RAMSADAY COLLEGE

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Caption: e-Material of BOT-A-CC-4-10-TH

GENETICS

7. Structural organisation of Gene:

7.2. Split gene.

Split genes

Introduction to Split Genes:

It has been generally believed that a gene is a continuous, uninterrupted sequence of nucleotides which codes for a single polypeptide chain. Some recent work has shown that this may not always be so. The 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.

Although these sequences are transcribed into hnRNA, they are later excised (spliced) and removed, so that they are not included in the mature mRNA that is translated into protein (Fig. 22.9). Such interruptions within the sequence of a gene have been variously called as introns, inserts, intervening sequences or 'silent' DNA by some.

The 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.

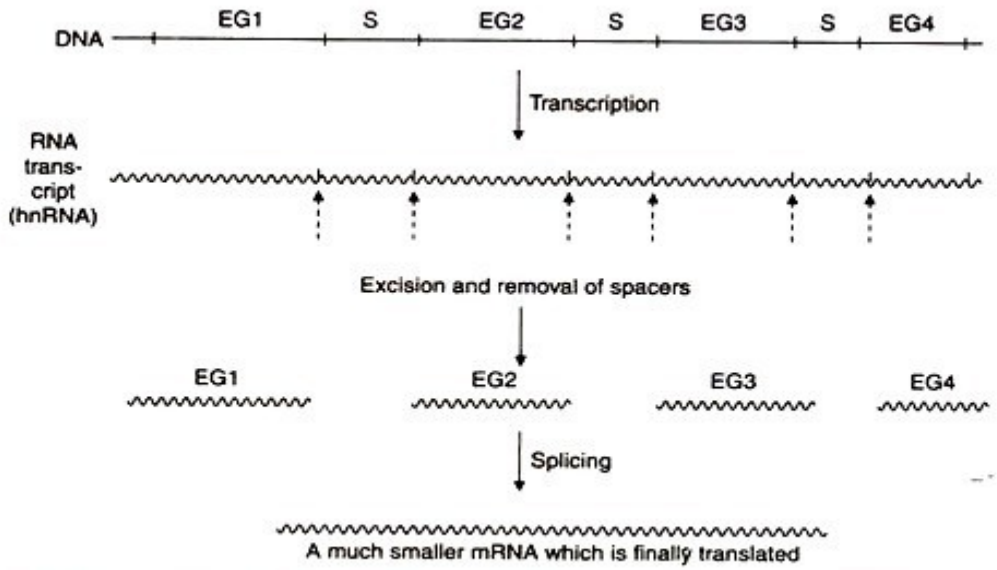

Fig. 22.9 Diagram to show relation between expressed genes (EG) and spacers(S) in split genes.

Intervening sequences were unequivocally demonstrated for the first time in the gene for β-globin in mouse and rabbit and then in chick ovalbumin. In the β-globin gene of mouse and rabbit a single intervening sequence is present about 200-250 nucleotides inside from one end of the gene, in both somatic and germ line cells of the animal.

White and Hogness (1977) have developed an R loop mapping technique for identifying the site and length of an intervening sequence in the electron microscope. The method involves hybridisation of the RNA transcript with double helical DNA of the gene at high temperature and increased concentrations of formamide.

Under these conditions the RNA-DNA hybrid molecules are more stable than the DNA duplexes. In those regions where RNA is complementary to a DNA strand, the two form a hybrid duplex; the unpaired DNA strand in the region forms a single- stranded loop visible in the electron microscope (Fig. 22.10).

With this technique Tiemeier (1978) found out that the intervening sequence in the β-globin gene forms an R loop about 585 base pairs long and located about 200-250 base pairs inside of the 3'end. The formation of loops has also been observed in adenovirus and SV 40.

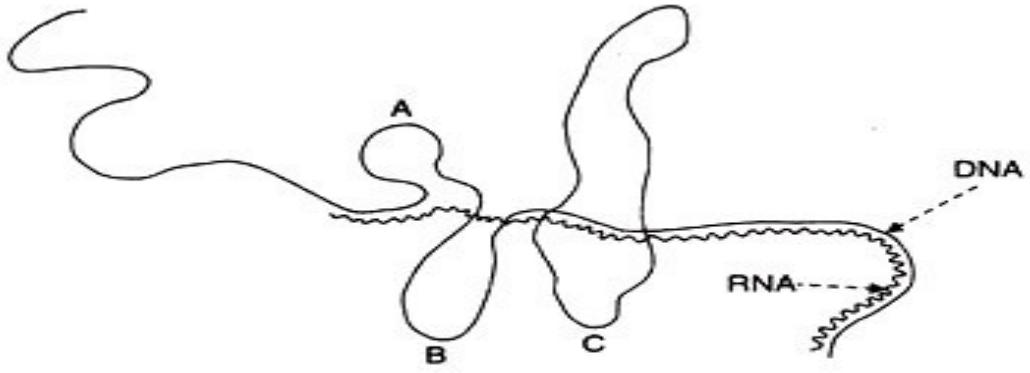

Fig. 22.10 Diagram from electron micrograph of the "hexon" gene of adenovirus. Loops *A*, *B* and *C* represent intervening sequences.

The ovalbumin gene in chicken has been shown to contain seven intervening sequences by the combined techniques of restriction enzyme and R loop mapping (Fig. 22.11). The insulin gene in mammals has two intervening sequences.

Unlike mammals, rat has two insulin genes I and II; gene for insulin II has two intervening sequences, one 119 base pairs the other 499 base pairs long. Even in the chloroplasts and mitochondria of eukaryotes, the genes for the larger rRNA contain intervening sequences.

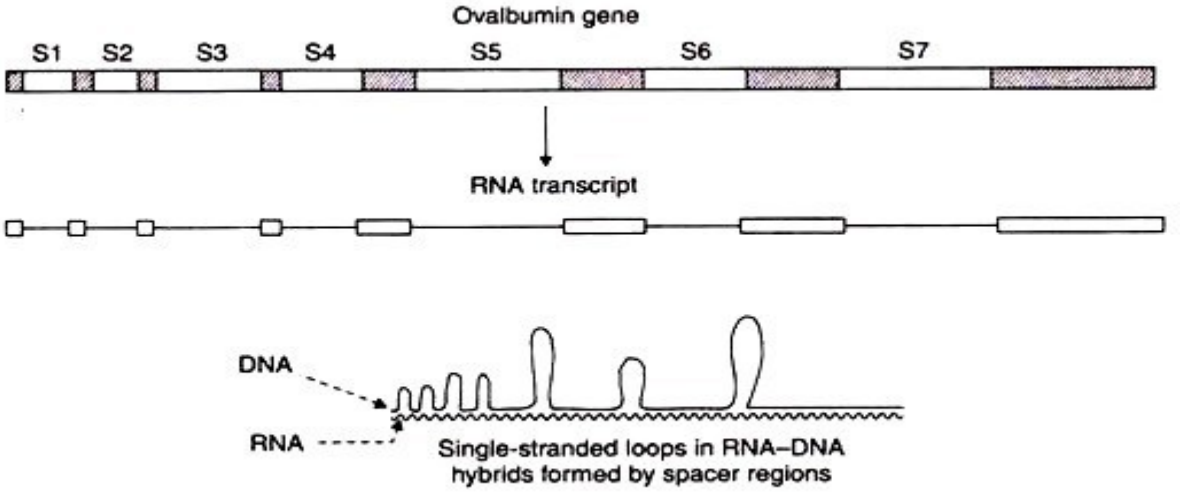

Fig. 22.11 Structure of ovalbumin gene and looping out of seven intervening sequences.

EM studies on cloned nucleolar rRNA genes have shown intervening sequences. In fact one of the first reports for split genes was on the 28S rRNA genes in D. melanogasterge. These

genes were found to be of variable lengths. When the complementary rRNA was hybridised to the rDNA, the longer genes were found to contain sequences that were not represented in rRNA.

The genes for tyrosine and phenylalanine tRNA in yeast contain a short intervening sequence of less than 20 bases in the region coding for the anticodon loop of tRNA. Lately split genes have been reported from various materials, and posttranscriptional removal of intervening sequences appears to be quite common in many eukaryotic genes.

Intron, Exon Structure of Split Genes:

Electron microscopic images of RNA-DNA hybrids and subsequent nucleotide sequencing of cloned genomic DNAs and complementary DNAs (cDNAs) demonstrated that the coding region of the mouse β-globin gene (encodes the β subunit of haemoglobin) is interrupted by two introns that are

In general, the intron-exon structure of eukaryotic genes is complicated, the amount of DNA in intron sequences usually exceeding that in the exons. For example, the chicken ovalbumin gene contains eight exons and seven introns distributed over 7700 base pairs (7.7 kilo-bases or 7.7 kb) of genomic DNA. The exons total only about 1.9 kb, which means that about 75% of the gene consists of introns.

A notable example is the human gene that encodes the blood clotting protein factor VIII. This gene contains about 186 kb of DNA and is divided into 26 exons. The mRNA is only about 9 kb long, which implies that about 175 kb of the gene contains introns. On average, introns constitute about 10 times more DNA than exons in the genes of higher eukaryotes.

Although most eukaryotic genes contain introns, their presence is not universal. Histone genes do not contain introns which shows that introns are not required for gene function in eukaryotic cells. Most genes of the simple eukaryote yeast lack introns. Introns are however, present in some rare genes of prokaryotes.

Most introns have no cellular function, although a few have been found to encode functional RNAs or proteins. In general, introns are considered to represent remnants of sequences that were important earlier in evolution. Introns may have helped to accelerate evolution by facilitating recombination between exons of different genes, the process is called exon shuffling.

Recombination between introns of different genes could produce new genes containing novel combinations of exons. This hypothesis is supported by studies that have demonstrated that some genes are chimaeras of exons derived from several other genes, thus indicating that new genes can be formed by recombination between intron sequences.