RAMA UNIVERSITY

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FACULTY OF AGRICULTURAL SCIENCES

AND ALLIED INDUSTRIES

TRANSCRIPTION AND TRANSLATION

ONE GENE – ONE POLYPEPTIDE CONCEPT

Ingram (1957) brought that each gene controls the production of a single polypeptide chain of protein molecule.

Crick (1958) proposed that the sequence of nucleotides in DNA and of aminoacids in proteins in co-linear. This means that there is a direct correspondence between the base pair sequence in DNA and aminoacid sequence in corresponding protein.

NON GENETIC RNA's

RNA is an intermediary between DNA base pair sequence and aminoacid sequence. Several kinds of RNA have been identified. RNA's differ from one another in molecular weight, structure and role in protein synthesis. The macro molecules are measured in Svedberg (S) units, determined by rate of sedimentation. The rate of sedimentation depends on the size and shape of the molecule.'

1. **Messenger RNA (m RNA):**

It is made up of single stranded molecule consisting of nucleotides ranging from 300 to 12,000. In Eukaryotis RNA molecules of variable length with sedimentation co-efficieint of 20 S to 100 S are involved. This is called heterogenous nuclear RNA (HnRNA). It is synthesised in nuclear and present out side the nucleus. The hn RNA has 2 fractions, one with coding sequence, that produce mature m RNA and another with non-coding sequences that are degraded in the nucleus. The coding sequences of hn RNA constitutes 'exon' and non-coding sequences constitute 'intron'.

Jacob and Monad (1961) first proposed the existence of m RNA. The life span of mRNA is 2 minutes at 37^{o}C in E coli. The two days in Eukaryotes and several months and years in dormant seeds of plants.

2. **Transfer RNA (t RNA)**

t RNA molelcules are smaller than m RNA molecule and contain 70 to 80 nucleotides. t RNA molecules have a completely folded tertiary structure due to hydrogen bonds between the constituent bases and due to the presence of a number of unusal bases such as A-U-G-C situated in the clover of the molecules where no base pairing occurs. Holley (1968) suggested a clover leaf model of alanine t RNA in yeast.

3. **Ribosomal – RNA (r RNA)**

Ribosomes are ribo molecules present in all types of cells. they occur as 60 s units inmitochondria, 70 S units in bacteria and chloroplast and 80 S units in eukaryotes.

r RNA is the insoluble RNA that constitutes the largest part, even upto 80 per cent of the total cellular RNA. r RNA molecules are sinlge polynucleotide stranded, unbranched and flexible and behave as a random coil or show helical regions with base paring between A-U and G-C. r RNA has a definite role in protein synthesis.

PROTEIN SYNTHESIS

Central dogma of molecular biology

The process of protein synthesis involves one of the central dogma of molecular biology, postulated by crick (1958) according to which genetic information flows from nucleic acid to protein. Protein synthesis involves two steps viz. , transcription and translation.

Transcription : Involves a sequential flow of information from DNA to RNA. This does not involve a change of code since DNA and m RNA are complementary. Translation involves a change of code from nucleotide sequences to aminoacid sequence.

Generally the flow of information in one way from DNA to RNA and from RNA to protein.

DNA ——Transcription→ RNA ——Translation→ Protein

In certain viruses, the existence of an enzyme 'RNA dependent DNA polymerase (also called in verse transcriptase) was reported and this enzyme could synthesize DNA from a single stranded RNA template.

The findings of Baltimore (1970) and others gave rise to the concept of 'Central dogma reverse'. According to this the sequence of information flower is not necessarily from DNA to RNA to protein , but can also take place from RNA to DNA.

DNA ——Transcription→ RNA ——Translation→ Protein

←—————— Inverse transcription.

TRANSCRIPTION

The process by which the information in the nucleotide sequence of DNA is transferred to complementary sequence of RNA is known as 'Transcription".

Transcription occurs throughout interphase and continues up to early prophase of cell division. 'DNA dependent rNA polymerase" or 'Transcriptase" is the enzyme involved in transcription. The locations of transcription are;

1. The nucleolus where genes from r RNA are transcribed.
2. The remaining chromatin where hnRNA (mRNA) is transcribed.

The system for invitro RNA synthesis contains;

i. Ribo nucleotide triphosphate (ATP, CTP, GTP, UTP)
ii. Enzyme RNA polymerase
iii. Mg^{++} or Mn^{++}
iv. Template DNA

The enzyme RNA polymerase acts only in the presence of DNA, against which the correct sequence of ribonucleotides is arranged and they are linked together by the enzyme. That is why the enzyme is known as 'DNA –dependent RNA polymerase.

The site of transcription is a cristron is called the promotor site. The template strand is called sense strand, while it's complementary strand is known as antisense strand. When only one strand of DNA is transcribed for a given region, it is called asymmetrical transcription. When both the strands of the DNA are transcribed, it is known as symmetrical transcription.

The enzyme RNA polymerase attaches itself at the promotes site. The DNA molecule unwinds over a short region. Then the free bases in the template stand of DNA determine the sequence of ribonucleotide in the newly formed in RNA. The RNA polymerase enzyme join the nucleotides together to produce RNA transcript.

RNA polymerase has give sub units of polypeptides chain (α, β, ,σ and ω) , and catalyze the linkage of ribose nucleotides by phosphodiester bonds. The Γ factor recognizes the start signal in the promotor region of DNA.

TRANSLATION

As soon as the mRNA is formed, it leaves the nucleus and reaches the cytoplasm where translation takes place.

Before the process of protein synthesis, the ribosomes occur in dissociated and inactive state. The m-RNA besides with 30 S ribosomal sub unit in the presence of a protein factor called 'Initiation factor ')IF). The mRNA carries tiplet codons for the synthesis of proteins. Proteins synthesis involves m RNA, ribosomes, aminoacid and their specific t RNAs.

Steps for Translation

i. **Attachment of m RNA with 30 S ribosomes and formation of polyribosomes**

The m-RNA transcribed inside the nucleus moves to the cytoplasm and binds itself with 30 S sub unit of the ribsome in the presence of initiation factor. Then the t RNA present in the cytoplasm binds itself with the first triplet codon 5' – AUG – 3' called the chain initiation codon of RNA to form the initiation complex. Later, the 30 S sub unit of ribosome unites with 50 S sub unit to form 70 S ribosome, in the presence of Mg++ ions. The message in the mRNA is not deciphered by one ribosome but many ribosomes are involved in the process and hence they are called polyribosomes.

ii. **Activation of the aminoacids**

Amino acids present in the cytoplasm are in a dormant stage. Each amino acid is activated by an activating enzyme called aminoacyl synthetase, beside the energy rich ATP. The aminocyl adenylate enzyme complex bound together with specific t

RNA molecule. As the enzyme is specific for specific aminoacid, the concerned aminoacid gets attached without error.

iii. Attachment of activated aminoacid to t RNA.

The DHU loop of tRNA recognises the synthetase enzyme. Then the aminoacid residue of the aminoacyl adenylate is transferred to the aminoacid attachment site of tRNA.

iv. Initiation of the polypeptide chain

In the m-RNA, the first riplet codon is AUG at its 5' end. AUG codes for methionine. Hence, protein synthesis commences with coding for methionine. The peptide chain formation starts in 5' end and proceeds towards 3' end and this helps in the correct sequence of protein synthesis.

The mRNA moves across the ribosome. A new codon of mRNA is brought in position. A new tRNA changed with specific amino acid is brought in position in such a way that the anticodon of t RNA pairs with the codon of mRNA. The attachment of two amino acids by polypeptile linkage involves enzymes translocase and peptidyl transferare along with energy rich GTP and t RNA is released.

This process of movement of mRNA from 5' to 3' direction and addition of amino acids to poly peptide chain continues till m RNA is no longer translated.

v. Transmission of the polypeptide chain

Any one of the three terminating codons in mRNA viz., UAA, UAG or UGA can signal the termination of chain elongation.

After chain termination, the enzyme peptidyl transferase hydrolyses the eesterbond between the chain and t RNA, releasign the poly peptide chain the last t RNA and m RNA.

Thus a polypeptide chain with a specific series of aminoacids is formed which results in synthesis of a specific protein that involves in a specific phenotypic expression in the organism.

IMPORTANT QUESTIONS:

1. Explain transcription process in detail with its importance.
2. What is translation? Discuss the steps and factors involved in translation.
3. Briefly explain one gene one polypeptide hypothesis.
4. Differentiate between transcription and translation.
5. What is the difference between translation in prokaryotes and eukaryotes.
6. Briefly explain central dogma.
7. What are different types of RNAs? Explain their roles.