Translation, Regulation of Gene Expression

Objectives

After going through this lesson, the learners will be able to understand the following:

- Structure of t-RNA
- Charging of t-RNA
- Process of translation
- *Lac*-operon

Content Outline

- Introduction
- Structure of t-RNA
- Translation
  - Charging of tRNA
  - Process of Translation
  - Untranslated regions
- Regulation of gene expression
- Operon concept
- *Lac*-operon
- Summary

Introduction

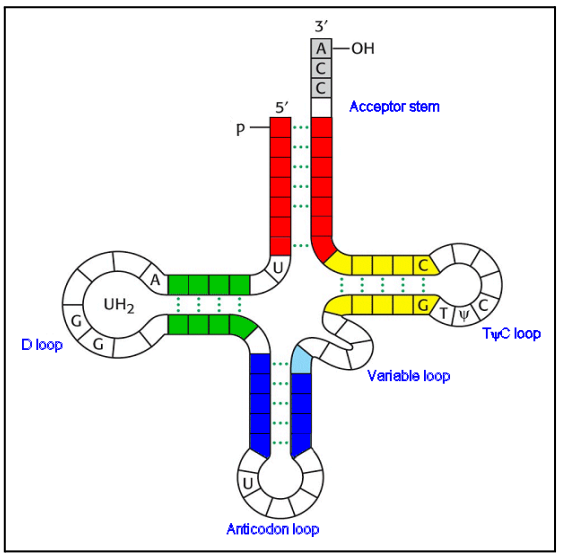
The process of replication of DNA and the transcription of RNA from DNA was experimentally proved and well explained by the scientists on the basis of complementarity. The biggest challenge was to explain the mechanism by which the polymer of nucleotides gets converted into a polymer of amino acids because no such complementarity could be envisaged among the two. Also, a lot of research in the field of microbiology was able to explain the process of translation as well as the mechanism by which the regulation of the expression of various genes occurred in the organisms. In this section we will discuss the genetic code which helps in converting the polymer of nucleotides into a polymer of amino acids. The content will also focus on the mechanism which helps in the regulation of the expression of genes in the organisms.

Structure of t-RNA

Francis Crick had an opinion that there has to be a mechanism in order to read the code on the mRNA and link it to the amino acids. He proposed that an adapter molecule is there that on the one hand reads the code and on other hand binds to the specific amino acids.

The transfer RNA is abbreviated as tRNA and was formerly referred to as sRNA, i.e., soluble RNA. It is an adapter molecule which is composed of RNA consisting of around 73 to 93 nucleotides. Transfer RNA or the so called tRNA serves as the physical link between the mRNA and the amino acid sequence of proteins. The tRNA's are specific for each amino acid. There are no tRNA for stop codons.For initiation, there is a specific tRNA that is referred to as initiator tRNA.

Robert William Holley isolated transfer RNA (tRNA) and then determined the sequence and structure of yeast alanine tRNA, which helps to incorporate the amino acid alanine into proteins. He gave the clover leaf model to explain the secondary structure of a tRNA. The tertiary structure of tRNA has a conserved inverted L-shaped appearance.



The structure of tRNA has the following parts-

i) A 5'-terminal phosphate group.

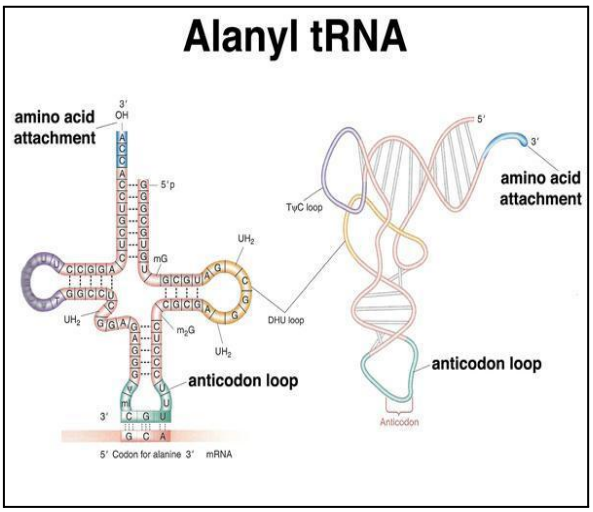
ii) A 3' end which always terminates with the sequence CCA. The amino acid gets attached to the 3' OH group of the ribose via an ester linkage.

iii) The DHU or D arm is a 4- to 6-bp stem ending in a loop. It has a recognition site for the specific enzyme aminoacyl-tRNA synthetase that activates the amino acid.

iv) One end of the tRNA contains a 5-bp stem called the anticodon loop which pairs with an mRNA specifying a certain amino acid. The anticodon loop has bases complementary to the code present on the mRNA.

v) The T arm is a 4- to 5- bp stem containing the sequence TΨC named for the presence of sequence TψC (thymine-pseudouridine(ψ)-cytosine. It functions as the ribosome-binding site.

vi) The variable arm is present in some tRNA between the anticodon loop and the TψC loop. It has 3 to 21 nucleotides depending upon the amino acid for which the tRNA encodes

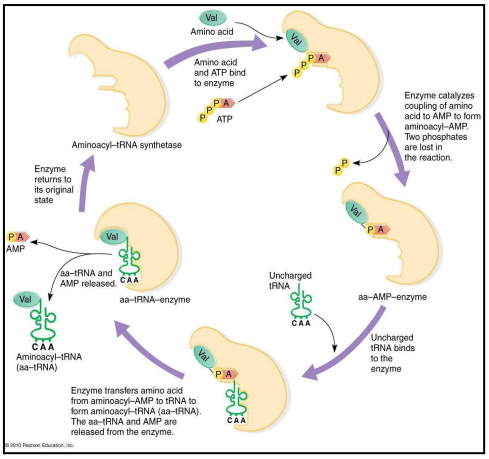


Translation

Translation is the process by which the polymerisation of amino acids to form a polypeptide occurs as per sequence of bases in mRNA.

Charging of tRNA- Amino acid activation stage

Amino acids get linked to their cognate tRNA on being activated in the presence of adenosine triphosphate (ATP). This process is called as charging of tRNA or aminoacylation of tRNA. The process of charging of tRNA begins when a specific amino acid and a molecule of ATP aminoacyl tRNA synthetase enzyme at its active site. This results in the formation of a covalent bond between AMP and the carboxyl group of the amino acid and the subsequent release of pyrophosphate from the enzyme. A specific tRNA having an anticodon that corresponds to the amino acid then binds to the synthetase.



The enzyme transfers amino acid from aminoacyl AMP to tRNA attached with it. The amino-acid binds with the 3' end of tRNA to form aminoacyl tRNA which gets released from the enzyme along with AMP. Now, the tRNA is called 'charged' t RNA. The enzyme returns to its original state after releasing the aminoacyl-tRNA or charged tRNA.

To summarize the amino acid activation takes place as follows:

AA+AT → AA –ATP

AA-ATP +tRNA (catalysed by the enzyme aminoacyl tRNAsynthetase)→AA-tRNA –AMP+2iP

The charged tRNA is used in the process of translation on a ribosome. The amino acids are joined by a bond which is known as a peptide bond which requires expenditure of energy. The purpose is solved by the charged t-RNA because if two such charged tRNAs are brought close to each other, the formation of peptide bonds between the amino acids would be favoured energetically.

Process of Translation

During the process of translation, the messenger RNA (mRNA) produced in the process of transcription gets decoded by a ribosome to produce a specific chain of amino acid or a polypeptide.

Ribosome, which consists of structural RNA and about 80 different proteins, acts as a cellular factory for synthesizing proteins. In its inactive state, ribosomes exist as two subunits; a large subunit (50 S in prokaryotes and 60S in eukaryotes) and a small subunit (30 S in prokaryotes and 40S in eukaryotes).

The process of translation occurs in three steps: initiation, elongation and termination.

i) **Initiation:** For initiation, the small subunit of ribosome binds to a site "upstream" (on the 5' side) of the mRNA and proceeds downstream (5' ☾ 3') until it encounters the start codon AUG. Three initiation factor proteins (known as IF1, IF2, and IF3) are required during the process of initiation in prokaryotes.

The binding of mRNA with the small subunit of ribosome is facilitated by the initiation factor-3 and a sequence called as the Shine Dalgarno sequence Shin-Dalgarno sequence is present on mRNA and is complementary to the 16S ribosomal RNA of the small subunit. The 1st AUG sequence down-stream from the Shine-Dalgarno sequence is recognised as the start codon. Then, a tRNA carrying amino acid formylated methionine binds to the mRNA at the start codon (normally AUG rarely GUG)forming the initiation complex. Initiation factor-2 helps in the binding of initiator tRNA with the complex. Initiation factor-1 is required for the dissociation of the complex.

After the formation of the initiation complex, the large ribosomal subunit binds to this complex. The large subunit of the ribosome has three sites for the attachment of the tRNAmolecules.The A-site (amino acid site) at which the aminoacyl-tRNA pairs up with the mRNA codon with its complementary anticodon. The P-site (polypeptide site) at which the tRNA carrying the growing polypeptide chain is present. The two sites A-site and the P site are required for amino acids to bind and be close enough for the formation of a peptide bond. The third site is the E-site (exit site) which gets occupied by the empty tRNA that gets shifted from the P site. From the E-site the tRNA gets released back into the cytoplasm to bind to another amino acid and repeat the process.

The only aminoacyl-tRNA that can bind directly at the P site of the ribosome is the initiator fMethionine tRNA. The A site of the ribosome is aligned with the second codon on the mRNA. All AA –tRNA complexes except the initiator AA-tRNA,bind to the A-site of the ribosome. The second aminoacyl-tRNA comes and occupies the A site and its amino acid which gets linked to the initiator fMethionine by the first peptide bond.

In eukaryotes, initiator tRNA is methionine (Met) whereas in bacteria a modified formylated methionine (fMet) is the initiator tRNA.

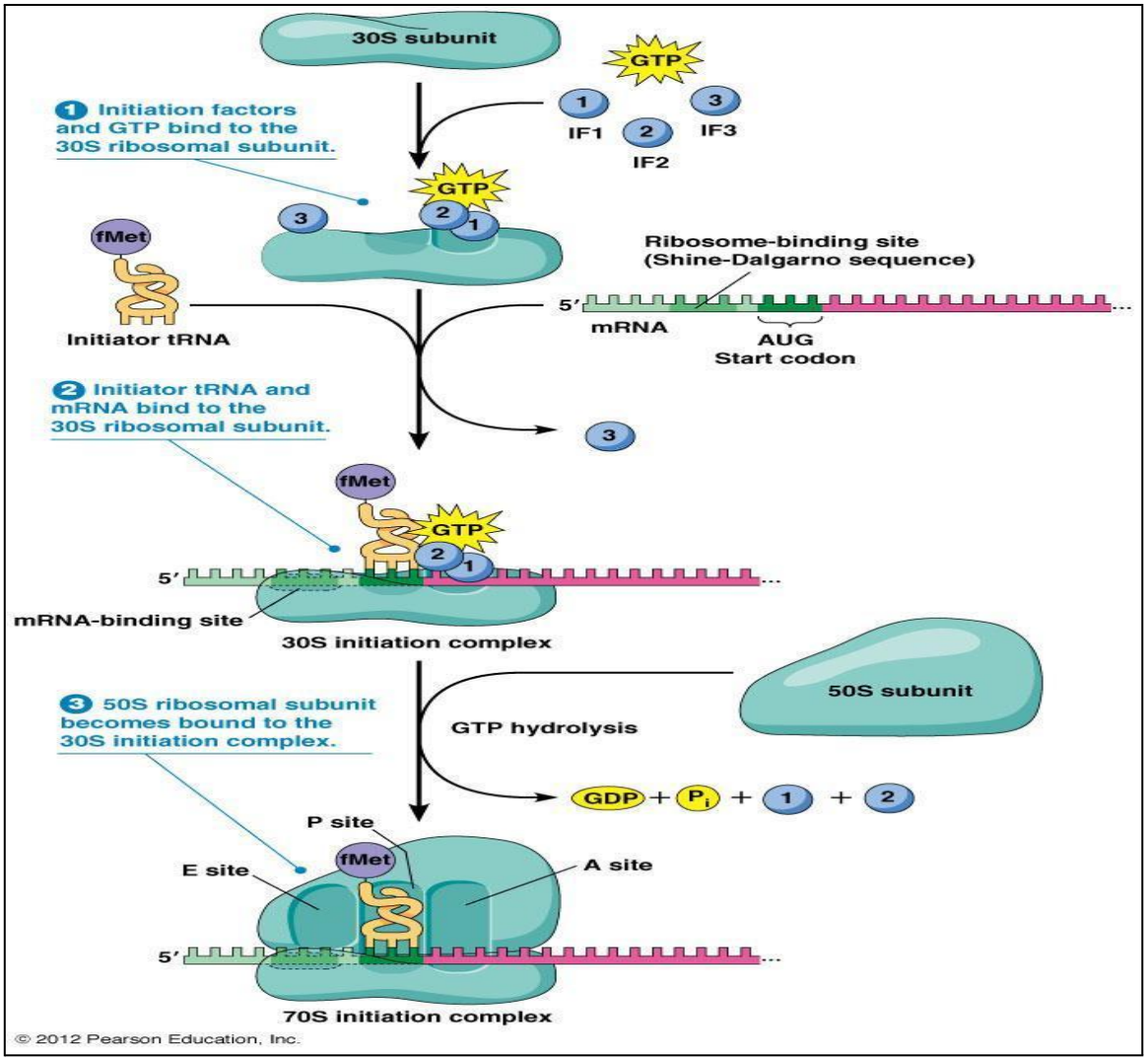
ii) Elongation

The ribosome moves along the mRNA in 5'-to-3'direction. The entire polypeptide chain is synthesised by a single ribosome.

There are 3 steps in the elongation process.

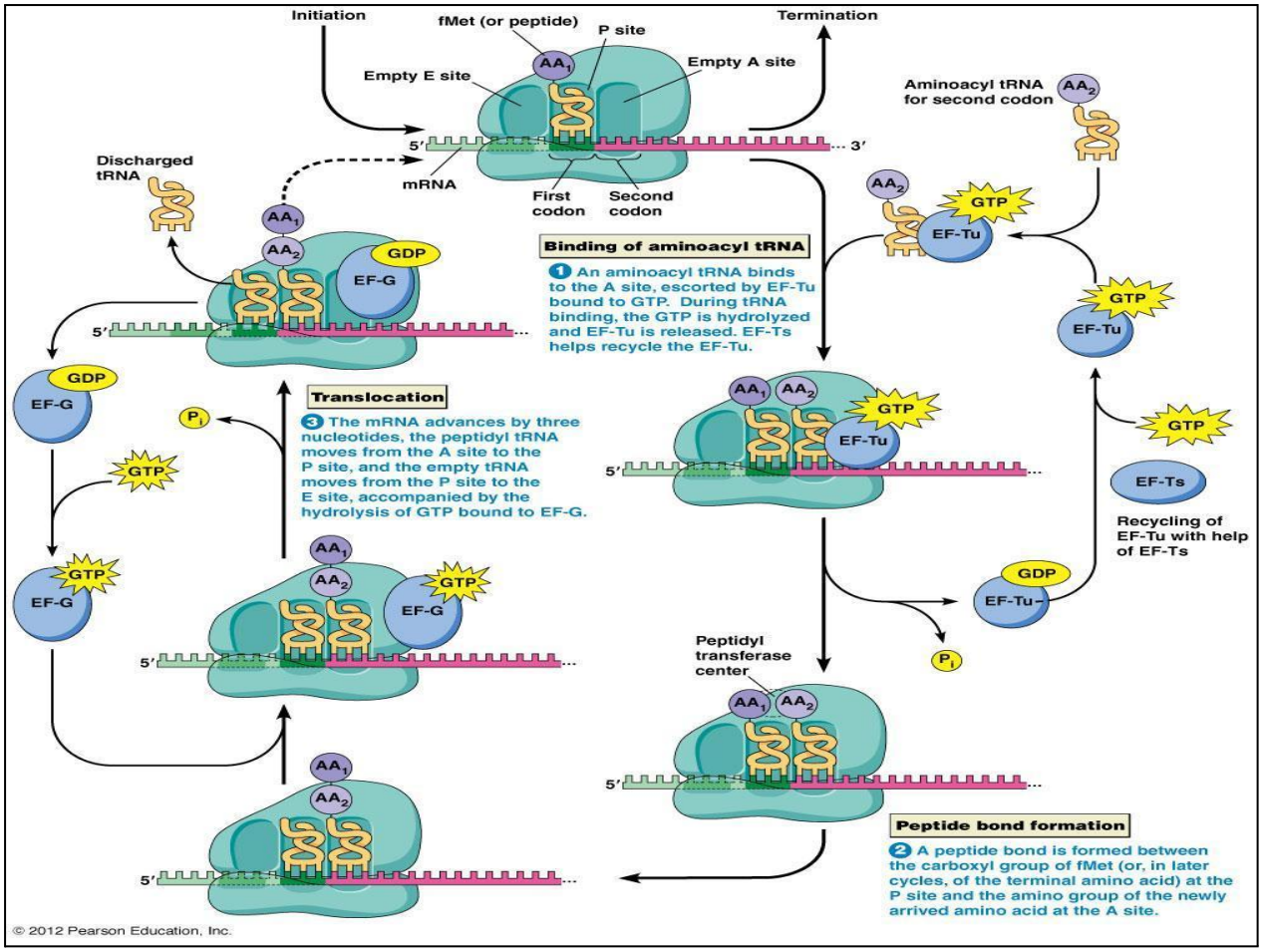
Placing the incoming aa-tRNA complex at the a site of the ribosome Formation of the peptide bond Translocation

Step 1. Placing incoming AA-tRNA complex at A-site of ribosome. An aminoacyl-tRNA (consisting of amino acid covalently bonded to tRNA) arrives and base pairs with the codon of mRNA present at the A-site. This step occurs in the presence of an elongation factor (called EF-Tu in bacteria and EF-1 in eukaryotes) and GTP which acts as a source of energy. GTP forms guanosine diphosphate (GDP). GDP and EF-Tu get released to get recycled by EF-Ts for the next round.



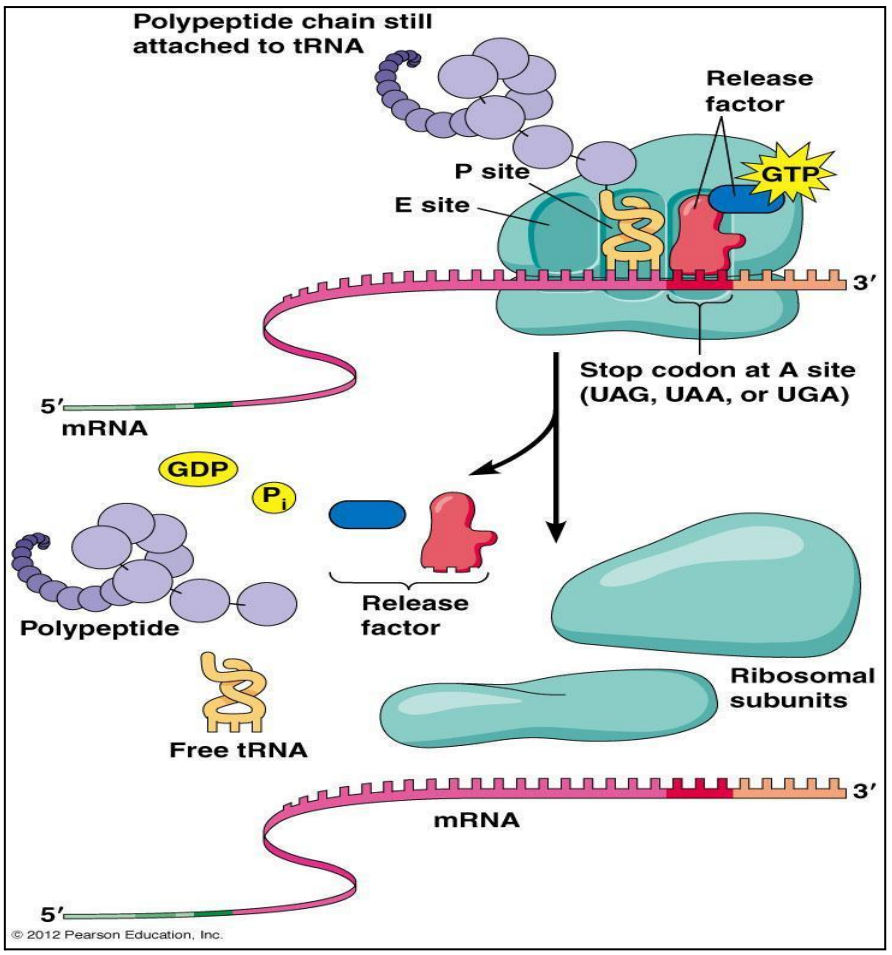
Step2:

Formation of peptide bonds. Peptide bonds are formed between amino acids of the adjacent tRNA by peptidyl transferase activity of the 23S rRNA (a ribozyme) molecule in the large ribosomal subunit.



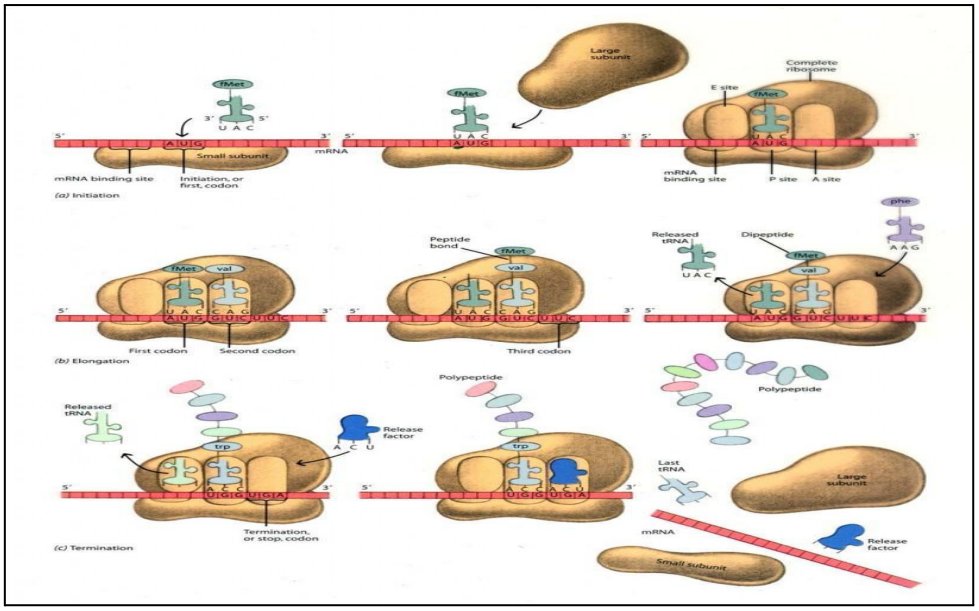
Step3:

Translocation. After the peptide bond is formed, the ribosome shifts or translocates along the mRNA. This shifts the tRNA at the A-site to the P site along with its attached peptide. This opens the A site for the arrival of a new aminoacyl-tRNA. This is promoted by another protein elongation factor called EF-G in bacteria and EF-2 in eukaryotes in the presence of a molecule of GTP which gives energy for the process. The tRNA which was at P-site gets shifted to the E site and later gets released into the cytoplasm to pick up another amino acid. The process gets repeated and all the codons in the mRNA get read by tRNA molecules. Hence, during the process, the amino acids attached to the tRNA get linked together in the appropriate order to form a growing polypeptide chain.This process of elongation is repeated until the ribosome reaches the stop codon on mRNA.



iii) Termination

The termination process occurs when the ribosome reaches the stop codon (UAA, UAG or UGA) because there are no tRNA molecules with anticodon for stop codons. These stop codons are recognized by protein release factors when they arrive at the A-site. These proteins along with a molecule of GTP help in the release of polypeptide from the ribosome. The two subunits of the ribosome also get disassociated after release of the polypeptide chain.

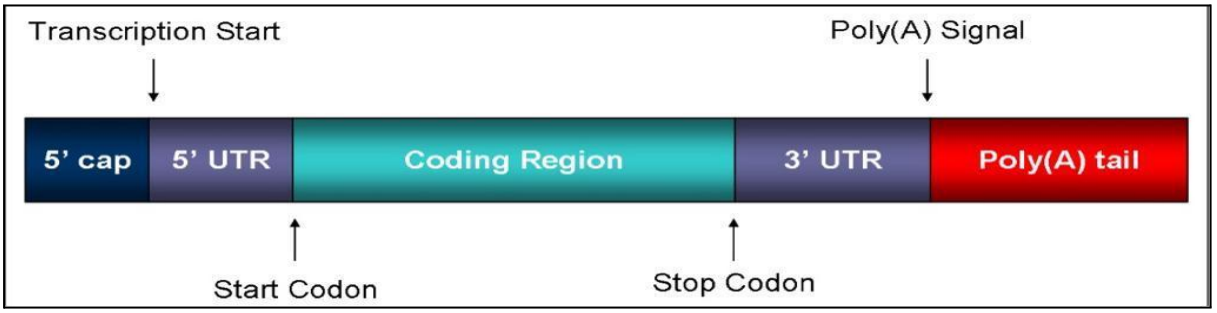


Untranslated Regions

A translational unit in mRNA is the sequence of RNA which has the start codon (AUG) and the stop codon on either side. The region between these gets translated into a polypeptide.

The regions of mRNA at 5′-end before the start codon and at 3′-end after the stop codon do not get translated and are called the untranslated regions (UTR). The region between the mRNAcap and the start codon is known as the 5'-untranslated region

[5'-UTR] whereas the region between the stop codon and the poly A tail of mRNA is called the 3'-untranslated region [3'-UTR]. The untranslated regions (UTR) are required for an efficient translation process.



Regulation of Gene Expression

A gene is said to be expressed if it is able to manufacture its corresponding protein. This multilayered process occurs in two major steps. In the first step called as transcription, the information in DNA is transferred by an enzyme called RNApolymerase II, to a messenger RNA (mRNA).

In eukaryotes, a pre-mRNA molecule called hnRNA is formed which is then processed to

form mature mRNA. In prokaryotes, mRNA is present in the cytoplasm whereas in eukaryotes mRNA moves out of the nucleus into the cytoplasm of the cell. In the next step, the mRNA which is a single-stranded copy of the gene gets translated into a protein molecule.

The process of regulation of genes helps in expression of only those genes which are required and switches off the genes which are not required. The expression of genes gets affected by the metabolic, physiological or environmental conditions.

In eukaryotes, the gene expression can be regulated at four levels:

1) Transcriptional level (during formation of primary transcript in the form of hnRNA),
2) Processing level (regulation of splicing of hnRNA to form mRNA),
3) Transport of mRNA from nucleus to the cytoplasm of the cell,
4) Translational level where mRNA is used to form a polypeptide

In prokaryotes, the regulation of gene expression occurs predominantly at transcriptional initiation.

Operon Concept

In 1961, Jacob and Monod gave the concept of a transcriptionally regulated system called as an operon. A polycistronic structural gene regulated by a common promoter and regulatory genes is referred to as **operon**. Example: *lac* operon, *trp* operon, *ara* operon, *his* operon, *val* operon, etc.

An operon consists of 3 basic DNA components:

1) **Promoter -** It is a nucleotide sequence recognized by RNA polymerase to initiate transcription and enables a gene to get transcribed.
2) **Operator -** It is a segment between the promoter and the structural genes of the operon at which the repressor binds. The operator region is adjacent to the promoter elements.
3) **Structural genes -** The region consists of genes that are co-regulated by the operon.

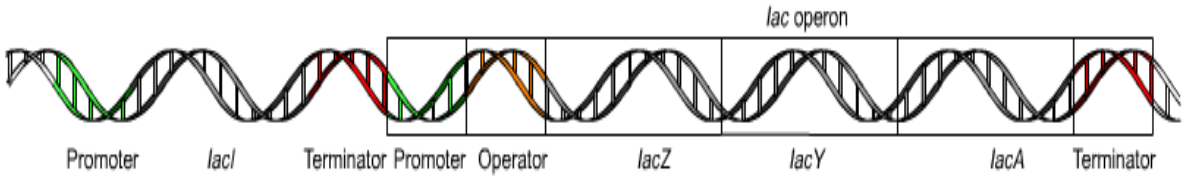
In a transcription unit, the activity of RNA polymerase at a given promoter is regulated by interaction with accessory proteins as they affect its ability to recognise start sites.

A regulatory gene which gets constantly expressed to form repressor proteins is present along

with the operon. Repressor proteins produced by the repressor mRNA bind the operator region in most operons. Each operon has its specific operator and specific repressor.

The regulation of an operon can be either negative or positive by induction or repression.These regulatory proteins which can act both positively (activators) and negatively (repressors) help to regulate this process. Negative control involves the binding of a repressor to the operator to prevent transcription. In positive control, transcription occurs when an activator protein binds to DNA at a site which is usually other than the operator.

The Lac Operon



The *lac* operon consists of one regulatory gene called the *lacI*gene or*i* gene' where 'i' refers to inhibitor and three structural genes (*lac*Z or 'z gene', *lac*Y or 'y gene', and *lac*A or 'a gene'). The '*i*gene' forms a repressor mRNA which codes for the repressor protein of the *lac* operon.

The '*z* gene' codes for the enzyme beta-galactosidase (β-gal) which results in hydrolysis of lactose into galactose and glucose. The '*y* gene' codes for the permease enzyme which increases permeability of the cell to β-galactosides. The '*a* gene' encodes a transacetylase enzyme which helps in transfer of an acetyl group from acetyl-CoA to β-galactosides. Hence, products of all the three genes i.e., *lacZ*, *lac*Y and *lac*A are required for metabolism of lactose in a *lac* operon.

Glucose is the preferred carbon source for bacterium. If instead of glucose, lactose is provided to the bacterium then lactose is transported into the cells through the action of permease and acts as an energy source for the bacterium. The expression of the genes involved in the catabolism of lactose to derive energy is controlled by the *lac*-operon.

Lactose plays a dual role in the lac operon as it is the substrate for enzyme β-galactosidase and also acts as 'inducer' to switch on the operon for the expression of the three structural genes. So, Lac operon is an example of regulation of enzyme synthesis by its substrate.The repressor of the operon gets synthesized continually i.e., all-the-time . Such genes which are transcribed continuously are called the constitutive genes. Housekeeping genes are always constitutive.

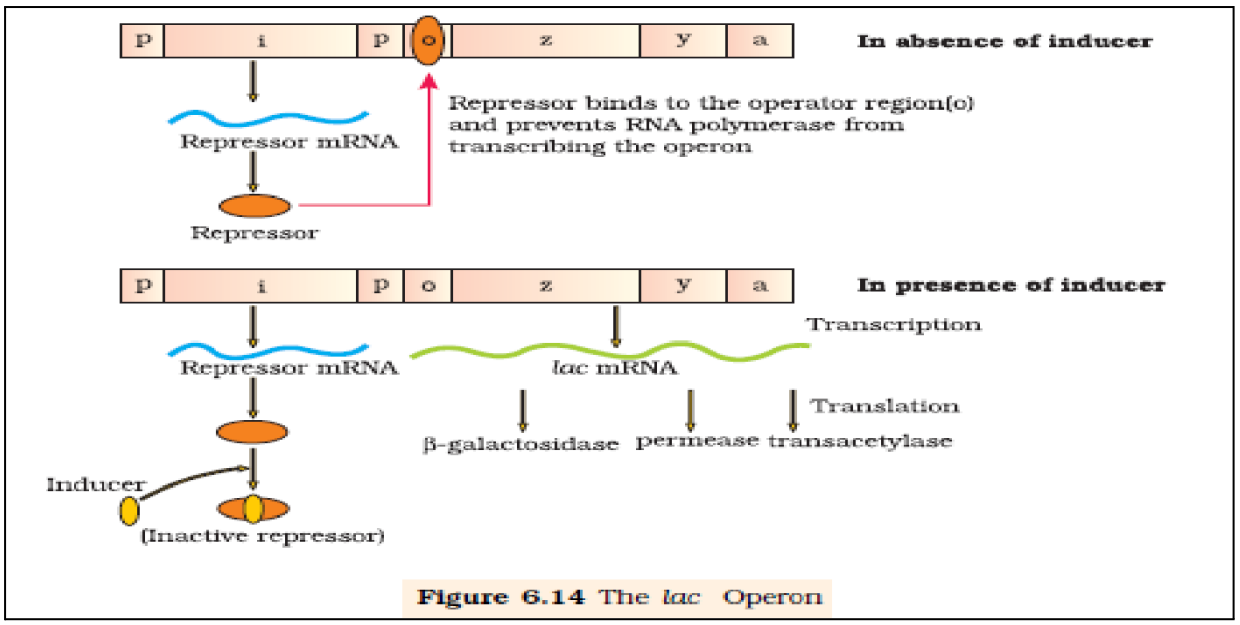


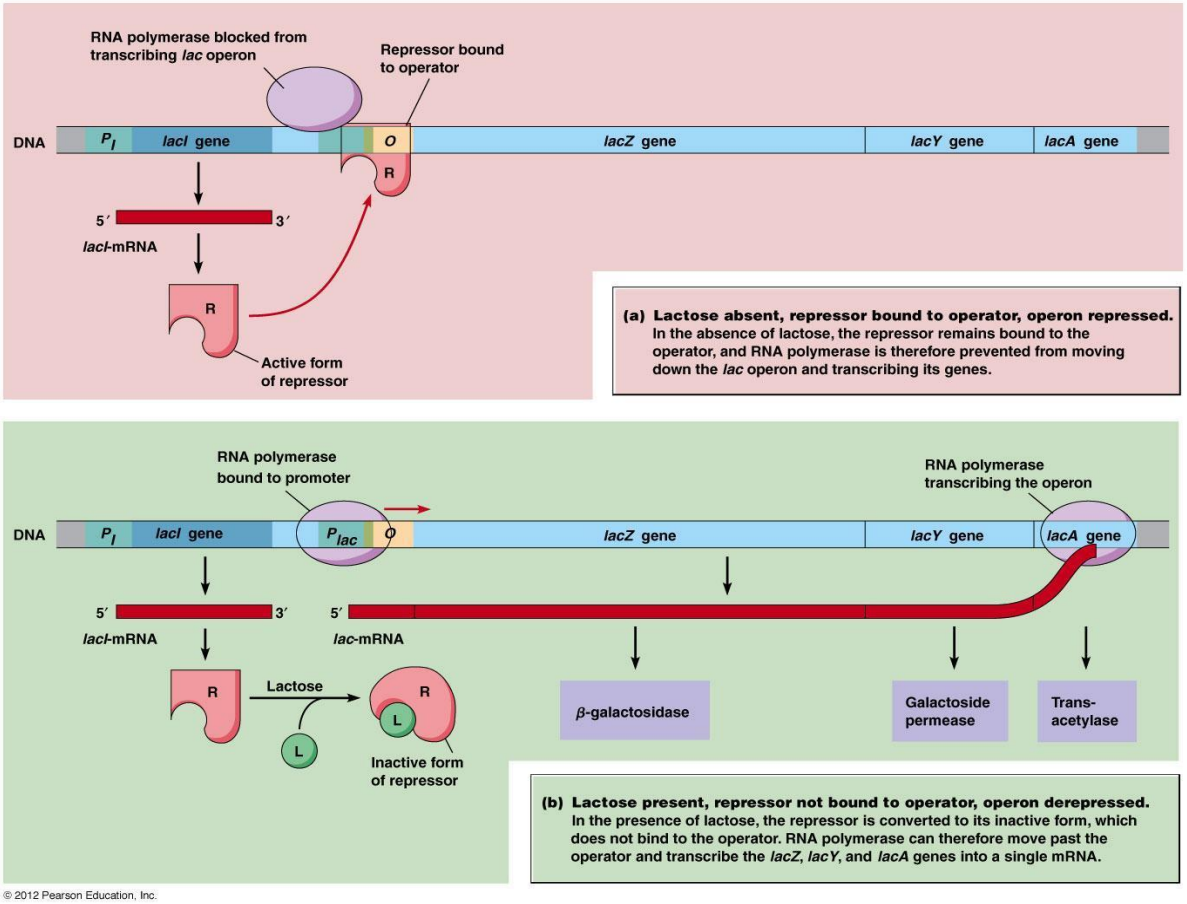
Figure 6.14 The *lac* Operon

In the absence of lactose, the repressor protein binds to the operator region of the operon and in order to prevent RNA polymerase from binding to the promoter. This inhibits the transcription of structural genes of *lac*-operon.

In the presence of lactose or allolactose which act as inducer, the repressor is inactivated as the inducer binds to it and RNA polymerase gains access to the promoter to carry out the transcription of the three structural genes required for the breakdown of lactose. The regulation of *lac* operon by repressor protein is referred to as **negative regulation**.

Summary

The genetic code which helps in the polymerisation of amino acids on the basis of the codons present on the mRNA was proposed by George Gamow. The genetic code is non-overlapping, unambiguous, degenerate, universal and gets read in a contiguous fashion. The secondary structure of tRNA which resembles a clover-leaf was given by Robert William Holley. The tRNA looks like an inverted-L in its tertiary 3D structure.



The process of translation of mRNA to form a polypeptide occurs in the cytoplasm of the

cell. Translation involves charging of tRNA and the three steps called initiation, transcription and termination.

Ribosomes and various factors help in the process of translation. The expression of genes can be regulated in order to form proteins only when they are required. A good example of regulation of the expression of genes is lac-operon which is present in bacteria. The operon model to explain the regulation of expression of genes was given by Francis Jacob and Jacques Monad.