Paper – 6.2

Genetics, Biotechnology and Nanotechnology

Unit – 10, Genetic Code and Gene Expression

Continued from Central Dogma...

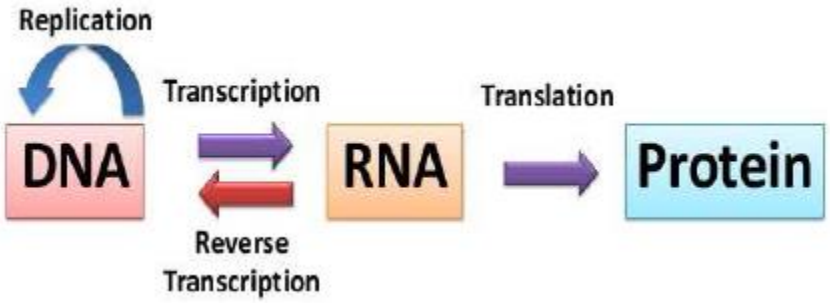
Central Dogma of Molecular Cell Biology

In 1958 F.Crick proposed that the concept of central dogma, which states that **"when a particular gene is expressed, (control a function or a reactions) its information is copied into another nucleic acid (mRNA) which in turn directs the synthesis of specific proteins".** So the central dogma was proposed as unidirectional flow of molecular information from DNA to mRNA and finally to polypeptide.

Later a reverse of central dogma was also found in retroviruses. **H. Temin and D. Baltimore** (1970) reported that retro viruses operate a central dogma in reverse manner (inverse flow of information) or **teminism** inside host cells. This discovery was important in understanding cancer and hence, these two scientists were awarded Nobel Prize.

Genetic RNA of these viruses first synthesizes DNA through reverse transcription. This process is catalyzed by the enzyme reverse transcriptase. DNA then transfers information to messenger RNA which takes part in translation of the coded information to from polypeptide.

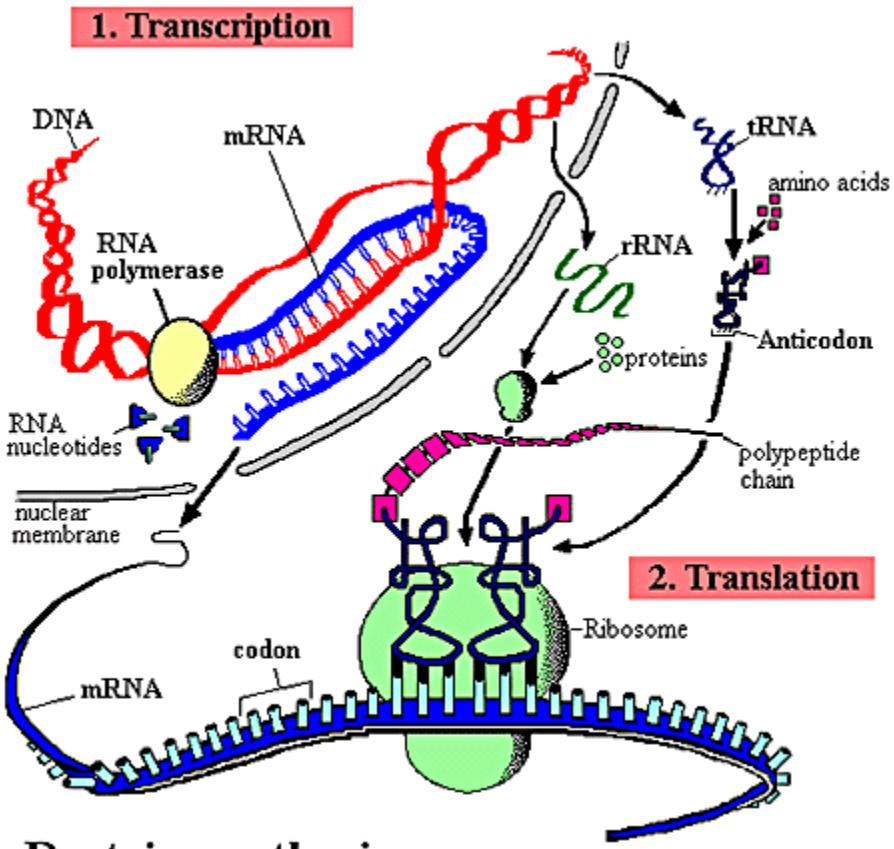
Central Dogma of a cell



Mechanism of Protein Synthesis

Two major steps are involved in protein synthesis are:-

- **Transcription**- involving transfer of genetic information from DNA to mRNA
- **Translation**- involving translation of the language of nucleic acid into that of a Polypeptide



Protein synthesis

Transcription

"The transfer of genetic information from DNA to mRNA in general is known as transcription". The segment of DNA that takes part in transcription is called transcription unit. It has three components a) A promoter b) The structural gene c) A terminator

a) **A promoter**- promoter sequences are present upstream (5'end) of the structural genes of a transcription unit. The binding sites for RNA polymerase lies within the promoter sequence. In prokaryotes 10bp upstream from the start point lies a conserved sequence described as 10 nucleotide sequences **TATAAT or "pribnow box**" and 35 nucleotide sequences **TTGACA as "recognition sequence".**

b) **The structural gene**- structure gene is part of that DNA strand which has 3' to 5' polarity as transcription occur in 5' to 3' direction. The strand of DNA that directs the synthesis of mRNA is called **template or non-coding strand**. The complementary strand is called **non-template or coding strand,** it is identical in base sequence to RNA transcribed from the gene, only with U in place of T.

c) **A terminator**- terminator is present at 3' end of coding strand and defines the end of the process of transcription.

- The base sequence of the mRNA molecule is complementary to that of the antisense strand which served as it template.
- Like DNA synthesis RNA synthesis also proceeds from 5' to 3' direction.

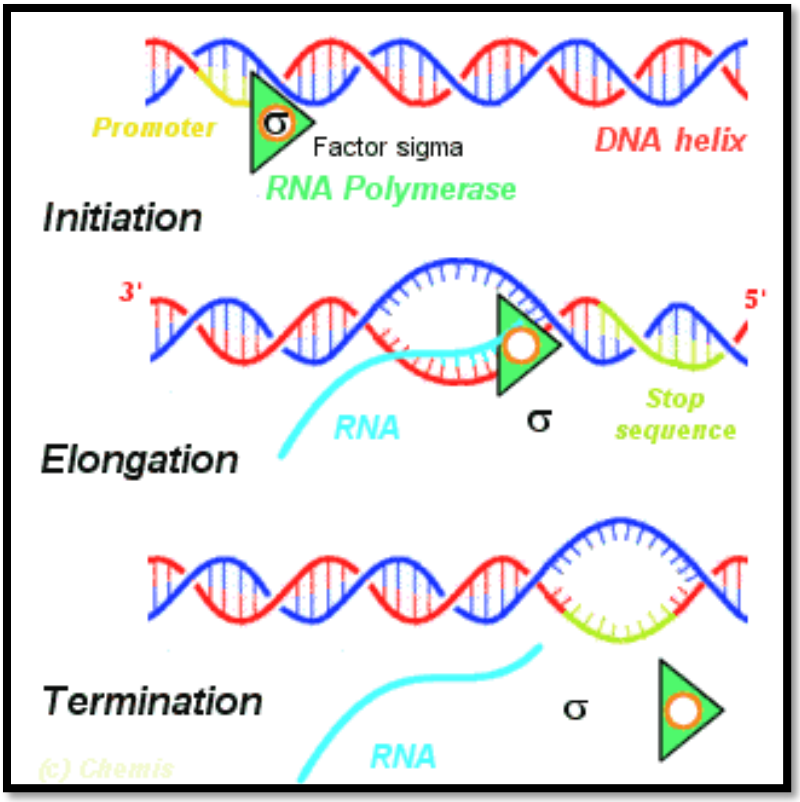
Mehanism Transcription in Eukaryotes

1) **Initiation**- binding of **RNA polymerase** to the **promoter region** with the help of an **Initiation Factor- Sigma factor** (binding of σ-factor alter the property of enzyme; make to function as an initiation enzyme).

2) **Elongation**- RNA polymerase will keep on making a complementary strand against template strand with the help of ribonucleotides. The newly transcribed strand keeps separating and the DNA duplex keep on folding back instantaneously. During elongation, same RNA polymerase acts as elongation enzyme due to separation of σ- factor from it. **The direction of transcription is also from 5'---- 3'like replication**. So the template against which it is transcribed has polarity of 3'—5'.

3) **Termination**- after reaching the terminator region newly formed or nascent RNA falls off along with RNA polymerase. Termination is assisted by Rho-factor(ρ-factor)

In eukaryotes the promoter site is recognized by presence of specific nucleotide sequence called **TATA box or Hogness box or Pribnow Box** (7 base pair long- TATAAA or TATAATs) located 19-27bp upstream to the start point. Another sequence is CAAT box present between -70 and -80bp. The nucleotide sequence at the two ends of all mRNA molecules is the same. Normally mRNA carries the codons of signal complete protein molecule (monocistronic mRNA) in eukaryotes, but in prokaryotes, it carries codons from several adjacent DNA cistron and becomes much longer in size (polycistronic mRNA).



Translation

"The process of formation of protein from mRNA is known as translation".

Translation has 3 steps

1) **Initiation of translation**- In the first step there is binding of mRNA with smaller sub unit of ribosome. Translation of Initiation codon (AUG) by a charged tRNA with Methionine (n-formyl methionine, f-Met, in prokaryote) amino acids takes place. It is followed by the translation of second codon by 2nd charged tRNA. After the translation of first two codons, the association of bigger subunit of ribosome takes place to form a complete translational complex. When two such charged tRNA comes close, the peptide bond between two amino acids, they carry, will take place with the help of a ribozyme called- Peptidyltransferase (23SrRNA molecule) enzyme. Formation of peptide bond between 1st& 2nd amino acid takes place. UTR- (Un- Translated-Regions) is the flanks of mRNA before Initiation and after the stop codon, which are not to be translated, but they play role in efficient translation .There are three initiation factors in prokaryotes- IF3, IF2, IF1. Eukaryotes have 9 initiation factors – eIF2, eIF3, eIF1, eIF4A, eIF4B, eIF4C, eIF4D, eIF5, eIF6.

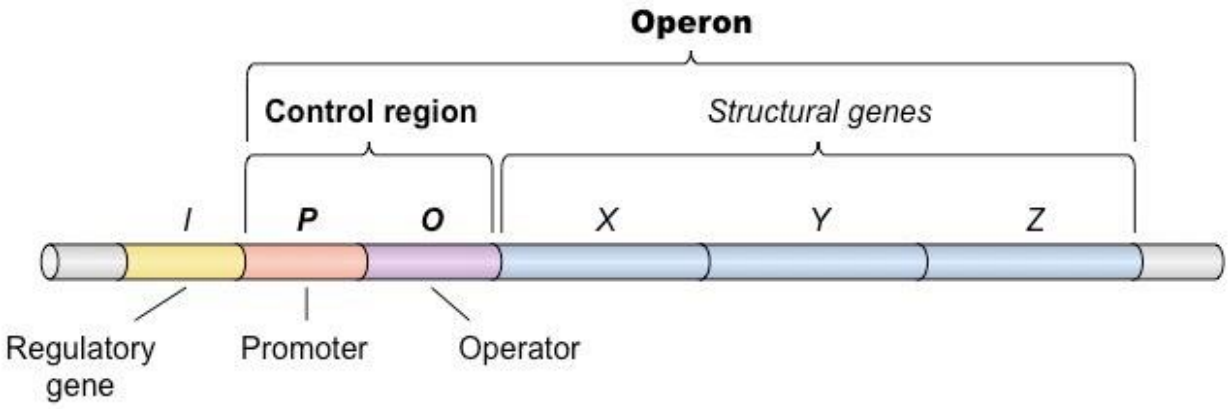
2) **Elongation-** The translated part of mRNA translocates out and ribosome moves From one to next codon. Regular addition of new amino acids takes place at A-site. Polypeptide chain (PPC) keeps elongating at the expence of energy provided by GTP. PPC hangs in the groove of bigger sub unit of ribosome on the P-site.

3) **Termination-** Binding of releasing factors to the stop codon helps in the release of polypeptide and terminates translation. Synthesis of polypeptide terminates when a nonsense codon of mRNA reaches the A-site. There are three nonsense codons- UAA, UAG & UGA. These codons are not recognized by any of the tRNAs. There is no tRNA having anticodon complementary to stop codon i.e., none of the tRNA has AUU, AUC or ACU anticodon. Finally the ribosome encounters a stop codon. The polypeptide, tRNA and mRNA are released. The small and large subunits dissociate from one another.

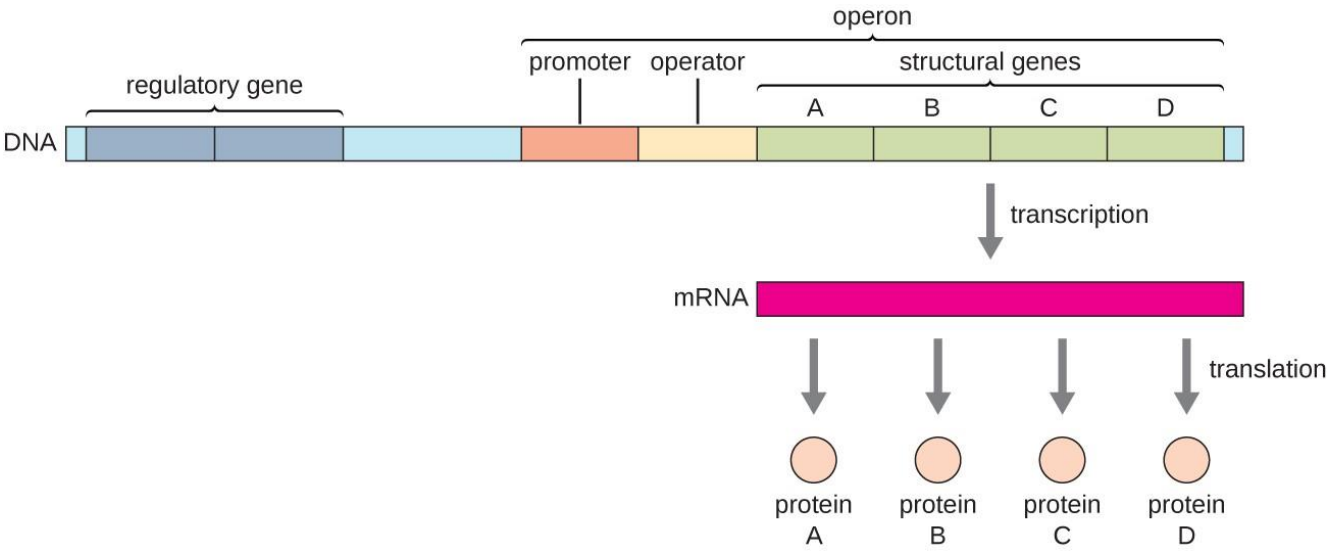
Some special features of Translation-

1. Translation is the ultimate step in gene expression.
2. The energy cost for protein synthesis is very high
    a. Only a small fraction of the energy input of translation is needed to form the peptide bond.
    b. The majority of energy is invested to assure that the sequence of the polypeptide is correct.
    c. If incorrect polypeptides (e.g. enzymes) are made by the cell it could have divesting effect affects cell fraction.
3. The mRNA is always read from 5' to 3'. The polypeptide is always synthesized in the direction of amino terminus to carboxyl terminus.

Operon Concept



- An operon is a group of genes that are transcribed together.
- They usually control an important biochemical process.
- They are only found in prokaryotes.
- Structure of operon consist of Regulatory gene, Structural gene, Promoter and Operator.



Francis Jacob and Jacques Monad (1961), explained that gene regulation is by operon model, while studying catabolism of lactose in *E.coli* suggested that the action of most genes is regulated at transcription level by Induction & Repression. Jacob & Monad won Nobel prize for discovering control of gene expression.

INDUCTION

In induction synthesis of enzyme takes place in response to appearance of specific substrate in the medium. The phenomenon of induction can be explained by taking the example of working of Lactose operon (*lac* operon).

REPRESSION

In repression synthesis of enzyme stops in response to appearance of specific substrate in the medium It can be explained by taking working of Tryptophan operon (*trp* operon).

The *lac* operon

- The operon model for lactose catabolism is called *lac* operon.
- The *lac* operon consist of regulatory gene, structural gene, operator and promoter site.

- The structural gene of *lac* operon consists of **three genes** each involved in processing the sugar lactose
- One of them is the gene for the enzyme **β- galactosidase**
- This enzyme hydrolyses lactose into glucose and galactose.

STRUCTURE OF *LAC* OPERON

1. **Regulatory gene:**
   - The regulatory gene is the **i** gene that code for the repressor protein .
   - This i gene is expressed in all the time hence it is also known as a constitutive gene.

2. **Structural genes:**
   - Three structural genes **lac z, lac y, lac a** involved in the synthesis of enzymes for the lactose catabolism.
   - The 3 genes as transcribed as a polycistronic mRNA.
     1. Lac z code for Betagalactosidase
     2. Lac y code for Lac permease
     3. Lac a code for Transacetylase

3. **Promoter:**
   - DNA sequence that define where transcription of a gene by RNA polymerase begins

4. **Operator:**
   - A segment of DNA to which a transcription factor binds to regulate gene expression by repressing it.