Arrows indicate reaction of activated

A proposed mechanism of splicing



The pre-mRNA (precursor mRNA) plus assorted snRNPs assemble and disassemble a spliceosome, which carries out the splicing reaction. The various snRNPs are designated U1, U2, and so on. In step 1 U1 is bound, followed by U2 binding (step 2). Factor U4/U6 and U5 then bind (step 3) and cleavage and transfer then occur (steps 4 and 5). The spliceosome dissassembles, releasing the joined exons (6) and the excised intron as a lariat structure (7). The excised intron is ultimately degraded into oligonucleotides (step 8).

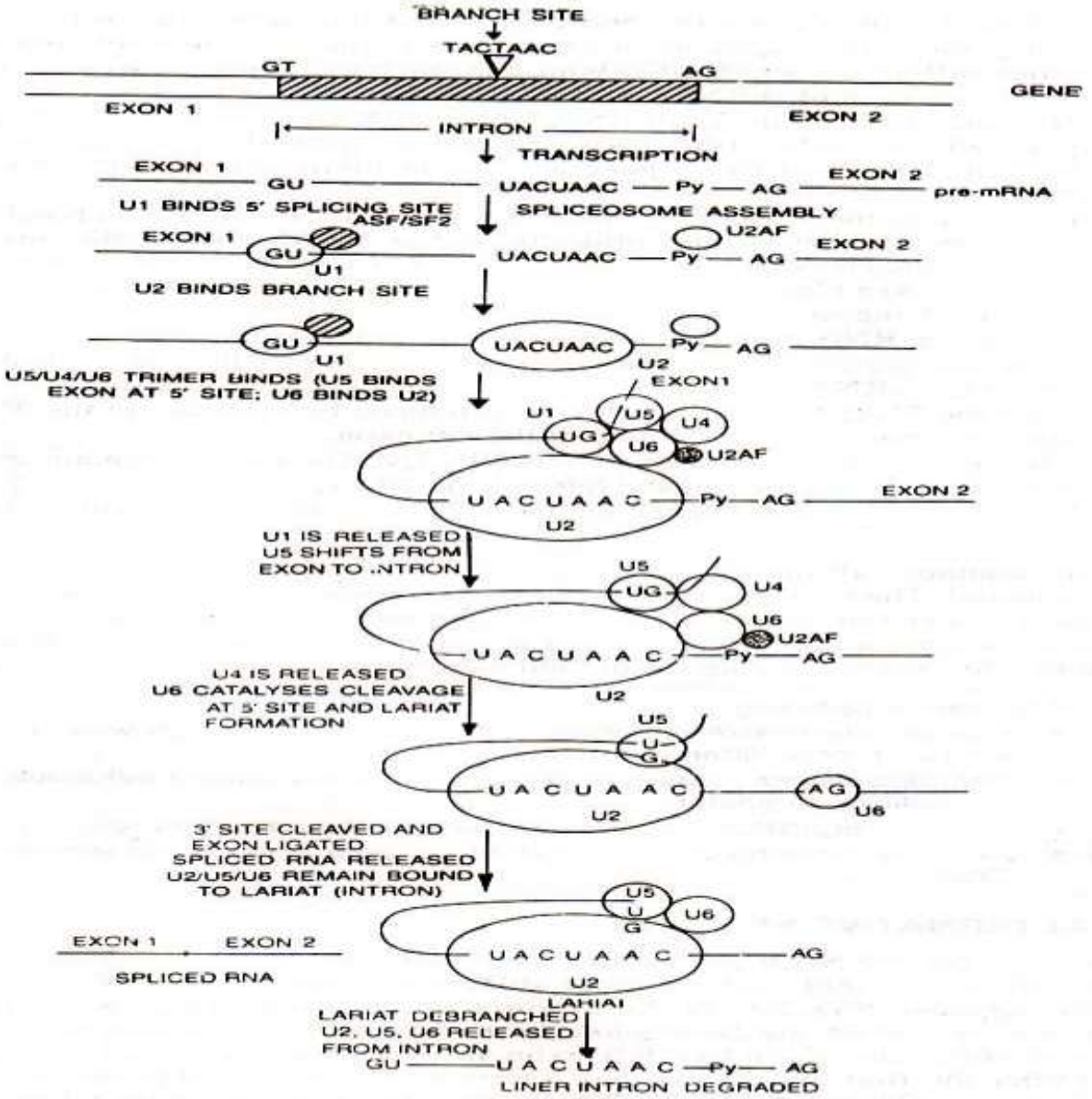
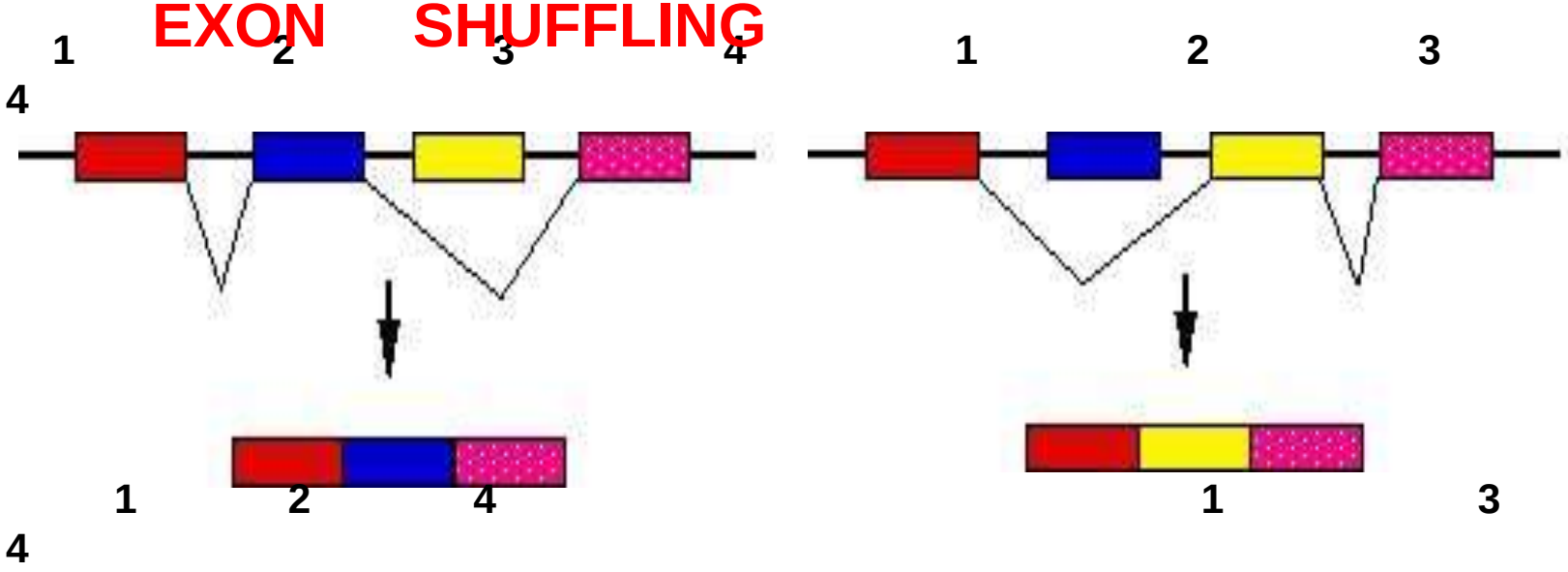


Fig. Formation of spliceosome and splicing of pre-mRNA in eukaryotic nuclei. Py represents the pyrimidine of branch site.

EXON SHUFFLING



mRNA may have different exons interrupted by introns. During splicing the exons are joined with each other and Introns are removed. During splicing which exons are retained in mRNA, decide the type of protein formed. Some genes have exons that can be exchanged in a process known as exon shuffling.

For example a gene with four exons might be spliced differently in two different cell types (In Cell type 1 exons 1,2 & 4 and in Cell type 2 exons 1,3 & 4 will be spliced) so different protein types will be produced by two cell types and it is known **Exon shuffling.**

Life span of mRNA or mRNA longevity

(Regulation of RNA Longevity)

mRNA transcribed by different genes show diversity in their life span or longevity. mRNA which has longer lifespan is able to produce more copies of polypeptides by involving many ribosomes subsequently and may form a polyribosome structure. Life span of mRNA regulates how much of a particular polypeptide is produced .

The information for lifespan is found in the 3' UTR. The sequence AUUUA, when found in the 3' UTR, is a signal for early degradation (and therefore short lifespan). The more times the sequence is present, the shorter is the lifespan of the mRNA.

This varies the amount of gene product that is produced (as a mRNA that's degraded quickly won't express much protein).

Some Messenger RNAs can last for a long time.

For example, mammalian red blood cells even after ejecting their nucleus continue to synthesize hemoglobin for several months.

Messenger RNA has non-coding nucleotides at either end of the molecule. These segments contain information about the number of times mRNA is translated before being destroyed by ribonucleases. Hormones stabilize certain mRNA transcripts.

Example Prolactin is a hormone that promotes milk production because it affects the length of time the mRNA for casein (a major milk protein) remains active.

Degradation and Half life of mRNA

Messenger RNA is transcribed as a complementary copy of DNA that will eventually be translated into an amino acid chain.

In eukaryotic cell mRNA travels from the nucleus to the cytoplasm for translation. Because mRNA is constantly being degraded in the cytoplasm, it is synthesized at a much higher rate than necessary for maintenance of a steady amount.

Djfferent mRNAs in a cell have different life time.

In bacterial cells individual mRNA can survive from a few seconds to more than a hour.

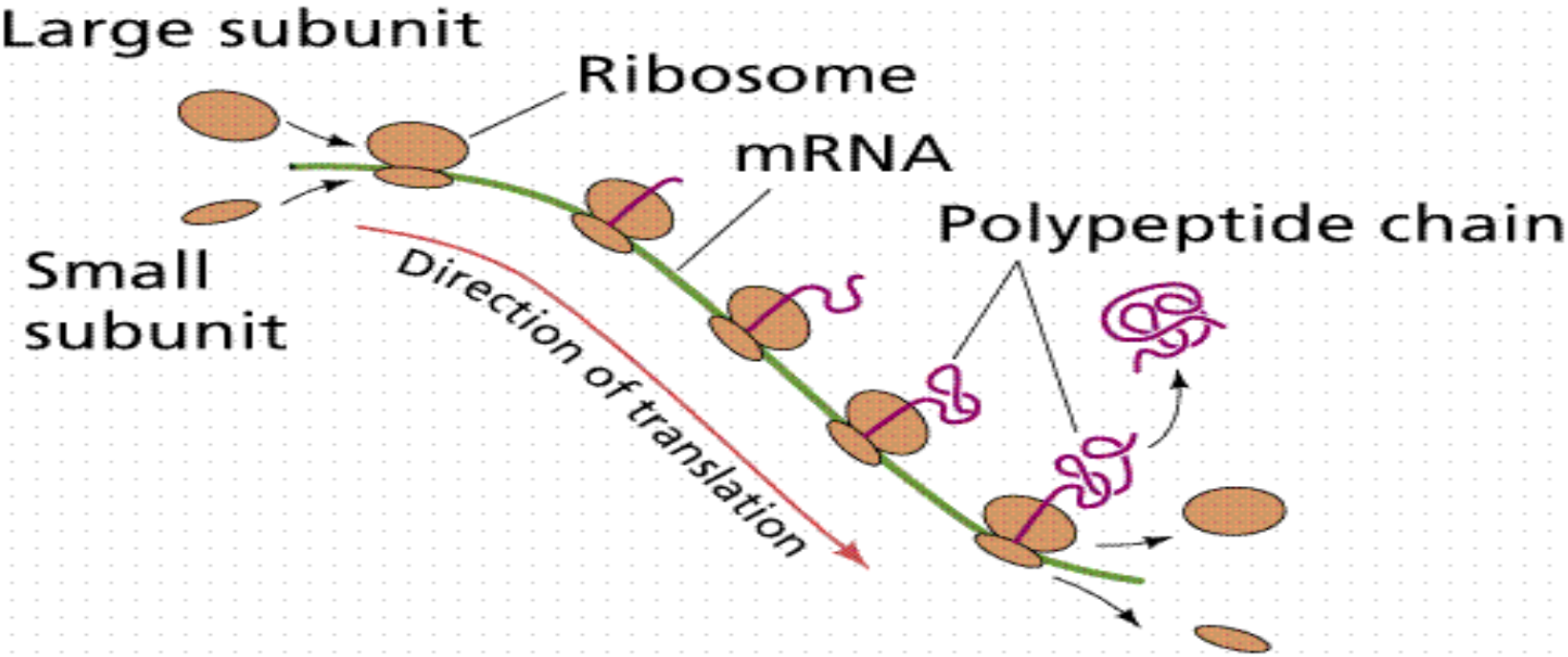
Short half life of mRNA enables a cell to produce altered protein in response to changing needs.

mRNAs with longer survival are able to produce more polypeptide chains of the same type.

There are different mechanisms for degradation of mRNA in Prokaryotes

Eukaryotic mRNA acquire a longer stability due to addition of 5’Cap and 3’ Poly-A tail

In some instances many ribosomes can bind to same mRNA successively ,all producing similar polypeptide chain only differing in length. The structure is known as polysome or polyribosome . mRNA is us



MONOCISTRONIC AND POLYCISTRONIC mRNA

A mRNA may be monocistronic if it contains single Open Reading Frame (ORF) for translating only one polypeptide chain as it occurs in most of eukaryotic cells.

In most of Prokaryotic cells mRNAs are polycistronic, are involved in translation of many different but related polypeptide chains . Such mRNAs are formed by transcription of many structural genes forming an Operon . The transcription of such genes is regulated as a unit.

In polycistronic mRNA each ORF has its own Initiation and Termination codons along with coding region.

Most of the mRNA found in Bacteria and Archaea are polycistronic.

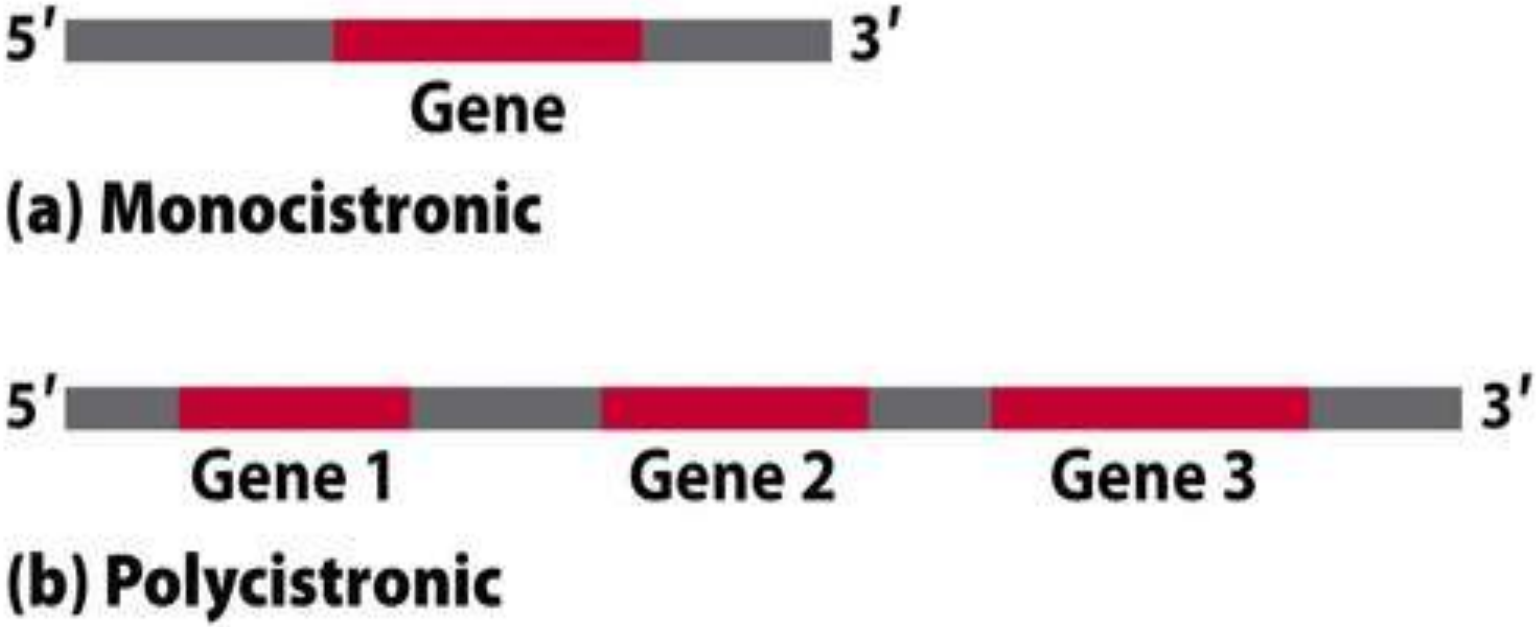
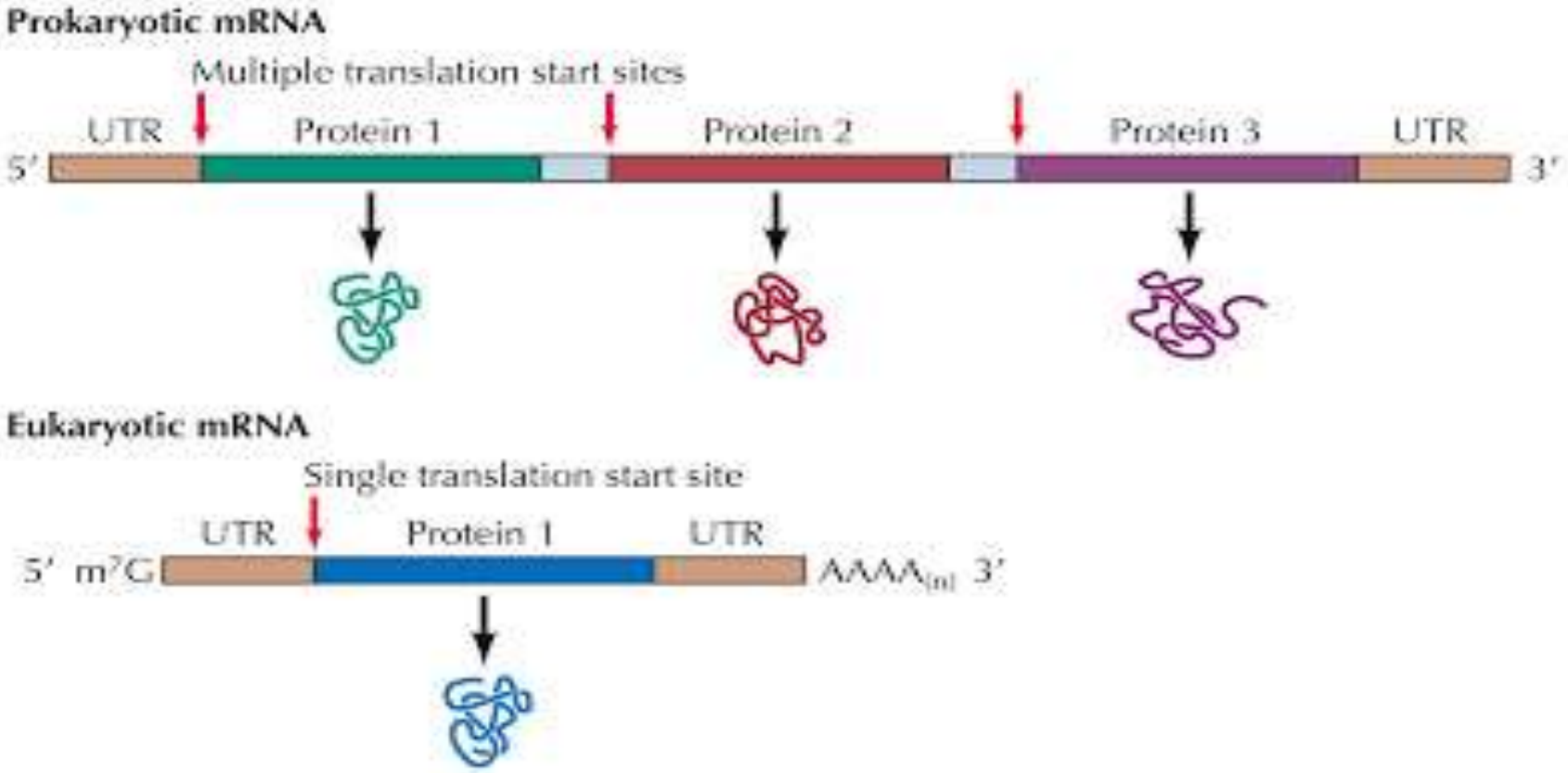


Figure 8-21
Lehninger Principles of Biochemistry, Fifth Edition

FIGURE 8-21 Bacterial mRNA. Schematic diagrams show (a) monocistronic and (b) polycistronic mRNAs of bacteria. Red segments represent RNA coding for a gene product; gray segments represent noncoding RNA. In the polycistronic transcript, noncoding RNA separates the three genes.

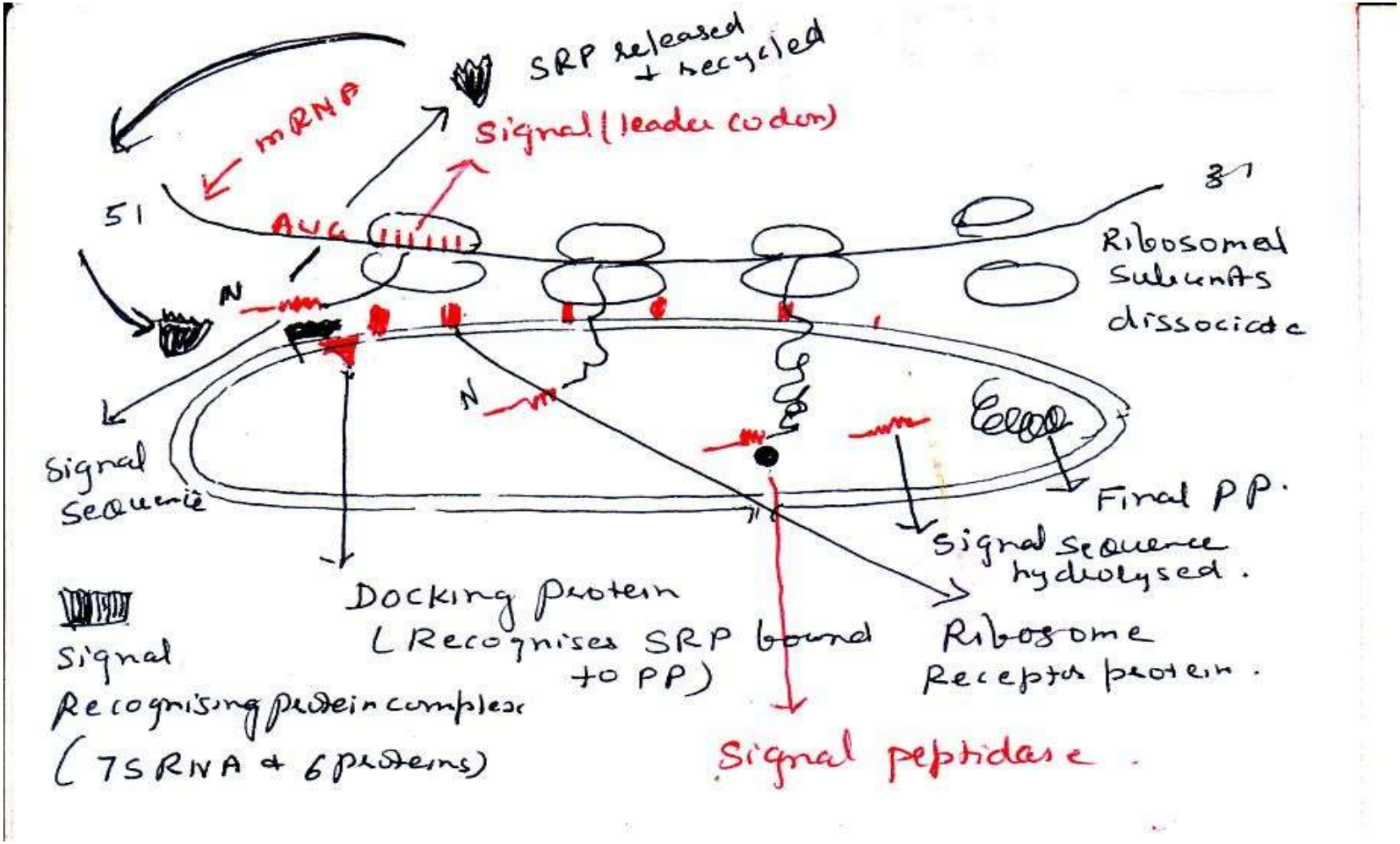

Prokaryotic mRNA
Multiple translation start sites
UTR
Protein 1
Protein 2
Protein 3
UTR
5'
3'
Eukaryotic mRNA
Single translation start site
UTR
Protein 1
UTR
5' m7G
AAAA(n) 3'

SOME EUKARYOTIC MESSENGER RNAs HAVE SIGNAL CODONS

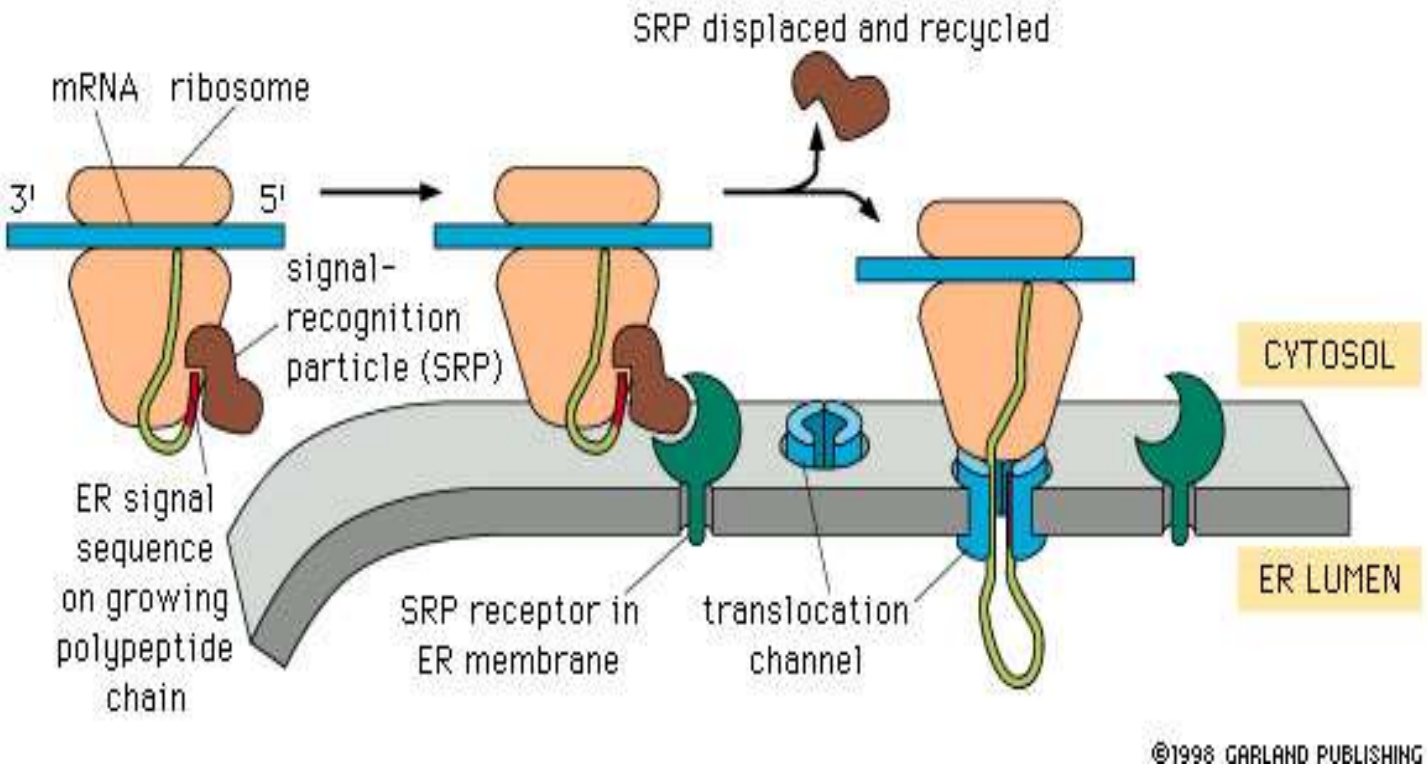
In Eukaryotes Some messenger RNAs possess signal codons just after the Initiator codon on the 5' end terminity. Because of the signal codon, a signal sequence appears at the amino terminus of growing poly peptide chain.

Signal Sequence is recognised by Signal Receptor Protein (SRP)which directs the ribosome to bind with Endoplasmic Reticulum Surface and protein Synthesis continues in its lumen resulting in secretory proteins

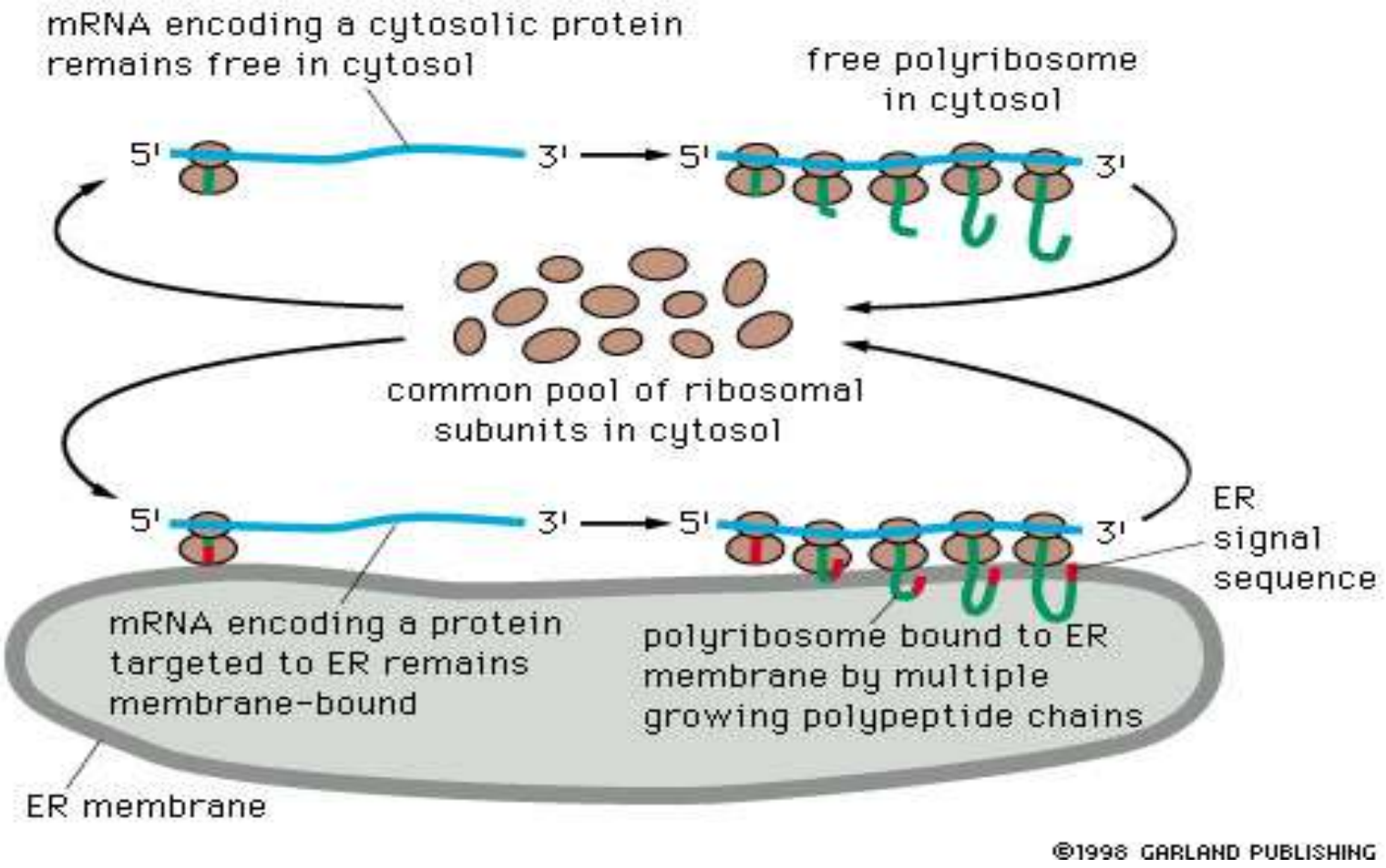
Those mRNAs which donot contain the signal codon complete protein synthesis after binding to ribosome free in the cytoplasm itself and give



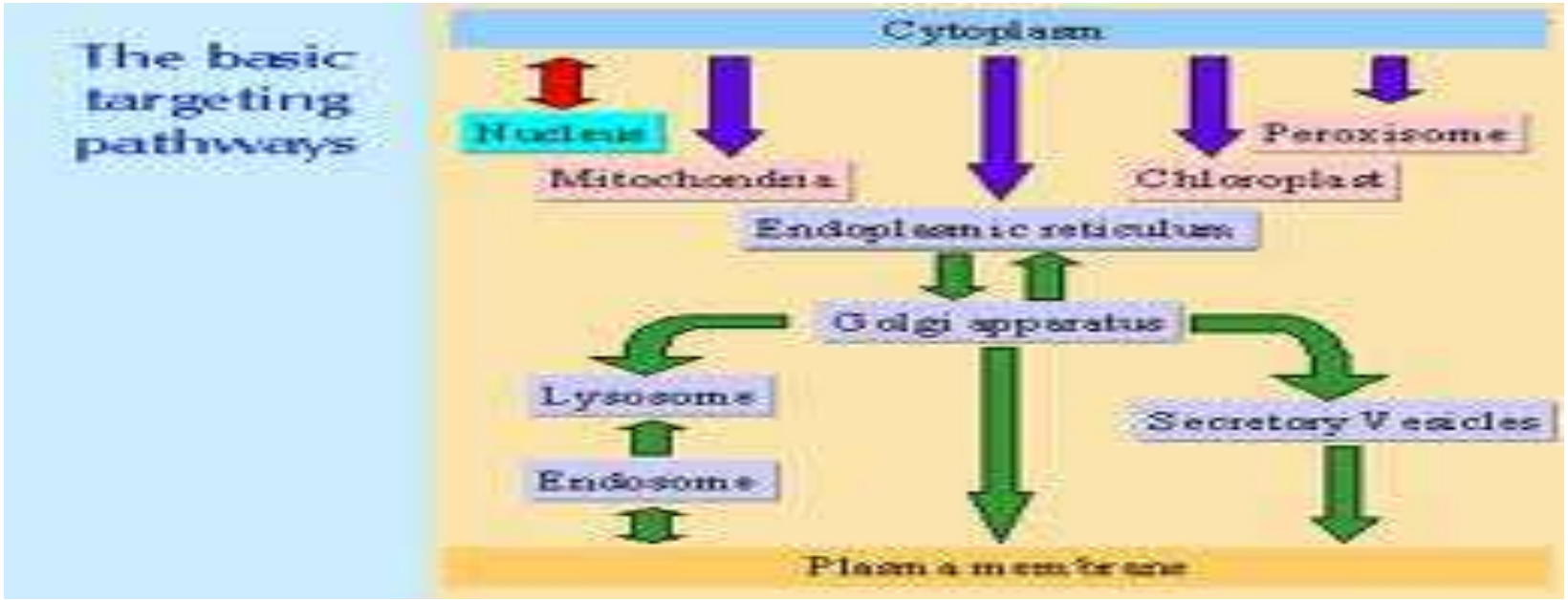
Schematic diagram showing the mechanism by which signal sequence directs the destiny of protein through lumen of Endoplasmic Reticulum



Signal Sequence directing Protein Targetting



Protein synthesis on free ribosomes or on ribosomes attached to E R membrane



Fifgure : Protein sorting and Transport from the book : Cell -A Molecular Approach by G M Cooper

mRNA Circularization

Although m RNA is generally a linear single stranded structure but some eukaryotic mRNA become circular by having interaction between 5' end cap and poly-A tail present at 3'end . Circularizatioin promotes recycling of ribosomes on same messenger RNA leading to efficient translation .

Transport of mRNA after transcription

Only some RNAs function within the nucleus whereas all other RNAs which are meant for protein synthesis after transcription have to transfer or migrate from the nucleus to the cytoplasm via nuclear pores.

One potential target for gene regulation is the nuclear envelope. mRNA mature transcript must be carried through the nuclear envelope and receptor molecules in nuclear pores must recognize the mRNA transcript.

The poly-A tail is important in recognition.

Splicing enzymes when added the primary transcript then transport is blocked.

FUNC TION OF MESSENGER RNA

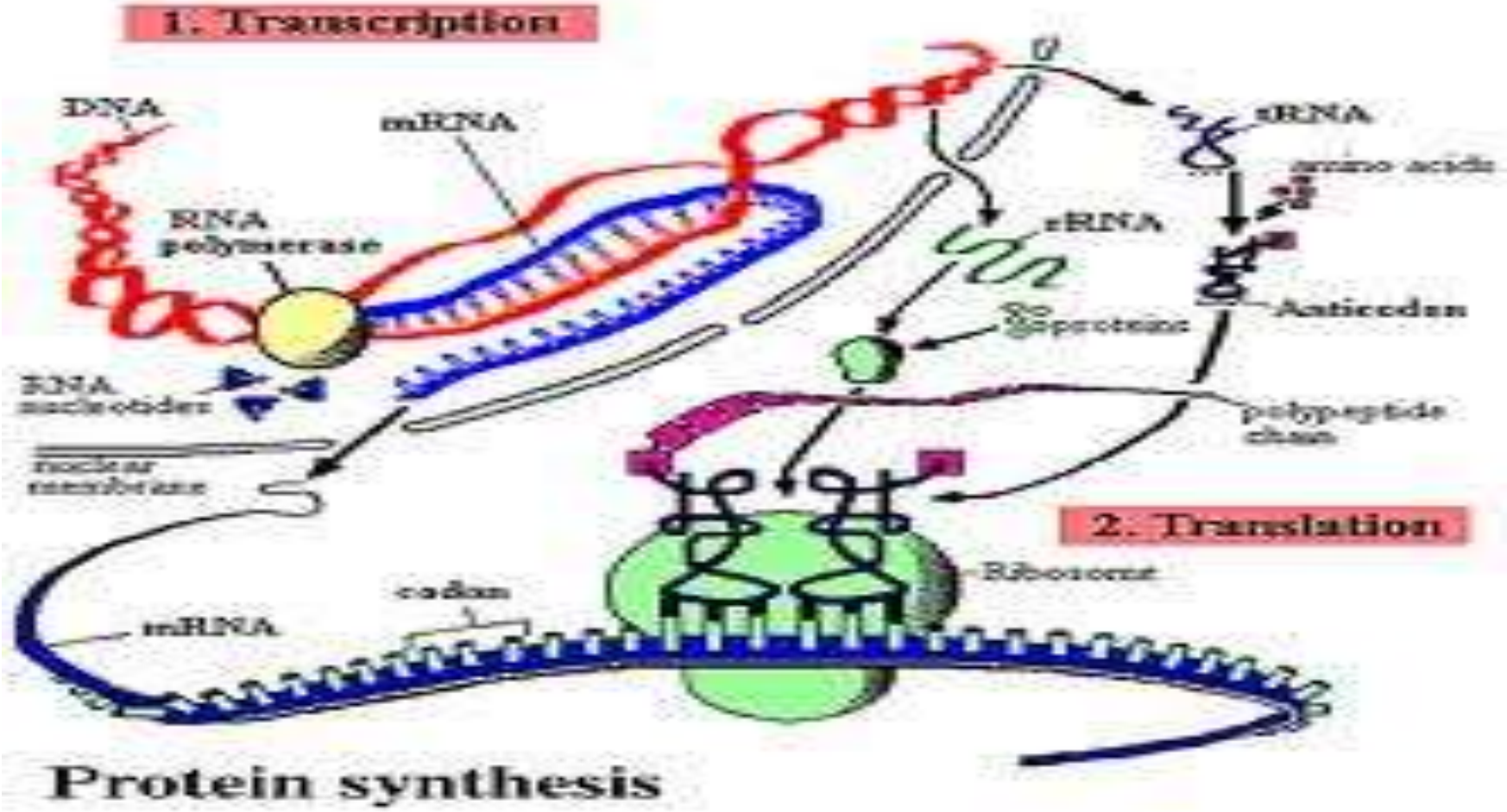
Messenger RNA(mRNA)-is the only coding RNA as it bears a complementary copy of deoxyribonucleotide sequence present on Gene (DNA segment) as ribonucleotide sequence. It encodes chemical blueprint for protein synthesis in 5'-3' direction.

The incorporation of a particular amino acid in the correct position is wonderfully controlled and directed by a template or message ,which starts right from a segment of a strand of DNA (Gene) .

The gene transcribes mRNA which carries the message (Genetic code) to the site of Protein synthesis , the ribosome present in cytoplasm

The genetic code is the total sum of codons present on mRNA. Each codon consists of sequence of 3 bases.

In the ribosome the message carried by mRNA is translated with the help of a number of loaded tRNA to produce a polypeptide chain.



Protein synthesis

REQUIREMENTS FOR PROTEIN SYNTHESIS

Protein Synthesis Machinery involves

- mRNA
- tRNAs
- Ribosome (site of protein synthesis)
- Enzymes & coenzymes (ATP,GTP)
- Numerous initiation , elongation and release factors.
- Activated Amino Acids

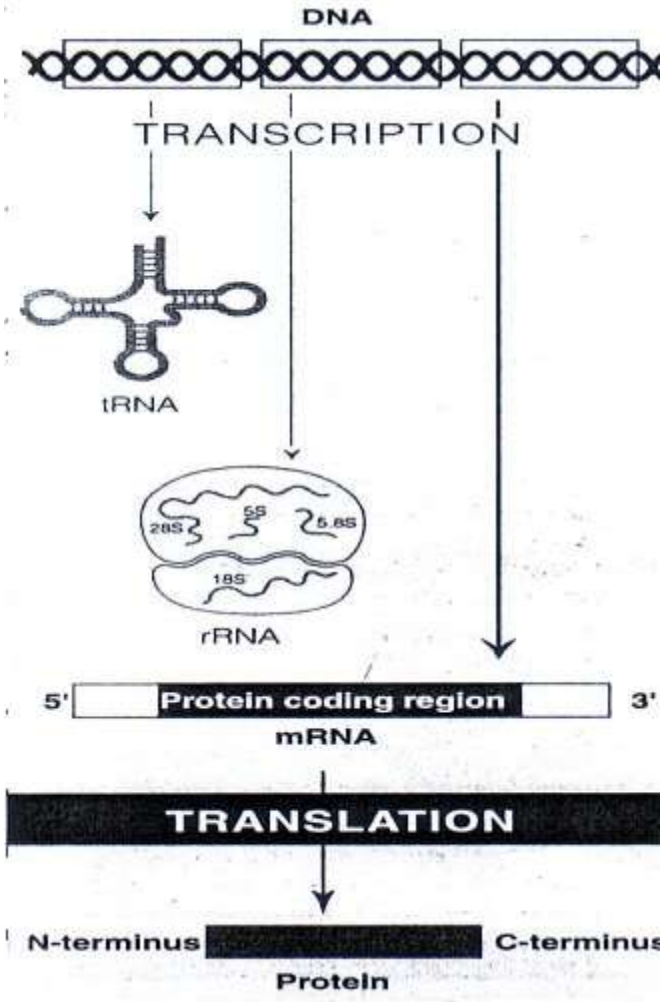


Fig. 30.1 Expression of genetic information by transcription
From : Lippincott's illustrated Reviews Biochemistry : Third Edition

END OF PRESENTATION

THANK YOU