Replication and Transcription

Objectives

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

- Reasons for stability of DNA
- Semi-conservative mode of replication of DNA
- Process of replication of DNA
- Process of transcription
- Difference between prokaryotic and eukaryotic transcription

Content

- Introduction
- Reasons for DNA being more stable than RNA
- RNA world
- Meselson-Stahl experiment
- Replication of DNA
- Transcription
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- Transcription in prokaryotes
- Transcription in eukaryotes
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Introduction

The Hershey-Chase experiment was able to unravel the mystery behind the nature of the genetic material, but very soon it came to light that some organisms like the viruses i.e., tobacco mosaic virus, retroviruses, QB bacteriophage etc have RNA as the genetic material. The overall findings suggested that most of the organisms have, so DNA is the predominant genetic material whereas RNA which is the genetic material in some organisms performs diverse functions in the living organisms.

In this section we will discuss what makes DNA a better genetic material than RNA, the process of replication of DNA, the process of transcription of DNA into RNA and the various intricacies involved in the process of replication and transcription.

Reasons for DNA Being More Stable than RNA

The characteristics of an ideal genetic material are that it should be

1. Able to generate its replica (Replication).
2. Chemically and structurally be stable.
3. Provide the scope for slow changes (mutation) that are required for evolution.
4. Able to express itself in the form of 'Mendelian Characters'.

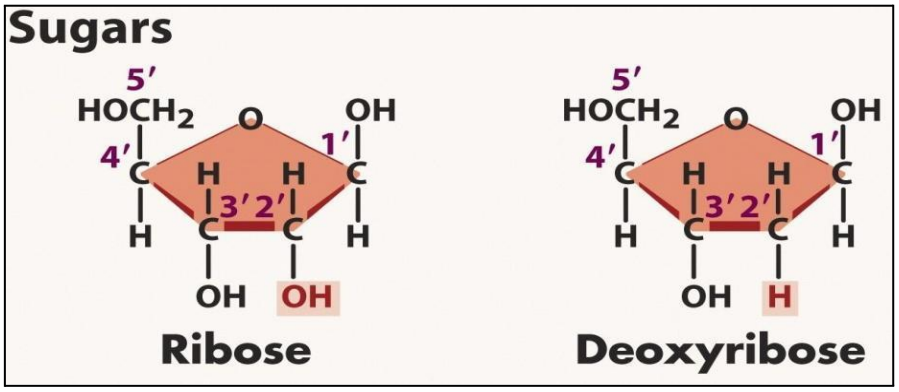
Proteins are not able to generate their replicas, so they cannot be considered as genetic material. Both DNA and RNA fulfill the characteristics which are essential for a genetic material.

The genetic material should not change with different stages of life cycle, age or with change in physiology of the organism i.e., it should be stable.

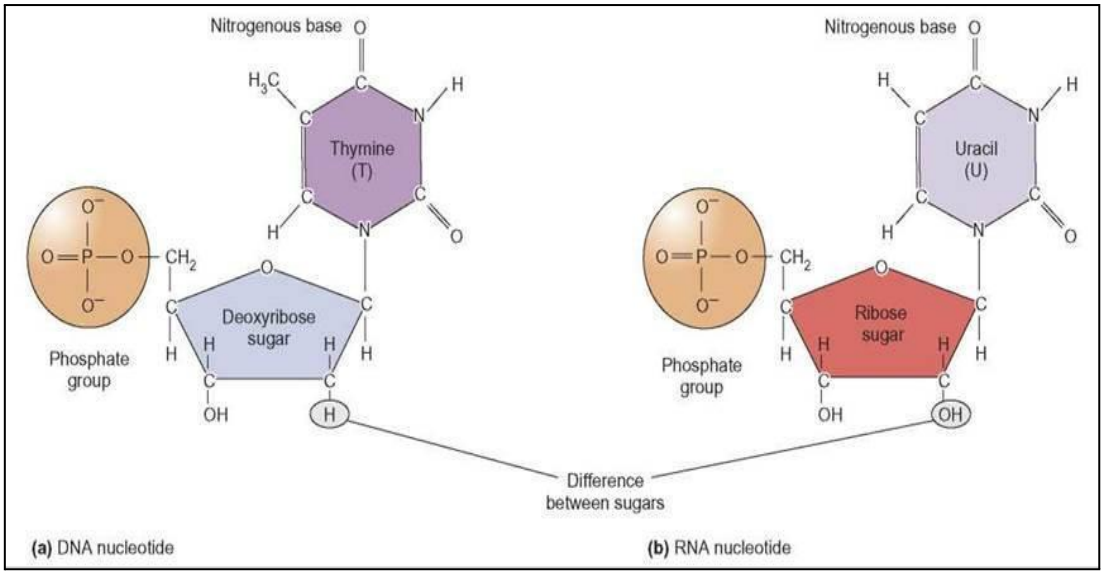
DNA is chemically less reactive, structurally more stable and a stable carrier of genetic information as compared to RNA which is less stable and more prone to mutations. RNA undergoes hydrolysis almost 100 times faster than DNA under normal cellular conditions. The unstable nature of RNA helps the RNA containing viruses to mutate and evolve faster.

This RNA is less stable than DNA due to the presence of two features viz.,

1. RNA has a 2′-OH group present at every nucleotide which is a reactive group and makes RNA labile and easily degradable. This property enables it to become more reactive due to which it can act as a catalyst i.e., ribozyme.



2. Presence of thymine (5'-methyl Uracil) in DNA at the place of uracil in RNA offers additional stability to DNA.



RNA World

In 1986, a Harvard molecular biologist, Walter Gilbert for the first time used the term "RNA world" in his article. The RNA world hypothesis says that RNA was the dominant genetic material when life evolved on Earth. RNA is considered a precursor to all current life on Earth. RNA is believed to have performed most jobs in the cell like storing genetic information, having ability to self-replicate and perform basic metabolic functions.

Today, different sorts of molecules like DNA, RNA, and proteins perform these jobs.

All the essential life processes like metabolism, translation, splicing etc. evolved around RNA. It was prescient of Francis Crick to make a guess that RNA could act as an enzyme, which was ultimately proven by Nobel prize-winning researcher Thomas R. Cech and others in the 1980s.

Hence, RNA has the ability to act as both genetic material as well as enzymes called ribozymes. RNA has the ability to self-replicate, undergo ligation with amino-acid and help in peptide bond formation. All the protein synthesising machinery has evolved around RNA. Also, RNA can be transcribed into DNA by the process of reverse transcription.

Experimental Proof of Semi-Conservative Mode of Replication of DNA

Watson and Crick once stated that ‘‘It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material’’.

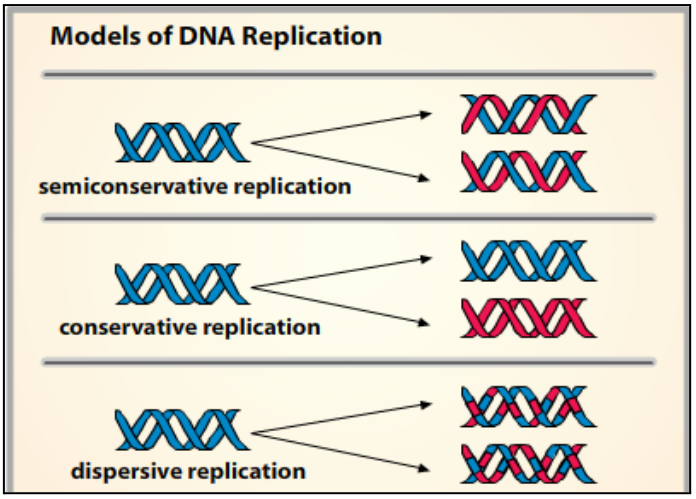
They suggested that during replication, the two strands of DNA would separate from each other and each strand would act as a template for the synthesis of new complementary strands. This mode of replication of DNA was called as semi-conservative mode of replication because after the completion of the process of replication, each DNA molecule would consist of one parental strand and one newly synthesised strand.

The pattern of replication of DNA suggested by Watson and Crick remained controversial for around 5 years and three patterns of replication were hypothesized meanwhile i.e.,

Semi-conservative - The original double strand of DNA get separated from each other and each strand acts as a template for the synthesis of a complementary strand of DNA.

Conservative replication - the original double strand of DNA is utilized as a template to create a new double stranded molecule of DNA. The original DNA strand remains intact during the process.

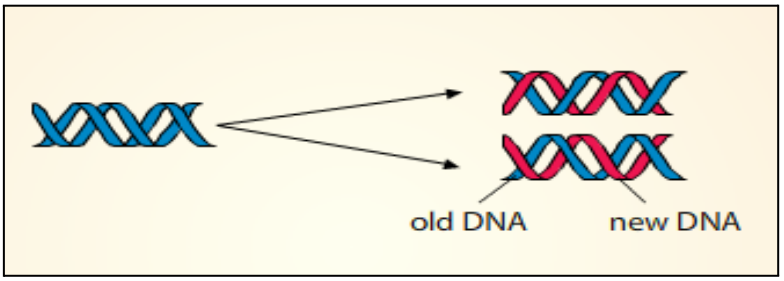
Dispersive replication- It resembles conservative replication as the original double strand does not separate and acts as template while remaining intact. The difference arises prior to cell division when the strands recombine in such a way that each daughter cell gets a mix of new and old DNA. In this mode, the original DNA gets cut up and then gets dispersed evenly between each copy with each round of replication.



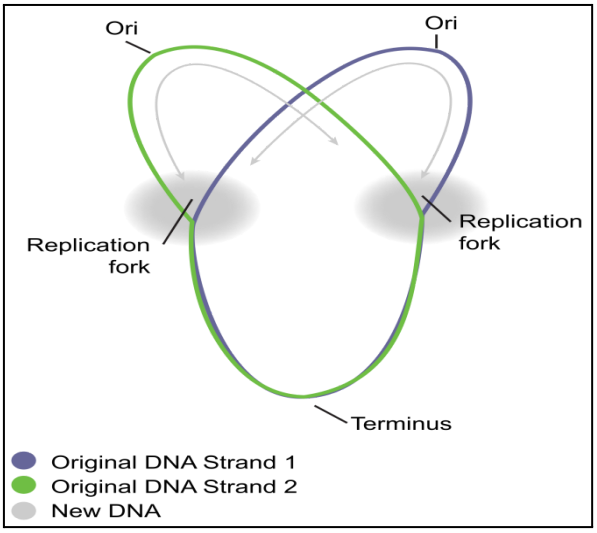
Meselson-Stahl Experiment

The support for the dispersive mode of replication remained strong till a definitive experiment to prove the semi-conservative nature of replication of DNA was performed in 1958 by Matthew Meselson and Franklin Stahl. They utilized methods that allowed them to

track new and old DNA from the samples of DNA collected over several rounds of replication and to distinguish existing DNA from newly synthesized DNA.



They worked on a bacterium *Escherichia coli* which has a single double stranded circular chromosome that undergoes replication bi-directionally from a single origin (*oriC*) to the terminus during a division cycle. *E. coli* (and certain other bacteria) shows a very rapid growth in rich medium and shows doubling in 20 minutes.

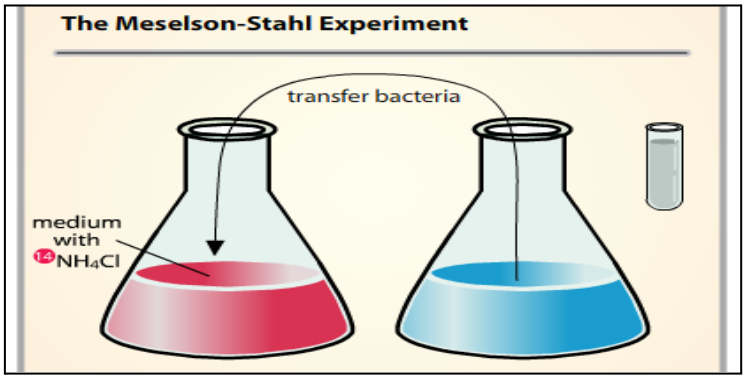


First, they grew *E.coli* bacteria in a medium containing $^{15}NH_4Cl$ which had ^{15}N, the heavy isotope of nitrogen as the only source of nitrogen, for several generations. After this period of growth, the entire DNA in the cells contained ^{15}N because it got incorporated into the entire newly synthesized DNA. This heavy DNA molecule was distinguished from the normal DNA by subjecting it to cesium chloride (CsCl) density gradient centrifugation.

They observed that 100% of the sample was found at the bottom of the tube used for density gradient centrifugation as it had only heavy nitrogen ^{15}N.

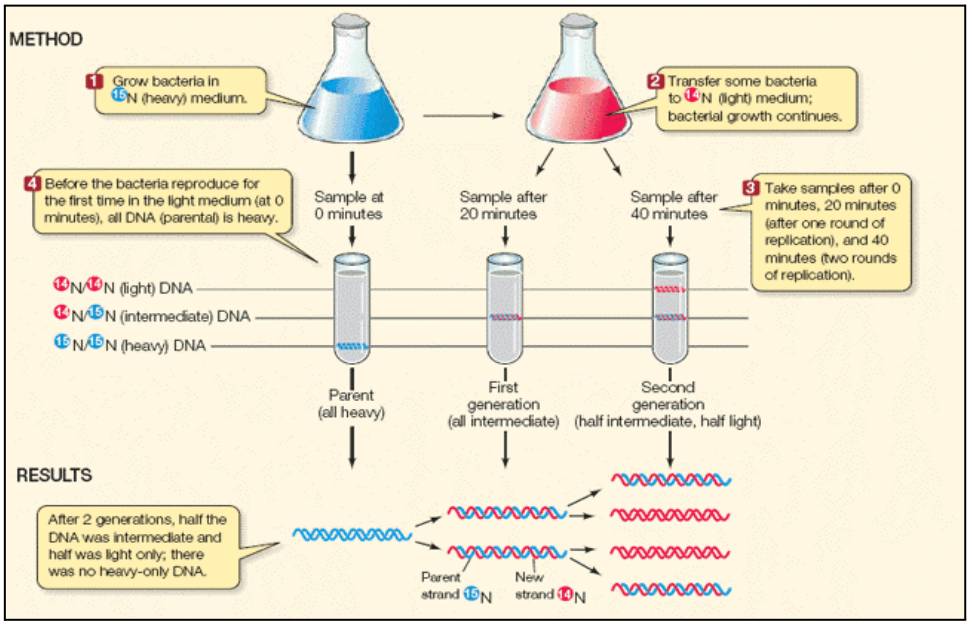
The *E.coli* cells from the ^{15}N containing medium were then transferred into a medium with normal $^{14}NH_4Cl$ (^{14}N is the normal, lighter isotope of nitrogen). The samples were taken after

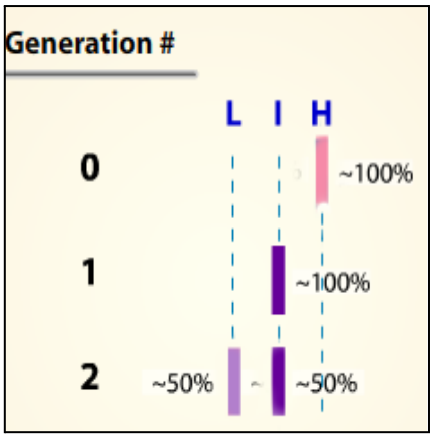
each replication cycle from the medium. The samples taken after each cycle were separated independently on CsCl gradients to measure the densities of samples of DNA.

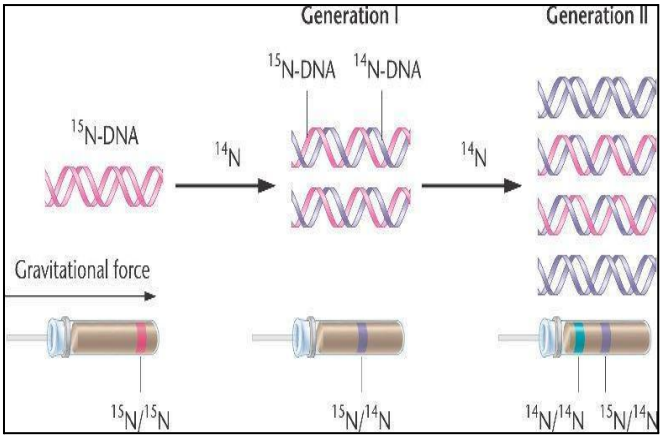


The DNA that was extracted from the ^{14}N culture medium after one replication cycle in the ^{14}N containing medium (i.e., first generation sample extracted after 20 minutes as *E. coli* divides in 20 minutes) had 100 % hybrid or intermediate density DNA in the CsCl density gradient.

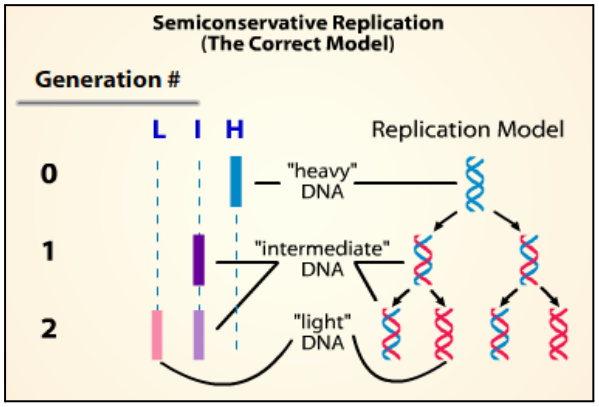
The DNA extracted from the culture after another replication cycle i.e., second generation (second generation sample extracted after 40 minutes) was composed of 50% hybrid DNA and 50 % light DNA i.e., equal amounts of hybrid DNA and 'light' DNA.

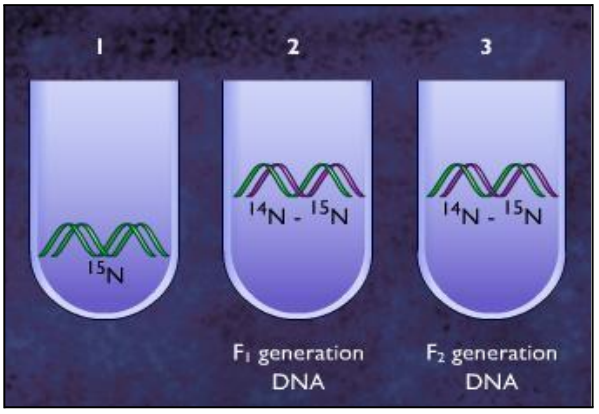




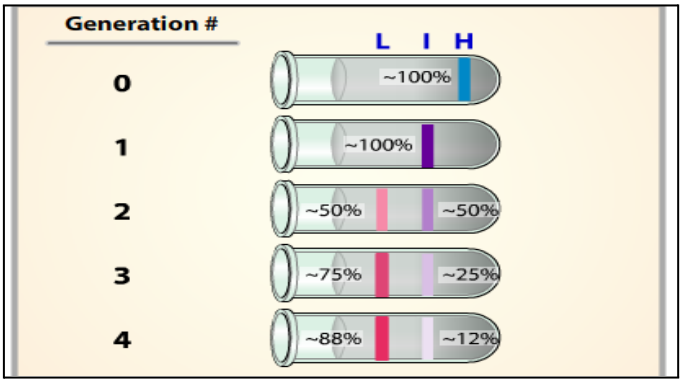


Hence, based on the experiment, Meselson and Stahl proposed that the DNA replicates semi-conservatively.





The results obtained after the third generation (i.e.,60 minutes) and the fourth generation (i.e.,80 minutes) in the Meselson and Stahl experiment would be as shown below-



Taylor and colleagues (1958) used radioactive thymidine ^{3}H to demonstrate that the replication of DNA present in chromosomes of root tip cells of *Vicia faba* occurs in semi-conservative mode. After the incorporation of radioactive thymidine ^{3}H, the root tips of

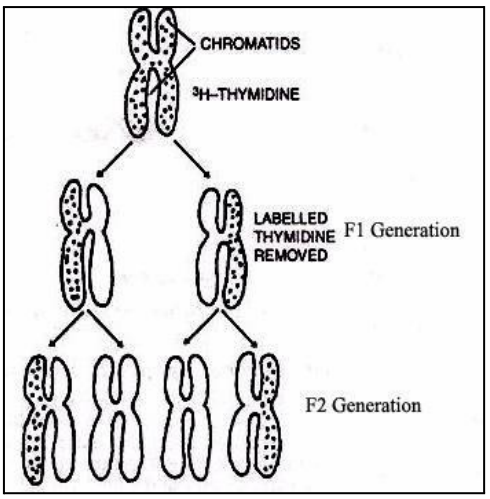
Vicia faba were transferred to an unlabelled medium which contained colchicine. It was observed that after the first generation, radioactivity was uniformly distributed in both the chromosomes because the original strand of DNA double helix was labelled with ^{3}H and the newly formed strand was non- labelled.

After second division (second generation), only one of two chromosomes exhibited radioactivity as it had one radioactive strand (original strand) and other non-radioactive strand (newly formed, non-labelled strand). On the basis of this, Taylor and colleagues proved that the DNA present in the eukaryotic chromosomes also replicates semi-conservatively.

Replication of DNA

DNA replication is the biological process of producing two identical copies of DNA from one original DNA molecule. In eukaryotes, the replication of DNA takes place at S-phase of the cell-cycle.

The process of replication requires enzymes like the DNA dependent DNA polymerase which uses a DNA template to catalyze the polymerization of deoxynucleotides. These enzymes are highly efficient, accurate and fast enzymes which can catalyze polymerization of a large number of nucleotides in a very short time. This is quite evident in *E. coli* which has 4.6×10^{6} base pairs and completes the process of replication within 38 minutes which means that the average rate of polymerization is approximately 2000 base pairs per second.



The Deoxyribonucleoside triphosphates (DNTP) serve dual purposes during the process of polymerization of DNA because the process of replication is energetically a very expensive process. They act as substrates (a deoxynucleotide part is added to form a new strand) while the two terminal phosphates are used to provide energy for polymerization reactions.

Apart from the DNA-dependent DNA polymerases, many additional enzymes are required to complete the process of replication. The various enzymes and molecules involved in the process of replication are summarized below-

Helicase- Helps to unwind the DNA helix at the replication fork.

DNA polymerase- Polymerizes the new strand of DNA in 5'-3' direction by adding nucleotides to the template strand. Also performs proof reading of newly formed DNA.

Single-strand binding proteins- Prevent the DNA separated by helicase enzymes from snapping back into a helix.

Topoisomerase- relaxes the super-coiled structure of the DNA

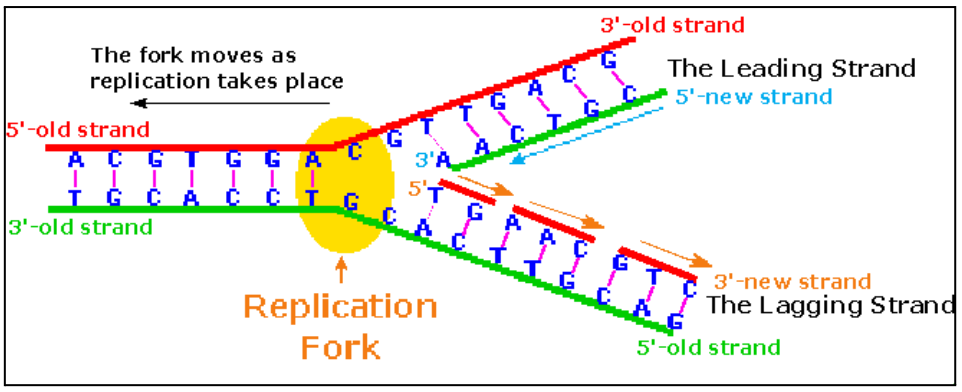
DNA gyrase- A type of topoisomerase which relieves the strain of the unwinding by DNA helicase

RNA Primers- Provide a starting point for DNA polymerase to carry the polymerization of the new strand of DNA from the template strand

DNA ligase- helps to join the fragments of DNA called Okazaki fragments in the lagging strand.

The process of replication of DNA takes place in three enzymatically catalyzed and coordinated steps namely initiation, elongation and termination.

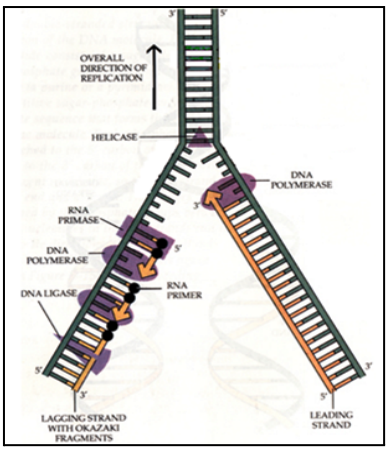
Initiation- The first step is the separation of the two antiparallel strands of DNA by the breaking of the hydrogen bonds which are present between the nitrogenous bases. The special proteins called as the initiator proteins target the DNA helix at regions called as the 'origins' which tend to be rich in adenine and thymine (AT rich) as it is easier to break the two hydrogen bonds present between A-T rather that the three hydrogen bonds present between G-C pair. The DNA strand gets unzipped to form a Y-shaped structure called the replication fork. The two strands of DNA cannot be separated in its entire length due to very high energy requirements. So, an enzyme called helicase untwists the DNA helix at the points of 'origin' called 'origin of replication' or Ori site.



Single-strand binding proteins (SSB) work with helicase to keep the parental DNA helix unwound and prevent it from snapping back in the form of a helix. They help the DNA to remain available for base-pairing with the newly synthesized daughter strands.

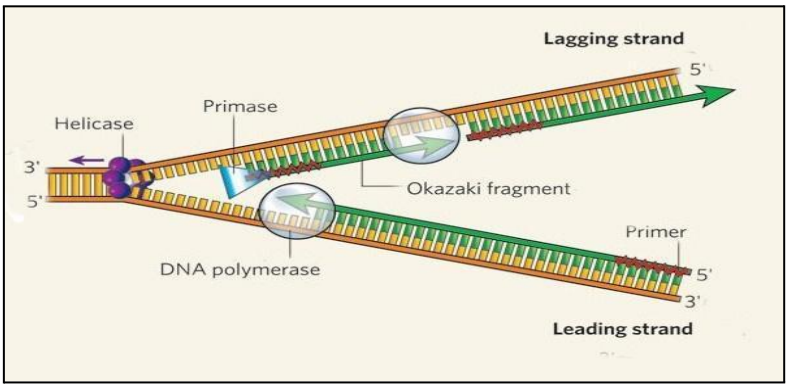
Elongation- All known DNA replication systems require a free 3' hydroxyl group before synthesis of new strand is initiated. So, the RNA primase enzyme binds to the parent chain and adds some RNA nucleotides called RNA primers for binding of DNA nucleotides. The RNA primers act as starters for the DNA polymerase enzyme to carry out the process of replication.

The DNA polymerase enzyme can add nucleotides only in 5'-3' direction, so the elongation process is different for the 5'-3' template DNA strand and the 3'-5' template DNA strand.

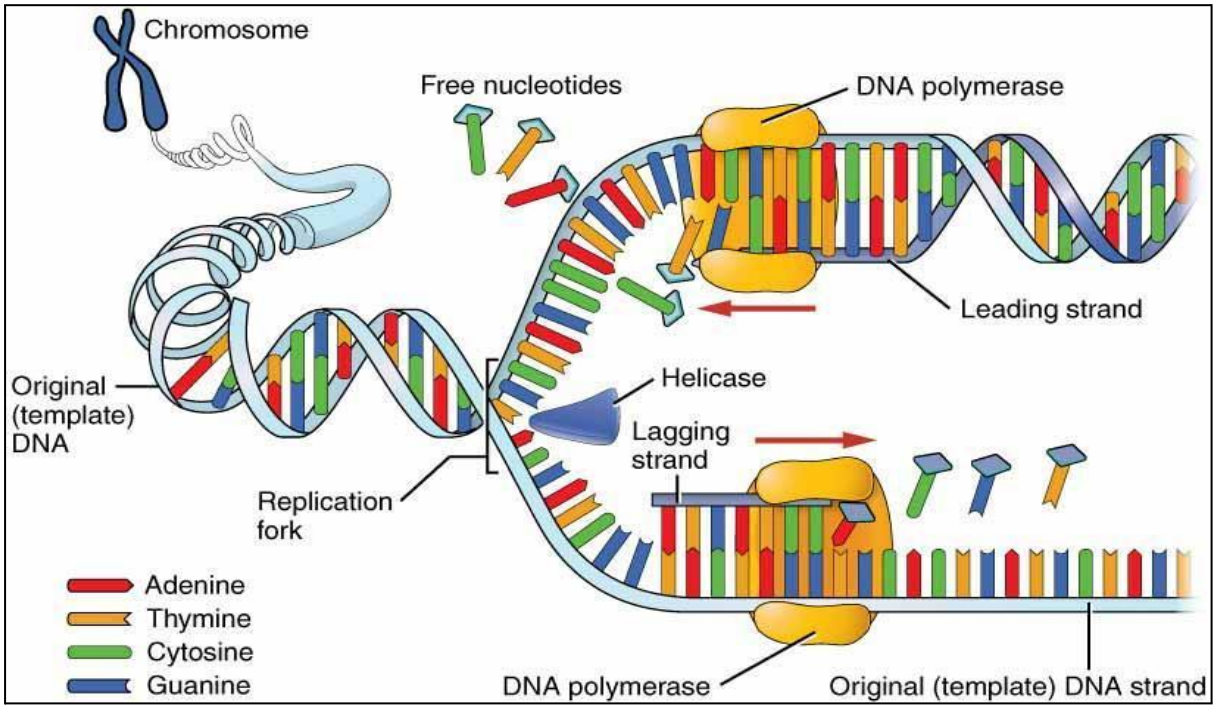


The replication is continuous on the template DNA with polarity 3' -5'. The 3'-5' template DNA strand has a single RNA primer and the DNA polymerase enzyme adds nucleotides to it in the 5'-3' direction so that the new strand moves in the direction of the replication fork. This newly synthesized strand is called the leading strand as it is synthesized continuously. Leading strand is synthesized rapidly and no gaps are formed in it so it does not require DNA ligase.

The replication is discontinuous on the template DNA which has a polarity 5′-3′. The 5’-3’ template DNA strand requires many RNA primers and gets replicated in the 5’-3’ direction by the DNA polymerase in the form of small segments called Okazaki fragments. These fragments are later joined by the DNA ligase enzyme to form a single continuous strand. The DNA polymerase enzyme acts in a direction away from the fork and the newly replicated strand is called the lagging strand.



Termination- This is the last step of DNA Replication. The termination occurs when the DNA polymerase reaches to an end of the strands.



The origin of the replication site is very important to begin the process of replication of DNA. During recombinant DNA procedures a vector is used to provide the origin of the replication site to carry out replication of a piece of DNA attached to it. Also, the process of replication of DNA and cell division cycle should occur in a highly coordinated way because a failure in cell division after DNA replication results in polyploidy which is a chromosomal anomaly.

Transcription

The process by which the synthesis of RNA occurs by using a DNA template is called transcription. In this process the genetic information stored in the DNA is copied into a RNA strand on the basis of the complementarity which exists between the nitrogenous bases. The only difference regarding the pairing is that Adenine in the DNA template strand pairs with Uracil in the mRNA instead of Thymine during transcription.

Some other differences which make the process of transcription different from the process of replication are that during transcription-

1. Only a segment of DNA is transcribed instead of the complete strand of DNA which gets copied during replication.
2. Only one strand of DNA serves as a template for transcription of RNA at any given time. The strand of DNA which gets transcribed is called the template strand or the non-coding strand. The other strand of DNA which does not get transcribed is called the non template or the coding strand because its sequence will be the same as that of the new RNA molecule except for the Uracil which will come in place of Thymine present on the coding strand.

It is also interesting to know that in most organisms, the strand of DNA that serves as the template for one gene may be the non-template strand (coding strand) for other genes within the same chromosome. Both the strands of DNA are not transcribed into RNA due to the following reasons-

(i) The first reason is that if both the DNA strands act as a template then two RNA molecules with different sequences of bases would be formed. If both of them code for proteins then the sequence of amino acids in the proteins would be different. This will mean that one segment of the DNA would be code for two different proteins which would complicate the machinery of the transfer of genetic information.

(ii) The other reason is that the two RNA molecules formed from the transcription of both the DNA strands would be complementary to each other. They will form double stranded RNA whereas a single stranded RNA is needed for getting translated into proteins. This would hamper the translation of RNA into proteins and the whole process of transcription would become of no use.

Transcription Unit and Gene

The specific segment of DNA which gets transcribed into RNA is called the transcription unit. A transcription unit consists of three regions

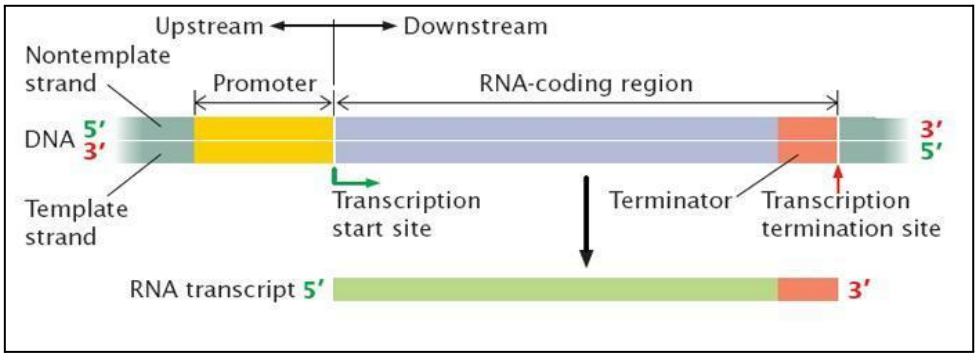
(i) A Promoter (ii) The Structural gene (iii) A Terminator

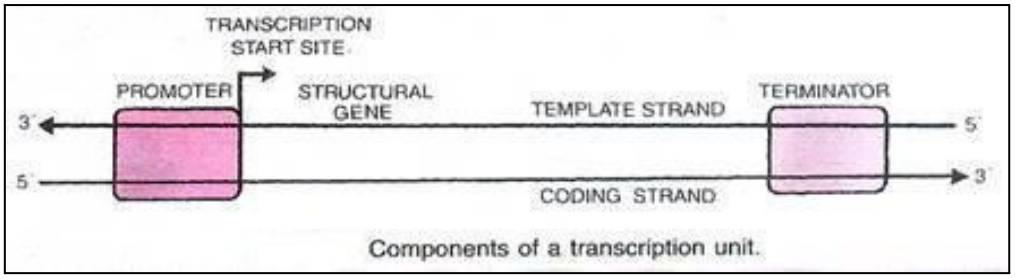
Eukaryotes also require an enhancer apart from the promoter. The enzyme DNA-dependent RNA polymerase can catalyze the polymerization of RNA in only 5'-3' direction. So, the strand that has the polarity 3′-5′ acts as a template, and is also referred to as template strand, master strand, antisense, or (-) strand. The other strand which has the polarity (5′-3′) and the sequence same as RNA (except thymine at the place of uracil), is referred to as the coding strand, sense or plus (+) strand.

All the reference points while defining a transcription unit are made with the coding strand. It is a convention that the location of the promoter is considered as the 5' end of the coding strand and that of the terminator as the 3' end of the coding strand. The promoter and the terminator regions flank the structural gene. Hence, the promoter is located upstream of the structural gene while the terminator region is present at the 3′ end of the coding strand, downstream of the structural gene (which corresponds to the 5′ end of the template strand). By switching the position of promoter with the terminator, the definition of coding and template strands could be reversed.

The RNA polymerase binds the promoter which has different parts where the various transcription factors can get attached.

Usually, the promoter has an AT rich region called TATA box which is a DNA sequence that is a type of promoter sequence which specifies to other molecules the point where the transcription begins.





Components of a transcription unit.

Gene: A gene is defined as the functional unit of inheritance.

Cistron: A segment of DNA which codes for a polypeptide. Depending on this the structural gene is referred to as either monocistronic or polycistronic.

Monocistronic: Found in Eukaryotes as the structural genes have interrupted coding sequences (means genes are called split).The coding sequences called Exons appear in the processed mRNA whereas the non coding sequences called Introns are removed by process of splicing. Introns do not appear in processed RNA.

Polycistronic: Structural gene found in Prokaryotes is called polycistronic.

Types of RNA: There are three major types of RNAs: messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA). All the three RNAs are needed to synthesize a protein in a cell. The mRNA acts as a template, tRNA brings amino acids to mRNA and reads the genetic code whereas the rRNAs play structural and catalytic roles during the process of translation.

Transcription in Prokaryotes

In prokaryotes, no proper nucleus is there due to which their DNA lies in the cytoplasm and transcription occurs in cytoplasm of the cell. The process of transcription requires an enzyme called DNA dependent RNA polymerase. The process of transcription in three steps namely initiation, elongation and termination.

Initiation: RNA polymerase binds to promoter and initiates transcription. RNA polymerase enzyme transiently associates with the initiation-factor called the sigma factor (σ) to initiate transcription.

Elongation: RNA polymerase uses nucleoside triphosphates as substrate and polymerizes RNA from DNA in a template dependent fashion following the rule of complementarity. Only a short stretch of RNA remains bound to the enzyme. The RNA polymerase is only capable of catalyzing the process of elongation.

Termination: Once the RNA polymerase reaches the terminator region it associates with a termination-factor called the rho-factor (ρ) to terminate the process of transcription. The nascent RNA as well as the RNA polymerase falls off which results in termination of transcription.

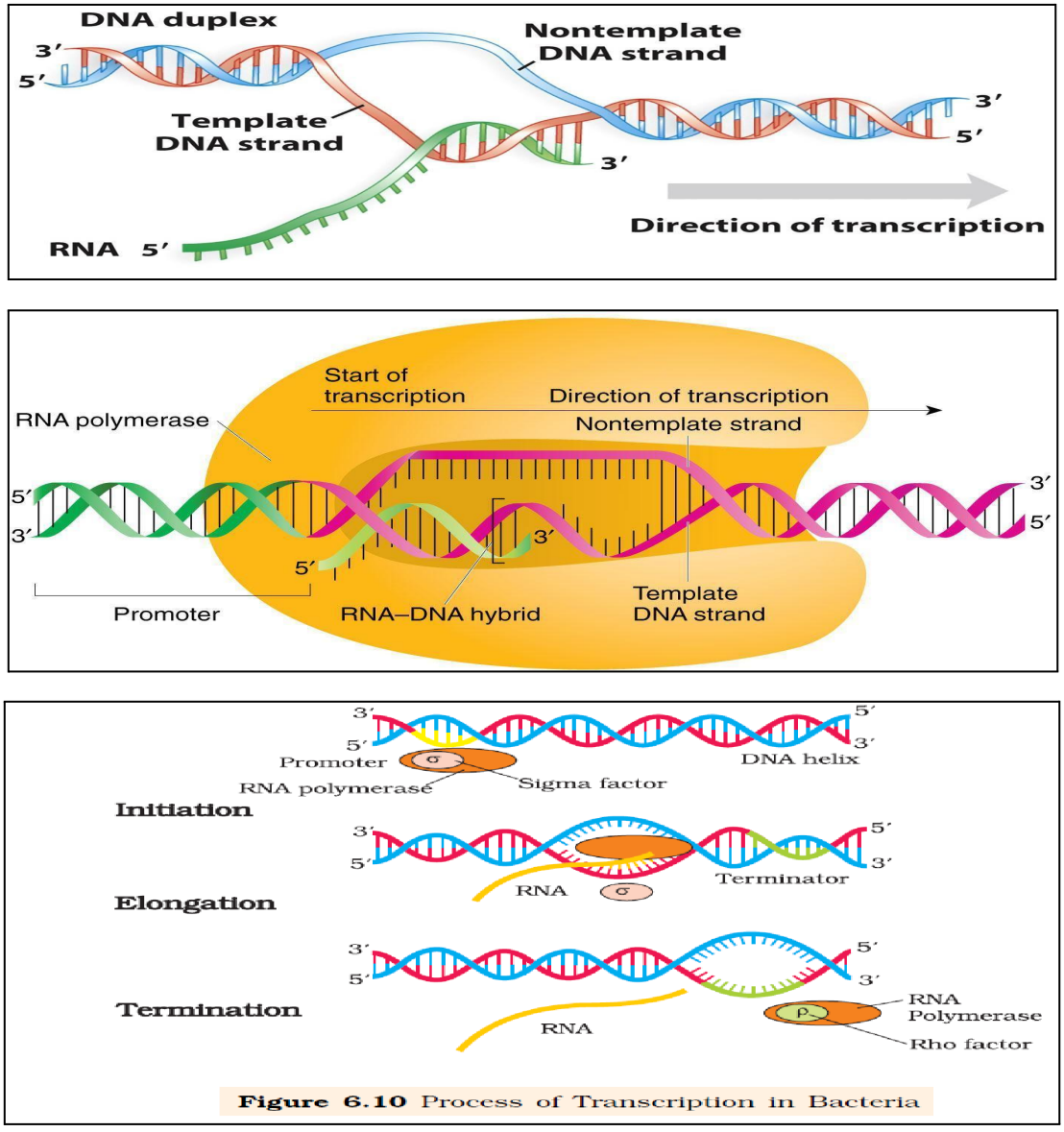


Figure 6.10 Process of Transcription in Bacteria

Transcription in Eukaryotes

Transcription occurs inside the nucleus in the eukaryotes and then the products of transcription move out into cytoplasm through the nuclear pores for undergoing translation. Though the basic features of transcription of RNA are shared between prokaryotes and eukaryotes, the transcription in eukaryotes is more complex than that in prokaryotes.

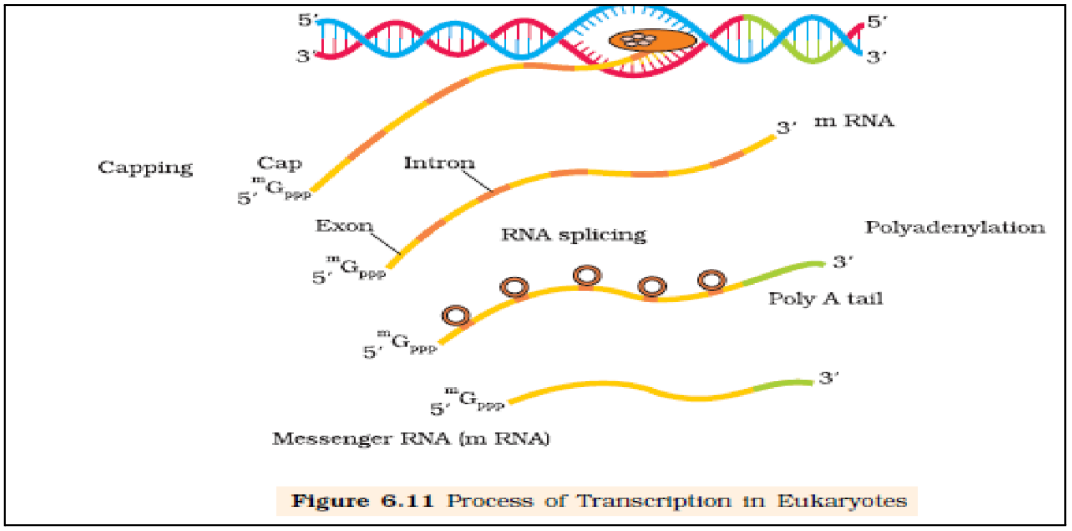
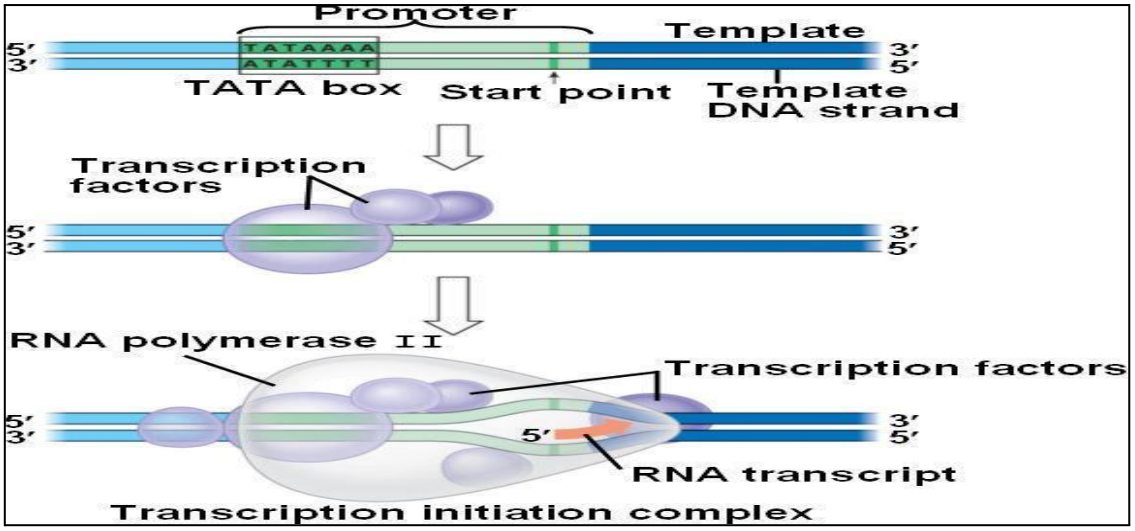


Figure 6.11 Process of Transcription in Eukaryotes

Differences between Transcription in Prokaryotes (Bacteria) and Eukaryotes

a) <u>Difference in RNA Polymerase</u>

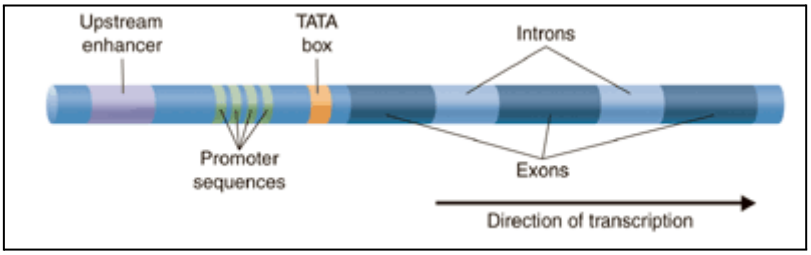
A single RNA polymerase helps in the transcription of all types of RNA in bacteria but eukaryotes have three different RNA polymerases. RNA polymerase-I helps in transcription of rRNAs (28S, 18S, and 5.8S). RNA polymerase-III catalyzes the transcription of tRNA, 5srRNA, and the snRNAs (small nuclear RNAs). The RNA polymerase-II carries out the transcription of the precursor of mRNA referred to as the heterogeneous nuclear RNA (hnRNA) hnRNA is processed(spliced) in nucleus, the introns are removed and the exons joined and sometimes reshuffled (alternate splicing). The mRNA transcript moves out of the nucleus into the cytoplasm. Eukaryotic RNA polymerases need the help of a set of proteins called the basic transcription factors as they cannot initiate transcription by themselves. A number of functions are provided by the basic transcription factors like binding to the promoter regions of the gene, attracting the appropriate RNA polymerase to the initiation site and unwind the DNA double helix to allow access to the incoming ribonucleotides required for the polymerization of RNA.



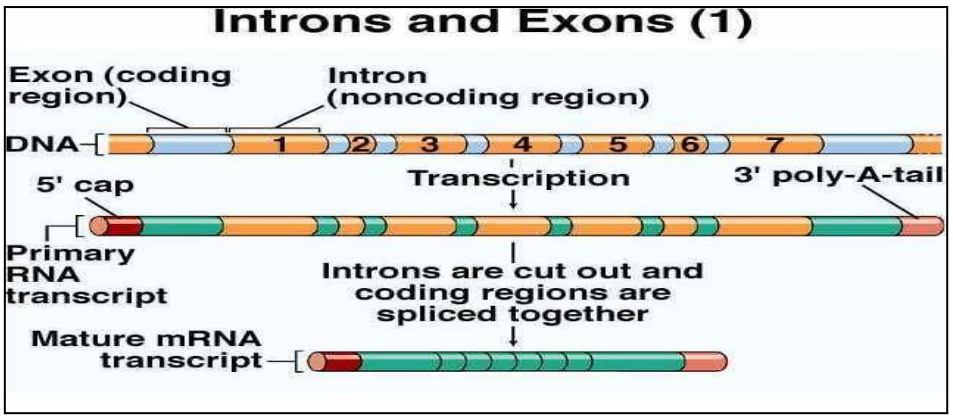
Difference in Processing of RNA

In bacteria, many times the translation can sometimes begin much before the mRNA is fully transcribed. This is because the mRNA does not require any processing to become active. Also, there is no separation of cytosol and nucleus in bacteria, so the process of transcription and translation take place in the cytoplasm of the cell and can occur simultaneously in the bacteria.

In eukaryotes the primary transcript of RNA has Introns and Exons. Most eukaryotic genes are split into segments. The stretches of DNA, which get transcribed into RNA but do not get translated into protein, are called introns. Those stretches of DNA which code for amino acids in the protein are called exons. The split-gene arrangement and the presence of introns indicate an ancient feature of the genome whereas the process of splicing reminds about the dominance of RNA-world. The introns are removed by the process of splicing and the exons are joined to get processed mRNA from the unprocessed hnRNA.



During its processing, the hnRNA undergoes two additional processes called capping and tailing. An unusual nucleotide, methyl guanosine triphosphate is added to the 5′-end of hnRNA during the process of capping. Adenylate residues (approximately 200-300) are added at 3′-end in a template independent manner during the process of tailing.



After hnRNA gets fully processed it is called mRNA which gets transported out of the nucleus for translation.

Summary

The DNA and RNA are both capable of acting as the genetic material, but RNA is more reactive and less stable than DNA due to which DNA is considered a better genetic material. However, when life evolved on Earth, RNA was preferred as the genetic material as RNA could mutate at a faster rate depending upon the conditions prevalent at that time . Even today RNA is capable of carrying out the process of protein synthesis on its own. The DNA can make its own copy by the process of replication which takes place in semi-conservative manner. The DNA can also be used as a template to synthesize RNA by the process of transcription. The process of transcription is significantly different from the process of replication of DNA. Also, the process of transcription also differs among the prokaryotes and the eukaryotes as it is much more complex in eukaryotes than that in the prokaryotes.