CSIR NET Life Science Unit 3

Transcription in Eukaryotes

Introduction

A eukaryote is referred to as any cell or an organism with a clear and defined nucleus. The eukaryotic cell possesses a clear nuclear membrane that edges the nucleus in which pairs of chromosomes are encountered. Eukaryotic cells also consist of organelles such as mitochondria, Golgi apparatus, endoplasmic reticulum, lysosomes and so on.

Eukaryotic transcription

Eukaryotic transcription is inferred as a process in which the eukaryotic cells replicate the data in a strand of DNA and copy the genetic knowledge into a new molecule of RNA. Eukaryotic transcription is referred to as the first step towards gene expression in which some segments of DNA are traced into RNA by a special enzyme called RNA polymerase.

This results in forming an antiparallel RNA fibre known as a primary transcript.

Procedure of eukaryotic transcription

Transcription eukaryotes take place in the following manner;

Transcription is the formation of RNA over the template of DNA. It creates single stranded RNA which has coded information similar to the sense or coding strand of DNA with the exception that T is replaced by U. The DNA strand which functions as a template for RNA synthesis is called template or antisense strand.

The segment of antisense DNA that takes part in transcription is called the transcription unit. It may consist of one or more cistrons (¨ genes).

Each transcription unit has a promoter region, generally in the beginning and terminator region where transcription ends.

Mode of transcription

The enzyme taking part in transcription is called RNA polymerase. There is single RNA polymerase (RNAP) in case of prokaryotes.

Eukaryotes have three types of RNA polymerase.

(i) RNA polymerase I – For synthesis of ribosomal RNAs except 5 S

(ii) RNA polymerase II – For mRNA and many snRNAs

(iii) RNA polymerase III – For tRNAs, 5S rRNA and some snRNAs

Prokaryotic RNA polymerase has a sigma factor for recognising the start signal of the promoter region. The remaining part of RNA polymerase is called core enzyme. In eukaryotes separate protein factors take part in recognition and initiation. They are called transcription factors, e.g., TFII, TFIII. Promoter region of the transcription unit has a separate recognition site and polymerase binding site.

Terminator region of the transcription unit has either palindromic sequences or poly A sequences.

Termination of transcription requires a separate termination factor called rho (r) factor. The various steps in transcription are as follows –

Activation of Ribonucleotides

Four types of ribonucleotides take part in the synthesis of RNA over DNA. They are AMP, GMP, UMP and CMP. The nucleotides are available in the nucleoplasm. Before their incorporation, the nucleotides are converted into activate through phosphorylation.

It produces triphosphates. Energy, phosphate and enzyme phosphorylase are required.

DNA Template

Only one DNA strand functions as a template strand. It is also called an antisense strand. The unit of transcription begins with a promoter and ends in a terminator. The separation of template strand does not require specific chemicals as in case of DNA replication.

Instead, the core enzyme of RNA polymerase travels along the template strand with the help of a protein factor called Nus A protein.

Initiation

RNA polymerase reaches the promoter region. Sigma factor (s) recognises the promoter region. In eukaryotes there are separate transcription factors for

recognition of promoter regions. As soon as the enzyme RNA polymerase gets attached, the template DNA of the transcription unit begins to unzipper.

Base Pairing

The activated phosphorylated ribonucleotides come to lie opposite complementary nitrogen bases of the template strand – A opposite T, U opposite A, G opposite C and C opposite G.

With the help of enzyme phosphatase, the extra phosphate radicals of nucleotides are hydrolysed.

It releases energy which is helpful in establishing temporary bonds between complementary base pairs.

Formation of RNA chain (Elongation)

The core enzyme with the help of energy and Mg2+ builds phosphodiester bonds between adjacent ribonucleotides forming the RNA chain.
As the enzyme moves along the DNA template, the RNA chain elongates.
Synthesis continues till the enzyme reaches the termination region.

In the terminator region RNA polymerase is separated from the DNA template by means of rho factor (r) and Nus G.

Chain Separation (Termination)

Rho factor has ATPase activity. This separates RNA polymerase as well as the newly built RNA strand. As soon as the RNA strand separates, the sense and antisense strands of DNA re-establish hydrogen bonds between their complementary base pairs. The duplex nature is restored.

Post Transcription Changes

Freshly synthesised RNA strand is called primary transcript.

It is often bigger than the functional RNA.

The various modifications which occur in primary transcript are as follows –

(i) Cleavage

A bigger primary transcript is often cleaved to form RNA of desired length. For example, the primary transcript of rRNA is 45 S.

It undergoes cleavage with the help of RNA enzyme ribonuclease P.

(ii) Splicing

Most of the eukaryotic mRNAs contain non–coding segments called introns or intervening sequences. They are removed. The process of removal of introns through cutting and joining the essential sequences or exons is called splicing. This is carried out with the help of snRNPs (snurps). The latter are formed by association of proteins with small nuclear RNAs (snRNAs).

SnRNP binds to 5' end of the intron while other attaches to 3' end of the intron. A spliceosome develops there. With the help of energy from ATP, cuts are made at both 5' and 3' ends of introns. This releases the introns. The ends of the adjacent exons are joined together to produce processed RNA.

(iii) Terminal Additions

The additions are made at the ends of newly formed RNAs for developing faculty to recognise the area of their action. For example, CCA is added at 3' end of tRNA. It is the area of attachment of amino acids. mRNA develops a cap at its 5' end.

The cap is 7–methyl guanosine (7mG). It develops at the 3' end and consists of repeated A–nucleotides.

(iv) Nucleotide Modifications

Nucleotides are modified post transcriptionally to perform different functions. Maximum modifications occur in tRNA. They include methylation, ethylation, deamination, etc. It produces such chemicals as dihydro–uracil, pseudo–uracil, methyl cytosine and inosine.

Difference Between Eukaryotic and Prokaryotic Transcription

<u>Prokaryotic Transcription</u>

- In this method, both transcription and translation take place simultaneously.
- The system of prokaryotic transcription occurs inside the cytoplasm of the cell.
- RNAs are always acquitted and processed in the cytoplasm of the cell.
- RNA polymerases are a complex formation comprising of five polypeptides.
- It does not require any extra proteins for the initiation of transcription.

Eukaryotic Transcription

- In this method, transcription and translation does not take place simultaneously.
- The eukaryotic transcription system occurs in the nucleus of the cell, and translation occurs inside the cytoplasm of the cell.
- RNAs are acquitted and processed in the nucleus of the cell together.
- RNA polymerases are a complex formation comprising of 10 -15 polypeptides.
- It requires extra proteins known as transcription factors in the initiation phase of transcription.

Eukaryotic transcription is inferred as a process in which the eukaryotic cells replicate the data in a strand of DNA and copy the genetic knowledge into a new molecule of RNA. Eukaryotic transcription is referred to as the first step towards gene expression in which some segments of DNA are traced into RNA by a special enzyme called RNA polymerase.