Hello students, this lecture series is

for Bachelor of Science second year

students subject microbiology semester

for paper code MIC104 paper titled
Nucleic acids
module name is eukaryotic DNA

Part 2 split genes and nucleosomes

Module number 6 and Miss Leena Gawas.

Assistant Professor,

Government College of Arts,

Science and Commerce khandola marcela goa the outline of this lecture

series is Introduction and types of Split genes sequences
Features and evolution and significance of split genes
RNA splicing
Nucleosomes
Chromosomal proteins: types and functions
Packaging and formation of nucleosomes
Solenoid model of nucleosomes
Chromatin replication and nucleosome assembly

the learning outcomes of student will

be able

to explain types of Split genes sequences.
write a short note on evolution and significance of split genes.
explain RNA splicing.
explain chromosomal proteins, types and its functions.
write a short note on nucleosomes formation and packaging.
explain solenoid model of nucleosomes.

Introduction

Gene is a continuous, uninterrupted sequence of nucleotides which codes for a single polypeptide chain. Sequences of some eukaryotic genes (globin, ovalbumin) are found to be interrupted by nucleotides that are not represented within the amino acid sequence of the protein. They are transcribed into hnRNA (heterogeneous RNA), later excised (spliced) and removed, they are not included in the mature mRNA -translated into protein. Such interruptions within the sequence of a gene variously called - introns, inserts, intervening sequences or 'silent' DNA. Sequences which are included in the mRNA and translated have been called exons; the eukaryotic genes being a mosaic of introns and exons. Although the coding regions are interrupted, they are present in the same order in the genome as in the mRNA. Hence the name split genes. Split genes are first observed in eukaryotes. No split genes are reported yet in prokaryotes.

Split Genes sequences

There are two types of sequences

1) Normal sequences (exons)

2) Interrupted sequence (introns)

1) Normal sequence (exons)

This represents the sequence of nucleotides which is included in the mRNA and is translated from DNA of split gene. These sequences code for a particular polypeptides chain and are known as exons.

2) Interrupted sequence (introns)

The intervening or interrupted sequences of split gene are known as introns. These sequences do not code for any peptide chain. Moreover, interrupted sequences are not included into mRNA which is transcribed from DNA of split genes.

Important features of split genes

Each interrupted gene begins with an exon and ends with an exon. The exons occur in the same precise order in the mRNA in which they occur in the gene. The same interrupted gene organisation is consistently presented in all the tissues of organisms. Most introns are blocked in all reading frames. i.e. termination codons occur frequently in their three reading frames. Therefore, most introns don't not have coding functions.

Significance of split genes

In some cases, different exons of a gene code for different active regions of the protein molecule e.g. antibodies. Thus, introns are relics of evolutionary processes that bring together different ancestral genes to form new larger genes. Introns may provide increased recombination rates between exons of a gene and may be of significance in genetic variation. Introns are known to code for enzymes involved in the processing of hn RNA.

RNA Splicing

Split Genes is a gene that when transcribed results in pre-mRNA containing exons interrupted by introns. Splicing is generally performed by endonuclease enzymes cleaving the introns at both ends. Phosphodiester bond between sugar and phosphate at the junction between intron and exon is cleaved. The freed 5′-end of the intron joins the branch point sequence of form lariat. Splicing is performed by a large complex called spliceosome. Spliceosome is made up of small nuclear ribonuclear proteins (sn RNP) called snurps. These consist of RNAs which are rich in uracil and are of several types U1, U2, U4, U5 and U6 which are collectively called small nuclear RNAs (sn RNA). Prokaryotic genes are contiguous. Eukaryotic genes, in contrast, contain segments of DNA that are expressed (called exons) interrupted by segments that are not expressed (called introns). During gene expression, the resulting mRNA contains both exons and introns. It then undergoes splicing by which introns are removed so that it would only contain exon sequences before it is transported to the cytoplasm.

Nucleosomes

Are the fundamental repeating subunits of all eukaryotic chromatin (except when packaged in sperm). They are made up of DNA and four pairs of proteins -histones, and resemble "beads on a string of DNA" when observed with an electron microscope. They represent the first order of DNA compaction in the chromosome. In order to fit DNA into the nucleus, it must be packaged into a highly compacted structure known as chromatin. In the first step of process DNA is condensed into an 11 nm fiber that represents an approximate 6-fold level of compaction-achieved through nucleosome assembly.

Chromosomal proteins

The chromosomes of eukaryotes are made up of DNA and proteins.There are 2 major types of proteins associated with DNA in the chromatin.

1. Histones.
2. Non Histones proteins

Histones

They are the most abundant proteins associated with the chromosomes. They are very rich in basic proteins. At normal pH of the cell the histones have net positive charge that facilitates their binding to the negatively charged DNA. This positive charge is found mainly on the amino group of the side chains of the basic amino acids lysine and arginine. Histones lack tryptophan**.**

Histones

Histones are highly modified proteins, and the modifications include acetylation, methylation and phosphorylation.5 major types of histones associated with eukaryotic DNA .Each class of histone consists of a:N – terminal, which is hydrophobic.C – terminal, which is hydrophilic.Central globular structure, which forms the central molecule.

H2A, H2B, H3 and H4 together form an octamer while H1 links to the linker DNA outside the octamer.

Functions of histones

Depress the genetic activity: As the histones increase the compaction of the DNA , it depresses the genetic activity. Structural role: They play a structural role in the packaging of DNA molecules and hence render them more compact.

Non histones

They are all the proteins associated with the DNA apart from the histones. They are very different from histones. They are acidic proteins i.e. have a net negative charge and likely to bind the positively charged histones.

Functions of non - histones

Structural: They play a structural role in the shape of the chromosome.Regulatory: They positively regulate the gene expression and stimulate genetic activity. They play an essential role in the transcriptional and translational activity.Enzymatic: many enzymatic

activities are associated with chromatin. Enzymes of chromosomal metabolism [nucleic acid polymerases, nucleases] and enzymes of histone metabolism are all non – histone proteins.

Formation of nucleosomes

Core particle: chromatosome . Consists of 146 bp of DNA wrapped 1.8 times in a left handed helix around the outside of an octamer of histones. Interacts with one molecule of histone H1 to form a particle containing ~166 bp of DNA called chromatosome. The chromatosome links with the linker DNA forming a nucleosome containing ~200 bp of DNA.

Packaging of DNA in Chromosomes

An eukaryotic chromosome consists of a linear DNA molecule. This DNA is condensed into a complex structure with histones and non histone proteins.

Why packaging is required

DNA is about 3 meters long and it has to be packed in a nucleus, which is only a few micrometers in a diameter. Hence highly coiled structure is required.

Packaging DNA in Chromosomes

There are various orders of packaging

- ✓ First order of packaging : nucleosomes
- ✓ Second order of packaging : solenoid fiber
- ✓ Scaffold loop.
- ✓ Chromatid.
- ✓ Chromosome.

Interaction between the DNA and histones

Take place between negatively charged phosphates of DNA and positively charged groups of histone. The major electrostatic interaction of DNA phosphates are with the globular part of the core. The other interactions include hydrogen bonding between oxygen of phosphates of the DNA and histones. Thus this shows that DNA doesn't appear to be buried into the core but contacts it at widely separated points.

Solenoid model of nucleosomes

According to this model , the 10 nm fibre of nucleosomes gets coiled upon itself to form a 30 nm wide helix. This 30 nm structure is called as solenoid. It has 5 or 6 nucleosomes per helix. The histone N – terminal tails direct the DNA to wrap around the histone octamer disc. These N – terminals are thus required for the formation of 30 nm fibre as they interact with adjacent nucleosomes by making multiple H – bonds and thus stabilising the 30 nm fibre.

Chromatin replication and nucleosome assembly

A. Phasing and modification of nucleosomes in active genes phasing of nucleosomes

Some specificity in the distribution of DNA sequences on the nucleosomes and a lack of their random distribution is called as Nucleosome phasing. Nucleosomes are phased along the length of the DNA. Spacing of nucleosomes is not random but is regular. e.g. In chromosome of SV40 a DNA segment of 400 bp encompassing the replication region and the promoters is naked , i.e completely devoid of nucleosomes showing phasing of nucleosomes. E.g. 5' ends of many genes are naked suggesting that the active genes have to uncoil and loose their nucleosome structure temporarily.

B. Modification of nucleosomes in active genes

e.g. Acetylation of Conserved Lysines.