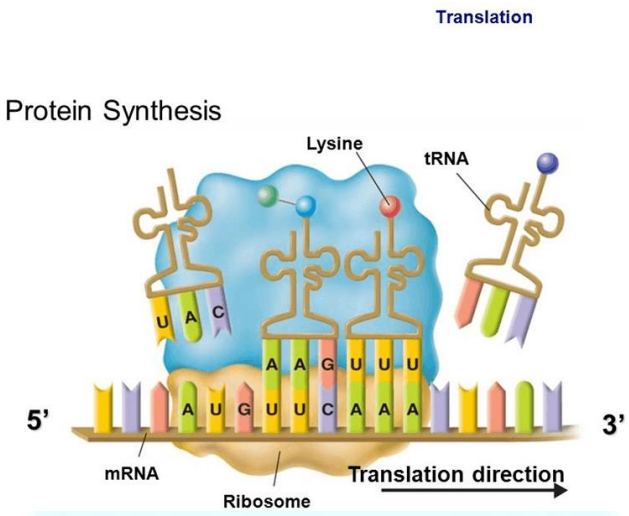
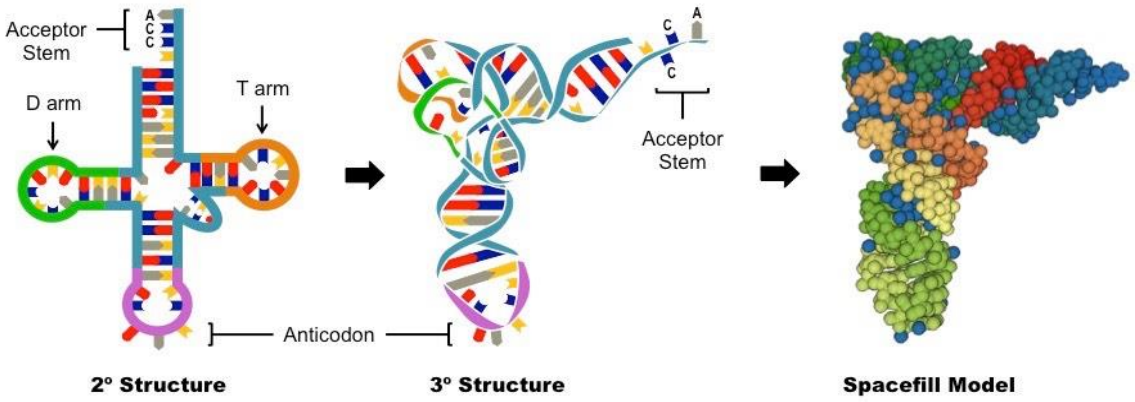
TRANSLATION

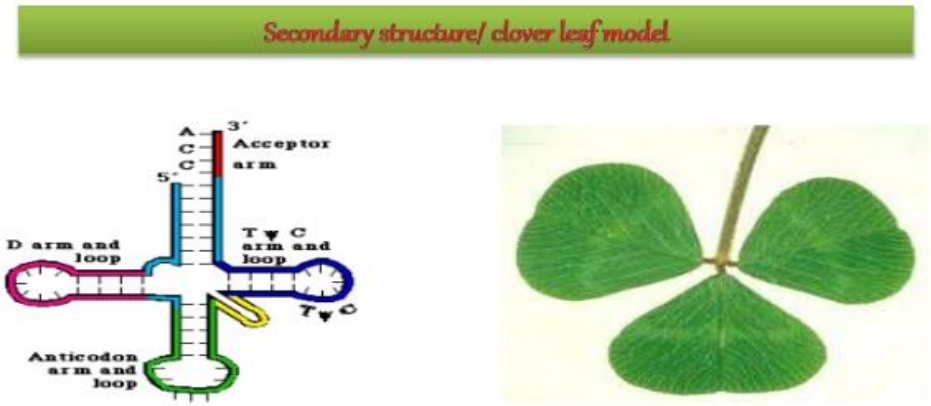


Machinery

- Translation begins when the mRNA codon "AUG" is read by a ribosome.
- Ribosome reads one codon at a time .
- tRNA carries over the proper amino acid.
- tRNA anticodon matches with the mRNA codon, prevents delivery of wrong amino acid.
- One by one, amino acids are linked together
- Translation ends when a "stop" codon is reached



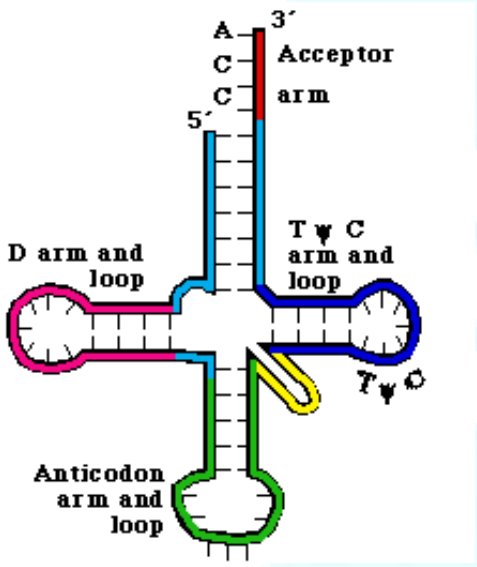
- Detailed structure of tRNA is elucidated from alanine tRNA of Yeast (Robert Holley 1965)
- Besides normal nucleotides it contains some unusual nucleotides.
- Unusual bases- Inosinic acid, Methy inosinic acid Pseudouridine,and Methyl ribo thymidic acid.



- Robert Holley proposed clover leaf model for the first time in 1968.
- It is a two dimensional description of the t-RNA.

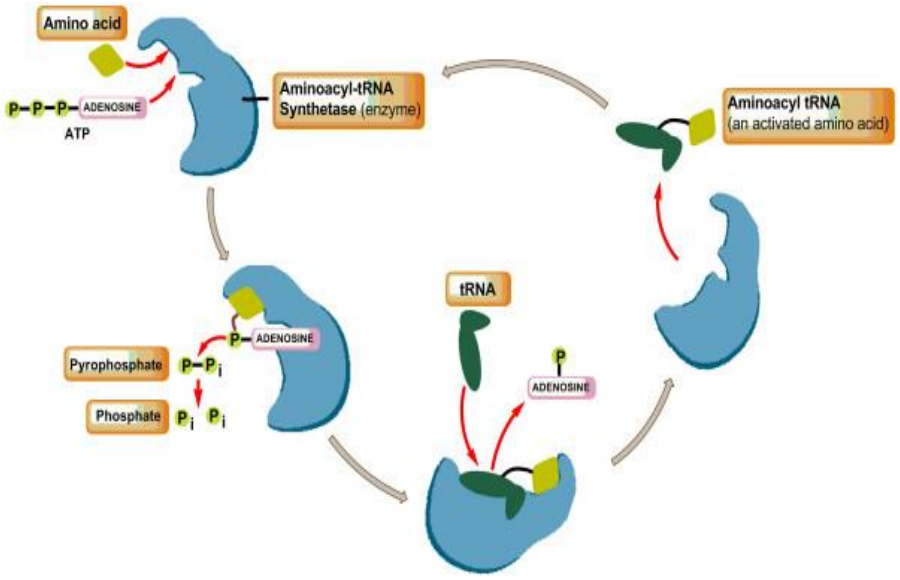
- Nucleotides at the 3 prime end are always unpaired and consists of CCA Amino acids are attached to the adenine of 3 prime end.

- Moving away from the 3’ end the first loop encountered is T phi C loop (TΦC Loop).
- Next to this small arm is the Variable arm.
- The next loop is the is the anticodon arm responsible for recognizing the codon of mRNA.
- It lies at a distance of 66 Angstrom from the 3 prime terminal.
- The third loop is the D loop consists of 8-12 unpaired bases and is thought to be involved in building to specific enzymes.
- The 5 Prime end is always terminated with guanine



Aminoacyl tRNA synthetase

- Amino acids enter in to protein synthesising pathway through Aminoacyl tRNA synthetase.
- It mainly catalyses the process of charging the tRNA with amino acids to form Aminoacyl tRNA.
- The acceptor, D stem and loop and the anticodon stem of the tRNA are involved in recognizing its specific synthetase enzyme.



Stages of Translation

There three main stages in translation process

I. INITIATION

II. ELONGATION

III. TERMINATION

Translation initiation in Eukaryotes.

- Begins with methionine that is not formylated tRNA (tRNAiMet) different from the one that is used for internal methionine codons.
- Translation start determined by the AUG and surrounding sequence
- Translation start site also affected by RNA structure at the 5' end of the mRNA

Prokaryotic initiation

- Initiation of translation in prokaryotes involves the assembly of the components of the translation system, which are: the two **ribosomal subunits** (50S and 30S subunits); the **mature mRNA to be translated**; **the tRNA charged with N-formylmethionine** (the first amino acid in the nascent peptide); **guanosine**

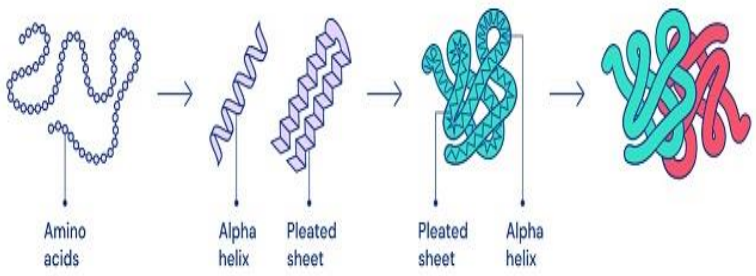
triphosphate (GTP) as a source of energy, and the three prokaryotic initiation factors **IF1, IF2, and IF3**, which help the assembly of the initiation complex.

- The initiation of translation is ensured by IF2 complex.
- Incoming aminoacyl tRNA will enter into the A site of the ribosomes Additional factors are required for the elongation of peptide chains .
- The peptide bond is formed enzymatically between alpha amino group of the incoming amino acid and the carboxyl group of the aminoacid attached to the tRNA at the p site

POST TRANSLATIONAL MODIFICATIONS

- The polypeptide that emerges out from the ribosome after translation are inactive.
- To become functional it must undergo certain modifications- Which can be called as 'Post translational modifications'
- It includes
    i. Protein folding
    ii. Proteolytic cleavage
    iii. Chemical modifications
    iv. Intein splicing

Protein folding



- Done with the help of special proteins called "chaperons" (eg; Hsp 70 and Hsp 40 in E.coli)

- Molecular chaperons donot specify the tertiary structure of proteins the help the protein to find the correct structure.
- Hsp 70 binds to the hydrophobic amino acids of the polypeptide chain by helping in shielding of hydrophobic regions of the proteins GroEL/GroES complex in E.coli.
- They form a multi subunit structure that looks like a hollowed out bullet with a central cavity.
- A single unfolded protein. Enters the cavity and emerges folded.

Processing by proteolytic cleavage

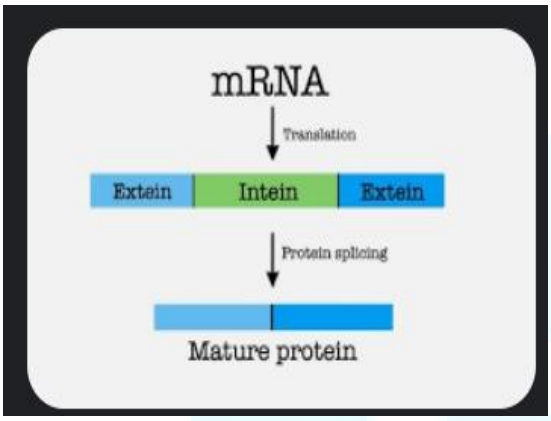
- 2 functions in post translational processing of proteins.
- Cleavage of the ends of polypeptidy segmenting polypeptides in which each segment can act as a functional protein.
- It is common in secreted polypeptides.
- Example : Melittin which causes lyses of cells in bees as well as in animals , which is produced as premellit in (inactive precursor). The 22 additional aa residue at the N terminus is removed by an extra cellular protease and the active protein (venom) is released.
- Insulin which are produced by islets of langerhans is also an example
- Some proteins are produced initially as polyproteins
- Cleavage of polyproteins releases individual proteins.
- Some eukaryotic viruses use this mechanism to reduce their genome size
- Eg; proopiomelanocortin synthesised in pituatary gland

Chemical modifications

- Simple type of modifications include additions of chemical groups (acetyl /methyl groups/phosphate groups)
- Complex type of modifications include glycosylations(attachment of large carbohydrate chains to the ends of polypeptides)

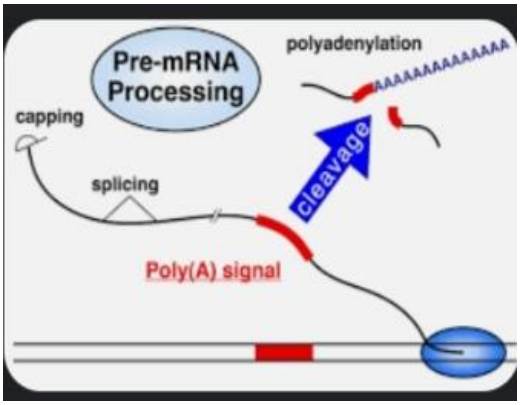
- Glycosylations are of two types
  1 N-glycosylations
  2 O-glycosylations

Intein splicing



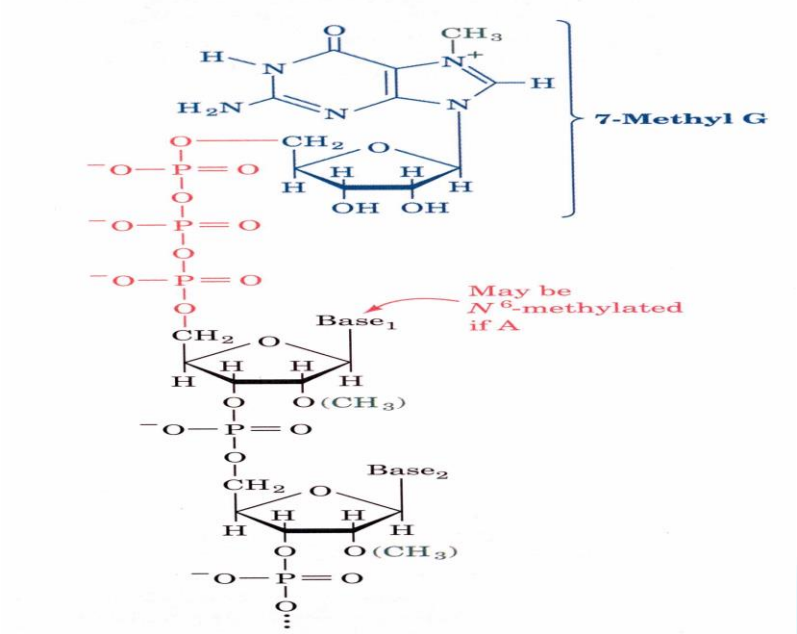
- Inteins are internal segments of protns.
- That are removed soon after translation.
- The two external segments or exteins become linked together.
- Most inteins falls in b/w 300-600 aminoacids in length.
- The first aa is usaually cys. Or less frequently ser. And the last two are always His. followed by Asn.
- Few aa. are conserved in between them , which is assumed that this conserved aa are responsible for the intei splicing.

Post transcriptional modification



- Primary transcript much larger than finished product.
- Precursor and partially processed RNA called heterogeneous nuclear RNA (hnRNA)
- Processing occurs in nucleus
- It includes;
    - **i.** Splicing
    - **ii.** Capping
    - **iii.** Polyadenylation
    - **iv.** Capping mRNA
- 5’ cap is a reversed guanosine residue so there is a 5’-5’ linkage between the cap and the first sugar in the mRNA.
- Guanosine cap is methylated.

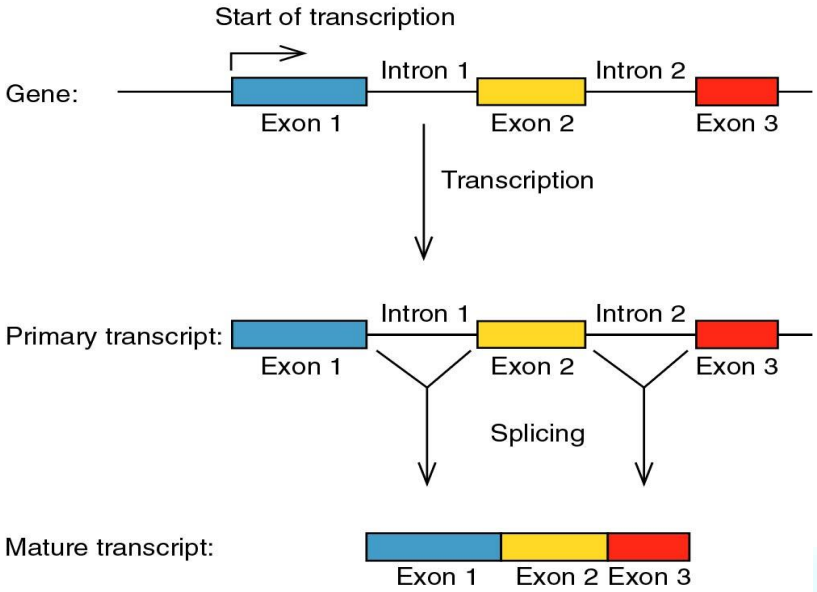
- First and second nucleosides in mRNA may be methylated



Introns and Exons

- Introns--Untranslated intervening sequences in mRNA
- Exons– Translated sequences
- Process-RNA splicing

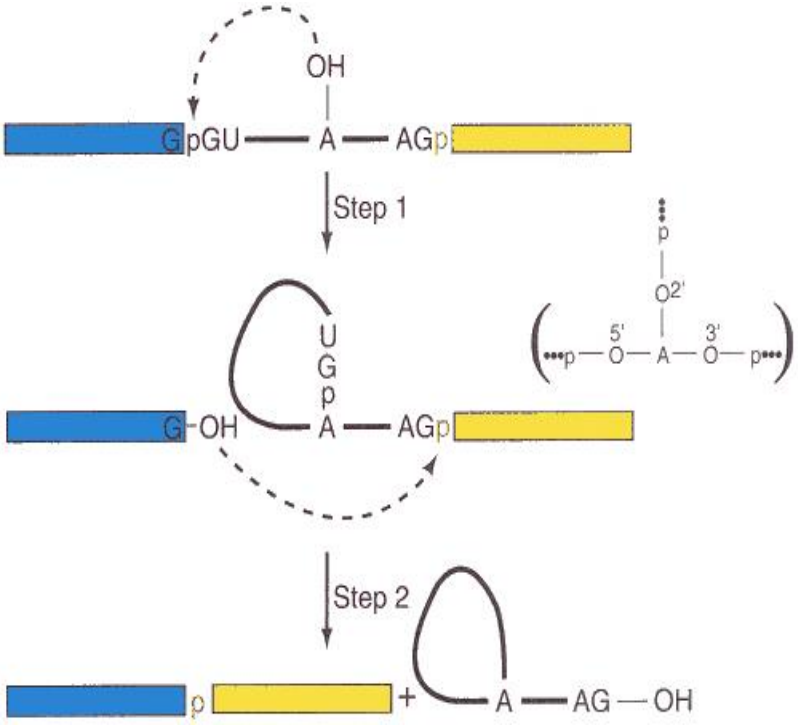
- Heterogeneous nuclear RNA (hnRNA)-Transcript before splicing is complete



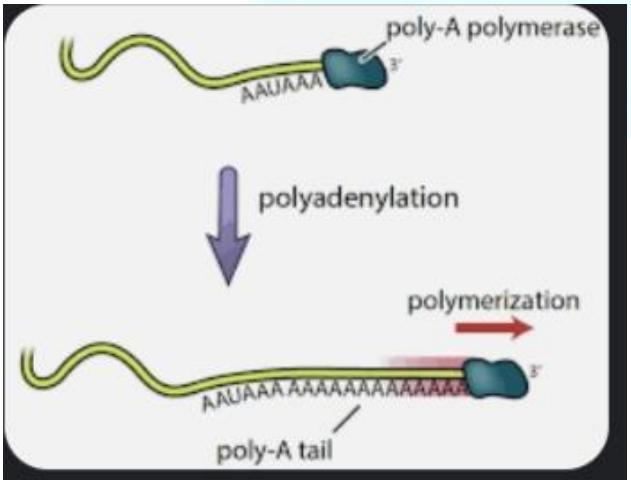
Splicing Overview

- Occurs in the nucleus.
- hnRNAs complexed with specific proteins, form a ribonucleoprotein particle (RNP)
- Primary transcripts assembled into hnRNP
- Splicing occurs on spliceosomes consist of
- Small nuclear ribonucleoproteins (SnRNPs)
  are components of spliceosomes ,contain small nuclear RNA (snRNA)
- Many types of snRNA with different functions in the splicing process

Simplified Splicing Mechanism



Polyadenylation



- Occurs on the 3' end of virtually all eukaryotic mRNAs.

- Occurs after capping.
- Catalyzed by polyadenylate polymerase
- Polyadenylation associated with mRNA half-life.
- Histones not polyadenylated