THE EUKARYOTIC GENE

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ABSTRACT

Eukaryotic genes, their expression and regulation are flexible enough to cope with, probably all possible situations. The genes are generally split with the complexity of introns becoming exons in some cases. They have the GT: AG junction. The common regulatory elements are the Goldberg-Hogness box, CAAT box, GATCC motif, cap site and enhancer sequences. The genes for rRNA, tRNA and histones are repeated to provide the large demands of the gene products.

THE regulation of expression of eukaryotic genes is far more complex than their prokaryotic counterparts. Bacteriophages have evolved an ingenious mechanism by which they can exercise extreme economy in utilizing their coding capacity by the arrangement of genes in an overlapping fashion[1-3]. This phenomenon is not limited to small phages and is found even in *Escherichia coli*[3], which indicates that overlapping genes may have some other functions to perform. In contrast, the eukaryotes have a large excess of coding capacity and this is in part achieved by the elaborate structural characteristics of these eukaryotic genes and their messages as well as certain regulatory elements.

Introns and Exons

Most of the eukaryotic genes are split, that is, the protein coding sequences, exons, are interspersed with sequences called introns, which do not code for protein. Recent evidences, at least in the cases of mitochondrial cytochrome b gene[4] and chicken αA-crystallin gene[5] show that introns can, indeed, contain protein coding sequences. An internal sequence in yeast nuclear mRNA introns is involved in splicing[6]. The eukaryotic genes which do not have such intervening sequences are interferon, histones with the exception of chicken histone H3[7], *Neurospora crassa* histones H3 and H4[8] and the adenovirus polypeptide IX gene[9]. The varying degrees of occurrence of intervening sequences can be seen from the non-allelic insulin genes in rat[10,11] which has only one intron and the α-2 collagen gene of chicken[12] which has a length of about 38 kb for a messenger size of 5000 nucleotides with at least 50 introns. More than one mRNAs can arise by alternative splicing within the coding region as demonstrated in the case of fibronectin mRNAs[13].

An analysis of the intron-exon junction sequences has revealed certain consensus sequences describing eight or nine nucleotides around the junctions. The consensus sequence deduced for the donor site is 5′ $^{A}_{C}AGGT^{A}_{G}AGT$ 3′ and for the acceptor site is 5′ $PyPyPyPyPyXCAGG^{G}_{T}$ 3′. Thus, the intron always begins with the dinucleotide GT at its 5′ end and ends with the dinucleotide AG at its 3′ end[14] for RNA polymerase II-read genes. The occurrence of the GT-AG consensus sequence does not apply for split ribosomal RNA[15,16] or tRNA genes[17] whose transcripts may probably be spliced by different enzymes. These sequences are present in organisms as diverse as yeasts, insects, chicken and humans.

Many of the eukaryotic split genes are also present in the genome in forms lacking the intervening sequences and are often deemed defective copies of functional genes[18]. These genes, variously called cDNA genes, pseudogenes or processed genes, appear to be some of a wide variety of DNA sequences generally present in the chromosome of multicellular organisms, which are of no apparent function. These pseudogenes are thought to have arisen by reverse transcription of the spliced RNA transcript of the homologous genes[19,20] possibly by incorporation into the genome of a retrovirus.

Common regulatory elements

The Goldberg-Hogness box: Prokaryotic promoters are DNA sequences to which RNA polymerase binds and are indispensable for specific initiation of transcription. Called the 'Pribnow' box[21], they contain a sequence of homology related to 5′ TATAAG 3′ and is located about 10 bp upstream from the mRNA start site[22,23]. A second sequence of homology, the 'recognition region' has also been noted in some cases in a region centered about 35 bp upstream from the mRNA start site. A search for possible eukaryotic counterparts of these sequences among several cellular

and viral protein coding genes has revealed the existence of an AT-rich region of homology centered about 25–30 bp upstream from the 'cap binding' site[24]. This sequence, known as the TATA box, was first noticed by Goldberg and Hogness in the case of histone genes of *Drosophila* and has come to be known as the Goldberg-Hogness box. It has been found upstream from the known or putative mRNA start sites of all as yet sequenced eukaryotic mRNA-coding genes transcribed by RNA polymerase II with the exception of the papovavirus late genes and the adenovirus early region 2[25,26]. The exact location of the TATA box varies from gene to gene with the T in position 1, falling between positions −34 to −26 from the mRNA start site. However, for a majority of these genes, this T falls within 2 bases of position −31. There are no obvious additional homologies within 10 bp around the TATA box, with the possible exception of a preference for G in the antisense strand. The TATA box is situated at roughly three turns of the DNA helix from the mRNA start site, whereas the prokaryotic Pribnow box is located at about one helix turn from this site. Studies using deletion mutants of conalbumin and adenovirus 2 major late genes indicate that the TATA box region is essential to promote specific transcription[27]. In addition, it has been found that the −12 to −32 adenovirus 2 major late gene fragment alone, when cloned in the plasmid pBR 322, can direct specific *in vitro* initiation of transcription, starting about 25 bp downstream from the TATA box[28]. *In vitro* transcription of SV 40 early gene mutants bearing various deletions located downstream from the TATA box also initiates about 25 bp downstream from the TATA box[29]. These results indicate that sufficient information for specific initiation of transcription *in vitro* is contained within a 20 bp region including the TATA box, and that transcription initiates about 25 bp downstream from the box. In contradiction, initiation still occurs on the SV 40 early gene region in the absence of the TATA region, but at multiple specific sites; deletion of the TATA box region of a sea urchin H2A gene did not abolish transcription completely, but reduced it and also generated varied 5′ termini for mRNA[30]. Thus 'TATA' box appears to play the role of a 'selector' in positioning RNA polymerase II precisely for the correct initiation of transcription to occur.

In marked contrast with what has been reported about the sequence requirements for specific *in vitro* initiation of transcription by RNA polymerase II, RNA polymerase III has an essential sequence component directing specific initiation of transcription located within the 5S RNA or tRNA genes[31–33]. The homologous sequences that are found upstream from the mRNA start site are not found upstream from the start sites of genes transcribed by RNA polymerase I[34] or RNA polymerase III[35] which indicates that the specific transcription of different genes by the distinct classes of RNA polymerases could be due to the specific recognition of sequences characteristic of a class of gene.

The 'CAAT' box: A second region of homology 5′ GG$^{C}_{T}$CAATCT 3′, has been noticed at positions −70 to −80 of several cellular and viral protein coding genes[36,37]. It occurs as such or in modified forms in most of the genes. It is present in all β-like globin genes[36], mouse α-globin gene[38] and human α-globin gene[39] and present as CCAAC in the case of δ-globin gene. Such a 'CAAT' box is present in many other eukaryotic genes. The widespread occurrence and location with respect to capping site suggests that this homology block should be important for transcription initiation. Contrary to this hypothesis, deletion of a region containing a 'CAAT' sequence 5′ to the sea urchin H2A histone gene does not abolish, and may even slightly increase, transcription of this gene in the *Xenopus* oocyte system[30]. Similarly deletion of the corresponding sequence 5′ to the chicken conalbumin gene[40] does not prevent specific *in vitro* transcription.

The 'GATCC' motif and the 'cap' site: There are sequence motifs that appear to be characteristics of histone genes and that are lacking from other RNA polymerase II transcribed genes. One such motif is a sequence realted to 5′ GATCC 3′ in histone genes located about 10 bp upstream from the TATA box, in the cases of sea urchin, *Drosophila*, chicken and a wide variety of other organisms[41]. Another sequence motif which appears to be specific to histone genes is the cap box sequence 5′ PyCATTCPu 3′ located at the mRNA start site. This sequence shows considerably less divergence than the analogous region of globin genes separated by a comparable evolutionary period[36]. However, DNA sequences downstream from the TATA box, including the natural β-globin mRNA cap site, are dispensable for transcription *in vivo*[42].

The Enhancer Elements

The presence of certain positive regulatory sequences called enhancer or activator sequences, increases the efficiency of transcription of some eukaryotic viral promoters[43,44]. Although there are variations in sequence among enhancers from various viruses, they generally have several properties in common: (i) they act on promoters only in *cis*, (ii) they

can act from a location either 3' or 5' to the promoter, (iii) they usually work in either orientation with respect to the promoter and (iv) they can act on heterologous promoters. For instance, SV 40 genome could be activated by an enhancer element from Moloney sarcoma virus[45]. The activity of some mammalian promoters such as the rabbit β-globin promoter, is significantly increased in the presence of a viral enhancer sequence[46]. The deletion of one of the two 72 bp repeat of the enhancer element has very little effect on the early gene expression, but deletion of all or part reduces early transcription considerably[47].

Repeated genes

Although most organisms contain repeated genes, they are more frequently present in eukaryotic than prokaryotic cells. Repeated genes can be broadly classified into two classes based on structural considerations and implied functional and evolutionary causes for gene repetition. They are, genes showing 'dosage repetition' and genes with 'variant repetition', two phenomena which are not mutually exclusive. Dosage repetition means that the genes in question occur in many copies per nucleus because the cell requires a large amount of the particular product which a single copy could not produce at the appropriate time. Dosage repetition is exhibited primarily by genes encoding rRNAs and tRNAs. These RNAs are present in every cell in high concentrations and a single copy gene, clearly, could not produce the required amounts of these products. Histone genes, in many organisms, also exhibit such dosage repetition because of the need for very rapid histone synthesis during early cleavage in animals like sea urchins or *Drosophila*.

Except for histone genes other protein coding genes do not need dosage repetition even if the protein is required in very large amounts, because one gene can direct the synthesis of much protein through the accumulation of mRNA. Highly differentiated cells may contain 50,000 copies of mRNA for their main protein product even though only a single copy of the gene is present. However, protein coding genes frequently occur in families of sequences in a pattern which came to be called 'variant repetition'.

Some of the gene families exhibiting variant repetition are the globin, actin, immunoglobulin, vitellogenin, ovalbumin, chorion protein etc[48]. Each of these represents a family of related proteins that have analogous but often clearly distinct sequences.

Ribosomal RNA genes: The best examples of gene dosage repetition are the genes coding for the 18S and 28S rRNA molecules. They are encoded on a single transcription unit synthesized by RNA polymerase I and this pre-rRNA is then processed in several steps to yield mature rRNA[49]. In many eukaryotic organisms, rRNA genes are tandemly repeated, transcription units alternating with nontranscribed regions called spacers. The extent of reiteration ranges from 45 genes per haploid genome in the insect *Sciara coprophila*[50] to 13,400 copies in the cone-bearing plant *Larix decidua*[51]. Ribosomal RNA genes are found on homologous chromosomes in closely related species like chimpanzee and man; on the other hand, individual differences in the chromosomal sites for rRNA genes have been reported in man[52].

The rDNA comprises of a repeating unit that includes the pre-rRNA gene and a spacer. In some cases the repeating unit also codes for 5S rRNA, but in general pre-rRNA and 5S RNA genes are not linked in eukaryotes. The transcription unit of pre-rRNA has sizes varying from about 8 kb in most eukaryotes including yeast[53], *Drosophila*[54] and frogs[55] to 10.5 kb in birds[56] and 13 kb in mammals[57]. The polarity of transcription always proceeds 5' end-nonconserved region-18S RNA-nonconserved region-28S RNA-3' end[58]. This polarity is universal, including bacterial and most organelle rRNA genes where analogous molecules substitute for 18S and 28S RNAs.

The genes for 5S rRNA occur in tandemly repeated clusters in all eukaryotic organisms studied. Initiation of transcription takes place at the 5' end of the mature RNA; there is no 5' terminal precursor sequence[49]. A larger molecule that extends beyond the end of the mature RNA at the 3' end has been found in *D. melanogaster*[59], rat and HeLa cells[60]. In *Xenopus* there is in general no precursor to 5S RNA. In *Xenopus*, two separate sets of genes encode oocyte type and somatic type 5S RNA. The major oocyte-specific set of 5S RNA gene in *X. laevis* is a large family with 25,000 members occurring in clusters at the telomeric regions of most chromosomes. The somatic 5S RNA genes constitute a much smaller set of several hundred genes[61].

TransferRNA genes: The total number of tRNA genes always exceeds by far the number of different tRNA sequences; this indicates that each tRNA gene is repeated. In *E. coli*, most tRNA genes are clustered in polycistronic transcription units and processed from large precursor molecules; some are on individual transcription units and some are found between the genes for rRNAs[62]. The arrangement of tRNA genes shows highly variable patterns in the genome of cell organelles. Eukaryotic tRNA genes, transcribed by

RNA polymerase III, do not give rise to large precursors or polycistronic transcription units, but yield short precursors that are processed at both ends[62]. Genes for tRNAs are clustered in many cases in two different fashions: homoclustering, where groups of genes for a single tRNA are present and heteroclustering, where interspersed genes for several tRNAs are present.

The 60 or so different tRNA genes in *Drosophila* are repeated about 12-fold on an average. *In situ* hybridization with total tRNA revealed about 140 loci distributed throughout the three autosomes and the X chromosome. Each of the thirteen purified tRNA species that hybridized to giant chromosomes labelled between two and six specific sites[63]. The sites for a particular tRNA species can be distributed either on the same or on different chromosomes. In one instance, two tRNA species, $tRNA_2^{Lys}$ and $tRNA_2^{Arg}$ labelled a common site at the same position. In *X. laevis* each tRNA gene is reiterated on an average 200-fold[64]. The reiteration can be different for various genes, 310 for $tRNA_1^{Met}$ and 170 for $tRNA_2^{Met}$[65].

Histone genes: In general, histone genes are clustered together in families and in many cases are tandemly repeated. The coding regions for five histone proteins are interdigitated with noncoding spacer sequences. Histone genes are highly repeated in sea urchin whereas only moderately repeated in other systems. While the repetition frequency is of the order of several hundred fold for various sea urchin species[66], for *Xenopus* it is 20–50 fold[67], for mouse 10–20 fold[68], for HeLa cells 3040 fold[69] and for chicken 10 fold[70]. In the case of the newt *Notophthalmus viridescens*, the histone gene cluster is 600–800 fold repeated. It is clear that although most vertebrates tend to have low copy number of the histone genes, exceptions, as in the case of newt, do occur. The genes coding for each of the five major histone proteins in sea urchin are interspersed with one another, the spacer DNA being located between each of the coding regions. In sea urchin, all five histone mRNAs hybridize within a single repeat unit, each one hybridizing only once per repeat unit.

Histone genes are present in the same order, are transcribed off the same strand and separated by similar lengths of A + T-rich spacer DNA. All these structural characteristics enable the organism to accomplish coordinate regulation of expression of all the histone genes. *L. pictus* proved to be different from other sea urchins in that although the two nonallelic major repeats of histone genes are homogenous in their coding sequence, they have divergent sequences in the spacers[71, 72]. The first histone 'orphon', a gene found outside the parental repeat, was observed for H3 gene of *L. pictus*[73]. However, histone genes organization has been studied from several other systems and they bear little resemblance to sea urchins. For instance it was found that histone genes are distributed on both the strands, thereby necessitating bidirectional transcription in the case of *Drosophila*[74], chicken[75], rice[76] and several other systems[41]. Histone genes are generally not split, however, H3 gene of chicken[7] and H3 and H4 genes of *N. crassa*[8] have been found to have intervening sequences.

The organization of histone genes in rice embryos was studied by using recombinant DNA technology. Rice DNA was restricted with *Bam*H1 and cloned in the plasmid pBR322. The clones containing the histone genes were identified by colony hybridization using radioactive labelled histone mRNAs as probe[77]. The size of the insert in one clone was 6.64 kb. A restriction map of the insert was determined. Messenger RNAs hybridized to this insert was translated into histones H2A, H2B and H4, thereby showing the presence of these genes on the inserted DNA. By translation of the mRNAs hybridized to *Eco*R1, *Pst*I and *Hpa*II restriction fragments as well as with the separated DNA strands, it was demonstrated that H2A and H2B histone genes are at one end of one strand and H4 histone gene is at the opposite end on the other strand. Thus H2A & H2B, and H4 histone genes in rice are transcribed in opposite directions[76]. The DNA from H2A histone gene end was sequenced[78]. Among the 320 nucleotide sequence obtained, typical eukaryotic homology blocks like CAT, TATA and the cap binding site were detected at the 5′ upstream region. In addition, it showed a GATCC motif which is specific for histone genes. The H2A coding sequence showed an abundance of basic amino acids and a tripeptide sequence Arg-Lys-Lys.

To obtain the histone gene cluster and other single copy genes, a large number of clones obtained by inserting larger size rice DNA fragments have to be screened. Hence the rice DNA fragments were cloned in lambda Charon phage DNA and *in vitro* packaged.

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ANNOUNCEMENT

NATIONAL SEMINAR ON HIMALAYAN CRYSTALLINES, METAMORPHICS AND STRUCTURES

With a view to arousing interest and capabilities amongst Himalayan Geologists for an integrated understanding the seminar mentioned above, is sponsored by the University Grants Commission and the Geological Society of India. The seminar will be held from 28th to 30th December, 1984. The venue is Department of Geology, University of Delhi. Interested scientists and scholars may correspond with Dr P. S. Saklani, Head of the Department of Geology, University of Delhi, Delhi 110 007 for further details.